MISSOURI VETERINARY EMERGENCY AWARENESS MANUAL

Introduction

The Missouri Veterinary Medical Association (MVMA), Missouri Department of Agriculture (MDA), and Missouri Department of Health and Senior Services (DHSS) are working collaboratively to develop the Missouri Veterinary Emergency Awareness Program. This is a training and information-sharing program for veterinarians that will allow them to respond to outbreaks of animal disease that might originate through natural means or by an act of bioterrorism. The program utilizes multiple methods of communication, including videoconferencing to regional sites across the state, personal presentations at MVMA district meetings, newsletter articles, direct mailings to veterinarians, and material made available on the World Wide Web.

The impetus for this training is the growing threat to animal and human health presented by emerging/re-emerging infectious diseases as well as by the threat of a bioterrorist act. The risk of disease introduction into populations of companion animals, livestock, and poultry has increased dramatically as the global movement of animals and people has accelerated. The impact of the introduction of disease into these populations could be catastrophic. For example, the occurrence of foot-and-mouth disease in the United States would have enormous economic ramifications and its effect on the livestock industry would involve a long period of recovery. Even if a foreign animal disease that might be introduced into our country could be eradicated, it would most likely be at a very high cost. The outbreak of exotic Newcastle disease in Southern California in 2003 was successfully controlled, but only because of early detection and extremely intensive investigation and follow-up.

It is estimated that 75 percent of the emerging/re-emerging pathogens are zoonotic. Therefore, the public health impact of these diseases must always be considered. In some instances, sick animals may serve as a source of infection for humans. At other times, both animals and humans might fall ill from a common environmental exposure. The detection of illness in animals may be used as a "sentinel" event, presenting a warning of possible impending human cases that could be prevented or minimized through implementation of effective intervention measures. This situation would have the highest likelihood of occurring in an urban area in which there are higher concentrations of people, companion animals, and veterinarians.

In addition to the spread of disease by natural mechanisms, the possibility of introduction of infectious disease by terrorist activity must also be considered. This problem is compounded by the fact that many potential agents of bioterrorism (e.g., anthrax, tularemia, plague) also occur naturally. This necessitates the rapid reporting and investigation of these cases (in animals and humans), so that naturally occurring cases can be differentiated from those caused by an act of bioterrorism. Since almost 80 percent of the Category A and B bioterrorist agents are zoonotic, investigations of animal and human cases of these diseases will often be closely linked to each other.

The purpose of this manual is to provide veterinarians with condensed reference material regarding the major high consequence livestock pathogens and potential agents of bioterrorism. It is not intended to be comprehensive either in the number of diseases included in the manual or in its treatment of individual diseases. The manual consists primarily of fact sheets derived from the Iowa State University Center for Food Security and Public Health (CFSPH), which is a Centers for Disease Control and Prevention (CDC) Center for Public Health Preparedness. The CFSPH is CDC's only Center for Public Health Preparedness that focuses on veterinary medicine and zoonotic diseases.

Additional materials in the manual include:

➢ Emergency response protocol for reporting foreign animal diseases.

➢ Listing of reportable diseases.

➢ Summary of general signs of reportable diseases in animals and poultry.

➢ Recommendations regarding biosecurity measures, methods for protecting livestock operations, and use of disinfectants.

➢ Reference chart to distinguish exotic Newcastle disease from highly pathogenic avian influenza.

➢ Reference chart to distinguish various vesicular diseases.

➢ Photographs to help in the diagnosis and differentiation of various infectious diseases, particularly vesicular diseases.

Recommendations and comments pertaining to the content of this manual may be addressed to representatives from the MVMA (telephone 573-636-8612), MDA (telephone 573-751-3377), and DHSS (telephone 573-751-6231).

The use of trade names in this manual is for the information of the reader. Such use does not constitute an official endorsement or approval by the MVMA, MDA, or DHSS.

Acknowledgements

➢ Photographs of animal diseases in this manual are from Foreign Animal Diseases (the "Gray Book"), 7th Ed., United States Animal Health Association (USAHA), 4221 Mitchell Avenue, Saint Joseph, MO, 64507. The participation of the USAHA is gratefully acknowledged. Other information on foreign animal and zoonotic diseases is available on the Internet at www.usaha.org.

➢ Technical Factsheets and Fast Fact Disease Summaries have been generously provided by the Iowa State University Center for Food Security and Public Health (CFSPH). In the interest of space and economy of printing, complete lists of references are not included for all of these documents. Complete lists of references are available from the CFSPH on the Internet at http://www.cfsph.iastate.edu. The cooperation of the CFSPH in production of this manual is greatly appreciated.

Third Edition, 2013

Changes to this edition include updated Technical Factsheets and the addition of Fast Fact Disease Summaries from the Iowa State CFSPH in Sections 1 and 2, and updated emergency reporting procedures and updated Vesicular Diseases Reference Chart in Section 3.

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*The classification system chosen for this manual consists of the primary Category A and Category B bioterrorist agents (Section I), as listed by the Centers for Disease Control and Prevention, plus additional high-consequence livestock pathogens (Section II). These listings are not mutually exclusive, as some "high-consequence livestock pathogens" could be classified as "potential bioterrorist agents" and vice versa.

Disease From Potential Bioterrorist Agents

Anthrax

*Woolsorters' Disease,
Cumberland Disease,
Maladi Charbon,
Malignant Pustule,
Malignant Carbuncle,
Milzbrand, Splenic Fever*

Last Updated: March 2007

Importance

Anthrax is a serious zoonotic disease that can affect most mammals and several species of birds, but is particularly important in herbivores. This disease is caused by a spore-forming bacterium, *Bacillus anthracis*. Anthrax spores are extremely resistant to inactivation by heat or chemicals, and can survive in the environment for decades. Susceptibility to clinical disease varies, with domesticated and wild ruminants most susceptible, horses somewhat less susceptible, and omnivores and carnivores relatively resistant. In endemic regions, anthrax can be a serious problem in unvaccinated ruminants. Although antibiotics may be effective if started early, the course of disease is usually rapid in these animals, and symptomatic infections are often fatal. Epizootics in wildlife are also a concern. In 2004, an outbreak in the Malilangwe Wildlife Reserve in Zimbabwe killed almost all of the approximately 500 kudu in the reserve, as well as large numbers of other wild ruminants.

Human cases usually develop after exposure to infected animals and their tissues. In most countries, human anthrax occurs infrequently and sporadically, mainly as an occupational hazard among veterinarians, agricultural workers and workers who process hides, hair, wool and bone products. In humans, the three forms of anthrax are cutaneous, gastrointestinal and inhalational. Cutaneous anthrax accounts for more than 95% of natural infections, and is rarely fatal if treated with antibiotics. The gastrointestinal form is less common but more serious, and can occur in outbreaks associated with contaminated meat. Inhalational anthrax is the most serious form, and has a very high case fatality rate even when treated. Natural cases of inhalational anthrax are rare; however, anthrax has been used as a weapon by bioterrorists, and weaponized anthrax can form aerosols readily. In 2001, weaponized anthrax was delivered in letters through the United States mail, resulting in 11 cases of inhalational anthrax and 11 cases of cutaneous anthrax. Five people with inhalational anthrax died. Because ruminants are particularly sensitive to anthrax, widespread disease in animals might serve as an early warning of a bioterrorist attack under some circumstances.

Etiology

Anthrax results from infection by *Bacillus anthracis*, a spore forming, Gram positive aerobic rod in the family Bacillaceae. Fully virulent *B. anthracis* isolates have two plasmids: pX01, which codes for a tripartite protein exotoxin complex, and pX02, which encodes the capsule genes. *B. anthracis* is genetically very homogeneous; however, researchers have identified several genetically distinct groups that appear to be derived from clones. Some of these clones are distributed worldwide, while others are found in limited geographic areas.

B. anthracis is a member of the *Bacillus cereus* group, which also contains *B. cereus* and *Bacillus thuringiensis*. These three organisms are very closely related. Based on genetic analysis, some authors consider them to be a single species; however, this idea is controversial. Plasmids closely related to pX01 and/or pX02 have recently been found in a few *B. cereus* isolates that caused anthrax-like diseases in people, chimpanzees or gorillas.

Geographic Distribution

Although *B. anthracis* can be found worldwide, anthrax cases usually occur only in limited geographic regions. Outbreaks are most common in areas characterized by alkaline, calcareous soil, a warm environment, and periodic episodes of flooding. Anthrax is particularly common in parts of Africa, Asia and the Middle East. In the United States, this disease has been reported from most states, but it occurs most often in the Midwest and West. Endemic foci are currently located in the Dakotas, Texas, northwest Minnesota and Nevada, with smaller areas in other U.S. states.

Transmission

In animals, transmission occurs by ingestion and possibly inhalation of spores, although entry through skin lesions has not been ruled out. Herbivores usually become infected when they ingest sufficient numbers of spores in soil or on plants in pastures. Outbreaks are often associated with heavy rainfall, flooding or drought. Contaminated

bone meal and other feed can also spread this disease. Carnivores usually become infected after eating contaminated meat. Vultures and flies may disseminate anthrax mechanically after feeding on carcasses.

In infected animals, large numbers of bacteria are present in hemorrhagic exudates from the mouth, nose and anus. When they are exposed to oxygen, these bacteria form endospores and contaminate the soil. Sporulation also occurs if a carcass is opened; however, this process requires oxygen and does not occur inside a closed carcass. Anthrax spores can remain viable for decades in the soil or animal products such as dried or processed hides and wool. Spores can also survive for two years in water, 10 years in milk and up to 71 years on silk threads. Vegetative organisms are thought to be destroyed within a few days during the decomposition of unopened carcasses.

Humans usually develop the cutaneous form of anthrax after skin contact with infected animal tissues such as hides, wool, bone meal and blood. Experiments in mice suggest that anthrax spores may germinate and enter the skin in abraded but not intact skin. Biting flies that feed on infected animals or carcasses may be able to transmit this form mechanically. Inhalational anthrax occurs after inhaling spores from animal products, laboratory cultures or other sources. Gastrointestinal anthrax results from the ingestion of raw or undercooked meat containing viable spores.

Anthrax has been weaponized by a number of countries. This agent has been used by terrorists. Weaponized anthrax forms aerosols readily and is often inhaled, but cutaneous exposure or ingestion can also occur.

Ecology

The ecology of anthrax is controversial. *B. anthracis* has long been considered an "obligate pathogen." Unlike other members of the genus *Bacillus*, which are saprophytes, *B. anthracis* is thought to multiply almost exclusively inside the body. In the environment, it may exist only as dormant spores. If this idea is correct, spores originally derived from carcasses are the only source of exposure for animals, although carnivores, rain and other agents can disperse the spores to other locations. Heavy rains, alternating with dry periods, may concentrate the spores and result in outbreaks among grazing animals. Alternatively, the "incubator hypothesis" suggests that anthrax spores can germinate and divide to a limited extent in the environment, if certain conditions are met. This is thought to increase the concentration of *B. anthracis* in "incubator areas," where outbreaks then occur. Although the incubator hypothesis is controversial, *B. anthracis* spores were recently shown to germinate on and around the roots of grass in a simple plant/ soil system. Plasmid transfer between *B. anthracis* isolates was also described in this system. As of March 2007, spore germination on plant roots had not been described outside the laboratory.

Disinfection

Anthrax spores are resistant to heat, sunlight, drying and many disinfectants. They can be killed with formaldehyde or 2% glutaraldehyde; overnight soaking is recommended. A 10% NaOH or 5% formaldehyde solution can be used for stockyards, pens and other equipment. Sodium hypochlorite has also been recommended for some purposes. The sporicidal effectiveness of hypochlorite solutions varies with the pH and the concentration of free available chlorine. To become an effective sporicidal agent, household bleach must be diluted with water to increase the free available chlorine, and adjusted to pH 7. Prolonged contact is recommended. Gaseous sterilization can be accomplished with chlorine dioxide, formaldehyde gas and other methods, under specific conditions of humidity and temperature. Sterilization is also possible by heating to 121°C (250°F) for at least 30 min. Gamma radiation has been used to decontaminate animal products, as well as mail from contaminated postal facilities. Exposed arms and hands can be washed with soap and hot water then immersed for one minute in a disinfectant such as an organic iodine solution or 1 p.p.m. solution of mercuric perchloride. Clothing should be cleaned and boiled.

There is little information on the time and temperatures needed to destroy *B. anthracis* spores in food; however, these spores are less resistant to heat inactivation than *Clostridium botulinum* spores, and methods to kill botulism spores are probably sufficient to destroy anthrax spores.

Comprehensive information regarding the efficacy of specific sterilization methods for *B. anthracis* and other *Bacillus* species is available at http://www.cdc.gov/ncidod/eid/vol9no6/02-0377.htm. Disinfectant sensitivity information for *B. anthracis* is limited*;* however, a recent study suggests that decontamination data for solid surfaces can be extrapolated from closely related *Bacillus* species.

Infections in Humans

Incubation Period

The incubation period in humans is usually 1 to 7 days, but varies with the form of the disease. Typically, symptoms of cutaneous anthrax appear after 2 to 3 days. The incubation period for the gastrointestinal form is usually 2 to 5 days, but may be as short as 15 hours. In one outbreak, the mean incubation period for the oropharyngeal form of gastrointestinal anthrax was 42 hours (range 2-144 hours). The incubation period for inhalational anthrax is highly variable. This disease can appear after two days, but spores may remain viable in the lungs for several weeks, and they can germinate and cause inhalational anthrax during that time. After accidental aerosol release in the Soviet Union, cases continued to appear for up to six weeks.

Clinical Signs

Three forms of disease are seen in humans: cutaneous anthrax, gastrointestinal anthrax and inhalational anthrax.

Cutaneous anthrax is characterized by a papular skin lesion, which becomes surrounded by a ring of fluid-filled vesicles. The central papule ulcerates, dries and develops a firmly adherent, depressed black scab. The skin lesion is usually painless, but it is often surrounded by significant edema. There may be regional lymphadenopathy. Swelling on the face or neck may occlude the airways. Lesions on the face or neck can also develop into meningitis. Fever, pus and pain are seen only if secondary infections occur. Cutaneous lesions often resolve spontaneously but disseminated, fatal infections occur in approximately 20% of cases. Small anthrax lesions usually heal with minimal scarring.

Gastrointestinal anthrax develops after eating contaminated meat. When spores germinate in the intestinal tract, they cause ulcerative lesions. These lesions can occur anywhere and may, in severe cases, result in hemorrhage, obstruction or perforation. There is limited information on this form of anthrax, but reported cases range from asymptomatic infections to fatal disease. Gastrointestinal anthrax has been divided into two syndromes: abdominal and oropharyngeal anthrax. The initial symptoms of the abdominal form may be mild and can include malaise, a low fever and mild gastrointestinal symptoms such as nausea, vomiting, diarrhea and anorexia. This may be followed by the acute onset of severe gastrointestinal symptoms such as severe abdominal pain, hematemesis and bloody diarrhea. Massive ascites can also occur. In addition, there may be high fever, dyspnea, cyanosis, disorientation and other signs of septicemia. Severe gastrointestinal anthrax rapidly progresses to shock, coma and death. Abdominal anthrax may not always be severe. In an outbreak in Thailand, seven of 74 people with gastrointestinal anthrax had severe symptoms; acute diarrhea was the only symptom in the others.

The oropharyngeal form is little known. The initial symptoms may include a sore throat, dysphagia, fever, hoarseness and swelling of the neck. The neck swelling is caused by edema and cervical lymphadenopathy, and can result in airway compromise. Mouth lesions have been reported on the tonsils, pharynx and hard palate. In one report, these lesions were initially edematous and congested. A central whitish area, caused by necrosis and ulceration, developed by the end of the first week. During the second week, a pseudomembrane developed over the ulcer.

Inhalational anthrax occurs after inhaling spores. The clinical signs develop gradually and are nonspecific. Early symptoms may include fever, chills, tiredness and malaise; a nonproductive cough and mild chest pain may be present. The symptoms sometimes improve for several hours to three days. The prodromal period ends with the acute onset of severe respiratory distress, tachycardia, diaphoresis, stridor and cyanosis, followed by fatal septicemia and shock within one to two days. Hematogenous spread of *B. anthracis* can also cause gastrointestinal lesions and symptoms.

Anthrax meningitis can be a complication of any of the other three forms of disease. After a prodromal period of 1-6 days, typical signs of meningoencephalitis develop rapidly. Patients quickly lose consciousness and die, many within 24 hours. Blood is often found in the cerebrospinal fluid.

Communicability

Person to person transmission of anthrax is extremely rare and has been reported only in cases of cutaneous anthrax.

Diagnostic Tests

Anthrax is often diagnosed by finding the characteristic organisms in clinical samples or by isolating *B. anthracis* in culture. Blood, fluid samples from skin lesions, aspirates of lymph nodes or spleen, ascitic fluid, pleural effusions or cerebrospinal fluid (in cases of meningitis) are stained with polychrome methylene blue (M'Fadyean's stain). Using this stain, *B. anthracis* organisms are square-ended, blue-black bacilli surrounded by a pink capsule. In a Gram stain, *Bacillus anthracis* is a large Gram positive rod that may occur singly, in pairs or in chains . Spores are not found in host tissues unless they have been exposed to air.

B. anthracis colonies on blood agar are white or gray, flat, approximately 3-5 mm in diameter and nonhemolytic, with a rough, ground-glass appearance and a very tacky, butyrous consistency. Tails - wisps of growth trailing back toward the parent colony – may sometimes be seen; this characteristic has been described as a "medusa head" appearance. Unlike the other members of the *B cereus* group, *B anthracis* is non-motile. Capsules are not found when the this organism is grown aerobically *in vitro,* but may be demonstrated in mucoid colonies from cultures grown on nutrient agar with 0.7% sodium bicarbonate, incubated overnight under CO_2. *B. anthracis* is also susceptible to specific bacteriophages (the gamma bacteriophage) and exhibits a characteristic 'string–of–pearls' formation when grown with penicillin; however, the latter characteristics may be absent with some isolates. Antibiotic treatment of patients may prevent isolation of the organism.

Polymerase chain reaction (PCR) techniques can also be used to identify *B. anthracis*.

Antibodies develop late in the course of disease, and serology is only useful in retrospective studies. Both acute and convalescent sera must be taken. Serologic tests include enzyme linked immunosorbent assays (ELISAs) and other tests. Approximately 68-92% of patients with cutaneous anthrax develop antibodies to protective antigen or the capsule. A skin hypersensitivity test using AnthraxinT is used in some countries for retrospective diagnosis.

Treatment

Natural strains of *B. anthracis* are usually susceptible to several antibiotics; most but not all natural strains are susceptible to penicillin. Some strains, particularly those used in bioterrorist attacks, may be resistant to penicillin. For this reason, the U.S. Centers for Disease Control and Prevention (CDC) recommends other antibiotics as the initial treatment, particularly for systemic disease, until antibiotic susceptibility has been determined. Antibiotics are effective only against the vegetative stage of *B. anthracis*, and not against spores. Treatment is continued for at least 60 days in inhalational anthrax, as spores may be able to remain dormant in the lungs and germinate during that time. Supportive therapy may also be necessary, particularly for the inhalational and gastrointestinal forms.

Effective treatment depends on early recognition of the symptoms: treatment for cutaneous anthrax is usually effective, but the inhalational and gastrointestinal forms are difficult to recognize early and the mortality rates are higher.

Prevention

Humans can be protected by preventing disease in animals. Veterinary supervision of animal production and slaughter also helps prevent contact with infected livestock or animal products. Trade restrictions may be placed on certain animal products from countries where anthrax is common and uncontrolled. Improvements in industry standards have decreased the occupational risks for people exposed to imported hides, wool, bone meal, and other animal products. In laboratories, good safety practices, including the use of biological safety cabinets, should be employed. Veterinarians should use protective clothing and equipment when examining sick animals. They should also avoid opening the carcasses of suspected cases. Vaccines are available for humans at a high risk of infection. Human anthrax vaccines in the U.S. are based on an inactivated, cell-free extract of cultivated *B anthracis*. New vaccines are in development.

Postexposure antibiotic prophylaxis is recommended for people who have been exposed to aerosolized anthrax spores. Treatment is continued for at least 60 days in inhalational anthrax, as spores may remain dormant in the lungs and germinate during that time. Simultaneous antibiotics and vaccination can be used in humans exposed to aerosols, as human anthrax vaccines are not live. Postexposure prophylaxis may also be needed for anyone who has eaten contaminated undercooked or raw meat. It is not generally recommended after cutaneous exposure; however, any exposed areas should be washed immediately, and the skin should be monitored for early signs of infection.

Morbidity and Mortality

Anthrax is still a significant risk in some countries, and outbreaks occasionally occur in humans. In Africa, estimates suggest that each cow with anthrax can result in up to ten human cases. However, the incidence of anthrax has declined sharply in developed nations. In the U.S., approximately 130 human cases occurred annually during the early 1900's, but only one or two cases of cutaneous anthrax are now generally seen in a year. In many countries, cases of anthrax occur infrequently and sporadically, mainly as an occupational hazard among veterinarians, agricultural workers, and workers who process hides, hair, wool and bone products.

The cutaneous form accounts for at least 90-95% of natural anthrax infections. The gastrointestinal form seems to be uncommon, but can occur in outbreaks associated with contaminated meat. Natural cases of inhalational anthrax are rare; however, aerosolized biological weapons would be expected to produce a high percentage of this form. In 2001, 11 cases of inhalational anthrax and 11 cases of cutaneous anthrax were associated with a bioterrorist attack via anthrax-contaminated mail.

The mortality rate varies with the form of the disease. Cutaneous anthrax is thought to be fatal in 5-20% of untreated cases, and less than 1% of patients treated with antibiotics. In contrast, the mortality rate is high for inhalational anthrax, even when treated appropriately. Earlier estimates suggested that the case-fatality rate for this form approached 90-100% but newer, more intensive treatment regiments may decrease the mortality rate. In the 2001 mail-associated bioterrorist attack, six of eleven patients with inhalational anthrax recovered with treatment (case fatality rate of 45%). However, once a patient reaches the fulminant stage, one study suggests that the mortality rate is 97% regardless of treatment. Anthrax meningoencephalitis is also deadly, with an estimated case fatality rate of 95-100%.

Only limited information exists for gastrointestinal anthrax. The case fatality rate for the abdominal form is unknown, but it is estimated to be from 25% to 60-75%. Asymptomatic or mild infections have been described among adults in some outbreaks, with higher mortality rates in children. In one report from Uganda, gastroenteritis occurred in 134 of 155 people (92%) who ate meat from an infected zebu. Twelve adults remained asymptomatic. Nine deaths occurred, all in children. The remaining people were treated with antibiotics and rehydration therapy, and all recovered. The overall case fatality rate in this outbreak was 7%. In Thailand, 28 cases of cutaneous anthrax and 74 cases of gastrointestinal anthrax were reported in one outbreak. Seven people with gastrointestinal anthrax had severe symptoms; acute diarrhea was the only symptom in the remainder. Three patients died, for a case-fatality rate of 4%. Reports of the oropharyngeal form of gastrointestinal anthrax are rare; however, published case fatality rates range from 12% during an outbreak in Thailand to 50% in a report from Turkey.

Infections in Animals

Species Affected

Virtually all mammals and some birds can contract anthrax, but susceptibility varies widely and most clinical cases occur in wild and domesticated herbivores. Cattle, sheep and goats are considered to be highly susceptible, and horses somewhat less so. Pigs, other omnivores and carnivores are more resistant to disease, but they may become ill if the dose is high. Birds are highly resistant.

Incubation Period

The incubation period varies from 1 to 20 days. In herbivores, infections become apparent after 3 to 7 days. The incubation period in pigs is usually 1 to 2 weeks.

Clinical Signs

In animals, anthrax can be a peracute, acute, subacute or chronic disease.

In ruminants, peracute systemic disease is common, and sudden death may be the only sign. Staggering, trembling and dyspnea may be seen in some animals, followed by rapid collapse, terminal convulsions and death. In the acute form, clinical signs may be apparent for up to 2 days before death. In this form, fever and excitement may be followed by depression, stupor, disorientation, muscle tremors, dyspnea and congested mucous membranes. Pregnant cows may abort, and milk production can drop severely. Bloody discharges from the nose, mouth and anus are sometimes seen. Occasionally, infections in ruminants are characterized by subcutaneous edematous swellings, most often in the ventral neck, thorax and shoulders Anthrax in wild herbivores varies with the species, but tends to resemble the disease in cattle.

Horses typically develop acute disease. Common symptoms in this species include fever, chills, anorexia, depression, severe colic and bloody diarrhea. Swellings may be seen in the neck, sternum, lower abdomen and external genitalia. Dyspnea can occur due to the swelling of the neck. Affected animals usually die within 1 to 3 days but some animals can survive up to a week.

Septicemia and sudden death occurs occasionally in pigs. More often, pigs have mild subacute to chronic infections characterized by localized swelling and systemic signs such as fever and enlarged lymph nodes. Some animals develop rapidly progressive swelling of the throat, with dyspnea and difficulty swallowing; these animals may suffocate. Intestinal involvement with anorexia, vomiting, diarrhea or constipation is less common. Some pigs with anthrax recover. Recovered, asymptomatic animals may have signs of localized infections in the tonsils and cervical lymph nodes at slaughter.

Clinically apparent anthrax in dogs, cats and wild carnivores resemble the disease in pigs. A recent review of published cases in dogs suggests that massive swelling of the head, neck and mediastinum is the most common symptom in this species. In the published cases, death was usually the result of toxemia and shock, but swelling of the threat and suffocation could also have been a factor. Hemorrhagic gastroenteritis was reported in one dog, in addition to a swollen foreleg and ptyalism. Severe acute gastroenteritis has also been reported in other carnivores and omnivores.

Communicability

Large numbers of bacteria are present in the carcass and in bloody discharges from body openings. These bacteria can contaminate the environment or be a source of exposure for humans and other animals. Skin and wool can contain spores, which remain viable for long periods.

Post Mortem Lesions   Click to view images

Rigor mortis is usually absent or incomplete, and the carcass is typically bloated and decomposes rapidly. Dark, tarry blood may ooze from the body orifices; some sources suggest this is not a common sign. Edema may be noted, particularly around the throat and neck, in horses. **Necropsies should be avoided, to prevent contamination of the surrounding area with spores.**

If a ruminant carcass is opened, signs of septicemia will be evident. The blood is dark, thick and does not clot readily. Edematous, blood-tinged effusions may be seen in the subcutaneous tissues, between skeletal muscles and under the serosa of organs. Petechiae and ecchymoses are often noted in the lymph nodes, the serosal surfaces of the abdomen and thorax, and the epicardium and endocardium. Hemorrhages and ulcers are also common in the intestinal mucosa; ulcers occur most often over Peyer's patches, but can also be found in other locations. Peritonitis and excessive peritoneal fluid may be noted. The spleen is usually enlarged and has a 'blackberry jam' consistency. The lymph nodes, liver and kidneys may be swollen and congested. Meningitis can also occur. Similar internal lesions can be seen in some horses; in others, the lesions may be limited to edema of the neck and throat.

Septicemic lesions may also be found in omnivores and carnivores, but are less common than edema and inflammation of the pharyngeal area, or gastrointestinal lesions. Pigs with chronic anthrax usually have lesions only in the pharyngeal area. The tonsils and cervical lymph nodes are typically enlarged and have a mottled salmon to brick-red color on cut surface. The tonsils may be covered by diphtheritic membranes or ulcers. The pharynx is usually edematous. A chronic intestinal form, with inflammation and lesions in the mesenteric lymph nodes, is also reported in pigs. Severe gastrointestinal inflammation, sometimes accompanied by hemorrhages and necrosis, has been reported in some omnivores and carnivores. Peritonitis can also occur.

Diagnostic Tests

A presumptive diagnosis can be made if the characteristic bacteria are found in blood or other tissues. Blood clots poorly in anthrax, and samples may be obtained by making a small cut in an ear vein or with a syringe from any available vein. In pigs, bacteremia is rare and a small piece of aseptically collected, affected lymphatic tissue is often used. Tissue aspirates and pharyngeal swabs have been examined in some animals. Air-dried, fixed smears should be stained with polychrome methylene blue (M'Fadyean's stain) or Giemsa stain. With M'Fadyean's stain, *B. anthracis* organisms are square-ended, blue-black bacilli surrounded by a pink capsule. In a Gram stain, *Bacillus anthracis* is a large Gram positive rod that may occur singly, in pairs or in chains. Endospores are not found in host tissues unless they have been exposed to air. Antibiotic treatment may result in false negatives.

Bacterial culture may be used for diagnosis. After overnight incubation, *B. anthracis* colonies on blood agar are white or gray, approximately 3-5 mm in diameter, and nonhemolytic, with a rough, ground-glass appearance and a very tacky, butyrous consistency. Tails – wisps of growth trailing back toward the parent colony – may sometimes be seen; this characteristic has been described as a "medusa head" appearance. Capsules are not found when this organism is grown aerobically *in vitro,* but may be demonstrated in mucoid colonies from cultures grown on nutrient agar with 0.7% sodium bicarbonate, incubated overnight at 37ºC (98.6°F) under CO_2. Capsules can also be induced by incubating the bacteria in blood for several hours. Unlike the other members of the *B cereus* group, *B anthracis* is non-motile. *B. anthracis* is susceptible to specific bacteriophages (the gamma bacteriophage) and exhibits a characteristic 'string-of-pearls' formation when grown with penicillin; however, the latter characteristics may be absent with some isolates.

In decomposing carcasses, putrefactive bacteria may outcompete and eliminate *B. anthracis* inside the body. In this case, anthrax may be confirmed by isolatng the organism from soil contaminated by the terminal discharges. However, recovery of this organism from decomposed carcasses, processed animal products such as bone meal or hides, and environmental samples can be difficult, and may require specialized laboratory procedures.

PCR is also used for diagnosis. This technique detects the bacterial toxin and capsule genes. Mouse or guinea pig inoculation to confirm virulence has largely been replaced by PCR; however, animal tests may be considered if other tests have failed. Although it has been superceded in many locations, some countries use a thermoprecipitin test (Ascoli test) to detect thermostable anthrax antigens in decomposed carcasses and animal products. Research laboratories may use immunofluorescence to detect *B. anthracis* capsules in blood or tissues, but this method is not generally used for diagnosis.

Immunoblotting (Western blotting) and ELISAs are available; however, serology is mainly used in research and rarely used for diagnosis. A skin hypersensitivity test using AnthraxinT is widely used in some countries for the retrospective diagnosis of anthrax in animals and humans.

Treatment

Antibiotics may be effective if treatment is started early. Supportive therapy may also be necessary.

Prevention

In endemic areas, modified live vaccines can prevent anthrax in livestock. Livestock are vaccinated annually, before the season when outbreaks generally occur. Livestock vaccines have also been used to protect cheetahs and endangered ruminants including black rhinoceros.

Anthrax is a reportable disease. Quarantines, effective carcass disposal techniques, and decontamination can help prevent dissemination during outbreaks. Sick animals should be isolated. To prevent sporulation, carcasses should not be opened. Scavengers should also be prevented from accessing the carcass. Local regulations determine carcass disposal; however, incineration is considered to be the most effective disposal method for carcasses, contaminated manure, bedding and other materials. Deep burial may also be used, but is less desirable. Barns, pens and equipment should be cleaned and disinfected. Once the soil has been contaminated by spores, it is very difficult to decontaminate; however, procedures such as soil removal and/or treatment with formaldehyde may be used in some circumstances. Insect repellents help prevent flies from spreading the organism. If a pet has been exposed to anthrax, the fur should be decontaminated by repeated bathing to mechanically remove the organism.

During an outbreak, prophylactic antibiotics are given to exposed and at-risk animals. Prophylactic treatment is well understood for outbreaks due to natural causes, but prolonged treatment could be necessary in a bioterrorist attack with aerosolized spores. Simultaneous vaccination and antibiotic treatment are not used in animals, because animal vaccines are live. However, animals can be vaccinated after antibiotic treatment. Grazing animals should be moved away from areas of possible contamination, and contaminated feed should be removed.

Good hygiene should be observed by anyone exposed to infected animals or contaminated areas, both to avoid spreading the disease and for personal protection.

Morbidity and Mortality

The worldwide incidence of anthrax is difficult to determine; however, this disease has been reported from nearly every continent. Outbreaks occur periodically in some countries. These epizootics may be seen in domesticated or wild animals, and are typically associated with droughts, heavy rains or flooding. Outbreaks are uncommon among domesticated animals in developed

nations. Approximately 25 outbreaks were reported in the U.S. between 1994 and 2000. Sporadic cases occur between outbreaks. Sporadic cases of anthrax are reported almost every year in domesticated or wild animals in the U.S.; the estimated annual mortality rate in U.S. livestock is one animal per one million animals at risk. Recently, more severe outbreaks have been reported on game ranches with non-traditional species, such as white-tailed deer. This may be related to difficulties in vaccinating exotic ungulates.

The mortality rate for anthrax varies with the species. Clinical infections in ruminants and horses are usually fatal; pigs often recover. In carnivores, mortality is also relatively low. Mortality rates are not widely available for wild animals; however, in 2004, an outbreak in the Malilangwe Wildlife Reserve in Zimbabwe killed almost all of the approximately 500 kudu in the reserve, as well as 68% of the nyala (*Tragelaphus angasi*), 48% of the bushbuck (*Tragelaphus scriptus*), 44% of the waterbuck (*Kobus ellipsiprymnus*) and 42% of the roan antelope (*Hippotragus equinus*). Approximately 6% of the buffalo (*Syncerus caffer*) in the area also died.

Internet Resources

American College of Physicians. Physicians Information and Education Resource (PIER). Anthrax
http://pier.acponline.org/physicians/public/d892/d892.html

Centers for Disease Control and Prevention (CDC)
http://www.cdc.gov/nczved/divisions/dfbmd/

CDC Emergency Response and Preparedness: Anthrax
http://www.bt.cdc.gov/agent/anthrax/

Food and Agriculture Organization of the United Nations. Manual on Meat Inspection for Developing Countries
http://www.fao.org/docrep/003/t0756e/t0756e00.htm

Medical Microbiology
http://www.ncbi.nlm.nih.gov/books/NBK7627/

Public Health Agency of Canada.
Material Safety Data Sheets
http://www.phac-aspc.gc.ca/msds-ftss/index.html

Spotts Whitney EA et al. Inactivation of *Bacillus anthracis* spores.
http://www.cdc.gov/ncidod/eid/vol9no6/02-0377.htm

The Merck Manual
http://www.merck.com/pubs/mmanual/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

World Health Organization. Guidelines for the Surveillance and Control of Anthrax in Humans and Animals
http://www.who.int/csr/resources/publications/anthrax/WHO_EMC_ZDI_98_6/en/

World Organization for Animal Health (OIE)
http://www.oie.int/

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

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Botulism

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Importance

Botulism is caused by botulinum toxins, neurotoxins produced by *Clostridium botulinum* and a few other species of *Clostridium*. By binding to nerve endings, these toxins cause progressive flaccid paralysis in humans and animals. Many untreated cases end in death from paralysis of the respiratory muscles. *C. botulinum* spores are common in the environment, but they can germinate and grow only in anaerobic environments under specific conditions. Foodborne botulism results from the ingestion of the preformed toxin after the organism has grown in food. Botulinum-producing organisms may also grow in the immature gastrointestinal tracts of human infants and foals, in human gastrointestinal tracts with certain abnormalities, and in anaerobic wounds. In addition, these toxins are a concern in bioterrorism.

Sporadic cases and outbreaks of botulism occur in both humans and animals. This disease is an important cause of death in unvaccinated ranched mink, and it can cause large outbreaks among wild birds such as waterfowl and gulls. Livestock may be accidentally fed the toxin in contaminated feed. Botulism seems to be increasing in cattle, possibly due to the increased use of plastic-packaged grass silage, and these outbreaks can cause significant economic losses. Cattle in areas with phosphorus-deficient soils may also chew on toxin-contaminated bones and scraps of flesh in the environment to satisfy the deficiency. In Senegal, which has such soils, botulism is thought to cause more deaths than any other cattle disease. In addition, botulinum has been reported in a variety of other species including poultry, dogs, cats, foxes, captive lions and sea lions, turtles, farmed fish and wild bighorn sheep. Botulism can be treated successfully, but patients may require weeks or months of intensive care, sometimes including mechanical ventilation, while the nerve endings regenerate. Treatment may be impractical in adult livestock unless the case is mild.

Etiology

Botulism is caused by botulinum toxin, a potent neurotoxin produced by *Clostridium botulinum*, a few strains of *C. baratii* and *C. butyricum*, and the recently reclassified species *C. argentinense* (formerly known as the type G toxin producing strains of *C. botulinum*). All of these organisms are anaerobic, Gram-positive, spore-forming rods.

The organisms that produce botulinum toxin are diverse, and can produce seven types of toxins (A through G). Researchers have also described a mosaic C/D toxin from outbreaks in birds. Most clostridial strains only produce one toxin type. All of the botulinum toxins cause the same clinical signs, although there may be some differences in the severity of the disease. However, knowing the toxin type is important in selecting an antiserum for treatment; antiserum produced against one type is not protective against others. Different toxin types also tend to cause botulism in different species. In people, botulism is usually caused by types A, B and E, although rare cases or outbreaks caused by types C, D, F and G have been described. Types C and D are the most common causes of disease in other mammals and birds, but types A, B and E can also be involved. Type C is especially common in birds, mink, horses in most parts of the world, cattle that have been fed poultry litter, and dogs that have eaten contaminated bird carcasses. Type C or type D may be found in livestock that have eaten feed contaminated with the carcasses of small animals. Types B and A are reported regularly from horses in the U.S., and can also affect other species. Type E toxin is often associated with aquatic environments, and can cause botulism in farmed rainbow trout (*Oncorhynchus mykiss*) and other fish, as well as in fish-eating birds. In addition to botulinum toxin, *C. botulinum* type C can produce a C2 toxin, which is an enterotoxin and causes gastrointestinal signs.

The species *C. botulinum* is very diverse, and has been divided into four genotypically and phenotypically distinct groups. In humans, botulism is usually caused by group I or group II organisms. Group I contains proteolytic strains that produce toxins A, B or F, while group II consists of nonproteolytic strains that make B, E or F toxins. Group I and II *C. botulinum* strains differ in heat resistance (spores from group I organisms are more heat resistant), growth temperatures, and other characteristics, which can influence the types of foods where they tend to grow.

Group III *C. botulinum* strains, which produce toxins C or D, are usually associated with botulism in animals. Group IV *C. botulinum* produces the type G toxin and has been reclassified as the new species *Clostridium argentinense*. This organism has caused a single outbreak among humans in Switzerland. *C. butyricum* produces type E toxin, and *C. baratii* produces type F.

Equine grass sickness

Botulinum toxin has also been implicated in equine grass sickness, a neurodegenerative disease that occurs among grazing equids. This disease, which is often fatal, is seen most often in the United Kingdom, but it has also been reported from other locations. A very similar disease occurs in South America, where it is called mal seco. Equine grass sickness affects the gastrointestinal tract and some other organs, can be acute or chronic, and tends to be seen in young horses on pastures in the spring. Its cause is still uncertain, but *C. botulinum* toxins have been implicated.

Geographic Distribution

C. botulinum is found worldwide. Although botulism can occur anywhere, the distribution of the organism is not homogeneous, and cases tend to be more common in certain geographic areas. Environmental factors can also influence where botulism is seen. For example, this disease tends to common in cattle from areas with phosphorus-poor soils, such as those in southern Africa.

The distribution of strains can also vary with the geographic area. In one study from the U.S., strains that produce type A toxin were detected mainly in neutral or alkaline soils in the western states, while type D-producing strains were found in some alkaline soils in these states. The distribution of type B strains was more uniform, but these organisms were especially common in the eastern states. Type C strains were detected in acidic soils in the Gulf Coast states. In North America, type E strains are most common along the shores of the Great Lakes and in the Pacific Northwest. In one study from the former U.S.S.R., strains that produced type E toxin accounted for 61% of the isolations. Knowing the toxin types that are prevalent in an area may be helpful in selecting an antitoxin, as this must often be done before the laboratory results are complete.

Transmission

All species of *Clostridium* can produce spores, dormant forms of the organism that are highly resistant to disinfectants, heat and environmental conditions that kill vegetative cells. These spores can survive for many years until favorable conditions allow them to germinate and grow. *C. botulinum* spores are common in the environment. In soil studies, this organism was detected in approximately 18-23% of the samples in the U.S. and 10% of the samples in the former U.S.S.R. In addition to soils, *C. botulinum* can be found in sediments in lakes, streams and coastal waters. This organism has been reported from the intestinal tracts of some healthy fish, birds and mammals, and the gills and viscera of shellfish such as crabs. Botulinum toxin has been detected in snails, earthworms, maggots feeding on contaminated carcasses, and nematodes. Because invertebrates are not affected by the toxin, they can be important in transmitting it to species such as birds.

The vegetative (active) form of *C. botulinum* can only grow and produce toxins under anaerobic conditions. A wide variety of plant and animal material can support its growth, but the conditions it needs are strict. In addition to the food or tissue being anaerobic, it must have a relatively high water content, the pH must be greater than 4.6, and ingredients such as salt or preservatives must not be present at high enough levels to inhibit germination and/or growth. Different strains or groups of *C. botulinum* may have somewhat different requirements. For instance, the acidity necessary to inhibit *C. botulinum* type C strains is reported to be pH 5.1 to 5.6, but other organisms can survive a lower pH. Each group also has an optimal, minimal and maximal temperature for growth. For example, some group II organisms may be able to grow in foods at refrigeration temperatures (3-4°C/ 37-39°F), but group I organisms and type C toxin-producing strains are inhibited. Because *C. botulinum* does not compete well with other microorganisms, growth is more likely to occur if other bacteria and molds have been killed or inhibited. *C. botulinum* spores can survive cooking and some food-processing conditions that kill vegetative cells, then germinate and grow in the cooked food.

Botulism usually occurs when people or animals ingest the toxins in food or water, or when the spores germinate in anaerobic tissues and produce toxins as they grow. Botulinum toxin does not pass through intact skin, but it can cross mucous membranes and broken skin. Laboratory accidents can cause botulism by inhalation or other means, and bioterrorism is a possibility.

Botulism in humans

In humans, the three major forms of disease are foodborne, wound, and infant or intestinal botulism. Foodborne botulism occurs when humans ingest preformed toxins in various foods. Modern industrial canning techniques were designed to kill *C. botulinum* spores, and most cases are caused by home-canned, low acid foods (pH > 4.6), as well as meat products such as sausages and ham, and fermented fish, seal and whale meat. However, many different foods can be involved if the conditions are favorable; botulism has been caused by products as diverse as yogurt, garlic oil and foil-wrapped baked potatoes. Baked foods that have been left at room temperature or in a warm oven overnight can cause this disease if the baking kills competing microorganisms, and anaerobic conditions occur in the interior of the food. Commercial foods are occasionally involved.

Wound botulism occurs when an anaerobic wound is contaminated with *C. botulinum*, and the organism is able

to grow and produce toxin. Wound botulism is rare except in injecting drug abusers, where it is caused by contaminated needles or drugs. It is especially common among people who inject "black tar heroin" directly into the subcutaneous tissues.

Infant botulism is seen in children less than a year of age. In this form, *C. botulinum* spores germinate in the intestinal tract and produce toxin. Infants are thought to be predisposed because their intestinal flora is immature and because they produce reduced amounts of bile acids, which inhibit clostridial organisms. Honey has been associated with some cases of infant botulism, but spores can also be found in many other sources including dust. Botulinum spores from the environment can be ingested by most older children and adults without harm; these spores simply pass through the intestines without germinating. However, there are rare cases of intestinal colonization botulism in people who have altered intestinal conditions from gastrointestinal surgery, intensive antibiotic therapy or abnormalities such as achlorhydria.

Botulism can also occur from laboratory accidents (e.g., by inhalation or accidental injection of the toxin). The toxin is used therapeutically to treat some muscle movement disorders and other conditions, and a few cases of iatrogenic botulism have been reported.

Botulism in animals

Preformed toxins in a variety of sources including decaying vegetable matter (e.g., grass, hay, haylage, grain, spoiled silage), meat and fish, carcasses, invertebrates and contaminated water can cause botulism in animals. Carnivores are usually fed the toxins in contaminated meat or fish, or ingest the toxins in carcasses (possibly including iguanas) or decaying, high protein garbage. Cattle in phosphorus-deficient areas may develop pica and chew bones and scraps of attached meat; a gram of dried flesh may have enough botulinum toxin to kill a cow. Similar cases occur in Australia, where protein-deficient sheep sometimes eat the carcasses of rabbits and other small animals. Herbivores also become ill when they are fed the toxin in forage such as hay or insufficiently acidified silage. These feeds may be contaminated by the toxin-containing carcasses of birds and mammals, or from other sources of *C. botulinum*. The feeding of poultry litter containing type C spores has been linked to some outbreaks in ruminants. Birds can ingest botulinum toxins in maggots that have fed on contaminated carcasses or invertebrates from water with decaying vegetation. Fish have been suggested as the source of the toxin in some outbreaks among birds. Contaminated feed can also result in cases in poultry. In addition, botulism has been described in animals that drank water contaminated by a carcass or other source of toxin.

The toxicoinfectious form of botulism in animals corresponds to the wound and intestinal forms in humans.

Similarly to human infants, botulism in foals (the shaker foal syndrome) seems to be caused by the growth of *C. botulinum* in the gastrointestinal tract. Rare cases of wound botulism have been seen in some species such as horses. Toxicoinfectious botulism is also seen in chickens, when broilers are intensively reared on litter; the cause of this phenomenon is unknown.

Botulism is not contagious by casual contact, but it can be transmitted between animals by predation or cannibalism. Contaminated foods usually contain spores as well as the toxin. Spores that are passing through the gastrointestinal tract may germinate and grow if the animal dies. This can perpetuate the cycle, and may result in large outbreaks in birds or other species. Outbreaks of botulism can also contaminate the environment with spores, making future outbreaks more likely.

Botulinum and Bioterrorism

In a bioterrorist attack, botulinum toxin could be delivered by aerosols, as well as in food or water. Aerosolization is the most likely form. After aerosol transmission, the clinical disease is expected to be similar to foodborne botulism.

Disinfection/Inactivation

Botulinum toxins are large, easily denatured proteins. Toxins exposed to sunlight are inactivated within 1 to 3 hours. They can also be inactivated by treating with 0.1% sodium hypochlorite or 0.1 N NaOH, as well as by heating to 80°C (176°F) for 20 minutes or to greater than 85°C (185°F) for at least 5 minutes. The toxin's heat resistance varies with the medium, its pH and the concentration of the toxin. In beef broth, type E toxin was reported to be inactivated within 1 to 9 minutes at 80°C (176°F), with the longest survival time at pH 5.0 and the shortest survival time at the pH extremes (pH 3.5 or 6.8). The World Health Organization (WHO) recommends boiling food for a few minutes to inactivate botulinum toxins. Chlorine and other disinfectants can destroy the toxins in water.

The vegetative cells of *Clostridium botulinum* are susceptible to many disinfectants, including 1% sodium hypochlorite and 70% ethanol. In contrast, spores are very resistant to environmental conditions. The spores of group I *C. botulinum* strains are highly heat resistant; 121°C (250°F) for 3 min is used to destroy them during commercial canning. Spores of group II strains are often damaged by heating to 90°C (194°F) for 10 min, 85°C for 52 min, or 80°C for 270 min; however, these treatments may not be sufficient in some foods. For example, lysozyme or other lytic enzymes present in the food may help damaged spores germinate. *C. botulinum* spores can be destroyed by autoclaving with moist heat (120°C/ 250°F for at least 15 minutes).

Botulism in Humans

Incubation Period

The incubation period for foodborne botulism can be a few hours to 8 days; most cases become symptomatic in 12 to 72 hours. Wound infections may become evident within a few days to two weeks, with an average incubation period of 10 days. The incubation period for adult intestinal colonization or infant botulism is unknown; some cases in adults have occurred up to 47 days after ingesting food that contained the organism. Botulism acquired by inhalation usually develops 12 to 36 hours after exposure, but in some cases the incubation period may be as long as several days.

Clinical Signs

The neurological signs caused by botulinum toxin are similar in all forms of the disease. Additional symptoms (e.g., gastrointestinal signs in foodborne cases) may also be seen in some forms.

Foodborne botulism

In foodborne cases, gastrointestinal disturbances such as nausea, vomiting and abdominal pain are often the first signs. The neurotoxin causes constipation; however, diarrhea may also occur with contaminated food. As the disease progresses, a symmetric, descending flaccid paralysis develops in the motor and autonomic nerves. The clinical signs may include blurred or double vision, photophobia, drooping eyelids, an expressionless face, slurred speech, dysphagia, urine retention, a dry mouth, somnolence and muscle weakness. Untreated cases may progress to descending paralysis of the respiratory muscles, arms and legs. Fatal respiratory paralysis can occur within 24 hours in severe cases. The pharynx may also collapse from cranial nerve paralysis, resulting in respiratory dysfunction even if the respiratory muscles are not affected. Fever is not usually seen, and cognitive function and the senses are almost always unaffected. Death is usually the result of respiratory compromise. Recovery can take weeks or months. In some cases, survivors report fatigue and shortness of breath for years.

Wound botulism

Wound botulism is very similar to the foodborne form; however, gastrointestinal signs are not usually present and patients may have a wound exudate or develop a fever. The abscess can also be minor (e.g., a small furuncle or mild cellulitis).

Infant botulism

Most cases of infant botulism occur in 2-week to 6-month-old babies, but infants up to a year of age can be affected. The first symptom is usually constipation, which can persist for several days before flaccid paralysis develops. Lethargy, weakness, excessively long sleep periods, difficulty in suckling and swallowing, diminished gag reflexes, dysphagia with drooling, drooping eyelids and poor pupillary light reflexes may also be seen. Some babies develop a weak or altered cry. In progressive cases, the infant may develop flaccid paralysis; a "floppy head" is typical. In severe cases, there may be respiratory dysfunction or arrest. Botulism might also be responsible for some cases of sudden death in infants. The symptoms and severity of this disease vary considerably in different babies. In infants that must be hospitalized, supportive care is usually required for several weeks; however, some mildly affected babies may recover quickly. Relapses are seen occasionally after the clinical signs have resolved.

Intestinal colonization botulism in adults

The initial symptoms of intestinal colonization botulism in adults may include lassitude, weakness and vertigo. As the disease progresses, patients may experience blurred or double vision, progressive difficulty speaking and swallowing, descending flaccid paralysis, and other symptoms characteristic of botulism. Abdominal distention and constipation may also be seen. Although this form of botulism resembles foodborne botulism, the symptoms may be prolonged, and relapses may be seen.

Inhalational botulism

Inhalational botulism was reported in laboratory workers in 1962. It resembled foodborne botulism.

Communicability

Person–to–person transmission has not been reported. Nevertheless, care should be taken when handling clinical samples that may contain botulinum toxin, such as feces, gastric contents or body fluids.

Diagnostic Tests

Botulism can be tentatively diagnosed by the clinical signs and the exclusion of other neurologic diseases. The definitive diagnosis relies on identifying the toxin and/ or bacterium in feces, blood/ serum, vomitus, gastric aspirates, wounds or food samples.

Botulinum toxin is usually identified with a mouse bioassay (the mouse neutralization test), but enzyme-linked immunosorbent assays (ELISAs) can also be used. Because ELISAs detect both active and inactivated (e.g., heat treated) toxins, false positives are possible with this test. Only active toxins are detected in mice. Polymerase chain reaction (PCR) assays that detect the neurotoxin genes can be helpful in identifying *C. botulinum*; however, some genes (silent toxin genes) do not produce active toxin, and the results from this assay are confirmed using the mouse bioassay. Botulinum toxins can be typed with neutralization tests in mice. Serology is not used in diagnosis, as small amounts of toxin are involved and survivors rarely develop antibodies.

C. botulinum can be isolated from food or clinical samples in anaerobic culture. Heat or ethanol treatment can aid recovery in highly contaminated samples such as food or feces. These treatments destroy competing

microorganisms while allowing clostridial spores to survive. The temperature used varies with the group; 80°C (176°F) can be used for 10 minutes with group I spores, but 60°C (140°F) for 10 to 20 minutes is less likely to injure group II spores. Some solid media that may be used are blood or egg yolk agar, *Brucella* agar with 5% sheep blood, and phenyl ethyl alcohol blood agar. Suitable liquid media include chopped-meat-glucose-starch medium, cooked-meat medium, reinforced clostridial medium, anaerobe broth and others. On solid media, *C. botulinum* colonies are usually grayish-white with an irregular edge. The colonies are generally beta-hemolytic on blood agar, while on egg yolk medium, they usually display surface iridescence that extends beyond the colony (lipase positive), and are variable for lecithinase activity.. The iridescent zone around the colony tends to be larger for C, D and E toxins. (Lipase is not specific for *C. botulinum*; many other *Clostridium* species and other bacteria also produce this enzyme.) The stained organism is a Gram positive rod that develops oval subterminal spores, especially on media such as chopped meat medium incubated for 5 to 7 days at 30°C (86°F). Biochemical tests and the detection of volatile metabolic products, using gas-liquid chromatography, are helpful in identification. The metabolic patterns and other characteristics vary with the strain/ group. Definitive identification is by demonstration of the toxin. Molecular genetic techniques are helpful in tracing the source of an outbreak.

Other diagnostic or clinical tests may be helpful in excluding other causes of flaccid paralysis, or supporting the diagnosis. Electromyography suggests neuromuscular junction blockage, normal axonal conduction, and potentiation with rapid repetitive stimulation in the affected muscles.

Treatment

The binding of botulinum toxins to the presynaptic endplates of neurons cannot be reversed; however, axons can produce new endplates if the patient can be kept alive while they regenerate. Supportive treatment is the cornerstone of treatment. Depending on the severity of the illness, the respiratory system may need to be sustained with oxygen treatment, intubation to keep the airway open and/or mechanical ventilation. Supportive care may be necessary for up to several weeks or months.

Botulinum antitoxin, given while the toxin is still circulating in the blood, may prevent the disease from progressing and decrease the duration of the illness. Once the toxin has bound to the nerve endings, antitoxin cannot reverse the binding. For this reason, it should be given as soon as possible, preferably within the first 24 hours. Recent studies and cases suggest that botulinum toxin may be found in the circulation for up to 12 days in some patients with the foodborne form. How late the administration of antitoxin should be considered is uncertain. Botulinum antitoxin is usually produced in

equines, and it can produce adverse effects such as serum sickness and sensitization to equine proteins, with the possibility of anaphylaxis. Equine source antitoxin is not used in infants for this reason; instead, infant botulism can be treated with human-derived antitoxin (BIG-IV/ Baby-BIG). This antitoxin is produced in human donors immunized with pentavalent botulinum toxoid (A to E). BIG-IV/ Baby-BIG can decrease the hospitalization time significantly in infants. It may also be used for older patients who cannot tolerate equine serum. In the U.S., equine botulinum antitoxin is available from the Centers for Disease Control and Prevention (CDC) through state health departments, and Big-IV/Baby-BIG is available from the California Department of Health Services' Infant Botulism Treatment and Prevention Program.

Additional treatments depend on the form of the disease. In foodborne illness, the amount of toxin in the gastrointestinal tract may be reduced with stomach lavage, emetics, enemas and/or cathartics. Treatment for wound botulism includes surgical debridement of the wound and antibiotics. Aerobic conditions may be induced in the wound by the use of hydrogen peroxide or hyperbaric oxygen therapy. Antibiotics are not recommended in infant botulism because the death of the microorganisms might release additional toxins from lysed cells. If antibiotics are used to treat botulism, drugs that have neuromuscular blocking properties, such as aminoglycosides, should be avoided.

Prevention

Procedures used to heat treat foods in commercial canning can destroy *C. botulinum* spores. The risk of botulism can also be reduced by acidification, reductions in the amount of moisture in the product, and treatment with salt or other compounds known to inhibit the organism's germination and/or growth. Refrigeration can prevent the growth of group I strains, but some nonproteolytic group II strains may grow at 3-4°C (37-39°F). Foods with "off" odors or flavors should not be eaten, but *C. botulinum* may grow without changing the food's flavor, odor or appearance. Preformed toxins in foods can be destroyed by boiling the food before serving.

Because some batches of honey may contain *C. botulinum* spores, this food should not be fed to children less than a year of age. To prevent toxins in sick animals from affecting people, meat from affected animals is not used for food. Whether the toxin can be transported from the bloodstream to the milk in ruminants is uncertain, but milk from these animals is not allowed to enter the human food chain.

In laboratories, *C. botulinum* must be handled under BSL-2 conditions at a minimum, with BSL-3 precautions used for some procedures. Investigational vaccines may be available for people who have a high risk of exposure (e.g., laboratory workers), and improved vaccines are in development. Person-to-person transmission of botulinum

has never been described, but precautions should be taken to avoid exposure to toxins in body fluids and feces, and anyone who has been exposed should remain alert for the onset of symptoms.

Morbidity and Mortality

Botulism tends to occur as sporadic cases or small outbreaks that affect a few people, but large outbreaks can also be seen, especially when commercially prepared foods are involved. Since 1980, infant botulism has been more common than foodborne botulism in the U.S. In 2006, 107 cases of infant botulism, 19 cases of foodborne botulism and 45 cases of wound botulism were reported to the CDC. Wound botulism, which was once very rare, has been increasing with certain types of drug abuse.

Untreated cases are often fatal, but supportive care has a high success rate when the disease is diagnosed in time. Before 1950, the case fatality rate for foodborne botulism was 60-70%; currently, it is approximately 5-10% in developed countries. Patients in some risk groups, such as those older than 60 years of age, have a higher case fatality rate. The severity of the illness and time before recovery may also be influenced by the dose of the toxin and the toxin type. Cases caused by type A toxin tend to be more severe than those caused by types B or E. People may also have differing sensitivity to the toxin. In one case, a person had detectable toxin in the circulation but was asymptomatic.

The case fatality rate for infant botulism is 2%. In infants that must be hospitalized, recovery usually requires several weeks in the hospital. Antitoxin (Baby-BIG/ BIG-IV) can significantly decrease this time. Estimates of the case-fatality rate for wound botulism vary widely from 1% to 15%.

People who survive botulism do not become immune to the disease. Even in the most severe cases, the amount of toxin in the body is usually too low to stimulate antibody production.

Botulism in Animals

Species Affected

Botulism has been reported in a variety of vertebrates including mammals, birds, reptiles and fish. This disease occurs in horses, cattle and sheep, as well as in ranched mink and foxes. It has also been documented in ferrets, laboratory rodents, nonhuman primates, captive mammals including lions and sea lions, and wild species such as bighorn sheep. Dogs, cats and pigs are relatively resistant to the ingestion of this toxin. Nevertheless, there are occasional reports of botulism in dogs and pigs, and an outbreak was reported in cats that had eaten highly contaminated tissues from a pelican. Botulism has also been seen in more than a hundred species of birds in 22 families, including chickens, pheasants, turkeys, ducks, geese, gulls, loons, mergansers, herons, horned grebes and cormorants.

Outbreaks have been reported in farmed rainbow trout, and other fish are susceptible in experimental studies. Botulism has also been documented in turtles.

Incubation Period

The incubation period can be 2 hours to 2 weeks; in most cases, the clinical signs appear in 12 to 48 hours. Mink are often found dead within 24 hours of ingesting the toxin, and in an outbreak among foxes, the incubation time was 8 to 96 hours. In dogs, it is reported to be 24-48 hours, but in one experiment, dogs fed the toxin became ill in 2-4 days.

Clinical Signs

Botulism is characterized by progressive motor paralysis. In animals, botulism usually affects the hind legs first and ascends. In addition to muscle paralysis, animals may have difficulty chewing and swallowing, experience visual disturbances, and develop generalized weakness and incoordination. Autonomic dysfunction may also be seen. Death usually results from paralysis of the respiratory muscles. Mildly affected animals may recover with minimal treatment.

Ruminants

Muscle weakness and incoordination, progressing to paralysis, is the most apparent sign in ruminants. Weakness is seen in the hind legs first. The animal may also have difficulty chewing and swallowing food, the tongue may protrude, drooling may be seen, and the head may be held abnormally low. Restlessness and urine retention can also occur. In cattle that become recumbent, the head is often turned toward the flank, similarly to a cow with hypocalcemia. Laterally recumbent animals are usually very close to death. Some sheep and goats have been found dead.

In Germany, a cattle disease characterized by lethargy, constipation alternating with diarrhea, edema, decreased milk yield, non-infectious chronic laminitis, engorged veins, a retracted abdomen and emaciation has been linked to the presence of botulinum toxin in the colon and cecum. Affected cattle may die unexpectedly. Most cases have been seen during the peripartum period, but slow growth and wasting was reported in heifers. This disease has been tentatively been named "visceral botulism."

Horses

The clinical signs in horses are similar to cattle. They may include restlessness, knuckling, incoordination, dysphagia, paralysis of the tongue, drooling, decreased muscle tone in the tail and/or tongue, and recumbency. The muscle paralysis is progressive; it usually begins at the hindquarters and gradually moves to the front limbs, head and neck. As in other species, paralysis of the respiratory muscles can result in death.

The "shaker foal syndrome" appears to be similar to botulism in human infants. The most characteristic signs are

a stilted gait, muscle tremors and the inability to stand for more than a few minutes. Dysphagia, constipation, reduced eyelid, tongue and tail tone, mydriasis, sluggish pupillary light reflexes and frequent urination may also be seen. In the later stages, foals usually develop tachycardia and dyspnea. Without treatment, death from respiratory paralysis generally occurs 24 to 72 hours after the initial signs. Some foals may be found dead.

Pigs

Pigs are relatively resistant to botulism. Reported clinical signs include anorexia, refusal to drink, vomiting, pupillary dilation and muscle paralysis.

Foxes and Mink

During outbreaks of botulism in mink, many animals may be found dead, while others have various degrees of flaccid paralysis and dyspnea. The clinical picture is similar in commercially raised foxes. In some mildly affected foxes, only the hind legs are paralyzed. These animals may sit and drag the hind part of their bodies.

Dogs

Limited studies in dogs suggest that this species is relatively insensitive to the ingestion of toxin. Clinical signs reported in dogs include vomiting and anterior abdominal pain, as well as signs related to the effects of the toxin on nerves, such as salivation, incoordination, weakness of the hind legs, flaccid paralysis, a depressed gag reflex, and a diminished withdrawal reflex and/or pupillary reflexes. Congestion of the mucous membranes of the mouth, brownish fetid saliva, cheilitis and an unusual, hoarse, suppressed bark or whine were reported in some experimentally exposed dogs. Some dogs recover, but others have died of respiratory failure.

Cats

In the single outbreak described in cats, anorexia and mild depression were the first signs, followed by flaccid paralysis, and in some cases, dyspnea. Similarly to other animals, the paralysis was evident first in the hindlegs, followed by the forelegs. Some severely affected cats died, others recovered spontaneously and rapidly.

Ferrets

Experimentally exposed ferrets developed botulism signs including weakness, ataxia, ascending paralysis, blepharospasm, photophobia and urinary incontinence, with death resulting from respiratory failure.

Sea lions

Inactivity and dysphagia, followed in some cases by unexpected deaths, were reported in sea lions. Although some animals appeared to be hungry, chewing fish and attempting to swallow, they eventually released the partially chewed fish from their mouths.

Birds

In poultry and waterfowl, botulism is an ascending flaccid paralysis and affects the legs first, followed by the wings and neck. Mild cases may have only paresis or leg paralysis. In gulls, the wing muscles seem to be affected before the legs, and delayed or uncoordinated flight may be an early sign. Mildly affected gulls are able to stand and run, but not fly. Diarrhea with excess urates and paralysis of the nictitating membrane have also been reported in some species. The feathers of chickens may be ruffled, and they may be shed easily when the birds are handled. Birds may die from respiratory dysfunction, and waterfowl with paralyzed necks may drown.

