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What Veterinarians Need to Know About West Nile Virus

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The identification of West Nile virus (WNV) in New York City during the summer of 1999 was the first report of West Nile virus in the Western Hemisphere. Documented West Nile virus infections in the United States for 1999 and 2000 totaled 83 cases in humans. Four hundred thirteen confirmed cases and three unconfirmed equine cases were reported from January through Nov. 20, 2001.

At least 71 of 295 horses, for which an outcome has been reported, died or were euthanatized. Seven human fatalities were reported in 1999 and 2000. Since 1999, West Nile virus infection in horses has been detected in 19 states (through November 2001), including Iowa, Missouri, Illinois, and Indiana. With mosquito season soon approaching and the recent detection of WNV in several Midwestern states, the arrival of the virus in Kansas may be imminent.

History

West Nile virus is an emerging disease in the United States that is known to infect humans, birds and horses. The method of spread of West Nile virus to the Western Hemisphere is unknown, but is has been speculated that migration or importation of infected birds and/ or mosquitos to the United States is likely. Controversy exists whether viremic migratory birds contribute to the movement of West Nile virus because determining the intensity and duration of viremia in naturally infected wild birds is problematic.

Classification

West Nile virus is a member of the genus *Flavivirus*, family Flaviviridae, and a member of the Japanese encephalitis complex. The Japanese encephalitis complex is composed of Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, and Kinjin virus, based on amino acid homology of the viral envelope proteins. A high level of cross-reactivity may be observed during serologic testing due to the close antigenic relationship between these viruses.

Transmission

Transmission of West Nile virus has only been proven to occur through the bite of infected mosquitoes. No experimental evidence has shown that the virus can be transmitted between humans, horses, and birds by direct or indirect contact. A mosquito vector appears to be necessary for transmission of the disease, but raptors dying with WNV are suspected to have been infected with the disease from ingestion of infected birds. A high, persistent level of viremia, high tissue viral titers, and significant morbidity and mortality are reported for infected birds, which serve as amplifying hosts for the disease.

A recent report of experimental infection of 12 horses exposed to the bites of infected *Aedes albopictus* mosquitoes with West Nile virus indicates that the viremias of West Nile virus- infected horses are of low magnitude and short duration. Human infections with WNV also typically have a short duration with a low level of viremia. Therefore, researchers have concluded that WNV-infected horses and humans are unlikely to serve as important amplifying hosts of natural infections.

But despite the lack of evidence for direct contact from horse to human and bird to human, it is recommended to avoid bare-handed contact with alive or dead animals.

According to the Centers for Disease Control, West Nile virus does not appear to cause extensive illness in dogs or cats. The seroprevalence of West Nile virus in New York in 1999 was reported to be low in dogs and cats. The CDC also reported that there has been no documentation of infection in dogs and cats by eating dead infected birds or other animals. Reuters Health Information recently issued a report that the viral load in frogs and lizards is low, and most likely not infectious to feeding mosquitoes. Low infection rates of other naturally infected animals have been observed, which include cats, bats, chipmunks, domestic rabbits, squirrels, and skunks. The full spectrum of species susceptible to West Nile virus is as yet unclear.

Transmission of West Nile virus typically occurs from June to September, and maximal transmission peaks in July, depending on the geographical area. It has been shown that the virus persists year-round in some species of overwintering mosquitoes. The mosquito species that is most frequently implicated in WNV transmission cycles is *Culex pipiens pipiens*, the common house mosquito. West Nile virus also has been isolated from *Aedes* sp. and *Anopheles* sp. of mosquitoes. The role

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Also in this issue

Thank you to the Pfizer Animal Health Group, Livestock Division, Cattle Products Group, for financial assistance in publishing this newsletter.

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of other mosquito species and other blood feeding arthropods (ticks, blackflies, mites, lice) is still unknown. WNV has been isolated from a few species of ticks.

Human infections

Humans may have asymptomatic infections or present with an acute, mild flu-like illness. Clinical signs frequently reported include fever, headache, conjunctivitis, lymphadenopathy, myalgia, rashes, altered consciousness and severe muscle weakness. Flaccid paralysis, encephalitis, meningitis, and meningoencephalitis were rarely reported, but do occur. The incubation period for WNV in humans is usually 3 to 15 days. Immunocompromised individuals infected with WNV, including the young and elderly, are more likely to develop clinical disease.

Avian infections

Clinical signs of West Nile virus in birds may include no signs to erratic behavior from encephalitis and/or meningoencephalitis. Crows and other closely related *Corvus* sp. seem to be most susceptible to the virus. Birds that are found dead may show evidence of trauma, and should still be submitted for West Nile virus testing. A high avian fatality rate was first observed in New York in 1999, which differed from other known West Nile infections. The cause for the high avian mortality rate is unknown, but it has been hypothesized that the birds in the Western hemisphere are not evolutionarily selected for West Nile virus resistance or possibly the strain(s) of the virus circulating in the United States have enhanced virulence. If a wild bird is found dead, it is recommended to call the West Nile virus information hotline for information on how to submit the bird for testing. Phone numbers are listed at the end of this article. Currently, many states, including Kansas, are using chickens as sentinel animals to monitor enzootic transmission and to predict epizootic and epidemic West Nile virus activity.

West Nile virus infection in horses

Clinical signs

The most frequent clinical signs reported in horses, include twitching of the face and/or muzzle, muscle fasciculations, ataxia, limb weakness, circling, proprioceptive deficits, difficulty rising or recumbency, facial paralysis, teeth grinding, dysphagia, blindness, or acute death. Results of experimental infections indicate anti-West Nile virus antibodies become detectable 8 to 10 days after infection.

Prognosis

The onset, progression, and outcome of the disease are believed to depend on a number of factors, including the level of viremia, age of the horse, and the presence of other concurrent diseases. A few case fatality rates were reported for West Nile infected horses with clinical disease, which ranged from 40 to 45 percent.

Differential diagnoses in horses should include rabies (rabies should be considered the primary differential), equine protozoal encephalomyelitis (EPM), western equine encephalomyelitis (WEE), eastern equine encephalomyelitis (EEE), Venezuelan equine encephalomyelitis (VEE), other arbovirus encephalitidies, equine herpes virus type 1 (EHV-1), equine degenerative myeloencephalopathy, botulism, toxicities, and cervical vertebral stenotic myelopathy.

Gross lesions in horses may be absent, but if lesions are observed they typically consist of hemorrhages present in the gray matter of the pons, medulla oblongata, cerebellum, thalamus, caudal brain stem, and the ventral horns of the spinal cord. Petechial to ecchymotic hemorrhages may also extend multifocally throughout the entire spinal cord.

Diagnosis

Serum samples from horses (collected in a 10 ml red-top tube or clot-separator tube) and CSF samples (in a red-top tube labeled with the site of collection) should be submitted for detection of antibodies against WNV.

Several laboratory methods are used to detect WNV infections in horses. The IgM-capture enzyme-linked immunosorbent assay (ELISA) on serum and CSF fluid is designed to detect antibodies to WNV. The first serum sample should be drawn as soon as possible after the onset of clinical signs, and the second serum sample should be drawn at least seven days after the first. The IgM capture ELISA test may cross-react with other closely related flaviviruses. IgM antibody in equine serum is relatively short-lived, and a positive IgM-capture ELISA indicates that exposure to WNV or a closely related flavivirus has occurred, probably within the last three months.

Several laboratory methods are used, in addition to the IgM-capture ELISA, to detect WNV infections in horses. Virus neutralization antibody titers, polymerase chain reaction testing (PCR), plaque assays, plaque reduction neutralization tests (PRNT), virus isolation, immunofluorescent antibody testing on tissues and isolates, and immunohistochemistry are used for WNV diagnosis, but are not available in all laboratories.

Prevention and control

Vaccination

A conditional license for a killed West Nile Virus vaccine was issued by the Animal and Plant Health Inspection Service (APHIS) to Fort Dodge pharmaceuticals to aid in the prevention of the disease caused by West Nile virus in horses. APHIS will continue to evaluate potency and efficacy data throughout the conditional licensure period of one year. The product is licensed for intramuscular use in horses. Two, 1cc doses are required, which should be given three to six weeks apart. Annual and biannual (spring and fall) vaccination is recommended thereafter.

Mosquito control

The main aim when attempting to reduce mosquito-breeding sites is to remove potential sources of stagnant water. Advise owners to thoroughly clean livestock and horse watering troughs monthly. Larvicides and larvicidal fish may be used in ponds or ornamental pools. Local mosquito control authorities may be able to help in assessing the mosquito breeding risks and recommend methods to reduce mosquito populations associated with a specific property. Reduced exposure to adult mosquitoes may be attempted by using insect repellents. Products containing DEET are not recommended for horses. Less practical recommendations for some horse owners include using screened housing and reducing outdoor exposure.

Sampling methods and safety precautions

West Nile virus is categorized as a biosafety level 3 agent, which limits laboratory testing by some laboratories. Safety precautions when working with horses or any mammal with central nervous system clinical signs should include all precautions normally taken when rabies virus is suspected.