Reptiles

Loss of equilibrium and flaccid paralysis of the legs, followed by drowning, have been reported in green sea turtles (*Chelonia mydas*).

Fish

Loss of equilibrium and erratic swimming have been seen in fish. Increased swimming bursts were the first sign of botulism in experimentally exposed rainbow trout (*Oncorhynchus mykiss*). Some fish including rainbow trout, walleye (S*tizostedion vitreum*) yellow perch (*Perca flavescens*), tilapia and coho salmon (*Oncorhynchus kisutch*) may attempt to swim in a head up/ tail down orientation, with breaching of the water surface. Hyperpigmentation, which can be dramatic, occurs in some species. In round goby (*Neogobius melanostomas*), the first sign of botulism was a faint, black band behind the pectoral fins, which darkened and spread toward the tail until the entire posterior of the fish was darkened. This was followed by darkening of the anterior body, until the entire fish was almost black. Goby did not develop abnormal swimming behavior until the late stages of hyperpigmentation. Hyperpigmentation was also an early sign in yellow perch, and it has been reported in carp (family Cyprinidae). In contrast, decreased color intensity was seen in tilapia and rainbow trout in one experiment, while rainbow trout did not have obvious changes in pigmentation in another study. Similarly to other vertebrates, death occurs in the late stages from respiratory compromise. Fish are usually immobile at this stage. A few fish with mild clinical signs such as slight loss of equilibrium and increased swimming bursts may recover completely.

Communicability

Botulism is not communicable by casual contact, but tissues from dead animals can be toxic if ingested by other animals.

Diagnostic Tests

Botulism can be difficult to diagnose, as the toxin is not always found in clinical samples or the feed. Diagnosis is often a matter of excluding other diseases. A definitive diagnosis can be made if botulinum toxin is identified in the

feed, serum/ blood, stomach, crop or intestinal contents, vomitus, feces or tissues. The toxin can usually be found in the blood or serum only in the early stages of the disease. Botulinum toxin is typically detected with a mouse bioassay. ELISAs may also be used, but they detect both active and inactivated (e.g., heat treated) toxins, and false positives are possible with this test. Botulinum toxins can be typed using neutralization tests in mice.

C. botulinum organisms may also be isolated from the feed. In toxicoinfectious botulism, the organism may be cultured from the gastrointestinal contents, feces, wounds or other tissues. Because healthy animals can have *C. botulinum* spores in the gastrointestinal tract, finding them in this location should be interpreted with caution.

C. botulinum must be isolated in anaerobic culture. Heat or ethanol treatment can aid recovery in highly contaminated samples such as food or feces. These treatments destroy competing microorganisms while allowing clostridial spores to survive. Some solid media that may be used are blood or egg yolk agar, *Brucella* agar with 5% sheep blood, and phenyl ethyl alcohol blood agar. Suitable liquid media include chopped-meat-glucose-starch medium, cooked-meat medium, reinforced clostridial medium, anaerobe broth and others. On solid media, *C. botulinum* colonies are usually grayish-white with an irregular edge. The colonies are generally beta-hemolytic on blood agar, while on egg yolk medium, they usually display surface iridescence that extends beyond the colony (lipase positive), and are variable for lecithinase activity.. The iridescent zone around the colony tends to be larger for C, D and E toxins. (Lipase is not specific for *C. botulinum*; many other *Clostridium* species and other bacteria also produce this enzyme.) The stained organism is a Gram positive rod that develops oval subterminal spores, especially on media such as chopped meat medium incubated for 5 to 7 days at 30°C (86°F). Biochemical tests and the detection of volatile metabolic products, using gas-liquid chromatography, are helpful in identification. The metabolic patterns and other characteristics vary with the strain/ group. Definitive identification is by demonstration of the toxin. Molecular techniques can be been used for the genetic characterization of *C. botulinum*, and may be helpful in determining the source of an outbreak.

Serology is not used routinely in diagnosis, but antibodies to botulinum toxin have been reported in some animals that recovered, including horses, cattle and a dog. In the dog, paired serum samples revealed a fourfold increase in titer. Antibodies to type C and/or D botulinum toxin have also been reported in a few golden jackals (*Canis aureus syriacus*) in Israel.

Treatment

Treatment is supportive, and may include nursing, nutritional support, oxygen and the use of mechanical ventilation until the nerve endings regenerate. Mechanical ventilation has significantly reduced the death rate in foals,

but it is impractical and/or unavailable for some animals such as adult livestock. In one study, foals that required hospitalization could be discharged in approximately 2 weeks, although they were not fully recovered and stall rest or confinement in a small paddock was recommended for an additional period. Feed that might be contaminated should be removed. Gastric lavage, emetics, cathartics and/or enemas may be used to eliminate some of the toxin from the gastrointestinal tract, and. activated charcoal or other substances may help prevent absorption. Where the water supply has high salinity, giving fresh water to prairie wildfowl can improve their condition. The supraorbital gland in these birds, which functions in osmoregulation, is innervated by nerves affected by the toxin.

Monovalent or polyvalent botulinum antitoxin is sometimes used in animals, but it can be expensive, especially in adult livestock. Antitoxin against one toxin type does not provide any significant cross-protection to other types. Decisions on antitoxin treatment must often be made before the results from typing are available. The use of guanidine hydrochloride, which might help the neuromuscular blockade, could also be considered.

Various treatments including antibiotics and citric acid (which chelates the iron needed by *C. botulinum* to grow) have been used in the toxicoinfectious form in birds, with varying success. In foals, antibiotics may be given to prevent complications such as aspiration pneumonia. If antibiotics are used, drugs that have neuromuscular blocking properties, such as aminoglycosides, should be avoided.

Some animals with mild disease can survive with minimal treatment, or recover on their own.

Prevention

In areas where botulism is relatively common, vaccines may be used in animals including horses, cattle, sheep, goats, mink and birds. Vaccine availability varies with the country. In the U.S., commercial vaccines are licensed for horses and mink. There is no cross-protection between toxin types. Surviving a case of botulism does not protect an animal from later exposure to this toxin or eliminate the need for vaccination. Foals born to vaccinated dams have occasionally developed the shaker foal syndrome.

During an outbreak, carcasses should be collected to prevent animals from eating contaminated tissues or the invertebrates that feed on them. Flies should be controlled to prevent the occurrence of "toxic" maggots (maggots that have ingested botulinum toxin), which may be eaten by birds. If possible, litter should also be removed from poultry houses in an outbreak. If this cannot be done, commercial acid disinfectant or granular sodium bisulfate treatment may help suppress the growth of the organism. During outbreaks in chickens, it may also be helpful to clean and disinfect the environment with products effective against spore-forming bacteria. Waterfowl should be chased away from contaminated areas when botulism occurs in

wild birds. It may also be helpful to stabilize water levels (fluctuations have been linked to the proliferation of *C. botulinum*) and eliminate large shallow areas where vegetation decays.

Feed for mink and other ranched animals may be heat processed and/or acidified to reduce the risk of botulism. Care should be taken in the preparation of feed for herbivores. Carcasses should not be allowed to contaminate the feed, and silage should be monitored for proper acidification. Ruminants should be given feed supplements to reduce the incidence of pica when dietary deficiencies exist.

Morbidity and Mortality

Outbreaks of botulism occur regularly in wild waterfowl and shorebirds. They can be preceded by fluctuations in the temperature and/or level of the water, which may increase the proliferation of *C. botulinum*. An estimated 10 to 50 thousand wild birds , especially ducks, are killed annually. In some large outbreaks of type C botulism in western North America, a million or more birds may die. Large numbers of gulls and other birds have been affected in some other areas, including coastlines in Europe. Between 2000 and 2004, more than 10,000 seabirds, mainly herring gulls (*Larus argentatus*) died from type C outbreaks in Sweden. Since 1999, type E botulism affecting large numbers of fish-eating birds, such as gulls and loons, has been reported regularly in the Great Lakes of North America.

The incidence of botulism in domesticated animals is not known with certainty. Carnivores are said to be relatively resistant to this disease; however, contaminated feed can cause outbreaks affecting hundreds or thousands of mink or ferrets. Mink are often vaccinated. Botulism seems to be uncommon in ranched foxes, but one outbreak resulted in the death of more than 44,000 animals. The mortality rate in this outbreak was 22%, and some lots of contaminated feed caused the death of more than 40% of the foxes. The majority of the animals affected were blue foxes (*Alopex lagopus*) and shadow foxes (a color variant of this species), while silver foxes and blue silver foxes, which are color variants of *Vulpes vulpes,* had mortality rates of less than 4%. Cats were relatively resistant to the ingestion of botulinum toxin in limited experimental studies. In the only outbreak reported in this species, the dose of toxin appeared to very high. Four of eight cats fed the contaminated meat died, but the surviving cats recovered quickly. Limited studies in dogs also suggest that this species is relatively insensitive to toxin ingestion. In one experiment, dogs did not become ill after feeding botulinum toxin unless food was withheld first for 48 hours.

In most parts of the world, botulism seems to be relatively uncommon in herbivores; however, it can occur more frequently where conditions such as phosphorus-deficient soils are conducive to this disease. Large numbers of animals may be affected in some outbreaks. The prognosis is poor in recumbent adult livestock. In cattle, death generally occurs within 6 to 72 hours of sternal recumbency. Mortality rates of up to 90% have been described in adult horses. Toxicoinfectious botulism in foals also had a case fatality rate of 90% at one time; however, the use of intensive care, mechanical ventilation and antitoxin has significantly improved survival. In two recent series, 87.5% of the mechanically ventilated foals in one study and 96% of the treated foals in another study (some of which did not require respiratory support) recovered.

Outbreaks of botulism in broiler chickens are uncommon. These birds become less susceptible to botulism with age, and most cases occur in intensively reared flocks between the ages of 2 and 8 weeks. The mortality rate varies from a few birds to 40% of the flock.

Among fish, susceptibility seems to vary with the species. In one experiment, the mortality rate at various oral doses was 92-100% in round goby, 83-92% in walleye, 42-92% in rainbow trout, and 25%-67% in yellow perch. Yellow perch also survived significantly longer than the other three species.

Post Mortem Lesions   **Click to view images**

There are no pathognomonic lesions; any lesions are usually the result of general muscle paralysis, debilitation, the inability to eat and drink, or other secondary effects, and may include signs such as congestion in a variety of tissues. Respiratory paralysis may cause nonspecific signs in the lungs. In shaker foal syndrome, the most consistent lesions are excess pericardial fluid with strands of fibrin, pulmonary edema and congestion.

Internet Resources

Botulism Toolkit
http://botulismtoolkit.com/

California Department of Health Services' Infant Botulism Treatment and Prevention Program
http://www.cdph.ca.gov/programs/ibtpp/Pages/default.aspx

Centers for Disease Control and Prevention (CDC)
http://www.cdc.gov/nczved/divisions/dfbmd/diseases/botulism/

eMedicine.com. Botulism
http://emedicine.medscape.com/article/829125-overview

Food and Drug Administration (FDA). Bacteriological Analytical Manual Online
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

FDA. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook (Bad Bug Book)
http://www.fda.gov/Food/FoodSafety/FoodborneIllness

Brucellosis
Undulant Fever

What is brucellosis and what causes it?

Brucellosis is an infectious disease caused by bacteria called **Brucella** (bru-CELL-a). Many different animal species and humans can become ill. Brucellosis is primarily a reproductive disease in animals, but it can also cause reoccurring fevers, arthritis or udder infection (mastitis).

What animals get brucellosis?

Brucellosis can affect sheep, goats, cattle, pigs, horses, and dogs. Brucellosis can also affect rats and wild animals including deer, bison, elk, moose, camels, water buffalo, and marine mammals.

How can my animal get brucellosis?

In animals, *Brucella* are usually spread through contact with infected birthing tissues and fluids (e.g., placenta, aborted fetuses, fetal fluids, vaginal discharges). The bacteria can also be found in the milk, blood, urine and semen of infected animals.

Animals can get the bacteria by ingestion (**oral**), **direct contact** with mucous membranes (eyes, nose, mouth), or breaks in the skin. *Brucella* can also be transmitted by contaminated objects (**fomites**) such as, equipment, clothing, shoes, hay, feed or water.

Some animals are carriers; they will have the bacteria but show no signs of illness. These animals can shed the bacteria into the environment for long periods of time, infecting other animals in the herd.

How does brucellosis affect my animal?

Brucellosis causes reproductive problems (e.g. abortions, stillbirth, infertility) in most species of animals. Other signs can include arthritis in cows and pigs, mastitis and lameness in goats, and oozing skin lesions in horses ("fistulous withers").

Can I get brucellosis?

Yes. People can become infected by eating or drinking (**oral**) raw milk or unpasteurized milk products that contain the *Brucella* bacteria. **Direct contact** or **aerosol** exposure to infected animal fluids are additional ways to be infected. People who work with animals (e.g., livestock producers, veterinarians) may be at higher risk of exposure to *Brucella*.

Infection in people causes flu-like signs (fever, night sweats, headaches, back pain). Arthritis (joint pain) and re-occurring fevers may occur with long term infection. Rarely, cases of brucellosis can involve the nervous system, eyes, or heart.

Who should I contact, if I suspect brucellosis?

In Animals – Contact your veterinarian immediately.

In Humans – Contact your physician immediately.

How can I protect my animals from brucellosis?

Brucella can survive for months in the environment under optimum conditions but can be destroyed by heat and some disinfectants. Thoroughly clean and disinfect areas exposed to infected animals, their urine, blood, milk, or discharges. Keep sick animals away from other animals to avoid spreading the disease.

In the United States, a vaccination program is used to control brucellosis in cattle. Control programs exist for wildlife (bison and elk) in Yellowstone National Park.

How can I protect myself from brucellosis?

Do not eat or drink raw milk or unpasteurized dairy products. Wear protective clothing (gloves, masks) when handling reproductive tissues (assisting delivery of newborn animals). Always wash your hands after touching animals.

For More Information

CFSPH Technical Fact Sheets. Brucellosis at http://www.cfsph.iastate.edu/DiseaseInfo/

CDC website. Brucellosis at http://www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellosis_g.htm

USDA-APHIS-VS. Brucellosis at http://www.aphis.usda.gov/vs/nahps/brucellosis/

Brucellosis
is a bacterial disease that can affect many animals and humans.

Photo from USDA ARS Photo Gallery

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Equine Encephalitis
Eastern Equine Encephalitis, Western Equine Encephalitis, Venezuelan Equine Encephalitis

What is equine encephalitis and what causes it?

The equine encephalitis viruses are mosquito transmitted diseases that can cause severe inflammation of the brain (encephalitis) in horses and humans. As the names suggest, Eastern equine encephalitis (EEE) most commonly occurs in the Eastern United States and Canada. Western equine encephalitis (WEE) has been isolated from Argentina to Western Canada and in U.S. states west of the Mississippi River. Venezuelan equine encephalitis (VEE) is primarily found in Central and South America, although it has been reported in Mexico and the U.S.

What animals can get EEE, WEE, or VEE?

These viruses primarily cause disease in equine species (e.g., horses, mules, donkeys, zebras), but a number of other animals such as pigs, llamas, bats, reptiles, amphibians, and rodents can also be infected. Birds are reservoirs for the virus, often being infected without signs of disease. Some birds (e.g., pheasants, emus, whooping cranes, partridges) can have illness or death once infected with EEE, WEE, or VEE.

How can my animal get EEE, WEE, or VEE?

These viruses are spread through the bite of an infected mosquito.

How does EEE, WEE, or VEE affect my animal?

Viral encephalitis viruses affect the nervous system, so affected animals will have fever, depression and changes in behavior. Signs of infection may also include impaired vision, muscle twitches, circling or head pressing behaviors, the inability to swallow, paralysis and convulsions. Horses infected with EEE often do not survive. Survival rates of horses infected with WEE is 70-80%. For VEE, death rates are variable but can be as high as 90%.

Can I get EEE, WEE, or VEE?

Yes. People can be infected from the bite of an mosquito carrying the virus. Disease will vary depending on the specific virus involved. Signs include the sudden onset of fever, chills, body and joint aches. Infection can develop into severe encephalitis, resulting in headache, disorientation, tremors, seizures and paralysis. Permanent brain damage, coma and death may also occur in some cases. VEE infection can also include coughing, sore throat, nausea, vomiting and diarrhea.

Who should I contact, if I suspect EEE, WEE, or VEE?

In Animals –
Contact your veterinarian.
In Humans –
Contact your physician.

Equine Encephalitis Viruses are spread by mosquitoes and can affect the nervous system of animals and humans.

Photo: Aedes mosquito, CDC Public Health Image Library

How can I protect my animal from equine encephalitis viruses?

Vaccines for EEE, WEE, and VEE are available for horses. Measures to control mosquito populations and minimize mosquito exposure will decrease chances of infection.

How can I protect myself from equine encephalitis viruses?

You can reduce the chances of becoming infected with EEE, WEE, and VEE by taking measures to decrease mosquito exposure and prevent mosquito bites such as using mosquito repellent (that contains DEET) and avoiding outdoor activities when mosquitoes are most active (dusk and dawn).

For More Information

CFSPH Technical Fact Sheets. Equine Encephalomyelitis at http://www.cfsph.iastate.edu/DiseaseInfo/

CDC website. Eastern Equine Encephalitis at http://www.cdc.gov/ncidod/dvbid/arbor/eeefact.htm

CDC website. Western Equine Encephalitis at http://www.cdc.gov/ncidod/dvbid/arbor/weefact.htm

USDA-APHIS website. Venezuelan Equine Encephalitis at http://www.aphis.usda.gov/publications/animal_health/content/printable_version/fs_ahvee.pdf

USDA-APHIS website. Eastern Equine Encephalitis at http://www.aphis.usda.gov/publications/animal_health/content/printable_version/fs_eastern_equine_enceph.pdf

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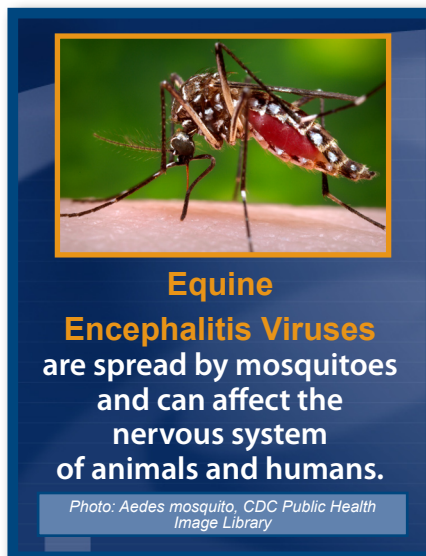
Glanders

Farcy, Malleus, Droes

Last Updated: August 31, 2007

Importance

Glanders is a serious zoonotic bacterial disease that primarily affects horses, mules and donkeys. Some animals die acutely within a few weeks. Others become chronically infected, and can spread the disease for years before succumbing. Although human disease is uncommon, it is life threatening and painful. Without antibiotic treatment, the case fatality rate can be as high as 95%. Occasionally, glanders also occurs in other mammalian species, particularly members of the cat family.

Glanders was a worldwide problem in equids for several centuries, but this disease was eradicated from most countries by the mid-1900s. Outbreaks are now uncommon and reported from limited geographic areas. In non-endemic regions, cases may be seen in people who work with the causative organism, *Burkholderia mallei,* in secure laboratories. An infection was reported in a U.S. researcher in 2000. Glanders is also considered to be a serious bioterrorist threat. *B. mallei* has been weaponized and was used as a biological weapon against military horses, or animals and humans, during the first and second world wars. If this organism is aerosolized during a biological attack or in a laboratory accident, the morbidity rate could be high.

Etiology

Glanders results from infection by *Burkholderia mallei*, a Gram negative rod in the family Burkholderiaceae. This organism was formerly known as *Pseudomonas mallei.* It is closely related to and appears to have evolved from the agent of melioidosis, *Burkholderia pseudomallei*.

Geographic Distribution

Glanders is thought to be endemic in parts of the Middle East, Asia, Africa and South America. Between 1998 and 2007, cases were reported from Brazil, Turkey, the former U.S.S.R., Eritrea, Ethiopia, Iran, Iraq, United Arab Emirates and Mongolia. This disease may also exist in Pakistan. The geographic distribution of *B. mallei* can be difficult to determine precisely, as cross-reactions with *B. pseudomallei* interfere with serological surveys.

In countries that have eradicated glanders, cases may occur in researchers who work with this agent. In 2000, this disease was reported in a U.S. researcher.

Transmission

Glanders is mainly transmitted by contact with skin exudates and respiratory secretions from infected equids. Latently infected as well as clinically ill animals can spread the disease. Horses, mules and donkeys often become infected when they ingest *B. mallei* in contaminated food or water. This organism can also be spread in aerosols, and by entry through skin abrasions and mucous membranes. Carnivores usually become infected when they eat contaminated meat. *B. mallei* is readily spread on fomites including harnesses, grooming tools, and food and water troughs. Although this organism is inactivated by heat and sunlight, its survival is prolonged in wet or humid environments. *B. mallei* remains viable in room temperature water for up to a month. Some sources suggest that it might be able to survive for more than a year in the environment, under some circumstances. Others state that it may survive for up to a few months in favorable environments, but it is likely to be inactivated within two weeks in unfavorable conditions.

Humans are infected by contact with sick animals, contaminated fomites, tissues or bacterial cultures. Transmission is often through small wounds and abrasions in the skin. Ingestion or inhalation can also occur. Transmission through unbroken skin has been reported, but not proven. Most laboratory-acquired infections have occurred during routine handling of cultures or samples, rather than after injuries or accidents. Rare cases of person-to-person transmission have been reported in family members who nursed sick individuals. Two cases were thought to have been sexually transmitted.

Aerosols may be the major route of infection in a bioterrorist attack.

Disinfection

Burkholderia mallei is susceptible to many common disinfectants including benzalkonium chloride, 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, iodine, mercuric chloride in alcohol and potassium permanganate. It is less susceptible to phenolic disinfectants. This organism can also be destroyed by heating to 55°C [131°F] for 10 minutes, or with ultraviolet irradiation.

Infections in Humans

Incubation Period

The incubation period is a few days to several weeks. It varies with the form of the disease: septicemia or localized disease usually becomes apparent after 1 to 5 days, while the pulmonary form typically develops after 10 to 14 days.

Clinical Signs

The symptoms of glanders vary with the route of exposure. Four forms of the disease - septicemia, pulmonary infection, acute localized infection and chronic infection – have been described in humans. One form of the disease can progress to another, and combinations of syndromes occur.

Localized infections are characterized by nodules, abscesses and ulcers in the mucous membranes, skin, lymphatic vessels and/or subcutaneous tissues at the site of inoculation. The nodules are white or gray and firm, with a caseous or calcified center. They are surrounded by areas of inflammation. When the mucous membranes are involved, a mucopurulent, sometimes blood–tinged discharge may be seen. These lesions are accompanied by fever, sweats, malaise and swelling of the regional lymph nodes. Abscesses often develop in the lymph nodes, and may drain. Mucosal or skin infections may disseminate after one to four weeks; symptoms of disseminated infections include a papular or pustular rash and abscesses in the internal organs. These abscesses are often found in the liver, spleen and lungs, but any tissue including the subcutaneous tissues and muscles can be affected. Disseminated infections often progress to septicemia.

The pulmonary form occurs after inhalation of *B. mallei*, or by hematogenous spread from other forms. It is characterized by pulmonary abscesses, pleural effusion and pneumonia. The onset is usually acute. The symptoms include fever, sweats, coughing and chest pain, progressing to dyspnea. Ulcers and nodules, accompanied by a mucopurulent discharge, can occur in the nose. Skin abscesses may also be seen; these abscesses can develop up to several months after the organisms were inhaled. Untreated pulmonary disease often develops into septicemia.

In the septicemic form, fever, chills, myalgia, headache and pleuritic chest pain develop acutely. Flushing, a pustular or papular rash, lymphadenopathy, cellulitis, cyanosis, jaundice, photophobia, diarrhea and granulomatous or necrotizing lesions may be seen. Tachycardia and mild hepatomegaly or splenomegaly have also been reported. Multi-organ failure is common, and death often occurs 24 to 48 hours after the onset of symptoms.

Chronic glanders is characterized by multiple abscesses, nodules and ulcers in a variety of tissues, with periodic recrudescence and milder symptoms than acute disease. A wide variety of organs can be affected including the skin, subcutaneous tissues, liver, spleen, gastrointestinal tract, respiratory tract and skeletal muscles. Weight loss and lymphadenopathy are often seen. This form of the disease can last up to 25 years.

Communicability

Person to person transmission has been reported, but appears to be rare. Some cases have been reported in family members who nursed sick individuals. Two other cases were thought to have been sexually transmitted.

Diagnostic Tests

Glanders can be diagnosed by culturing *B. mallei* from lesions. This organism may also be found in sputum, blood or urine, although blood cultures are often negative.

B. mallei can be stained with methylene blue, Wright or Gram stains, but the staining may be weak or irregular. Some authors report that this organism stains best with Giemsa. *B. mallei* is a nonmotile, Gram negative, straight or slightly curved rod; organisms from clinical samples and young cultures are rods, while bacteria from older cultures can be pleomorphic. Bipolar staining may be seen. *B. mallei* is not always found in smears from clinical samples; few bacteria may be present, and they may not stain well.

B. mallei can be isolated on ordinary culture media including blood agar and meat nutrient agar, but it grows slowly; a 48-hour incubation is recommended. On glycerol agar, a smooth, slightly cream-colored, moist, viscid confluent layer is seen after a few days; this layer eventually becomes thicker, tougher and darker brown. *B. mallei* also grows well on glycerol-potato agar, and a selective medium has been described. *B. mallei* is usually identified by biochemical tests, but this approach can take more than seven days. Automated bacterial identification systems do not always correctly identify this organism. In some commercial kits, cross-reactions can occur with nonvirulent bacteria. If necessary, *B. mallei* can also be isolated by inoculation into guinea pigs or hamsters.

Polymerase chain reaction (PCR) assays may be available in some laboratories. One recently published PCR assay can differentiate *B. mallei* from *B. pseudomallei*. Other genetic techniques used to

distinguish these two organisms include PCR–restriction fragment length polymorphism, pulse-field gel electrophoresis, 16S rRNA sequencing, variable number tandem repeat polymorphism and multilocus sequence typing (MLST). These specialized techniques may be available mainly in research laboratories.

Serology is sometimes helpful, but high background titers in normal serum complicate interpretation. In addition, serologic reactions to *B. mallei* cannot be differentiated from reactions to *B. pseudomallei*. Serologic tests include agglutination, indirect hemagglutination, enzyme-linked immunosorbent assays (ELISAs), immunofluorescence and complement fixation; these tests are not available in all countries. Positive reactions in agglutination tests develop after 7 to 10 days.

Radiography is helpful in the pulmonary form. The lesions may include bilateral bronchopneumonia, miliary nodules, segmental or lobar infiltrates, and cavitating lesions.

Treatment

Glanders is treated with antibiotics. Few studies have been published on the antibiotic susceptibility of *B. mallei*, but some treatment recommendations are available. This organism is usually resistant to some classes of antibiotics. Long-term treatment or multiple drugs may be necessary. Abscesses may need to be drained.

Prevention

Strict precautions should be taken when handling infected animals and contaminated fomites. Protective clothing including heavy gloves and face shields should be worn when working with infected animals. Protection from aerosols may also be appropriate. Biosafety level 3 practices are required for manipulating infected tissues and cultures. Postexposure prophylaxis with antibiotics may be used in some situations. No vaccine is available.

Although person-to-person transmission is rare, human glanders patients should be isolated. Infection control precautions should be taken, and disposable surgical masks, face shields, and gowns should be used as appropriate during nursing.

Morbidity and Mortality

Glanders is a sporadic disease that usually occurs in people who work with clinical samples or have frequent, close contact with horses and their tissues. Risk groups include veterinarians, animal caretakers or other equestrian personnel, laboratory workers and abattoir workers. Human epidemics have not been seen. Transmission from horses to humans may be inefficient; even when morbidity rates in horses are 5-30%, zoonotic disease remains uncommon. However, some infections might be subclinical or mild; autopsy studies conducted in endemic areas found glanders-associated nodules in many people who had contact with horses. In laboratories, *B.*

mallei is highly infectious, particularly when it is aerosolized. With aerosolized bacteria, morbidity rates up to 46% have been reported.

The mortality rate for glanders is high, particularly when effective antibiotics are not given. In the septicemic form, the case fatality rate is 95% or higher in untreated cases and more than 50% when the infection is treated. The mortality rate in the pulmonary form is 90-95% if untreated, and 40% if treated. In chronic glanders, the case fatality rate can reach 50% even in treated cases. The mortality rate for localized disease is 20% when treated; untreated cases often progress to other forms. Intensive therapy with newer antibiotics may result in lower mortality rates than have been reported in the past.

Infections in Animals

Species Affected

The major hosts for *B. mallei* are horses, mules and donkeys, but other species of mammals can also be infected. Glanders has been reported in dogs, cats, goats, sheep and camels. Members of the cat family seem to be particularly susceptible to this disease. Outbreaks have been reported in captive large felids, and occasional cases are seen in domesticated cats. Cattle, pigs and birds are highly resistant to this disease.

Most domesticated animals other than cattle, pigs and rats can be infected experimentally. Wildlife species including bears, wolves, field mice, rabbits and voles have also been infected. Hamsters and guinea pigs are the most susceptible rodents. Mice are resistant to disease unless the dose of organisms is high.

Incubation Period

In animals, glanders may appear immediately or become latent. The incubation period varies from a few days to many months; two to six weeks is typical. Experimental infections can result in clinical signs after three days.

Clinical Signs

Horses, donkeys and mules

In equids, glanders is traditionally categorized into nasal, pulmonary and cutaneous forms. In the nasal form, deep ulcers and nodules occur inside the nasal passages, resulting in a thick, purulent, yellowish discharge. This discharge may be unilateral or bilateral, and can become bloody. Nasal perforation is possible. The regional (submaxillary) lymph nodes become enlarged and indurated, and may suppurate and drain. Healed ulcers become star–shaped scars. In the pulmonary form, nodules and abscesses develop in the lungs. Some infections are inapparent; others vary from mild dyspnea to severe respiratory disease. In more severe cases, the clinical signs include coughing, dyspnea, febrile episodes

and progressive debilitation. Diarrhea and polyuria may also be seen. Discharges from pulmonary abscesses can spread the infection to the upper respiratory tract. In the cutaneous form, the skin contains nodules that rupture and ulcerate, discharging an oily, purulent yellow exudate. The regional lymphatics and lymph nodes become chronically enlarged; the lymphatics are filled with a purulent exudate. In addition, there may be swelling of the joints and painful edema of the legs. Glanderous orchitis is a common symptom in males.

Clinical cases are usually a combination of these forms, and can occur as acute, chronic or latent disease. Acute disease is more likely to occur in donkeys, and chronic or latent disease is more common in horses. Sources disagree whether mules are more likely to develop acute or chronic disease.

Nasal and pulmonary signs are usually seen in the acute form. The symptoms include a high fever, decreased appetite, coughing, progressive dyspnea, nasal discharge, and ulcers and nodules on the nasal mucosa. Bloody crusting may be seen on the nostrils, and there may be a purulent ocular discharge. The submaxillary lymph nodes are usually swollen and painful. Neurological signs have been reported in experimentally infected horses, possibly as the result of secondary bacterial infections from a compromised blood-brain barrier. Animals with the acute form of glanders usually die in a few days to a few weeks.

The chronic form develops insidiously, and results in progressive debilitation. The symptoms may include coughing, malaise, dyspnea, an intermittent fever, enlargement of the lymph nodes, a chronic nasal discharge, and ulcers, nodules and stellate scars on the nasal mucosa. The skin and lymphatics may also be involved. The chronic form is slowly progressive and often fatal; however, affected animals may live for years before succumbing to the disease.

In the latent form, there may be few symptoms other than a nasal discharge and occasional labored breathing. Lesions may be found only in the lungs.

Cats

In cats, nodules and ulcers may be found in the nasal passages and on the conjunctivae, as well as deeper in the respiratory tract. Affected cats typically have a purulent yellowish nasal discharge that may become bloody. The lymph nodes are swollen, and dyspnea may be seen. Affected cats usually die in 1 to 2 weeks.

Communicability

Horses, donkeys and mules can transmit glanders to other animals and humans; nasal discharges and exudates from lesions may contain large numbers of organisms.

Post Mortem Lesions

Ulcers, nodules and/or stellate scars may be found in the nasal passages, trachea, pharynx and larynx. Gray nodules can also be found in other tissues, particularly the lung, liver, spleen and kidneys. Glanders nodules are firm, round and approximately 1 cm. in diameter, with a caseous or calcified center. They are usually surrounded by areas of inflammation. Catarrhal bronchopneumonia with enlarged bronchial lymph nodes may also be seen, particularly in acute disease. In recent experiments, horses with acute infections developed severe diffuse pulmonary edema with areas of hemorrhage, congestion or pneumonia. The lymph nodes may be enlarged, congested and/or fibrotic, and can contain abscesses. Swollen lymphatics, with chains of nodules and ulcerated nodules, may be noted in the skin. Orchitis can be seen in males.

In cats, nodules and ulcers have been reported in the nasal cavity, conjunctivae, larynx, trachea and bronchi.

Diagnostic Tests

Glanders can be diagnosed by culturing *B. mallei* from lesions or respiratory exudates.

This organism can sometimes be found in smears from fresh lesions, where it is usually present in large numbers. It can be difficult to find in older lesions or tissue sections. *B. mallei* can be stained with methylene blue, Wright or Gram stains, but the staining may be weak or irregular. Some authors report that this organism stains best with Giemsa. *B. mallei* is a nonmotile, Gram negative, straight or slightly curved rod; organisms from clinical samples and young cultures are rods, while bacteria from older cultures can be pleomorphic. Bipolar staining may be seen.

B. mallei can be isolated on ordinary culture media including blood agar, but it grows slowly; a 48-hour incubation is recommended. On glycerol agar, a smooth, slightly cream-colored, moist, viscid confluent layer is seen after a few days; this layer eventually becomes thicker, tougher, and darker brown. This organism also grows well on glycerol-potato agar, and a selective medium has been described. *B. mallei* is usually identified with biochemical tests. Automated bacterial identification systems do not always correctly identify this organism. In some commercial kits, cross-reactions can occur with nonvirulent bacteria. If necessary, *B. mallei* can also be isolated by inoculation into guinea pigs or hamsters.

Polymerase chain reaction (PCR) assays are available in some laboratories. One recently published PCR assay can differentiate *B. mallei* from *B. pseudomallei*. Other genetic techniques used to distinguish these two organisms include PCR–restriction fragment length polymorphism, pulse-field gel electrophoresis, 16S rRNA sequencing, variable number tandem repeat polymorphism, and multilocus sequence typing (MLST). These specialized techniques may be available mainly in research laboratories.

A hypersensitivity reaction to *B. mallei,* called the mallein test, is also used to identify infected equids. In reactors, marked eyelid swelling occurs 1 to 2 days after

intrapalpebral injection of a protein fraction of *B. mallei.* Conjunctivitis occurs after administration in eyedrops, and a firm, painful swelling with raised edges is seen within 24 hours after subcutaneous (non-ocular) injection. Positive reactions by any of these three routes are accompanied by fever. The subcutaneous mallein test may interfere with future serologic testing, and the other two routes of administration are generally preferred. Mallein tests can give inconclusive results in acute glanders, or in the late stages of chronic disease.

A variety of serologic tests may be available, but the most accurate and reliable tests in equids are complement fixation and ELISA. A rose bengal plate agglutination test is sometimes used in Russia .Agglutination and precipitin tests are unreliable for horses with chronic glanders and animals in poor condition. Serological tests cannot distinguish reactions to *B. mallei* from reactions to *B. pseudomallei*.

Treatment

Some antibiotics may be effective against glanders, but treatment is given only in endemic areas. Treatment is risky even in these regions, as infections can spread to humans and other animals, and treated animals can become asymptomatic carriers.

Prevention

Animals that test positive for glanders are euthanized except in endemic areas. In an outbreak, the premises should be quarantined, thoroughly cleaned and disinfected. All contaminated bedding and food should be burned or buried, and equipment and other fomites should be disinfected. Carcasses should be burned or buried. Whenever possible, susceptible animals should be kept away from contaminated premises for several months.

In endemic areas, susceptible animals should be kept away from communal feeding and watering areas, since glanders is more common where animals congregate. Routine testing and euthanasia of positive animals can eradicate the disease. Vaccines are not available.

Morbidity and Mortality

Glanders can spread widely when large numbers of animals are in close contact. In Chinese experiments conducted during World War II, 30% of exposed horses became infected. Acute infections are usually fatal within a few days to two weeks. Animals with the chronic form can sometimes survive for years.

Internet Resources

Centers for Disease Control and Prevention (CDC
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/
glanders_g.htm

eMedicine. Glanders and Melioidosis
http://www.emedicine.com/emerg/topic884.htm

Food and Agriculture Organization of the United Nations (FAO). Manual for the Recognition of Exotic Diseases of Livestock
http://www.spc.int/rahs/

FAO. Manual on Meat Inspection for Developing Countries
http://www.fao.org/docrep/003/t0756e/t0756e00.htm

Public Health Agency of Canada. Material Safety Data Sheets
http://www.phac-aspc.gc.ca/msds-ftss/index.html

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

United States Animal Health Association. Foreign Animal Diseases
http://www.vet.uga.edu/vpp/gray_book02/fad/index.php

World Organization for Animal Health (OIE)
http://www.oie.int/

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/eng/normes/mmanual/a_summry.htm

OIE Terrestrial Animal Health Code.
http://www.oie.int/eng/normes/mcode/A_summry.htm

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Melioidosis

*Pseudoglanders,
Whitmore Disease*

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Importance

Melioidosis is a bacterial disease that affects humans and many species of animals. While some infections are subclinical, others result in localized acute or chronic disease, or fatal septicemia. Because it can affect almost any organ, melioidosis can mimic many other diseases; it is sometimes called "the great imitator." A misdiagnosis may be fatal; the causative organism, *Burkholderia pseudomallei,* is susceptible to a limited number of antibiotics. In endemic areas, melioidosis is an important cause of illness and death in humans and animals. It is also a serious concern in imported animals. In 1975, a panda apparently introduced melioidosis to the Paris Zoo, where it caused a severe outbreak. The epidemic spread to other zoos in Paris and Mulhouse, and to equestrian clubs throughout France. It decimated some zoo populations and caused at least two human deaths. In addition, there are fears that *B. pseudomallei* might be used as a biological weapon.

Etiology

Melioidosis results from infection by *Burkholderia pseudomallei*, a Gram negative bacillus in the family Burkholderiaceae. This organism was formerly known as *Pseudomonas pseudomallei*. It is closely related to *Burkholderia mallei*, the agent of glanders, as well as to *B. thailandensis* and *B. oklahomensis*.

Geographic Distribution

Melioidosis is endemic in Southeast Asia, China, the Indian subcontinent and parts of Australia. It has also been reported from the Caribbean, the Middle East, South America, Singapore and Taiwan. The situation in Africa is uncertain. Although isolated cases were reported from some African countries in the past, melioidosis is not a commonly reported disease in Africa. However, laboratory support is absent or weak in some countries, and this disease may be underdiagnosed.

Only non-indigenous cases of melioidosis have been reported in the U.S. Cases formerly thought to be *B. pseudomallei* were reported from Oklahoma after a farming accident and in Georgia after a car accident; these organisms have been reclassified as *Burkholderia oklahomensis* sp. nov.

Transmission

Animals and humans usually acquire melioidosis from organisms in the environment. *B. pseudomallei* is a saprophytic bacterium that is widespread in soil and muddy water in endemic areas. It is particularly common in moist clay soils.

Infections can occur by ingestion, by inhalation, or through wounds and abrasions. All three routes are thought to occur in animals. Infected animals can shed the organism in wound exudates and, depending on the site of the infection, from other sources including nasal secretions, milk, feces and urine. Transplacental transmission has been reported in goats, a pig and a spider monkey. Nosocomial transmission was reported in four cats at a veterinary hospital, possibly via contamination of a multidose injectable solution. Vector-borne transmission by mosquitoes (*Aedes aegypti)* and rat fleas (*Xenopsylla cheopsis*) has been reported, but the role of insect bites remains uncertain.

There have been a few reports of zoonotic transmission, often after contamination of skin lesions by exposure to infected animals, tissues including meat, or milk. However, most people become infected directly from the environment. Inoculation through skin wounds is thought to be the major route of transmission to humans. Inhalation, which usually leads to the pneumonic form of the disease, may be particularly important during periods of heavy rainfall and strong winds. The importance of ingestion is controversial. Person-to-person transmission has been described rarely, generally to family members in close contact (e.g. family members who nursed patients). Sexual transmission has also been suggested in some cases. Vertical transmission has rarely been proven, but a few cases have been described in newborns. One infant may have been infected by nursing culture-positive breast milk.

In non-endemic areas, contamination of the environment from infected animals or humans is a concern. Shed organisms can survive for months or years in soil and

water. In one report, *B pseudomallei* remained viable in triple distilled water for more than three years. This experiment is ongoing, and unpublished reports suggest that the organism is still present fourteen years later. Other laboratories have reported that *B pseudomallei* can survive in room temperature water for as long as eight weeks, in muddy water for up to seven months, and in soil for up to 30 months. This organism can also survive in some antiseptic and detergent solutions, and resists pH 4.5 for up to 70 days. One outbreak was associated with a contaminated container of commercial hand-washing detergent. *B. pseudomallei* is also relatively resistant to desiccation in soil, and can survive soil water content of less than 10% for up to 70 days. In addition, it can enter the cells of protozoa (*Acanthamoeba* or the dinoflagellate *Alexandrium minutum*) or the mycorrhizal fungus *Gigaspora decipiens*. This characteristic may help it survive environmental stresses.

B. pseudomallei seems to be capable of existing in a viable but non-cultivable state in the environment; although these organisms cannot be cultured, they can still cause disease. This phenomenon occurs in acid pH, as well as under other conditions. *B. pseudomallei* has an optimal pH range of 5-8. Below pH 4.5, there is a rapid reduction in the number of cells able to produce colonies, but a parallel increase in viable but non-cultivable organisms, which appear as Gram-positive, coccoid organisms. These organisms revert to conventional Gram-negative-bacilli if the acidic medium is replaced with fresh medium with a neutral pH.

Disinfection

B. pseudomallei is stated to be susceptible to numerous disinfectants including 1% sodium hypochlorite, 70% ethanol, glutaraldehyde and formaldehyde. However, unpublished experiments suggest that it can remain viable for some time in 0.3% chlorhexidine. Disinfectants may not completely eliminate this organism from drinking water, particularly when it is protected within protozoa or found in biofilms. Chlorination reduces the number of *B. pseudomallei* in water, but small numbers of bacteria have been isolated from water containing up to 1000 ppm. free chlorine.

There is little information on the susceptibility of this organism to sunlight or other sources of UV irradiation. Although one report suggested that *B. pseudomallei* is more resistant to UV light than most bacteria, several authors feel that its resistance is probably similar to other soil bacteria. Moist heat of 121°C (249°F) for at least 15 min or dry heat of 160-170°C (320-338°F) for at least one hour can also kill this organism.

Infections in Humans

Incubation Period

In naturally acquired infections, the incubation period varies from less than a day (after very high exposure) to several months or years. Incubation periods of more than two months are common. A few cases have remained subclinical for up to 29 years, and one infection apparently became symptomatic after 62 years. Infections from aerosolized forms in biological weapons are expected to have an incubation period of 10 to 14 days.

Clinical Signs

B. pseudomallei can cause a wide spectrum of clinical diseases. For this reason, melioidosis is sometimes called "the great imitator." While some infections are inapparent, others result in acute pulmonary disease, septicemia, or localized acute or chronic suppurative infections. The frequency of the various syndromes can vary with the region; for example, parotid abscesses are common among children in Thailand, but rare in Australia. One syndrome can develop into another if the organisms spread to other sites.

Acute localized infections sometimes occur at the site of inoculation. In the skin, these infections appear as gray or white, firm nodules and ulcers. The nodules may caseate, and are often surrounded by inflammation. Regional lymphadenopathy and lymphangitis may also be seen. Other forms of acute localized disease include suppurative parotitis/ parotid abscesses, destructive corneal ulcers seen after corneal trauma, and cellulitis. Some infections may resemble necrotizing fasciitis. Genitourinary infections often manifest as prostatic abscesses. Localized infections can disseminate, but systemic infections are not always preceded by localized signs. Skin and subcutaneous infections can also result from the hematogenous spread of the organisms from other sites.

Pulmonary disease is the most common form of melioidosis. It can occur as either the primary syndrome or as a component of septicemia. The symptoms usually include fever, coughing, pleuritic chest pain and, in some cases, hemoptysis. Ulcerative lesions and nodules are sometimes found in the nose, and the septum may perforate. Severe weight loss may be seen. Pulmonary signs can develop suddenly, or may occur gradually after a prodromal syndrome characterized by headache, anorexia and generalized myalgia. Complications include pneumothorax, empyema and pericarditis. Untreated cases often progress to septicemia.

Septicemia is the most serious form of melioidosis. It is most common in people with pre-existing diseases such as diabetes, cancer and kidney failure. The onset is usually acute, with fever, rigors and other typical signs of sepsis. However, in some patients septicemia may develop more gradually, with a fluctuating fever often

associated with severe weight loss. Common symptoms of septicemic melioidosis include fever, severe headache, disorientation, pharyngitis, upper abdominal pain, diarrhea, jaundice and notable muscle tenderness. Pulmonary signs including dyspnea are common, and arthritis or meningitis may be seen. Some patients have a disseminated pustular rash with regional lymphadenopathy, cellulitis or lymphangitis. Septic shock is common, and it is usually fatal once it develops.

Chronic melioidosis is characterized by abscesses and suppurative lesions, which can occur in a variety of organs. Although the liver, spleen, skeletal muscle and prostate gland are affected most often, lesions can occur in any organ including the skin, lung, myocardium, bone, joints, lymph nodes and testes. Mycotic aneurysms are also seen. Rarely, melioidosis can result in brain abscesses, encephalomyelitis (often accompanied by flaccid paralysis) or meningitis. Fever may or may not be present in chronic melioidosis.

Some infected patients remain asymptomatic for years. These chronic carriers may eventually develop clinical disease, typically when they become immuno-suppressed from another condition.

Communicability

In general, *B. pseudomallei* is not spread by casual contact. However, rare cases of person-to-person transmission have been described, usually between family members in close contact. Depending on the site of the infection, bacteria may be found in exudates from abscesses, urine, feces, nasal secretions and milk. Although vertical transmission has rarely been proven, a few cases of melioidosis have been described in newborns. Another infant was thought to be infected by nursing contaminated milk. Sexual transmission has been suggested in some cases, but it has not been proven. Transmission from humans to animals is theoretically possible.

Diagnostic Tests

Melioidosis can be diagnosed by recovering *B. pseudomallei* from blood, sputum, throat swabs, tissues or wound exudates. In the septicemic form, blood cultures may be negative until just before death. The soil and/ or water may also be sampled during outbreaks. *B. pseudomallei* grows on most media including blood agar. Selective media such as Ashdown's selective medium are also used frequently. Mature colonies often have a wrinkled form; these colonies may be mixed with smooth colonies. A few strains, which are usually isolated from human sputum samples, form mucoid colonies. *B. pseudomallei* colonies have a characteristic putrid, earthy odor. (Due to the risk of infection, directly sniffing the plates is dangerous and not recommended.) On microscopic examination, the organisms are motile, short Gram negative bacilli, with bipolar or irregular staining in young cultures. *B. pseudomallei* can be identified by

biochemistry, or with latex agglutination tests to detect antigens. There are conflicting reports on the reliability of automated identification systems; however, some systems might misidentify *B. pseudomallei* as another organism. This is a particular concern in non-endemic areas where the isolation of *B. pseudomallei* is unexpected.

B. pseudomallei antigens can be identified directly in tissues, wound exudates or body fluids by direct immunofluorescence or latex agglutination. Antigen tests including enzyme-linked immunosorbent assays (ELISAs) have also been developed for the exotoxin and other bacterial components. PCR assays have been reported, and may be able to differentiate *B. mallei* DNA from *B. pseudomallei*. Other genetic techniques used to distinguish these two organisms include PCR–restriction fragment length polymorphism, pulse-field gel electrophoresis, 16S rRNA sequencing, variable number tandem repeat polymorphism, and multilocus sequence typing (MLST). These specialized genetic techniques may be mainly available in research laboratories.

Serologic tests may be helpful in some circumstances, particularly when paired sera are available. In some endemic areas, most of the population is seropositive, which limits the value of single tests. However, a high single titer in the presence of clinical signs may be suggestive. Serologic tests include agglutination, indirect hemagglutination, immunofluorescence, ELISAs, dot immunoassay, immunoblotting (Western blotting) and the immunochromatographic test (ICT). Complement fixation is uncommonly used. Cross-reactions can occur in serologic tests with closely related organisms including *Burkholderia mallei*, the causative agent of glanders, and *Burkholderia cepecia*. False positives have also been reported from other Gram negative bacteria including *Legionella* spp.

Treatment

B. pseudomallei is variably susceptible to antibiotics; this organism is intrinsically resistant to many drugs. Long-term treatment may be necessary. Multiple drugs were generally used in the past, but some newer single antibiotics are equally effective. Pulmonary resection or drainage of abscesses is sometimes necessary. Relapses can occur after apparently successful treatment, and lifelong monitoring is often recommended.

Prevention

B. pseudomallei is widely distributed in soil and standing water in endemic regions. People with diabetes or other predisposing conditions should take special precautions to avoid skin contact with these sources. In addition, gloves and rubber boots are recommended for anyone doing agricultural work. Skin wounds including abrasions or burns should be promptly and thoroughly cleansed. A few outbreaks have been linked to

contaminated drinking water supplies. Although small numbers of organisms may survive, chlorination of the water supply decreases the risk of infection. Because *B. pseudomallei* can be found in milk from infected ruminants, only pasteurized dairy products should be consumed. Veterinarians should take precautions to avoid exposure, including the use of gloves and protective clothing, when working with infected animals or collecting diagnostic samples. People who process meat should also wear gloves and disinfect knives regularly. In endemic areas, infected carcasses intended for human consumption are condemned and destroyed.

Laboratory workers may be exposed in clinical samples from patients, even where melioidosis is not endemic. Practices such as sniffing opened culture plates should be discouraged. Postexposure prophylaxis may be given after laboratory exposure to aerosols or contact with skin wounds, or to people with risk factors for septicemia.

In hospitals, ordinary precautions to prevent transmission in blood and body fluids should be taken. No vaccine is available.

Morbidity and Mortality

Melioidosis can occur as sporadic cases or outbreaks. A few outbreaks have been linked to contaminated drinking water supplies. In one outbreak, the source was a container of contaminated handwashing detergent. Increased numbers of cases are seen after heavy rainfall or flooding. In Australia, the risk for septicemia peaks two weeks after the beginning of the summer rains. Melioidosis is an underdiagnosed disease, because it mimics other diseases and because diagnostic facilities may be limited in some endemic areas. In some countries, cases are usually reported only from foreign travelers or in autopsy studies. Approximately 0-5 cases are seen annually in the U.S. These infections typically occur in immigrants and travelers, but clinical disease can also be seen in people who were exposed months or years earlier.

More than 70% of all cases of melioidosis occur in people who have other illnesses. This disease is particularly common in people with diabetes. Other chronic conditions including thalassemia, kidney disease, chronic lung disease, cancer and alcoholism, as well as the use of steroids, also increase the risk of disease. However, some clinical infections occur in previously healthy people, including laboratory workers who are occupationally exposed. The severity of the disease and the clinical signs are influenced by the strain of the organism, the host's immunity, the form of the disease and the dose of organisms. For example, acute suppurative parotiditis is common in children in Thailand, and usually has a good prognosis. However, even when treatment is optimal, the case fatality rate for acute severe melioidosis is 30% to 47%. The case fatality rate is greater than 90% in untreated septicemia, and 40-75% when it is treated. Once septic shock develops, the case

fatality rate is approximately 95%. Although the mortality rate is influenced by the availability of health care, melioidosis is a significant disease even when treatment is optimal. In Australia, the mortality rate for all patients with melioidosis is close to 20%.

Infections in Animals

Species Affected

Many terrestrial and aquatic mammals, as well as birds and fish, can be affected by melioidosis. In Australia, goats, sheep and pigs are infected most often; sheep and goats seem to be particularly susceptible to this disease. Cases of melioidosis have also been reported in dogs, cats, cattle, buffalo, camels, alpacas, horses, mules, zebra, deer, tree kangaroos, wallabies, koalas, various nonhuman primates, captive marine mammals, crocodiles, snakes, tropical fish and some species of birds including parrots. Rodents and rabbits can be infected in the laboratory.

Incubation Period

The incubation period ranges from days to months or years. Some abscesses are carried asymptomatically.

Clinical Signs

Subclinical infections are common in animals, and asymptomatic abscesses may be found at slaughter. Symptomatic melioidosis may be acute, subacute or chronic, and mild or severe. The lungs, spleen, liver and associated lymph nodes are often involved in animals, but any organ can be affected. The effects vary with the site. Acute melioidosis, which is most often seen in young animals, often occurs as septicemia. Localized respiratory signs, gastrointestinal symptoms, septic arthritis with lameness, osteomyelitis, mastitis, orchitis, aortic aneurysms and other syndromes may be also seen. Neurological signs have been reported in many species including cows, goats and horses. Septicemia or extensive involvement of the vital organs can be fatal.

Forms of melioidosis reported in mammals and birds

Pulmonary melioidosis is common in sheep; typical symptoms are fever, severe coughing, respiratory distress and profuse mucopurulent yellow nasal and ocular discharge. Some sheep become arthritic and lame. In others, the only symptoms may be fever and generalized weakness. Neurological signs including circling, incoordination, blindness, hyperesthesia, nystagmus and spasms have also been reported. Orchitis with testicular nodules can occur in rams. In goats, respiratory disease is less severe than in sheep, and coughing is not a prominent sign. Progressive emaciation, lameness or hindleg paresis, and abortions have also been reported in goats. Mastitis and aortic aneurysms may be particularly common in this species.

Pigs may be relatively resistant to melioidosis when husbandry and nutrition are good. Adult pigs tend to develop chronic infections with few symptoms; however, enlarged lymph nodes (particularly the submandibular nodes) may be palpable. Progressive emaciation, neurological signs, incoordination, multiple skin ulcers and diarrhea have also been reported. Young pigs can develop acute septicemia with fever, anorexia, coughing and nasal and ocular discharge. Occasional abortions or stillbirths have been seen in sows, and orchitis can occur in boars. In endemic regions, asymptomatic splenic abscesses are often found in pigs at slaughter.

Various forms of melioidosis have been reported in horses. Generally, the disease lasts approximately three weeks to three months. The symptoms may include weakness, emaciation, edema and lymphangitis of the limbs, mild colic, diarrhea, and signs of pneumonia including coughing and nasal discharge. Skin infections can initially resemble fungal eczema, but later become papular. Hyperacute septicemia with high fever, limb edema, diarrhea and rapid death has also been reported. Acute meningoencephalitis can be seen in rare cases.

Melioidosis is rarely reported in cattle. Most cases in adult cattle have been chronic. Fever, dyspnea, continuous profuse salivation and neurologic signs were reported in one animal. In two other cases, abscessation or acute, localized arthritis was seen after wound contamination. Acute melioidosis has been reported in a calf.

Camels may develop chronic respiratory disease with a hacking cough, purulent nasal discharge and dyspnea. Hindleg ataxia and a wasting disease with severe emaciation have also been reported. Acute septicemia has been seen in both camels and alpacas.

Acute, subacute or chronic melioidosis can occur in dogs. Acute cases are characterized by septicemia with fever, severe diarrhea and fulminant pneumonia. Subacute disease can begin as a skin lesion with lymphangitis and lymphadenitis; untreated cases may progress to septicemia over a week to several months. Respiratory disease can also be the initial syndrome. In addition, chronic disease can occur in any organ; it may be accompanied by anorexia, myalgia, edema of the limbs and skin abscesses. Abscesses have also been reported in various organs in cats. In two recently described cases, the symptoms were not strongly suggestive of an infectious disease. One cat presented with jaundice and anemia, and died soon after it was seen. Fatal neurological disease was reported in the second cat, possibly after dissemination from an infected foot wound.

Most cases in captive marine mammals have been characterized by acute septicemia with fever, inappetence, anorexia and listlessness followed by death. Unlike other species, respiratory distress was not reported. Enteric disease with diarrhea and liver abscesses has been seen in some dolphins.

Although birds may be relatively resistant to melioidosis, fatal cases with lethargy, anorexia and diarrhea have been reported in various avian species in Australia. Experimentally infected chickens remained asymptomatic.

Communicability

Most cases of melioidosis are acquired from the environment, and direct animal-to-animal transmission is uncommon. However, infected animals can shed *B. pseudomallei* in wound exudates, urine, feces and nasal secretions. This organism has been found in mastitic milk from goats and cattle. Subclinically infected animals can shed *B. pseudomallei*.

Rare cases of zoonotic transmission have been reported. Humans can be infected by ingesting contaminated milk, or by touching contaminated tissues and secretions with broken skin.

Post Mortem Lesions   🖼 Click to view images

At necropsy, the major findings are multiple abscesses containing thick, caseous greenish-yellow or off-white material. These abscesses are generally not calcified. The regional lymph nodes, lungs, spleen, liver and subcutaneous tissues are most often involved, but abscesses can occur in most organs. In animals with respiratory disease, exudative bronchopneumonia, consolidation and/or abscesses may be found in the lungs. Suppurative lesions including nodules and ulcers may also be found on the nasal mucosa and septum, as well as on the turbinate bones. These nodules may coalesce to form irregular plaques. Meningoencephalitis, severe enteritis, suppurative polyarthritis and other syndromes have also been reported. Aortic aneurysms and mastitis are common in goats. Splenic abscesses are often found in asymptomatic pigs at slaughter.

Diagnostic Tests

Melioidosis is diagnosed by isolating *B. pseudomallei* from infected animals. This organism can be found in abscesses and wound exudates, in the milk of animals with mastitis, and in the feces of animals with diarrhea. Throat swabs, sputum, blood and urine are also expected to contain bacteria in some cases. Because the number of bacteria in the latter samples is usually small, particular care should be taken to preserve them during sample shipment to the laboratory. Environmental samples may be taken from soil and/ or water during outbreaks.

B. pseudomallei grows on most media including blood agar. Selective media such as Ashdown's selective medium are also used often. Mature colonies often have a wrinkled form; these colonies may be mixed with smooth colonies. A few strains form mucoid colonies. *B. pseudomallei* colonies have a characteristic putrid, earthy odor. (Due to the risk of infection, directly sniffing the plates is dangerous and not recommended.) On microscopic examination, the organisms are motile, short Gram negative bacilli, with bipolar or irregular staining in

young cultures. *B. pseudomallei* can be identified by biochemistry, or with latex agglutination tests to detect antigens. There are conflicting reports on the reliability of automated identification systems; however, some systems might misidentify *B. pseudomallei* as another organism. This is a particular concern in non-endemic areas where the isolation of *B. pseudomallei* is unexpected.

B. pseudomallei antigens can be identified directly in tissues, wound exudates or body fluids by direct immunofluorescence or latex agglutination. Antigen tests including ELISAs have also been developed for the exotoxin and bacterial components. PCR assays have been reported, and may be able to differentiate *B. mallei* from *B. pseudomallei*. Other genetic techniques used to distinguish these two organisms include PCR–restriction fragment length polymorphism, pulse-field gel electrophoresis, 16S rRNA sequencing, variable number tandem repeat polymorphism and multilocus sequence typing (MLST). These specialized genetic techniques may be available mainly in research laboratories.

Serology is used in some species including horses, goats and dairy cattle. Animals in endemic areas often have pre-existing titers. Available serologic tests include indirect hemagglutination, immunofluorescence, complement fixation and other assays. Cross-reactions can occur in serologic tests with closely related organisms including *Burkholderia mallei*, the causative agent of glanders, and *Burkholderia cepecia.* False positives have also been reported from other Gram negative bacteria including *Legionella* spp.

Treatment

B. pseudomallei is susceptible to some antibiotics; however, this organism is intrinsically resistant to many drugs. Relapses can occur when treatment is stopped.

In non-endemic regions, infected animals are euthanized rather than treated. Livestock may also be culled in endemic areas, as treatment can be expensive and protracted, and it is often unsuccessful; however, pets are sometimes treated. To minimize the risk of relapses, lifelong monitoring may be advisable.

Prevention

Melioidosis is usually acquired from the environment, particularly after contact with soil or water. To minimize contact with dirt, animals can be raised on wooden slats, concrete or paved floors. Providing safe drinking water is important in endemic areas. *B. pseudomallei* is particularly common in muddy water, and it is less likely to be found in fresh or clear water. Although small numbers of bacteria may survive treatment, chlorination of the water supply decreases the risk of infection. Carnivores and omnivores should not be allowed to eat contaminated carcasses. Licensed vaccines are not available.

Euthanasia of infected animals is often recommended even in endemic areas, because melioidosis is difficult to treat and can be zoonotic. After culling infected animals, the premises should be disinfected. If infected animals are not euthanized, precautions should be taken to protect people and other animals. Strict hygiene is necessary to prevent transmission from infected horses in stables. The feces from infected horses should be removed several times a day, and the premises should be disinfected regularly with potassium hypochlorite and cresol solutions. The hooves and lower legs of the animals should also be disinfected. Food and water should be provided as aseptically as possible. Standing water should be allowed only in limited quantities or disinfected immediately.

In non-endemic areas, infected animals are usually euthanized to prevent the introduction of disease, and the premises are disinfected.

Morbidity and Mortality

The susceptibility to clinical disease may vary between species. Pigs generally seem to be more resistant to symptomatic melioidosis than sheep and goats, and infections in cattle are very rare. Immunosuppression may predispose cats and dogs to clinical disease. Approximately 19% of a group of military dogs that served in the Vietnam War was seropositive but asymptomatic. One outbreak occurred in marine mammals at a Hong Kong oceanarium after heavy summer rains washed soil into the animals' water. However, melioidosis does not appear to be a problem in marine mammals in the wild.

The mortality rate varies with the site of the lesions, but can be high in sheep. Extensive abscesses and involvement of the vital organs can be fatal. Septicemia has a high case fatality rate, but it seems to be less common in animals than humans. Most cases of septicemia are seen in young animals.

Internet Resources

Centers for Disease Control and Prevention (CDC)
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/melioidosis_g.htm

eMedicine. Glanders and Melioidosis
http://www.emedicine.com/emerg/topic884.htm

Food and Agriculture Organization of the United Nations (FAO). Manual for the Recognition of Exotic Diseases of Livestock
http://www.spc.int/rahs/

FAO. Manual on Meat Inspection for Developing Countries
http://www.fao.org/docrep/003/t0756e/t0756e00.htm

Public Health Agency of Canada. Material Safety Data Sheets
http://www.phac-aspc.gc.ca/msds-ftss/index.html

Plague

Peste, Black Death, Bubonic Plague, Pneumonic Plague, Septicemic Plague, Pestis Minor

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Importance

Plague is an important zoonotic bacterial disease, and a cause of significant mortality in wild rodents and rabbits. In some animals such as prairie dogs, outbreaks may kill nearly all of the animals in a colony. Sporadic cases also occur in other wild and domesticated mammals, particularly felids. Infections in animals can be transmitted to humans, resulting in life-threatening disease. Pneumonic plague, which is a particularly deadly form of the disease, is usually fatal if antibiotics are not started very soon after the symptoms appear. Bubonic plague is less fulminant, but also has a high mortality rate if left untreated.

At least three major plague pandemics have been seen in human populations. The Justinian plague occurred in the Mediterranean region in the 6th century AD and caused an estimated 100 million deaths, and the Black Death killed a third of the European population beginning in the 14th century. The most recent pandemic, which began in China in the late 1800s, spread worldwide and caused an estimated 12 million fatalities by 1930. The biovars that caused these three pandemics still exist in wild animal reservoirs in parts of the world. The Antiqua biovar, which caused the Justinian plague, occurs in Africa and Central Asia. The Medievalis biovar, associated with the Black Death, is now found only in Central Asia, but the Orientalis biovar, which caused the last pandemic, is widespread. These pathogens occasionally spill over from their reservoirs to affect people or other animals. Approximately 1,000 to 5,000 human cases and 100 to 200 deaths are reported annually to the World Health Organization (WHO), and many additional cases are probably not diagnosed. Most outbreaks occur in Asia and Africa, but sporadic cases and outbreaks can be seen in any endemic region. Plague may reoccur after a long period when the disease seems to disappear; recent outbreaks in India, Indonesia and Zambia followed quiescent periods of 30 to 50 years. This disease is also important because it might be used as a weapon by bioterrorists.

Etiology

Plague results from infection by *Yersinia pestis*, a Gram negative bacillus in the family Enterobacteriaceae. Only one serotype is recognized. *Y. pestis* can be divided into three biovars: Antiqua, Medievalis, and Orientalis. The Antiqua strains are more variable than isolates in the other two biovars. Other classification schemes have also been proposed, including three host-related varieties: ratti (borne by rats), marmotae (borne by marmots), and citelli (borne by susliks [Eurasian ground squirrels]).

Geographic Distribution

Y. pestis can be found in parts of Africa, the Middle East, Asia, and Nortb and South America, as well as on Madagascar. The distribution of this organism is patchy. In North America, *Y. pestis* occurs in the western third of the continent, from British Columbia and Alberta, Canada to Mexico, and as far east as Dallas and the western borders of Kansas, Nebraska, Oklahoma and South Dakota. In South America, active foci are found mainly in Brazil and the Andes mountain region of Bolivia, Peru and Ecuador. In Asia, plague has been reported from areas in the former U.S.S.R. east through China, and south to Southwest and Southeast Asia. In Africa, this disease occurs primarily in the eastern and southern regions, but foci are also found in the west and north. Plague is not endemic in Europe or Oceania.

The distribution of each biovar varies. The Antiqua biovar occurs in Africa and Central Asia, and the Medievalis biovar is found in Central Asia. The Orientalis biovar, which caused the last pandemic, is distributed almost worldwide.

Transmission

Plague is usually transmitted by the bites of infected fleas. More than 30 species of fleas are capable of transmitting *Y. pestis*, but they vary in their efficiency as vectors. The oriental rat flea, *Xenopsylla cheopis*, is a particularly effective biological vector. In this flea, *Y. pestis* blocks the gastrointestinal tract, causing the starving flea to bite its host repeatedly and regurgitate the pathogen as it does. Other species of rodent fleas, including some that are not readily blocked, are also important in transmission. Dog and cat fleas (*Ctenocephalides* spp) can be infected, but are poor

vectors compared to species such as *X. cheopis*. Human fleas (*Pulex irritans*) can also carry *Y. pestis*. Fleas are usually short-lived; however, some may survive for several months, or even a year or more, in rodent burrows after their host have died. During epizootics, there is a high risk that fleas leaving dead animals will bite species they do not usually infest, such as humans.

Other arthropods have also been proposed as potential vectors. *Y. pestis* has been detected in human lice during outbreaks in people, and lice were able to transmit the infection between rabbits in the laboratory. Ticks have been suggested as possible mechanical vectors in China and the former USSR. *Y. pestis* has been found in *Ornithodoros* spp. from Brazil and several ixodid and argasid ticks in Russia.

Direct transmission can also occur between animals. *Y. pestis* is present in tissues, draining lesions and some body fluids (depending on the form of the disease); these bacteria can be transmitted through mucous membranes and broken skin. People or animals with the pneumonic form of plague may transmit *Y. pestis* in respiratory droplets. In humans, transmission by inhalation is most common in crowded, poorly ventilated conditions. Animals can transmit bacteria in bites. Carnivores and omnivores, including humans, may also be infected by eating tissues from infected animals. In camels and other herbivores, this may occur when dead rodents or their excretions contaminate the animal's feed.

Y. pestis can be transmitted on fomites at least for short periods; however, its long-term survival in the environment, particularly in soil, is still poorly understood. This organism is not resistant to desiccation or heat, and on surfaces such as glass and steel, it usually survives for less than 72 hours. However, it is reported to survive for long periods of time in organic material; it may remain viable for up to 100 days in blood and for as long as 9 months in human bodies. Viable *Y. pestis* was recently found after 24 days in soil that had been contaminated by the blood of a dead mountain lion. In the laboratory, this organism can survive for many months, and possibly years, in autoclaved soil, and for long periods in water. Rodents have been infected experimentally by burrowing in or running over recently contaminated soil, but whether this is an important maintenance mechanism for plague remains to be determined.

Epidemiology

In the wild, *Y. pestis* seems to be maintained in cycles between wild rodents or lagomorphs (e.g., pikas) and fleas. Periodically, these animals experience epizootics, increasing the risk of transmission to other species. What triggers these epizootics, and how *Y. pestis* persists during interepizootic periods, is poorly understood. Whether this organism circulates in its epizootic hosts between outbreaks, or in a different 'maintenance' host, is controversial.

Sporadic cases of plague occur in people who are exposed to tissues from wild animals, or to their fleas. Domesticated animals can act as 'bridges' that carry *Y. pestis* closer to humans. These animals may become infected themselves, or they can simply act as temporary hosts for infected fleas. Infection of rodents in urban areas, particularly rats, can result in epidemic plague in humans. The importance of different transmission routes during human epidemics is still incompletely understood.

Disinfection

Y. pestis is susceptible to a number of disinfectants including 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, formaldehyde, and iodine–based and phenolic disinfectants. It can also be inactivated by moist heat (121° C for at least 15 min) or dry heat (160-170° C for at least 1 hour).

Infections in Humans

Incubation Period

The incubation period for pneumonic plague is 1 to 4 days. The symptoms of bubonic plague appear after 2 to 6 days.

Clinical Signs

Three major forms of plague are seen in humans: bubonic plague, septicemic plague and pneumonic plague. All three are acute diseases.

Bubonic plague is the most common form. It begins with the sudden onset of high fever, chills, headache, malaise and myalgia. Dizziness, nausea and vomiting may also be seen. Patients with bubonic plague typically develop an infected, swollen and very painful draining lymph node, called a bubo. Although it can occur anywhere, the bubo is often one of the femoral or inguinal lymph nodes. In some cases, a pustule, vesicle, eschar or papule may be found at the site of the flea bite. People who become infected by ingestion can develop severe pharyngitis and tonsillitis, with swelling of a submandibular lymph node and the neck. Vomiting and abdominal pain may also be seen. If it is not treated, bubonic plague often progresses to septicemia and/or secondary pneumonia.

Approximately 10-25% of plague cases are characterized by primary septicemia. In addition to high fever and other signs in common with bubonic plague, this form has signs of sepsis, but there may be no obvious involvement of the lymph nodes. Epistaxis, hematuria petechiae, disseminated intravascular coagulation (DIC) and neurological signs may also be seen, and the course of the disease can be rapid. Secondary septicemia is similar, but results from disseminated bubonic plague. Meningitis is a relatively rare form of plague; it occurs in approximately 6% of people with the septicemic or pneumonic forms.

Pneumonic plague occurs after the inhalation of bacteria (primary pneumonic plague) or after blood–borne spread to the lungs. The symptoms of pneumonic plague develop acutely and include high fever, chills, headache, myalgia, malaise and an increased respiratory rate. Within 24 hours, a cough develops; it is initially dry but becomes productive, then bloodstained and/or purulent. The sputum contains only specks of blood at first but eventually becomes foamy and pink or red from blood. Other symptoms may include pleuritic chest pain, nausea, vomiting, diarrhea and abdominal pain. Pneumonic plague is rapidly fatal, with dyspnea, stridor and cyanosis ending in respiratory failure and circulatory collapse.

Pestis minor is a benign form of bubonic plague, usually seen only among people in regions where plague is endemic. Pestis minor is characterized by fever, lymphadenitis, headache and prostration, which resolve spontaneously within a week.

Communicability

Pneumonic plague can be transmitted from person to person in respiratory droplets, particularly under crowded, poorly ventilated conditions. This form of plague is most contagious during its final stages, when the number of bacteria in the sputum increases. In the earlier stages, transmission does not seem to occur as readily. Transmission between patients with bacteremia is theoretically possible via ectoparasites.

Person-to-person spread of bubonic plague seems to be rare or nonexistent; however, affected tissues such as draining buboes can contain viable bacteria.

Diagnostic Tests

A presumptive diagnosis can be made by identifying the characteristic organisms in sputum, bronchial/tracheal washings, blood, lymph node (bubo) aspirates, cerebrospinal fluid (CSF) or postmortem tissue samples; *Y. pestis* is a Gram negative, facultative intracellular coccobacillus or bacillus with bipolar staining. Bipolar staining is particularly evident when Wright-Giemsa or Wayson stains are used. In some samples such as lymph node aspirates, a relatively homogenous population of bacteria can be found, but samples such as sputum are contaminated by a wide variety of other organisms. *Y. pestis* in clinical samples can be identified by immunofluorescence. Rapid immunoassays can also detect antigens from this organism in clinical samples, and polymerase chain reaction (PCR) assays may be used to detect nucleic acids.

Plague can also be diagnosed by isolating *Y. pestis*. Organisms may be recovered from respiratory secretions, blood and/or aspirates of affected lymph nodes, depending on the form of the disease, as well as from lungs and other tissues postmortem. Organisms are usually present in blood only during septicemia; however, bacteria are sometimes released intermittently from lymph nodes into the blood, and a series of blood samples collected 10-30 minutes apart may be diagnostic. Specimens for culture should be collected before antibiotics are started. *Y. pestis* will grow on ordinary media including blood agar, MacConkey agar, nutrient agar or brain-heart infusion broth. *Yersinia*-specific CIN agar can also be used; this medium is particularly helpful with contaminated samples. *Y. pestis* colonies are small, gray and nonmucoid, and may have a 'hammered copper' appearance. Colonies may take up to 48 hours to appear. *Y. pestis* can be identified with routine biochemical tests and other methods. Automated systems may misidentify this bacterium, as it grows slowly and biochemical reactions may be delayed. A specific bacteriophage that lyses only *Y pestis* and not *Y pseudotuberculosis* is used as a rapid diagnostic test in reference laboratories. *Y. pestis* may also be recovered in laboratory animals such as mice, particularly when the sample is contaminated with other organisms.

Serology is occasionally helpful. Serological tests include enzyme-linked immunosorbent assays (ELISAs), passive hemagglutination, hemagglutination-inhibition, latex agglutination and complement fixation. A fourfold rise in titer is diagnostic.

Treatment

Antibiotics are effective for the treatment of plague; in pneumonic plague, their efficacy is often limited if the symptoms have been present for more than 20 hours. Buboes are occasionally drained but usually resolve with antibiotic treatment. Antibiotic resistant strains seem to be rare, but have been isolated in Madagascar.

Prevention

In endemic areas, rodents should be controlled around human homes, workplaces and recreational areas. Buildings should be rodent-proofed, and access to food sources should be prevented. Brush, rock piles, junk and cluttered firewood should not be allowed to accumulate, as they may provide nesting places for rodents. Campers and hikers should not approach rodents or their carcasses, and should avoid sleeping beside rodent burrows. To prevent pets from serving as a link between wild animal hosts and humans, a good flea control program should be established for dogs and cats, and these animals should be kept from hunting or eating tissues from animals that may be infected. Game meat, as well as tissues from domesticated animals that might be infected, should be cooked thoroughly. Die-offs of rodents or lagomorphs should be reported.

Personal protective equipment (PPE) including gloves should be worn when handling animals or tissues if there is any risk that they might be infected. Good hygiene, including frequent hand washing, should be practiced. Insect repellents can also be applied to clothing and skin if exposure to rodent fleas is expected.

Veterinarians and their staff should use good infection control procedures and PPE with suspected cases of plague. More stringent precautions are necessary when pneumonic plague is suspected or higher risk procedures such as necropsies are performed. Specific recommendations for protective measures are available from the U.S. Centers for Disease Control (CDC) and other groups (see Internet Resources, below).

In endemic regions, rodents that host *Y. pestis* may be monitored and/or controlled. Concurrent insecticidal treatment is often necessary when hosts die or are killed, as fleas leave the carcasses to seek new hosts. People who have been exposed to *Y. pestis* are treated prophylactically with antibiotics. Good infection control procedures, including the use of disposable surgical masks, are used to prevent transmission from patients with pneumonic plague.

Morbidity and Mortality

Y. pestis is endemic in populations of wild rodents and lagomorphs, and occasionally spills over to affect people or other animals. Worldwide, approximately 1,000 to 5,000 human cases of plague and 100 to 200 deaths reported annually to the World Health Organization, and many additional cases are probably not diagnosed. Most outbreaks occur in Asia and Africa, but sporadic cases and outbreaks can be seen in any endemic region. On average, fewer than 20 cases of plague are reported annually in the U.S., but up to 40 cases have been reported in some years. Plague may reoccur after a long period when the disease seems to disappear; recent outbreaks in India, Indonesia and Zambia followed quiescent periods of 30 to 50 years.

Bubonic plague accounts for 80–95% of the cases seen worldwide. Without treatment, the case fatality rate for this form is estimated to be 40-70%; some sources suggest it may be as high as 90%. The availability of treatment lowers the case fatality rate in bubonic or septicemic plague to approximately 5–15%. Untreated pneumonic or septicemic plague is almost always fatal, often within a few days. If appropriate treatment is given very soon after the onset of symptoms, most people survive; however, the narrow window for treatment means that the case fatality rate for the pneumonic form remains greater than 50%.

Infections in Animals

Species Affected

Rodents and lagomorphs are the most important host species for plague. These animals are infested with fleas that can transmit *Y. pestis,* and develop bacteremia high enough to infect those fleas. Infections have been documented in more than 200 species and subspecies of rodents. In the U.S., significant hosts include prairie dogs (*Cynomys* spp.), ground squirrels (*Spermophilus* spp.),

antelope ground squirrels (*Ammospermophilus* spp.), chipmunks (*Tamias* spp.), wood rats (*Neotoma* spp.) and mice (*Peromyscus* spp.) in the southwestern states, and ground squirrels, chipmunks, and wood rats in Pacific coast states. In Asia, important hosts include pikas (*Ochotona* spp.), which are lagomorphs, and rodents including various species of susliks (*Spermophilus* spp.), rats (*Rattus* spp.), Siberian marmots (*Marmota sibirica*), voles (*Microtus* spp.), jerboas, and some gerbils (*Rhombomys opimus* and *Meriones* spp.). Rats are considered to be the primary hosts for *Y pestis* in Madagascar. In some geographic areas, the hosts are not known. Among rodents and lagomorphs, clinical signs are more likely to be seen in some species than others.

Many other species of mammals also become infected, but the majority are incidental hosts. Some species are more likely to develop clinical signs than others. Felids seem to be particularly susceptible to plague; fatal disease has been reported in housecats and wild cats including bobcats and mountain lions. Black-footed ferrets (*Mustela nigripes*) are also very susceptible. Infrequent cases of plague have been described in ungulates including camels (*Camelus bactrianus* and *Camelus dromedarius*), various species of deer, prong-horn antelope, llamas and goats. *Y. pestis* infections have also been reported in dogs, coyotes, foxes, badgers, skunks and nonhuman primates.

Incubation Period

Clinical signs develop within 1 to 4 days in cats.

Clinical Signs

Bubonic plague, septicemic plague and pneumonic plague seem to occur in animals as well as humans; however, plague should be a consideration in any animal with a systemic infection and a history of potential exposure in an endemic area.

Most cats infected with *Y. pestis* develop the bubonic form of plague. This form is usually characterized by fever, anorexia and lethargy, with an enlarged lymph node (bubo) near the site of inoculation. Many cats are probably infected by ingestion, and the submandibular lymph nodes are most often involved. The affected lymph node may develop abscesses, ulcerate and drain. Some cats also have cellulitis, abscesses at sites other than lymph nodes, mouth lesions including ulcers, or necrotic tonsillitis. Vomiting, diarrhea, ocular discharges, dehydration and weight loss have been reported. Bubonic plague can progress to septicemic plague, with systemic signs including tachycardia, pale or brick red mucous membranes, a prolonged capillary refill time and a weak pulse. DIC and/or respiratory distress may also be seen. Cats with primary septicemic plague have similar clinical signs, but without a bubo. Pneumonic plague can develop in cats with bubonic or septicemic plague, and is characterized by respiratory signs including dyspnea and

hemoptysis. Neurological signs such as incoordination have also been reported in infected cats. Studies in experimentally infected cats and serological surveys suggest that some animals might have mild or asymptomatic infections.

Dogs seem less likely to become ill than cats, and subclinical infections may be more common. Only rare descriptions of plague in naturally infected dogs have been published: the clinical signs included fever, lethargy, submandibular lymphadenitis, lesions in the mouth and coughing. Experimentally infected dogs inoculated by the subcutaneous or oral routes developed a fever and other signs of illness, but recovered spontaneously during the next week. Two dogs exposed via aerosols died.

In rodents, the outcome varies from subclinical infection or mild illness to severe, rapidly fatal disease. Epizootics with high mortality rates are reported among some rodents and lagomorphs. Infections in other wild animals are poorly understood. Wild felids including mountain lions and bobcats seem to be relatively susceptible to plague, and may be found dead. Other wild carnivores or omnivores might be less susceptible. Fever and lethargy, without bacteremia, were reported in experimentally infected raccoons in one study. In another experiment, neither fever nor deaths were seen in this species, or in coyotes and striped skunks infected by the oral route. However, some individual animals may be more susceptible; *Y. pestis* has been found in the carcasses of dead coyotes, as well as in foxes and other species.

Occasional cases of plague have been reported in domesticated or wild ungulates. Ocular plague, characterized by keratoconjunctivitis, endophthalmitis and panophthalmitis, has been documented in mule deer (*Odocoileus hemionus*) and black-tailed deer (*Odocoileus hemionus columbianus*). Septicemia and pneumonia have also been seen in mule deer, either with or without ocular signs. Overall, plague is not reported to be an important cause of morbidity and mortality in this species. Goats and camels can become ill and die, and a death was reported in a llama in New Mexico. Clinical cases have not been reported in the literature in cattle, horses or pigs.

Communicability

Animals with the pneumonic form can transmit *Y. pestis* in respiratory droplets. Bacteria can also be found in draining lesions, in some other secretions and excretions, and in tissues. These organisms can cross mucous membranes or broken skin. They may also be ingested by predators. Some animals, including cats, have transmitted the organism in bites.

Most human cases are associated with wild rodents or lagomorphs, but other species including bobcats, coyotes, mountain lions, foxes and badgers have also been involved. Among domesticated animals, cats seem to be most likely to transmit plague to humans. Small outbreaks have also been reported in people who ate uncooked tissues from infected hosts (e.g., uncooked camel liver or guinea pig flesh). Cases transmitted by direct contact with dogs have not been published; however, a recent study suggests that extended contact with dogs may increase the risk of plague, possibly by bringing infected rodent fleas into the household.

Post Mortem Lesions   Click to view images

In cats, necrotic foci may be found in the liver, spleen, lungs and other internal organs. The liver may be pale and the spleen enlarged. Affected lymph nodes can be markedly swollen, with necrosuppurative inflammation, edema and hemorrhagess. Diffuse interstitial pneumonia, focal congestion, abscesses and hemorrhages may be found in the lungs.

In wild animals, the lesions may include hemorrhagic buboes and splenomegaly in some acute cases, or caseous buboes and necrotic lesions in the spleen, liver and lungs when the disease progresses more slowly. Keratoconjunctivitis, endophthalmitis and panophthalmitis, as well as septicemic lesions, pneumonia and lymphadenitis have been reported in deer.

Diagnostic Tests

In the U.S., plague diagnosis is usually carried out by state public health laboratories or the Centers for Disease Control and Prevention (CDC). These laboratories should be contacted before collecting samples. Plague is a serious zoonotic disease; samples should be collected, handled and shipped with all appropriate precautions, including appropriate personal protective equipment (PPE) during their collection.

A presumptive diagnosis can be made by identifying the characteristic organisms in clinical samples such as lymph node (bubo) aspirates or swabs of draining lesions. Some types of samples, including lymph nodes, may contain a relatively homogenous population of bacteria. *Y. pestis* is a Gram negative, facultative intracellular coccobacillus or bacillus with bipolar staining. Bipolar staining is particularly evident when Wright-Giemsa or Wayson stains are used. Bacteria in clinical samples can be identified by immunofluorescence. Rapid immunoassays can also be used to detect *Y. pestis* antigens in clinical samples, and PCR may be used to identify nucleic acids. *Y. pestis* can sometimes be detected by PCR or other techniques in fleas collected from the animal.

Plague can also be diagnosed by isolating *Y. pestis* from blood, nasal/oral swabs, lymph node aspirates, swabs of draining lesions, transtracheal aspirates and/or tissue samples including the liver, spleen, lungs and affected lymph nodes. Specimens for culture should be collected before antibiotics are started. *Y. pestis* will grow on ordinary media including blood agar, MacConkey agar, nutrient agar or brain-heart infusion broth. *Yersinia-*

specific CIN agar can also be used; this medium is particularly helpful with contaminated samples. *Y. pestis* colonies are small, gray and nonmucoid, and may have a 'hammered copper' appearance. Colonies may take up to 48 hours to appear. *Y. pestis* can be identified with routine biochemical tests and other methods. Automated systems may misidentify this bacterium, as it grows slowly and biochemical reactions may be delayed. A specific bacteriophage that lyses only *Y pestis* and not *Y pseudotuberculosis* is used as a rapid diagnostic test in reference laboratories. *Y. pestis* may also be recovered in laboratory animals such as mice, particularly when the sample is contaminated with other organisms.

Serology using paired serum samples can be helpful. A single sample, together with consistent clinical signs, may also be supportive. Various serological tests including latex hemagglutination and passive hemagglutination tests may be available.

Treatment

Early treatment with antibiotics can be successful.

Prevention

A good flea control program should be established for dogs and cats, and they should be kept from hunting or eating tissues from animals that may be infected. Animals that become ill should be examined by a veterinarian. Barrier precautions are necessary during examination and treatment, and suspected cases are isolated. The most stringent measures are needed before antibiotics are begun and during the initial stages of treatment. PPE may include gloves, surgical masks to prevent droplet infection, protective clothing, and eye protection if splashes or sprays are expected. Excellent hygiene should be practiced.

Vaccination has been used to protect endangered black-footed ferrets, which are highly susceptible to plague, during epizootics. Vaccines (in food bait) were also given to prairie dogs, which are the food source for these ferrets, and prairie dog burrows were dusted with an insecticide. Vaccines might also be promising for controlling plague in rodents near human environments. Vaccines are not currently available for domesticated animals.

Morbidity and Mortality

In endemic areas, epizootics occur periodically in susceptible rodents and lagomorphs. The mortality rate may approach 100%. Between epizootics, plague persists in wild animals without causing high mortality. Resistance to plague varies between rodent species. Highly susceptible hosts include California ground squirrels (*Spermophilus beecheyi*), rock squirrels (*S. variegatus*) and prairie dogs (*Cynomys* spp.) in North America, and some suslik populations (*Spermophilus* spp.) in Asia. Other species are more resistant. The percentage of individuals who survive *Y. pestis* infection is reported to be 40-80% in the great gerbil (*Rhombomys opimus*), 50%–70% in little susliks (*S. pygmaeus*) and 44%–60% in midday gerbils (*Meriones meridianus*). In North America, kangaroo rats (*Dipodomys* spp.) are reported to be highly resistant, while northern grasshopper mice (*Onychomys leucogaster*), deer mice (*P. maniculatus*) and California voles (*Microtus californicus*) vary in their susceptibility. Populations that live in endemic areas may be more resistant than those that live outside these regions.

Among other mammals, felids seem to be particularly susceptible to plague; fatal disease has been reported in housecats and wild cats including bobcats and mountain lions. One study reported that the mortality rate was 14% in housecats with bubonic plague, 70% in cats with septicemic plague (or cases that were not classified into a form), and 83% in the pneumonic form. In experimentally infected cats with bubonic plague, the case fatality rate can be as high as 60% if the disease is left untreated. Subclinical infections also seem to occur. Surveillance has reported antibodies in healthy cats, and some cats have survived experimental infections. In one study, 20 of 25 cats inoculated by ingestion or subcutaneous inoculation became ill, but three cats seroconverted without clinical signs. Dogs do not seem to be as susceptible to plague as cats. Ten dogs that were infected by subcutaneous or oral inoculation experienced only a brief illness and recovered on their own. Pneumonic infections may be more serious: two dogs infected by aerosols died.

Serological evidence suggests that wild carnivores are frequently exposed to *Y. pestis*, probably through hunting. Seroprevalence rates are reported to be 13-14% in raccoons and coyotes, and 55% in badgers. Experimentally infected raccoons, coyotes and striped skunks survived the infection. However, fatal infections have occasionally been reported in some species, including coyotes, in the wild. Black-footed ferrets (*M. nigripes*) are very susceptible to plague, and have a high mortality rate. In contrast, experimentally infected domesticated ferrets (*Mustela putorius furo*) and Siberian polecats (*M. eversmanni*) did not become ill. .

Internet Resources

Centers for Disease Control and Prevention (CDC) Plague
http://www.cdc.gov/ncidod/dvbid/plague/index.htm

Material Safety Data Sheets–Canadian Laboratory Center for Disease Control
http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php

Medical Microbiology
http://www.gsbs.utmb.edu/microbook

The Merck Manual
http://www.merck.com/pubs/mmanual/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

Psittacosis/ Avian Chlamydiosis

Ornithosis, Parrot Fever

Last Updated: June 2009

Importance

Avian chlamydiosis is a zoonotic disease of birds caused by the intracellular bacterium *Chlamydophila psittaci*. This disease is called psittacosis in humans. It may be called either avian chlamydiosis or psittacosis in psittacine birds; the term avian chlamydiosis is generally used in other avian species. Infections are particularly common among psittacine birds and pigeons, but most or all species of birds are probably susceptible. Some birds carry this organism asymptomatically. Others become mildly to severely ill, either immediately or after they have been stressed. Significant economic losses may be seen in turkeys and ducks, and high mortality can occur in clinically affected psittacines.

Humans are readily infected by *C. psittaci*. In 1929, exposure to imported pet psittacines caused a pandemic in the U.S. and Europe. Since that time, improved screening and control of avian infections have decreased the incidence of human disease. However, *C. psittaci* is difficult to eliminate entirely; sporadic cases and outbreaks continue to occur. Unusual sources of outbreaks have also been reported. In Australia, one cluster of cases was linked to outdoor activities in an environment contaminated by wild birds. In people, psittacosis is readily treated with antibiotics, but it can be fatal if it is left untreated.

Etiology

Psittacosis/ avian chlamydiosis results from infection by *Chlamydophila psittaci,* a Gram negative, coccoid, obligate intracellular bacterium in the family Chlamydiaceae. *C. psittaci* can be divided into serotypes/ serovars, or alternatively, into genotypes. At least six serotypes, named A through F, have been recognized with specific monoclonal antibodies. *C. psittaci* genotypes are based on genetic differences in the outer membrane protein A (ompA). Each genotype generally corresponds with the serotype of the same name. Genotyping also recognizes a seventh type, E/B, which is indistinguishable from types E or B using serology. Each genotype/ serotype tends to be associated with certain species of birds (see "Species Affected," below). Strains that cause severe disease in one avian species can be mildly virulent or asymptomatic in others. Humans can be infected with any of the genotypes.

The species *Chlamydophila psittaci* includes some but not all of the organisms that were previously called *Chlamydia psittaci*. In 1999, the Chlamydiaceae were reorganized, based on analyses of ribosomal RNA. The new genus *Chlamydophila* was established, and all avian strains of *Chlamydia psittaci* were reassigned to *Chlamydophila psittaci*. Most mammalian strains of *Chlamydia psittaci* were reclassified as *Chlamydophila abortus*, *Chlamydophila felis* or *Chlamydophila caviae*, but two mammalian isolates, WC and M56, were placed in *Chlamydophila psittaci*. WC was isolated from one epizootic in cattle, and M56 was found during a single outbreak in muskrats.

Geographic Distribution

C. psittaci can be found worldwide. This organism is particularly common among psittacine birds in tropical and subtropical regions.

Transmission

C. psittaci can be transmitted between birds by the inhalation of infectious dust or airborne particles such as feathers, and by the ingestion of infectious material including carcasses. Large quantities of this organism are excreted in feces, and can become aerosolized when the fecal material dries. *C. psittaci* is also found in respiratory and oral secretions. Some birds carry the organism asymptomatically, and can shed it intermittently for long periods. Shedding can be stimulated by concurrent infections or stressors such as nutritional deficiencies, handling, overcrowding or egg laying. *C. psittaci* can also be transmitted on fomites including contaminated feed or water. The infectious form found outside cells, which is called the elementary body, is resistant to drying and can remain viable for months if protected by organic debris. It is reported to survive in bird feed for up to two months, on glass for 15 days, and in straw for 20 days. Biting flies, mites and lice may be involved in mechanical transmission.

Vertical transmission has been reported in turkeys, ducks, chickens, parakeets, seagulls and snow geese, but appears to be infrequent. More often, young birds may be infected in the nest via regurgitated food from the parents, by exposure to environmental contamination, or from ectoparasites. Nestlings that survive can become carriers. Epizootics tend to occur when large numbers of birds are in close contact.

Humans usually become infected after inhaling contaminated dust, feathers or aerosolized secretions and excretions. Direct contact with infected birds, including bites, can also spread the disease. Rare cases of person–to–person transmission have been reported, possibly via aerosol spread during paroxysmal coughing. Dogs can be infected with *C. psittaci* if they eat bird carcasses or feces. They are probably also infected via inhalation.

Disinfection

C. psittaci is susceptible to many disinfectants including quaternary ammonium compounds, chlorophenols, iodophore disinfectants, formaldehyde, glutaraldehyde, isopropyl alcohol and sodium hydroxide (bleach). This organism is resistant to acid or alkali. It is susceptible to moist heat of 121°C (250°F) for a minimum of 15 minutes, and dry heat of 160-170°C (320-338°F) for one hour or longer.

Infections in Humans

Incubation Period

The incubation period in humans can be as long as one month; most infections become symptomatic in 5-14 days.

Clinical Signs

Psittacosis can be acute or insidious in onset. The disease varies from a mild, flu–like illness with fever, chills, headache, myalgia, anorexia, malaise, sore throat and/ or photophobia, with or without respiratory symptoms, to severe atypical pneumonia with dyspnea. Some patients have a dry cough, which may become mucopurulent. Gastrointestinal signs, arthralgia, joint swelling and nonspecific rash have also been reported. In uncomplicated infections, the illness usually lasts approximately 7-10 days, and may be self-limiting. Complications occur in some cases. Pregnant women may give birth prematurely, and fetal death is possible. Endocarditis, myocarditis, renal disease, hepatitis, anemia, and neurologic signs such as encephalitis, meningitis and myelitis may also be seen. Multiorgan failure is possible. Atypical forms of psittacosis have been reported. One patient experienced severe abdominal pain, vomiting, constipation, headache and weight loss over six months, with no history of respiratory disease. Death can occur in untreated cases, but it is rare in patients treated with appropriate antibiotics. Some infections are asymptomatic.

Infection of the eye can result in ocular signs including progressive follicular keratoconjunctivitis. *C. psittaci* has been linked to ocular lymphoma, although this is controversial.

Communicability

Person–to–person transmission is rare, but has been reported. *C. psittaci* might be spread in aerosols during paroxysmal coughing.

Diagnostic Tests

In humans, psittacosis is usually diagnosed using a combination of clinical signs and serology. The most common confirmatory test is a rising titer to *C. psittaci* in paired sera with the micro-immunofluorescence (MIF) test or enzyme-linked immunosorbent assay (ELISA). Complement fixation can also be used. Some cross-reactivity with other chlamydiae including *Chlamydia pneumoniae*, *Chlamydia trachomatis*, and *Chlamydophila felis* can occur in all serological tests. Treatment with antibiotics can delay or diminish the antibody response.

Polymerase chain reaction (PCR) assays are used to detect nucleic acids in clinical samples. These tests can distinguish *C. psittaci* from other species of *Chlamydia* or *Chlamydophila*. Antigen-capture ELISAs may also be used to detect the organism.

Because biosafety level 3 facilities are required, culture is not widely available. Where this test is performed, *C. psittaci* can be isolated from sputum, pleural fluid or blood during the acute stage of the disease. In the past, this organism was sometimes recovered in 6-day-old embryonated eggs, or less often, by animal inoculation into mice; these techniques have been replaced in most diagnostic laboratories by cell culture. *C. psittaci* can be isolated in many cell types including buffalo green monkey (BGM), McCoy, HeLa, African green monkey (Vero) and L-929 cells. The identity of the organism can be confirmed with immunofluorescence, immunoperoxidase staining or other techniques. Isolates can be serotyped with monoclonal antibodies, or genotyped with genotype-specific real-time PCR, DNA microarrays or DNA sequencing.

Treatment

Tetracycline antibiotics combined with supportive care are effective. Other antibiotics such as macrolides may be used in some patients. Relapses are possible.

Prevention

Prevention and testing programs in birds help protect humans. Pet birds should be bought from reputable suppliers, and examined by a veterinarian when they are first acquired. Good hygiene, including frequent hand washing, should be used when handling birds. Birds and cages should be kept in a well-ventilated area to prevent the accumulation of infectious dust. Cages should be cleaned regularly to prevent the build-up of wastes. Dampening the

cage first with cleaning solution or disinfectant reduces aerosolization. Any bird that has regular contact with the public (e.g., birds in schools and long-term care facilities) should be routinely screened for *C. psittaci*. Children should be warned not to touch sick or dead birds. Because asymptomatic birds can shed *C. psittaci*, anyone who has been in contact with birds and develops symptoms consistent with psittacosis should consult a physician.

Humans can be infected during a transient exposure, and precautions should be taken during any contact with infected birds. Personal protective equipment (PPE) should be used when handling birds or cleaning their cages. A respirator (N95 or higher rating) protects the wearer from inhaled organisms; surgical masks might not be effective. Gloves and protective clothing should also be worn. Carcasses, tissues and contaminated fomites should be handled carefully. Dead birds should be immersed in disinfectant solutions to reduce the risk of aerosolization. Carcasses should be wet with disinfectant, or detergent and water, during necropsy. Necropsies should be done in a laminar flow hood. If a hood is unavailable, PPE should be worn.

Construction workers and others should use PPE when removing accumulations of pigeon feces. PPE may include gloves, protective clothing, boots, and where appropriate, a respirator. Wetting the wastes before removal decreases aerosolization. One outbreak in Australia was apparently caused by environmental contamination from wild birds; the organism may have spread to people during lawn mowing and other outdoor activities. In areas where such outbreaks have been reported, PPE should be considered during activities that might result in exposure.

Morbidity and Mortality

The risk of psittacosis is highest among people who are exposed to birds or their tissues; this group includes bird owners, veterinarians, laboratory workers, pet shop employees and poultry workers (including workers in processing plants). Since 1996, countries around the world have reported psittacosis cases ranging from fewer than 10 to more than 200 per year; additional undiagnosed or unreported cases are thought to occur. The annual incidence fluctuates due to outbreaks. Currently, fewer than 50 confirmed cases are reported each year in the U.S.

Human infections are relatively common after exposure to infected birds. In one outbreak, 31% of households that received pet birds from an infected flock either became ill or developed antibodies to *C. psittaci*. Many infections have been associated with pet birds, aviaries or pigeon lofts. Poultry are also linked to human disease, and some recent cases occurred after exposure to farmed ducks. One unusual outbreak in Australia was apparently caused by organisms carried in wild birds.

Clinical cases may be mild or severe, depending on the age and health of the individual, as well as other factors; more serious cases are usually seen in the elderly and those who are debilitated or immunocompromised. Before the use of antibiotics, the case fatality rate was 15-20% in the general population and as high as 80% in pregnant women. Properly treated cases are rarely fatal. Convalescence may be slow after severe disease.

Infections in Animals

Species Affected

C. psittaci has been reported in at least 30 orders of birds. It is particularly common in the orders Psittaciformes (psittacine birds) and Columbiformes (pigeons and doves). Infections are infrequent in canaries and finches, which are members of the order Passeriformes. Among poultry, avian chlamydiosis is sometimes seen in ducks and turkeys but occurs rarely in chickens. Common wild bird reservoirs include gulls, ducks, herons, egrets, pigeons, blackbirds, grackles, house sparrows and killdeer. Infections have also been reported in raptors. Outbreaks have been reported in shorebirds and migratory birds. Gulls and egrets can be subclinical carriers for strains that are highly virulent for other birds.

Each genotype of *C. psittaci* tends to be associated with certain species of birds. Genotype A is usually found in psittacine birds, and can cause severe disease in these species. Genotype B is most often associated with pigeons, but it has also been reported in psittacines and turkeys. Genotype C occurs in ducks and geese. Genotype D has primarily been isolated from turkeys, and it is considered to be the most virulent type for this species. This genotype has also been found in egrets and gulls. Genotype E has a diverse host range: it occurs in pigeons, and has also been isolated from sick ratites (ostriches and rheas), ducks and turkeys. Genotype F, which has been found in one turkey and a psittacine bird, is apparently rare among domesticated species. Genotype E/B, which was first described in 2005, has been reported in ducks, turkeys and pigeons; additional hosts may be discovered.

Infections with *Chlamydophila psittaci* have been reported occasionally in mammals including dogs, cats, horses, cattle (WC strain) and muskrats (M56 strain).

Incubation Period

The incubation period in cage birds is usually three days to several weeks. In carriers, active disease can occur any time, and may be seen years after infection.

Clinical Signs

C. psittaci produces a systemic disease in birds. Depending on the strain of the organism and the species, age and condition of the bird, infections may be asymptomatic or result in mild to severe clinical signs.

Acute or chronic disease can be seen in psittacines. Many infected birds remain asymptomatic until they become stressed. The clinical signs may include anorexia, lethargy, ruffled feathers, serous or mucopurulent oculonasal discharge, and weight loss. Some birds develop

respiratory signs ranging from sneezing to respiratory distress. Conjunctivitis and diarrhea with green to yellowish droppings may also be seen. Neurological signs can be found, especially in subacute to chronic cases; torticollis, opisthotonos, tremors, convulsive movements, and flaccid paralysis or paresis of the legs have been reported. Severely affected birds may become emaciated and dehydrated before death. Recurrent kerotoconjunctivitis, often without generalized signs of disease, has been seen in small Australian parakeets. Conjunctivitis, with or without other signs, is also common in some finches. Residual disturbances in feathering may be apparent in survivors.

The clinical signs are similar in other species of birds. Turkeys are especially likely to develop pneumonitis and myocarditis when infected. Egg production is decreased. Conjunctivitis, blepharitis and rhinitis are common in pigeons. Neurological signs may include transient ataxia in pigeons, and trembling or gait abnormalities in ducks. One study has linked *C. psittaci* with cystic oviducts among laying hens; this remains to be confirmed.

Infections are reported occasionally among mammals that have been in contact with birds. In horses, *Chlamydophila psittaci* has been linked to some abortions. A variety of syndromes have been attributed to this organism in dogs. *Chlamydophila psittaci* genotype C, possibly acquired from a pet bird, was isolated from a group of dogs with recurrent respiratory and reproductive problems, including episodes of severe dyspnea and keratoconjunctivitis. These dogs produced litters that were smaller than normal, with unusually large numbers of dead pups. In another outbreak, the introduction of an infected cockatiel into a household caused illness in two of three dogs. One dog developed acute disease with fever, shivering, coughing, retching, dyspnea and a slight oculonasal discharge. Another dog had a mild fever, lethargy, anorexia, congestion of the mucous membranes, and evidence of bacterial endocarditis, which resolved upon antibiotic treatment. A third dog was clinically unaffected but seropositive. Bird-associated isolates of *Chlamydia psittaci* were reported from a 5-month-old dog with fever, pleural effusion and shifting leg lameness, and from a dog with a spasmodic exercise-induced cough and loss of condition. An avian strain of *Chlamydia psittaci* was isolated from the 5-month old dog, while the latter case was linked to the ingestion of infected budgerigar carcasses and infectious feces. Cats are generally infected with *Chlamydophila felis* rather than *Chlamydophila psittaci*; however, one case of conjunctivitis was apparently acquired from a macaw.

Communicability

Infected birds can shed *C. psittaci* for weeks to months. Shedding may be continuous or intermittent, and can be precipitated by stress.

Diagnostic Tests

C. psittaci infections can be diagnosed by culture, the detection of antigens or nucleic acids, histochemistry, immunohistochemical staining and serology. A combination of techniques may be necessary, especially when only one bird is tested. It is easier to make a diagnosis in birds that are acutely ill.

C. psittaci can be detected in a variety of secretions, excretions including feces, and tissues such as the liver, spleen, lung, kidney, pericardium and colon contents. Repeated fecal sampling over 3-5 consecutive days is helpful in birds suspected to be carriers; these birds may shed organisms intermittently. *C. psittaci* can be cultured only in laboratories with biosafety level 3 facilities. This organism may be isolated in many cell types including buffalo green monkey, McCoy, HeLa, Vero and L-929 cells. It can also be recovered in 6-7 day old embryonated eggs. It is often identified by immunofluorescence or immunoperoxidase staining, but genetic techniques can also be used. Isolates can be serotyped with monoclonal antibodies, or genotyped with genotype-specific real-time PCR, DNA microarrays or DNA sequencing. Isolation may be unsuccessful in birds treated with antibiotics during the 2-3 weeks before testing.

Avian chlamydiosis can also be diagnosed by demonstrating *C. psittaci* directly in clinical samples, using immunohistochemistry or PCR. Antigen capture ELISAs for use with human clinical samples can be employed in birds; however, their sensitivity and specificity in avian species is unknown, and they are best used in conjunction with other tests. Histochemistry can give a tentative diagnosis or be used to support other diagnostic techniques. Chlamydiae are small coccoid organisms that stain red or pink against a counterstained blue or green background. They can be detected in tissues with Giemsa, Gimenez, Ziehl–Neelsen and Macchiavello's stains.

Serological tests include complement fixation, ELISAs, latex agglutination, elementary body agglutination (EBA), microimmunofluorescence, and agar gel immunodiffusion tests. The EBA test detects only IgM and can be used to diagnose current infections. A four–fold rise in titer should be seen in paired samples. A presumptive diagnosis can be made if single high titers are found in several birds in a population.

Treatment

Antibiotics can be used to treat avian chlamydiosis, but some birds may remain infected. Prolonged treatment, with isolation of the bird, is necessary.

Prevention

No vaccine is available, and complete eradication appears to be impractical due to the large number of potential hosts. However, steps can be taken to reduce the risk of infection. To prevent the introduction of avian chlamydiosis into a facility, new birds should be examined

for signs of illness, quarantined for at least 30 days, and tested *for C. psittaci*. Birds that have returned from events such as shows or fairs are also isolated. Wild birds should be excluded from the facility, and wild rodents, which might act as mechanical vectors, should be controlled. Regular cleaning and disinfection of the premises and equipment also aids control. Cages should be positioned so that nothing including feces, food or feathers is readily transferred between them. Cross-contamination between areas or units should be minimized. Good exhaust ventilation can help reduce the build-up of aerosols and prevent cross-contamination. All-in/ all-out management of units, where appropriate, can be helpful. The routine use of prophylactic antibiotics is discouraged, because it may favor the development of antibiotic-resistant strains of *C. psittaci* and other bacteria. Records should be kept of bird-related transactions for at least one year. Breeders can also participate in a voluntary certification program for pet birds.

Infected premises are usually quarantined. Poultry may be euthanized. Infected pet birds and their contacts can be isolated and treated. During treatment, measures such as frequent wet-mopping of the floor with disinfectants can reduce the circulation of dust and feathers. Before restocking or releasing treated birds from quarantine, the premises should be thoroughly cleaned and disinfected.

Morbidity and Mortality

C. psittaci is frequently found in psittacine birds and pigeons. The reported prevalence is 16-81% in psittacines and 23-85% in pigeons, with a seroprevalence rate of 10-96% in feral pigeons. In some regions, this organism is also common in domesticated ducks and turkeys.

Morbidity and mortality rates vary with the host species, condition of the bird, and virulence of the isolate. In psittacine birds, which are often infected with virulent genotype A strains, the mortality rate can be 50% or higher. Clinical signs tend to be less severe in pigeons, which are usually infected with the milder genotypes B and E, and deaths are often caused by secondary infections. In turkeys, the mortality rate for untreated infections is 5-40%. Genotype D is the most virulent type in this species, with an overall morbidity rate of 50–80% and a mortality rate of 5–30% or higher. In broiler turkeys, up to 80% of infections with this genotype may be fatal. Other genotypes in turkeys usually result in 5–20% morbidity, with much lower mortality rates. In ducks, genotype C has a morbidity rate of 10-80% and a mortality rate of 0-30%. Some duck farms are infected with few or no clinical signs. Outbreaks of severe disease have also been seen in shorebirds and migratory birds. Concurrent infections or stress increase the severity of the disease in all species. Age can also be a factor; young birds tend to be more susceptible than older birds.

C. psittaci is uncommonly reported in mammals, and the morbidity and mortality rates are unknown.

Post Mortem Lesions   Click to view images

Post–mortem lesions in birds may include nasal adenitis, congestion of the lungs, fibrinous pneumonia, fibrinous airsacculitis, splenomegaly and hepatic enlargement with multifocal hepatic necrosis. Fibrinous perihepatitis, pericarditis, peritonitis and vascular congestion may also be seen. In some turkeys, an enlarged and congested spleen may be the only gross lesion. Enteritis, hepatomegaly, airsacculitis and conjunctivitis with swollen and encrusted eyelids are common in pigeons. Asymptomatically infected birds often have no gross lesions.

Internet Resources

Centers for Disease Control and Prevention (CDC). Psittacosis.
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/psittacosis_t.htm

Medical Microbiology
http://www.ncbi.nlm.nih.gov/books/NBK7627/

National Association of State Public Health Veterinarians (NASPHV)
http://www.nasphv.org

NASPHV Compendium of Measures to Control *Chlamydophila psittaci* Infection among Humans (Psittacosis) and Pet Birds (Avian Chlamydiosis)
http://www.nasphv.org/documentsCompendiaPsittacosis.html

The Merck Manual
http://www.merck.com/pubs/mmanual/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

Public Health Agency of Canada. Pathogen Safety Data Sheets and Risk Assessment
http://www.phac-aspc.gc.ca/msds-ftss/index.html

World Organization for Animal Health (OIE)
http://www.oie.int/

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals http://www.oie.int/international-standard-setting/terrestrial-manual/access-online

OIE Terrestrial Animal Health Code
http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

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Acha PN, Szyfres B (Pan American Health Organization [PAHO]). Zoonoses and communicable diseases common to man and animals. Volume 2. Chlamydiosis, rickettsioses and viroses. 3rd ed. Washington DC: PAHO; 2003. Scientific and Technical Publication No. 580. Zoonotic chlamydiosis; p. 42-51.

Q Fever

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Importance

Q fever is a highly contagious zoonotic disease caused by the intracellular pathogen *Coxiella burnetii*. Although this infection was first described in the 1930s, it is still poorly understood. Many domesticated and wild animals including mammals, birds, reptiles and arthropods can carry *C. burnetii*. In most cases, the infection is asymptomatic, but abortions or stillbirths can occur in ruminants. In sheep, 5-50% of the flock may be affected. Both symptomatic and asymptomatic animals shed *C. burnetii* in large quantities at parturition. Shedding can also occur in feces, milk and urine. These organisms persist in the environment for long periods and can be spread long distances by the wind.

Human outbreaks can result from the inhalation of aerosolized organisms. More often, sporadic cases occur in people who are occupationally exposed. These cases tend to result from exposure to parturient ruminants; however, cats, dogs, rabbits and other species have also been implicated. Although Q fever is usually asymptomatic or mild in humans, a few people develop serious disease. Pneumonia or hepatitis may occur in acute cases, and chronic infections can result in endocarditis or a wide variety of other diseases.

Etiology

Q fever results from infection by *Coxiella burnetii*. This organism is an obligate intracellular pathogen and has been traditionally placed in the family Rickettsiaceae; however, recent phylogenetic studies have demonstrated that *C. burnetii* is more closely related to *Legionella*, *Francisella* and *Rickettsiella.* This organism is now classified in the family Coxiellaceae and order Legionellales in the gamma subdivision of Proteobacteria.

C. burnetii forms unusual spore-like structures that are highly resistant to environmental conditions. This organism also has two distinct antigenic phases, phase I and phase II. Phase I and II cells are morphologically identical, but differ in some biochemical characteristics including their lipopolysaccharide (LPS) composition. Organisms isolated from infected animals or humans express phase I antigens and are very infectious. Organisms expressing phase II antigens are less infectious and are recovered after the bacteria are passaged repeatedly in cell cultures or eggs. Experimentally infected animals first produce antibodies to phase II antigens and later produce antibodies to phase I antigens. A similar response occurs in humans, and is used to distinguish acute from chronic infections.

Geographic Distribution

Q fever has been found worldwide, except in New Zealand.

Transmission

C. burnetii can be transmitted by aerosols or direct contact; it is also spread by ingestion. Infections in animals can persist for several years and possibly lifelong. Organisms localize in the mammary glands, supramammary lymph nodes, uterus, placenta and fetus in animals; bacteria can be shed in milk, the placenta and reproductive discharges during subsequent pregnancies and lactations. *C. burnetii* can also be found in the feces and urine, and in the semen of bulls. Sexual transmission has been demonstrated in mice. Ticks may be important in transmission among wildlife, and can also spread infections to domesticated ruminants. In addition, *C. burnetii* has been found in lice, mites and parasitic flies.

Most human infections are associated with cattle, sheep and goats, and often occur when the animal gives birth. Cases have also been linked to other species including cats, dogs, rabbits and wild animals. One outbreak was associated with pigeon feces. Humans are usually infected via aerosols, but transmission may also occur by the ingestion of unpasteurized milk or other contaminated material. In addition, transmission has been documented in blood transfusions and can probably occur by sexual contact. Vertical (transplacental) transmission appears to be possible but rare, and tick-borne infections are thought to be uncommon or nonexistent. Persistent (dormant) infections can occur in humans; these organisms may be reactivated by immunosuppression or other factors.

C. burnetii is highly resistant to environmental conditions and is easily spread by aerosols; infectious airborne particles can travel up to 11 miles. Viable organisms can be found for up to 30 days in dried sputum, 120 days in dust, 49 days in dried urine from infected guinea pigs, and for at least 19 months in tick feces. At 4-6°C (39-43°F), organisms can survive for 42 months in milk and 12 to 16 months in wool.

Disinfection

C. burnetii is highly resistant to physical and chemical agents. Variable susceptibility has been reported for hypochlorite, formalin and phenolic disinfectants; 0.05% hypochlorite, 5% peroxide or a 1:100 solution of Lysol® may be effective. *C. burnetii* is also susceptible to glutaraldehyde, ethanol, gaseous formaldehyde, gamma irradiation or temperatures of 130°C (266°F) for 60 minutes. High temperature pasteurization destroys the organism.

Infections in Humans

Incubation Period

In humans, the incubation period for acute Q fever varies from 2 to 48 days; the typical incubation period is approximately 2 to 3 weeks. Chronic Q fever can occur from months to many years after infection.

Clinical Signs

Symptomatic infections can be acute or chronic. Many cases of acute Q fever are asymptomatic or very mild, and remain unnoticed. The symptoms of acute disease are flu-like and can include high fever, chills, a headache, fatigue, malaise, myalgia, sore throat and chest pain. The headache may be very severe. The illness is often self-limiting, and generally lasts from a week to more than three weeks. Some patients with Q fever develop atypical pneumonia. These patients usually have a nonproductive cough, with pneumonitis on X–ray. In severe cases, lobar consolidation and pneumonia may be seen; severe infections are particularly common in elderly or debilitated patients. Patients with atypical pneumonia can be ill for up to three months. Hepatitis can also occur in acute Q fever. Three forms of hepatitis may be seen. In one form, an infectious hepatitis-like form is accompanied by hepatomegaly. The clinical signs may include fever, malaise and right upper abdominal pain. Jaundice can occur, but it is uncommon. Other patients with hepatitis experience prolonged fever of unknown origin with granulomas on liver biopsy. Clinically asymptomatic hepatitis is also seen. The syndromes that accompany acute Q fever vary with the geographic region, with atypical pneumonia more common in some countries and hepatitis the predominant form in others.

Other, less common, signs can also occur in acute disease. Rashes have been reported in a few patients. Complications are uncommon but may include pericarditis and/or myocarditis, aseptic meningitis and/or encephalitis, polyneuropathy, optic neuritis, hemolytic anemia, transient hypoplastic anemia, thyroiditis, gastroenteritis, pancreatitis, lymphadenopathy that mimics lymphoma, erythema nodosum, bone marrow necrosis, hemolytic-uremic syndrome, splenic rupture and others. A serious systemic infection was reported in an acute case of Q fever in a transplant patient.

Chronic Q fever is an uncommon condition that develops months or years after the acute syndrome. Endocarditis is the most commonly reported syndrome. It usually occurs in people who have pre-existing damage to the heart valves or are immunosuppressed. The symptoms are nonspecific and similar to subacute or acute bacterial endocarditis. Arterial embolisms occur in some patients. Other syndromes that have been reported in chronic Q fever include infections of aneurysms or vascular grafts, osteoarthritis, osteomyelitis, tenosynovitis, spondylodiscitis, paravertebral abscesses, psoas abscess and hepatitis. Rare cases of pericardial effusion, amyloidosis, pulmonary interstitial fibrosis, pseudotumor of the lung, lymphoma-like presentation, and mixed cryoglobulinemia have also been reported. *C. burnetii* has also been linked to chronic fatigue syndrome by some authors.

Approximately 98% of cases in pregnant women seem to be asymptomatic; however, *C. burnetii* has been linked to premature delivery, abortion, placentitis or lower birth weight in some women. Pregnancy complications have been reported with both acute and chronic Q fever. The consequences of congenital Q fever are unknown.

Communicability

Person to person spread is very rare. Generally, isolation is not considered necessary.

Diagnostic Tests

In humans, Q fever is usually diagnosed by serology or PCR. Serologic tests can be done as early as the second week of illness; they may include immunofluorescence, enzyme-linked immunosorbent assay (ELISA), microagglutination or complement fixation. Antibodies to the protein antigens found in phase II organisms predominate in acute Q fever; high levels of antibodies to the lipopolysaccharide of phase I organisms, combined with steady or falling titers to phase II, indicate chronic Q fever. Polymerase chain reaction (PCR) assays can detect the organism in a wide variety of samples including blood, cerebrospinal fluid, various tissue samples and milk.

Isolation of *C. burnetii* is dangerous to laboratory personnel and is rarely done. In specialized laboratories, organisms can be recovered from blood or tissue samples; bacteria are isolated in cell cultures, embryonated chicken eggs or laboratory animals including mice and guinea pigs.

C. burnetii antigens can be detected in tissues by immunoperoxidase staining, capture ELISA, an enzyme-linked immunosorbent fluorescence assay system, or

other assays. Antigen assays are particularly useful in patients with chronic Q fever.

Treatment

Antibiotics can shorten the course of acute illness and reduce the risk of complications; treatment is most effective if it is begun early. Treatment of chronic cases is more difficult and may require long-term antibiotic therapy. Surgical replacement is sometimes necessary for damaged valves and some other conditions.

Prevention

Most human cases are associated with exposure to ruminants, particularly when the animal has given birth. Whenever possible, the placenta from sheep, goats and cattle should be removed and destroyed immediately. Pens should be cleaned. In a recent outbreak, a pregnant sheep that gave birth at a market was responsible for nearly three hundred human cases. The authors recommend not displaying sheep in public places during the third trimester, and testing susceptible animals in petting zoos for *C. burnetii*. Because ingestion is a potential route of exposure, unpasteurized milk and milk products should be avoided. Manure from contaminated farms should not be spread in suburban areas and gardens. Facilities that study susceptible ruminants should use good laboratory practices, and the animals should be negative for *C. burnetii*. Biosafety level 3 is required for the manipulation of contaminated specimens and cultivation of this organism.

Effective vaccines may be available for people who are occupationally exposed. A licensed vaccine is available in Australia. In the United States, an investigational vaccine can be obtained from special laboratories such as the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).

People at high risk for chronic Q fever, such as those who are immunosuppressed, should consider staying away from susceptible ruminants, particularly parturient ruminants. It may be advisable to avoid all animals that have recently given birth, as cases have also resulted from exposure to cats and other species.

Morbidity and Mortality

The worldwide incidence of Q fever is unknown; however, this disease is common in some countries. The mean annual incidence in Germany ranges from 0.1-3.1 per million people, and varies with the region. In southern France, the prevalence of acute Q fever is 50 cases per 100,000 inhabitants. Symptomatic cases are rarely reported in the U.S., but this may be the result of underreporting and poor recognition of the disease. Until 1999, reporting of Q fever was optional in the U.S. Between 1948 and 1977, 1,164 infections were reported to the Centers for Disease Control and Prevention (CDC). Fewer than 30 cases were reported annually between 1978 and 1986. Twenty-one cases were reported in 2000, after reporting became mandatory. Twenty-six cases were

reported in 2001, 61 cases in 2002, 71 in 2003 and 70 in 2004.

In endemic areas, Q fever can occur sporadically or in outbreaks. Most cases are seen in people occupationally exposed to farm animals or their products: farmers, abattoir workers, researchers, laboratory personnel, dairy workers and woolsorters have an increased risk of infection. Outbreaks have also been reported when pregnant animals were displayed in public, or strong winds dispersed organisms from infected farms. In an outbreak in 1954, approximately 500 people were infected by exposure to a pregnant cow that aborted at a farmer's market. Recently, nearly 300 people were infected when a pregnant sheep gave birth at a market in Germany.

Acute Q fever is usually self–limiting, and most people recover spontaneously within a few weeks. Most acute infections are mild or subclinical. Up to 60% are thought to be asymptomatic, most of the remainder experience mild illness, while 2-5% develop severe disease and require hospitalization. The overall mortality rate is 1-2% in untreated cases and lower in those who are treated. The mortality rate in patients with atypical pneumonia is 0.5% to 1.5%.

Chronic Q fever usually occurs in people who are immunosuppressed or have predisposing conditions such as cardiac valvular disease or vascular grafts. The incidence of chronic disease is stated to be less than 1% by one source, and 5% by another. Estimates of the mortality rate in chronic Q fever vary widely, with one source suggesting a mortality rate of 1-11%, and another stating that as many as 65% may die of the disease.

Infections in Animals

Species Affected

C. burnetii can infect many species of domesticated animals and wildlife; in many species, the infection appears to be asymptomatic. Its reservoirs may be only partially known. Sheep, goats and cattle seem to be the most common domesticated animal reservoirs. Wild rodents may be important reservoirs in some areas, and cats are suspected in urban outbreaks. *C. burnetii* has also been isolated from dogs, rabbits, horses, pigs, camels, buffalo, deer, pigeons, swallows, parrots, crows, geese and other mammals and birds. Antibodies have been found in coyotes, raccoons, opossums, badgers, jackrabbits, black bears, musk ox and other species. There are also reports of *C. burnetii* in fish and snakes.

Incubation Period

The incubation period is variable; reproductive failure is usually the only symptom. Abortions generally occur late in pregnancy.

Clinical Signs

Many species are susceptible to infection, but most species seem to be infected asymptomatically. Abortion,

stillbirth, retained placenta, endometritis, infertility and small or weak offspring can be seen in sheep, goats and cattle. Most abortions occur near term. Several abortions may be followed by uncomplicated recovery, particularly in small ruminants; in other cases, the disease may recur yearly. In dogs and cats, infections have been associated with stillbirths and weak offspring. Abortions and perinatal death occur after experimental (intraperitoneal) infection of pregnant mice.

With the exception of reproductive disease, animals are usually asymptomatic. Goats sometimes have a poor appetite and are depressed for 1 to 2 days before an abortion. Placental retention for 2 to 5 days and agalactia have also been reported. Clinical signs including fever, anorexia, mild coughing, rhinitis and increased respiratory rates occur in experimentally infected sheep but have not been reported in natural infections. Experimentally infected cats develop fever, lethargy and anorexia that last for several days. Experimentally infected mice may have pneumonia, hepatitis or splenomegaly, depending on the route of inoculation.

Communicability

Large numbers of organisms are found in the placenta, fetal fluids, aborted fetus, milk, urine and feces. Asymptomatic seropositive and seronegative animals, as well as symptomatic animals, may shed organisms.

Post Mortem Lesions   Click to view images

Placentitis is a characteristic sign in ruminants. The placenta is typically leathery and thickened, and may contain large quantities of white-yellow, creamy exudate at the edges of the cotyledons and in the intercotyledonary areas. In some cases, the exudate may be reddish-brown and fluid. Severe vasculitis is uncommon, but thrombi and some degree of vascular inflammation may be noted. Fetal pneumonia has been seen in goats and cattle and may occur in sheep; however, the lesions in aborted fetuses are usually non-specific.

Diagnostic Tests

C. burnetii can be detected in vaginal discharges, the placenta, placental fluids and aborted fetuses (liver, lung or stomach contents), as well as milk, urine and feces. Organisms are not shed continuously in milk and colostrum. In the placenta, organisms can be identified in exudates or areas of inflammation with a modified Ziehl–Neelsen, Gimenez, Stamp, Giemsa or modified Koster stain; *C. burnetii* is an acid-fast, pleomorphic, small coccoid or filamentous organism. This organism is not usually detected by Gram stains. The presence of organisms, together with serological tests and clinical findings may be adequate for a diagnosis at the flock or herd level. Bacterial identity can be confirmed by immunohistochemistry or capture ELISA. PCR techniques are also available in some laboratories. Fresh, frozen or paraffin-embedded samples of blood, milk, feces, vaginal exudates, placenta, fetal tissue and other tissues can be tested by PCR.

A number of serologic tests are available; the most commonly used assays include indirect immunofluorescence, ELISA and complement fixation. Serology may be more helpful in screening herds than in individual animals. Some animals do not seem to seroconvert, and others shed organisms before they develop antibodies. Animals can also remain seropositive for several years after an acute infection. Cross–reactions have been seen between some strains of *C. burnetii* and *Chlamydia* in ELISA and immunoblot assays.

C. burnetii can be isolated in cell cultures, embryonated chicken eggs or laboratory animals including mice and guinea pigs; however, isolation is dangerous to laboratory personnel and is rarely used for diagnosis.

Treatment

Little is known about the efficacy of antibiotic treatment in ruminants or other domestic animals. Prophylactic treatment is sometimes recommended to reduce the risk of abortion. Antibiotics may suppress rather than eliminate infections.

Prevention

In a *C. burnetii*-free flock, introduction of new stock should be minimized, and contact with wildlife should be prevented as much as possible. Good tick control should also be practiced. Prevention may be difficult, as this organism can also be introduced on fomites or in aerosols over long distances. In an infected flock, isolating infected pregnant animals and burning or burying the reproductive membranes and placenta can decrease transmission. The amount of *C. burnetii* in the environment can also be reduced by regular cleaning, particularly of areas where animals give birth. Cleaning can be followed by disinfection with 10% bleach. Antibiotics may be given prophylactically before animals give birth.

Vaccines are not available for domesticated ruminants in the United States but are used in some countries. Vaccines may prevent infections in calves, decrease shedding of organisms and improve fertility in infected animals. They do not eliminate shedding of the organism.