Although horses are not believed to play a significant role in amplification of the virus, precautionary measures should be taken when performing postmortem examinations of horses and submitting samples from these horses. Safety precautions when performing postmortem examinations should be focused on preventing viral contact with open wounds and mucous membranes. Aerosol transmission is thought to be very unlikely, however it is recommended by APHIS to take precautions

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Three-step program targets herd eradication of BVDV infection

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Studies have established that persistently infected (PI) cattle are the major source of bovine viral diarrhea virus (BVDV) in cattle populations. Persistent infections occur when a fetus is infected with a noncytopathic strain of BVDV during approximately the first 125 days of gestation. PI calves are a constant threat to herd health and profitability because they typically shed BVDV on a lifelong basis, often in large amounts, and are the principal means by which BVDV herd infection and enzootic disease are perpetuated. If BVDV is present before the fetus develops immunocompetence, the virus is recognized as self and is allowed to survive and replicate. Should the fetus survive, the calf will be born viremic, shedding BVDV in all body secretions and excretions. Protection of the dam may or may not correlate with protection of the fetus against subsequent persistent infection if viremia of the dam occurs. True prevention and control of BVD presupposes elimination of PI cattle.

An effective BVDV control program is made up of three distinct disease management practices that will dramatically reduce losses associated with BVDV and will result in eventual eradication of the virus from herds. Close communication with diagnostic laboratory personnel will help to assure appropriate sampling and testing are accomplished.

Step 1: Herd Monitoring

Monitoring (screening) of herds that do not have a history of clinical BVDV infection is done to assess whether BVDV is circulating in the herd, to assess whether PI cattle are present, and to serve as a tool for the ongoing detection of lapses in execution of any BVDV control practice.

The use of paired serum samples, single serum sample surveys (including use of sentinel animals) and use of diagnostics from animal losses all can be used in effective herd monitoring plans. The use of BVDV RNA probes (PCR) to detect virus in bulk milk tanks is effective for monitoring status of lactating dairy cows.

Step 2: Testing and Culling Infected Cattle

Whereas herd monitoring is conducted to determine whether BVDV is circulating through a herd, herd-based laboratory diagnostic testing is done to identify PI cattle so that they can be removed from the herd. This second control step is initiated when a herd monitoring program suggests that BVDV is present. Any of the following diagnostic tests can be used to identify PI cattle in beef or dairy herds:

- Virus isolation using standard microplate isolation techniques. This highly specific test requires a serum sample taken from calves that are at least 2 months of age, when the level of interfering maternal antibodies have declined.
- Immunohistochemistry (IHC) on skin samples. The required biopsy sample is an ear notch that can be collected from any age animal.
- BVDV RNA probes (PCR) on serum, blood or bulk-tank milk samples. Also highly sensitive, RNA tests can be used to test individual animals using serum or whole blood samples, or to test entire herds using pooled serum samples or bulk-tank milk samples. Test sensitivity is unaffected by the presence of antibody; however, false positive results may occur.
- ELISA tests on serum and skin are offered or are under development in some laboratories. Please communicate with your diagnostic laboratory about these and other tests that may become available.

Tests should be employed on any new additions to a herd, including new bulls. Elimination of PI animals before they are commingled with the herd is an important control point that should be incorporated into every herd biosecurity plan.

In a beef herd where monitoring procedures show that BVDV is circulating, laboratory testing to identify and remove PI carriers should be conducted at least two weeks before the start of the breeding season to prevent contact between PI cattle and pregnant cows. All bulls, females without calves, and calves from the current calf crop (but not their dams) should be tested. Calves that are BVDV positive should be sold for slaughter. Their dams must be tested, with those testing positive (indicating PI status) sold for slaughter.

Testing in a dairy operation also focuses most heavily on the calf population. Initially all cattle should be grouped by age to prevent contact between calves and pregnant cows until PI testing is complete. Nonlactating cattle should be tested individually. Animals testing positive should be sold for slaughter. Initial testing of lactating cattle can be accomplished inexpensively through bulk-tank milk testing. If results of the bulk-tank test are positive, lactating cows, replacement heifers, calves, and all other herd cattle must be tested individually.

Step 3: Targeted BVDV Vaccination

The final step in a BVDV control program is the use of effective BVD vaccines. Even in herds where screening and testing procedures are used to identify and remove PI cattle, BVD vaccination should continue so that susceptible cattle will be protected if BVDV is somehow reintroduced to the farm or ranch.