Morbidity and Mortality

Information on the prevalence of Q fever in the U.S. is limited. Surveys report infection rates that vary with the state, testing method and year the study was done. Across the U.S., reported seroprevalence rates in cattle range from 1% to 82%. The highest rates have generally been reported in California. In one area of California, 18% to 55% of sheep had antibodies to *C. burnetii*; the number of seropositive sheep varied seasonally and was highest soon after lambing. In other surveys, 82% of the individual

cows in some California dairies were seropositive, (with a herd infection rate of 98-100%) as well as 78% of the coyotes, 55% of the foxes, 53% of the brush rabbits and 22% of the deer in Northern California. Some studies suggest that the incidence of Q fever has been increasing, and this infection may currently be common throughout the U.S. A recent study found *C. burnetii* by PCR in 94% of bulk tank milk samples throughout the country, with little regional variation. Another survey reported antibodies in 92% of the dairy herds associated with U.S. veterinary schools.

C. burnetii infections may also be common in Canadian livestock. In Ontario, infections were found in 33-82% of cattle herds and 0-35% of sheep flocks. Close contact with sheep appears to increase the risk of infection in dogs.

Significant morbidity can be seen in some species. In sheep, abortions can affect 5-50% of the flock. In one California study, Q fever may have been responsible for 9% of all abortions in goats. Deaths are rare in natural infections.

Internet Resources

Centers for Disease Control and Prevention (CDC)
http://www.cdc.gov/ncidod/diseases/submenus/ sub_q_fever.htm

Public Health Agency of Canada. Material Safety Data Sheets
http://www.phac-aspc.gc.ca/msds-ftss/index.html

Medical Microbiology
http://www.gsbs.utmb.edu/microbook

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/eng/normes/mmanual/a_summry.htm

The Merck Manual
http://www.merck.com/pubs/mmanual/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

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Tularemia

*Rabbit Fever, Deer Fly Fever,
Meat-Cutter's Disease
Ohara Disease, Francis Disease*

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Importance

Tularemia is a zoonotic bacterial disease that can affect many mammals. It is most prevalent among wild animals, but clinical cases occur regularly in cats, and outbreaks have been reported among sheep, captive prairie dogs and ranched mink. A variety of syndromes can be seen, but fatal septicemia is common in some animal species. In humans, the disease varies from a localized illness to fulminant, life-threatening pneumonia or septicemia. Tularemia has recently emerged in some areas. It was recognized for the first time in Spain in 1997-1998, when two outbreaks, one associated with hares and the other linked to crayfish, affected more than 500 people. In 2000, a human epidemic in Kosovo was associated with a population explosion and epizootic among rodents, which occurred after large numbers of people had been displaced from their homes by the conflict. In 2002, an unexpected outbreak was seen among captive prairie dogs in the U.S., and the disease entered the Czech Republic in a shipment of these animals. Tularemia was detected for the first time in the Southern Hemisphere the same year, when a case caused by *Francisella tularensis* subsp. *novicida* was identified in Australia. In addition, tularemia is considered to be a potential bioterrorism agent.

Etiology

Tularemia results from infection by *Francisella tularensis* (formerly known as *Pasteurella tularensis*), a Gram negative coccobacillus in the family Francisellaceae. Four subspecies are currently recognized. Although all four cause tularemia, they tend to be associated with different animal hosts, and their propensity to cause severe disease varies. *F. tularensis subsp. tularensis* (also known as type A) has been found almost exclusively in North America. It is the most virulent species, particularly in humans. *F. tularensis* subsp. *holarctica* (type B) is much more widely distributed in the Northern Hemisphere, but it is less virulent. The two remaining species, *F. tularensis subsp. mediasiatica* and *F. tularensis subsp. novicida*, have been recognized in limited geographical regions, are rarely found in people, and seem to cause relatively mild disease when they occur. Additional subspecies have also been proposed.

Geographic Distribution

With rare exceptions, tularemia occurs only in the Northern Hemisphere. This disease has been reported from North America, Europe, parts of Asia and the Middle East, and northern Africa. A single human case, caused by *F. tularensis novicida*, was acquired in Australia.

F. tularensis tularensis is seen almost exclusively in North America, and has very rarely been isolated in Europe. *F. tularensis holarctica* is widely distributed in the Northern Hemisphere. This species is responsible for nearly all cases of tularemia outside North America. It co-exists with *F. tularensis tularensis* in North America. *F. tularensis mediasiatica* occurs in a limited area of Central Asia, and *F. tularensis novicida* has been reported from North America, Australia and Spain.

Francisella tularensis persists in some locations without outbreaks being reported, and the distribution of clinical cases among humans is patchy. In the U.S., Missouri, Arkansas, Oklahoma, Massachusetts and South Dakota accounted for 58% of the human cases from 2000 to 2005. Parts of Europe such as Finland and Sweden, and foci in northern Asia, also seem to be disproportionately affected.

Transmission

F. tularensis can be transmitted by ingestion, inhalation, arthropod–borne transfer, or direct contact with mucous membranes and broken skin. This organism is found in the blood and tissues of infected animals, and it can survive for long periods on fomites including food and water. Aquatic animals may develop tularemia after being immersed in contaminated water, and some human outbreaks have been linked to drinking from natural springs and wells. Carnivores can be infected by ingesting carcasses, and cannibalism seemed to be the primary route of transmission during an outbreak in captive prairie dogs.

A variety of arthropods can transmit *F. tularensis*. Ixodid ticks are biological vectors; species that are important in transmission include *Dermacentor andersoni*, *D. variabilis* and *Amblyomma americanum in North America*, and *Haemaphysalis flava* and *Ixodes japonesi* in Japan. *F. tularensis* is transmitted transstadially, and ticks can remain infected throughout their lifetimes; however, the occurrence of transovarial transmission is controversial. Biting flies in the family Tabanidae (particularly the deer fly, *Chrysops discalis*) can act as mechanical vectors. Individual flies can carry the organism for two weeks. Mosquitoes can also be vectors. The arthropods that are most important in transmission vary with the geographical area. For example, tabanid flies have been implicated as vectors in both the western U.S. and northern Europe, while mosquitoes can spread the organism in northern Europe. *F. tularensis* also occurs in other arthropods, although their role in transmission is speculative in many cases. In Europe, *F. tularensis holarctica* has been isolated from mites (family Gamasidae) collected from rodents. Mites can also transmit tularemia between rodents in the laboratory. Fleas are thought to be of little importance as vectors. Although they can remain infected for weeks, they do not transmit the organism readily between animals. Ceratopogonids (biting midges) and Simulidae (blackflies) have been proposed as potential vectors. Fruit flies (*Drosophila melanogaster*) can be infected in the laboratory, and bedbugs are reported to harbor the organism for 136 days. *F. tularensis holarctica* seems to be associated with lakes, streams and aquatic animals in some areas, and it might also be maintained in aquatic protozoans.

F. tularensis can survive for long periods in the environment. Viable bacteria can be found for weeks to months in the carcasses and hides of infected animals, and in fomites including grain dust, straw, water and soil. This organism is highly resistant to freezing; live bacteria have been found after 3 years in rabbit meat stored at -15°C.

Humans acquire *F. tularensis* from various sources including lakes and streams, food contaminated by the carcasses of infected animals or their excretions, laboratory cultures or clinical samples, and tissues from infected animals. Hunting or skinning animals, or contact with meat when preparing food, are important routes of exposure. Respiratory infections sometimes occur in farmers who are exposed through activities such as piling hay. Cases have been reported after mowing lawns, possibly from running over an animal carcass. Human infections have also been linked to handling infected animals or being bitten by them. Person-to-person transmission has not been reported.

Disinfection

F. tularensis can be killed by variety of disinfectants including 1% hypochlorite, 70% ethanol, glutaraldehyde and formaldehyde. It can also be inactivated by moist heat (121° C for at least 15 min) and dry heat (160–170° C for at least 1 hour).

Infections in Humans

Incubation Period

The incubation period in humans can be 3 to 15 days; most often, clinical signs appear after 3 to 5 days.

Clinical Signs

Six forms of tularemia are seen in humans: ulceroglandular, glandular, oculoglandular, oropharyngeal, respiratory and typhoidal. The form of the disease depends on the inoculation site.

Ulceroglandular tularemia, the most common form, occurs after infection through the skin or mucous membranes. The initial clinical signs are nonspecific and may include fever, chills, headache, body aches and malaise. An inflamed papule usually develops where the bacteria entered the body. This papule turns into a pustule that ulcerates. In some cases, the skin lesion may heal by the time the patient seeks medical care; in others, it persists. The regional lymph nodes become enlarged and painful, and may suppurate and drain profusely. Unusual cases with a vesicular skin rash have also been reported. The vesicles contain clear fluid that becomes turbid with time. Vesicles may also be found around an ulcerated eschar. Glandular tularemia is identical to the ulceroglandular form, but there is no lesion to indicate where the organism might have been inoculated.

Inoculation of the conjunctiva, often by touching the eye with contaminated fingers, results in oculoglandular tularemia; this form is characterized by unilateral, painful, purulent conjunctivitis with preauricular and/or cervical lymphadenopathy. In some cases, there may be chemosis, periorbital edema and multiple small nodules or ulcerations on the conjunctiva. Corneal perforation and iris prolapse are possible complications.

Oropharyngeal tularemia occurs after eating or drinking the bacteria. In addition to nonspecific symptoms such as fever and malaise, patients develop exudative stomatitis and pharyngitis with pustules and ulcers. In some cases, the tonsils may also be inflamed. The lymph nodes in the neck are usually enlarged and tender, and abscesses may develop in these nodes. Lymph node enlargement often occurs on only one side. Gastrointestinal signs such as abdominal pain from mesenteric lymphadenopathy, as well as vomiting, diarrhea and gastrointestinal bleeding, are occasionally reported.

Respiratory (pneumonic) tularemia occurs after inhalation. The outcome varies with the organism. Inhalation of *F. tularensis holarctica* does not usually cause fulminating disease or pneumonia, and cases are not generally fatal; however, *F. tularensis tularensis* is more

likely to causes serious disease. Sometimes, the only signs of respiratory tularemia are a cough, decreased breath sounds and substernal discomfort. In other cases, there may be a high fever, chills, malaise, coughing, chest pain, dyspnea and other signs of pneumonia. Patients may be weak, and in some cases, delirious. Some patients have severe systemic signs such as high fever, nausea and vomiting, without obvious signs of respiratory disease. Unless treated promptly, the more severe forms of respiratory tularemia, especially those caused by *F. tularensis tularensis,* have a high case fatality rate. Pulmonary signs can also occur with any other form of tularemia, when the lungs are affected via hematogenous spread.

Typhoidal tularemia is the term used for severe cases without an obvious route of exposure. Most cases are probably the result of inhalation, but this form can also develop after skin inoculation or ingestion. The symptoms may include nonspecific signs such as fever, prostration, headache, nausea and weight loss. Some patients become extremely weak and develop recurring chills and drenching sweats. A nonspecific rash may be seen, but lymphadenopathy is usually absent. Pneumonia is particularly common in the typhoidal form and can be severe.

Complications of tularemia may include meningitis, endocarditis, osteomyelitis, kidney failure, hepatitis, disseminated intravascular coagulation and acute respiratory distress. People who have recovered from tularemia can develop localized papules without generalization of the lesions, if they are later exposed to large amounts of the bacterium.

Communicability

Person-to-person transmission has not been reported; however, infectious organisms can be found in the blood, lesions and tissues.

Diagnostic Tests

In humans, tularemia is often diagnosed by serology. Commonly used serological tests include tube agglutination, microagglutination and enzyme–linked immunosorbent assays (ELISA). Significant, detectable titers begin to appear at the end of the second week of infection, although some specific antibodies may be present within the first 7 days. Cross–reactions can occur with *Brucella* spp., *Legionella* sp., *Proteus* OX19, and *Yersinia* spp., usually at low titers.

Tularemia can also be diagnosed by isolating *F. tularensis* from blood or affected tissues/ exudates such as sputum, pharyngeal or conjunctival exudates, ulcers, lymph nodes and gastric washings. *F. tularensis* subsp. *novicida* can be isolated on standard media such as blood agar; however, *F. tularensis tularensis and F. tularensis holarctica* are fastidious and require thiol compounds such as cysteine. The latter two organisms can be isolated

on cysteine heart agar with 9% chocolatized blood (CHAB), buffered charcoal yeast extract (BYCE), McCoy and Chapin medium, modified Thayer/Martin agar amd other specialized media. On CHAB, *F. tularensis* colonies are distinctive and appear green, opalescent, raised and shiny after 24 to 48 hours. Some bacteria appear to inhibit the growth of *F. tularensis*, a problem in contaminated samples. An antibiotic supplemented CHAB medium (CHAB-A) can be helpful. *F. tularensis* from tissues may also form colonies on sheep blood agar, but it will not grow well on this medium after subculture. *F. tularensis* colonies can be identified using slide agglutination, immunofluorescence or polymerase chain reaction (PCR) assays. This organism is a non–motile, Gram negative coccobacillus, with bipolar staining in young cultures. Because it is very small and stains faintly with conventional stains such as Gram stain, it can be difficult to detect. *F. tularensis tularensis* can be distinguished from *F. tularensis holarctica* by glycerol fermentation and molecular methods including PCR. Culture and identification of the organism can take from 2 days to more than 2 weeks. Because it must be done at biosafety level 3 (BSL-3), this test not available in all diagnostic laboratories.

PCR can also identify *F. tularensis* in clinical samples. Some PCR tests can distinguish the subtype. Other molecular techniques such as restriction fragment linked polymorphism (RFLP) Southern blot, pulsed-field gel electrophoresis (PFGE) and multi-locus variable number tandem repeat assays (MLVA) can be used to identify strains for epidemiological studies. 16S rDNA sequencing can be helpful in areas where *F. tularensis* has not been reported before.

Immunohistochemical staining is also used in diagnosis. Histopathology can be helpful. ELISAs are occasionally used to detect antigens in tissues and secretions, but this technique is not used routinely to diagnose tularemia in humans.

Treatment

F. tularensis is susceptible to a number of antibiotics such as tetracyclines and quinolones. Early treatment avoids complications such as suppuration of the lymph nodes.

Prevention

Tularemia can be acquired in bites from arthropods, through contaminated food and water, or by direct contact with infected animals, their tissues or excretions.

Long pants, shirts with long sleeves, and long socks can reduce bites from a variety of insects including ticks, tabanid flies and mosquitoes. Any attached ticks should be removed promptly. Insect repellants that contain chemicals such as DEET can fend off ticks and mosquitoes. They are not effective against deer flies and horse flies. Because deer flies and horse flies are attracted

to dark, moving objects, wearing light colors and a light colored baseball cap can be helpful, especially when it is warm and the insects are active. Additional methods of control include deer fly patches that attach to the backs of caps, and deer fly traps.

Hunters and others who handle wildlife and their carcasses should use gloves, and make sure that any breaks in the skin are covered. This precaution is particularly important with rabbits, hares, beavers, muskrats, prairie dogs and other rodents. The hands should be washed with soap and water after handling the animal, and any equipment should be cleaned well. Game meat should be cooked completely. Veterinarians and their staff, as well as sheep ranchers, should use good infection control procedures and precautions when working with animals that may be infected. Particular care should be taken to avoid bites and scratches. Die-offs of rodents and lagomorphs should be reported. In endemic areas, dust masks may be helpful during activities such as piling hay or mowing the lawn. Water should be filtered or treated before drinking.

Precautions have also been published for laboratories that work with *F. tularensis*. At one time, a live attenuated vaccine was used in the U.S. to protect laboratory workers. This vaccine is under review by the US Food and Drug Administration and it is no longer available.

Controlling a tularemia outbreak relies on finding the source of the organism and applying specific measures to stop transmission. In the former USSR, widespread outbreaks after WWII were controlled by massive vaccination campaigns, aided by improvements in sanitary conditions after the war.

Morbidity and Mortality

Tularemia is an occupational hazard for hunters, butchers, farmers, fur/ wool handlers, veterinarians, laboratory workers and others who come in contact with infected animals. In most countries, tularemia outbreaks are interspersed with periods during which only a few sporadic cases occur. Some outbreaks in humans have been linked to epizootics among animals. Epidemics can be seen during or after wars, when population explosions occur among rodents that have access to unharvested grain and other food sources, and these animals contaminate human food. One outbreak occurred in a sugar plant, and may have been caused by sugar beets contaminated with rodent excretions or carcasses. Other epidemics have been reported in people who hunted lagomorphs, hamsters or other species during epizootics in these animals, or who drank contaminated water. Human cases have also been associated with outbreaks in sheep. One unusual outbreak occurred among people who had been exposed to contaminated freshwater crayfish while fishing.

Infections caused by *F. tularensis tularensis* are more virulent than those caused by *F. tularensis holarctica;* the course is more likely to be fulminant and deaths are more common. Estimates vary, but before antibiotics, the overall case fatality rate for tularemia caused by *F. tularensis tularensis* was 5-15%, or possibly as high as 30%. Far fewer deaths were caused by *F. tularensis holarctica*. Antibiotics have reduced the overall case fatality rate from tularemia to 1-3%.

Ulceroglandular and glandular tularemia are the most common forms of disease caused by both organisms, occurring in at least 75 to 85% of cases. The case fatality rate for ulceroglandular tularemia is estimated to be 5% if it is untreated. The oculoglandular form is uncommon, and the prevalence of the oropharyngeal form varies with the region. The respiratory form of tularemia is often linked to farming activities. The case fatality rate varies widely for this form, depending on the subspecies of the organism, but it can be greater than 50% for untreated, severe cases. The typhoidal form may occur in 5 to 15% of cases. It is most often caused by *F. tularensis tularensis* and has the highest case fatality rate. Typhoidal tularemia would be expected to be the predominant form after an attack that used aerosolized *F. tularensis* in a biological weapon.

Infections in Animals

Species Affected

Nearly two hundred species of animals can be infected with *F. tularensis*. Common wild animal hosts include lagomorphs (cottontail rabbits, jack rabbits and hares), muskrats, beavers, and a variety of rodents including voles, field mice, vole rats, squirrels and lemmings. Among domesticated animals, sheep and cats seem to be particularly susceptible to clinical disease. Tularemia has also been seen in dogs, pigs, horses, nonhuman primates, ranched mink and captive prairie dogs; cattle seem to be relatively resistant. Infections have been reported occasionally in birds, reptiles, amphibians, crayfish, mollusks and fish; it is not known whether some of these cases were true infections or if the organism was acquired only temporarily from contaminated water.

Each subspecies of *F. tularensis* tends to be associated with certain host species. Lagomorphs are important hosts for *F. tularensis tularensis* in North America. *F. tularensis holarctica* is linked to muskrats (*Ondatra zibethicus*), American beavers (*Castor canadensis*) and voles (*Microtus* spp.) in North America, and to hares (*Lepus* spp.) and rodents in Europe. Little is known about the preferred hosts for *F. tularensis mediasiatica,* which has been detected in hares and Gerbillinae (gerbils and their relatives), or *F. tularensis* subsp. *novicida*.

Incubation Period

The incubation period is 1 to 10 days.

Clinical Signs

The full spectrum of clinical signs is not known in animals, but syndromes corresponding to the typhoidal, respiratory, ulceroglandular and oropharyngeal forms have been reported. Septicemia is often reported in susceptible animals, but asymptomatic infections also seem to be common, especially in resistant species such as dogs and cattle.

Signs of septicemia may occur in wild mammals, domesticated rabbits and pet rodents, but many animals are found dead. Rabbits and rodents may be depressed, with anorexia and ataxia, a roughened coat and a tendency to huddle. In an outbreak among captive prairie dogs, which probably spread by cannibalism, all of the animals had signs of the oropharyngeal form. The clinical signs also included emaciation, dehydration and lethargy.

In cats, infections often begin with the sudden onset of fever, lethargy, anorexia, and regional or generalized lymphadenopathy. The lymph nodes may suppurate and drain. The submandibular lymph nodes are often affected, and oral lesions including white patches or ulcers may be found. Pneumonia, icterus, hepatomegaly, splenomegaly, weight loss and vomiting have also been reported. Other syndromes can be seen. One infected cat had a chronic draining cutaneous lesion and swelling of the mandibular lymph nodes, but no systemic signs, for about a year before tularemia was diagnosed. In general, the case fatality rate seems to be high.

In contrast, dogs appear to be relatively resistant and may recover spontaneously. Clinical signs that have been reported in this species include anorexia, depression, mild fever, lymphadenopathy (which may be mild), draining abscesses, and mucoid ocular discharge or conjunctivitis. Experimentally infected dogs that were fed *F. tularensis* developed a self-limited illness with fever and mucopurulent discharge from the nose and eyes. Dogs that were inoculated intradermally had pustules at the inoculation site and regional lymphadenopathy.

Outbreaks in sheep are usually characterized by late term abortions in ewes, and illness and deaths among lambs. Fever, listlessness, regional lymphadenopathy and diarrhea may be seen. Adult sheep can have systemic signs, but this is uncommon. Although serological evidence suggests that infections are fairly common in cattle, a specific syndrome has not been described, and many cases may be asymptomatic. Tularemia has been diagnosed rarely in sick calves.

Nonspecific signs including lethargy, anorexia, vomiting, diarrhea, generalized lymphadenopathy, pale mucous membranes and cutaneous petechiae have been reported in nonhuman primates. Some animals have died acutely.

Communicability

F. tularensis occurs in blood and tissues. This organism has also been reported in the feces and urine of some animals. Infected animals can transmit tularemia to arthropod vectors. In an outbreak among prairie dogs, *F. tularensis* was probably transmitted between the animals by cannibalism. Outbreaks in sheep might follow epizootics among lagomorphs or rodents, and infections appear to be acquired via ticks.

Human cases have been associated with cats, pet rodents, dogs, prairie dogs and sheep, as well as wild animals. Tularemia has been transmitted to people in bites from cats, a pet hamster and a dormouse, as well as in a scratch by a buzzard. In some cases, cats that transmitted the bacterium were not ill, and may have been colonized temporarily after contact with a rodent. Human infections linked to dogs include a case of typhoidal tularemia in a child who had probably been infected via saliva from a sick puppy, and seven cases of respiratory tularemia in people who had been exposed to dogs that hunted rabbits. In the latter case, people were apparently infected when the wet dogs shook themselves.

Post Mortem Lesions   [Click to view images]

Animals with acute tularemia are often in good body condition, but they may also be dehydrated, thin or emaciated. Oral lesions can occur in animals that were infected by ingestion. The liver, spleen and lymph nodes are frequently enlarged, and the affected lymph nodes may contain areas of caseous necrosis. Miliary, grayish–white, white or light yellow necrotic foci are often noted in the liver, spleen, bone marrow, lungs and/or lymph nodes. Some of these foci may be barely visible. In rabbits, the pale necrotic foci on a dark, congested liver and spleen have been compared to the Milky Way. The lungs are often congested and edematous, and they may contain areas of consolidation and fibrinous pneumonia or pleuritis. Ulcerative enteritis, associated with necrosis of Peyer's patches, can also be seen, and icterus occurs in some cats. More resistant species may have chronic granulomas that resemble tuberculosis lesions.

Diagnostic Tests

F. tularensis is zoonotic, and samples must be collected, handled and shipped with care. The receiving laboratory should be consulted before collecting or shipping samples.

Impression smears of the liver, spleen, bone marrow, kidney, lung or blood may be helpful for a presumptive diagnosis; small Gram negative coccobacilli can sometimes be found inside cells and scattered among tissue debris. *F. tularensis* is very small (0.2–0.7 μm) and easy to miss, it stains faintly with conventional stains such as Gram stain, and it can look like stain precipitates. Organisms are more likely to be found in impression smears with immunofluorescence.

Immunohistochemistry can detect antigens in tissue sections, and ELISAs have been used to find antigens in tissues and secretions from animals, or in environmental samples such as water and mud.

F. tularensis can be isolated from enlarged lymph nodes, blood, and tissues including liver, spleen and bone marrow; the overgrowth of other bacteria may prevent recovery from animals that are found dead. *F. tularensis* subsp. *novicida* can be isolated on standard media such as blood agar; however, *F. tularensis tularensis and F. tularensis holarctica* are fastidious and require thiol compounds such as cysteine. The latter two organisms can be isolated on cysteine heart agar with 9% chocolatized blood (CHAB), buffered charcoal yeast extract (BYCE), McCoy and Chapin medium, modified Thayer/Martin agar and other specialized media. On CHAB, *F. tularensis* colonies are distinctive and appear green, opalescent, raised and shiny after 24–48 hours. On McCoy and Chapin medium, the colonies are small, prominent, round and transparent. Confluent, milky, mucoid colonies develop on Francis medium and modified Thayer/Martin agar. Organisms from tissues may also form colonies on sheep blood agar, but they will not grow well on this medium after subculture. Some bacteria seem to inhibit the growth of *F. tularensis*, a problem in contaminated samples. An antibiotic supplemented CHAB medium (CHAB-A) can be helpful in this situation. *F. tularensis* colonies can be identified using slide agglutination, immunofluorescence or PCR. The organism is a Gram negative coccobacillus with bipolar staining in young cultures, but bacteria from older cultures may be pleomorphic. *F. tularensis tularensis* can be distinguished from *F. tularensis holarctica* by glycerol fermentation and molecular methods including PCR. Culture and identification of the organism can take from 2 days to more than 2 weeks. Because it must be done at biosafety level 3 (BSL-3), this test is not available in all diagnostic laboratories.

PCR can also identify *F. tularensis* in clinical samples. Some PCR tests can distinguish the subtype. Other molecular techniques such as RFLP Southern blot, PFGE and MLVA can be used to identify strains for epidemiological studies. 16S rDNA sequencing can be helpful in areas where *F. tularensis* has not been reported before.

Serology is occasionally useful in animals. Species sensitive to tularemia typically die before specific antibodies develop; however, significant titers may be found in more resistant animals such as sheep, cattle, pigs and dogs. Serological tests include tube agglutination, microagglutination and ELISA. A rising titer should be seen. Cross-reactions may occur with other bacteria including *Yersinia* spp., *Brucella* spp. and *Legionella* spp.

Treatment

Tularemia can be treated with various antibiotics including tetracyclines and quinolones. Supportive therapy may also be required. Early treatment is expected to be most effective.

Prevention

Good tick control programs can reduce the risk of infection. Some repellants for use in livestock are effective against deer flies and horse flies. Cats and dogs should be kept from hunting rodents and rabbits in areas where tularemia is endemic. This disease is also less likely to occur in domesticated rabbits and pet rodents that are housed inside. Good infection control procedures should be used with infected animals. Vaccines are not available for any species.

Morbidity and Mortality

Tularemia is relatively common and highly fatal in some species of wild animals; epizootics occur regularly among rabbits and rodents. Susceptibility varies among domesticated animals. Epizootics of tularemia were once common among range sheep in Idaho, Montana and Wyoming, and occasional outbreaks can still occur. The morbidity rate/ abortion rate in this species can be as high as 50%. Mortality rates up to 10-15% are seen in untreated lambs, but adult sheep do not usually develop systemic signs. Cats seem to be relatively susceptible to tularemia. Sick cats often have severe clinical signs, and the mortality rate is high if the disease is not treated early. However, milder cases are reported occasionally, and some cats with no history of disease are seropositive. Dogs, cattle and some other species seem to be relatively resistant, and may have milder clinical signs.

Internet Resources

Centers for Disease Control and Prevention (CDC). Tularemia
http://www.cdc.gov/Tularemia/

CDC. Emergency Preparedness and Response. Tularemia
http://www.bt.cdc.gov/agent/tularemia/index.asp

Public Health Agency of Canada. Material Safety Data Sheets
http://www.phac-aspc.gc.ca/msds-ftss/index.html

Medical Microbiology
http://www.gsbs.utmb.edu/microbook

The Merck Manual
http://www.merck.com/mmpe/index.html

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

University of Rhode Island. Deer and horse flies.
http://www.uri.edu/ce/factsheets/sheets/deerhorseflies.html.

World Organization for Animal Health (OIE)
http://www.oie.int

Viral Hemorrhagic Fevers–Ebola and Marburg

African Hemorrhagic Fever

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Importance

Ebola and Marburg hemorrhagic fevers are severe zoonotic diseases seen in humans and non-human primates. Most species of ebolavirus and the only known species of marburgvirus occur in Africa. Primates are infected sporadically from an unknown source; current evidence suggests that the reservoir hosts are probably bats. Humans seem to become infected directly from bats in caves, as well as when they contact tissues from infected apes and other species. Once the virus has entered the population, it can spread from person to person. Some epidemics affect hundreds of people and decimate entire villages, particularly where hospital facilities and medical supplies are inadequate and nosocomial spread occurs. Although the mortality rate varies, the most pathogenic viruses kill up to 90% of those who become infected. No vaccine is available, and the only treatment is supportive. Epizootics in gorillas and chimpanzees are equally serious, and appear to threaten the survival of these species in the wild. Other wild mammals including duikers also seem to be killed during outbreaks.

One species of ebolavirus, *Reston ebolavirus*, occurs in the Philippines. Until 2008, this virus was known only as an infection of nonhuman primates. It does not appear to be a human pathogen; although some people in contact with this virus have seroconverted, they remained asymptomatic. Between 1989 and 1996, *Reston ebolavirus* was isolated repeatedly at primate quarantine facilities in the U.S. and Italy; in all but one instance, infected monkeys had been imported from a single facility in the Philippines. The source of the virus was never found, but infected monkeys do not seem to have been exported since this facility was closed in 1997. In December 2008, *Reston ebolavirus* was discovered in pigs during an unusually severe outbreak of porcine reproductive and respiratory syndrome (PRRS) in the Philippines. Genetic evidence suggests that the virus may have been circulating in these swine populations since 1989 or earlier. Whether *Reston ebolavirus* can cause disease in swine or is an incidental finding is unknown. Its full host range, reservoir host(s) and geographic range also remain to be discovered.

Etiology

Ebola and Marburg hemorrhagic fever are caused by members of the genera *Ebolavirus* and *Marburgvirus,* respectively. These viruses are the only members of the family Filoviridae. The genus *Ebolavirus* contains four recognized species: *Zaire ebolavirus* (formerly Zaire Ebola virus), *Sudan ebolavirus* (formerly Sudan Ebola virus), *Ivory Coast ebolavirus* (formerly Cote d'Ivoire Ebola virus) and *Reston ebolavirus* (formerly Reston Ebola virus). At least 13 strains of these viruses have been identified. A fifth species, tentatively named *Bundibugyo ebolavirus*, was isolated from a recent outbreak in Uganda. *Marburgvirus* contains a single species, *Lake Victoria marburgvirus* (formerly Marburg virus). Six strains of *Lake Victoria marburgvirus* had been recognized as of 1990; at least nine genetically distinct strains were identified from a more recent outbreak in the Democratic Republic of the Congo.

Geographic Distribution

Zaire ebolavirus, *Sudan ebolavirus*, *Ivory Coast ebolavirus* and *Bundibugyo ebolavirus* are endemic in several African countries south of the Sahara desert. The pattern of outbreaks seems to suggest that each filovirus may have a distinct geographic range. *Ivory Coast ebolavirus* has been reported only from West Africa, while *Sudan ebolavirus* tends to occur in eastern Africa (Sudan and Uganda), and *Zaire ebolavirus* has been seen mainly in the west-central region (Gabon, Republic of the Congo and Democratic Republic of the Congo [formerly Zaire]). *Bundibugyo ebolavirus* was reported from an outbreak in Uganda. However, recent serological surveys suggest that some of these viruses may be more widespread. Antibodies to *Zaire ebolavirus* have been found in nonhuman primates and bats in much of central Africa; seropositive animals were found in some countries, such as Cameroon, where outbreaks of Ebola hemorrhagic fever have never been reported.

Lake Victoria marburgvirus has been found in bats, nonhuman primates and/or humans from eastern Africa to the far western edge of the Congo. This virus has

Viral Hemorrhagic Fevers-Ebola and Marburg

caused epidemics in African countries including Angola and Uganda. One case reported from South Africa may have been acquired in Zimbabwe. In 1967, *Lake Victoria marburgvirus* caused an outbreak in Germany and Yugoslavia, when humans were exposed to tissues from imported green (vervet) monkeys (*Cercopithecus aethiops*).

Reston ebolavirus is the only filovirus known to be endemic outside Africa. This virus occurs in the Philippines, but outbreaks have been seen in imported, non-human primates at quarantine facilities in the United States and Italy. In each case, the virus was eradicated.

Transmission

Filoviruses seem to emerge in primates after infection from an outside source. The reservoir hosts have not been definitively identified, but bats are the most likely candidates. Although virus isolation has not yet been successful, RT-PCR and serologic evidence suggest that *Zaire ebolavirus* infections occur in African fruit bats (*Hypsignathus monstrosus*, *Epomops franqueti* and *Myonycteris torquata*), and *Lake Victoria marburgvirus* infections occur in fruit bats (*Rousettus aegyptiacu*s) and insectivorous bats (*Rhinolophus eloquens* and *Miniopterus inflatus)*. Bats that are experimentally infected with ebolavirus become viremic for up to four weeks but remain asymptomatic. How viruses are transmitted from bats to primates or other mammals is unknown. In addition to bats, there may be other reservoir and/or amplifying hosts.

Humans often become infected with ebolaviruses after handling the carcasses of animals found in the forest, particularly nonhuman primates and duikers (forest antelope). The virus is thought to enter the body through mucous membranes and broken skin. Filoviruses are probably also transmitted directly from bats to humans. Some marburgviruses infections have been acquired by exposure to primate tissues, while others were associated with transmission within caves, possibly from infected bats. Filoviruses can be spread from person to person. High viral titers occur in blood, which can contaminate the environment during the hemorrhagic stage of the disease. These viruses are also found in many secretions and excretions that are not visibly contaminated with blood, including saliva, tears, breast milk, semen and feces. Urine may be a source of virus; however, *Zaire ebolavirus* was absent from patients' urine during a recent outbreak. Filoviruses disappear from blood and most tissues after the acute stage of the disease, but some body fluids can contain these viruses for a few months. In one patient, *Lake Victoria marburgvirus* was transmitted sexually 13 weeks after the onset of disease. *Zaire ebolavirus* was also isolated from the semen of a convalescent patient up to 82 days after the onset of clinical signs, and detected by RT-PCR up to 91 days. The latter virus was recovered from the breast milk of a convalescing patient, 15 days after the onset of disease.

Filoviruses have been reported to survive for some time in blood and tissues at room temperature. Fomites, particularly those contaminated by blood, can transmit these viruses. Aerosol transmission has been reported in nonhuman primates, but it does not seem to be important during human outbreaks. Arthropod-borne transmission is theoretically possible, but most authors suggest it is unlikely.

Disinfection

Filoviruses can be destroyed by autoclaving. Treatment with sodium hypochlorite (1:100 dilution of household bleach) or standard hospital disinfectants can also be used. Ebolaviruses are reported to be susceptible to 2% sodium hypochlorite, 2% glutaraldehyde, 5% peracetic acid and 1% formalin. These viruses can also be inactivated by ultraviolet light, gamma irradiation, 0.3% betapropiolactone for 30 minutes at 37°C [98.6°F], or heating to 60°C [140°F] for 1 hour. Marburgvirus is susceptible to 1% sodium hypochlorite, 2% glutaraldehyde or formaldehyde, ultraviolet light and heat.

Infections in Humans

Incubation Period

The incubation period for filoviruses can be as short as two days or as long as 21 day; in most cases, symptoms appear in 4 to 10 days.

Clinical Signs

Lake Victoria marburgvirus and the Zaire, Sudan and Ivory Coast species of *ebolavirus* usually cause similar diseases; however, the mortality rate, speed of onset and severity of disease vary with the virus. The symptoms usually appear abruptly; the initial signs may include fever, chills, headache, severe malaise, muscle aches, abdominal pain, nausea, vomiting and diarrhea. Some patients develop conjunctivitis or pharyngitis with a nonproductive cough. A purplish-red, maculopapular rash may also be seen; this rash is especially common on the trunk and shoulders of patients infected with *Zaire ebolavirus*. After a few days, patients may develop mild to severe bleeding tendencies. In mild cases, this may be limited to bruising, bleeding of the gums, epistaxis, petechiae and/or mild oozing from venipuncture sites. In severe cases, patients have hemorrhages from the gastrointestinal tract or other sites, go into shock, and develop multi-organ failure. Although many patients die, some begin to recover after a week or two. Recovery is more likely if the bleeding tendencies were mild. During convalescence, which can be slow, some patients develop joint pain, deafness, orchitis or pericarditis.

Unlike other filoviruses, *Reston ebolavirus* does not seem to be pathogenic for humans. Asymptomatic seroconversion can be seen.

Viral Hemorrhagic Fevers-Ebola and Marburg

Communicability

Filoviruses can spread between humans by contact with blood, body fluids and excretions, and tissues. Large quantities of ebolavirus have been found in blood as soon as two days after the onset of symptoms. During the acute stage of the disease, filoviruses are also found in many secretions and excretions that are not visibly contaminated with blood, including saliva, tears, breast milk, semen and feces. Aerosol transmission may be possible, but it does not seem to be a significant route of transmission during between humans.

Some body fluids can contain viruses for up to a few months after recovery. *Zaire ebolavirus* has been isolated from the semen of convalescent patients up to 82 days after the onset of clinical signs, and detected by RT-PCR up to 91 days. In one case, *Lake Victoria marburgvirus* was transmitted sexually 13 weeks after the onset of symptoms. *Zaire ebolavirus* was isolated from the breast milk of a recovering patient, 15 days after the onset of disease, and transmission to a nursing child may be possible. How long filoviruses can be found in milk is unknown. Some authors speculate that filoviruses might also occur within the eye, which is an immunologically privileged site, during convalescence. With these exceptions, filoviruses do not seem to persist after recovery from acute disease, and one study reported that the risk of transmission by casual contact after discharge from the hospital appears to be low.

Diagnostic Tests

Ebola or Marburg hemorrhagic fever can be diagnosed by detecting antigens with an antigen-capture enzyme-linked immunosorbent assay (ELISA) or immunostaining, and by detecting viral RNA with reverse transcription polymerase chain reaction (RT-PCR) assays. Virus isolation is also used. Ebolaviruses and marburgvirus can be recovered in many cell lines, including Vero or Vero E6 cells. In humans, filoviruses are most reliably isolated from the blood during the acute-stage of the disease, but they may also be found in throat washes, urine, semen, anterior eye fluid and other fluids, and in many tissues including the skin. Skin biopsies are often taken at post-mortem for immunohistochemistry. Serology is valuable, particularly in later stages of the disease. Serological assays include ELISA tests that detect IgM and IgG, as well as the indirect immunofluorescence assay (IFA). Neutralization tests are unreliable for filoviruses. Because the consequences of misdiagnosis (including false positive diagnosis) are severe, multiple techniques are used to confirm the infection whenever possible.

The remote location of most epidemics can make the initial diagnosis challenging. Most diagnostic specimens must be sent to BSL-4 laboratories, which are usually unavailable where outbreaks occur. Virus isolation is sometimes unsuccessful because a cold chain cannot be maintained. Diagnostic techniques that can be used in remote areas without extensive laboratory facilities, including methods that use recombinant antigens, are in development.

Treatment

No specific treatment is available. Supportive therapy generally consists of intravenous fluid replacement to maintain blood volume and electrolyte balance, as well as analgesics and standard nursing care. Although more specific treatments have been attempted, most have apparently had little effect on the outcome. Some techniques being tested in laboratory animals are promising when used early in the incubation period, but none have entered human clinical trials. The antiviral drug ribavirin does not seem to be effective. Strict infection control measures and barrier nursing precautions must be used during treatment, to prevent infection of medical staff.

Prevention

In Africa, the index case is often associated with exposure to the tissues of an infected animal during butchering. Most cases have been linked to exposure to chimpanzee, gorilla or duiker carcasses, but one person butchered mandrills shortly before becoming ill. Because the full host range may not be known, all sick and dead wild animals should be avoided. These animals should not be touched, eaten or fed to other animals. To prevent infection from animals that appear healthy but are incubating the disease, good personal hygiene should be used when handling and preparing meat, and the meat should be thoroughly cooked. Meat inspection and testing, where available, also protects consumers. Some filovirus infections have been linked to exposure to caves. Particular care should be taken to avoid areas infested with bats. If contact with bats or caves is unavoidable, personal protective equipment and good hygiene should be used; however, the means of transmission from bats to humans is still unknown. Surveillance for deaths and illness in wild animals, particularly nonhuman primates, may provide an early warning to prevent human epidemics.

Human epidemics can be stopped by isolating patients in facilities with barrier nursing procedures and strict infection control measures. Healthcare workers should use good hygiene and personal protective equipment including gloves, gowns, masks and eye protection to prevent exposure to blood and body fluids. Burial practices should avoid all contact with the body or fomites. In some remote areas of Africa, where disinfectants and routine medical supplies are not readily available, these measures are challenging to achieve. Precautions should also be taken to avoid spreading the disease during convalescence. Sexual transmission is possible during this period, and abstinence should be practiced for at least three months after recovery. If possible, mothers should avoid breastfeeding for a time; ebolavirus may be found in the milk for at least 15 days

after the onset of disease. No vaccines are currently available for humans, but some promising vaccines are in development.

The *Reston ebolavirus*, which was recently discovered in pigs in the Philippines, is not known to affect humans. However, as a precaution, tissues from infected animals should not be eaten or handled. Good hygiene and appropriate personal protective equipment should be used by individuals who must handle these animals or their tissues.

Morbidity and Mortality

Ebola and Marburg hemorrhagic diseases are most likely to occur in people who butcher carcasses or enter caves and mines. Once infected, people often transmit the disease to family members and others in contact. Healthcare workers are at high risk, as hospital supplies are often limited or nonexistent in areas where filoviral diseases occur. In some cases, hundreds of people may become infected through nosocomial transmission, unsafe self-treatment at home, and other routes. Strict isolation, good infection control measures and barrier nursing can halt the spread of the disease: in some cases, infections have been limited to a few contacts.

Outbreaks of Ebola hemorrhagic fever are reported periodically in Africa. The number of outbreaks has increased in the last ten years, due either to a higher incidence or better recognition of the disease. Marburg hemorrhagic fever was only recently recognized as a serious and recurring problem in humans. *Lake Victoria marburgvirus* was first discovered in Europe in 1967, when laboratory workers were exposed to infected tissues from imported primates. Between 1967 and 1994, only six cases of Marburg were reported, all from Africa; three cases occurred in travelers and three in their contacts. However, in 1998, this virus caused an epidemic that affected hundreds of people in the Democratic Republic of the Congo (DRC). This outbreak was associated with a mine where infected bats were later discovered. Several different strains of *Lake Victoria marburgvirus* were isolated during the epidemic, suggesting that this virus had been introduced repeatedly into the population via infected miners. This outbreak also uncovered a pattern of hemorrhagic disease in the mine dating to 1987 or earlier, and one survivor of an earlier outbreak was found to have antibodies to this virus. In 2004-2005, another large Marburg outbreak was reported in Angola, where *Lake Victoria marburgvirus* was not thought to exist.

In human populations, the African filoviruses usually have high mortality rates. *Zaire ebolavirus* is the most pathogenic virus. The case fatality rate for this infection was 59% to 88% in all outbreaks to 2008; in five of seven epidemics, it was at least 78%. *Sudan ebolavirus* is less virulent, with a case fatality rate of 41-65%. Only one outbreak of *Bundibugyo ebolavirus* has been reported; the case fatality rate was 36%. *Ivory Coast ebolavirus* was only reported once; in 1999, a scientist developed a fatal

hemorrhagic illness after performing an autopsy on a wild chimpanzee that had died from a similar disease. The mortality rate for *Lake Victoria marburgvirus* varies widely. The 1967 outbreak in Europe had a case fatality rate of 22-23%, and three of the six patients reported between 1967 and 1994 died. However, the case fatality rate may have been as high as 83% (56% in laboratory-confirmed cases) during the 1998-2000 outbreak in DRC, and it was 88% during the 2004-2005 outbreak in Angola. It is not known whether the high mortality rates in recent outbreaks are associated with more virulent strains of the virus, higher doses, concurrent malnutrition and disease, or the availability and quality of healthcare. It is also uncertain whether African filoviruses can cause mild or asymptomatic infections. Antibodies have been reported in people who have no history of Ebola or Marburg hemorrhagic disease, but in some cases, the illness may have been misdiagnosed as another disease such as malaria. Cross-reactivity with other viruses may also be a problem. Seroprevalence rates tend to be higher in groups that have more contact with wild animals.

Reston ebolavirus can infect humans but has never been associated with disease. Seroconversion is possible, but does not seem to be common. In the Philippines, antibodies to this virus were reported in 6 of 141 individuals tested.

Infections in Animals

Species Affected

Bats are the probable reservoir hosts for filoviruses. Viral RNA and antibodies to *Zaire ebolavirus* have been found in three species of arboreal fruit bats: *Hypsignathus monstrosus*, *Epomops franqueti* and *Myonycteris torquata*. *Lake Victoria marburgvirus* RNA and antibodies to this virus have been found in a cave-dwelling fruit bat (*Rousettus aegyptiacus*) and an insectivorous bat (*Rhinolophus eloquens*). RNA alone was detected in an insectivorous bat of the species *Miniopterus inflatus*. Attempts to isolate either virus from wild bats have been unsuccessful, but two genera of bats (*Epomophorus* and *Tadarida*) remained viremic for up to four weeks after intravenous inoculation with *Zaire ebolavirus*. Reservoirs have not been reported for *Sudan ebolavirus*, *Reston ebolavirus* and *Ivory Coast ebolavirus*, but these viruses may also be transmitted by bats. Other reservoir or amplifying hosts may also exist, but there is little evidence to implicate any species. In 1998, *Zaire ebolavirus* RNA was detected in six mice (*Mus setulosus* and *Praomys* sp) and a shrew (*Sylvisorex ollula*). These species were proposed as possible reservoir hosts, but the results have not been confirmed by other groups, and virus isolation was unsuccessful.

Although bats seem to carry filoviruses asymptomatically, these viruses are pathogenic in incidental hosts. The African filoviruses (all filoviruses except *Reston ebolavirus*) cause severe disease in both

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humans and nonhuman primates. Domesticated animals do not seem to be affected by these viruses, but wild primates and other wildlife are susceptible. In Africa, Ebola outbreaks have been linked to reports of dead and dying gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), mandrills (*Mandrillus* sp.), guenon (*Cercopithecus* sp.) and other monkeys, as well as duikers (a species of forest antelope, *Cephalophus dorsalis*), bush pigs (red river hog, *Potamochoerus porcus*) and other species. Attempts to isolate ebolaviruses or detect viral RNA have been successful only in chimpanzees, gorillas and duikers. It is not known whether other species were affected by Ebola or other diseases. However, antibodies to ebolaviruses have been reported in mandrills, drills (*Mandrillus* sp.), baboons (*Papio* sp.), colobus monkeys (*Colobus badius*) and guenon, as well as chimpanzees and gorillas, suggesting that these species might become infected. One study detected antibodies in dogs, but death or illness has not been reported in this species. Experimental ebolavirus infections have been established in nonhuman primates, newborn mice and guinea pigs, which become ill, and bats, which remain asymptomatic. *Lake Victoria marburgvirus* seems to affect only humans and non-human primates. Antibodies to this virus have been reported in primates including captive vervet monkeys (*Cercopithecus aethiops*) and baboons.

Reston ebolavirus causes hemorrhagic fever in nonhuman primates including cynomolgus macaques (*Macaca fascicularis*). This virus was recently reported in domesticated swine in the Philippines. *Reston ebolavirus* does not seem to be pathogenic for humans, although seroconversion can occur.

Incubation Period

The incubation period varies with the virus and dose. In cynomolgus macaques, inoculation of *Zaire ebolavirus* by the oral or conjunctival route causes clinical signs within 3 to 4 days. The incubation period for *Lake Victoria marburgvirus* or *Zaire ebolavirus* infection in rhesus macaques and vervet monkeys is 3 to 16 days. In guinea pigs, the incubation period is 4 to 10 days.

Clinical Signs

Filoviruses cause hemorrhagic fever in nonhuman primates. Wild chimpanzees and gorillas are often found dead. Clinical signs observed in dying wild animals (of various species) during Ebola outbreaks include vomiting, diarrhea, hair loss and emaciation, as well as bleeding from the nostrils. Whether all of these signs are associated with filovirus infections or some were caused by other diseases is unknown. During the 1989 *Reston ebolavirus* outbreak in Virginia, anorexia was the first sign of disease in cynomolgus monkeys. Some monkeys had swollen eyelids or increased lacrimation. Nasal discharge, coughing and splenomegaly were also seen. Fever, subcutaneous hemorrhages, epistaxis and/or bloody diarrhea were less common signs. In experimentally infected monkeys, the signs of Ebola or Marburg

hemorrhagic fever include fever, anorexia, vomiting, splenomegaly, weight loss and a skin rash. Hemorrhagic signs may include petechiae, bleeding into the gastrointestinal tract, or bleeding from puncture wounds and mucous membranes. Shock and hypothermia are soon followed by death. African species of ebolaviruses are usually more pathogenic than *Reston ebolavirus*; the clinical signs are more severe, hemorrhages are more common, and the mortality rate is higher.

Guinea pigs infected with unpassaged virus from primates usually develop a fever and weight loss but recover; animals infected with serially passaged virus may develop fatal liver disease. No clinical signs have been reported in infected wild bats, and experimentally infected bats remain asymptomatic.

Whether *Reston ebolavirus* causes disease in swine is unknown. This virus was found during an unusually severe outbreak of porcine reproductive and respiratory syndrome caused by an atypical PRRS virus. The clinical signs were consistent with atypical PRRS. These pigs were also infected with porcine circovirus type 2.

Communicability

Blood, secretions and excretions, and tissues may contain infectious virus; filoviruses can probably be found almost anywhere in the body. Aerosol transmission has been reported in experimentally infected primates. The extent of transmission between animals in the wild depends on the interactions between members of the population, as well as the infectivity of body fluids and carcasses. Gorilla to gorilla transmission probably occurs, but the extent to which is responsible for the spread of disease is controversial. Unpublished data suggests that carcasses decomposing in the African forests are infectious for only three or four days after death.

Post Mortem Lesions

At necropsy, petechiae, ecchymoses and frank hemorrhages may be present. Hemorrhages can occur in any organ, but they are particularly common in the gastrointestinal tract, kidneys, and pleural, pericardial and peritoneal spaces. The liver and spleen may be swollen and friable, and the liver may be severely reticulated and discolored. Other potential lesions include interstitial pneumonia, nephritis and a maculopapular rash, as well as necrosis of the liver, lymphoid tissue, adrenal cortex or pulmonary epithelium.

Diagnostic Tests

Filovirus infections can be diagnosed by detecting antigens with an antigen-capture ELISA or immunostaining, and by detecting viral RNA with RT-PCR. Virus isolation is also used: ebolaviruses and marburgvirus can be recovered in many cell lines, including Vero or Vero E6 cells. Electron microscopy can identify virus particles in tissues: filoviruses are pleomorphic, long and filamentous and may be branched. Some may be U-shaped, b-shaped or circular. In primates,

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filoviruses are found in high concentrations in the liver, spleen, lungs and lymph nodes. Skin biopsies collected into formalin may be helpful; large amounts of ebolavirus antigens have been found in skin. In bats, filoviruses have been found in liver and spleen. For surveillance in wild animals by RT-PCR, liver, spleen, muscle and skin have been taken from animals in good condition. RT-PCR can sometimes detect ebolavirus RNA in the bones of decomposed carcasses. Virus isolation is more difficult: unpublished data suggests that carcasses decomposing in the African forests may contain infectious virus for only 3 to 4 days after death.

Serologic tests include IFA and ELISAs. Immunoblotting may be used in research. Neutralization tests are unreliable for filoviruses. Paired serum samples should be tested if possible; low IFA titers in single samples are difficult to interpret. Cross-reactions can occur, and the significance of antibody titers in asymptomatic primates is controversial.

Treatment

No specific therapy is available. Because most filovirus infections are highly fatal in both humans and nonhuman primates, infected animals are usually euthanized.

Prevention

Quarantine of nonhuman primates during importation protects humans and healthy nonhuman primates from exposure. To prevent the exportation of *Reston ebolavirus*, the government of the Philippines has banned wild-caught monkeys from export and established a 45-day quarantine. During outbreaks, suspects and exposed animals should be isolated, and euthanized after confirmation of the disease. Strict infection control procedures are necessary to prevent virus transmission on fomites. Prevention of human exposure is vital. No vaccine is commercially available, but some vaccines being tested in nonhuman primates have been promising.

Measures to prevent infection of swine with *Reston ebolavirus* have not yet been established, but normal biosecurity measures including the prevention of contact with bats or nonhuman primates are appropriate. Eradication procedures including quarantines, testing and culling are being established in infected pigs, and exports have been suspended from affected areas.

Morbidity and Mortality

In Africa, high mortality rates have been reported in gorilla, chimpanzee and duiker populations during human ebolavirus epidemics. Among wild animals, outbreaks occur suddenly and may cause widespread mortality on one area while having little or no impact on other regions. The effect on local populations can be severe. In one preserve, gorilla and duiker numbers fell an estimated 50% and chimpanzee populations decreased by 88% during one outbreak. Another study estimated 90-95% mortality (5000 animals) in a population of gorillas.

Experimental inoculation of gorillas or chimpanzees is not done, but infection of other nonhuman primates is often fatal. The mortality rate varies with the dose and virus. Nearly all macaques inoculated with *Zaire ebolavirus* die, but many animals inoculated with *Sudan ebolavirus* survive. Antibodies have been reported in some wild primate populations, suggesting that some animals may recover or are resistant to disease. In one survey, none of 145 captive-born mandrills and chimpanzees had antibodies to ebolavirus, but 13% of wild-born chimpanzees, 3% of mandrills, 7% of gorillas, 4% of baboons, and 1% of guenon were seropositive. In chimpanzees, the seroprevalence rate varied from 4% to 18%, depending on the area. Ebolavirus outbreaks in Africa have also been linked to reports of other dead and dying primates including mandrills and guenon, as well as bush pigs and possibly other species. Because filoviruses have not been demonstrated in these species, whether they died of Ebola or other diseases is unknown. Few species other than nonhuman primates have been successfully inoculated with filoviruses. Experimentally infected bats remain asymptomatic.

Reston ebolavirus has been reported only in captive nonhuman primates and domesticated pigs; this virus has never been isolated from wild animals. *Reston ebolavirus* spreads rapidly among susceptible primates. During the first outbreak to be recognized, 82% of the cynomolgus monkeys (*Macaca fascicularis*) at a quarantine facility in Reston, Virginia died. (This outbreak was complicated by the discovery of simian hemorrhagic fever virus, which is also pathogenic for this species, in the same population.) Experimental infection of cynomolgus monkeys resulted in a mortality rate greater than 80%. Although control measures were established in the Philippines, *Reston ebolavirus* outbreaks recurred at primate quarantine facilities in the U.S. in 1989, 1990 and 1996, and in Italy in 1992. Although some monkeys in the 1989 outbreaks came from an illegal exporter, most incidents were linked to the one breeding facility in the Philippines. In 1996, a study found viral antigens in 32% of dead or moribund monkeys and 4% of healthy monkeys at this facility. The mortality rate was 14%, significantly higher than the 2% average mortality reported at other sites. The source of the infection was not found, but *Reston ebolavirus* was not reported in imported primates after this facility was closed in 1997. In December 2008, *Reston ebolavirus* was detected in domesticated pigs investigated during an outbreak of PRRS in the Philippines. Although high morbidity and mortality were reported in populations infected with both *Reston ebolavirus* and PRRS virus, the contribution of the ebolavirus (if any) has not yet been determined.

Section 2

High-Consequence Livestock Pathogens

African Swine Fever

Pesti Porcine Africaine,
Peste Porcina Africana,
Pestis Africana Suum,
Maladie de Montgomery,
Warthog Disease,
Afrikaanse Varkpes,
Afrikanische Schweinepest

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Importance

African swine fever (ASF) is a serious viral disease of pigs, endemic in Africa. The African swine fever virus (ASFV) is highly contagious, and can spread very rapidly in pig populations by direct or indirect contact. This virus can persist for long periods in pig products and the environment. It can also become endemic in feral or wild Suidae, and in *Ornithodoros* ticks. ASFV isolates vary in virulence from highly pathogenic strains that cause near 100% mortality to low–virulence isolates that can be difficult to diagnose. There is no vaccine or treatment.

African swine fever is a serious problem in many African countries. Disease outbreaks have also occurred in Europe, South America and the Caribbean, and the cost of eradication has been significant. During outbreaks in Malta and the Dominican Republic, the swine herds of these countries were completely depopulated. In Spain and Portugal, ASFV became endemic in the 1960s and complete eradication took more than 30 years. Changes in production practices and increasing globalization have increased the risk of introducing African swine fever into North America.

Etiology

African swine fever results from infection by the African swine fever virus. Formerly classified as a member of the family Iridoviridae, this virus is currently the sole member of the new genus *Asfivirus* in the family Asfarviridae. ASFV is the only DNA virus transmitted by arthropods. Distinct antigenic types have not been identified for this virus, but restriction enzyme analysis has been used to identify viral genotypes. ASFV isolates can vary greatly in their virulence, from highly virulent isolates that kill most pigs to viruses that result only in seroconversion.

Species Affected

African swine fever affects members of the pig family (Suidae). Species that can be infected include domesticated swine, European wild boars, warthogs (*Phacochoerus africanus*), bush pigs *Potamochoerus porcus*), giant forest hogs (*Hylochoerus* spp.), and peccaries (*Tayassu* spp.). Symptomatic infections occur in domesticated pigs, feral pigs and European wild boars. ASFV infections are generally asymptomatic in warthogs, bush pigs and giant forest hogs; these species are thought to be the reservoirs for the virus in Africa. Other species that may be able to carry the virus asymptomatically include the collared peccary (*Tayassu tajacu*) and the white-lipped peccary (*Tayassu albirostris*), both found in the Americas.

Geographic Distribution

African swine fever is endemic in most of sub–Saharan Africa including the island of Madagascar; the highest incidence of disease is seen from the equator to the northern Transvaal. Outbreaks have been reported periodically outside this region; however, in most cases, the disease was eventually eradicated. Outside Africa, ASFV is endemic in feral pigs in Sardinia, Italy. It was also introduced into the Caucasus in 2007, and has apparently become endemic among wild boars in the region. The virus has caused outbreaks among domesticated swine in the Republic of Georgia, Russia, Armenia, Azerbaijan and other countries in the region.

Transmission

African swine fever can be transmitted by direct contact with infected animals, indirect contact on fomites, and tick vectors. Transmission during direct contact is usually by oronasal spread. Aerosol transmission is thought to be unimportant, as it only seems to occur over short distances when pigs are in close contact. African swine fever virus can be found in all tissues and body fluids, but particularly high levels are found in the blood. Massive environmental contamination may result if blood is shed during necropsies or pig fights, or if a pig develops bloody diarrhea. The virus can also spread on fomites, including vehicles, feed and equipment. There is evidence that some pigs may become carriers.

African swine fever often spreads to new areas when pigs are fed uncooked scraps that contain ASFV–infected pork. In one outbreak, pigs became infected after

being fed the intestines of guinea fowl that had eaten infected ticks. The African swine fever virus is highly resistant to environmental conditions. It can survive for a year and a half in blood stored at 4º C, 11 days in feces at room temperature, and at least a month in contaminated pig pens. The virus will also remain infectious for 150 days in boned meat stored at 39º F, 140 days in salted dried hams, and several years in frozen carcasses.

African swine fever is also spread through the bite of infected *Ornithodoros* spp. soft ticks. In tick populations, transstadial, transovarial and sexual transmission occur. In Africa, ASFV is thought to cycle between newborn warthogs and the soft ticks (*Ornithodoros moubata*) that live in their burrows. Individual ticks can apparently remain infected for life, and infected soft tick colonies can maintain this virus for years. *Ornithodoros erraticus* became infected with ASFV when the virus was enzootic in Spain and Portugal, and additional *Ornithodoros* spp have been infected in the laboratory.

Other bloodsucking insects such as mosquitoes and biting flies may also be able to transmit the virus mechanically. Stable flies (*Stomoxys calcitrans*) can carry high levels of the virus for 2 days. Under experimental conditions, these flies could transmit ASFV 24 hours after feeding on infected pigs.

Incubation Period

The incubation period is 5 to 19 days after direct contact with infected pigs, but can be less than 5 days after exposure to ticks. Acute disease typically appears in 5 to 7 days.

Clinical Signs

African swine fever can be a peracute, acute, subacute or chronic disease. Highly virulent strains produce peracute or acute disease, and may affect the entire herd within a few days. Less virulent strains produce milder symptoms that are easily confused with other diseases, and can take several weeks to spread through the herd.

Sudden deaths with few lesions are characteristic of the peracute form, and may be the first sign of an infection in a herd. Acute disease is characterized by a high fever, anorexia, lethargy, weakness and recumbency. Erythema can be seen, and is most apparent in white pigs. Some pigs develop cyanotic skin blotching on the ears, tail, lower legs or hams. Pigs may also have abdominal pain, constipation or diarrhea; the diarrhea is initially mucoid and later may become bloody. Hemorrhages can occur in the skin, as well as the internal organs. Dyspnea, vomiting, nasal and conjunctival discharges, and neurologic signs have also been reported. Pregnant animals frequently abort; in some cases, abortions may be the first signs of an outbreak. Leukopenia can be seen in laboratory tests. In acute African swine fever, death often occurs within 7 to 10 days. Subacute African swine fever, which is caused by moderately virulent isolates, is similar to acute ASF but less

severe. In this form of the disease, the death rate is generally lower in adult swine, but may still be very high in very young animals. In subacute disease, fever, thrombocytopenia and leukopenia may be transient, and affected pigs usually die or recover within 3 to 4 weeks.

Animals infected with isolates of low virulence may seroconvert without symptoms, abort or develop chronic African swine fever. The symptoms of chronic disease are intermittent low fever, appetite loss and depression. Pigs may become emaciated. They can also develop respiratory problems and swollen joints. Coughing is common, and diarrhea and occasional vomiting have been reported. Ulcers and reddened or raised necrotic skin foci may appear over body protrusions and other areas subject to trauma. In some cases, the only symptoms may be emaciation and stunting. Chronic African swine fever can be fatal.

Post-Mortem Lesions   🖻 **Click to view images**

The gross lesions of African swine fever are highly variable, and are affected by the virulence of the isolate and the course of the disease.

In pigs with peracute or acute disease, the carcass is often in good condition. Animals that die peracutely may have few or poorly developed lesions. In acute disease, there may be bluish-purple discoloration and/or hemorrhages in the skin, and there may be signs of bloody diarrhea or other internal hemorrhages. The major internal lesions are hemorrhagic, and occur in the spleen, lymph nodes, kidneys and heart. In animals infected with highly virulent isolates, the spleen can be very large, friable, and dark red to black. The lymph nodes are often swollen and hemorrhagic, and may look like blood clots; the nodes most often affected are the gastrohepatic and renal lymph nodes. Petechiae are common on the cortical and cut surfaces of the kidneys, as well as in the renal pelvis. Perirenal edema may also be present. Hydropericardium with hemorrhagic fluid may be noted. Less consistent clinical signs include hemorrhages, petechiae and ecchymoses in other organs including the urinary bladder, lungs, heart, stomach and intestines. Congestion or edema may be seen in the liver, gall bladder or lungs, and the pleural, and peritoneal cavities may contain straw-colored or blood-stained fluid. The brain and meninges can be congested, edematous or hemorrhagic. Aborted fetuses may be anasarcous and have a mottled liver. They may have petechiae or ecchymoses in the skin and myocardium. Petechiae can also be found in the placenta.

Similar but less pronounced lesions are seen in pigs infected with moderately virulent isolates. The spleen may be enlarged but not friable, and the color may be closer to normal. The lymph nodes are typically enlarged and can be hemorrhagic, and slight petechiation may be found on the kidneys.