BVDV biosecurity programs, utilizing all appropriate tools and strategies, should be implemented in all cow-calf and dairy herds to reduce or eliminate risk for losses from BVDV, especially from fetal infections resulting in PI calves.

Herds shown to be free of PI BVDV animals and BVDV infections by monitoring methods are still at risk for BVDV losses, including the fetal infections that result in PI calves. The biosecurity plans also may be appropriate for beef herds at low risk for PI infections since complete BVDV control programs may be economically unfeasible for some of these operations. In these low-risk herds, producers can effectively reduce the threat of losses related to BVDV by using an acceptable monitoring plan, testing for PI status of all herd additions, and specially targeting the BVD vaccination program to the prevention of fetal infections.

Classical Swine Fever Potentially Devastating for U.S.

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Editor's Note: The following article is part of a continuing series on foreign animal diseases. Previous issues of the Kansas Veterinary Quarterly have reviewed foot and mouth disease, vesicular stomatitis and rinderpest. With all suspect foreign animal diseases, the attending veterinarian should immediately contact state or federal animal health authorities.

Historically, the disease referred to as swine fever in most of the world has been called hog cholera in the United States. More recently, the word "classical" was added to the name to differentiate swine fever (hog cholera) from African swine fever, which is clinically indistinguishable from classical swine fever. Because the use of the acronym HCV for hog cholera virus could be confused with hepatitis C virus of humans, the use of the name classical swine fever virus is increasingly preferred.

Since the early 1800s, classical swine fever has been one of the world's most economically important diseases of pigs because of losses due to disease, eradication, control measures, and restrictions on importation of pork from infected countries. Classical swine fever has the potential to cause devastating losses in countries, such as the United States, that are free of the disease and do not vaccinate. The disease occurs in acute, subacute, chronic, and subclinical forms. Clinical signs and postmortem lesions range from sudden death with generalized hemorrhages to inapparent infections.

Etiology: Classical swine fever virus is caused by a *Pestivirus* in the family Flaviviridae and is closely related to and cross reacts with the viruses that cause bovine virus diarrhea in cattle and border disease in sheep. There is one serotype, but there are marked antigenic variations and differences in virulence between strains. Pigs, domestic and wild, are the only hosts and reservoir. The virus is enveloped and thus susceptible to lipid solvents, many disinfectants, and drying, but it survives for long periods in the cold, in uncooked meat, and in some processed meat, cured and smoked.

Distribution: Classical swine fever occurs worldwide. The eighth edition of *Diseases of Swine* (1999) lists only the following countries as free of classical swine fever: Austrailia, Belgium, Canada, France, Great Britain, Iceland, Ireland, New Zealand, Portugal, the Scandinavian countries, Spain, Switzerland, and the United States. Several western European countries have eliminated classical swine fever from their domestic hogs but the virus is endemic in their wild pigs, which leads to periodic outbreaks. In recent years the virus has been very active. In 1996-1997 there were outbreaks in several Western European countries, Haiti and the Dominican Republic. The Netherlands slaughtered more than 10 million hogs and spent more than $2 billion in eradicating the disease. Haiti and the Dominican Republic, with the help of the United States Department of Agriculture, hope to eradicate the classical swine fever by the end of 2002. During their foot and mouth outbreak, Great Britain had an outbreak of classical swine fever in 2000. They have since eradicated the disease. Cuba had an outbreak in 2001.

Transmission: The virus is present in saliva, nasal secretions, blood, semen, feces and urine. Direct contact between infected and susceptible pigs is the most important means of transmission. Transplacental infection also occurs. Vehicles and trailers that are not properly disinfected, multiple use needles, birds, mosquitoes, and people can act as mechanical vectors. Uncooked, infected pork is an important means of spread into new areas. Airborne transmission is not important.

Clinical Signs: Virus replication is initially in the lymphoid tissue of the upper respiratory tract and tonsils. Within 16 hours there is viremia with systemic infection of lymphoid tissues; within three to four days the virus invades endothelial and epithelial cells. Young pigs can die peracutely without clinical signs. The acute or classical form is caused by high-virulence strains and is characterized by fever of 41℃ to 42℃ (105.8 -107.6℉), huddling, anorexia, vomiting, coughing, conjunctivitis, diarrhea or constipation and central nervous system signs that include paresis, paralysis, lethargy, circling, tremors, and convulsions. There is purplish discoloration of the skin of the ears, legs and abdomen of lightly pigmented hogs. Severe leukopenia occurs early and reaches levels unmatched by other swine diseases. Mortality may be 90 to 100 percent, with most pigs dying 10 to 20 days after infection. The subacute or chronic form is caused by moderate- or low-virulence strains. The clinical signs are similar to the acute form, but they are milder, and the disease progresses and spreads more slowly. Death occurs in one to three months. Low-virulence strains may cause inapparent infections making diagnosis especially difficult. Abortions, stillbirths, and fetal malformations occur in pregnant animals. With low-virulence strains, fetuses infected

Mortality may be 90 to 100 percent, with most pigs dying 10 to 20 days after infection.

before 70 days of gestation may be born infected with and immunotolerant to the virus. These pigs remain lifelong carriers and viral shedders. Congenital tremors or shaker pig syndrome also can result from *in utero* infection. The late onset form occurs in congenitally infected, chronic carriers that are born and remain clinically normal for fairly long periods, but which after a month or more of age become sick with signs of acute or subacute classical swine fever. Most die by six months of age.

Lesions: Gross lesions can be sparse in the peracute form. Hemorrhages may involve any serosal surface, but are most consistently present in the lymph nodes and kidneys. Lymph nodes are usually swollen. Splenic infarction is highly suggestive of acute classical swine fever; but the spleen is not enlarged as it is with African swine fever. Button ulcers can occur in the colon (they also occur with salmonellosis). Microscopically, degeneration, necrosis, and proliferation of endothelial cells can occur in any organ, but are best demonstrated in the brain and lymphoid tissues. Necrosis due to vascular thrombosis may be present in many organs.

Diagnosis: Diagnosis requires confirmation by a reference laboratory. Virus isolation and/or fluorescent antibody (FA) staining using kidney, spleen, tonsils, lymph nodes, thymus, brain and blood are used to confirm the presence of virus. The tonsils are the tissue most consistently positive. The USDA Foreign Animal Disease Laboratory at Plum Island, N.Y., recommends collecting heparinized

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blood and tissues from several pigs in different stages of the disease. Collect a full set of tissues, including brain, and submit them both fresh and in 10 percent buffered formalin. All fresh tissues should be individually packaged in plastic bags and submitted on ice, but not frozen.

Differential diagnoses: The differential diagnoses include any acute, highly contagious febrile diseases of pigs, such as African swine fever, salmonellosis, erysipelas, acute pasteurellosis, virulent porcine reproductive and respiratory syndrome virus, and viral encephalomyelitis such as pseudorabies. In the United States, the most important differential is salmonellosis, which can very closely mimic classical swine fever, especially when caused by moderately virulent strains. Evidence of this is the fact that for almost 20 years classical swine fever was thought to be caused by *Salmonella choleraesuis* because infection with the bacterium resulted in a disease that could not be differentiated from natural outbreaks of classical swine fever.

Prevention and Control : Vaccination is not allowed in the United States or Canada where strict quarantine and slaughter are used for eradication. Inactivated and attenuated live vaccines are used for control in many endemically infected countries.

African Swine Fever

African swine fever is a highly contagious and tick-transmitted disease of swine that affects the mononuclear phagocytic system and which clinically cannot be differentiated from classical swine fever (hog cholera). Like classical swine fever, African swine fever can occur in acute, subacute, and chronic forms.

Etiology: African swine fever virus is a DNA virus and the type species of the genus *Asfivirus* and the sole member of the newly created family *Asfarviridae* (derived from African swine fever and related viruses). The virus is highly resistant to environmental inactivation and can survive for months in frozen, uncooked, cured, and dried meats. The main target cells are monocytes, macrophages and dendritic cells.

Hosts: In Africa, warthogs and bushpigs are the natural hosts as they develop subclinical infections and are usually lifelong carriers. The virus also is maintained in the soft tick *Ornithodoros moubata* that lives in warthog burrows. Domestic swine, the European wild boar, American feral and wild pigs, and the giant forest pig of Europe are all susceptible and develop clinical disease.

Distribution: African swine fever is enzootic in most countries of sub-Saharan Africa. Outside of Africa outbreaks have occurred in Portugal, Spain, France, Italy, Netherlands, Belgium, Cuba, Haiti, the Dominican Republic, and Brazil. Currently, the disease has been eradicated from all countries outside of Africa except for the Italian island of Sardinia.

Transmission: In endemic countries transmission is primarily pig-to-pig by contact with blood or bloody exudates. The most frequent means of entrance of ASF virus into free areas has been feeding pigs uncooked, infected pork products. In Africa, Sardinia, and the Iberian Peninsula soft ticks of the genus *Ornithodoros* act as biological reservoirs and vectors. African swine fever virus is transmitted transovarian in ticks, and tick bites are capable of infecting pigs making ASF virus the only DNA arbovirus. Ticks capable of serving as biological vectors for African swine fever virus have been identified in the United States.