In animals with chronic African swine fever, the carcass may be emaciated. Other possible post–mortem lesions are focal areas of skin necrosis, skin ulcers,

consolidated lobules in the lung, caseous pneumonia, nonseptic fibrinous pericarditis, pleural adhesions, generalized lymphadenopathy and swollen joints.

Morbidity and Mortality

In domesticated pigs, the morbidity rate approaches 100% in naïve herds. The mortality rate depends on the virulence of the isolate, and can range from 0% to 100%. Highly virulent isolates can cause nearly 100% mortality in pigs of all ages. Less virulent isolates are more likely to be fatal in pigs with a concurrent disease, pregnant animals and young animals. In subacute disease, the mortality rate may be as high as 70-80% in young pigs but less than 20% in older animals. Asymptomatic infections or mild disease is usually seen in warthogs and bush pigs.

Diagnosis

Clinical

African swine fever should be suspected in pigs with a fever, when the necropsy findings include a very large, friable, dark red to black spleen, and greatly enlarged and hemorrhagic gastrohepatic and renal lymph nodes. Less virulent isolates can be difficult to diagnose clinically or at necropsy, and often resemble other diseases.

Differential diagnosis

The differential diagnosis includes classical swine fever (hog cholera), acute porcine reproductive and respiratory syndrome, porcine dermatitis and nephropathy syndrome, erysipelas, salmonellosis, eperythrozoonosis, actinobacillosis, Glasser's disease (*Haemophilus parasuis* infection), Aujeszky's disease, thrombocytopenic purpura, warfarin poisoning other generalized septicemic or hemorrhagic conditions, and heavy metal toxicity.

Laboratory tests

African swine fever can be diagnosed by virus isolation. ASFV is usually isolated by inoculating blood or tissue samples from suspect pigs into pig leukocyte or bone marrow cultures. Porcine alveolar macrophages and blood monocyte cultures also support ASFV replication. Most isolates of ASFV induce hemadsorption of pig erythrocytes to the surface of infected cells. A few non-hemadsorbing isolates can be missed with this test; most of these viruses are avirulent, but some do produce symptomatic disease. ASFV can also be detected in peripheral blood leukocytes from infected pigs using a hemadsorption "autorosette" test.

ASFV antigens can be found in tissue smears or cryostat sections, as well as in the buffy coat, with the fluorescent antibody test (FAT). The World Organization for Animal Health (OIE) does not consider this test alone to be sufficient for diagnosis, although it is useful in conjunction with other assays. Nucleic acids can be detected with a polymerase chain reaction (PCR) assay or by the hybridization of nucleic acid probes to tissue sections. PCR is particularly useful in putrefied samples that cannot be used for virus isolation and antigen detection. A rapid, real time PCR technique using tonsil scraping samples has recently been published. This test can detect the virus a few days before the onset of symptoms.

Serology is also useful for diagnosis, particularly in endemic regions. Antibodies to ASFV persist for long periods after infection. Many serologic tests have been developed for the diagnosis of African swine fever, but only a few have been standardized for routine use in diagnostic laboratories. These tests include the enzyme–linked immunosorbent assay (ELISA), immunoblotting, indirect fluorescent antibody (IFA) and counter immunoelectrophoresis (immunoelectro–osmophoresis) tests. The ELISA is prescribed for international trade.

Animal inoculation, performed in pigs, was used in the past to distinguish African swine fever from classical swine fever. This test is no longer be recommended by the OIE due to humane considerations and the complexity of the test.

Samples to collect

Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease.

For virus isolation from live animals, blood should be collected into an anticoagulant (heparin or EDTA) At necropsy, samples of the spleen, kidney, tonsils and lymph nodes should be collected. ASFV is not found in aborted fetuses; in cases of abortion, a blood sample should be collected from the dam. Samples for virus isolation should be transported as cold as possible, but kept from freezing. If a cold chain is impossible to maintain, samples may be submitted in glycerosaline, although this can result in a small decrease in the probability of virus identification.

Tissue samples should also be collected for the FAT and histology. Serum and/ or tissue fluids should be submitted for serology. Paired serum samples are useful when they are available.

Recommended actions if African swine fever is suspected

Notification of authorities

African swine fever is reportable to the World Organization for Animal Health (OIE). Disease notification requirements for OIE member nations and import/export guidelines can be found in the OIE Terrestrial Animal Health Code [http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/]. Veterinarians who encounter a case of African swine fever should follow their national and/or local guidelines for disease reporting and diagnostic testing.

African swine fever should be reported to state or federal authorities immediately upon diagnosis or suspicion of the disease.

Federal: Area Veterinarians in Charge (AVIC):

http://www.aphis.usda.gov/animal_health/area_offices/

State Veterinarians:

http://www.usaha.org/Portals/6/StateAnimalHealthOfficials.pdf

Quarantine and Disinfection

To prevent introduction of the African swine fever virus into areas free of the disease, all garbage fed to pigs should be cooked. Unprocessed meat must be heated to at least 70ºC for 30 minutes to inactivate the virus; 30 minutes at 60ºC is sufficient for serum and bodily fluids.

African swine fever is a highly contagious disease. Eradication is by slaughter of infected and in–contact animals, and disposal of carcasses, often by burying, rendering or burning. Rapid diagnosis and the prevention of disease spread to feral or wild pigs are very important. Strict quarantines must be imposed. ASFV can survive for long periods on fomites and in the environment, and sanitation and disinfection are important in preventing further spread. Many common disinfectants are ineffective; care should be taken to use a disinfectant specifically approved for African swine fever. Sodium hypochlorite and some iodine and quaternary ammonium compounds are effective.

Potential tick vectors should be controlled with acaricides. In outbreaks, a detailed entomological investigation should be conducted, to investigate the possible roles of local soft tick vectors and their potential for becoming long term carriers. Although *Ornithodoros moubata* is an important long-term vector in Africa, and. *Ornithodoros erraticus* became chronically infected in Spain and Portugal, *Ornithodoros* ticks never became chronically infected during outbreaks in South America. In addition, biting insects that may be able to transmit the virus mechanically should be controlled. No treatment or vaccine exists for this disease, and an ASF vaccine is unlikely to be developed soon.

Public Health

Humans are not susceptible to African swine fever virus.

Internet Resources

African Swine Fever Network
http://pigtrop.cirad.fr/en/worldwide/afrique_ASFnetwork.html

Food and Agriculture Organization of the United Nations. Recognizing African Swine Fever. A Field Manual.
http://www.fao.org/docrep/004/X8060E/X8060E00.HTM

Manual for the Recognition of Exotic Diseases of Livestock
http://www.spc.int/rahs/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

United States Animal Health Association. Foreign Animal Diseases
http://www.aphis.usda.gov/emergency_response/downloads/nahems/fad.pdf

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

OIE Terrestrial Animal Health Code

http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

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Classical Swine Fever

Hog Cholera, Swine Fever,
Peste du Porc, Colera Porcina,
Virusschweinepest

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Food Security
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Importance

Classical swine fever (CSF) is a highly contagious and economically significant viral disease of pigs. The severity of this disease varies with the strain of the virus, the age of the pig, and the immune status of the herd. Acute infections, which are caused by highly virulent isolates and have a high mortality rate, are likely to be diagnosed rapidly. However, infections with less virulent isolates can be more difficult to recognize, particularly in older pigs. These infections may be relatively mild, and can resemble septicemias caused by other agents, as well as other diseases. In some herds, the only symptom may be poor reproductive performance or the failure of some pigs to thrive. The wide range of clinical signs and similarity to other diseases can make classical swine fever challenging to diagnose.

Although classical swine fever was once widespread, many countries have eradicated this disease from domesticated swine. Reintroduction of the virus can be devastating. In 1997-1998, an outbreak in the Netherlands spread to involve more than 400 herds and cost $2.3 billion to eradicate. Approximately 12 million pigs were killed, some in eradication efforts but most for welfare reasons associated with the epidemic. The United Kingdom experienced an outbreak in 2000, and minor outbreaks were reported in Romania, Slovakia, Spain and Germany in 2001. North America is also at risk for the introduction of this disease, which is still endemic in much of South and Central America.

Etiology

Classical swine fever (hog cholera) results from infection by classical swine fever virus (CSFV), a member of the genus *Pestivirus* and family Flaviviridae. Only one CSFV serotype has been found, but minor antigenic variability has been demonstrated between viral strains. This virus is closely related to the ruminant pestiviruses that cause bovine virus diarrhea and border disease. Other pestiviruses have also been described recently.

Species Affected

Classical swine fever affects domesticated and wild pigs. All feral and wild pigs, including European wild boar and collared peccaries, are thought to be susceptible.

Geographic Distribution

Classical swine fever is found in much of Asia, some Caribbean islands, the African countries of Madagascar and Mauritius, and much of South and Central America. This disease has been eradicated from the United States, Canada, New Zealand, Australia and most of western and central Europe.

CSFV is endemic in wild boar in parts of Europe; the significance for domesticated pigs is controversial.

Transmission

Classical swine fever is highly contagious. Infected pigs are the only reservoir of virus. Blood, secretions and excretions (including oronasal and lacrimal secretions, urine, feces and semen) and tissues contain infectious virus. Virus shedding can begin before the onset of clinical signs, and occurs throughout the course of acute or subclinical disease. Chronically or persistently infected pigs can shed virus continuously or intermittently for months.

Transmission between pigs occurs mainly by the oral or oronasal routes, via direct or indirect contact. CSFV is often spread by feeding uncooked contaminated garbage. Animals can also be infected through the mucus membranes, conjunctiva and skin abrasions. CSFV can be spread by genital transmission or artificial insemination. Infected carrier sows may give birth to persistently infected pigs. The virus can also be spread on fomites, and mechanical spread by insects, birds and other wild or domesticated animals may occur. Airborne transmission seems to be possible over short distances; however, the maximum distance the virus can spread is unclear. While aerosol transmission occurred only within a radius of 250 meters in one study, transmission could occur up to 1 km in another.

CSFV is moderately fragile in the environment; this virus is reported to survive for 3 days at 50ºC and 7 to 15 days at 37ºC. Estimates of its survival in pens and on fomites under field conditions vary. Some studies suggest that virus inactivation occurs within a few days, while others describe survival, under winter conditions, for up to four weeks.

CSFV can remain infectious for nearly three months in refrigerated meat and for more than four years in frozen meat. In this proteinaceous environment, this virus does not appear to be inactivated by smoking or salt curing. Reported virus survival times in cured and smoked meats vary with the technique, and range from 17 to more than 180 days.

Incubation Period

The incubation period can range from 2 to 15 days, depending on the virulence of the strain, the route of inoculation and the dose. Under field conditions, disease may not become evident in a herd for 2 to 4 weeks or longer.

Clinical Signs

The symptoms of classical swine fever vary with the strain of virus, and the age and susceptibility of the pigs. More virulent strains cause acute disease; less virulent strains can result in a high percentage of chronic, mild or asymptomatic infections. Although highly virulent strains were once more prevalent, most epizootics are now caused by moderately virulent strains. Older animals are less likely to show severe symptoms than younger pigs. Some breed-specific differences have also been reported.

Acute swine fever is the most severe form of the disease. In this form, common symptoms include a high fever, huddling, weakness, drowsiness, anorexia, conjunctivitis, and constipation followed by diarrhea. Pigs may be incoordinated or exhibit an unsteady, weaving or staggering gait, which progresses to posterior paresis. Some pigs may vomit yellow, bile-containing fluid, or develop respiratory signs. The abdomen, inner thighs, ears and tail may develop a purple cyanotic discoloration. Hemorrhages can also occur in the skin. Severe leukopenia usually occurs soon after disease onset, and convulsions may be seen in the terminal stages. Pigs with acute classical swine fever often die within one to three weeks.

Subacute disease can be caused moderately virulent strains of CSFV. It may also occur in older pigs. The subacute form is similar to acute classical swine fever; however, the symptoms are less severe, and the fever may persist for two to three weeks. Some pigs with subacute classical swine fever may survive; others die within a month.

Chronic disease tends to be seen with less virulent strains or in partially immune herds. In the initial stages, chronic disease can resemble acute or subacute disease, with anorexia, depression, elevated temperatures, leukopenia, and periods of constipation or diarrhea. Affected pigs usually improve after several weeks; however, after a period where they appear relatively normal, they develop recurrent symptoms that may include intermittent fever, anorexia, periods of constipation or diarrhea, wasting or stunted growth, alopecia and skin lesions. Immunosuppression may lead to concurrent infections. The symptoms of chronic infections can wax and wane for weeks to months, and may affect only a few animals in the herd. Affected pigs may survive for one to three months, but chronic infections are always fatal.

In some breeding herds infected with less virulent strains, poor reproductive performance may be the only sign of disease. Sows may abort or give birth to stillborn, mummified, malformed, weak or dead piglets. Some piglets may be born with a congenital tremor or congenital malformations of the visceral organs and central nervous system. Other piglets may be asymptomatic at birth, but persistently infected. These animals are persistently viremic and become clinically ill after several months ("late onset" disease). The symptoms can include inappetence and depression, as well as stunted growth, dermatitis, diarrhea, conjunctivitis, ataxia or posterior paresis. Although affected pigs usually survive for more than six months, all typically die within a year. Congenital infections may be limited to a few piglets in the herd.

Post Mortem Lesions **Click to view images**

The lesions of classical swine fever are highly variable. During outbreaks, the likelihood of observing the characteristic necropsy lesions is better if four or five pigs are examined. In acute disease, the most common lesion is hemorrhage. The skin may be discolored purple and the lymph nodes may be swollen and hemorrhagic. Petechial or ecchymotic hemorrhages can often be seen on serosal and mucosal surfaces, particularly on the kidney, urinary bladder, epicardium, larynx, trachea, intestines, subcutaneous tissues, and spleen. Straw–colored fluid may be found in the peritoneal and thoracic cavities and the pericardial sac. Severe tonsillitis, sometimes with necrotic foci, is common. Splenic infarcts are occasionally seen. The lungs may be congested and hemorrhagic. In some acute cases, lesions may be absent or inconspicuous.

The lesions of chronic disease are less severe and may be complicated by secondary infections. In addition, necrotic foci or "button" ulcers may be found in the intestinal mucosa, epiglottis and larynx. In growing pigs that have survived for more than a month, bone lesions can also occur at the costochondral junction of the ribs and the growth plates of the long bones.

In congenitally infected piglets, common lesions include cerebellar hypoplasia, thymic atrophy, ascites, and deformities of the head and legs. Edema and petechial hemorrhages may be seen in the skin and internal organs.

Morbidity and Mortality

The severity of the disease varies with the viral strain; while some strains cause acute disease with high mortality rates, others can result in mild or even subclinical disease. The morbidity and mortality rates are high during acute infections, and the case fatality rate can approach 100%. Morbidity and mortality are lower in subacute disease. Chronic infections are always fatal, but may affect only a few animals in a herd. The age and immune status of the animals also affects the course of disease, with lower mortality rates in adult pigs than younger animals.

Diagnosis

Clinical

Classical swine fever should be suspected in pigs with signs of septicemia and a high fever, particularly if uncooked scraps have been fed, unusual biological products have been used, or new animals have been added to the herd. This disease may also be considered in herds with other symptoms, including breeding herds with poor reproductive performance and disease in piglets. It can be difficult to differentiate classical swine fever from other diseases without laboratory testing.

Differential diagnosis

The differential diagnosis varies with the form of the disease, and includes African swine fever, porcine dermatitis and nephropathy syndrome, postweaning multisystemic wasting syndrome, hemolytic disease of the newborn, porcine reproductive and respiratory syndrome, thrombocytopenic purpura, anticoagulant (e.g. warfarin) poisoning, salt poisoning, Aujeszky's disease and parvovirus infections. Septicemic diseases such as erysipelas, eperythrozoonosis, salmonellosis, pasteurellosis, actinobacillosis, and *Haemophilus suis* infections must also be considered. Congenital infection with the pestiviruses that cause bovine virus diarrhea or border disease can resemble classical swine fever.

Laboratory tests

Classical swine fever can be diagnosed by detecting the virus, its antigens or nucleic acids in whole blood or tissue samples. Viral antigens are detected by direct immunofluorescence (FAT or FATST test) or enzyme–linked immunosorbent assays (ELISAs). The virus can also be isolated in several cell lines including PK–15 cells; it is identified by direct immunofluorescence or immunoperoxidase staining. Reverse transcriptase polymerase chain reaction (RT–PCR) tests are used in some laboratories.

The ruminant pestiviruses that cause bovine virus diarrhea and border disease can occasionally infect pigs. Serum neutralization tests, or immunoperoxidase procedures that use monoclonal antibodies, can differentiate CSFV from these viruses. They can also be distinguished using genetic methods such as RT-PCR.

Serology is used for diagnosis and surveillance. Antibodies develop after 2 to 3 weeks, and persist lifelong. For this reason, serology is most useful in herds thought to have been infected 30 or more days previously. It is particularly helpful in herds infected with less virulent strains, where viral antigens may be more difficult to find. The most commonly used tests are virus neutralization tests, which include the fluorescent antibody virus neutralization (FAVN) test and the neutralizing peroxidase–linked assay (NPLA), and various ELISAs. Antibodies against ruminant pestiviruses may be found in breeding animals; only tests that use monoclonal antibodies can differentiate between these viruses and CSFV. The definitive test for differentiation is the comparative neutralization test. Congenitally infected pigs are immunotolerant and are negative on serology.

Companion ELISAs have been developed for marker vaccines, but have limitations in their sensitivity and/or specificity.

Samples to collect

Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease.

Blood (collected into EDTA), or tissue samples taken at necropsy, should be taken from a few febrile animals for virus isolation, antigen detection or nucleic acid detection. Additionally, whole blood samples may be taken from a larger group of pigs.

Serum samples are particularly useful in herds that have been infected for 30 days or more. Antibodies usually develop during the second or third week after infection, and persist for life. Serum samples should be taken from convalescent or recovered animals, or sows that have been in contact with suspected cases.

At necropsy, the tonsils should be submitted for virus isolation or antigen detection. Other organs to collect include the maxillary, submandibular and mesenteric lymph nodes, spleen, kidneys, and the distal part of the ileum. Samples for antigen detection and virus isolation should be refrigerated but not frozen; they should be kept cold during shipment to the laboratory.

Recommended actions if classical swine fever is suspected

Notification of authorities

Classical swine fever should be reported immediately upon diagnosis or suspicion of the disease.

Federal: Area Veterinarians in Charge (AVICS)
http://www.aphis.usda.gov/animal_health/area_offices/
State Veterinarians:
http://www.aphis.usda.gov/vs/sregs/official.html

Control

CSFV is moderately fragile in the environment. This virus is sensitive to drying and ultraviolet light. It is stable at pH 5-10, but is rapidly inactivated by pH 3 or less, or pH greater than 11. Sodium hypochlorite and phenolic compounds are effective disinfectants. Detergents, organic solvents, quaternary ammonium compounds, and aldehydes (formaldehyde, glutaraldehyde) are also reported to destroy this virus.

CSFV can survive for several months in refrigerated meat and years in frozen meat. In this proteinaceous environment, the virus is not inactivated by smoking or salt curing. However, it can be destroyed by cooking. Techniques reported to be effective include heating the meat to 65.5ºC or greater for 30 minutes, or 71ºC for one minute. The virus can also be inactivated in swill by heat treatment. Many countries have completely banned the practice of feeding swill to pigs.

In countries where classical swine fever is endemic, vaccines may be used to protect animals from clinical disease. Vaccines can also be used to reduce the prevalence of infections during an eradication program. Both modified live and subunit (marker) vaccines are manufactured, although availability varies with the country.

Quarantines, movement bans and good surveillance are important in controlling outbreaks. Strict biosecurity on a farm can reduce the risk of infection. During an outbreak, confirmed cases and contact animals may be slaughtered. Although CSFV can be spread over long distances by animal transportation and other forms of dissemination, farms within a 500 meter radius of an infected farm have a particularly high risk of infection. Culling of all pigs in an area may be practiced, due to this 'neighborhood effect.' Infected premises are thoroughly cleaned and disinfected. Vaccination may be used as a tool to assist in controlling an outbreak and eradicating the disease. In countries free of classical swine fever, periodic serologic sampling is necessary to monitor for the potential reintroduction of disease.

Controlling endemic infections in wild populations is difficult. Oral vaccination has been attempted in wild boar in Germany. Contact between domesticated herds and wild pigs should be avoided.

Public Health

Classical swine fever does not affect humans.

Internet Resources

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

United States Animal Health Association.
Foreign Animal Diseases
http://www.usaha.org/pubs/fad.pdf

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/eng/normes/mmanual/a_summry.htm

OIE International Animal Health Code
http://www.oie.int/eng/normes/mcode/A_summry.htm

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Foot and Mouth Disease

Fiebre Aftosa

Last Updated: September 24, 2007

Importance

Foot-and-mouth disease (FMD) is a highly contagious viral disease that primarily affects cloven-hooved livestock and wildlife. Although adult animals generally recover, the morbidity rate is very high in naïve populations, and significant pain and distress occur in some species. Sequelae may include decreased milk yield, permanent hoof damage and chronic mastitis. High mortality rates can be seen in young animals. Although foot-and-mouth disease was once found worldwide, it has been eradicated from some regions including North America and most of Europe. Where it is endemic, this disease is a major constraint to the international livestock trade. Unless strict precautions are followed, FMD can be readily re-introduced into disease-free livestock. Once this occurs, the disease can spread rapidly through a region, particularly if detection is delayed. Outbreaks can severely disrupt livestock production, result in embargoes by trade partners, and require significant resources to control. Direct and indirect economic losses equivalent to several billion US dollars are not uncommon. Since 1997, a PanAsia lineage virus has caused a series of outbreaks in Asia, Africa, the Middle East and Europe. Some outbreaks, particularly those in Taiwan and the United Kingdom, have been devastating.

Etiology

The foot-and-mouth disease virus (FMDV) is a member of the genus *Aphthovirus* in the family Picornaviridae. There are seven immunologically distinct serotypes - O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1 - and over 60 strains within these serotypes. New strains occasionally develop spontaneously.

FMDV serotypes and strains vary within each geographic region. Serotype O is the most common serotype worldwide. This serotype is responsible for a pan-Asian epidemic that began in 1990 and has affected many countries throughout the world. Other serotypes also cause serious outbreaks. Immunity to one serotype does not provide any cross-protection to other serotypes. Cross-protection against other strains varies with their antigenic similarity.

Species Affected

FMDV can infect most or all members of the order Artiodactyla (cloven-hooved mammals), as well as a few species in other orders. Each species varies in its susceptibility to infection and clinical disease, as well as its ability to transmit the virus to other animals. Livestock susceptible to FMD include cattle, pigs, sheep, goats, water buffalo and reindeer. Lllamas, alpacas and camels can be infected experimentally, but do not appear to be very susceptible. FMDV can also infect at least 70 species of wild animals including African buffalo (*Syncerus caffer*), bison (*Bison* spp.), elk, moose, chamois, giraffes, wildebeest, blackbuck, warthogs, kudu, impala, and several species of deer, antelopes and gazelles. Susceptible non cloven-hooved species include hedgehogs, armadillos, kangaroos, nutrias, capybaras, guinea pigs, rats and mice. Infections have been reported in African and Asian elephants in zoos; however, African elephants are not considered susceptible to FMD under natural conditions in southern Africa.

On most continents, cattle are usually the most important maintenance hosts for FMDV, but some virus strains are primarily found in pigs, sheep or goats. Cattle and African buffalo are the usual maintenance hosts for FMDV in Africa; African buffalo are currently thought to carry only the SAT serotype. With this exception, wildlife hosts do not seem to be able to maintain FMD viruses, and are usually infected by contact with domesticated livestock. Early reports suggested that transmission also occurred between cattle and European hedgehogs, but there is no evidence that this species has helped to propagate FMDV in the last 40 years.

Geographic Distribution

Foot-and-mouth disease is endemic in parts of Asia, Africa, the Middle East and South America. In parts of Africa, virus persistence in wild African buffalo makes eradication unfeasible. North America, New Zealand, Australia, Greenland, Iceland

and most of Europe are free of this disease. Sporadic outbreaks have occurred in disease-free countries, with the exception of New Zealand, Greenland, Iceland and the smaller islands of Oceania. The last U.S. outbreak occurred in 1929.

Transmission

FMDV can be found in all secretions and excretions from acutely infected animals, including expired air, saliva, milk, urine, feces and semen. Pigs, in particular, produce large quantities of aerosolized virus. Animals can shed FMDV for up to four days before the onset of symptoms. This virus is also found in large quantities in vesicle fluid, and peak transmission usually occurs when vesicles rupture. Transmission can occur by direct or indirect contact with infected animals and contaminated fomites; routes of spread include inhalation of aerosolized virus, ingestion of contaminated feed, and entry of the virus through skin abrasions or mucous membranes. The importance of each of these routes varies with the species. For example, pigs are less susceptible to aerosolized virus than cattle or sheep. Sheep may have less obvious symptoms than other species, and have been important in disseminating the virus in some outbreaks. Sexual transmission could be a significant route of spread for the SAT type viruses in African buffalo populations.

Some animals carry FMDV for prolonged periods after recovering from acute disease. Animals with natural or vaccine-induced immunity can also become carriers if they are later exposed to virus; these animals can remain asymptomatic. FMDV can persist for up to nine months in sheep and up to four months in goats. Most cattle carry this virus for six months or less, but some animals remain persistently infected for up to 3.5 years. Individual African buffalo have been shown to be carriers for at least five years, and the virus can persist in a herd of African buffalo for at least 24 years. Llamas do not become carriers. A single study suggested that pigs may become carriers, but many other studies have found that this species cleared the infection within 3 to 4 weeks. In carriers, FMDV is found only in the esophageal-pharyngeal fluid. The amount of virus is small, and it may be found only intermittently. Carriers might be able to transmit FMDV to other animals if they come in close contact; the importance of this route of transmission is controversial. Unequivocal evidence for transmission from carriers has been reported only from Africa, where African buffalo can spread the disease to cattle. With the exception of African buffalo, wildlife seems to be infected by contact with domesticated animals; FMDV disappears from the wildlife populations when outbreaks in livestock are controlled. Persistent infections have been reported in some experimentally infected wildlife including fallow (*Dama dama*) and sika deer (*Cervus nippon*) and kudu (*Tragelaphus strepsiceros*), and occasionally in red deer (*Cervus elaphus*). Deer could carry FMDV for up to 11 weeks.

Foot and Mouth Disease

FMDV can be transmitted on fomites including vehicles, as well as mechanically by animals and other living vectors. Airborne transmission can occur under favorable climatic conditions. FMDV is thought to have been transmitted via aerosols from Brittany to Jersey (approximately 30 miles or 48 km) and for approximately 70 miles (113 km) from Jersey to the Isle of Wight. There is limited information on the survival of FMDV in the environment, but most studies suggest that it remains viable, on average, for three months or less. In very cold climates, survival up to six months may be possible. Virus stability increases at lower temperatures; in cell culture medium at 4°C (39°F), this virus can remain viable for up to a year. It was reported to survive on bran and hay for more than three months in a laboratory. It can also remain viable for approximately two months on wool at 4°C, with significantly decreased survival when the temperature increases to 18°C (64°F), and for 2 to 3 months in bovine feces. Organic material protects the virus from drying, and enhances its survival on fomites. Virus survival is also enhanced when FMDV is protected from sunlight. FMDV is inactivated at pH below 6.5 or above 11. This virus can persist in meat and other animal products when the pH remains above 6.0, but it is inactivated by acidification of muscles during rigor mortis. It can survive for long periods in chilled or frozen lymph nodes or bone marrow.

In humans, FMDV may be carried in the nasal passages for a period of time. In one study, this virus was detected in the nasal passages of one of eight people 28 hours after exposure to infected animals, and from none of the eight at 48 hours. More recent studies have found that FMDV is not transmitted by people when personal hygiene and biosecurity protocols are followed, and suggest that nasal carriage of the virus may be unimportant. The discrepancy between these studies remains to be resolved.

Incubation Period

In cattle, the incubation period varies from two to 14 days, depending on the dose of the virus and route of infection. In pigs, the incubation period is usually two days or more, but can be as short as 18-24 hours. The incubation period in sheep is usually 3 to 8 days. Incubation periods as short as 24 hours and as long as 12 days have been reported in this species after experimental infection.

Clinical Signs

Foot-and-mouth disease is characterized by fever and vesicles (blisters) on the feet, in and around the mouth, and on the mammary gland. Occasionally, vesicles may occur at other locations including the vulva, prepuce or pressure points on the legs. Vesicles often rupture rapidly, becoming erosions. Pain and discomfort from the lesions leads to a variety of symptoms including depression, anorexia, excessive salivation, lameness and reluctance to move or rise. Lesions on the coronary band may cause

growth arrest lines on the hoof. In severe cases, the hooves may be sloughed. Although FMDV does not cross the placenta, abortion may occur in pregnant animals. Most adults recover in two to three weeks, although secondary infections may lead to a longer recovery time. Possible complications include temporary or permanent decreases in milk production, chronic lameness or mastitis, weight loss and loss of condition. Deaths usually occur only in young animals, as the result of multifocal myocarditis; vesicles are not always found. In some outbreaks, the mortality rate in young animals can be high. Severe disease may also cause sudden deaths among older animals, particularly some species of wildlife, but this is rare.

The symptoms and severity of FMD vary with the species of animal, and the serotype and strain of the virus. Cattle usually become febrile and develop lesions on the tongue, dental pad, gums, soft palate, nostrils or muzzle. The vesicles on the tongue often coalesce, rupture quickly, and are highly painful, and the animal becomes reluctant to eat. Profuse salivation and nasal discharge are common; the nasal discharge is mucoid at first, but becomes mucopurulent. Affected animals become lethargic, may lose condition rapidly, and have gradual or sudden, severe decreases in milk production. Hoof lesions occur in the area of the coronary band and interdigital space. Foot lesions cause reluctance to rise, or stamping or shaking of the feet. Pregnant animals may abort. Young calves can die of heart failure without developing vesicles. In areas where cattle are intensively vaccinated, the entry of FMD into the herd can sometimes cause swelling of tongue and severe symptoms that resemble an allergic disease.

In pigs, the most severe lesions usually occur on the feet. The first symptoms may be lameness and blanching of the skin around the coronary bands. Vesicles develop on the coronary band and heel, and in the interdigital space. The lesions may become so painful that pigs crawl rather than walk. The horns of the digits are sometimes sloughed. Lesions at other sites are less common and less severe. Vesicles are sometimes found on the snout or udder, as well as on the hock or elbows if the pigs are housed on rough concrete floors. Mouth lesions are usually small and less apparent than in cattle, and drooling is rare. Affected pigs may also have a decreased appetite, become lethargic and huddle together. Fever may be seen, but the temperature elevation can be short or inconsistent. In some cases, the temperature may be near normal or even below normal. Young pigs up to 14 weeks may die suddenly due to heart failure; piglets less than eight weeks of age are particularly susceptible.

Foot-and-mouth disease tends to be mild in sheep and goats. Common symptoms include fever and mild to severe lameness of one or more legs. Vesicles may develop in the interdigital cleft and on the heel bulbs and coronary band, but they may rupture and be hidden by foot lesions from other causes. Mouth lesions are often not noticeable or severe, and generally appear as shallow erosions. Vesicles may also be noted on the teats, and rarely on the vulva or prepuce. Milk production may drop, and rams can be reluctant to mate. Ewes may abort. Up to 25% of infected sheep remain asymptomatic, and 20% have lesions only at one site. Young lambs and kids may die due to heart failure, without vesicles. In some epidemics, large numbers of lambs may fall down dead when stressed.

Minimal lesions and fever have been reported in llamas, which rarely become anorexic or demonstrate pain and discomfort.

The symptoms in wildlife resemble those seen in domesticated livestock. Vesicles and erosions may be found at various sites, particularly on the feet and in the mouth. More severe lesions occur where there is frequent mechanical trauma, e.g. on the feet and snout of suids or the carpal joints of warthogs. Loss of horns has also been seen. Some wildlife species typically experience subclinical infections or mild disease, while others develop severe, acute disease. Infections with SAT-type viruses in African buffalo are often subclinical, although small mouth and/or foot lesions have been reported. Severe disease has been documented in mountain gazelles, impala, blackbuck, white tailed-deer, warthogs and a kangaroo. In one outbreak in mountain gazelles, at least half the animals died due to heart failure or pancreatic atrophy and emaciation. Young animals of any species can die suddenly of myocarditis.

Post Mortem Lesions   📷 Click to view images

The characteristic lesions of foot-and-mouth disease are single or multiple, fluid-filled vesicles or bullae from 2 mm to 10 cm in diameter. The earliest lesions can appear as small pale areas or vesicles. Some vesicles may coalesce to form bullae. Vesicles are generally present for only a short period. Once they rupture, red, eroded areas or ulcers will be seen. These erosions may be covered with a gray fibrinous coating, and a demarcation line of newly developing epithelium may be noted. Loss of vesicular fluid through the epidermis can lead to the development of "dry" lesions, which appear necrotic rather than vesicular. Dry lesions are particularly common in the oral cavity of pigs.

The location and prominence of FMD lesions varies with the species. In cattle, numerous erosions, ulcers or vesicles may be found in the oral cavity. In pigs, sheep and goats, these lesions may be more common on the heel, coronary band and interdigital cleft of the feet. Some lesions may extend to the skin. Coronitis may be seen on the hooves, and animals with severe disease may slough their hooves or claws. In addition, vesicles may be found in other locations including the teats or udder; pressure points of the legs, ruminal pillars, prepuce or vulva. In young animals, cardiac degeneration and necrosis can cause gray or yellow streaking in the myocardium; these lesions are sometimes called "tiger heart" lesions.

Morbidity and Mortality

The morbidity rate varies with the species, immunity and other factors. Recovery from infection leads to immunity against the infecting virus, but little or no immunity develops to other serotypes. If several serotypes are endemic in a region, periodic episodes of disease may be seen. If only a single, persistent serotype circulates in a population, clinical disease may be mild and mainly occurs in young animals as they lose their protection from maternal antibodies. Carriers occur in endemic areas. In wild African buffalo populations, 50-70% of the animals may become carriers. Carrier rates from 15% to 50% have been reported in cattle and sheep.

In regions where FMD is not endemic, the morbidity rate can be as high as 100%. All susceptible species may not be affected during an outbreak. During one Asian epidemic, only pigs were infected. The mortality rate is generally less than 1% in adult livestock, but it can be much higher in young animals. Mortality rates of 40-94% have been reported in lambs. During one epidemic in Taiwan, the overall mortality rate in piglets was 40%. Up to 100% of suckling pigs may die.

Among wildlife, impala seem to be particularly susceptible to disease; regular epidemics of FMD occur in this species in southern Africa. Most outbreaks in wildlife are similar to those in domesticated species, with animals usually recovering in a week or two; however, higher mortality rates have occasionally been reported. A case fatality rate of at least 50% was reported in mountain gazelles in Israel. The same virus caused the usual symptoms and few deaths in cattle.

Diagnosis

Clinical

The symptoms of FMD vary with the species, but vesicles and erosions in the oral cavity or on the feet, teats or other areas are suggestive. In cattle, suspicion should be raised by simultaneous salivation and lameness, particularly when a vesicular lesion has been seen or is suspected to exist. Profuse salivation is uncommon in pigs or sheep, where lameness is more typical. Suspect or febrile animals should be examined closely for lesions. When sudden death is observed in young cloven-hooved livestock, older animals should also be examined; young animals that die of heart disease may not have vesicular lesions. Tranquilization may be necessary for a thorough examination as vesicles are painful and may be difficult to see. Laboratory confirmation is necessary, as all vesicular diseases have almost identical clinical signs.

Differential diagnosis

FMD cannot be distinguished clinically from other vesicular diseases including vesicular stomatitis, swine vesicular disease and vesicular exanthema. In domesticated animals, the symptoms may also resemble foot rot, traumatic stomatitis, and chemical and thermal burns. In cattle, oral lesions can resemble rinderpest, infectious bovine rhinotracheitis, bovine viral diarrhea, malignant catarrhal fever and epizootic hemorrhagic disease. In sheep, the lesions can be confused with bluetongue, contagious ecthyma, and lip and leg ulceration.

Laboratory tests

Foot-and-mouth disease can be diagnosed by virus isolation, detection of viral antigens or nucleic acids, and serology. FMDV can be isolated in primary bovine thyroid cells or primary pig, calf or lamb kidney cells. BHK-21 or IB-RS-2 cells can also be used, but cell lines are less sensitive than primary cells. If necessary, unweaned mice may be inoculated with the virus. In cell cultures, FMDV is identified using enzyme-linked immunosorbent assay (ELISA), complement fixation or reverse transcription polymerase chain reaction (RT-PCR) tests. ELISAs can also identify viral antigens directly in tissues; complement fixation is less specific and sensitive. RT-PCR techniques are also available. The virus serotype can be determined with either ELISA or RT-PCR. Electron microscopy is sometimes used to distinguish FMDV from other viruses in lesions.

Serological tests can be used for diagnosis as well as to certify animals for export. Antibodies to FMDV structural proteins are used to diagnose previous or current infections in unvaccinated animals. These tests include ELISAs and virus neutralization tests, and are serotype specific. Serological tests that detect antibodies to nonstructural proteins (NSP) can diagnose previous or current infections in vaccinated animals. Anti-NSP tests include ELISAs, and are not serotype specific. Some vaccinated animals that become persistently infected may not be detected by anti-NSP tests.

Carrier animals can be identified by isolating FMDV from the esophageal-pharyngeal fluids, but the virus may be present in low amounts and shed only intermittently. Repeated sampling may be necessary. RT-PCR can also be used to identify these animals.

Samples to collect

Before collecting or sending any samples from vesicular disease suspects, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent spread of the disease. Since vesicular diseases cannot be distinguished clinically, and some are zoonotic, samples should be collected and handled with all appropriate precautions.

In acute disease, the preferred sample for virus detection is epithelium from unruptured or freshly ruptured vesicles, or vesicular fluid. Sedation is generally advisable before these samples are collected. FMDV is extremely sensitive to low pH, and virus isolation is dependent on good buffering; epithelial samples should be shipped in a transport medium, and kept refrigerated or on ice. If vesicles are not available, blood (serum) and esophageal-pharyngeal fluid samples can be collected for

virus isolation or RT-PCR. Esophageal–pharyngeal fluids are taken by probang cup from ruminants, or as throat swabs from pigs, and are shipped in transport medium. These samples should be refrigerated or frozen immediately after collection. Vesicles are the preferred sample from animals that died of heart failure, but myocardial tissue or blood can be collected if vesicles are not present. FMDV may also be found in milk, other secretions and excretions, and other organs. Serum should be collected for serology.

In animals suspected to be carriers, esophageal-pharyngeal fluids should be collected.

Recommended actions if foot and mouth disease is suspected

Notification of authorities

A quick response is vitally important in containing an outbreak of FMD. State and federal veterinarians should be immediately informed of any suspected vesicular disease.

Federal: Area Veterinarians in Charge (AVIC): http://www.aphis.usda.gov/animal_health/area_offices/

State Veterinarians: http://www.aphis.usda.gov/vs/sregs/official.html

Control

FMDV is usually introduced into a country in contaminated feed or infected animals. Waste food (swill) fed to swine is a particular concern. In countries where foot-and-mouth disease is not endemic, the importation of animals and animal products from FMD-endemic areas is strictly controlled. Heat-treatment of all swill fed to pigs reduces the risk of an outbreak. Some countries have banned swill feeding altogether, due to difficulties in ensuring that adequate heat-treatment protocols are followed. Low-temperature pasteurization [72°C (162°F)] for 15 seconds) does not inactivate FMDV. High temperature short time (HTST) pasteurization greatly reduces the amount of viable FMDV in milk, but some studies suggest that residual virus may sometimes persist.

FMD outbreaks are usually controlled by quarantines and movement restrictions, euthanasia of affected and in-contact animals, and cleansing and disinfection of affected premises, equipment and vehicles. Effective disinfectants include sodium hydroxide (2%), sodium carbonate (4%), citric acid (0.2%) and Virkon-S®. Iodophores, quaternary ammonium compounds, hypochlorite and phenols are less effective, especially in the presence of organic matter. Infected carcasses must be disposed of safely by incineration, rendering, burial or other techniques. Milk from infected cows can be inactivated by heating to 100°C (212°F) for more than 20 minutes. Slurry can be heated to 67°C (153°F) for three minutes. Rodents and other vectors may be killed to prevent them from mechanically disseminating the virus.

Good biosecurity measures should be practiced on uninfected farms to prevent entry of the virus.

Vaccination may be used to reduce the spread of FMDV or protect specific animals (e.g. those in zoological collections) during some outbreaks. The decision to use vaccination is complex, and varies with the scientific, economic, political and societal factors specific to the outbreak. Vaccines are also used in endemic regions to protect animals from clinical disease. FMDV vaccines must closely match the serotype and strain of the infecting strain. Vaccination with one serotype does not protect the animal against other serotypes, and may not protect the animal completely or at all from other strains of the same serotype. Currently, there is no universal FMD vaccine. Vaccine banks contain a wide variety of strains, particularly those judged to be the greatest threat of introduction, for use in an outbreak. Some countries maintain individual vaccine banks. There are also three international vaccine banks: the North American FMD Vaccine Bank (for Canada, the U.S. and Mexico), the E.U. Vaccine Bank (for all EU countries) and the International Vaccine Bank (for a variety of countries including Australia, New Zealand and some European nations).

Humans are thought to carry FMDV mechanically for a short period of time, based on a study that found this virus in the nasal passages of one of eight people 28 hours after they had been exposed to infected animals and none of the eight people at 48 hours. People who have been exposed to infected animals should avoid susceptible livestock for a designated period, usually a few days to a week. Some recent studies suggest that extended avoidance periods may not be necessary if good biosecurity practices, including effective personal hygiene protocols (showering or washing hands, and changing clothing), are followed. The discrepancy between these studies remains to be resolved, and government authorities should be consulted for the most recent waiting period recommendations.

Transmission of FMDV from wildlife in southern Africa is controlled by separating wildlife from domesticated livestock with fences, and by vaccination of livestock.

Public Health

Foot-and-mouth disease is not considered to be a public health problem. FMDV infections in humans are very rare, with approximately 40 cases diagnosed since 1921. Vesicular lesions and influenza-like symptoms can be seen; the disease is generally mild, short-lived and self-limiting.

[Note: Foot=and-mouth disease is not related to hand, foot and mouth disease, a condition seen only in humans.]

Influenza

*Flu, Grippe, Avian Influenza,
Grippe Aviaire, Fowl Plague,
Swine Influenza, Hog Flu, Pig Flu,
Equine Influenza, Canine Influenza*

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Importance

Influenza viruses are RNA viruses in the family Orthomyxoviridae that can affect birds and mammals including humans. Influenza B and C viruses are maintained only in human populations, although they are isolated occasionally from other mammals.[1-9] Influenza A viruses can affect many species, with the vast majority of these viruses occurring among birds. Waterfowl and shorebirds seem to the reservoir hosts for avian influenza viruses, which are usually carried asymptomatically in these populations.[1,10-17] Avian influenza viruses can also become established among poultry, causing two forms of disease. Low pathogenicity avian influenza (LPAI) viruses, the form usually carried in wild birds, generally cause asymptomatic infections, mild respiratory disease or decreased egg production in poultry.[11,13,14,18,19] Some LPAI viruses can mutate in poultry populations to become high pathogenicity avian influenza (HPAI) viruses. HPAI viruses cause a severe illness that can kill up to 90-100% of a flock.[10,11,13] A few influenza A viruses have become adapted to mammals including humans, swine, horses and dogs, and circulate in these populations. These viruses are called human influenza A viruses, swine influenza viruses, equine influenza viruses and canine influenza viruses, respectively. In the mammalian species to which they are adapted, influenza viruses cause respiratory disease with high morbidity but low mortality rates. More severe cases can occur in conjunction with other diseases or debilitation, as well as in infancy or old age.

Influenza A viruses that circulate in birds and mammals are occasionally transmitted from one species to another. In most cases, these infections do not spread efficiently; they remain limited to an individual or a small group, and soon disappear from the novel host population.[1-4,11,20-24] However, some of these viruses can become adapted to a new species and cause outbreaks, epidemics or pandemics.[1,2,16,20,25-32] One equine influenza virus recently began circulating in dog populations, becoming the first canine influenza virus.[28-30] Similarly, avian influenza viruses have caused or contributed to past pandemics in humans and pigs.[2,20,27,31] Avian and swine influenza viruses, which are the only animal influenza viruses known to recurrently affect humans, are of the greatest concern as zoonotic agents.

The effects of avian influenza viruses on people are highly variable. Although many human infections are limited to conjunctivitis or mild respiratory disease, some viruses can cause severe disease and death.[2,11,12,15,26,33-41] Currently, HPAI H5N1 avian influenza viruses seem to be the greatest threat to people, as well as to poultry. These viruses first emerged in the late 1990s. They have since become established among birds in Asia, have spread to other geographic regions, and continue to threaten new areas.[11,12,42] As of December 2009, H5N1 viruses have been responsible for approximately 450 human infections, generally as the result of close contact with poultry; about 60% of all laboratory confirmed cases have been fatal.[43] Asian lineage H5N1 viruses have also caused disease in housecats, several species of large felids, palm civets, raccoon dogs, stone martens, a dog and a mink.[9,11,44-55] Some of these infections were fatal. In addition, H5N1 viruses have been detected in pigs and pikas, and experimental infections have been established in a variety of species including foxes, ferrets, rodents and rabbits.[34,51,56-58,58-70] Unusually, numerous deaths have been reported in wild birds, which are rarely affected by avian influenza viruses.[11,12,51,54,71-73] There are fears that an Asian lineage H5N1 strain could eventually become adapted to humans, possibly resulting in a severe human pandemic.

Other avian influenza viruses can also undergo cross-species transmission. H9N2 (LPAI) viruses, which have become endemic in poultry in parts of Asia and the Middle East, may be of particular concern.[36,74,75] These viruses have caused outbreaks among poultry in many countries,[75-77] and it has been recently recognized that some isolates share internal genes with H5N1 viruses.[74,78,79] H9N2 viruses have been detected in pigs with respiratory disease and fatal paralysis in China.[74] Antibodies to H9N2 viruses, as well as rare clinical cases, have also been reported in humans.[36-38,74,74,80,81] As of December 2009, known human H9N2 infections have been relatively mild, and fatal cases have not been reported.[36-38,74]

Swine influenza virus infections also occur sporadically among people. Most of these cases have been relatively mild and some may have been asymptomatic, but

Influenza

severe illnesses and a few deaths have been reported.[1-3,21-24,82-94] Swine influenza viruses are usually not well-adapted to humans, and little or no person-to-person transmission usually occurs.[1,2,22,24,89] Nevertheless, these viruses appear to have been responsible for the first human pandemic of the 21st century. In April 2009, a novel virus with the subtype H1N1 began circulating in people.[93,95,96] The genetic analysis of this virus suggests that it originated from North American and Eurasian swine influenza viruses that reassorted.[97-99] The novel H1N1 virus has also been transmitted from people to animals. Pigs are susceptible to this virus, and sporadic outbreaks have been reported among swine herds in a number of countries.[100-116] Outbreaks have also been reported in turkey flocks, and a few cases have been recognized in pet ferrets, cats and dogs, as well as in a cheetah in a zoo.[117-129]

Etiology

Viruses in the family Orthomyxoviridae cause influenza. There are three genera of influenza viruses: *influenzavirus A*, *influenzavirus B* and *influenzavirus C*.[130] Separate viral species are not recognized within these genera; the members of each genus belong to the three species "influenza A virus," "influenza B virus" or "influenza C virus," respectively.[130] These viruses are also called type A, type B and type C influenza viruses.

Influenza A viruses

Influenza A viruses include avian, swine, equine and canine influenza viruses, as well as the human influenza A viruses. Influenza A viruses are classified into subtypes based on two surface antigens, the hemagglutinin (H) and neuraminidase (N) proteins. There are 16 hemagglutinin antigens (H1 to H16) and nine neuraminidase antigens (N1 to N9).[11,13,19,23] These two proteins are involved in cell attachment and release from cells, and are also major targets for the immune response.[2,20,131] Wild birds carry most of the known hemagglutinin and neuraminidase antigens, but some, such as H14 and H15, are uncommon and seem to occur only in limited geographic areas.[17] Only limited subtypes are found in each species of mammal.[3] Influenza A viruses are also classified into strains. Strains of influenza viruses are described by their type, host, place of first isolation, strain number (if any), year of isolation, and antigenic subtype.[1,3] [e.g., the prototype strain of the H7N7 subtype of equine influenza virus, first isolated in Czechoslovakia in 1956, is A/eq/Prague/56 (H7N7).] For human strains, the host is omitted.

Antigenic shift and drift in influenza A viruses

Influenza A viruses change frequently. Strains evolve as they accumulate point mutations during virus replication; this process is sometimes called 'antigenic drift.'[3] A more abrupt change can occur during genetic reassortment. Reassortment is possible whenever two different influenza viruses infect a cell simultaneously; when the new viruses (the 'progeny') are assembled, they

may contain some genes from one parent virus and some genes from the other.[20] Reassortment between different strains results in the periodic emergence of novel strains. Reassortment between subtypes can result in the emergence of a new subtype. Reassortment can also occur between avian, swine, equine, canine and human influenza A viruses. This type of reassortment can result in a 'hybrid' virus with, for example, both avian and human influenza virus proteins.

An abrupt change in the subtypes found in a host species is called an 'antigenic shift.' Antigenic shifts can result from three mechanisms: 1) genetic reassortment between subtypes, 2) the direct transfer of a whole virus from one host species into another, or 3) the re-emergence of a virus that was found previously in a species but is no longer in circulation.[1,2] For example, human viruses can continue to circulate in pigs and could re-emerge into the human population.[2] Antigenic drift and antigenic shifts result in the periodic emergence of novel influenza viruses. By evading the immune response, these viruses can cause influenza epidemics and pandemics.

Avian influenza viruses

Avian influenza viruses circulate in a variety of domesticated and wild birds.[1,11,14,17,34] They are also isolated occasionally from mammals including humans.[1,9,11,25,26,35,37,44-53,55-57,132,133] Avian influenza viruses are classified as either high pathogenicity (HPAI) or low pathogenicity (LPAI) viruses, based on the genetic features of the virus and the severity of disease in experimentally inoculated chickens.[11,13,19] Although there are exceptions (e.g., viruses that fit the genetic description of HPAI viruses but cause mild illness), HPAI viruses usually cause severe disease in poultry, while LPAI infections are generally much milder. To date, all HPAI viruses have contained the H5 or H7 hemagglutinin; subtypes that contained other hemagglutinins have been found only in the LPAI form.[12,18,19] H5 and H7 LPAI viruses can evolve into high pathogenicity strains, typically while they are circulating among poultry.[11,12,15] When a subtype has become established and circulates for a time, numerous variants may occur in the population. For example, multiple genotypes and a number of clades of Asian lineage H5N1 viruses are currently found among poultry.[11,40,42,134,135]

In wild species, avian influenza viruses are especially common among birds that live in wetlands and other aquatic environments.[17] Waterfowl (order Anseriformes) and shorebirds (order Charadriiformes) seem to be the natural reservoirs for influenza A viruses, and carry all of the known subtypes, usually in the LPAI form.[1,12-17,23] Important reservoir hosts include ducks, geese, swans, gulls, terns and waders.[17] The LPAI viruses found in wild birds can be divided into Eurasian and American lineages.[17] Although viruses occasionally cross between these two geographic regions, this is uncommon.[17] The predominant subtypes in wild ducks change periodically.[1] H3, H4 and H6 are detected most often in North

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American and northern European wild ducks, but nearly all hemagglutinin and neuraminidase antigens can be found.[17] Waders (families Charadriidae and Scolopacidae) seem to have a wider variety of hemagglutinin/ neuraminidase combinations than ducks. In the eastern U.S., H1 through H12 (LPAI) viruses have been isolated from these birds; H1, H2, H5, H7 and H9-H12 viruses are particularly common.[17] Gulls are often infected with H13 LPAI viruses, which are rare in other avian species.[17] They can also carry H16 viruses.[17] Most, though not all, infections in wild waterfowl and shorebirds are asymptomatic.[1,2,11,12,136,137]

Limited information is available on the subtypes found in other species of birds. Subtypes that have been detected in ratites include H3N2, H4N2, H4N6, H5N1, H5N2, H5N9, H7N1, H7N3, H9N2, H10N1, H10N4 and H10N7.[18,51,138-140] Isolates from cage birds usually contain H3 or H4; however, infections with high pathogenicity subtypes containing H7 or H5 can also occur.[18,51,60,71,141,142] Very few avian influenza viruses were found in wild passerine birds, pigeons and doves in one survey.[143]

Swine influenza viruses

Swine influenza viruses mainly affect pigs, but they can cause disease in turkeys.[1,3] Outbreaks have also been described recently in ferrets and mink.[91,144] Other species may also be infected, although this seems to be rare. One H1N1 swine influenza virus, which was avirulent for both poultry and pigs, was isolated from a duck in Hong Kong, and experimental infections have been reported in calves.[145,146]

The most common subtypes currently found in pigs are H1N1, H1N2 and H3N2; however, the situation is complex, as two or more viruses of each subtype are circulating in swine populations.[2,16,20,147] One H1N1 virus found in North America is the 'classical' H1N1 swine influenza virus. This virus, the first influenza virus known to have infected pigs, was first detected in swine populations in 1918.[1,2,16,20] Reassortant H1N1 viruses, which contain the same neuraminidase and hemagglutinin as the classical H1N1 virus, but have internal proteins from triple reassortant H3N2 viruses (see below), have recently become prominent among pigs in North America.[148-150] An 'avian-like' H1N1 virus circulates mainly in European pigs.[2,16,20] This virus seems to be an avian influenza virus that was transmitted whole to pigs.[16,20,151] It has, in some locations, replaced the classical H1N1 virus.[16,20] A different 'avian-like' H1N1 virus has been detected, together with the classical H1N1 virus, among pigs in Asia.[9,16] Other variants have also been found. For example, H1N1 reassortant viruses consisting of classical swine influenza virus genes and a human PB1 polymerase gene have been detected in pigs in Canada[152] and a wholly human lineage H1N1 virus was reported from pigs in China in 2007.[153]

In North America, some of the most important swine influenza viruses are the triple reassortant H3N2 viruses.

These viruses first emerged in U.S. pigs in the late 1990s, mainly in the Midwest,[20,34,152,154,155] and they have been detected in Canada since 2005.[89,144,156] The North American H3N2 triple reassortant viruses contain hemagglutinin and neuraminidase proteins from a human influenza virus, and internal proteins from the classical swine influenza virus, an avian influenza virus and a human influenza virus.[154] The particular combination of internal genes carried by these viruses is known as the triple reassortant internal gene (TRIG) cassette. This cassette seems to be especially efficient in generating swine influenza virus recombinants with new hemagglutinin and neuraminidase genes, including some from human influenza viruses.[149,150] Viruses with this cassette have had increased antigenic drift compared to other swine influenza viruses.[149]

H3N2 viruses also occur in Europe and Asia, but these viruses seem to be the result of reassortment between a human H3N2 virus, circulating there in pigs since the 1970s, and the H1N1 'avian-like' virus.[2] The European H3N2 viruses contain human H3 and N2 proteins, and internal proteins from the avian virus.[2] In China, H3N2 viruses that have been detected include double reassortants that contain human H3 and N2 and internal genes from avian influenza viruses, and triple reassortants with human H3 and N2 and internal gene segments from both swine and avian influenza viruses.[157] Some wholly human-like H3N2 viruses have also been found among pigs in China.[157]

The H1N2 virus in the U.S. is a reassortant of the classical H1N1 swine influenza virus and the North American triple reassortant H3N2 virus.[2] Other variants have also been detected. Some H1N2 viruses isolated from Canadian pigs contained neuraminidase and hemagglutinin genes from two different human influenza viruses, the polymerase gene from human H1N2 viruses, and other internal genes from classical H1N1 swine influenza viruses.[152] The H1N2 virus in Europe is a reassortant of a human H1N1 virus and the 'human-like' European H3N2 virus.[2,16] In China, both the H1N2 swine influenza virus from North America, and apparent reassortants between the H1N1 classical swine influenza virus and North American H3N2 human influenza viruses have been reported.[158] Other novel reassortants of swine influenza viruses continue to be discovered.[159,160]

New subtypes have also been found in some swine populations. The novel subtype H3N1 has recently been isolated from pigs in the U.S.[161,162] This subtype appears to contain genes from human, swine and avian influenza viruses.[161,162] A different H3N1 influenza virus, containing human and swine influenza virus genes, has been found in Korea[163] and an H3N1 virus which may be a novel reassortant between H3N2 and H1N1 swine influenza viruses has been reported in Italy.[164] An H2N3 virus isolated from pigs with respiratory disease in the U.S. contained genes from avian and swine influenza viruses.[165] An avian H9N2 virus has been reported from

outbreaks of respiratory disease and paralysis in pigs in southeastern China, and may circulate in swine populations there.[74] This subtype appears to contain neuraminidase and hemagglutinin genes from avian H9N2 viruses and internal genes from an H5N1 virus (Sw/SD/2/03) that also infects pig populations in the area.[74] Avian (LPAI) H5N2 and avian/swine H5N2 reassortant viruses have been isolated from pigs in Korea.[166] The avian H5N2 virus appears to have been circulating among pigs since 2006.[166]

The novel H1N1 virus of swine origin

Swine influenza viruses are occasionally found in humans.[1-4,21-24,82-94,144] In most cases, these viruses are poorly adapted to humans, and little or no person-to-person transmission occurs.[1,2,22,24,89] In 2009, a novel H1N1 virus, which seems to have originated from one or more swine influenza viruses, emerged in human populations.[97-99] This virus appears to be a reassortant between North American and Eurasian swine influenza viruses; it contains a hemagglutinin gene that is most closely related to swine influenza viruses in North America, a neuraminidase gene that is related to swine influenza viruses in Eurasia, and internal genes from two or more swine influenza viruses including the North American triple reassortant H3N2 viruses and a Eurasian virus.[97-99] Similarly to some of the swine influenza viruses described above, the parental swine influenza viruses include some gene segments that originally came from avian and human influenza viruses.[98,99] In 2009, the novel H1N1 virus was the dominant influenza virus being transmitted in human populations in most parts of the world.[167] It has also been transmitted to animals, including pigs, apparently from infected humans.[100-117,119-129]

Equine influenza viruses

Equine influenza viruses mainly infect horses and other Equidae (i.e., donkeys, mules and zebras).[1,25,168,169] The two subtypes known to circulate in equine populations and cause disease are H7N7 (equine virus 1) and H3N8 (equine virus 2).[1,3,25] There is less antigenic drift in these viruses than human influenza A viruses.[3,25] H7N7 equine influenza viruses have become extinct or are present at only very low levels in most parts of the world where surveillance is conducted.[1,25,170] In contrast, H3N8 viruses circulate widely. H3N8 viruses have diverged into distinct Eurasian and American evolutionary lineages.[171,172] The American lineage contains the classical American lineage (also called the Kentucky lineage), the Florida sublineage (originally called the Florida lineage) and the South American lineage.[172] Some viruses of the American lineage (Florida sublineage) have also become established in Europe and Asia.[171,172]

In 1989, a novel strain of equine influenza [A/eq/Jilin/89 (H3N8)] caused a serious epidemic, with high morbidity and mortality rates, in Chinese horses.[25,170]

This virus appears to be an avian influenza virus. A related virus caused influenza in a few hundred horses the following year but there were no deaths. The avian-like virus continued to circulate in horses in China for at least five years without further fatalities.

One equine H3N8 virus recently jumped into dogs in North America.[28,29,173] Equine influenza viruses can also infect dogs without becoming established in canine populations. A limited outbreak with an equine H3N8 (American lineage) virus was reported among foxhounds in the U.K. in 2002,[32] and equine H3N8 viruses were shown to infect dogs asymptomatically during close contact with horses in an experimental study.[174] Infections with equine H3N8 viruses have been reported among pigs in China.[175]

Canine influenza viruses

An H3N8 canine influenza virus has been reported in canine populations in a number of U.S. states.[30,176-183] This virus appears to be an equine influenza virus (Florida sublineage) that recently jumped species, and it bears a close resemblance to an isolate seen in horses in Wisconsin in 2004; however, the canine influenza virus has diverged genetically from equine influenza viruses.[28-30]

An H3N2 virus, isolated during an outbreak of canine respiratory disease in Korea in 2007, has the potential to become a second canine influenza virus.[133] There is evidence that this virus may have been transmitted between dogs during the outbreak, and dog-to-dog transmission occurs readily in experimentally infected dogs.[133,184] The H3N2 virus seems to have originated in birds.[133] It contains gene segments that may have come from several different avian viruses.[133] At least three different isolates of this virus have been recovered.[133]

Human influenza A viruses

Human influenza A viruses are mainly found in people, but they can also infect ferrets and sometimes swine.[1,3,5,16,152,153,185-188] Experimental infections have been reported in raccoons.[189] Human viruses can also replicate, to a limited extent, in the nasal epithelium of experimentally infected horses.[170] H1N1, H1N2 and H3N2 viruses are currently in general circulation in humans.[11,190] H1N2 viruses were first seen in human populations in 2001, probably as a result of genetic reassortment between the H3N2 and H1N1 viruses.[190,191] H2N2 viruses circulated in the human population between 1957 and 1968.[1] A novel H1N1 virus (see above) emerged in human populations in 2009.

Human influenza viruses change frequently as the result of antigenic drift, and occasionally as the result of antigenic shift. Epidemics occur every few years, due to small changes in the influenza viruses.[2,192] Human pandemics, resulting from antigenic shifts, were most recently reported in 1918, 1957, 1968 and 2009.

Influenza A viruses in other species

Influenza A viruses are occasionally isolated from outbreaks or isolated cases in other species of mammals. Avian influenza viruses have infected pinnipeds, cetaceans and mink,[1,9] and swine influenza viruses have caused outbreaks in mink and ferrets.[91,144] Antibodies to influenza viruses have been detected in other species including raccoons, cattle, yak, sheep, goats, reindeer and deer,[9,189,193] and a variety of mammals have been infected experimentally.[9,189,194-196] There are also some indications, including the detection of viral nucleic acids by RT-PCR, that reptiles and amphibians can be infected with influenza viruses.[9] The Asian lineage H5N1 avian influenza viruses appear to have an unusually wide host range, and can infect housecats, several species of large felids, dogs, foxes, stone martens, mink, palm civets, raccoon dogs, pigs, ferrets, rodents, pikas, rabbits and macaques.[9,34,44,45,47-49,51-58,60-64,67-69,132] Unpublished research suggests that some raccoons in Japan have antibodies to H5N1 viruses.[193] With the possible exception of H5N1 viruses in pikas,[57] there is currently no evidence that influenza viruses have become adapted to, and are circulating in, any species other than birds, swine, humans, dogs and horses.