Clinical signs and pathogenesis: Infection can be by tick bite, fighting, or through abrasions and cuts, but it is usually by the oral or nasal routes. The primary virus replication is in the tonsils and mandibular lymph nodes before viral spread by lymph and blood to the target organs (lymph nodes, bone marrow, spleen, lung, liver, and kidney). Virulent ASF virus infects and destroys most antigen presenting cells (monocytes, macrophages, and dendritic cells) resulting in a failure of the immune system. There also is necrosis of lymphocytes, although viral replication does not occur in lymphocytes. Less virulent strains result in destruction of fewer antigen presenting cells and some antibody production, but the antibody is not always protective. Virus titers in the blood are very high and can be prolonged.

In the acute form, the incubation period is 5-15 days and the disease course is 6 to 12 days. Clinical signs include anorexia, high fever, leukopenia, cyanosis of the extremities, petechiae and ecchymoses of the skin. Disseminated intravascular coagulation and thrombocytopenia contribute to coagulation deficiencies, and prolonged bleeding from wounds and needle pricks is common. Mortality with the acute form is often 90 to 100 percent. Recovered animals often remain carriers for long periods. In Spain and Portugal, subacute and chronic forms characterized by respiratory signs, abortion, and low mortality were common, but eradication of African swine fever from the Iberian Peninsula resulted in the disappearance of moderate and low-virulence strains.

Lesions: Necropsy lesions consist of severe edema of the lungs and fluid in the pleural and pericardial spaces, in which changes are not present with hog cholera. There are prominent hemorrhages in the alimentary tract, larynx, bladder, kidney, and other viscera. The gastrohepatic and renal lymph nodes are very enlarged and hemorrhagic. The spleen is enlarged 6 to 8 times and dark black and friable; with hog cholera the spleen is normal size. Often death appears to be due to spontaneous hemorrhage into the abdomen or intestines, or hemorrhage from a gastric ulcer. Pigs may have bloody diarrhea, but button ulcers like those of hog cholera and salmonellosis are not present. Microscopic lesions consist of hemorrhages and necrosis of endothelial cells and lymphoid tissue; lymphoid necrosis is not a major feature of hog cholera.

Diagnosis: Identification of African swine fever virus in swine tissues or blood is required for initial diagnosis and can be by virus isolation, fluorescent antibody or immunohistochemical staining, or polymerase chain reaction (PCR). The preferred specimens are spleen, lymph nodes, tonsil, lung, liver, kidney, brain, heparinized blood, and serum. If possible, sample several pigs, especially if they are autolyzed. Tonsil biopsies, heparinized blood, and serum can be submitted from live animals. Serology is not useful in diagnosing virulent African swine fever because there is no immune response, but is used for detecting pigs infected by low or moderately virulent strains in endemic areas.

Differential Diagnoses: It is not possible to differentiate African swine fever from classical swine fever by clinical or postmortem examination. As with classical swine fever, most systemic, contagious, and febrile diseases of pigs should be considered.

Prevention and Control: Restricting the importation of pigs and uncooked pork products from areas with African swine fever is important in prevention. All pork waste from ships and planes should be incinerated. There are no effective treatments or vaccines available. In infected areas, control is by serologic testing and slaughter of infected animals.

Diagnostic lab continues to expand toxicology services

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The comparative toxicology laboratory in the diagnostic laboratory offers a wide variety of services to the practicing veterinarian. Among these are rapid, sensitive assays for toxic materials, development of new methods for analysis, and consultation services 24 hours a day, seven days a week.

Available Assays

Heavy metals: Lead is one of the most frequently diagnosed poisonings in veterinary medicine. The laboratory offers rapid, sensitive assays for lead in blood and tissues. Heparinized or EDTA-treated blood can be analyzed in a matter of hours; tissues in one day. Other heavy metals measured are copper, cadmium, zinc and selenium. Selenium also poses the potential for dietary deficiency. Research in recent years has identified a number of selenium-containing enzymes that can serve as indicators of a deficiency in this mineral. We are adapting and refining methods for measuring the levels of these enzymes, so we can give a meaningful assessment of the extent of selenium deficiency in farm animals and pets.

Organophosphate and carbamate insecticide poisoning: These compounds pose a health threat to both animals and humans by inhibiting the enzyme acetylcholinesterase. The laboratory provides rapid assays for these poisonings in serum, blood and brain. We also are developing methods for distinguishing the nature of the poisoning agent and detecting possible interfering effects of drugs used in treatment of affected animals.

Poisonings due to other compounds: The laboratory offers rapid assays for nitrate, cyanide (overnight), urea and common mycotoxins in feed. We also can measure nitrate in ocular fluid, urea and ammonia in rumen contents, and strychnine, drugs of abuse, and ethylene glycol in blood and urine. We offer identification of blue-green algae, mushrooms and toxic plant specimens.

Assays Under Development

The laboratory is in the process of developing assay methods for additional heavy metals, anticoagulant rodenticides, chlorinated hydrocarbon insecticides, specific mycotoxins, and enzymes of selenium metabolism and function. In the meantime, we will relay samples for these specific assays to select reference laboratories.

Toxicologists are available 24 hours a day, seven days a week for consultation on poisonings, preparation of materials for analysis and costs. Questions concerning tests, availability of new assays, and other specifics can be directed to 785-532-5678 or 785-532-5679.

Research Roundup

The following abstracts are from the American Association of Veterinary Laboratory Diagnosticians Annual meeting, Fall 2001.

Potential impact of diagnostic delays on the magnitude of a foot and mouth disease epidemic in the United States.

Should foot and mouth disease (FMD) enter the United States, diagnosis of the first case will most likely be based on identification of the virus and/or FMD antibodies in tissues or serum by the Foreign Animal Disease Diagnostic Laboratory at Plum Island, N.Y. The time between collection of tissues from an animal with FMD and laboratory confirmation could be quite variable depending on such factors as the geographic location of suspect animals, transportation time to Plum Island, and delivery and testing priority assigned to the samples. Because control programs may not be initiated until a laboratory diagnosis has been confirmed, considerable transmission of FMD could take place in the interim.

The objective of this investigation was to examine the potential impact of the time from sample collection to diagnosis on the magnitude of a FMD epidemic, using a SLIR-type simulation model of FMD transmission for three California counties for which direct and indirect contact among livestock facilities have been characterized. Three hundred epidemics were simulated for each of one-, seven-, 14-, and 21-day delays between the time the first herd was first infectious and a laboratory diagnosis of FMD was confirmed. The model indicates that the average projected number of additional herds affected per day after the first herd was first infectious was 12.9 (0.5 herds/hour) for delays between seven and 14 days, and 42 (1.8 herds per hour) for delays between 14 and 21 days. These preliminary estimates suggest that delays in the first diagnosis of FMD by only a day or two could substantially escalate an FMD epidemic by increasing the overall number of herds affected by perhaps one to two herds for each hour the diagnosis and simultaneous implementation of control measures are delayed. Other models have suggested that delays in the slaughter of affected animals even after controls were in place, can be expected to contribute substantially to increasing the magnitude of an FMD epidemic. Results here suggest that new approaches and strategies for FMD diagnosis should be considered to minimize potential impact of FMD should it get into the U.S.

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Early exposure to and fecal shedding of *Clostridium perfringens* in 10 healthy foals.

Clostridia associated enterocolitis has been increasingly recognized in neonatal foals and young adult horses with colitis. *Clostridium perfringens* is most commonly implicated as the cause of hemorrhagic enterocolitis in neonatal foals. The disease is associated with a high mortality.

Clostridium perfringens types A and C are the primary genotypes isolated from foals with neonatal enterocolitis. Although recent studies indicate that the majority of foals are shedding *C. perfringens* by 8 to 12 hours of age, acquisition of the organism and predisposing factors necessary for the disease remain unclear.

The purpose of this study was to further elucidate the environmental source of and the earliest age at which *C. perfringens* was shed by 10 healthy foals. No *C. perfringens* was detected in any environmental samples or samples from the foaling area (stall, colostrum, hands, mare body samples). There was no detectable *C. perfringens* from the meconium or foal fecal samples taken at birth or 4 hours of age. Four of the 10 foals were shedding *C. perfringens* type A by 10 hours of age and 90 percent were shedding by 72 hours of age. None of the foals developed classic *C.*

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against aerosol spread in laboratory and field settings.