Influenza B viruses

Influenza B viruses are known to circulate only in human populations. These viruses can cause epidemics, but they have not, to date, been responsible for pandemics.[1] They have also been found occasionally in animals.[1,2,4,5,9,197] Influenza B viruses are categorized into lineages rather than subtypes. They are also classified into strains.[11] Influenza B viruses undergo antigenic drift, though it occurs more slowly than in influenza A viruses.[1,190] Until recently, the B/Victoria/2/87 lineage predominated in human populations, and influenza B viruses were said not to undergo antigenic shifts.[11,198] In the 1990s, viruses of the B/Yamagata/16/88 lineage circulated to a very limited extent in Asia.[198] This lineage emerged in various parts of the world in 2001, and it is now co-circulating with the B/Victoria/2/87 lineage.[198,199] Recent evidence suggests that recombination between these two lineages is resulting in antigenic shifts.[199,200]

Influenza C viruses

Influenza C viruses circulate in human populations, and are mainly associated with disease in people.[1,131,192,201] Until recently, they had never been linked to large-scale epidemics.[1,131,192,201] However, a nationwide epidemic of influenza C was reported in Japan between January and July 2004.[202] Influenza C viruses have also been found in animals.[1-8] Influenza C viruses are not classified into subtypes, but they are classified into strains.[11] Each strain is antigenically stable, and accumulates few changes over time.[203] Recent evidence suggests that reassortment occurs frequently between different strains of influenza C viruses.[203,204]

Geographic Distribution

Human influenza viruses, including the novel H1N1 virus that entered human populations in 2009, are found worldwide.[1,10,205,206] Avian influenza (LPAI) viruses also occur worldwide in wild birds and poultry.[1,3,10,14] HPAI viruses have been eradicated from domesticated poultry in most developed nations. The Asian lineage H5N1 HPAI outbreak began among poultry in Southeast Asia in 2003.[12] From 2003 to 2007, HPAI H5N1 viruses spread into domesticated or wild birds in other regions of Asia as well as into parts of Europe, the Pacific, the Middle East and Africa.[11] Although some countries (e.g., all countries in Europe) eradicate these viruses whenever they occur in domesticated birds, this epizootic is ongoing and worldwide eradication is not expected in the short term.[11] Unusually, some Asian lineage H5N1 HPAI viruses are also circulating in wild bird populations in Eurasia.[11,12,71-73,207,208] As of December 2009, wild bird surveillance has not detected these viruses in North America or New Zealand.[209,210]

Swine influenza viruses are enzootic in most areas that have dense populations of pigs.[211] This disease is common in North and South America, Europe and parts of Asia, and it has been reported from Africa.[4,16] Although the subtypes of the swine influenza viruses found in the U.S. and Europe are the same, they are actually different viruses (see 'Etiology').

Equine influenza occurs in nearly all countries with substantial numbers of horses.[169] Only a few countries such as New Zealand and Iceland are known to be free from this disease.[168-170,212] The H3N8 subtype is widespread in horse populations.[25,170] The H7N7 subtype is either extinct or present at very low levels.[1,25,168,170]

The H3N8 canine influenza virus has been reported in the U.S. In 2004-2006, infections were seen in racing greyhounds in a number of states including Florida, Texas, Arkansas, Alabama, Arizona, West Virginia, Kansas, Iowa, Colorado, Rhode Island and Massachusetts.[177,180] Infections were first reported in the general canine population in Florida, but the virus later spread to other states.[177,178,181-183,213] The distribution of this virus in the U.S. is patchy; in some cases, it caused an outbreak or was detected serologically in an area, but later disappeared from that region.[213] There is no evidence that the canine influenza virus is currently circulating outside the U.S. However, infections with equine influenza viruses are occasionally reported among dogs in other regions. In the U.K., an equine H3N8 virus was responsible for an outbreak of respiratory disease in a foxhound kennel in 2002.[32,214] Limited serological evidence also suggests that some U.K. foxhounds were exposed to an H3N8 virus in 2003.[215] These cases appear to have been caused by H3N8 equine influenza viruses that did not become established in the canine population.[32,213,214] H3N8 infections were reported from dogs in Australia during an equine influenza outbreak in 2007; these were also equine viruses that did not become

adapted to dogs.[213] As of December 2009, the H3N2 influenza virus has been reported only from dogs in Korea.[133]

Transmission

Transmission of mammalian influenza viruses

In mammals, influenza viruses are transmitted in aerosols created by coughing and sneezing, and by contact with nasal discharges, either directly or on fomites.[1,3,16,25,147,190-192] Close contact and closed environments favor transmission. In ferrets, *in utero* transmission can occur with high viremia after experimental infection.[188]

Transmission of avian influenza viruses among birds

In birds, avian influenza viruses are shed in the feces as well as in saliva and nasal secretions.[1,3,11,13] The feces contain large amounts of virus, and fecal-oral transmission is the predominant means of spread for LPAI viruses in wild bird populations.[17,73] Fecal-cloacal transmission might also be possible.[17] Fecal transmission is facilitated by the persistence of avian influenza viruses in aquatic environments for prolonged periods, particularly at low temperatures.[1,2,17,216,217] Respiratory transmission of LPAI viruses is thought to be unimportant in most wild birds; however, it is possible that is might play a role in some species, particularly those that live on land.[17] Some recent isolates of Asian lineage H5N1 (HPAI) viruses have been found in higher quantities in respiratory secretions than the feces.[73,218,219] This suggests that, at least in some wild birds, these strains may no longer be transmitted primarily by the fecal-oral route.

Once an avian influenza virus has entered a poultry flock, it can spread on the farm by both the fecal–oral route and aerosols, due to the close proximity of the birds. Fomites can be important in transmission and flies may act as mechanical vectors.[12,13,15] Avian influenza viruses have also been found in the yolk and albumen of eggs from hens infected with HPAI viruses.[13,220] Although infected eggs are unlikely to hatch, broken eggs could transmit the virus to other chicks in the incubator. It might also be possible for LPAI viruses to be shed in eggs, but the current evidence suggests this is very rare, if it occurs at all.[221,222]

In countries where HPAI has been eradicated from domesticated poultry, the disease could be introduced into flocks by migratory waterfowl or shorebirds, as well as infected poultry or fomites.[3,12,13] Migrating birds, which can fly long distances, may exchange viruses with other populations at staging, stopover or wintering sites.[17] Wild birds usually carry only the low pathogenicity form of avian influenza viruses.[1,12,13,17] Once they are introduced into poultry, these viruses reassort and/or mutate to produce HPAI viruses. However, the Asian lineage HPAI H5N1 strains appear to occur regularly in wild birds, although their importance in transmitting these viruses to poultry is controversial.[12,207,208,223,224] HPAI H5N2 viruses have also been detected recently in some asymptomatic wild ducks and geese in Africa.[225]

Survival of influenza viruses in the environment

The survival of avian influenza viruses in the environment is influenced by temperature, pH, salinity and the presence of organic material.[216,217,226] These viruses, which are often transmitted between birds in feces, may persist for relatively long periods in aquatic environments.[217,226] They appear to survive best at low temperatures and in fresh or brackish water rather than salt water.[216,217,226] LPAI viruses are reported to persist in distilled water for more than 100 days at 28°C (82°F) and 200 days at 17°C (63°F).[216] These viruses also remained viable for at least 35 days in peptone water at 4°C (39°F), 30°C (86°F) or 37°C (98.6°F).[216] Various avian influenza viruses were reported to survive for four weeks at 18°C (64°F).[216] One recent study suggested that H5 and H7 HPAI viruses may survive for shorter periods in water than LPAI viruses; however, they still persisted in fresh water for 100 days or more at 17°C (63°F) and for approximately 26-30 days at 28°C (82°F).[217] Avian influenza viruses might survive indefinitely when frozen.[15,216]

A few studies have examined virus persistence in feces. In one study, LPAI viruses (H7N2) persisted for up to two weeks in feces and on cages.[227] These viruses could survive for up to 32 days at 15-20°C (59-68°F), and for at least 20 days at 28-30°C (82-86°F), but they were inactivated more quickly when mixed with chicken manure.[227] In other studies, LPAI viruses were reported to survive for at least 44 or 105 days in feces.[216]

Mammalian influenza viruses (which are shed in respiratory secretions) are relatively labile, but can persist for several hours in dried mucus.[192] There is little information on the survival of mammalian influenza viruses in water or organic material. In one study, swine influenza viruses were inactivated in untreated pig slurry in 1-2.5 hours at 50-55°C (122-131°F), two weeks at 20°C (68°F), and 9 weeks at 5°C (41°F).[228]

Routes of transmission of avian influenza viruses to mammals

Some avian influenza viruses can be transmitted to mammals by direct or indirect contact. Transmission is best understood for the Asian lineage H5N1 (HPAI) viruses. Close contact with dead or sick birds seems to be the principal way this virus is spread to humans, but a few cases may have resulted from indirect exposure via contaminated feces, and swimming in contaminated water is theoretically a source of exposure.[11,12,41] Ingestion of H5N1 viruses has been reported in naturally infected housecats, other felids and dogs; experimentally infected cats, pigs, ferrets, mice and foxes; and rarely in humans.[11,45,49,63,65,66,229] One Asian lineage H5N1 infection occurred in a dog that had eaten infected duck carcasses.[49,] Similarly, leopards and tigers in zoos, as well

as some housecats, were apparently infected when they ate raw birds.[45,46,48,50,53,63] Infected housecats in an animal shelter probably ingested contaminated feces from a swan while they were grooming, but aerosol transmission could not be ruled out.[132] Infected raccoon dogs in China were fed chicken carcasses, and might have acquired the H5N1 virus from this source.[55] In humans, the strongest evidence for oral transmission is that two people became infected with an Asian lineage H5N1 virus after eating uncooked duck blood.[11,229] There are other human cases where ingestion probably occurred, but additional routes of exposure also existed.[230]

Experimental studies suggest that Asian lineage H5N1 viruses can be transmitted to mammals by the respiratory, oral and intraocular routes; however, all routes have not been reported in each species. Infections have been established in cats by intratracheal inoculation with Asian lineage H5N1 viruses and by feeding them H5N1-infected chicks.[63,65] Cats appear to shed these viruses from the intestinal tract as well as the respiratory tract.[53,65] Pigs and foxes can also be infected by feeding them H5N1-infected poultry, as well as by intranasal or intratracheal inoculation.[58,66,69] Infected foxes can excrete this virus in both respiratory secretions and feces, but pigs are known to shed it only from the respiratory tract.[58,66,69] In experimentally infected dogs, Asian lineage H5N1 viruses have been found in respiratory secretions, but fecal shedding has not been reported.[67,68] In one experiment, cattle excreted small amounts of H5N1 viruses from the respiratory tract after intranasal inoculation; a high dose of the virus, which had been recovered from cats, was used to inoculate the cattle.[70] Fecal shedding of Asian lineage H5N1 virus may also be possible in humans: this virus has been recovered from a child with diarrhea.[231] In addition, it may be found in the urine of some mammals.[9]

The eye might act as an entry point for some HPAI viruses. After intraocular inoculation of mice and ferrets with H7 and H5N1 (HPAI) isolates, the viruses could be detected in the respiratory tract and caused systemic disease.[196,232,233] Transplacental transmission of avian influenza viruses is not well studied in mammals; however, viral antigens and nucleic acids were detected in the fetus of a woman who died of an Asian lineage H5N1 infection.[234]

There are few detailed reports of mammalian infections with avian LPAI viruses. Raccoons that were intranasally inoculated with LPAI H4N8 viruses shed virus from the respiratory but not the digestive tract.[189] These raccoons could transmit this virus to uninfected raccoons.[189]

Transmission of influenza viruses between species – sporadic cases, limited transmission and cross-species jumps

Ordinarily, swine influenza viruses circulate only among pigs, equine influenza viruses among the Equidae,

avian influenza viruses among birds, and human influenza viruses among people. Although these viruses occasionally infect species other than their normal host, the virus is usually poorly adapted to the new host population and often affects only one or a few individuals.[1,3,4,11,20] Occasionally, one of these viruses may cause an outbreak. For example, avian influenza viruses have affected mink, horses, seals and pigs, swine influenza viruses have caused outbreaks in ferrets, mink and turkeys, and equine influenza viruses have infected dogs.[1,3,9,16,25,26,28,32,91,133,144] Generally, efficient transmission requires a novel hemagglutinin and/or neuraminidase protein to evade the immune response, together with viral proteins that are well adapted to the new host's cells.[20] Many outbreaks end without permanent adaptation of the virus to the species. In most of the cases mentioned above, the virus eventually disappeared from the novel host population.

It is, however, possible for a virus to become established in the new species. This has happened occasionally with whole viruses that jump to new hosts. The canine influenza virus, which jumped from horses to dogs, is a good example. Some evidence also suggests that the H1N1 virus, which caused the deadly 1918 'Spanish flu' pandemic, was probably an intact avian virus that became adapted to humans.[20,27,31] Dissemination is more likely if the new virus reassorts with a virus that is already adapted to the species.[11] Reassortment can occur in the new host's own cells.[11,12,20] It could also occur in an intermediate host, particularly a pig.[2,11,12,20] Pigs have receptors that can bind swine, human and avian influenza viruses.[2,16,23,147] For this reason, they have been called 'mixing vessels' for the formation of new viruses. Repeated reassortment between human, avian and swine influenza viruses has resulted in a wide variety of novel swine influenza viruses that contain segments originating from two or more species. (See 'Etiology' for a description of some of these viruses.) Recently, quail cells have also been shown to bind both human and avian influenza viruses.[235] Although reassortment can occur anywhere, many new viruses originate in Asia. In rural China and other regions, a variety of species including ducks are kept in close proximity to each other and to humans.[1,2,34] This results in an increased opportunity for virus reassortment.

Transmission of Asian lineage H5N1 viruses between mammals

Because Asian lineage H5N1 avian influenza viruses can cause fatal disease in humans and other mammals, there are grave concerns about the possibility that these viruses might become adapted to these species. Over the decade that H5N1 viruses have been circulating among birds, these viruses have changed and differentiated into a number of strains and clades.[11,40,42,134,135] An early study showed that, from 1999 to 2002, H5N1 avian influenza viruses isolated from healthy ducks in southern China acquired the ability to replicate and cause lethal disease in

mice.[34,62] As of December 2009, little or no host-to-host transmission has been seen in mammals, with the possible exception of pikas. Limited transmission of Asian lineage H5N1 viruses has been reported among zoo tigers and experimentally infected housecats.[50,63] No animal-to-animal transmission was reported in asymptomatic cats infected by exposure to a sick swan, or in experimentally infected pigs.[58,132] In one study, an Asian lineage H5N1 virus was not transmitted to one dog or three cats in contact with four experimentally infected dogs, or to three dogs in contact with infected cats.[67] However, there is recent evidence that Asian lineage H5N1 viruses might have become established among some pika populations in China; these viruses do not seem to cause severe clinical signs in these animals.[57]

In humans, only rare cases of limited person-to-person spread have been documented, and these cases occurred after close, prolonged contact.[11,12] In 2007, an Asian lineage H5N1 virus with the ability to bind human receptors was isolated from a person in Thailand.[236] Whether this modification would allow the virus to be transmitted more efficiently from person to person is unknown.[236] This particular isolate was found only once, to date, and may have been eliminated by infection control measures. Sustained person-to-person transmission has never been reported, as of December 2009.[11,12]

Zoonotic influenza viruses reported in humans

Infections with avian or swine influenza viruses are reported periodically in humans. With rare exceptions, these viruses have not become adapted to people.

Avian influenza viruses in humans

- Two of the last four human pandemics appear to have been the result of reassortment between avian and human influenza viruses.[20] The 1957 H2N2 ('Asian flu') virus contained avian hemagglutinin, neuraminidase and an internal protein, and five other proteins from a human H1N1 strain.[2,20] The H3N2 'Hong Kong flu' virus of 1968 had two new proteins from an avian virus – the new hemagglutinin and an internal protein – but kept the neuraminidase and remaining proteins from the H2N2 virus.[2,20]

- Illnesses caused by H5, H7 and H9 avian influenza viruses are documented occasionally in people.[11,12,23,37,38] Most of these infections have resulted from direct contact with infected poultry or fomites; however, during a 2003 outbreak in the Netherlands, three family members of poultry workers were also infected.[11,35] The virus subtype was H7N7. No sustained person-to-person transmission has been reported, to date, with any of the viruses currently circulating in bird populations.

- Asian lineage H5N1 avian influenza viruses have been responsible for nearly 450 confirmed clinical cases in humans, after contact with infected poultry.[2,11,12,15,34,43] Because exposure to these viruses can be high in some human populations, and clinical illness is typically severe [12,38,41] (thus more likely to be diagnosed), it is difficult to determine whether these viruses are more likely to infect humans than other subtypes.

- Some currently circulating H9N2 viruses might undergo relatively frequent cross-species transmission.[74] H9N2 (LPAI) viruses, which circulate among poultry in parts of Asia and the Middle East, have been associated with disease in Chinese pigs.[74,75] Humans can also be infected with H9N2 viruses. Surveys in China report that from 0% to 4.5% of the human populations studied have antibodies to H9 viruses.[74,80,81] In one study, the overall seroprevalence was 4.5%; 15.5% of poultry retailers, 2.6-5.7% of farmers and other poultry workers, and 1.3% of the general population were seropositive.[80] Another survey reported seroprevalences of 0% to 1.7% in poultry workers, depending on the geographic area.[81] Symptomatic infections have occasionally been reported in H9N2-virus infected humans.[11,12,34,36,37] In general, these cases appear to be clinically indistinguishable from human influenza virus infections.[36]

- Some serological evidence suggests that poultry workers, veterinarians and hunters may be regularly exposed to avian influenza viruses of various subtypes; antibodies to H4, H5, H6, H7, H9, H10 and H11 viruses have been found in healthy people.[38,80,81,240-242] These antibodies may be more common among people who are exposed to free-range or backyard poultry than workers in poultry confinement facilities.[242,243] Experimental infections, accompanied in some cases by mild respiratory signs and other influenza symptoms, have been established in human volunteers inoculated with some subtypes including H4N8, H10N7 and H6N1.[38]

Swine influenza viruses in humans

- Infections with swine influenza viruses are reported sporadically in humans.[1,2,21-24,82-90,92-94,244] Most of these infections occur after direct contact with pigs, but viruses may also spread to people through another host. For example, an H1N1 swine influenza virus, which had infected a turkey herd, was then transmitted to a laboratory technician who developed respiratory signs.[94,245] How often swine influenza viruses infect people is unknown. If most infections resemble human influenza, they may not be

investigated and recognized as zoonoses. Before 2005, when swine influenza in humans became reportable in the U.S., approximately one case was reported to the Centers for Disease Control and Prevention (CDC) every 1-2 years.[93] From December 2005 through February 2009, 12 cases were reported to the CDC.[93] Recent serological evidence suggests that swine influenza infections might occur regularly in people who have contact with pigs.[1,2,23,240,246-248]

- Although many swine influenza virus infections seem to be limited to a single person, sometimes cases are followed by a few infections among close contacts. In Czechoslovakia, five family members of an infected laboratory worker became ill.[21] Similarly, several health care workers developed influenza symptoms after exposure to a pregnant woman with swine influenza in Wisconsin.[21] One college student transmitted the virus to his roommate, who remained asymptomatic.[22] Until 2009, the most extensive person-to-person transmission was reported in 1976, when approximately 500 of 12,000 people on a military base in Fort Dix, New Jersey became seropositive to a swine influenza virus.[1,2,21,22] This virus remained limited to the base and did not spread to the surrounding community.

- In 2009, a novel H1N1 virus with genes of swine origin became established in human populations, causing a pandemic.[206,249,250] Genetic analysis suggests that this virus was probably transmitted to people very recently, and that it might have been circulating among pigs in an unknown location for years before it emerged in humans.[98,99] As of December 2009, this swine population has not been found, and it is not known how humans acquired the novel H1N1 virus.[98] There is no evidence that pigs are playing an significant role in the spread of this virus among people,[251] but people might be involved in disseminating the virus to pigs and other animals.[103,104,108,113,117,119-129,252]

Equine influenza viruses and canine influenza viruses in humans

- There are no published reports of equine or canine influenza viruses causing disease in humans after natural exposure. Serological evidence and one experiment in volunteers suggest that humans might be susceptible to equine viruses.[1]

Disinfection

Influenza viruses are susceptible to a wide variety of disinfectants including sodium hypochlorite, 70% ethanol, oxidizing agents, quaternary ammonium compounds, aldehydes (glutaraldehyde, formaldehyde), phenols, acids, povidone-iodine and lipid solvents.[3,130,192,216,253] They can also be inactivated by heat of 56°C (133°F) for a minimum of 60 minutes (or higher temperatures for shorter periods), as well as by ionizing radiation or low pH (pH 2).[3,130,192,216,227] Avian influenza viruses seem to be more resistant to high temperatures and low pH than mammalian influenza viruses.[9]

Infections in Humans

Incubation Period

The incubation period for seasonal human influenza is short; most infections appear after one to four days.[1,190-192] Infections with the novel H1N1 virus circulating in humans usually become apparent in two to seven days.[254,255]

The incubation period for avian influenza in humans is difficult to determine.[12] Limited data from Asian lineage H5N1 infections suggest that, for this virus, it may range from two to eight days and could be as long as 17 days.[12] In most cases, the first symptoms occur in two to five days.[40] The World Health Organization (WHO) currently suggests using an incubation period of seven days for field investigations and monitoring patient contacts.[12]

Clinical Signs

Seasonal human influenza

Uncomplicated infections with human influenza A or B viruses are usually characterized by upper respiratory symptoms, which may include fever, chills, anorexia, headache, myalgia, weakness, sneezing, rhinitis, sore throat and a nonproductive cough.[1,131,188,190-192] Diarrhea, abdominal pain and photophobia are also possible.[131,188] Nausea, vomiting and otitis media are common in children, and febrile seizures can occur in severe cases.[190,191] In young children, the initial signs may mimic bacterial sepsis.[190,191] Most people recover in one to seven days, but in some cases, the symptoms may last up to two weeks or longer.[131,190,192]

More severe syndromes, including pneumonia, can be seen in some individuals, especially those with chronic respiratory or heart disease.[131,190-192] Secondary bacterial or viral infections may also occur.[1,131,190,191] In addition, influenza A has been associated with encephalopathy, transverse myelitis, Reye syndrome, myocarditis, pericarditis and myositis.[190,192]

Because influenza C viruses are difficult to isolate, there are few reports on their clinical features. These viruses are mainly thought to cause mild upper respiratory disease in children and young adults, but more severe cases with lower respiratory signs including bronchitis or pneumonia can also occur; some recent descriptions suggest that clinical cases may be indistinguishable from influenza A or B.[1,201,203,204,256-258] In one recent study, the

most common clinical signs were fever, cough and rhinorrhea, but 29 of 179 children were hospitalized with more serious illnesses such as pneumonia, bronchitis or bronchiolitis.[259] Serious disease was most common in children less than two years of age.[259] Fever and cough were the most common signs in 14 patients from France, with rhinitis, pharyngitis, wheezing and/or otitis in some individuals.[257] This study also documented lower respiratory tract signs including pneumonia and bronchiolitis in a few patients.[257] Fever, cough, arthralgia, headache, sore throat and rhinorrhea were reported in four infected children in Cuba.[258] A study from Spain reported high fever and lower respiratory tract illness, severe enough to require hospitalization, in a few infants.[256] Gastrointestinal symptoms including diarrhea and vomiting have been reported in some patients; co-infections with gastrointestinal pathogens were present in some but not all cases.[256,257] Some influenza C infections may be asymptomatic.

Novel H1N1 virus of swine origin

In most people, the novel H1N1 virus causes a relatively mild illness, which resembles the disease caused by other human influenza viruses.[167,167,205,205,255,255,260-262] Vomiting and diarrhea have been reported in a significant number of cases.[205,255,261,262] Most people have a self-limiting illness, and recover within a week.[260] Severe primary viral pneumonia and/or acute respiratory distress syndrome occur in a small percentage of cases, and may be fatal.[167,255,262-265] Patients who become severely ill usually begin to deteriorate 3-5 days after the onset of the symptoms, and their condition rapidly becomes serious, often progressing to respiratory failure within 24 hours.[260,264] Multiple organ failure may be seen.[260,265] Like other influenza viruses, the novel H1N1 virus can also exacerbate chronic medical conditions, especially respiratory diseases such as asthma or chronic obstructive pulmonary disease, and some cases may be complicated by secondary bacterial infections.[260,261,265,266] Underlying health conditions, very young age or pregnancy increase the risk of severe disease.[167,260,264,267-270] A significant number of serious or fatal cases have been reported in healthy children or young adults, who would not be expected to have a high risk of complications.[167,260,264,267,268]

Avian influenza infections in humans

Infections with avian influenza viruses have occasionally been reported in humans. Healthy children and adults, as well as those with chronic medical conditions, have been affected.[12] Some infections have been limited to conjunctivitis and/or typical influenza symptoms; other cases, especially those caused by Asian lineage H5N1 viruses, were serious or fatal.[2,11,12,15,33-35,38]

Asian lineage H5N1 viruses

The Asian lineage H5N1 HPAI viruses appear to cause more severe disease than other HPAI viruses or LPAI viruses.[38] High fever and upper respiratory symptoms resembling human seasonal influenza tend to be the initial signs.[12,40,271] In some patients, there may also be mucosal bleeding, or gastrointestinal symptoms such as diarrhea, vomiting and abdominal pain.[12,40,271] Respiratory signs are not always present at diagnosis; two patients from southern Vietnam had acute encephalitis without symptoms to indicate respiratory involvement.[12] Similarly, a patient from Thailand exhibited only fever and diarrhea.[12] Many patients develop lower respiratory tract disease shortly after the first signs; the symptoms may include chest pain, dyspnea, tachypnea, hoarseness of the voice and crackles during inspiration.[12,40] The respiratory secretions and sputum are sometimes blood-tinged.[12] Most patients deteriorate rapidly.[12,40] Heart failure, kidney disease, encephalitis and multiorgan dysfunction are common in the later stages, and disseminated intravascular coagulation can occur.[12,40,271] Milder cases have been reported occasionally, particularly among children.[38,39] One H5N1 infection in a child with upper respiratory signs and an uncomplicated recovery after antibiotic treatment was recognized only by routine virus surveillance.[39] Asymptomatic infections with Asian lineage H5N1 viruses seem to be rare.[38,41]

The following human infections with Asian lineage H5N1 and other avian influenza viruses were reported between 1997 and 2009:

- In 1997, the first eighteen H5N1 infections in people were reported during an HPAI outbreak among poultry in Hong Kong.[2,11,12,15,34] The symptoms included fever, sore throat and cough and, in some cases, severe respiratory distress and viral pneumonia.[12] Eighteen people were hospitalized and six died.

- In 1999, avian influenza (LPAI H9N2) was confirmed in two children with upper respiratory signs, fever, sore throat, abdominal pain and vomiting in Hong Kong.[11,12,34,38] The illnesses were mild and both children recovered. No other cases were found. Six unrelated H9N2 infections associated with acute respiratory disease were also reported from mainland China in 1998-99; all six people recovered.[11,34,38]

- In 2002, antibodies to an avian H7N2 virus were found in one person after an LPAI outbreak among poultry in Virginia.[11]

- In 2003, two HPAI H5N1 infections were reported in a Hong Kong family that had traveled to China.[11,12,34] One of the two people died. Another family member died of a respiratory illness while in China, but no testing was done.

- In 2003, 347 total (suspected and confirmed) and 89 confirmed human infections were associated with an H7N7 HPAI outbreak among poultry in the Netherlands.[11,33,35] Most cases occurred in

poultry workers, but three family members also became ill.[11,35] In 78 of the confirmed cases, conjunctivitis was the only sign of infection.[35] Two people had influenza symptoms such as fever, coughing and muscle aches. Five had both conjunctivitis and influenza-like illnesses. (Four cases were classified as "other.") The single death occurred in an otherwise healthy veterinarian who developed acute respiratory distress syndrome and other complications.[35] His initial symptoms included a persistent high fever and headache but no signs of respiratory disease. The virus isolated from the fatal case had accumulated a significant number of mutations, while viruses from most of the other individuals had not.[35] This virus also caused severe or fatal infections in experimentally infected ferrets and mice, while other H7 viruses from milder human cases in North America were significantly less virulent.[196]

- Cases of conjunctivitis have been reported after contact with HPAI H7N7 avian viruses in infected seals.[26,35]

- In 2003, an H9N2 LPAI infection was confirmed in a child in Hong Kong. [11,12,36] The symptoms included mild fever, mild dehydration and cough.[36] The child was hospitalized but recovered.

- In 2003, an LPAI H7N2 infection with respiratory signs was reported in a patient in New York.[11] The person, who had serious underlying medical conditions, was hospitalized but recovered.

- In 2004, two cases of conjunctivitis and flu-like symptoms were confirmed in poultry workers in Canada.[11] One virus was LPAI; the other was HPAI. Both people recovered after treatment with an antiviral drug. Ten other infections were suspected but not confirmed; these cases included both conjunctivitis and upper respiratory symptoms. All of the infections were associated with an H7N3 virus outbreak in poultry.

- From 2004 to 2008, sporadic human illness and deaths were associated with widespread outbreaks of Asian lineage H5N1 high pathogenicity avian influenza among poultry. As of December 11 2009, 445 confirmed human cases had been reported to WHO; 263 cases were fatal.[43]

- In 2007, a mild LPAI H9N2 virus infection was reported in a 9-month-old child in Hong Kong. [11]

- In 2008, an H9N2 virus was found in a 2-month-old infant in China.[37]

- In 2009, an H9N2 virus infection was reported in a 3-year-old child with a fever, cough and rhinorrhea in Hong Kong.[37] She was hospitalized but recovered. There is no indication in the report that this case was more severe than the previously reported infections.

Swine influenza virus infections in humans

Serological evidence suggests that swine influenza virus infections might occur regularly among people who are occupationally exposed.[1,2,23,240,246-248] Because few infections with swine influenza viruses have been described, it is not known whether the symptoms caused by these viruses differ significantly from human influenza.[23] Reported swine influenza infections include the following cases. It should be kept in mind that severe or fatal cases are more likely to be investigated than mild illnesses that resemble human seasonal influenza.

- A localized outbreak was reported at Fort Dix, New Jersey in 1976. An H1N1 swine influenza virus was isolated from five recruits with respiratory disease, including one who died of pneumonia.[1,2,22] Other people on the base may also have been ill with the same infection.[21,244] Serological evidence suggests that approximately 500 people on the fort had been infected by person-to-person spread. (This virus is not the same virus involved in the swine-origin H1N1 pandemic of 2009.)

- A self-limiting illness with influenza symptoms was reported in a college student infected with an H1N1 virus in 1979.[22] There was evidence that his roommate had been infected but remained asymptomatic.

- In 1980, an H1N1 virus infection with influenza symptoms including diarrhea occurred in a young boy, who recovered.[22] There was no evidence of spread to his family.

- Swine influenza virus (H1N1) was isolated from an immunocompromised child with fulminant pneumonia who died in 1982.[82] Serological evidence of possible infection was found in five contacts, but the infection did not spread further.

- In 1986, an H1N1 virus caused severe viral pneumonia in a 29-year-old swine farmer in the Netherlands.[85] The farmer had been in contact with pigs showing signs of respiratory disease.

- In 1988, an H1N1 swine influenza virus was isolated from a pregnant woman with viral pneumonia in Wisconsin.[88] She apparently became infected while attending an agricultural fair, and died shortly after giving birth. Several health care workers developed influenza-symptoms after exposure.[21]

- In 1991, a healthy young laboratory animal caretaker in Maryland died of pneumonia caused by an H1N1 influenza virus.[83] He had close contact with pigs in a research facility. The virus appeared to be a reassortant, but all of the gene segments were of swine influenza virus origin. No one who had been in contact with the caretaker became ill, and only one person was seropositive.

- In 1993, an H1N1 swine influenza virus caused severe viral pneumonia in a 5-year-old child who lived on a pig farm in the Netherlands.[85]

- In 2004, an Asian H1N2 swine influenza virus was isolated in the Philippines from a 25-year-old man with symptoms of influenza including high fever, dizziness and occasional vomiting.[24] He recovered without complications. There was no evidence of person-to-person transmission.

- In 2005, an Asian H1N1 swine influenza virus was isolated from a 4-year-old boy in Thailand with rhinorrhea, fever and myalgia.[24] The child recovered without complications, and there was no evidence that the virus had infected others.

- In 2005, a recombinant swine influenza virus was recovered from a farm worker with influenza symptoms in Canada.[89] The virus, which was also found in sick pigs on the farm, was a triple reassortant H3N2 virus with genes from swine, human and avian influenza viruses. The infected individual was given antiviral drugs, and recovered uneventfully. Other workers on the farm were treated prophylactically and did not become ill.

- In 2007, an H3N2 swine influenza virus was isolated from an infant with respiratory disease in Canada.[90] The child was hospitalized but recovered. He had no direct contact with animals, but lived on a communal farm. Four of 7 household members and 4 of 46 other people on the farm had antibodies to this virus.

- In November 2008, a mild, self-limited case of H1N1 swine influenza was reported from a 50-year-old woman who worked on a swine farm in Spain.[86] This case was diagnosed only because the physician participated in an influenza surveillance program and collected a laboratory sample for virus identification.[87] The physician who treated her reported an influenza-like illness shortly afterward, but was not tested for the virus. No other potential cases were associated with this infection.

- Between 2005 and February 2009, 11 human infections with triple reassortant H1N1 swine influenza viruses were reported to the U.S. Centers for Disease Control and Prevention (CDC).[84] The symptoms included fever, coughing, sore throat, headache, diarrhea, vomiting, myalgia, shortness of breath and conjunctivitis. Two children were hospitalized for dehydration, but recovered without other complications. Two patients, a 26-year-old previously healthy woman and a 48-year-old woman with asthma and a history of smoking, experienced severe illness with pneumonia and respiratory failure, but recovered. Nine of the patients had a history of contact with pigs, and one case was thought to have been transmitted from person to person. One patient had three family members with suspected but unconfirmed swine influenza virus infections.

- A recent literature review summarized 49 cases of swine influenza that had been documented in scientific journals as of April 2006 (including many of the cases described above), and one additional case identified in an ongoing survey of swine influenza among farmers.[21] Thirteen of the cases were from the outbreak at Fort Dix; the other 37 were described as 'cases in civilians.' Twenty of the 37 civilian patients were previously healthy; others had immunosuppressive conditions including cancer and pregnancy. Four cases involved H3N2 viruses; the remainder were H1N1. All cases were described in the literature as upper respiratory disease, acute respiratory disease or pneumonia. Most patients recovered, but seven deaths were reported.

Equine and canine influenza virus infections in humans

Antibodies to equine H3N8 viruses have been reported in humans.[1] Human volunteers inoculated with an equine virus became ill, and virus could be isolated for up to 10 days.[1] There are no reports of clinical cases caused by natural exposure to equine influenza viruses or canine influenza viruses.

Communicability

Human influenza viruses are readily transmitted from person to person. Infected adults usually begin to shed influenza A viruses the day before the symptoms appear, and are infectious for 3-5 days after the initial signs.[190,192] Young children can shed virus for up to six days before, and 10 or more days after they become ill.[190,191] Severely immunocompromised individuals may remain infectious for weeks to months.[190,191] Humans have transmitted influenza viruses to ferrets and occasionally to swine.[1,3,153,185,187]

For the novel (2009 pandemic) H1N1 virus, the estimated period of communicability is from 1 day before the symptoms appear, to as long as 7 days after their

onset.[93,249] People may shed this virus for as long as they are ill, and in some cases, for 2-3 days after the fever has resolved.[93,272] Children and people who are immunocompromised might be infectious for longer.[249] One study presented at a recent conference found that viral nucleic acids could be detected by reverse transcription polymerase chain reaction (RT-PCR) assays in children for 1 to 13 days after they became febrile, and virus could be isolated for 1 to 7 days.[272] Atypical prolonged shedding up to 28 days (by PCR) has been reported in healthy adults with severe or relatively severe cases.[273] Humans can transmit the novel H1N1 virus to animals as well as people. Swine herds, turkeys, ferrets, felids and dogs have apparently been infected from human contacts.[103,104,108,110,113,117,119-129,252]

Rare cases of probable person-to-person transmission, and no cases of sustained transmission, have been reported in humans infected with avian influenza viruses.[11,35] Fecal shedding of the Asian lineage H5N1 virus has been documented in a child with diarrhea.[231] Transmission of this virus across the placenta may also be possible.[234]

Swine influenza viruses have typically been transmitted only to a few close contacts, at most.[21,22,82] There are two known outbreaks with more extensive spread. One was a localized outbreak among recruits infected with an H1N1 virus at a military base in Fort Dix, New Jersey.[1,2,22,93,94] Approximately 500 people on the base, which contained 12,000 people, were infected or exposed; however, the virus did not spread to the surrounding community.[1,2,22] The other is the 2009 H1N1 pandemic in humans.

Diagnostic Tests

Human influenza A and influenza B infections can be diagnosed by virus isolation or by the detection of antigens or nucleic acids. The viruses can be isolated in cell lines or chicken embryos, with identification by hemagglutination and neuraminidase inhibition tests or by RT-PCR. Antigens can be detected in respiratory secretions by immunofluorescence or enzyme-linked immunosorbent assays (ELISAs).[131,191] Commercial rapid diagnostic test kits can provide a diagnosis within 30 minutes.[191] RT-PCR techniques are also available.[190,191] Infections can also be diagnosed by serology; a rising titer must be seen. Serological tests include complement fixation, hemagglutination inhibition and immunodiffusion.[1,131,191] RT-PCR or culture can be used for the diagnosis of influenza C.[202]

Infections with the novel H1N1 virus can be confirmed by RT-PCR or virus isolation from respiratory secretions.[274,275] Samples should be collected as soon as possible after the onset of illness. The current immunofluorescence or rapid antigen tests for human influenza cannot distinguish other human influenza viruses from the novel H1N1 virus.[274,275] Serology is currently used mainly in epidemiology and research.[275]

Avian influenza viruses can be identified by RT-PCR, antigen detection or virus isolation from respiratory and throat swab samples.[11,40] RT-PCR is usually the primary test for infection with Asian lineage H5N1 viruses.[40] Virus isolation is done at World Health Organization (WHO) H5 Reference Laboratories.[40] In the U.S., samples that test positive by PCR or antigen tests are confirmed by the CDC. RT-PCR and antigen testing of avian influenza viruses must be carried out in Biosafety Level (BSL) 2 laboratory conditions.[11,40] Enhanced BSL 3+ laboratory conditions are needed for the isolation of H5N1 HPAI viruses.[11,40] Serology has been used for surveillance. The microneutralization assay is the most reliable test for detecting antibodies to avian influenza viruses.[38,40]

An influenza test that is positive for influenza A, but does not detect the hemagglutinins in common human influenza viruses suggests a novel, possibly zoonotic, influenza virus.[276]

Treatment

Supportive care for uncomplicated influenza in humans includes fluids and rest. More severe cases, or infections that have an elevated risk of complications, may be treated with antiviral drugs. Four drugs - amantadine, rimantadine, zanamivir and oseltamivir - are used to treat influenza.[131,190-192,277] Amantadine and rimantadine (adamantanes) are active against human influenza A viruses, if treatment is begun within the first 48 hours.[131,190-192,277] Zanamivir and oseltamivir are effective for both influenza A and influenza B.[179,277] Treatment usually results in milder symptoms and recovery, on average, one day sooner.[131,190,277] Side effects, including neuropsychiatric events, may occur.[191] Testing must be done to determine each individual virus's drug susceptibility. Drug resistance develops rapidly in viruses exposed to amantadine or rimantadine, and may emerge during treatment.[1,131,190] During the 2006-2008 flu seasons, human influenza viruses circulating in the U.S. and Canada exhibited high resistance to amantadine and rimantadine.[190,191,277] The CDC recommends that these two drugs be avoided until the circulating strains become susceptible again.[190,191,277] Laboratory studies have shown that influenza viruses can also become resistant to zanamivir and oseltamivir; however, this appears to be less common than resistance to adamantanes.[190,191,277] The novel (swine origin) H1N1 virus circulating among humans in 2009 is resistant to amantadine and rimantadine (adamantanes), but it is usually sensitive to oseltamivir and zanamivir.[261,278-280] Oseltamivir-resistant isolates of this virus have been reported sporadically, but they are currently uncommon.[167,278] Current recommendations for the treatment of infections with the novel H1N1 virus, including the use of antiviral drugs, are available on the CDC and WHO Web sites (see Internet Resources).[280,281]

Oseltamivir appears to increase the chance of survival in patients infected with Asian lineage H5N1 viruses, particularly if it is given early.[12,40,157,271,282] Further testing, particularly on the optimum dose and duration of treatment, is still needed.[12,40] These viruses are resistant to amantadine and rimantadine.[11] Although resistance to zanamivir and oseltamivir has also been reported in H5N1 viruses, it is currently uncommon.[11,40,283]

Prevention

Preventative measures for seasonal human influenza viruses

An annual vaccine is available for influenza A and B.[1,131,190] The vaccine is given in the fall before the flu season.[131] It contains the viral strains that are most likely to produce epidemics during the following winter, and it is updated annually. Details on vaccine efficacy, vaccine types, and recommendations for vaccination in specific population groups are available from the CDC.[190,191]

Three antiviral drugs - amantadine, rimantadine and oseltamivir - can be used for prophylaxis in high-risk populations such as the elderly or immunocompromised.[190,191,277] Due to the high resistance of currently circulating viruses to amantadine and rimantadine, the CDC recommends that these two drugs be avoided in the U.S. until the influenza strains become susceptible again.[190,191,277] Other preventative measures include the avoidance of contact with people with symptomatic disease, as well as hand washing and other hygiene measures.

To protect ferrets from infection with human influenza, people who are ill should avoid contact with these animals.[187] If contact is unavoidable, good hygiene and the use of face masks and/or other measures that prevent accidental droplet transmission from coughs and sneezes may be helpful. Avoidance of contact with swine should also be considered, as influenza viruses have been transmitted occasionally to or from this species, and recombination can occur between human and swine influenza viruses. Because infections with seasonal human influenza viruses cannot be distinguished clinically from infections with the novel (pandemic) H1N1 virus, anyone with an undiagnosed flu-like illness should also avoid unnecessary close contact with species susceptible to the latter virus.

Preventative measures for the novel H1N1 virus circulating in humans

Preventative measures are similar to those for seasonal human influenza, and include the avoidance of close contact (approximately 6 feet) with people who have flu-like illnesses, as well as frequent hand washing, the avoidance of unnecessary hand contact with the eyes, nose or mouth, and other common sense hygiene measures.[93,249,284] To protect others, the mouth and nose should be covered when coughing or sneezing.[93,249,284]

There appears to be little or no cross-reactivity with the H1N1 strains in the current seasonal human influenza vaccine,[285] but vaccines for the novel H1N1 virus became available in Fall 2009. Where limited quantities of these vaccines are available, specific risk groups may be targeted first for vaccination.[286] Antiviral drugs may be used for prophylaxis in some high risk populations after exposure.[287] In other cases, people may be monitored, and treated at the first sign of disease.[287] The CDC Web site has detailed information on the current recommendations.[287]

In areas where infections with the novel H1N1 virus are common, people at an increased risk for complications should consider avoiding crowded conditions or close contact with others.[288] The CDC currently recommends that anyone infected with the novel H1N1 virus and anyone who has an undiagnosed flu-like illness limit contact with others, and stay home except for necessities (for instance, seeking medical care).[249,289,290] The CDC has published specific guidelines for self-isolation and treatment, as well as recommendations for infection control measures in health care settings (see Internet Resources).[261,263,290,291] People who remain home should minimize contact with others in the household during their illness.[291] Face masks and respirators are no longer recommended in homes, communities or non-healthcare occupational settings, but they may be used voluntarily by individuals at risk for complications.[284] To prevent virus transmission to pigs, anyone who has a flu-like illness should avoid contact with this species. Care should also be taken to avoid spreading the virus to other animals, particularly turkeys, ferrets, cats (both housecats and other felines) and dogs.

Preventative measures for swine influenza viruses occurring in pigs

Good hygiene and sanitation, including frequent hand washing, can help prevent human infections with swine influenza viruses. Protective clothing, gloves and other personal protective equipment also reduce exposure.

There is no indication that any swine influenza virus can be acquired by eating well-cooked pork.[292,293] In pigs, swine influenza viruses replicate in the lungs and upper respiratory tract, and they are not ordinarily expected to occur outside these tissues (e.g., in meat).[211] Ordinary food safety precautions including hand washing before and after handling raw meat, the prevention of cross-contamination of foods or surfaces used for food preparation, and the use of hot soapy water to wash contaminated surfaces would be protective if any viruses survived long enough to reach consumers.[292] Influenza viruses are also killed by sanitizing cutting boards with 1 tbsp bleach in a gallon of water, and by cooking pork to an internal temperature of 160°F (71.1°C).[292]

Preventative measures for avian influenza viruses

Controlling avian influenza epidemics in poultry decreases the risk of exposure for humans.[12] People working with infected birds should follow good hygiene practices and wear appropriate protective clothing such as boots (or shoe covers), coveralls, gloves and respirators.[11] In addition, the World Health Organization recommends prophylaxis with antiviral drugs in people who cull birds infected with Asian lineage H5N1 HPAI viruses.[12] To prevent reassortment between human and avian influenza viruses, people in contact with infected birds should be vaccinated against human influenza.[12,20] They are also discouraged from having contact with sick birds while suffering flu symptoms.[20] H5N1 vaccines have also been developed.[11,294] In the U.S., these vaccines are stockpiled by the government and will be distributed by public health officials if they are needed.[11,294] Avian influenza vaccines for humans are not commercially available in the U.S.

In areas where H5N1 viruses might be present in domesticated poultry, poultry farms and live bird markets should be avoided.[11] Precautions should also be taken when handling raw meat and eggs. Sanitary precautions and cooking methods recommended to destroy *Salmonella* and other poultry pathogens are sufficient to kill avian influenza viruses.[11] The hands should be washed thoroughly with soap and warm water after handling poultry products.[11] Cutting boards and utensils should be washed with soap and hot water.[11] Poultry should be cooked to a temperature of at least 74°C (165ºF).[11] Eggs should be cooked until the whites and yolks are both firm.[11]

Avian influenza viruses can be carried in wild birds, and these birds could be the initial source of infection in an area. Wild birds should be observed from a distance; close contact is discouraged.[295] If birds or contaminated surfaces are touched, the hands should be washed with soap and water before eating, drinking, smoking, or rubbing the eyes.[295] Dead or diseased wildlife should be reported to state, tribal or federal natural resource agencies.[295] Hunters should not handle or eat sick game, and they should always wear rubber or latex gloves while handling and cleaning wild birds.[295] The hands, as well as equipment and surfaces, should be thoroughly washed after dressing the carcass.[295] All game should be cooked thoroughly.[295]

If an avian influenza pandemic occurs in humans, additional precautions will be necessary. During a pandemic, crowded conditions and close contact with other people should be avoided.[296] Respirators and other protective equipment may be advisable during close contact with an infected individual.[296] In addition, infection control measures such as good hygiene, cancellation of social events and voluntary quarantines of infected individuals can limit the spread of disease.[296,297]

Morbidity and Mortality

Seasonal human influenza

Although the morbidity rate for seasonal influenza is high, uncomplicated infections with human influenza viruses are rarely fatal in healthy individuals.[1,20,131,188,192] Infections are more severe in the elderly, young children (particularly infants), people with respiratory or cardiac disease, and those who are immunosuppressed.[131,190-192] Influenza-related deaths are usually the result of pneumonia or the exacerbation of a cardiopulmonary condition or other chronic disease.[191] Over 90% of these deaths occur in the elderly.[190] The estimated mortality rate from seasonal influenza is 0.0004 - 0.0006% in persons under 50 years old, 0.0075% between the ages of 50 and 64, and 0.1% in those over 65.[190,191] Deaths are rare in children, but can occur.[190,191] Immunity to the viral surface antigens (the hemagglutinin and neuraminidase) reduces the risk of infection and severity of disease. Antibodies offer limited or no protection against other virus types or subtypes.[190]

Human influenza can occur as a localized outbreak, an epidemic, a pandemic or as sporadic cases.[20] Although a new virus may spread among a population before the "flu season," epidemics in temperate regions usually do not begin until after school starts in the fall.[131] During a typical epidemic, influenza appears first among school-aged children, then spreads to preschool children and adults.[1,131] During epidemics, 15% to 40% of the population may be infected.[1,20] The outbreak usually lasts for three to six weeks.[1,131] Epidemics in tropical regions are not usually seasonal.[1]

Antigenic drift is usually responsible for small-scale epidemics and localized outbreaks.[2] In North America, an epidemic of influenza A usually occurs every 1- 3 years, and an epidemic of influenza B every 3-4 years.[192] Since 1968, the type A (H3N2) viruses have caused the most serious outbreaks with the highest mortality rates.[190,191] Pandemics, which last occurred in 1918, 1957, 1968 and 2009, are caused by antigenic shifts in influenza A viruses.[20,131] During influenza pandemics, the morbidity and mortality rates may increase dramatically in all age groups.[1,2,11,16,131,191] In the most severe pandemic, in 1918, the morbidity rate was 25-40% and the case fatality rate 2-5%.[20] Approximately 500,000 deaths were reported in the U.S. and an estimated 20-50 million deaths worldwide.[1,2,11,16,20,131] It should be noted that antiviral drugs and antibiotics were not available at the time, and intensive care procedures were less effective. After a pandemic, an influenza virus usually becomes established in the population and circulates for years.[11]

Less is known about influenza C than influenza A or B. Until recently, these viruses were thought to cause only sporadic cases of influenza and minor localized outbreaks.[1,131,192,201] However, in 2004, a nationwide influenza C epidemic was reported in Japan.[202] Influenza C infections seem to be most serious in very young

children. In one study, 30% of the children hospitalized with severe infections were less than two years old, and an additional 12% were between the ages of two and five.[259] Symptomatic influenza C infections are reported less often than illnesses caused by influenza A or B viruses;[256,257,259] however, serological studies suggest that a large percentage of the population is exposed to influenza C viruses in childhood.[298-301]

2009 pandemic: Novel H1N1 virus of swine origin

Morbidity and mortality information for the novel H1N1 virus are still preliminary. The initial outbreak with this virus occurred in Mexico in April.[93,95,96,205,302-304] This was followed by the identification of the virus among travelers in other countries, then by the recognition of sustained person-to-person transmission outside Mexico.[95,96,302-304] In June, a human pandemic was declared.[250] As of November 27 2009, more than 622,000 cases and 7,800 deaths attributed to this virus had been reported to the World Health Organization.[305] Because many countries no longer count or report individual cases, this underestimates the number of cases, particular those that are mild.[206,279,305]

Cases were reported in both the Northern and Southern Hemispheres during the initial stage of the outbreak. However, like other human influenza viruses, the novel H1N1 virus has been transmitted most widely during the traditional flu season, which begins in the autumn. Because the virus emerged in April, this occurred first in the Southern Hemisphere. During the flu season in the Southern Hemisphere, the reported hospitalization rates from various countries ranged from 2.0 to 31.8 per 100,000 population.[269] The mortality rate in the Southern Hemisphere was relatively low, with less than 1 death per 100,000 population in most countries; individual countries reported mortality rates from 0 to 36.1 per million population.[269] In Victoria, Australia, approximately 5% of the population is thought to have become ill, and 0.3% of those infected were hospitalized, with 20% of hospitalized patients transferred to an intensive care unit (ICU).[265] In Victoria, 85% of these critically ill patients survived.[265] In Taiwan, the mortality rate among 91 hospitalized patients was approximately 10%.[306] In New South Wales, Australia, the overall mortality rate from influenza was lower than in previous years, but severe illness was seen in some high risk groups.[269] Peru reported 8381 confirmed cases and 143 deaths, most (75%) in people who had other health issues.[269] A small number of H1N1 infections may be asymptomatic.[269] Transmission of the novel H1N1 virus appears to have declined normally after the flu season in the temperate regions of the Southern Hemisphere.[279] The autumn flu season in the Northern Hemisphere has been very active in the initial stages.[206,279,305,307] The impact of the novel H1N1 virus has been greater among indigenous people in both the Northern and Southern Hemispheres,

with hospitalization and mortality rates that were 3-7 times greater than in non-indigenous groups.[269]

Although the vast majority of cases have been mild and uncomplicated, viral pneumonia has been a significant concern with this virus.[167,260,264,306] Secondary bacterial infections have also contributed to some severe cases and deaths.[260,266] The risk of severe illness has been greatest in children under the age of 2 years (especially infants under a year of age), pregnant women, people with underlying health conditions such as chronic respiratory disease, some cardiovascular conditions or immunosuppression, and those who are obese.[167,260,262,264,265,267-270,279,284,306,307] HIV infection was linked to more severe illness in South Africa,[269] but data from other countries suggest that this is not necessarily the case among HIV-infected individuals who are receiving antiretroviral drugs.[167] Unusually, severe or fatal cases have also been reported among some young, previously healthy individuals, who are not ordinarily expected to be at high risk.[167,205,262,264,265,267,268,279,307] The reported percentage of hospitalized patients who have had no significant pre-existing conditions ranges from approximately 24% to 59%, depending on the country and the conditions that are defined as predisposing.[255,307,308] Some older people may have some immunity to the novel H1N1 virus,[309,310] and this group has had lower morbidity rates than expected, but they are more likely to have severe symptoms if they become ill.[167,249,260,264,269]

Zoonotic swine influenza

The overall prevalence of swine influenza virus infections in humans is unknown; however, serological evidence suggests that exposure may be relatively common among people who work with pigs.[1,2,23,240,246-248] Swine influenza infections have been reported among farm workers, laboratory workers, visitors at agricultural fairs or livestock shows, and a meat packer.[21] Infections not associated with swine contact have included instances of limited person-to-person transmission and some published cases with no known connection to swine.[21,22,84] Most sporadic cases of swine influenza have been relatively mild and some may have been asymptomatic, but some severe illnesses and a few deaths have been reported.[1,2,21,22,24,82-90] During the outbreak at the Fort Dix military base, one person died of pneumonia, at least twelve additional cases thought to be swine influenza were reported, other probable cases were suspected, and serological evidence of infection was found in approximately 500 of 12,000 people on the base.[1,2,21,22,244] One review reported that, of 37 other cases reported in the literature, six cases were fatal.[21] Four of these patients had primary viral pneumonia, one had secondary bacterial infection, and one had extensive involvement of the abdominal organs.[21] Two patients who died were described as previously healthy, one was pregnant, and two were immunosuppressed by cancer.[1,2,21,22,82,83] The health status of one person was not known. In a series of 11 infections with North American triple reassortant

H1N1 swine influenza viruses between 2005 and 2009, two children were hospitalized for dehydration, and severe illnesses were reported in a previously healthy 26-year-old woman and a 48-year-old woman with asthma and a history of smoking.[84] All of the patients in this study recovered.

Zoonotic avian influenza

The severity of avian influenza varies with the virus isolate. Particularly severe infections have been reported with Asian lineage H5N1 (HPAI) viruses.[11] Most patients infected with these viruses have been young and have had no predisposing conditions.[40] From 2003 through December 11 2009, 445 laboratory confirmed human H5N1 avian virus infections, 263 of them fatal, were reported to WHO.[43] The overall case fatality rate, as of December 11, was 59%.[11,43] Higher or lower case fatality rates have been reported in smaller series, varying with the country and the clade of the virus.[11,12,271,311-313] A few milder cases have been documented, particularly among children.[38,39] One H5N1 infection in a child with upper respiratory signs and an uncomplicated recovery after antibiotic treatment was recognized only by routine virus surveillance.[39] The prevalence of human infections with Asian lineage H5N1 viruses is unknown; however, asymptomatic infections seem to be rare.[38,41]

Human disease has also been reported occasionally after infection with various H7 viruses and H9N2 viruses.[11,26,33-38,74] The reported infections with H9N2 viruses have resembled human influenza, and they have not been fatal.[11,12,34,36-38,74] Most infections with H7 viruses have been limited to conjunctivitis, but influenza symptoms have also been seen. A single death was reported in an otherwise healthy veterinarian who became infected with an H7N7 virus.[35] Some isolates may also cause asymptomatic or mild, unrecognized infections. During an H7N3 LPAI outbreak in Italy in 2003, 3.8% of poultry workers tested developed antibodies to H7 viruses.[314] Interestingly, no seropositive individuals were identified in serum samples collected during H7N1 epidemics from 1999-2002.[314] In other studies, antibodies to H4, H5, H6, H7, H9, H10 and H11 avian influenza viruses have been found in poultry workers, veterinarians and waterfowl hunters.[240-242; Jia2009 /id; Cong2007 /id; Peiris2009 /id; Wang2009 /id] Whether these antibodies result from productive infections, exposure to antigens or cross-reactions with human influenza viruses remains to be determined.

Infections in Animals

Species Affected

Influenza A viruses

Influenza A viruses can cause disease in birds and many mammals including swine, horses, ferrets, dogs, cats, mink, pinnipeds and cetaceans. Influenza viruses circulate in some of these species; only individual cases or limited outbreaks have been reported in others.

Avian influenza viruses

Avian influenza viruses mainly infect birds, but some strains can also infect and/or cause disease in mammals.[1,9,11,16,25,26,44-53,55,57,58,61-63,65,67,69,194,196] Waterfowl and shorebirds, which tend to carry these viruses asymptomatically, appear to be the natural reservoir hosts.[1,2,12,13,16] Poultry can develop serious or mild disease, depending on the subtype and strain of the virus. A few isolates, such as the Asian lineage H5N1 viruses or an H5N3 virus isolated from terns in the 1960s, can also cause serious disease in other avian species including gulls, terns, wood ducks, farmed ostriches, emus and passerine birds.[51,71,136,138-142,315-318]

Host range of the Asian lineage H5N1 avian influenza viruses

Asian lineage H5N1 viruses can infect and/or cause disease in many species of birds in addition to poultry. Many H5N1 viruses have been isolated from birds in the order Anseriformes, particularly the families Anatidae (ducks, swans and geese) and Charadriiformes (shore birds, gulls and terns).[34,51,71,72,137,207,208,319] Symptomatic or fatal infections have also been reported in pheasants, partridges, quail, jungle fowl, guineafowl and peafowl (order Galliformes); egrets, storks and herons (order Ciconiiformes); pigeons (order Columbiformes); eagles, falcons, kites, kestrels, goshawks, and buzzards/ vultures (order Falconiformes); owls (order Strigiformes); crakes, cranes, moorhens, bustards, watercocks, coots and sultans (order Gruiformes); cormorants and pelicans (order Pelecaniformes), emus (order Struthioniformes), grebes (order Podicipediformes), budgerigars (order Psittaciformes), hornbills (order Coraciiformes) and flamingos (order Phoenicopteriformes).[51,54,60,71,73,136] Disease can also occur in passeriform birds; species that can be affected include finches, house sparrows (*Passer domesticus*), Eurasian tree sparrows (*Passer montanus*), mynahs, crows, ravens, jackdaws, Oriental magpie robins (*Copsychus saularis)*, munias, orioles, shrikes, starlings, mesias, red-billed leiothrix (*Leiothrix lutea*), Japanese white-eye (*Zosterops japonicus*) and magpies.[51,54,71,142,317] Asian lineage H5N1 viruses have also been found in a variety of birds that appeared healthy.[51,224] In a recent study from Thailand, there was no apparent difference in the prevalence of the H5N1 virus between waterfowl and other birds.[224]

Symptomatic infections with Asian lineage H5N1 viruses have also been reported in mammals including captive tigers (*Panthera tigris*), leopards (*Panthera pardus*), clouded leopards (*Neofelis nebulos*), lions (*Panthera leo*) and Asiatic golden cats (*Catopuma temminckii*), as well as housecats, a dog, stone martens (*Mustela foina*), a wild mink (*Mustela vison*), raccoon dogs and captive palm civets (*Chrotogale owstoni*).[9,11,44-55] Asymptomatic infections have been reported in some

housecats, and Asian lineage H5N1 viruses have been recovered from populations of apparently healthy wild pikas.[57,132] During outbreaks in poultry, serological evidence of infection or exposure has been reported in cats, dogs and swine, and viruses have been isolated rarely from pigs in China.[9,56,58,59] Unpublished research suggests that some raccoons in Japan also have antibodies to H5N1 viruses.[193] Experimental infections have been established in housecats, dogs, foxes, pigs, ferrets, rodents, cynomolgus macaques and rabbits.[34,51,58,60-70] Cattle can be experimentally infected with viruses isolated from cats.[70] The currently circulating H5N1 strains are continuing to evolve, and other species may also be susceptible to infection and/or disease.