Conducting necropsies of suspected cases

The followiing recommendations were taken from the APHIS Web site, *Guidelines for Investigating Suspect West Nile Virus Cases in Equine*:

- Keep the use of needles and sharp instruments to a minimum.
- Do NOT use mechanical saws to obtain spinal cord samples.
- Procedures that create an aerosol should be done in a way to minimize the dispersal of the aerosol particles. Although aerosol transmission is not thought to be important, it is still considered a prudent public health precaution to minimize aerosolization of the virus.
- Wear Tyvek® disposable coveralls or, at a minimum, a solid-front, water-resistant, long-sleeve gown.
- Wear three pairs of gloves. The innermost pair should be latex or other disposable gloves. Substantial waterproof gloves like Playtex® kitchen gloves, should be worn over the innermost pair. The gloves should be long enough for the gown sleeves to be tucked inside the gloves; duct tape may be useful for keeping sleeves inside gloves. The outermost pair of gloves should be metal or Kevlar®, e.g., a Whizard® Hand Guard (steel/Kevlar®) glove from Koch® (1-800-456-5624) or a locally purchased filleting glove. This outer pair of gloves must be worn throughout the necropsy procedure.
- Wear a face shield or goggles to protect mucous membranes, and wear a disposable "half mask" HEPA respirator (3M® 8293) to avoid aerosol infection.

Postmortem sample collection

Fresh brain tissue should be sent to the state diagnostic laboratory for rabies testing, initially. One half of the brain should be submitted in 10 percent formalin, while the other half should be submitted fresh, on ice.

The cervical, thoracic and lumbar spinal cord should be collected in 4 centimeter-long segments. Each cervical, thoracic, and lumbar spinal cord segment should be divided in half. One half of each segment should be fixed in 10 percent formalin and the other half should be submitted as a fresh sample. Spinal cord segments that are collected for West Nile virus testing should be sent with the brain samples to the state diagnostic laboratory. If a negative fluorescent antibody test for rabies is confirmed, the remaining brain and spinal cord samples will be sent by the state diagnostic laboratory to the National Veterinary Services Laboratory. West Nile virus testing will begin only after a negative rabies result is confirmed by the state diagnostic laboratory or state public health laboratory.

West Nile Virus Resources

- Centers for Disease Control and Prevention: *http://www.cdc.gov/ncidod/dvbid/westnile/index.htm*
- Animal and Plant Health Inspection Service: *http://aphis.usda.gov/oa/wnv/index.html*
- Emerging Infectious Diseases· July-August 2001 Special Theme Issue on West Nile Virus: *http://www.cdc.gov/ncidod/eid/index.htm*
- Epidemic/Epizootic West Nile virus in the United States: Revised Guidelines for Surveillance, Prevention, and Control (April, 2001) *http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-apr-2001.pdf*
- Kansas State University Web site with Kansas surveillance information: *http://www.oznet.ksu.edu/westnilevirus/wnv2001.htm*
- West Nile virus information hotline , Manhattan: 785-532-2569. Outside of Manhattan (toll free): 1-866-452-7810. (A recorded message about what to do with dead birds is given and you will be directed to a Web site address).
- Kansas Department of Health and Environment (Kansas surveillance program): 785-296-2951

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perfringens enterocolitis during the study. Data from this study indicates that exposure of foals to *C. perfringens* on this particular farm did not occur in the immediate foaling vicinity, but it had occurred by 10 hours in some of the foals.But because 90 percent of the foals were shedding *C. perfringens* without development of disease, further investigations are necessary to identify specific factors and conditions required for the development of disease in young foals.

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An outbreak of rabbit viral hemorrhagic disease in Utah.

Viral Hemorrhagic Disease (VHD) was first reported in China in 1984, probably associated with importation of Angora rabbits from Europe. Subsequently, this rabbit calicivirus has been reported in many countries around the world including Mexico.

The first confirmed report of VHD in domestic rabbits in the United States occurred in Iowa in March 2000. The outbreak affected 27 rabbits and the source of infection remains unknown. Mexico has succeeded in eradicating the disease. VHD was released in Australia to kill wild rabbits in the mid 1990s. The virus is very resistant to many physical and chemical agents and remains viable in the environment for weeks to months.

Rabbits may die without any clinical signs, but clinical signs reported include loss of appetite, lethargy, fever, bloody nasal discharge, neurologic signs and respiratory distress. Young rabbits are relatively resistant to infection. The virus is highly contagious and mortality rates approach 100 percent.

Transmission of the virus is horizontal by direct contact with an infected rabbit or feces of an infected rabbit. VHD can also be spread by contaminated fomites including clothing, shoes, feed, water, etc. as well as mechanical transmission by insects, birds, and animals.

Two dead rabbits were submitted to the Utah State Diagnostic Laboratory in August 2001. Tissues were forwarded to the USDA Plum Island Animal Disease Center where VHD was confirmed. Unfortunately rabbits had been shipped to other premises in Utah, in other states within the previous two weeks. All known and exposed and sick rabbits were euthanized. The source of the outbreak has not been determined.

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