Equine and canine influenza viruses

Equine influenza viruses mainly affect horses, donkeys and mules, but they can also occur in zebras.[25,168,169] Recently, infections with equine H3N8 viruses were reported among pigs in China.[175] Experimental infections have been established in cattle, dogs and humans, and antibodies to equine H3N8 viruses have been detected in dogs and humans.[1,1,32,170,174,174,215] Clinical cases have also been reported in dogs exposed to infected horses.[32,213]

Recently, an H3N8 equine influenza virus jumped into dogs, becoming the first canine influenza virus.[28,29,173] The new H3N8 canine influenza viruses have diverged genetically from the viruses found among horses,[30] and circulate only in dogs. To date, canine influenza virus infections have not been reported in other species, including humans.[29] An H3N2 virus has been reported in dogs in Korea.[133,184]

Swine influenza viruses

Swine influenza viruses mainly affect pigs, but they can also cause disease in turkeys.[1,3] Outbreaks have been described recently in ferrets and mink.[91,144] One H1N1 swine influenza virus, which was avirulent for both poultry and pigs, was isolated from a duck in Hong Kong.[146] Experimental infections have been reported in calves.[145]

Human influenza viruses

Human seasonal influenza A viruses mainly cause disease in people and ferrets.[185-188] They can also infect pigs, and have been reported in dogs, cattle and birds.[1,3,5,152,153] Experimental infections have been established in horses and raccoons.[1,170,189]

Novel H1N1 virus of swine origin

The novel (swine origin) H1N1 virus circulating in humans has infected pigs and turkeys.[100-118,125,320,321] A few clinical cases have also been reported in pet ferrets, cats, a cheetah in a zoo, and dogs.[119-124,126-129] Experimental infections have been established in ferrets, mice and cynomolgus macaques (*Macaca fascicularis*).[262,310,322] In one experiment, chickens exposed to infected pigs did not become infected.[323]

Influenza viruses in other species

In ferrets, clinical cases or outbreaks have been reported in animals infected with human influenza viruses,[185-188] the novel (swine origin) H1N1 virus,[119-122,262,310,322] and an H1N1 swine influenza virus.[91] Experimental infections with avian, swine and equine influenza viruses have also been established in this species.[165,194,196,232,238,324-327]

In 1984, an H10N4 virus was isolated from mink during an epidemic in Sweden.[1,9] This virus is thought to have been of avian origin. An H3N2 swine influenza virus caused a recent outbreak in mink.[144] Experimental infections with H1N1 and H3N2 human influenza viruses, H1N1 swine influenza virus, H3N8 equine influenza virus, and H3N8 and H4N6 avian influenza viruses have been established in mink, but the animals remained asymptomatic despite shedding virus.[9] Mink can also be infected with H5N3, H7N7, H8N4 and H11N4 viruses.[9]

Raccoons in the U.S. have serological evidence of infection with H1, H3, H4 and H10 viruses, and they can be infected experimentally with avian LPAI H4N8 viruses and human H3N2 viruses.[189] Unpublished research suggests that some raccoons in Japan have antibodies to H5N1 viruses.[193]

Influenza A viruses can infect pinnipeds and cetaceans. H3N3, H7N7, H4N5 and H4N6 viruses, closely related to avian viruses, have been isolated from seals.[1,9] Antibodies to H1, H3, H4, H6, H7, H8 and H12 viruses have also been found in these animals.[9,328] Influenza A infections have been reported sporadically in cetaceans, and H1N3, H13N2 and H13N9 viruses have been isolated from whales.[1,9] Antibodies to influenza A viruses have been reported in sea lions and porpoises.[9]

Serological evidence of infection with influenza A viruses has also been reported from some other mammals including cattle, yak, sheep, goats, reindeer and deer.[9] Human influenza viruses have been isolated from some of these species, and an H3N2 virus isolated from cattle caused an illness resembling influenza in calves.[9] Antibodies to influenza A viruses have been reported in reptiles and amphibians including snakes, crocodiles, alligators, caimans, toads and frogs, and influenza A viruses have been detected by RT-PCR in caimans, alligators and crocodiles.[9] There is evidence that some of these viruses were avian, human and equine influenza viruses.[9]

Influenza B viruses

Influenza B viruses can cause disease in humans, ferrets and seals, and these viruses have also been isolated from pigs and a horse.[1,2,4,197] Serological evidence of infection has been found in pigs, dogs, horses and seals.[1,5,328] Serological studies from the U.K. suggest that influenza B infections in swine are sporadic and do not spread to other pigs.[5]

Influenza C viruses

Influenza C viruses have been isolated from humans and swine.[1-4,6,201] These viruses can cause disease in experimentally infected dogs.[1] Serological evidence of infection has been found in pigs, dogs and horses.[1,5,7,8]

Incubation Period

In poultry, the incubation period can be a few hours to a week.[3,13,14] A 21-day incubation period, which takes into account the transmission dynamics of the virus, is used for an avian population in the context of disease control.[13] The incubation period for mammalian influenza viruses is also short. The clinical signs usually appear within 1-3 days in horses, pigs or seals,[1,3,4,25,147,168,170,253,323,329] although incubation periods up to 5 days have been reported in some horses.[330] The incubation period for H3N8 canine influenza can be two to five days, but most cases appear in 2-3 days.[178,213] Little is known about H3N2 influenza virus in dogs; however, fever first appeared at 24 hours in experimentally infected dogs, and other clinical signs began 2-8 days after inoculation.[184]

Clinical Signs

Avian influenza

HPAI viruses usually cause severe disease in poultry. These viruses can cause serious infections in some species of birds on a farm while leaving others unaffected.[1,13] The clinical signs are variable.[3,13,18,19] Sudden death of large numbers of birds is a common presentation.[13] Systemic signs, and in some cases, respiratory signs, may be noted in chickens, turkeys and other gallinaceous birds. The birds can be markedly depressed, with decreased feed and water consumption, and ruffled feathers.[13] Sinusitis, lacrimation, edema of the head, cyanosis of the head, comb and wattle, and green to white diarrhea may also be seen.[3,13-15,19] In addition, there can be coughing, sneezing, blood-tinged oral and nasal discharges, ecchymoses on the shanks and feet, neurological disease, decreased egg production, loss of egg pigmentation and deformed or shell-less eggs.[1,3,13-15] However, none of these signs is pathognomonic, and sudden death may occur with few other signs.[19] Most of the flock usually dies.[13] Because a virus can be defined as HPAI based on its genetic composition, it is also possible for an HPAI virus to be isolated from gallinaceous birds showing mild signs consistent with LPAI.[331]

LPAI viruses usually cause subclinical infections or mild illness in poultry.[13,18] Decreased egg production, misshapen eggs, decreased fertility or hatchability of the eggs, respiratory signs, lethargy, decreased feed and water consumption, or somewhat increased flock mortality rates may be seen.[11,13,14,332-342] More severe disease, mimicking high pathogenicity avian influenza, can occur if the birds are concurrently infected with other viruses or there are other exacerbating factors.[18,19]

Clinical signs tend to be minimal in ducks and geese infected with avian influenza viruses, including most HPAI viruses. In ducks, the most common signs are sinusitis, diarrhea and increased mortality.[3,10,72] However, some recent H5N1 isolates have caused severe acute disease with neurological signs and high mortality rates in domesticated ducks.[13,60,71-73,136,219,343] There are few descriptions of the clinical signs in other domesticated birds. Ostriches that were experimentally infected with an HPAI H7N1 virus developed mild depression and hemorrhagic diarrhea.[140] Green diarrhea was the only sign of illness in ostriches inoculated with an LPAI virus of the same subtype.[140] High mortality is occasionally seen in young ostriches infected with either LPAI or HPAI viruses.[140]

Avian influenza is often subclinical in wild birds, but some strains can cause illness and death.[1,11-13,17,54,136,137,319] Strains known to cause fatal illness include some of the currently circulating Asian lineage H5N1 viruses.[12,54,72,73,136,137] Some captive wild birds infected with these viruses have died suddenly, within a few hours, without apparent clinical signs.[54] In other cases, anorexia, extreme lethargy, dark green diarrhea, respiratory distress and/or neurological signs were seen, with death within 1-2 days.[54] Swans have been severely affected by H5N1 viruses; these birds are generally found dead.[137,319] Experimental infections with H5N1 viruses resulted in severe neurological disease in some mute swans and sudden death in others, while some birds shed virus subclinically.[344] Diving ducks, grebes and mergansers also seem to be highly susceptible to these viruses.[143] Experimental infections with H5N1 viruses in call ducks (*Anas platyrhyncha* var. *domestica*; a cross between wild and domesticated ducks) or wood ducks (*Aix sponsa*) resulted in drowsiness, severe weakness and neurological signs, but some indigenous North American ducks including mallards (*Anas platyrhynchos*), northern pintails (*Anas acuta*), blue-winged teals (*Anas crecca*) and redheads (*Aythya americana*) remained asymptomatic when inoculated with one of these strains.[136,343]

Symptomatic infections with H5N1 viruses have also been reported in experimentally infected gulls and passerine or psittacine birds.[60,142,317] Laughing gulls (*Larus atricilla*) developed severe neurological disease; the clinical signs included weakness, cloudy eyes, ruffled feathers, incoordination and torticollis.[136] Most infected gulls died. One gull that recovered had a persistent head tilt; another recovered completely. Anorexia and depression occurred in experimentally infected zebra finches, and all of the birds died within five days of inoculation.[142] House finches and budgerigars developed anorexia, depression and neurological signs, and died rapidly.[142] In one study, H5N1 infections were mild in house sparrows, which experienced only mild depression and survived, and starlings, which remained asymptomatic.[142] In another study, house sparrows but not starlings had severe, often fatal infections.[317]

Other subtypes can also be pathogenic to some wild birds. An H7N1 (HPAI) virus caused conjunctivitis, apathy and anorexia, with a high mortality rate, in canaries and a siskin[141] and an H5N3 HPAI virus caused an outbreak with a high mortality rate among South African terns in the 1960s.[318]

Other influenza viruses in birds

Turkeys infected with swine influenza viruses may develop respiratory disease, have decreased egg production, or produce abnormal eggs.[3]

Avian H5N1 influenza in mammals

Both symptomatic and subclinical Asian lineage H5N1 virus infections have been seen in felids. Although fatal infections have been reported in some housecats,[47,48,53] little is known about the clinical signs after natural exposure in this species. One cat had fever, depression, dyspnea, convulsions and ataxia,[48] and a few infected housecats were found dead.[47] One of the latter cats was apparently well up to 24 hours before its death.[47] In contrast, asymptomatic infections were reported in housecats that had been accidentally exposed to a sick, H5N1-infected swan.[132] In experimentally infected housecats, the clinical signs included fever, lethargy, conjunctivitis, protrusion of the third eyelid, dyspnea and death.[63,65,67] Fatal infections have also been reported in some captive tigers and leopards.[45,46,50] Some of these animals exhibited respiratory distress, serosanguineous nasal discharge, high fever and neurological signs before death.[9,46] During an outbreak in Cambodia, captive lions, tigers, leopards and Asiatic golden cats were lethargic and had decreased appetites without respiratory signs for 5-7 days, but recovered.[54]

Other mammals may also be affected by Asian lineage H5N1 viruses. A dog that ate infected poultry developed a high fever, with panting and lethargy, and died the following day.[49] Experimentally infected dogs have been asymptomatic or developed only transient fever and conjunctivitis.[11,67,68] Fatal respiratory disease was reported in infected raccoon dogs.[55] Other raccoon dogs on the same farm had died with respiratory signs and/or diarrhea before the virus was found.[55] Captive palm civets had neurological signs, with evidence of interstitial pneumonia, encephalitis and hepatitis at necropsy.[9] Some infections in palm civets were fatal.[9] A wild stone marten was also found with neurological signs.[9] HPAI H5N1 viruses have been isolated from wild pikas; however, there was no evidence that the pika population was seriously affected.[57]

Asian lineage H5N1 infections in pigs appear to be mild or asymptomatic. Mild respiratory signs including cough, fever and transient anorexia were observed in some experimentally infected pigs.[58] In another study, some Asian lineage H5N1 strains caused slight and transient weight loss, but other clinical signs were not seen, and lung lesions were much less severe than those

caused by swine influenza viruses.[66] One group reported that miniature pigs were resistant to infection.[60]

Experimental infections have been established in foxes, ferrets, mice and cattle, although no naturally infected animals have been reported. Some infected foxes developed fever but no other clinical signs; however, lung lesions were reported at necropsy.[69] In ferrets, the syndromes ranged from very mild upper respiratory infections to severe, fatal disease; the pathogenicity varied with the specific isolate and the route of inoculation (intranasal or intragastric).[61,66] The clinical signs in severe cases included high fever, extreme lethargy, anorexia, weight loss, respiratory disease, diarrhea and neurological signs.[61,66] Similarly, infections in mice varied with the isolate and the route of inoculation (respiratory or intragastric).[229] Cattle inoculated with high titers of H5N1 viruses isolated from infected cats remained asymptomatic but could transiently shed virus.[70]

Equine influenza

Equine influenza usually spreads rapidly in a group of animals. In naïve horses, the first sign is usually a high fever, followed by a deep, dry cough.[25,168,169,253,330] Other clinical signs may include a serous to mucopurulent nasal discharge, myalgia, inappetence, photophobia, corneal opacity and enlarged submandibular lymph nodes.[1,3,25,168,253,330] There may be edema of the legs and scrotum, and enteritis (spasmodic impaction colic) has been reported in some epidemics.[25,253] Animals with partial immunity can have milder, atypical infections with little or no coughing or fever.[25,330] Equine influenza is sometimes complicated by secondary bacterial infections.[168,169,330]

Healthy adult horses usually recover within 1-3 weeks, but the cough may persist longer.[1,25,168,253] In severely affected animals, convalescence can take up to six months.[168] Secondary bacterial infections prolong recovery.[3,25,168] Death in adult horses usually results from bacterial pneumonia, pleuritis or purpura hemorrhagica.[25] Sequelae may include chronic pharyngitis, chronic bronchiolitis and emphysema.[25,168,253] Interstitial myocarditis can occur during or after the infection.[1,253] Loss of eyesight has also been reported.[253] Young foals without maternal antibodies can develop rapidly fatal viral pneumonia.[1,25,253,330] Postinfection encephalopathy has also been reported in foals.[253]

Other influenza viruses in horses

Horses experimentally infected with human influenza virus (H3N2 'Hong Kong') developed a mild febrile illness.[1] The virus could be isolated for up to five days.

Canine influenza (H3N8)

Canine influenza is an emerging disease in dogs. The most common presentation seen with H3N8 viruses is relatively mild and resembles kennel cough.[28,176,177,179,213] In this form, an initial (usually low grade) fever is followed by a persistent cough and sometimes a purulent

nasal discharge.[28,30,178,213] The clinical signs can last for up to a month regardless of treatment.[30,178] The nasal discharge appears to resolve with antibiotics, suggesting that secondary bacterial infections may be important in this disease.[178] More severely affected dogs exhibit a high fever with an increased respiratory rate and other signs of pneumonia or bronchopneumonia.[30,173,177,179,213] Lethargy and anorexia are common.[213] Some dogs have been found dead peracutely with evidence of hemorrhages in the respiratory tract; this syndrome has been seen in racing greyhounds, but does not seem to be prominent in pets.[28,213] Asymptomatic seroconversion also occurs.[28]

Other influenza viruses in dogs

In the U.K., an H3N8 equine influenza (American lineage) virus caused a limited outbreak among foxhounds in 2002.[32] The disease, which was diagnosed as bronchointerstitial pneumonia, was characterized by coughing, lethargy and weakness, sometimes progressing to loss of consciousness.[32] One dog died and several were euthanized.[32] Asymptomatic infections were reported in an experimental study with a Japanese H3N8 (Florida sublineage) isolate, which was used to infect dogs via close contact with horses.[174]

The only known outbreak of H3N2 canine influenza was characterized by severe respiratory disease with fever, nasal discharge, sneezing, coughing and anorexia.[133] Four of five pet dogs seen at veterinary clinics died.[133] Fever, sneezing, coughing and nasal discharges occurred in experimentally inoculated dogs, and severe pathological changes were seen in the lungs.[133,184]

The clinical signs in dogs experimentally infected with influenza C virus included nasal discharge and conjunctivitis, which persisted for 10 days.[1]

Swine influenza

Swine influenza is an acute upper respiratory disease characterized by a variety of clinical signs, which may include fever, lethargy, anorexia, weight loss and labored breathing.[1-4,147,211] Coughing can occur in the later stages of the disease.[2,211] Sneezing, nasal discharge, conjunctivitis and/or abortions may also be seen.[2,4,147] Some outbreaks are more severe than others, and swine influenza viruses can circulate in pigs with few or no clinical signs.[1,2,16,149] Complications may include secondary bacterial or viral infections.[2,4,147] Severe, potentially fatal bronchopneumonia is occasionally seen.[3]

H9N2 influenza viruses in pigs

An avian H9N2 virus caused respiratory disease and paralysis in pigs in southeastern China.[74]

Novel H1N1 virus of swine origin in mammals

A number of swine herds have been infected with the novel H1N1 virus circulating in humans.[100-111,114-116,321] The illness has been mild, with little or no mortality, and the clinical signs have resembled those caused by other swine influenza viruses.[100,103,105,108,110,113-116,321,345] Coughing, nasal discharge, fever, weakness and decreased appetite have been reported.[103,107,108,111,113,114,252] Abortions or diarrhea have also been seen in some herds.[103,111] Experimentally infected pigs developed mild disease, with nasal discharge, sneezing and fever as the most prominent signs.[323,346] Diarrhea was reported in some experimentally infected animals.[323] In one study, miniature pigs remained asymptomatic although they shed the virus.[310]

Infected turkey flocks reported in Chile and Canada experienced only decreased egg production and reduced quality of the eggs, with no mortality or other clinical signs.[117,118] Decreased egg production was also reported in a turkey flock in the U.S.[125]

Upper or lower respiratory signs including sneezing and coughing in some cases, and pneumonia in others, have been described in cats.[123,127,129] Some cats with respiratory disease did not have a fever.[123] Although some animals apparently had milder cases, one cat became dyspneic and severely ill but recovered with medical care, and two cats died.[123,129] The illness lasted for several weeks in some animals.[127] An infected cheetah developed lethargy, anorexia and a cough, but recovered.[129] There is little information on cases in dogs, but the novel H1N1 virus was isolated from sick animals in China,[124] and a dog in the U.S. was ill with clinical signs of lethargy, anorexia, fever and coughing, and radiological evidence of pneumonia.[128] The dog in the U.S. was hospitalized and treated with supportive care including antibiotics, and recovered.[128]

Respiratory disease has been reported in naturally infected ferrets; the clinical signs included fever, coughing, sneezing, nasal discharge and weakness.[119,122] Several ferrets recovered, but one died.[119,120,122] In experimentally infected ferrets, lethargy, decreased appetite, sneezing, nasal discharge and ruffled fur were reported in one study,[262] and lethargy and weight loss, with little sneezing, in another.[322]

Influenza viruses in ferrets

Ferrets are susceptible to human influenza viruses. The clinical signs may include fever, anorexia, depression, listlessness, sneezing, purulent nasal discharge and coughing.[185,186,188] The infection is not usually fatal in adult animals, which generally recover in five days to two weeks.[185,187,188] More severe or fatal disease can be seen in neonates.[188] Infections with the novel 2009 H1N1 (swine origin) virus have also been reported after contact with humans. The clinical signs (see previous section) appeared to be similar to those caused by human seasonal influenza viruses, but one death was reported.[119,120,122]

A single outbreak caused by a swine influenza virus has been reported in ferrets.[91] The virus was a triple reassortant H1N1 swine influenza virus, and the clinical signs included sneezing, coughing, crusting of the nose and eyes, and severe dyspnea.[91] Some severely affected animals died.

Natural infections with avian influenza viruses have not been documented, but ferrets have been infected experimentally with a few of these viruses.[61,66,196,324,326] In one study, ferrets inoculated with influenza viruses from various species, including birds, developed rhinitis, with sneezing and shivering, but did not have an elevated temperature.[324] Animals inoculated with avian H7 (LPAI and HPAI) viruses from recent outbreaks developed illness of varying severity.[196] Although most viruses caused relatively mild illness with fever, transient weight loss and respiratory signs, the inoculation of an HPAI H7 virus from a fatal case in a Dutch veterinarian resulted in severe disease with fever, lethargy, anorexia, severe weight loss, nasal discharge, diarrhea, dyspnea and neurological signs.[196] In another experiment, ferrets infected with an H7N3 (LPAI) virus had only a transient elevation in temperature, and developed no other clinical signs.[326] Experimental Asian lineage H5N1 virus infections in ferrets ranged from very mild upper respiratory infections to severe, fatal disease; the pathogenicity varied with the specific isolate and the route of inoculation (intranasal or intragastric).[61,66] The clinical signs in severe cases included high fever, extreme lethargy, anorexia, weight loss, respiratory signs, diarrhea and neurological signs.[61,66] Some of these infections were fatal.[61,66]

Influenza in mink

In 1984, an H10N4 avian influenza virus caused an epidemic on 33 mink farms in Sweden.[1,9] The clinical signs included anorexia, sneezing, coughing, and nasal and ocular discharges, and many mink died. Mink naturally infected with a Canadian H3N2 swine influenza virus had respiratory signs including pneumonia, with increased mortality particularly on ranches where the mink were co-infected with other pathogens.[144]

Influenza in raccoons

Serological evidence of infection with H1, H3, H4 and H10 viruses has been reported in wild raccoons, but whether clinical signs occur is unknown.[189] Raccoons that were experimentally infected with avian LPAI H4N8 or human H3N2 viruses shed these viruses but remained asymptomatic.[189] According to yet unpublished research, antibodies to H5N1 viruses have also been found among raccoons in Japan.[193]

Influenza in marine mammals

Influenza A viruses have been associated with outbreaks of pneumonia in seals and disease in a pilot whale.[1,26,329] The viruses appeared to be of avian origin.[26] Clinical signs in seals included weakness, incoordination, dyspnea and subcutaneous emphysema of the neck.[9,329] A white or bloody nasal discharge was seen in some animals. Experimental infections with these viruses were milder or asymptomatic, suggesting that co-infections may have increased the severity of the clinical signs.[9] In the single known case in a whale, the signs were nonspecific and included extreme emaciation, difficulty maneuvering and sloughing skin.[329] Influenza B infections have been reported in some stranded seals.[9]

Communicability

Influenza viruses are readily transmitted between animals in the species to which they are adapted. Chickens can begin shedding avian influenza viruses as soon as 1-2 days after infection.[222] Most chickens shed LPAI influenza viruses for only a week, but a minority of the flock can excrete the virus in feces for up to two weeks, and shedding for as long as 36 days has been reported in experimentally infected birds.[226,227] Turkeys may excrete some avian influenza viruses for up to 72 days.[226] Waterfowl are often infected subclinically, and ducks can shed these viruses for up to 30 days.[2,226] Pigs may begin excreting swine influenza viruses within 24 hours of infection, and typically shed the viruses for 7-10 days.[16,94,147] Shedding up to four months has been documented in one pig.[16,94] Horses begin excreting equine influenza viruses during the incubation period, and usually shed these viruses for 4-5 days or less after the onset of clinical signs.[3,25,253]

Animals that have been infected with influenza viruses from other species may or may not transmit the virus. Limited animal-to-animal transmission seems to occur, under some conditions, with the currently circulating Asian lineage H5N1 viruses. Cats can shed these viruses from the intestinal tract as well as the respiratory tract.[53,65,67] Experimentally infected cats shed Asian lineage H5N1 viruses by the third day post-inoculation, and were able to infect sentinel cats in close contact.[63,65] In contrast, naturally infected, asymptomatic cats appeared to shed Asian lineage H5N1 viruses only sporadically, and for less than two weeks.[132] Horizontal transmission was not observed in this instance.[132] Limited animal-to-animal transmission was reported among tigers in a zoo.[50] Horizontal transmission of Asian lineage H5N1 viruses has not been reported inexperimentally infected dogs, pigs, foxes or cattle; however, virus shedding has been described.[58,66-69] In experimentally infected dogs and pigs, H5N1 viruses have been detected only in respiratory secretions,[66-68] but in experimentally infected foxes, these viruses were found in both respiratory secretions and feces.[69] As of December 2009, sustained or prolonged transmission of Asian lineage H5N1 viruses has not been reported in any of these mammals. However, the recent isolation of H5N1 viruses from pikas suggests that these viruses may be maintained in this population.[57]

A recent study suggests that raccoons may be able to transmit some influenza viruses. Raccoons that were experimentally infected with an avian LPAI H4N8 virus shed this virus in respiratory secretions but not from the digestive tract, and could infect other raccoons in contact.[189] Raccoons that were inoculated with a human H3N2 virus shed virus mainly from respiratory secretions, but minimal intestinal shedding was also reported.[189] The H3N2 virus was not transmitted to uninfected raccoons.[189]

Post Mortem Lesions

High pathogenicity avian influenza

The lesions in chickens and turkeys are highly variable and resemble those found in other systemic avian diseases.[10,347] Birds that die peracutely and young birds may have few or no lesions.[13,14,347] In other cases, the sinuses may be swollen, and the comb and wattle are often edematous, hemorrhagic, congested and/or cyanotic.[13,14,347] There may be subcutaneous edema on the head and neck, edema and diffuse subcutaneous hemorrhages on the feet and shanks, fluid (which may contain blood) in the nares and oral cavity, and congestion, swelling and hemorrhages of the conjunctivae.[13,14] Hemorrhagic tracheitis can be seen in some birds; in others, the tracheal lesions may be limited to excess mucoid exudate.[13] The lungs may be reddened from hemorrhages and congestion, and they may exude fluid when cut.[13] Petechiae may be noted throughout the abdominal fat, on serosal surfaces and on the peritoneum, and they can sometimes be found in the muscles.[13,14,347] Hemorrhages may also be seen on the mucosa and in the glands of the proventriculus, beneath the lining of the gizzard, and in the intestinal mucosa.[13] The kidneys can be severely congested and are sometimes plugged with urate deposits.[13] The ovaries may be hemorrhagic or degenerated, with areas of necrosis. The peritoneal cavity often contains yolk from ruptured ova, which may cause severe airsacculitis and peritonitis.[13] A study of the 2003 H7N7 outbreak in the Netherlands suggested that the occurrence of peritonitis, tracheitis, edema of the wattles and/or neck, or petechial hemorrhages in the proventriculus may be particularly suggestive of an HPAI infection, especially when there is acute high morbidity in the flock.[347]

Postmortem lesions have occasionally been described in wild birds infected with Asian lineage H5N1 viruses. Experimentally infected wood ducks had multiple petechial hemorrhages in the pancreas.[136] More extensive lesions were reported in experimentally infected laughing gulls; in these birds, petechial hemorrhages were found in the ventriculus, apex of the heart, cerebrum and pancreas.[136] In naturally infected swans, one study reported that the most consistent lesions were multifocal hemorrhagic necrosis in the pancreas, subepicardial hemorrhages, and pulmonary congestion and edema.[319] Pancreatic lesions alone or no gross lesions have also been seen in some swans.[348] Mild or absent gross lesions were reported in experimentally infected zebra finches, house finches and budgerigars despite high mortality rates in these species.[142]

Low pathogenicity avian influenza

Rhinitis and catarrhal to purulent sinusitis are often seen in young birds infected with LPAI viruses.[13] Congestion and inflammation may also occur in the trachea, and less frequently, the lungs.[13,14] Lower respiratory tract lesions such as pneumonia are usually seen only in birds with secondary bacterial infections.[13] The ovary can be hemorrhagic in laying hens, with involuted and degenerated ova.[13] Yolk may be present in the abdominal cavity, and can cause severe air sacculitis and peritonitis.[13] The oviduct may also be edematous, with exudates in the lumen.[13,14] Some birds may have signs of acute renal failure and visceral urate deposition.[14]

Avian H5N1 influenza viruses in mammals

Pulmonary edema; pneumonia; conjunctivitis; cerebral, renal and splenic congestion; multifocal hepatic necrosis; hemorrhages in the intestinal serosa, lymph nodes, perirenal tissue and/or diaphragm; and severe hemorrhagic pancreatitis have been reported in naturally infected cats. [47,48,53] The lungs were also affected in experimentally infected cats, with multiple to coalescing foci of pulmonary consolidation.[63,65] These lesions were similar whether the cats were infected intratracheally or by the ingestion of infected chicks. In one study, cats infected by ingestion also had enlarged tonsils with multifocal petechial hemorrhages, as well as enlarged mandibular and/ or retropharyngeal lymph nodes.[65] Petechial hemorrhages occurred in the liver of some cats, and in one cat, the liver lesions were accompanied by generalized icterus.[65] In naturally infected tigers and leopards, the gross lesions included severe pulmonary consolidation and multifocal hemorrhages in multiple organs including the lung, heart, thymus, stomach, intestines, liver and lymph nodes.[46]

Bloody nasal discharge, severe pulmonary congestion and edema, and congestion of the spleen, kidney and liver were reported in a naturally infected dog.[49] Pulmonary lesions including interstitial pneumonia have been noted in some experimentally infected pigs.[58] In one study, Asian lineage H5N1-infected pigs had mild to minimal gross lung lesions, with mild to moderate bronchiolitis and alveolitis detected on histopathologic examination.[66] Experimentally infected foxes also developed lesions mainly in the lung. [69] More severe lesions were seen in foxes inoculated intratracheally than in animals fed infected birds, and some of these animals also had histopathologic evidence of encephalitis and myocarditis. [69]

Swine influenza

In uncomplicated infections, the gross lesions are mainly those of a viral pneumonia.[2] Affected parts of the lungs are depressed and consolidated, dark red to purple-red, and sharply demarcated.[2,4] Lesions may be found throughout the lungs, but they are usually more extensive in the ventral regions.[2,4] Other parts of the lungs may be pale and emphysematous.[4] The airways are often dilated and filled with mucopurulent exudate.[4] The bronchial and mediastinal lymph nodes are typically edematous but not congested.[2,4] Severe pulmonary edema, as well as serous or serofibrinous pleuritis, may also be seen.[4] Some strains of swine influenza viruses produce more marked lesions

Swine influenza can be diagnosed by virus isolation, the detection of viral antigens or nucleic acids, and serology. Mammalian influenza viruses can be isolated in embryonated chicken eggs or cell cultures.[2,147] Swine influenza viruses are often recovered in Madin–Darby canine kidney cells, but other cell types can also be used.[147] These viruses can be isolated from lung tissues at necropsy, and from nasal or pharyngeal swabs taken from acutely ill pigs.[3,4,147] Recovery is best from an animal with a fever, 24-48 hours after the onset of illness.[147] Isolated viruses can be subtyped with hemagglutination inhibition and neuraminidase inhibition tests or RT-PCR.[2,147]

Immunofluorescent techniques can detect antigens in fresh lung tissue, nasal epithelial cells or bronchoalveolar lavage.[2,147] Other antigen detection tests include immunohistochemistry on fixed tissue samples, and ELISAs.[2,147] RT-PCR assays are used to detect viral RNA.[2,147]

Serology on paired samples can diagnose swine influenza retrospectively.[4] The hemagglutination inhibition test, which is subtype specific, is most often used.[2,4,147] It may not detect new viruses.[2] ELISA kits are available. Uncommonly used serological tests in swine include agar gel immunodiffusion, the indirect fluorescent antibody test and virus neutralization.[147]

Equine influenza

Equine influenza may tentatively be diagnosed based on the clinical signs.[168] As in swine, the disease is confirmed by virus isolation, the detection of viral antigens (e.g., by ELISA), or the detection of nucleic acids by RT-PCR.[25,168,169] Equine influenza viruses can be isolated from nasopharyngeal swabs or nasal and tracheal washes. In horses, peak virus shedding is thought to occur during the first 24 to 48 hours of fever; whenever possible, samples should be collected within the first 3-5 days after the onset of clinical signs.[25,169] Ideally, virus recovery should be attempted in both embryonated eggs and cell cultures.[169] Equine influenza can also be diagnosed retrospectively by serology, using paired serum samples.[25,168,169] The most commonly used serological tests in horses are the hemagglutination inhibition test and a single-radial hemolysis (SRH) test.[25,169]

Canine influenza

At this time, serology and RT-PCR are the most reliable methods for detecting H3N8 canine influenza.[177,213,350] Hemagglutination inhibition is the most commonly used serological test.[213] Virus neutralization (microneutralization test) can also be done, but this test is usually too cumbersome for routine use.[213] Acute and convalescent titers should be submitted if possible.[213,350] Because canine influenza is an emerging disease, most dogs are not expected to have pre-existing titers to the canine influenza virus; however, single titers are still considered to be less useful.[213,350]

RT-PCR is the most reliable method to detect the virus directly.[213] This test can be used on nasal swabs from live animals or lung tissue samples at necropsy.[213,350] Virus isolation may also be successful in some dogs, but only during the early stages of disease before antibodies develop.[213] The H3N8 canine influenza virus can be found in lung tissue samples taken post-mortem, but virus isolation fails to detect the virus in many infected dogs that do not die of the disease.[28,177,213,350] In experimental infections, nasal swabs have been more likely to yield virus than nasopharyngeal swabs.[213,350] The H3N8 canine influenza virus has been isolated in both embryonated eggs and cell cultures (MDCK cells)[213]. Antigen-capture ELISA tests do not seem to be reliable in individual dogs, probably because the amount of virus shed is low.[213] However, these tests may be able to detect H3N8 canine influenza during outbreaks at kennels or other large facilities.[213]

Little is known about testing for Korean H3N2 viruses in dogs. Some H3N2 viruses were isolated from nasal swabs taken from dogs during an outbreak.[133] In experimentally infected dogs, these viruses were shed in nasal secretions from one to six days after inoculation.[133] RT-PCR can also detect this virus.[133] Serology is expected to be useful.

Treatment

Animals with influenza are usually treated with supportive care and rest.[4,14,25,168] In horses, prolonged recovery and more severe disease have been associated with increased stress.[253] Antibiotics may be used to control secondary bacterial infections; they seem to be particularly important in the treatment of canine influenza.[4,14,25,168,179] Antiviral drugs are not generally given to most animals; however, ferrets have been treated with amantadine as well as antihistamines, antibiotics and other supportive therapy.[185] Antiviral drugs could also be of use in valuable horses.[10,351]

Poultry flocks infected with HPAI viruses are depopulated and are not treated.[3,11]

Prevention

Vaccines

Influenza vaccines are available for pigs, horses, dogs and, in some countries, birds.[1,3,4,13,25,147,168,169,352] The vaccines do not always prevent infection or virus shedding, but the disease is usually milder if it occurs. Influenza vaccines may change periodically to reflect the current subtypes and strains in a geographic area. In general, swine and equine viruses display less antigenic drift than human viruses, and these vaccines are changed less often.[2,3,25]

In the U.S., avian influenza vaccines are used most often in turkeys and are intended only to prevent infection by LPAI viruses.[19] HPAI vaccines are not used routinely in the U.S. or most other countries; however, nations may consider vaccination as a preventative or adjunct control measure during an outbreak.[19,353] Avian vaccines are usually autogenous or from viruses of the same subtype or

hemagglutinin type.[19] Currently licensed vaccines in the U.S. include inactivated whole virus and recombinant fowlpox- H5 vaccines. The use of these vaccines requires the approval of the state veterinarian and, in the case of H5 and H7 vaccines, USDA approval. Because vaccines may allow birds to shed virus while remaining asymptomatic, good surveillance and movement controls are critical in a vaccination campaign.[13,353-355] Methods used to recognize infections with field viruses in vaccinated flocks include "DIVA" (differentiating vaccinated from infected animals) strategies, and the use of sentinel birds.[13,353,354,356] Vaccination may place selection pressures on avian influenza viruses, and might eventually result in the evolution of vaccine-resistant isolates.[355,357]

Other preventative measures

Poultry can be infected by contact with newly introduced birds or fomites, as well as by contact with wild birds, particularly waterfowl and shorebirds.[3,12,15] Illegal poultry movements may be of primary importance in transmission in some regions.[358,359] The risk of infection can be decreased by all-in/ all-out flock management, and by preventing any contact with wild birds or their water sources.[10,15] Keeping flocks indoors is often recommended in areas where the H5N1 virus has been isolated from wild birds. Poultry should not be returned to the farm from live bird markets or other slaughter channels.[15] In addition, strict hygiene and biosecurity measures are necessary to prevent virus transmission on fomites.[10,12,15,216] Mammals should not be fed poultry or other birds that may be infected with the avian influenza viruses.[45] They should also kept from contact with potentially infected flocks and wild birds. During outbreaks of H5N1 avian influenza, cats and dogs should be kept indoors whenever possible.

In pigs and horses, influenza is usually introduced into a facility in a new animal.[2-4,16,94] Isolation of newly acquired animals can decrease the risk of transmission to the rest of the herd.[168] Similarly to birds, good biosecurity is important.[4,168] Pigs should also be protected from the influenza viruses found in other species, particularly birds and humans. To prevent human influenza viruses from entering a herd, swine workers who are ill should avoid contact with pigs, and the public should be restricted from entering swine operations.[360] Once a herd of swine has been infected with a swine influenza virus, the virus usually persists in the herd and causes periodic outbreaks; however, good management can decrease the severity of disease.[1,2,4,16,94] Infected swine herds can be cleared of influenza viruses by depopulation.[16,94]

Ferrets can be infected by human influenza viruses (including the novel H1N1 virus), and people with influenza should avoid contact with this species.[187] If contact is unavoidable, good hygiene, as well as the use of face masks and/or other measures to prevent droplet transmission from coughs and sneezes, may be helpful. Felids (including housecats and a cheetah) and dogs have also been infected with the novel H1N1 virus from humans.[119-124,127-129]

Eradication and prevention of virus transmission during outbreaks

During an outbreak of influenza among mammals, quarantines and isolation of infected animals help prevent virus dissemination.[3,25] Good hygiene can keep the virus from spreading on fomites. Rest decreases virus shedding in horses.[25] Infected facilities should be cleaned and disinfected after the outbreak.

In poultry, outbreaks of high pathogenicity avian influenza are controlled by eradication.[3,11] The outbreak is managed by quarantine, depopulation, cleaning and disinfection, and surveillance around the affected flocks. Strict hygiene is necessary to prevent virus transmission on fomites. Because H5 and H7 LPAI viruses can mutate to become HPAI viruses, these infections are reportable to the OIE, and are being controlled similarly in many countries.[19]

Morbidity and Mortality

The severity of an influenza virus infection varies with the dose and strain of virus and the host's immunity. In mammals, uncomplicated infections are usually associated with high morbidity rates, low mortality rates and rapid recovery.[1-4,25,147,168,170,330] Secondary bacterial infections can exacerbate the clinical signs, prolong recovery and result in complications such as pneumonia.

Avian influenza

Avian influenza outbreaks occur in most countries including the U.S. Low pathogenicity forms occur most often, but outbreaks with high pathogenicity H5 and H7 viruses are also seen occasionally.[10,11] Seasonality has been reported in the current H5N1 epidemic; this virus has tended to reemerge during colder temperatures in the Northern Hemisphere.[283] The reason for the seasonality is unknown, but it may be the result of multiple factors such as increased virus survival in the cold, increased poultry trade during winter festivals, and wild bird movements.[283] In domesticated poultry (particularly chickens), high pathogenicity avian influenza has very high morbidity and mortality rates, up to 90-100%.[11,13] Any surviving birds are usually in poor condition. LPAI viruses usually result in mild or asymptomatic infections, but may also mimic HPAI viruses.[13,18,19] High mortality is occasionally seen in young ostriches infected with either LPAI or HPAI viruses.[140]

Symptomatic infections are unusual in wild birds; however, some of the Asian lineage H5N1 viruses have caused outbreaks with high mortality rates.[1,2,10-12,54,72,73] In April 2005, an outbreak that began at Qinghai Lake in central China resulted in the death of more than 6000 migratory wild birds.[12] Asian lineage H5N1 viruses have also been isolated sporadically from other dead birds, including waterfowl, in a number of countries.[11,51,71,73,319] High mortality rates have been reported in some but not

all experimentally infected wild birds. In one study, all six laughing gulls infected with recent strains of H5N1 became severely ill, and four died.[136] Four of six infected wood ducks also became severely ill while two others remained asymptomatic.[136] Three of the sick ducks died and one recovered. Mallard, northern pintail, blue-winged teal and redhead ducks inoculated with the same viral strains did not become ill.[136] Morbidity and mortality rates in passerine and psittacine birds have varied with the species. In one study, mortality rates approached 100% in zebra finches, house finches and budgerigars, but all house sparrows experienced mild disease and survived, and all starlings remained asymptomatic.[142] In a study with a different Asian lineage H5N1 virus, the mortality rate was 66-100% in house sparrows, but no deaths were seen in starlings. [317]

Avian H5N1 influenza in mammals

Asian lineage H5N1 viruses have been reported in a variety of mammalian species. In an unpublished study from Thailand, antibodies to these viruses were found in 8 of 11 cats and 160 of 629 dogs.[59] In contrast, no antibodies were detected in 171 cats from areas of Austria and Germany where infections had been reported in wild birds.[361] Some infections with Asian lineage H5N1 viruses have been fatal; deaths have been reported in housecats, some large felids, a dog, raccoon dogs, palm civets and experimentally infected ferrets[9,45-50,53,55,61,66] However, both mild and severe cases have been reported in several of these species. Fatal cases were reported in some naturally infected housecats, and some experimentally infected cats exhibited severe disease and high mortality rates.[47,48,53,63,65] In contrast, asymptomatic infections were reported in cats exposed to an infected swan in an animal shelter.[132] Few of these cats shed virus, and none became ill despite the presence of other viral and bacterial infections, and high stress levels in this population.[132] Similarly, fatal cases were reported among captive tigers and leopards in Thailand, but captive leopards, tigers, Asiatic golden cats and lions at a wildlife rescue center in Cambodia all recovered after an illness lasting 5-7 days.[9,46,50,54] Asymptomatic or mild infections have been reported in experimentally infected dogs, but one death was reported in a naturally infected dog.[49,67,68] In experimentally infected ferrets and mice, the severity of the clinical signs varied with the specific isolate and the route of inoculation (intranasal or intragastric).[61,66] Interestingly, there is no evidence that HPAI H5N1 viruses are causing significant illness among infected pikas in China,[57] and Asian lineage H5N1 viruses isolated from Indonesian pigs were less virulent in mice than isolates from poultry.[56]

Although Asian lineage H5N1 viruses have been reported in pigs, severe disease does not seem to occur in this species. A serological study conducted in Vietnam found that a low percentage of pigs (0.25%) had been exposed to H5N1 influenza viruses in 2004.[58] Asian lineage H5N1 viruses have also been detected in swine in Indonesia,[56] and these viruses have been isolated rarely from pigs in China.[9,58] However, there are no reports of severe illness among swine. Experimental infections also suggest that the clinical signs may be mild in this species,[58,66] and miniature pigs were resistant to infection in one study.[60]

Swine influenza

Influenza is a major cause of acute respiratory disease in finishing pigs. Approximately 25-33% of 6-7 month-old finishing pigs and 45% of breeding pigs have antibodies to the classical swine H1N1 virus in the U.S.[1,16] High seroprevalence rates to swine influenza viruses have also been reported in other countries.[1,2,5,16] In addition, pigs can be infected with human influenza A, B and C viruses.[1-6,8,201] In the U.K., a study found antibodies to both swine and human influenza viruses in 14% of all pigs.[16] Approximately 10% of the pigs were seropositive for influenza C viruses, but only sporadic infections with the human influenza B viruses were found.[28] In Japan, a similar study found antibodies to the type C viruses in 19% of pigs.[8]

Swine influenza viruses are usually introduced into a herd in an infected animal, and can survive in a few carrier animals for up to four months.[3,4,16,94] In a newly infected herd, up to 100% of the animals may become ill but most animals recover within 3-7 days if there are no secondary bacterial infections or other complications.[2-4,147] In uncomplicated cases, the case fatality rate has ranged from less than 1% to 4%.[1,3,4]

Once the virus has been introduced, it usually persists in the herd.[1,2,16,94] Annual outbreaks are often seen, and in temperate regions, occur mainly during the colder months.[1,2,4,16,94] Many infections in endemically infected herds are subclinical; typical signs of influenza may occur in only 25% to 30% of the pigs.[2,16,94] Maternal antibodies decrease the severity of disease in young pigs.[2] Viruses may also infect the herd with few or no clinical signs.[1,2,16,94]

Influenza epidemics can occur if a virus infects a population without immunity to the virus, or if the infection is exacerbated by factors such as poor husbandry, stress, secondary infections or cold weather.[1,16] In the epidemic form, the virus spreads rapidly in pigs of all ages.[147] In the 1918 epizootic, millions of pigs developed influenza, and thousands of the infections were fatal.[1]

Novel H1N1 virus of swine origin

The H1N1 virus circulating in humans appears to cause mild disease in pigs.[103,107,108,345]. Morbidity rates from less than 1% to as high as 90% have been reported, but little or no mortality has been seen.[102-104,107,108,111-116,252,321] Experimental studies support this view; deaths have not been reported among experimentally infected pigs.[310,323,346]

Decreased egg production may be the main effect in turkeys. In a turkeys flock in Chile, the morbidity rate was

61%, but no deaths were seen.[117] Egg production in this flock dropped from 70% to 31%. Similarly, egg production dropped by approximately 80%, in affected turkey flocks in Canada.[118] Although a slight increase in flock mortality occurred in the latter case, it may have been unrelated to the H1N1 infection.[362] Decreased egg production and no mortality were reported in a U.S. turkey flock.[125]

A few cats, pet ferrets and dogs have been infected naturally with the novel H1N1 virus.[119-124,127-129] Several ferrets recovered, but one died.[119,120,122] Two infected cats died, another developed severe illness with dyspnea but recovered with medical care, and some cats apparently had milder cases.[121,123,129] An infected dog in the U.S. was ill with pneumonia, and required hospitalization and supportive care, but recovered.[128] Experimental studies in mice, ferrets and nonhuman primates suggest that this virus might cause more severe lung pathology and/or clinical signs than seasonal human H1N1 viruses, or that the illness might last longer.[262,310,322]

Equine influenza

In horses, influenza outbreaks are not as seasonal as they are in pigs or humans.[25] Most outbreaks are associated with sales, races and other events where horses congregate.[3,25] Close contact with other horses, crowding and transportation are typical risk factors for disease.[170] Widespread epidemics can be seen, with morbidity rates of 60-90% or greater, in naïve populations.[1,25,253,330] In 1987, an equine influenza epidemic in India affected more than 27,000 animals and killed several hundred.[25] In populations that have been previously exposed, cases are seen mainly in young and newly introduced animals.[1,25]

Unless there are complications, healthy adult horses usually recover within 1-3 weeks, although coughing can persist.[1,25,168,253] The H3N8 viruses usually cause more severe disease than the H7N7 viruses.[1,25] Deaths are rare in adult horses, and are usually the result of secondary bacterial infections.[1,25,168,330] Higher mortality rates have been reported in foals, animals in poor condition, donkeys and zebras.[25,168,330] In horses, tracheal clearance rates can be depressed for up to a month after infection.[25]

Avian influenza viruses have rarely been reported in horses. In 1989, a novel strain of equine influenza [A/eq/Jilin/89 (H3N8)] caused a serious epidemic in Chinese horses.[25,170] The morbidity rate was at least 80% and the mortality rate was 20-35%.[25,170] The virus appeared to be an avian influenza virus. A related virus caused influenza in a few hundred horses the following year but there were no deaths. The avian-like virus continued to circulate in horses for at least five years without further fatalities.

Canine influenza

Canine H3N8 influenza was first reported in racing greyhounds and, at first, appeared to be confined to this breed.[177,213] Although this disease was first reported in 2004, new evidence suggests that the H3N8 virus may

have been circulating in U.S. greyhound populations as early as 1999.[28,180,363] More recently, H3N8 canine influenza has been seen in a variety of breeds at veterinary clinics and animal shelters in several states.[28,30,179,181,183,213] All dogs regardless of breed or age are now considered to be susceptible.[178,179,213] The prevalence of this disease in the U.S. is not yet known. One study suggests that canine influenza is rare, if it exists at all, in Canada. In the province of Ontario, a survey found antibodies to the H3N8 virus in only one of 225 dogs in 2006.[364] This dog was a greyhound that had come from a racetrack in Florida, and may have been infected there. It had no recent history of respiratory disease.

Because dogs have not been exposed to the canine influenza virus before, most of the population is expected to be fully susceptible.[177,178] In kennels, the infection rate may reach 100%, and clinical signs can occur in 60-80% of the dogs infected.[178,213] Most dogs are expected to develop the less severe form of the disease, and recover; however, a more severe form with pneumonia may occur in a minority.[177,179,213] In dogs with severe disease, the overall mortality rate is thought to be 1-5%.[28,173,176,179] Higher case fatality rates have been reported in small groups of greyhounds.[213] At one Florida greyhound racetrack, the case fatality rate was 36%.[28] High case fatality rates are not expected in most canine populations; however, severe disease is more likely in dogs that are in poor condition or are concurrently exposed to other pathogens.

The H3N2 virus has been reported from outbreaks at three veterinary hospitals and a kennel in South Korea.[133] Cases were described in a miniature schnauzer, a cocker spaniel, a Yorkshire terrier and two Jindo dogs (a Korean breed of hunting dog), as well as 13 dogs of unknown breed at an animal shelter.[133] Only one of the five dogs seen at veterinary clinics survived. The fate of the dogs in the animal shelter was not stated.

Influenza in other mammals

In 1984, an avian H10N4 virus caused an outbreak on Swedish mink farms. It affected 33 farms and killed 3,000 mink.[1] The morbidity rate was nearly 100%, with a mortality rate of 3%.[9] In an outbreak caused by a triple reassortant H1N1 swine influenza virus in ferrets, the morbidity rate was 8% and the mortality rate was 0.6%.[91] In seals, the case fatality rate was estimated to be 20% in one outbreak with an H7N7 virus, and 4% in an outbreak with an H4N5 virus.[1] Explosive epidemics in seals are thought to be exacerbated by high population densities and unseasonably warm temperatures,[329] and they may be more severe if the animals are co-infected with other pathogens.[9]

Internet Resources

Newcastle Disease

Avian Paramyxovirus-1Infection,
Goose Paramyxovirus Infection

Last Updated: July 14, 2008

Importance

Newcastle disease is a viral disease of birds with a wide range of clinical signs from mild to severe. This disease is caused by a diverse group of viruses; the milder strains are endemic in the United States, while highly virulent strains are exotic. The highly virulent form of Newcastle disease is one of the most important poultry diseases worldwide. Chickens are particularly susceptible, and may experience morbidity and mortality rates up to 100%. Outbreaks of virulent Newcastle disease have a tremendous impact on backyard chickens in developing countries, where these birds are a significant source of protein and this disease is endemic. In developed countries, where the more virulent forms of the virus have been eradicated, trade embargoes and restrictions cause significant economic losses during outbreaks. In the United States, one epidemic in 2002-2003 resulted in the death of more than three million birds and caused industry losses estimated at $5 billion. Low pathogenicity isolates, which are common in poultry worldwide, can decrease productivity but have no impact on international trade.

Although the most significant impact of Newcastle disease is on chickens, other species can also be affected. Some pet and zoo birds become ill after infection, while other species can carry and shed virulent viruses asymptomatically. These birds, particularly illegally imported psittacines, can introduce Newcastle disease viruses to disease-free countries. Newcastle disease is also an important cause of death during the first three months of life in cormorant colonies. Since the late 1990s, novel strains have caused outbreaks among geese (a species that is usually resistant to disease) in China.

Etiology

Newcastle disease is caused by viruses in the serotype avian paramyxovirus type 1 (APMV-1). These viruses, which are called either APMV-1 or Newcastle disease viruses (NDV), are members of the genus *Avulavirus* in the family Paramyxoviridae. APMV-1 strains maintained in pigeon populations have some antigenic differences from other NDV isolates, and are sometimes called pigeon paramyxovirus type 1 (PPMV-1).

APMV-1 strains are classified into three pathotypes based on their virulence in chickens. Lentogenic strains are the least virulent, mesogenic strains are moderately virulent, and velogenic strains are the most virulent. Most strains cluster toward the two extremes of virulence, and are either lentogenic or velogenic. Velogenic viruses can be subdivided into a neurotropic form, which is typically associated with respiratory and neurologic signs, and a viscerotropic form with hemorrhagic intestinal lesions. These clinical forms overlap and are rarely clear-cut, even in specific pathogen free (SPF) chickens.

Several tests can be used to assess the virulence of an APMV-1 strain, and countries may use different criteria to define Newcastle disease. The OIE defines Newcastle disease as an infection caused by a highly virulent APMV-1 virus – an isolate that has either 1) an intracerebral pathogenicity index (ICPI) of at least 0.7 in day-old chicks, or 2) an amino acid sequence that resembles those seen in highly virulent viruses (multiple basic amino acids at the C-terminus of the F2 protein and phenylalanine at residue 117 of the F1 protein). Such viruses must be reported to the OIE and have severe repercussions for international trade. The U.S. defines "exotic Newcastle disease" (END) as the disease caused by velogenic viscerotropic strains.

APMV-1 isolates can also be separated into two clades, called class I and class II, based on the genetic relationship between viruses. The vast majority of APMV-1 strains belong to class II, which is divided into at least nine genotypes (I to IX). Class I isolates have been found mainly in wild waterfowl, and are usually of low pathogenicity.

Species Affected

Newcastle disease primarily affects birds. Some avian species become ill, while others carry these viruses asymptomatically. Infections also occur in humans, but have not been reported in other species of mammals.

APMV-1 viruses are known to infect more than 250 species of birds in 27 orders; other avian species may also be susceptible. Wild birds, particularly waterfowl (order Anseriformes), tend to carry these viruses asymptomatically. Most of the viruses found in wild birds are lentogenic; however, virulent APMV-1 has become established in some cormorant populations (*Phalacrocorax* sp.; order Pelecaniformes) and causes disease in juvenile birds.

Susceptibility to illness varies widely among poultry and pet birds. Members of the order Phasianiformes (gallinaceous birds), particularly chickens, are highly susceptible to disease. Turkeys are less likely to develop severe symptoms, and the susceptibility of game birds (pheasants, partridges, quail and guinea fowl) varies with the species. Ducks and geese usually have inapparent infections, but some isolates (in genotypes VII and VI) have caused outbreaks among geese in China since the 1990s. Clinical cases have been also been described occasionally in ducks. Outbreaks have been reported in ostriches (order Struthioniformes). Pigeons (order Columbiformes) are susceptible to disease, and lentogenic or mesogenic APMV-1 viruses (PPMV-1) are endemic in pigeon populations. Susceptibility to disease varies widely in psittacine birds (order Psittaciformes); cockatiels often die or develop neurological signs, but some species tend to carry velogenic viruses subclinically.

Some birds found in the wild or in zoos also become ill. Penguins (order Sphenisciformes) are highly susceptible to Newcastle disease, and birds often die acutely. Fatal or severe disease has been reported in some raptors (order Falconiformes) including a bearded vulture (*Gypaetus barbatus*), some species of falcons, a captive white-tailed sea eagle (*Haliaeetus albicilla)* and a wild osprey (*Pandion haliaetus*). Other raptors tend to be resistant to disease. Illness has also been reported in gulls (order Charadriiformes) owls (order Strigiformes) pelicans (order Pelecaniformes) and a Northern gannet (*Morus bassanus;* order Pelecaniformes). Susceptibility varies among passerine birds (order Passeriformes), with some species excreting virus subclinically and others developing severe clinical signs. Occasional deaths have also been reported in Corvidae (crows and ravens).

Geographic Distribution

Velogenic APMV-1 is endemic in Asia, the Middle East, Africa, Central and South America, and parts of Mexico. Virulent strains are endemic in wild cormorants in the U.S. and Canada, but commercial poultry are free of velogenic isolates. Lentogenic isolates are found in poultry throughout the world, including the U.S. Mesogenic strains may also be found, but are less common.

Transmission

APMV-1 can be transmitted by inhalation or ingestion (fecal/ oral route). Birds shed virus in both feces and respiratory secretions. Gallinaceous birds excrete APMV-

1 for only 1-2 weeks, but psittacine birds often shed these viruses for several months. Some species of psittacine birds can excrete virus for more than a year. Prolonged shedding has also been reported in some members of other orders, including owls (more than four months) and cormorants (one month). Shedding can be sporadic. APMV-1 is present in all parts of the carcass, and some outbreaks in raptors have been linked to eating infected chicken, pigeon or quails. When the temperature is just above freezing (1-2ºC [34-35ºF]), this virus is reported to survive on chicken skin for up to 160 days and in bone marrow for nearly 200 days. The importance of aerosols in long distance transmission is controversial. In one study, APMV-1 was found 64 meters but not 165 meters downwind of an infected farm. The survival of aerosolized virus is probably dependent on humidity and other environmental factors, as well as the concentration of infected poultry. Some isolates can be transmitted through the egg to hatching chicks. Egg-associated transmission of highly virulent isolates is possible but uncommon, as the embryo usually dies unless the viral titer in the egg is low. Other sources of virus for newly hatched chicks are feces-contaminated eggshells and cracked or broken eggs.

APMV-1 is readily transmitted on fomites. Survival is prolonged on eggshells and especially in feces, compared to an inorganic surface (filter paper). Published information on virus survival is highly variable, probably because it is affected by the humidity, temperature, suspending agent and exposure to light. One study reported that APMV-1 survived in contaminated, uncleaned poultry houses for up to 7 days in summer, as long as 14 days in the spring, and 30 days during the winter. Another group reported virus isolation up to 16 days after depopulation of an unvaccinated flock. However, one study found that APMV-1 remained viable for up to 255 days in a henhouse, at ambient temperatures of –11ºC (12ºF) to 36ºC (97ºF). At 23-29ºC (73-84ºF), APMV-1 is reported to survive in contaminated litter for 10 to 14 days, and at 20ºC (68ºF) in soil for 22 days. Virus has also been recovered from earthworms for 4 to 18 days, and from experimentally contaminated lake water for 11 to 19 days. Flies might be able to transmit APMV-1 mechanically, but it is still uncertain whether insects can carry enough virus to infect poultry. The importance of arthropod-borne transmission may vary with the type of housing and flock management.

The epidemiology of APMV-1 is incompletely understood; however, wild birds, particularly waterfowl, may be the reservoir hosts for lentogenic viruses. These viruses could become more virulent after becoming established in poultry. Some recent outbreaks were apparently caused by velogenic viruses that emerged from local, low pathogenic isolates. Acquisition of virulence has also been reported in experimentally infected birds. Psittacine birds have introduced APMV-1 to poultry flocks in some outbreaks. Although early reports suggested that virulent

strains might be endemic in wild psittacine populations, these birds are now thought to become infected after capture. Cormorants could transmit velogenic viruses to poultry; gulls associated with cormorant colonies could also be a source of virus, and are more likely to visit farms. Lentogenic or mesogenic APMV-1 viruses are endemic in pigeon populations, and can become more virulent if they enter and cycle in poultry flocks.

Incubation period

The incubation period in poultry varies from 2 to 15 days depending on the virulence of the strain and the susceptibility of the population. In chickens infected with velogenic isolates, an incubation period of 2 to 6 days is common. Incubation periods up to 25 days have been reported in some avian species.

Clinical signs

The clinical signs vary with the pathogenicity of the isolate and the species of bird. In chickens, lentogenic strains usually cause subclinical infections or mild respiratory disease with coughing, gasping, sneezing and rales. Mesogenic strains can cause acute respiratory disease and neurologic signs in some chickens, but the mortality rate is usually low. Lentogenic or mesogenic strains can produce more severe symptoms if the flock is co-infected with other pathogens.

Velogenic strains cause severe, often fatal, disease in chickens. The clinical signs are highly variable. Most birds are lethargic and inappetent, and the feathers may be ruffled. Conjunctival reddening and edema may be an early sign. Some birds develop watery, greenish or white diarrhea, respiratory signs (including cyanosis) or swelling of the tissues of the head and neck. Neurologic signs including tremors, clonic spasms, paresis or paralysis of the wings and/or legs, torticollis (twisted neck) and circling may also be seen. Nervous signs can occur concurrently with other symptoms but are generally seen later in the course of disease. Egg laying often declines dramatically, and eggs may be misshapen, abnormally colored, and rough or thin-shelled, with watery albumen. Sudden death, with few or no symptoms, is also common. Birds that survive for two weeks usually live but may have permanent neurological damage and/or a permanent decrease in egg production.. The symptoms may be less severe in vaccinated birds.

Similar clinical signs are seen in other species of birds; however, either neurological signs or respiratory signs can predominate in some species. Newcastle disease is generally milder in turkeys than chickens, but some strains may cause significant disease. Severe clinical signs can sometimes be seen in game birds, particularly pheasants. Respiratory signs have been reported in some but not all outbreaks in pheasants. Guinea fowl sometimes become ill, but they can also carry velogenic isolates subclinically.

In psittacine birds, Newcastle disease may be acute, subacute, chronic or inapparent. The clinical signs are highly variable, but may include respiratory and/or neurologic signs, as well as diarrhea and sudden death. Respiratory signs tend to predominate in ostriches and emus, and these birds are usually less severely affected than chickens. Diarrhea, polydipsia, conjunctivitis and neurological signs are generally seen in pigeons and doves. Neurological signs, particularly talon convulsions and the inability to coordinate flight, are prominent in raptors. Sudden death may also occur. Geese and ducks are usually infected subclinically (with most strains), but illness is occasionally reported. Neurological signs, diarrhea, anorexia and sudden death may be seen in these birds. Respiratory symptoms appear to be rare in waterfowl.

In cormorant colonies, Newcastle disease is usually characterized by neurological signs, and illness is almost always limited to juveniles. Affected birds may be weak, with paresis or paralysis of one or both legs and/or wings, incoordination, tremors, torticollis and/or drooping of the head. Sick or dead birds can be found in the same nest as apparently normal nestmates. Older fledged cormorants may be seen trying to walk, fly, swim or dive. Sick or dead gulls and juvenile white pelicans have been seen near affected cormorant colonies. Sick pelicans had neurological signs similar to cormorants, such as unilateral or bilateral wing and or leg paralysis/ paresis, drooping neck, and an inability or reluctance to move; however, it has not been proven that these symptoms were caused by APMV-1. In addition to increased mortality, the only clinical signs reported in gulls were wing and/or leg paralysis or paresis.

Post Mortem Lesions   Click to view images

Significant gross lesions are usually found only in birds infected with velogenic strains. The head or periorbital region may be swollen, and the interstitial tissue of the neck can be edematous, especially near the thoracic inlet. Congestion or hemorrhages may be found in the caudal pharynx and tracheal mucosa, and diphtheritic membranes sometimes occur in the oropharynx, trachea and esophagus. Petechiae and small ecchymoses may be seen in the mucosa of the proventriculus. Hemorrhages, ulcers, edema and/or necrosis often occur in the cecal tonsils and lymphoid tissues of the intestinal wall (including Peyer's patches); this lesion is particularly suggestive of Newcastle disease. Thymic and bursal hemorrhages may also be present, but can be difficult to see in older birds. The spleen may be enlarged, friable and dark red or mottled. Pancreatic necrosis and pulmonary edema can be found in some birds. The ovaries are often edematous or degenerated, and may contain hemorrhages. Some birds, particularly those that die suddenly, have few or no gross lesions. Similar lesions have been reported in geese, turkeys, pheasants and other species infected with virulent strains. In experimentally infected guinea fowl, the only

significant lesions were hemorrhages at the tip of the glands of the proventriculus and in the cecal tonsil.

In chickens infected with less virulent strains, the lesions may be limited to congestion and mucoid exudates in the respiratory tract, and opacity and thickening of the air sacs. More severe lesions can be seen in birds with secondary bacterial infections.

Morbidity and Mortality

Morbidity and mortality rates vary greatly depending on the virulence of the strain and susceptibility of the host. Lentogenic and mesogenic viruses usually kill few birds; in poultry, the mortality rate is approximately 10% for mesogenic strains and negligible with lentogenic strains. Concurrent illnesses may increase the severity of illness and result in a higher death rate. In contrast, velogenic isolates have morbidity and mortality rates up to 100% in unvaccinated chickens. The onset of disease is usually rapid, and the virus often spreads quickly, particularly in group-housed flocks. Some isolates can affect young birds more severely. Vaccinated poultry tend to have milder infections. In one epidemic mainly affecting vaccinated chickens, flock mortality rates were 30% to 90%.

Other species of birds are usually affected less severely than chickens. Velogenic isolates can kill up to 100% of experimentally infected pheasants, but some individual birds may be resistant to disease, and the mortality rate reported during outbreaks is highly variable. From 22% to 77% of the pheasants in affected flocks died during one epizootic in Denmark, but in another outbreak in the U.K., the mortality rate was less than 3% even in the most severely affected pen. In guinea fowl, the mortality rate was 21% during one outbreak, and 8-100% in experimental infected birds (depending on the strain of the virus). Mortality rates as high as 28% have been reported in ostriches in some outbreaks, but few birds died in others. Newcastle disease is rarely severe in waterfowl; however, some velogenic strains circulating in China have an average morbidity rate of 17.5% and an average mortality rate of 9% in geese.

APMV-1 (PPMV-1) is endemic in pigeons and doves in many countries. In these birds, highly virulent strains have morbidity rates as high as 70% and mortality rates that approach 40%. Velogenic strains are endemic in cormorants, but adult birds do not appear to develop clinical signs or die. The estimated mortality during several outbreaks in juvenile cormorants ranged from less than 1% to 92%. Up to 90% of juvenile white pelicans near these colonies have died in some outbreaks; however, it has not been proven that the disease in pelicans was caused by APMV-1.

Diagnosis

Clinical

Newcastle disease should be considered, especially in chicken flocks, when the morbidity and mortality rates are high, and the symptoms could be consistent with this disease. Unexpected deaths are sometimes the first sign. There are no pathognomonic gross lesions; however, some lesions may be suggestive, particularly when several carcasses are examined.

Differential diagnosis

The differential diagnosis for velogenic Newcastle disease includes other causes of septicemia, enteritis, respiratory disease and/or neurologic signs. In poultry, these diseases include fowl cholera, highly pathogenic avian influenza, laryngotracheitis, the diphtheritic form of fowl pox, psittacosis, mycoplasmosis, infectious bronchitis, aspergillosis, and management problems such as deprivation of water or feed, and poor ventilation. In pet birds, diseases to consider include psittacosis, Pacheco's disease, salmonellosis, adenovirus, and nutritional deficiencies, as well as other paramyxovirus infections. In cormorants, botulism, fowl cholera and traumatic skeletal abnormalities are among the differentials.

Laboratory tests

Newcastle disease can be diagnosed by isolating APMV-1 from affected birds. This virus is usually recovered by inoculating samples into 9-11 day old embryonated chicken eggs. Chorioallantoic fluid from the eggs is tested for hemagglutinating activity, and any agents that hemagglutinate are examined for hemagglutination inhibition (HI) with a monospecific antiserum to APMV-1. Some HI tests that use monoclonal antibodies can identify particular strains of APMV-1. APMV-1 can cross-react with some other avian paramyxoviruses, particularly APMV-3 and APMV-7, in the HI test.

The pathogenicity of the isolate can be quantified by 1) the mean death time (MDT) in chicken embryos, 2) the intracerebral pathogenicity index (ICPI) in 1-day old chicks, or 3) the intravenous pathogenicity index (IVPI) in 6-week old chickens. In the MDT assay, velogenic isolates have an MDT of less than 60 hours, mesogenic strains have an MDT of 60-89 hours, and lentogenic viruses have an MDT greater than 90 hours. The ICPI and IVPI tests are scoring systems that evaluate illness or death in chickens. The values in the ICPI test range from 0 to 2.0; the most virulent viruses approach 2.0, while lentogenic strains are usually close to 0.0. The values in the IVPI test are from 0 to 3.0; the IVPI for velogenic strains approach 3.0, while lentogenic strains and some mesogenic strains have IVPI values of zero. However, some viruses that can produce severe disease have IVPI values of zero; the ICPI test is generally preferred for this reason. Other variations of these tests are also used; some can distinguish viscerotropic (velogenic) from neurotropic strains.

Reverse-transcription polymerase chain reaction (RT-PCR), gene sequencing, restriction enzyme analysis and other molecular techniques are also used to identify APMV-1 in eggs or clinical specimens. Some of these tests can also determine the virus's pathotype. Most iso-

lates that are highly virulent for chickens have a particular sequence, 112R/K-R-Q-K/R-R116 (multiple basic amino acids) at the C-terminus of the F2 protein and phenylalanine at residue 117 of the F1 protein. The presence of this genetic sequence is enough to classify an isolate as highly virulent for the purposes of international trade. If this pattern is not present, the pathogenicity of the virus must be determined in the ICPI or other test. Rapid diagnostic tests, as well as tests using monoclonal antibodies, are optimized for more virulent viruses, and may not identify some lentogenic viruses (particularly Class I isolates).

Serological assays may be useful in some circumstances. Hemagglutination inhibition is the most commonly used serological test. Other tests include virus neutralization, hemagglutination and enzyme-linked immunosorbent assays (ELISA). Vaccination can interfere with serologic testing. In some species, immunohistochemistry may be used to detect antigens in tissues; this test is not performed routinely for diagnosis in chickens.

Samples to collect

Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease. Newcastle disease is zoonotic; samples should be collected and handled with all appropriate precautions.

Tracheal and cloacal swabs should be taken from live birds for virus isolation. If cloacal swabs might harm the bird, fresh feces may be collected instead. Whenever possible, samples should be taken in the early stages of disease. At necropsy, samples should be collected from the spleen, trachea, lung, intestines (particularly the cecal tonsil), intestinal contents, liver, kidneys, heart and brain. Oronasal swabs should also be taken. Samples for virus isolation should be collected from recently dead birds or moribund birds after euthanasia. Tissues may be collected separately or pooled; intestinal samples are generally processed separately. These samples should be kept cold (e.g. on wet ice), and swabs should be sent to the laboratory in transport medium. Similar tissues and feces are collected for RT-PCR and other molecular assays. Clotted blood or serum samples can be submitted for serology.

Recommended actions if highly virulent Newcastle disease is suspected

Notification of authorities

State and federal veterinarians should be informed immediately of any suspected cases of highly virulent (velogenic) Newcastle disease.

Federal: Area Veterinarians in Charge (AVIC):
http://www.aphis.usda.gov/animal_health/area_offices/
State Veterinarians:
http://www.aphis.usda.gov/vs/sregs/official.html

Control

Good biosecurity can help prevent Newcastle disease in poultry flocks. Flocks should not be allowed to contact domesticated poultry of unknown health status, any pet birds (particularly psittacines), and wild or feral birds (particularly cormorants, gulls and pigeons). Whenever possible, workers should avoid contact with birds outside the farm. Biosecurity measures include bird-proofing houses, feed and water supplies, minimizing travel on and off the facility, and disinfecting vehicles and equipment that enter the farm. Pests such as insects and mice should also be controlled. If possible, employees should shower and change into dedicated clothing for work. All in/ all out breeding (one age group per farm), with disinfection between groups, is also advisable. More detailed biosecurity guidelines can be found in the Internet Resources section of this factsheet.

Similar biosecurity measures can protect birds kept in zoos or aviaries, or as pets (see Internet Resources). Establishing an effective biosecurity program can decrease the risk that hobby or pet birds would be euthanized during a Newcastle disease outbreak. Pet birds should be bought only from suppliers who can certify that the birds have been imported legally or bred in the U.S., and are healthy. Legally imported pet birds have been quarantined and tested for velogenic strains of APMV-1. Domestically raised birds are usually closed-banded. Some species such as Amazon parrots are difficult to raise domestically; vendors who are selling large numbers of young birds of these species (particularly when they are bargain-priced) without adequate documentation should be viewed with caution. Newly acquired birds should be isolated or quarantined for at least 30 days, and they should be monitored closely for signs of illness. Avian carcasses (of any species) that could be infected with velogenic Newcastle disease should never be fed to raptors, chickens or other birds. Illegally imported psittacines should be reported, because many of them may be carrying velogenic APMV-1.

Vaccines are used in chickens, pheasants and other species. In addition, birds in aviaries, breeding farms and zoos are often vaccinated. Vaccination can protect birds from clinical signs but does not necessarily prevent virus replication and shedding. Sentinel chickens are sometimes used to monitor vaccinated flocks.

Outbreaks are eradicated with quarantines and movement controls, depopulation of all infected and exposed birds, and thorough cleaning and disinfection of the premises. Effective disinfectants include chlorhexidine, sodium hypochlorite (6%), phenolic disinfectants and oxidizing agents (e.g. Virkon®). APMV-1 can also be inactivated by heat (56°C [133°F] for 3 hours or 60°C [140°F] for 30 min), acid (pH 3), ether and formalin; the efficacy of formalin varies with the temperature. Whether flies are competent vectors for APMV-1 is still uncertain, but fly control is prudent on and near infected farms. Before eradication begins, the facilities should be treated

with insecticides that can kill adult flies. Insect control should be continued until disinfection is complete. Farms must generally remain empty for a few weeks before re-stocking; the specific time may vary with the climate, season and other factors. During some eradication programs, government agencies may collect and test birds that die suddenly in any facility. This measure can be helpful in recognizing new cases.

Public Health

Velogenic strains of APMV-1 can cause conjunctivitis in humans, usually when the person has been exposed to large quantities of virus. Laboratory workers and vaccination crews are affected most often. Poultry workers are rarely infected, and handling or consuming poultry products does not appear to be a risk. The conjunctivitis usually resolves rapidly without treatment, but APMV-1 is shed in the ocular discharges for 4 to 7 days. All direct or indirect contact with birds should be avoided during this time.

Mild, self-limiting influenza-like disease with fever, headache and malaise has also been reported in humans; in some cases, it is uncertain whether the illness was caused by APMV-1 or misdiagnosed by cross-reactions in serologic tests. A recent report, confirmed by virus isolation, suggests that APMV-1 could cause serious opportunistic infections in people who are immunosuppressed. A patient developed fatal pneumonia 18 days after receiving a peripheral blood stem cell transplant. There was no history of contact with poultry, and the isolate was most closely related to APMV-1 viruses from pigeons.

Internet Resources

California Department of Food and Agriculture.
Newcastle Disease Information
http://www.cdfa.ca.gov/ahfss/Animal_Health/Newcastle_Disease_Info.html

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

United States Animal Health Association.
Foreign Animal Diseases
http://www.vet.uga.edu/vpp/gray_book02/fad/index.php

United States Department of Agriculture (USDA).
Biosecurity for the Birds
http://www.aphis.usda.gov/animal_health/birdbiosecurity/

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/eng/normes/mmanual/a_summry.htm

OIE Terrestrial Animal Health Code
http://www.oie.int/eng/normes/mcode/A_summry.htm

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Rift Valley Fever

Infectious Enzootic Hepatitis of Sheep and Cattle

Last Updated: November 2006

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Importance

Rift Valley fever (RVF) is a zoonotic, arthropod-borne viral disease important in domesticated ruminants. This disease is characterized by high mortality rates in young animals and abortions in pregnant ruminants. Rift Valley fever is endemic in sub-Saharan Africa. Epidemics occur in this region when heavy rainfalls cause infected mosquito eggs to hatch, and large numbers of susceptible animals are present. Rift Valley fever first appeared outside Africa in 2000, when outbreaks were reported in Saudi Arabia and Yemen.

Rift Valley fever epizootics are often accompanied by human disease. Many human cases are caused by occupational exposure to blood and tissues from infected animals, but mosquito-borne transmission can cause epidemics. The most common form of the disease is a self-limiting, flu-like illness; however, ocular disease and rare cases of fatal hemorrhagic fever also occur.

Etiology

Rift Valley fever results from infection by the Rift Valley fever virus, an RNA virus in the genus *Phlebovirus* (family Bunyaviridae).

Geographic Distribution

The Rift Valley fever virus is found throughout most of Africa. The disease is endemic in southern and eastern Africa, where outbreaks occur at irregular intervals. Epidemics have also been reported in Egypt, Saudi Arabia and Yemen.

Transmission

Rift Valley fever is transmitted by mosquitoes and is usually amplified in ruminant hosts. In endemic regions, cases can occur sporadically or in epidemics. The virus appears to survive in the dried eggs of *Aedes* mosquitoes; epidemics are associated with the hatching of these mosquitoes during years of heavy rainfall and localized flooding. In Africa, outbreaks typically occur in savannah grasslands every 5 to 15 years, and in semi-arid regions every 25 to 35 years. Once it has been amplified in animals, the RVF virus can also be transmitted by other vectors, including many mosquito species and possibly other biting insects such as ticks and midges. The virus can be transmitted *in utero* to the fetus. It has also been found in semen and raw milk.

Humans do not seem to be infected by casual contact with live hosts, but can be infected by aerosols or direct contact with tissues during parturition, necropsy, slaughter, laboratory procedures or meat preparation for cooking. *In utero* transmission to a human infant was first reported in 2006.

Both animals and humans theoretically have the potential to introduce Rift Valley fever into new areas by infecting mosquitoes.

Disinfection

Under optimal conditions, the Rift Valley fever virus remains viable in aerosols for more than an hour at 25°C (77°F). In a neutral or alkaline pH, mixed with serum or other proteins, the virus can survive for as long as four months at 4°C (40°F) and eight years below 0°C (32°F). It is quickly destroyed by pH changes in decomposing carcasses. The Rift Valley fever virus is susceptible to low pH (≤ 6.2), lipid solvents and detergents, and solutions of sodium or calcium hypochlorite with residual chlorine content greater than 5000 ppm.

Infections in Humans

Incubation Period

In humans, the incubation period is 2 to 6 days.

Rift Valley Fever

Clinical Signs

Infection with the Rift Valley fever virus usually results in an asymptomatic infection or a mild to moderate, non-fatal, flu-like illness with fever and liver abnormalities

The symptoms of uncomplicated infections may include fever, headache, generalized weakness, dizziness, weight loss, myalgia and back pain. Some patients also have stiffness of the neck, photophobia and vomiting. Most people recover spontaneously within two days to a week.

Complications including hemorrhagic fever, meningoencephalitis or ocular disease occur in a small percentage of patients. Hemorrhagic fever usually develops two to four days after the initial symptoms. The symptoms may include jaundice, hematemesis, melena, a purpuric rash, petechiae and bleeding from the gums. Hemorrhagic fever frequently progresses to frank hemorrhages, shock and death.

Ocular disease and meningoencephalitis are usually seen one to three weeks after the initial symptoms. The ocular form is characterized by retinal lesions and may result in some degree of permanent visual impairment. Death is rare in cases of ocular disease or meningoencephalitis.

Communicability

Virus titers in infected humans are theoretically high enough to infect mosquitoes and introduce Rift Valley fever into new areas. The virus can be found in the blood and tissues.

Diagnostic Tests

The Rift Valley fever virus can be isolated from the blood, brain, liver or other tissues; in living hosts, viremia usually occurs only during the first three days of fever. The virus can be grown in numerous cell lines including baby hamster kidney cells, monkey kidney (Vero) cells, chicken embryo reticulum, and primary cultures from cattle or sheep. Hamsters, adult or suckling mice, embryonated chicken eggs or 2-day-old lambs can also be used.

Viral antigens and RNA can be detected in blood and tissue samples by various antigen detection tests and reverse transcription polymerase chain reaction (RT-PCR) assays. Enzyme-linked immunoassay (ELISA) and other serologic tests can detect specific IgM or rising titers.

Treatment

No specific treatment, other than supportive care, is available; however, ribavirin has been promising in animal studies. Interferon, immune modulators and convalescent-phase plasma may also prove to be helpful. Most cases of Rift Valley fever are relatively mild, brief illnesses and may not require treatment.

Prevention

Mosquito repellents, long shirts and trousers, bednets, and other arthropod control measures should be used to prevent transmission by mosquitoes and other potential insect vectors. Outdoor activities should be avoided, if possible, during periods of peak mosquito activity. Insecticides may be helpful. During epidemics, vaccination of susceptible animals can prevent amplification of the virus and protect people as well as animals.

Barrier precautions should be used whenever contact may occur with infectious tissues or blood from animals; recommended measures include personal protective equipment such as protective clothing, gloves and goggles. Diagnostic tissue samples should be processed by trained staff in appropriately equipped laboratories. Universal precautions are recommended for healthcare workers who care for patients with confirmed or suspected Rift Valley fever. Barrier techniques are recommended when nursing hospitalized patients.

A human vaccine has been developed, but has limited availability. Additional vaccines are under investigation.

Morbidity and Mortality

Humans are highly susceptible to Rift Valley fever. Most cases develop in veterinarians, abattoir workers and others who work closely with blood and tissue samples from animals. During outbreaks in animals, mosquitoes may spread the virus to humans and cause epidemics. In Egypt, approximately 200,000 human cases and 598 deaths occurred during an epidemic in 1977.

In December 2006, an outbreak of RVF in Kenya, Somalia and the United Republic of Tanzania resulted in substantial numbers of human and animal cases and deaths. As of May 18, 2007, over 1000 human cases and 300 deaths have been reported.

Most people with Rift Valley fever recover spontaneously within a week. Ocular disease is seen in approximately 0.5% to 2% of cases, and meningoencephalitis and hemorrhagic fever in less than 1%. The case fatality rate for hemorrhagic fever is approximately 50%. Deaths rarely occur in people with eye disease or meningoencephalitis, but 1% to 10% of patients with ocular disease have some permanent visual impairment. The overall case fatality rate for all patients with Rift Valley fever is less than 1%.

Infections in Animals

Species Affected

Rift Valley fever can affect many species of animals including sheep, cattle, goats, buffalo, camels, and monkeys, as well as gray squirrels and other rodents. The primary amplifying hosts are sheep and cattle. Viremia without severe disease may be seen in adult cats, dogs, horses and some monkeys, but severe disease can occur in

newborn puppies and kittens. Rabbits, pigs, guinea pigs, chickens and hedgehogs do not become viremic.

Incubation Period

The incubation period can be as long as 3 days in sheep, cattle, goats and dogs. In newborn lambs, it is 12 to 36 hours. Experimental infections usually become evident after 12 hours in newborn lambs, calves, kids and puppies.

Clinical Signs

The clinical signs vary with the age, species and breed of the animal. In endemic regions, epidemics of Rift Valley fever can be recognized by high mortality rates in newborn animals and abortions in adults.

Rift Valley fever is usually most severe in young animals. In lambs, a biphasic fever, anorexia and lymphadenopathy may be followed by weakness and death within 36 hours. Hemorrhagic diarrhea or abdominal pain can also be seen. The youngest animals are most severely affected; in neonates, the mortality rate may reach 90% to 100%. Similar symptoms occur in young calves: fever, anorexia and depression are typical, with mortality rates of 10% to 70%.

Abortions are the most characteristic sign in adult sheep and cattle. Other symptoms that may occur in adult sheep include fever, weakness, a mucopurulent nasal discharge (sometimes bloodstained), melena, hemorrhagic or foul-smelling diarrhea, and vomiting. In adult cattle, fever, anorexia, weakness, excessive salivation, fetid diarrhea and decreased milk production have been reported. Icterus may also be seen, particularly in cattle.

Similar but milder infections occur in goats. Adult camels do not develop symptoms other than abortion, but young animals may have more severe disease. Viremia without severe disease may be seen in adult cats, dogs, horses and some monkeys, but severe disease can occur in newborn puppies and kittens.

Post Mortem Lesions   **Click to view images**

The most consistent lesion is hepatic necrosis; the necrosis is more extensive and severe in younger animals. In aborted fetuses and newborn lambs, the liver may be very large, yellowish-brown to dark reddish-brown, soft and friable, with irregular patches of congestion. Multiple gray to white necrotic foci are usually present, but may only be visible microscopically. The liver lesions are usually less severe in adult animals and may consist of numerous pinpoint reddish to grayish-white necrotic foci.

Additional lesions may include jaundice, widespread cutaneous hemorrhages and fluid in the body cavities. The peripheral lymph nodes and spleen are typically enlarged and edematous, and often contain petechiae. The walls of the gallbladder are often edematous, with visible hemorrhages. A variable degree of inflammation or hemorrhagic enteritis can sometimes be found in the intestines. In lambs, numerous small hemorrhages typically

occur in the abomasal mucosa, and the small intestine and abomasum may contain dark chocolate-brown contents with partially digested blood. In addition, petechial and ecchymotic hemorrhages may be seen on the surface of other internal organs. Microscopically, hepatic necrosis is the most prominent lesion.

Communicability

Infections in animals are typically transmitted by mosquitoes and not by direct contact; however, during parturition, necropsy or slaughter, viruses in the tissues can become aerosolized or enter the skin through abrasions. The Rift Valley fever virus has also been found in raw milk and may be present in semen.

Diagnostic Tests

Rift Valley fever can be diagnosed by isolation of the virus from the blood of febrile animals. The RVF virus can also be recovered from the tissues of dead animals and aborted fetuses; the liver, spleen and brain are generally used. This virus can be grown in numerous cell lines including baby hamster kidney cells, monkey kidney (Vero) cells, chicken embryo reticulum and primary cultures from cattle or sheep. Hamsters, adult or suckling mice, embryonated chicken eggs or two-day-old lambs can also be used.

Viral titers in tissues are often high, and a rapid diagnosis can sometimes be made with complement fixation, neutralization or agar gel diffusion tests on tissue suspensions. Viral antigens can also be detected by immunofluorescent staining of impression smears from the liver, spleen or brain. Enzyme immunoassays and immunodiffusion tests can identify virus in the blood. RT-PCR testing can detect viral RNA.

Commonly used serologic tests include virus neutralization, ELISA and hemagglutination inhibition tests. Immunofluorescence, complement fixation, radioimmunoassay and immunodiffusion are used less frequently. Cross-reactions with other phleboviruses can occur in serologic tests other than virus neutralization.

Treatment

No specific treatment, other than supportive care, is available.

Prevention

Vaccines are generally used to protect animals from Rift Valley fever in endemic regions. During epidemics, vaccination of susceptible animals can prevent amplification of the virus and protect people as well as animals. Attenuated and inactivated Rift Valley fever vaccines are both available. Attenuated vaccines produce better immunity; however, abortions and birth defects can occur in pregnant animals. Subunit vaccines are in development.

Additional, less commonly used, preventative measures include vector controls, movement of stock to higher altitudes, and the confinement of stock in insect-proof stables. These control methods are often impractical, or are ineffective because they are instituted too late. The movement of animals from endemic areas to RVF-free regions can result in epidemics.

Morbidity and Mortality

Epidemics of Rift Valley fever tend to occur at intervals, when heavy rainfalls cause infected mosquito eggs to hatch and a susceptible animal population is present. Outbreaks are characterized by large numbers of abortions and high mortality in neonates. Indigenous cattle may have asymptomatic infections, while more severe disease is seen in exotic species.

The mortality rate can be very high in young animals including neonatal ruminants, puppies and kittens, with fatalities decreasing in older age groups. The mortality rate in newborn lambs is 90% to 100%, while the mortality rates in adult sheep can vary from 5% to almost 100% in different epidemics and on different farms. Deaths are most common in pregnant ewes that abort. The estimated mortality rate in calves varies from 10% to 70%, but fewer than 10% of infections in adult cattle are usually fatal.

Abortion rates vary from 5% to almost 100% in ewes. Although up to 85% of cattle have aborted in some outbreaks, the abortion rate is typically less than 10% in this species. Abortion rates in camels can be as high as in cattle.

Internet Resources

Centers for Disease Control and Prevention (CDC). Special Pathogens Branch
http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/rvf.htm

Manual for the Recognition of Exotic Diseases of Livestock
http://www.spc.int/rahs/*

Medical Microbiology
http://www.ncbi.nlm.nih.gov/books/NBK7627/

Food and Agriculture Organization of the United Nations (FAO). Preparation of Rift Valley Fever Contingency Plans. FAO Animal Health Manual No. 15
http://www.fao.org/DOCREP/005/Y4140E/y4140e00.htm#TopOfPage

Merck Veterinary Manual.
http://www.merckvetmanual.com/mvm/index.jsp

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

OIE Terrestrial Animal Health Code
http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

United States Animal Health Association. Foreign Animal Diseases.
http://www.aphis.usda.gov/emergency_response/downloads/nahems/fad.pdf

World Health Organization (WHO). Rift Valley Fever Fact Sheet
http://www.who.int/mediacentre/factsheets/fs207/en/index.html

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United States Animal Health Association [USAHA]. Foreign animal diseases [online]. Richmond, VA: USAHA; 1998. Rift valley fever. Available at: http://www.vet.uga.edu/vpp/gray_book02/fad/rvf.php.* Accessed 7 Nov 2006.

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Swine Vesicular Disease

What is swine vesicular disease and what causes it?

Swine vesicular disease is a contagious viral disease of pigs. Illness is characterized by the development of blisters, open sores and ulcers around the mouth and on the legs. The disease is not life-threatening and affected pigs usually recover on their own. This disease is important due to it similarity to a number of other vesicular diseases, particularly foot and mouth disease. Swine vesicular disease has been eradicated from many countries, but can be found in parts of Europe (southern Italy) and Asia. The disease is not found in North America, so in the U.S. it is referred to as a foreign animal disease.

What animals get swine vesicular disease?

Pigs are the only natural host for the swine vesicular disease virus.

How can my animal get swine vesicular disease?

Swine vesicular disease is highly contagious through **direct contact** with infected pigs or the contaminated environment. The virus is found in secretions from the mouth and nose, and in the feces of infected animals. The virus can enter healthy pigs through open skin wounds, contact with mucous membranes, or by **ingestion**. Transfer of the virus on **fomites**, (e.g. contaminated vehicles) and earthworms, play an important role in the spread of disease.

How does swine vesicular disease affect my animal?

Swine vesicular disease is characterized by the formation of blisters and ulcerations on the legs, and around the mouth. Sores and lesions can also occasionally be seen on the snout, lips, tongue, and teats. Affected pigs may also develop fever, stop eating for a few days, and lose weight. Some pigs do not show any signs of disease at all. Most pigs usually make a full recovery within 2 to 3 weeks.

Can I get swine vesicular disease?

Yes, however this is rare. Some laboratory personnel working closely with the virus have developed symptoms of disease. Producers and veterinarians in close contact with infected pigs have not developed the disease.

Who should I contact if I suspect swine vesicular disease?

In Animals – Contact your veterinarian immediately. Swine vesicular disease is not currently found in the U.S.; suspicion of disease requires immediate attention.

In Humans – Contact your physician.

Swine Vesicular Disease is a highly contagious viral disease of swine that has with severe economic consequences.

Photo: Ulcers on the snout and foot of a pig. From USDA Plum Island Animal Disease Center.

How can I protect my animal from swine vesicular disease?

Animals imported from countries where swine vesicular disease exists must be carefully examined by veterinarians upon entry into the United States. Any new animals entering the farm should be quarantined away from the rest of the herd until it is determined that they are healthy and there is no risk to the other animals on the farm. No vaccine is available.

In areas where the virus is found, pigs should be monitored closely for signs of disease and separated from the rest of the herd if vesicles are seen. Pigs should not be fed pork products that may contain the virus. Proper disposal of infected carcasses is important, as earthworms have been known to carry the virus when infected carcasses are buried in the soil. Disinfection of all contaminated areas, including transport vehicles, is essential to prevent further spread of disease.

How can I protect myself from swine vesicular disease?

Laboratory personnel working with swine vesicular disease virus must take all necessary precautions to prevent themselves from becoming infected.

For More Information

CFSPH Technical Fact Sheets. Swine Vesicular Disease at http://www.cfsph.iastate.edu/DiseaseInfo/

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Vesicular Stomatitis

What is vesicular stomatitis and what causes it?

Vesicular stomatitis (ves-ICK-u-lar st-OO-ma-TIE-tis) is an important viral disease of animals and can infect humans. It is caused by a virus and is found in the United States, Mexico, Central America and parts of South America. The vesicular stomatitis virus (VSV) causes blister-like sores on the mouths or feet of infected animals. The signs of this disease are almost identical to three other important diseases of animals: foot and mouth disease, swine vesicular disease and vesicular exanthema of swine.

What animals get vesicular stomatitis?

Horses, donkeys, mules, cattle, swine, and South American camelids can be affected by VSV. Horses are usually affected the most severely. Sheep and goats are resistant and rarely show signs of disease.

How can my animal get vesicular stomatitis?

VSV can be transmitted by insects (**vector**), especially sand flies and black flies. It can also be transmitted by **direct contact** with infected animals and contaminated objects known as **fomites**. Once VSV has entered a herd, the disease spreads between animals through contact with saliva or fluid from ruptured sores from infected animals.

How does vesicular stomatitis affect my animal?

In animals, VSV causes blister-like sores to form in the mouth, gums, tongue, lips, nostrils, hooves and teats. These blisters swell and break, leav-ing raw tissue that is so painful that infected animals often refuse to eat or drink. When blisters occur around the hooves, lameness can occur. Weight loss usually follows, and in dairy cows a severe drop in milk production is often seen.

Can I get vesicular stomatitis?

Yes. Humans can become infected with VSV when handling infected animals (**direct contact**).

In affected people, vesicular stomatitis causes a flu-like illness with symptoms of fever, muscle aches, headache and weakness. Rarely, humans can get oral blisters similar to cold sores. Recovery usually occurs in four to seven days.

Who should I contact, if I suspect vesicular stomatitis?

In Animals – Contact your veterinarian immediately. Since VSV is so similar to FMD, it requires immediate notification of veterinary authorities.

In Humans – Contact your physician and tell them you have been in contact with animals with VSV.

How can I protect my animal from vesicular stomatitis?

On-farm insect control programs may help reduce the likelihood of disease entering and spreading through a farm. When an outbreak of VSV occurs in a region, horse owners can protect their animals by keeping them on the farm and avoiding contact with other horses. Good sanitation and quarantine practices of affected farms will usually contain the infection until it dies out.
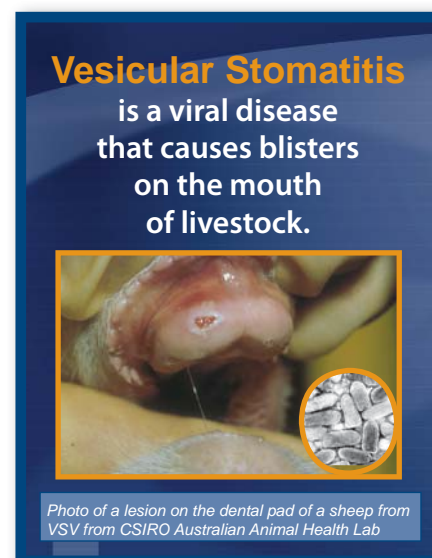
How can I protect myself from vesicular stomatitis?

Use protective measures such as gloves and a mask when handling animals suspected of having vesicular stomatitis. When working with animals, good personal hygiene with frequent hand washing is important in controlling most diseases that can spread from animals to humans.

For More Information

CFSPH Technical Fact Sheets. Vesicular Stomatitis at http://www.cfsph.ia-state.edu/DiseaseInfo/default.htm

U.S. Department of Agriculture. Vesicular Stomatitis at http://www.aphis.usda.gov/lpa/pubs/fsheet_faq_notice/fs_ahvs.html

Vesicular Stomatitis **is a viral disease that causes blisters on the mouth of livestock.**

Photo of a lesion on the dental pad of a sheep from VSV from CSIRO Australian Animal Health Lab

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Section 3

Additional Resources

This protocol for investigation of a foreign animal disease (FAD) gives the

emergency response contact numbers that can be used for all reportable diseases (including anthrax).

Missouri Emergency Response Protocol for Reporting a Foreign Animal Disease

Emergency Response Plan: This plan is for dealing with all foreign animal diseases, including foot-and-mouth disease. The goal of this plan is to detect, control, and eradicate all intentional (agroterrorism) or accidental introduction of the disease. This plan considers presumptive positive cases and confirmed positive cases of any foreign animal disease.

I. Foreign Animal Diseases, including vesicular diseases in cloven-hoofed animals, will be handled as an animal disease emergency. Any practicing veterinarian that suspects a foreign animal disease, including foot-and-mouth disease (FMD), will notify the State Veterinarian's Office at (573) 751-3377 or (573) 526-0860 or contact the State Emergency Management Agency (SEMA) 24-hour duty officer at (573) 751-2748.

II. Any case with an animal exhibiting clinical signs consistent with a foreign animal disease will be reported to the office of the State Veterinarian and/or the USDA Area Veterinarian In Charge (AVIC). The State Veterinarian will immediately dispatch a Foreign Animal Disease Diagnostician (FADD) to the premises. If the reporting veterinarian, based on clinical experience and reasonable judgment, determines that the disease is highly suspicious of an FAD, the State Veterinarian or APHIS Area Veterinarian in Charge may authorize an interim quarantine of the premises by telephone to the reporting veterinarian. The FADD will assess the situation upon arrival at the premises and may confirm the quarantine of the premises should the situation warrant such action in his/her opinion. The FADD will also collect appropriate samples for laboratory analysis. Any veterinarian reporting such an incident must remain on the premises until released by the FADD. The samples will be submitted with the highest

importance and given priority for analysis. The FADD will use clinical signs, history, and professional experience to assess the risk of the disease. Categories of risk will be assigned as (1) Unlikely, (2) Possible, or (3) Highly Likely.

"Unlikely" or "Possible" Risk

(1) An official state quarantine of the premises will be issued until the laboratory result rules out the foreign animal disease.

(2) If the laboratory test is negative the animals will be released from quarantine.

"Highly Likely" Risk

(1) FADD will immediately contact and consult with the AVIC and State Veterinarian.

(2) The submitted samples will be given the highest priority to reach a diagnosis within 24 hours.

(3) A quarantine will be placed on the farm of the index herd.

(4) The FADD will work with the producer on appropriate biosecurity and public health measures.

(5) A movement control zone quarantine of six miles will be placed around the index farm.

(6) Producers on adjacent farms will be notified of the movement control zone quarantine by other regulatory personnel (not the FADD).

The State Veterinarian will take the following actions:

(1) Notify Director, Missouri Department of Agriculture, of the suspicious case.

(2) Consider stopping movement of all animals within the state.

(3) Notify all field veterinarians, State Emergency Management Agency (SEMA), Food Safety and Inspection Service (FSIS), University Extension, and livestock industry partners.

(4) Prepare a press release and notify the Missouri Veterinary Medical Association.

III. A presumptive positive case (animal with clinical signs and initial laboratory positive test for the agent) will initiate the following actions:

(1) State Veterinarian and AVIC will:
- Stop all movement of susceptible species of livestock in the state for 72 hours.

- Initiate depopulation and disposal of infected herd(s). Identified burial sites will be selected to minimize negative environmental impact.

- Provide information to the Missouri Department of Natural Resources on the plan to dispose of dead animals.

- Keep accurate records of depopulated animals for possible indemnity payments at a later time.

- Coordinate with SEMA to achieve Governor's Declaration of Emergency.

- Continue quarantine and movement restrictions.

- Continue active epidemiological investigation and surveillance to detect new cases.

- If appropriate, make decision on use of foot-and-mouth disease (FMD) vaccine to control disease.

(2) SEMA Director will:
- Activate State Emergency Response Plan.

- Assist with coordination of movement control within the State.

- Coordinate with FEMA on Federal Emergency Response Plan Activation.

(3) USDA, APHIS will:
- Activate the National Incident Management System (NIMS).

- Coordinate with other federal agencies on emergency declaration.

- Impose a federal quarantine on the state for interstate commerce.

- Cooperate with the State Veterinarian in identification of a source of infection.

- Coordinate national surveillance.

Reportable Diseases and Follow-Up Guidelines

I. Animal and Livestock Diseases

Reportable Communicable Diseases

The following are diseases that must be reported to state (573) 751-3377 or federal (573) 636-3116 agriculture officials within 24 hours of suspicion or diagnosis:

Avian
- Avian infectious encephalomyelitis
- Avian influenza
- Fowl typhoid (salmonella gallinarum)
- Infectious laryngotracheitis
- Mycoplasma gallisepticum (MG)
- Mycoplasma meleagridis (MM)
- Mycoplasma synoviae (MS)
- Paramyxovirus infection (other than Newcastle Disease)
- Psittacosis (chlamydiosis and ornithosis)
- Pullorum disease (salmonella pullorum)
- Salmonellosis caused by Salmonella enteritidis
- Velogenic viscerotropic Newcastle disease

Bovine
- Akabane
- Anthrax
- Bluetongue
- Bovine babesiosis (Texas fever, piroplasmosis)
- Bovine spongiform encephalopathy (BSE)
- Brucellosis
- Contagious bovine pleuropneumonia
- East Coast fever (coastal fever, theileriosis)
- Ephemeral fever (three-day sickness)
- Foot-and-mouth disease
- Gonderiosis (theileriosis)
- Heartwater
- Hemorrhagic septicemia (Asiatic type 1 shipping fever)
- Ibaraki
- Infectious petechial fever

- Louping Ill
- Lumpy skin disease (pseudourticaria)
- Malignant catarrhal fever
- Paratuberculosis
- Pseudorabies
- Q fever
- Rift Valley fever
- Rinderpest (cattle plague)
- Scabies
- Screwworm
- Sweating sickness (tick-borne toxicosis)
- Tuberculosis
- Trypanosomiasis (nagana)
- Vesicular stomatitis
- Wesselborne disease

Caprine-Ovine
- Bluetongue
- Borna disease
- Brucellosis caused by Brucella meletensis and B. ovis
- Caseous lymphadenitis
- Contagious agalactia of sheep and goats
- Contagious caprine pleuropneumonia
- Foot-and-mouth disease
- Goat and sheep pox
- Gonderiosis (theileriosis)
- Heartwater
- Nairobi sheep disease
- Peste des petits ruminants (kata)
- Screwworm
- Tuberculosis
- Rift Valley fever
- Scabies
- Scrapie
- Vesicular stomatitis
- Visna-Maedi (chronic progressive pneumonia)

Equine

- African Horse sickness
- Babesiosis (piroplasmosis)
- Contagious equine metritis
- Dourine (equine trypanosomiasis)
- Eastern equine encephalomyelitis
- Epizootic lymphangitis
- Equine infectious anemia (EIA)
- Equine piroplasmosis
- Equine rhinopneumonitis
- Equine viral arteritis
- Glanders
- Potomac horse fever
- Venezuelan equine encephalomyelitis
- Vesicular stomatitis
- Western equine encephalomyelitis

All Species

- Anthrax
- Brucellosis
- Exotic myiasis
- Foot-and-mouth disease
- Paratuberculosis (Johnes disease)
- Rabies
- Tuberculosis
- Vesicular exanthema
- Vesicular stomatitis

Porcine

- African swine fever
- Brucellosis
- Foot-and-mouth disease
- Hog cholera
- Porcine babesiosis
- Pseudorabies
- Swine vesicular disease
- Teschen disease (porcine encephalomyelitis)
- Vesicular exanthema
- Vesicular stomatitis

Cervidae

- Chronic Wasting Disease (CWD)

II. Communicable Diseases

The following must be reported to the local public health agency or the Missouri Department of Health and Senior Services during business hours at (573) 751-6113 or after hours/weekends at 800-392-0272 within 24 hours of suspicion or diagnosis:

- Rabies, animal or human

III. Disease From Potential Agents of Bioterrorism:

These diseases are divided into three categories of decreasing priority. Some are considered to be "foreign animal diseases" (e.g., Venezuelan equine encephalomyelitis), and most are zoonotic. A number of these diseases are reportable to state or federal agriculture officials, as noted above. All diseases (animal or human) suspected to have resulted from an act of bioterrorism must be reported immediately to (1) state (573) 751-3377 or federal (573) 636-3116 agriculture officials and/or (2) the Missouri Department of Health and Senior Services (business hours: 573-751-6113; after hours/weekends: 800-392-0272).

Category A

- Anthrax (Bacillus anthracis)
- Botulism (Clostridium botulinum toxin)
- Plague (Yersinia pestis)
- Smallpox (Variola major)
- Tularemia (Francisella tularensis)
- Viral Hemorrhagic Fevers (Ebola, Marburg, Lassa, Machupo)

Category B

- Brucellosis (Brucella species)
- Glanders (Burkholderia mallei)
- Melioidosis (Burkholderia pseudomallei)
- Psittacosis (Chlamydophila psittaci)
- Q Fever (Coxiella burnetii)

- Typhus Fever (Rickettsia prowazekii)
- Viral Encephalitis (VEE, EEE, WEE)
- Toxins (Ricinus communis, Clostridium perfringens, Staphylococcus aureus)

Category C
- Nipah (Nipah virus)
- Hantavirus (Hantavirus)

IV. Recommendations for Veterinarians Examining Animals With Suspected Foreign Animal Disease or Disease Resulting From an Act of Bioterrorism

Veterinarians who examine or treat animals with suspected foreign animal disease (FAD) or disease resulting from an act of bioterrorism (BT) should use infection control precautions to protect the health of themselves, staff, and clients, as well as other animal patients in the area. Generally, animals suspected of having a FAD/BT disease should not be moved from their home premises. If a tentative diagnosis of FAD/BT disease is not made until the animal is brought to a clinic, the animal should be isolated immediately. In either event, veterinarians and staff should wear personal protective equipment (PPE) during the examination. The animal should not be taken to a common treatment room, and all treatments and diagnostics should be performed in the examination room. The number of staff allowed in the exam room and that come in contact with the animal should be limited to as few persons as possible. Veterinarians who do not wish to examine an animal with suspected FAD/BT disease should advise the animal's owner to contact the state agriculture or health department for further guidance.

Infection Control Precautions

The most common routes for transmission of FAD/BT diseases are through direct contact with infected animals and by airborne spread. In addition, all avenues of transmission for some of these agents are not totally understood. When examining animals with suspected FAD/BT disease, veterinarians and staff should use the following precautions:

1. Hand hygiene after all contact with a sick animal and contaminated surfaces.

2. Use of gown and gloves for any contact with the sick animal and contaminated surfaces.

3. Eye protection (e.g., tight-fitting goggles or face shield) if splash or spray of body fluids is likely.

4. Respiratory protection, including a NIOSH-certified N95 filtering disposable respirator (or other respirator offering comparable levels of respiratory protection), for entering the exam room or patient care area. If N95 or comparable respirators are not available, then surgical masks should be worn to protect against transmission through contact or large droplets.

5. Contain and dispose of contaminated waste after consultation with state or local health officials. Do not dispose of waste in landfills or dumps.

6. Handle used patient-care equipment in a manner that prevents contamination of skin and clothing. Ensure that used equipment has been cleaned and reprocessed appropriately.

7. Ensure that procedures are in place for cleaning and disinfecting contaminated environmental surfaces. EPA-registered detergent-disinfectants currently used by healthcare or veterinary facilities for environmental sanitation may be used. Manufacturer's recommendations for dilution (i.e., concentration), contact time, and care in handling should be followed.

8. Handling of laundry (e.g., towels, clothing) should be evaluated on a case-by-case basis. For many agents, laundry may be washed in a standard washing machine with hot water and detergent. The use of chlorine bleach during hot-water washing can provide an added measure of safety. Washing laundry contaminated with a resistant form of an organism (e.g., spore formers) may not be sufficient. The state agriculture or health department may be

consulted for guidance. Care should be used when handling soiled laundry to avoid direct contact with contaminated material. Soiled laundry should not be shaken or otherwise handled in a manner that may aerosolize infectious particles.

V. Risk Communication

In the event of a FAD/BT disease, it is highly advisable not to talk to media or to release information to other individuals or agencies that do not have the "need to know." It is important that information be communicated accurately and in a timely manner to the media, public, and decision-makers, but this is best accomplished by using public information resources available through state and federal agencies. Failure to control the message could result in misinterpretation of data, distortion of events, and information being taken out of context.

General Signs of Reportable Animal and Poultry Diseases

I. Vesicles/erosions on tongue, nose, lips, feet, teats
- Foot-and-mouth disease
- Vesicular stomatitis
- Swine vesicular disease
- Bluetongue of cattle
- Sore mouth (contagious ecthyma) of sheep and goats
- Bovine virus diarrhea
- Malignant catarrhal fever
- Vesicular exanthema of swine
- Rinderpest
- Contagious foot rot of sheep

II. High herd/flock morbidity, low fatality
- Foot-and-mouth disease
  - Cattle, swine, sheep, goats, all cloven hoofed susceptible
  - Does not occur in horses
  - 100 percent herd incidence in the U.S.
  - Less than 1 percent fatality, higher in calves
- Vesicular stomatitis
  - Higher morbidity and more severe in horses than cattle or swine
  - Lower morbidity and severity in cattle and swine than FMD
  - Sheep and goats rarely infected

III. High herd/flock morbidity, high fatality
- Hog cholera
- African swine fever
  - Eradicated from Western Hemisphere
  - Virus with lower virulence has emerged
- Exotic Newcastle disease
- High pathogenic avian influenza
- Rift Valley fever (morbidity and fatality variable among outbreaks)
- Rinderpest

IV. Low morbidity, high fatality
- Anthrax
- Scrapie
- Bovine spongiform encephalopathy

- Chronic wasting disease

V. Abortion storms not associated with known pathogens for the location
- Rift Valley fever, early sign in sheep and cattle, may be sentinels for impending human disease
- Q fever, abortions of sheep, goats and cattle, late pregnancy, usually the only sign in animals, shorter incubation than disease in humans so may precede human disease
- Brucella abortus in cattle (always remain vigilant!)

VI. Unusual respiratory sounds in a poultry house
- Avian influenza
- Exotic Newcastle disease

VII. Acute onset, rapid infection
- Foot-and-mouth disease
- Hog cholera
- Rift Valley fever
- African swine fever
- Swine vesicular disease
- Rinderpest

VIII. Central nervous system signs
- Viral encephalidities (eastern, western, Venezuelan, West Nile)
- Hog cholera
- Scrapie
- Bovine spongiform encephalopathy
- Botulism

IX. Fly larvae (maggots) in living tissue
- Screwworms

X. Sudden death without clinical signs
- Anthrax
- Rinderpest

Missouri Veterinary Medical Association
Emergency Management and Public Health Committee
Biosecurity of Veterinary Practices

Practitioners, their staffs and technicians must be aware of the clinical signs of the important foreign animal diseases so that they are able to suspect a potentially dangerous disease seen on a farm call, in the clinic or by client description over the telephone. Education of veterinarians and staffs should focus on vesicular diseases, all of which are reportable, hog cholera, which might look like any other highly contagious and deadly swine disease, highly contagious poultry diseases and anthrax.

Clinic and Hospital Biosecurity

· Carefully screen new employees; double check education and employment histories.

· Be aware of repeated visits by strangers and unrecognized vehicles in the vicinity.

· Build a perimeter fence; possibly install a security system

· Limit internal traffic between large animal and small animal facilities; place disinfectant tubs with boot brushes for use between facilities.

· Carefully screen unknown visitors, prohibit entry to animal facilities and be aware of animal extremist organizations.

· Livestock arriving at the large animal facility should be observed for signs of obvious abnormalities before unloading.

· Emergency contact phone numbers should be posted in the clinic and carried in practice vehicles. A wallet-size card with these contact numbers was mailed to all veterinarians in Missouri by the MVMA.

Vehicle and Livestock Facility Biosecurity

· Wear clean outer clothing and disinfect boots when entering and leaving livestock facilities. Livestock producers expect this level of biosecurity.

· Carry Virkon-S disinfectant, boot tub and brush, clean coveralls, disposable nitrile gloves, surgical masks and caps, and a two-gallon garden sprayer for external disinfection of the vehicle if necessary.

· Boots must be brushed clean with disinfectant. It is very difficult to sterilize fecal material.

· When entering a premises where vesicular or other highly contagious disease is suspected, wear disposable coveralls and plastic overboots which can be left at the facility for burning or other disposal.

· Elasta-A-Boots are tough quality plastic disposable boots, about $0.50 a pair, and Disposable Coveralls, about $1.25 a pair, are available from Nasco and other farm supply outlets. These items should be routine equipment in vehicles and clinics.

· If a reportable disease is suspected it is best to park the practice vehicle at the farm perimeter.

· If a reportable disease is suspected, state authorities must be contacted immediately beginning with the State Veterinarian, followed by others in order as listed in "Contacts for Animal Emergencies" if necessary. The first contact should always be the Office of the State Veterinarian.

· After reporting the disease, the veterinarian should remain on the farm until the arrival of the Foreign Animal Disease Diagnostician (FADD)..

· Contaminated clothing should be placed in a heavy plastic bag and washed in hot water with mild bleach. Plastic coveralls and boots left at the suspect farm for burning or other disposal.

· Veterinarians should emphasize farm biosecurity to clients.

Missouri Veterinary Medical Association
Emergency Management and Public Health Committee

Suggestions to Protect Your Livestock Operation

Restrict human traffic to farmstead
· Have a secure perimeter fence, limit entry to one gate.
· Post a sign forbidding entry without permission. Have visitors sign a register.
· Be aware of repeat sightings of unknown persons and vehicles near the farm
· Supply a tub of disinfectant, freshen daily, and a brush for scrubbing footwear.
· Provide plastic over-boots for visitors.
· Footwear worn away from the farmstead to any place where livestock are present should be
  scrubbed and disinfected before reentering the farm.

Restrict vehicle entry to farmstead
· Stop all nonessential vehicles from entering the farm and arrange whenever possible for collection
  and delivery of supplies to take place at farm boundary.
· If a vehicle must enter the farmstead make sure that prior to entry their wheels are sprayed with
  disinfectant.
· Livestock haulers should clean, disinfect and let dry as long as possible between loads.
· Identify an off-farm site for the livestock farm delivery and commercial pickup of animals for
  rendering.
· Keep a record of all deliveries. In the event of a disease being confirmed this may help in
  identifying the source.
· Ensure that sources of feed and bedding are protected and that samples of delivered feed are
  "banked" for future analysis in case of an animal disease outbreak.

Keep record of stock movement onto and off the farm
· Participate in the premises and livestock identification program.
· Verify health and origin of purchased livestock.
· New stock entering the herd should be quarantined, observed for 30 days and tested as suggested
  by your veterinarian prior to entry.
· Keep complete records of all stock movement onto and off of the farm.
· Each farm premises must be treated as a separate unit; record animal movement between units.
· Avoid contact of your farm animals with those of your neighbors.

Keep dogs, cats, birds, wild game and vermin under control
Since other animals and birds can serve as a source of disease entering the herd it is vitally
important to make every effort for their elimination or control.

Provide for family and animal health and comfort
The farm should have an emergency 3-day supply of food and drinking water
and feed for animals and poultry.

Report any unusual signs of animal sickness or death to your veterinarian.

Disinfection of Premises and Fomites

First, remove organic matter; scrub with soap and water.

Virkon S

- The only disinfectant labeled for foot-and-mouth disease. Works fairly well in organic matter
- Effective against hog cholera virus, many other viruses, bacteria, and fungi.
- Needs 5 – 10 minutes contact time, long activity on hard surfaces
- Comes as a powder, follow directions for 1 percent solution for all uses
- Should be mixed fresh, lasts about five days, color changes when losing potency, test strips included

Bleach/Hypochlorite

- 3 percent dilution, 1/2 cup to 1 gallon water, mix fresh for each use
- Effective against FMD and hog cholera viruses and most other viruses, bacteria, fungi, and spores; requires 10 minutes contact time, inactivated by organic matter
- Inexpensive, Clorox best brand for use

Iodophores and Iodine

- Not effective against foot-and-mouth virus
- Kills most viruses, bacteria, and fungi; inactivated by organic matter
- Requires 10 minutes, contact time
- Questionable efficacy against hog cholera and other swine viruses
- Expensive

Chlorhexidine; Nolvasan

- Questionable effect against FMD virus, not effective against some other bacteria and viruses, not effective against spores
- Requires 10-minute contact time
- Inactivated by organic material

Quaternary Ammonium Compounds

- Roccal D or Roccal
- Not effective against FMD virus; otherwise, kills a wide range of bacteria, viruses, and fungi; does not kill spores
- Requires 10 minutes, contact time
- Works well in organic matter at neutral or high pH
- May be combined with detergents
- Toxic to cats

White Vinegar

- Use 1 gallon per gallon water, works well against FMD virus
- 5-minute contact time

Sodium Carbonate (soda ash, washing soda)

- Strong alkalizing agent
- Effective in dry powder form or as 4 percent solution to disinfect FMD virus-contaminated barns, pens corrals, etc.; caution when applying (surgical mask, coveralls, gloves)

Vesicular Diseases Reference Chart

	Foot-and-Mouth Disease	Vesicular Stomatitis	Swine Vesicular Disease	Vesicular Exanthema of Swine
Etiology	Aphthovirus	Vesiculovirus	Enterovirus	Calicivirus
Geographic Distribution	Endemic in Asia, Africa, Middle East, parts of South America; U.S. free since 1929	North & Central America, northern South America	Many European countries	U.S. only (eradicated in 1956)
Transmission	Respiratory aerosols; oral consumption; direct and indirect (fomite) contact	Insect vectors (sand flies & black flies); contact, aerosol in humans	Ingestion of contaminated meat; contact with animals, feces	Ingestion of uncooked garbage contaminated with pork
Incubation Period	Ingestion 1-3 days, Exposure 3-5 days	Animals 3-5 (up to 21) days, Humans 24-48 hours	Ingestion 2-3 days, Exposure 2-7 days	18-72 hours
Clinical Signs by Species	All vesicular diseases produce a fever with vesicles that progress to erosions in the mouth, nares, muzzle, teats, and feet. These 4 diseases are clinically indistiguishable from each other, particularly in swine.			
Notification	State & Federal Veterinarians should be contacted IMMEDIATELY and informed of suspicions			
Cattle	**Disease Indicators** Oral & hoof lesions; salivation, drooling; lameness; abortions; death in young animals; "panters"	Vesicles in oral cavity, mammary glands, coronary bands, interdigital space	Not affected	Not affected
Pigs	**Amplifying Hosts** Severe hoof lesions; hoof sloughing; snout vesicles; less severe oral lesions	Same as cattle	Severe signs in animals housed on concrete; lameness; salivation; neurological signs; more severe in young	Deeper lesions with formation of granulation tissue on the feet
Sheep & Goats	**Maintenance Hosts** Mild signs if any	Rarely show signs	Not affected	Not affected
Horses, Donkeys, Mules	Not affected	Most severe with oral and coronary band vesicles; drooling; rub mouths on objects; lameness	Not affected	Not affected
Humans	Not common	Flu-like signs, headache, rare oral blisters	Not affected	Seroconversion and mild meningitis in one lab worker
Clinical Summary	Salivation and lameness with vesicles; Equidae not affected	Horses are affected; less contagious so spread is slower; lesions in one area of body	Pigs only; mild lesions; no mortality	Pigs only; deeper lesions; low mortality

Vesicular Diseases Reference Chart- Additional Information

|	Foot-and-Mouth Disease	Vesicular Stomatitis	Swine Vesicular Disease	Vesicular Exanthema of Swine
Morbidity & Mortality	Morbidity 100%; Mortality less than 1%, severe in young	Morbidity varies, up to 90%; Mortality low; death in young less common	Morbidity is low; lesions less severe; Mortality not a concern	Morbidity varies, up to 100%; Mortality is low
Differentials	Rinderpest, bovine herpes virus 1 (IBR), BVD, bovine papular stomatitis, malignant catarrhal fever, bluetongue, contagious ecthyma, lip and leg ulceration, foot rot, chemical and thermal burns			
Post-Mortem Lesions	Single or multiple vesicles, ruptured vesicles with demarcation line, "dry" lesions in pig oral cavity, coronitis, hoof wall separation, "Tiger heart" lesions, rumen pillar lesions	Similar to FMD, but without heart and rumen lesions	Similar to FMD	Similar to FMD
Sample Collection	Before collecting or sending any samples, the proper authorities should be contacted. Samples should only be sent under secure conditions to authorized laboratories to prevent spread.			
Prefer	Epithelium from unruptured or recently ruptured vesicles in proper medium			
Additional Samples/ Tissues	Esophageal-pharyngeal fluid (cattle) or throat swab (pigs), 5ml blood with anticoagulant; 10ml for serum, lymph nodes, thyroid, adrenal gland, kidney, heart in formalin	Vesicular fluid collected aseptically and frozen	Vesicular fluid collected aseptically and frozen; unclotted whole blood from febrile animals; fecal and serum samples from infected and noninfected animals	Vesicular fluid collected aseptically and frozen; unclotted whole blood from febrile animals; fecal and serum samples from infected and noninfected animals
Sample Packaging	Caution with dry ice as carbon dioxide will inactivate the virus	Virus inactivated by 1% formalin		
Disinfection	2% sodium hydroxide (lye), 4% sodium carbonate, 5% acetic acid; 6% hypochlorite; Resistant to iodophores, quaternary ammonium compounds, and phenol, especially with organic matter present.	2% sodium hydroxide (lye), 4% sodium carbonate, 2% iodophores, chlorine dioxide	10% formalin, 2% sodium hydroxide (lye), iodophores, chlorine dioxide	Organic matter: 1% sodium hydroxide combined with detergent No Organic Matter: oxidizing agents and iodophores with detergents
Prevention & Control	Destroy litter and susceptible animal products	Control insects, no movement of animals from farm for 30 days		

	Exotic Newcastle Disease (END) ▼	**Highly Pathogenic Avian Influenza (HPAI)** ▼
Importance	Highly contagious, often fatal disease	
Organism	Avian *paramyxovirus-1*	Type A Influenza virus, Orthomyxovirus; Classified by surface antigens H and N
Clinical Signs in Birds	END and HPAI are clinically indistinguishable from each other **Respiratory:** Coughing, sneezing, nasal discharge **Digestive:** Watery diarrhea **Nervous:** Depression, ataxia, torticollis Sudden death without clinical signs, decreased egg production, thin-shelled eggs	
Clinical Signs in Humans	Mild conjunctivitis	Mild to fatal disease
Transmission	Spread by feces and respiratory discharges, direct contact, aerosolization and fomites.	
Differential Diagnosis	**Poultry:** HPAI, fowl cholera, infectious coryza, fowl pox, avian chlamydiosis, infectious laryngotracheitis, mycoplasmosis, infectious bronchitis, management problems. **Psittacines:** Avian chlamydiosis, Pacheco's disease, avian influenza, salmonellosis, toxicosis.	END, infectious laryngotracheitis, acute bacterial diseases (eg. fowl cholera and *E. coli* infections)
Morbidity/ Mortality	Mortality can reach 100%; Morbidity can reach 90%	Mortality can reach 100%; Morbidity can reach 100%
Diagnosis	Virus isolation required for definitive diagnosis	
Sample Collection	Before collecting or sending any samples, the proper authorities should be contacted. Samples should only be sent under secure conditions to authorized laboratories to prevent spread.	
Prefer	Tracheal or cloacal swabs from live or dead birds, as well as feces.	
Notification	State & Federal Veterinarians should be contacted IMMEDIATELY and informed of suspicions	
Quarantine	Suspected animals, areas, farms will be quarantined by the state veterinarian.	
Vaccination	Routine in poultry flocks. Will not prevent infection or virus shedding.	Costly; no cross protection; may result in reassortment viruses. Inactivated H5 vaccine licensed in US for emergency use
Disinfection	Virus killed by extremes of pH, heat, dryness. Phenolics (eg. One Stroke Environ), oxidizing agents (eg. Virkon) and quaternary ammonium compounds (eg. Roccal-D Plus) Halogens (eg. 6% household bleach) Biguanides (eg. Nolvalsan-S) Ultraviolet and sunlight	Aldehydes in presence of organic matter Dilute acids (eg. paracetic acid)

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Section 4

Photographs
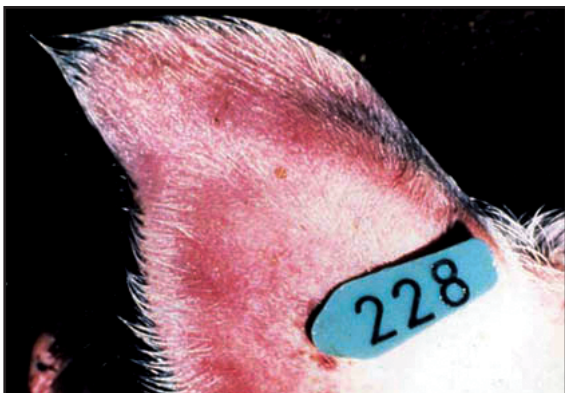
African Swine Fever

ASF 2—Pig with African swine fever. Reddened skin on the extremities is a non specific lesion associated with a septicemic/viremic condition.

African Swine Fever

ASF3—Pig with African swine fever. Necrosis of the skin is a frequent lesion in chronic ASF.

Avian Influenza, Highly Pathogenic

HPAI 1—Edema of the wattles of a chicken with highly pathogenic avian influenza.

Avian Influenza, Highly Pathogenic

HPAI 2—Cyanotic comb of a chicken on the left with highly pathogenic avian influenza compared with a normal chicken on the right.

Avian Influenza, Highly Pathogenic

HPAI 3—Congestion and petechiae in the skin on the hocks and shanks of a chicken with highly pathogenic avian influenza.

HPAI3
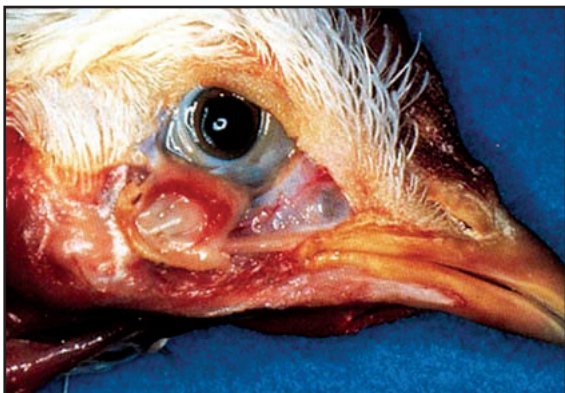
Exotic Newcastle Disease

ND 1—Edema and hemorrhage in the reflected lower eyelid of a chicken with exotic Newcastle disease.

ND1

Foot-and-Mouth Disease, Bovine

FMD 1—Excessive salivation in a cow 24 hours after experimental inoculation with foot-and-mouth disease virus. Note the ring of saliva on the floor.
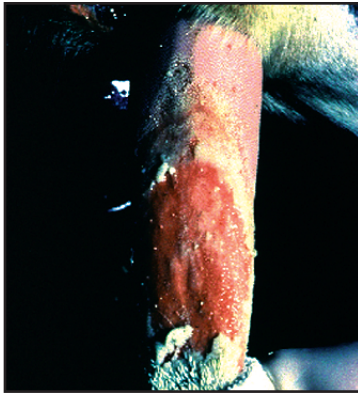
FMD1

Foot-and-Mouth Disease, Bovine

FMD 2—Tongue of the same cow with three small, unruptured vesicles about 36 hours after inoculation.
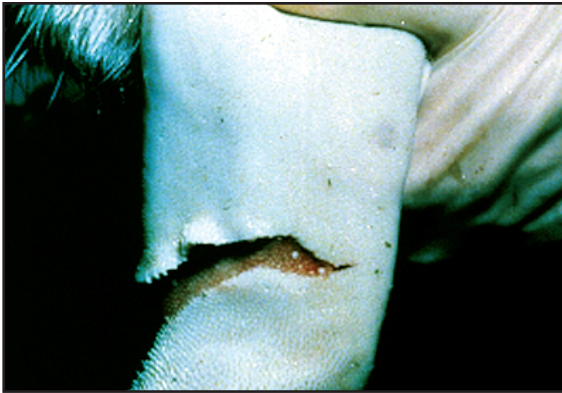
FMD2

135

Foot-and-Mouth Disease, Bovine

FMD 3—The tongue of the same cow five days after inoculation. Most of the white, blanched epithelium has sloughed off. There are still fragments of white, necrotic epithelium on the surface of the tip of the tongue.
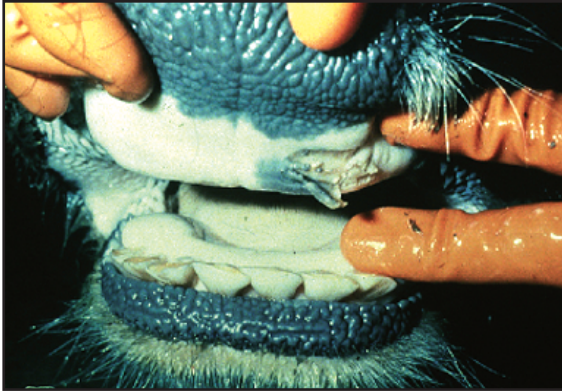

Foot-and-Mouth Disease, Bovine

FMD 4—Cow with foot-and-mouth disease. Tongue with a large ruptured vesicle — excellent specimen for diagnosis.

Foot-and-Mouth Disease, Bovine

FMD 5—Cow with foot-and-mouth disease. Ruptured vesicle in the gingiva of the dental pad. This sign could be confused with a traumatic injury. The tags of epithelium should be collected for a diagnostic specimen. This lesion is similar to bluetongue in cattle.

Foot-and-Mouth Disease, Bovine

FMD 6—Cow with foot-and-mouth disease. Blanching and vesicles along and above the coronary band of both claws. Note that the vesicles join over the interdigital space.

Foot-and-Mouth Disease, Swine

FMD 9—In swine the feet are more severely infected from foot-and-mouth disease virus than the nose and tongue. The primary clinical sign in swine is lameness, as shown by walking on the knees.

Foot-and-Mouth Disease, Swine

FMD 10—Pig with foot-and-mouth disease. The vesicle in the coronary band has ruptured, and the area above the coronary band has eroded.

Foot-and-Mouth Disease, Swine

FMD 12—Pig with foot-and-mouth disease. The whitish areas on the surface of the tongue are dry FMD lesions. Oral lesions are less frequent in pigs than in cattle, but when they do occur in pigs, the lesions are usually the dry type.

Hog Cholera/Classical Swine Fever

HC 1—Conjunctivitis and exudate in the medial canthus of a pig infected with hog cholera.

Rift Valley Fever

RVF 1—Fetuses can be aborted at any stage of gestation in cattle with Rift Valley fever.

Swine Vesicular Disease

SVD 1—Erosions on the tongue of a pig with swine vesicular disease are similar to those resulting from foot-and-mouth disease.
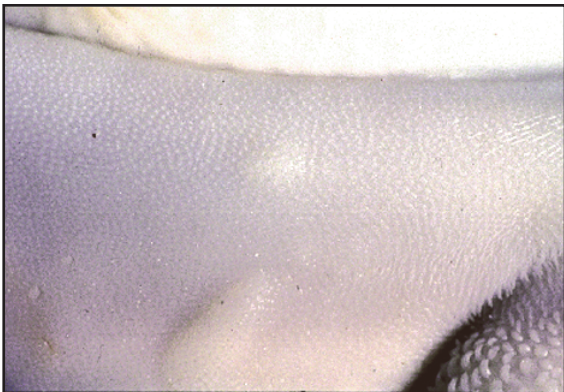

Swine Vesicular Disease

SVD 2—Ruptured vesicles on the heel of a pig with swine vesicular disease are indistinguishable from foot-and-mouth disease.

Vesicular Stomatitis

VS bov 2—Vesicles on the tongue of a cow with vesicular stomatitis.

Vesicular Stomatitis

VS bov 3—Vesicles and erosions on the teats of a cow with vesicular stomatitis.

Vesicular Stomatitis

VS eq 5—Erosions and ruptured vesicles on the gingiva of a horse with vesicular stomatitis.

Vesicular Stomatitis

VS eq 7—Erosions and dried exudate on the coronary band of a horse with vesicular stomatitis.

Vesicular Stomatitis

VS por 8—A large vesicle on the snout of a pig with vesicular stomatitis.

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Notes