In: **A Concise Review of Veterinary Virology**, Carter G.R., Wise D.J. and Flores E.F. (Eds.). International Veterinary Information Service, Ithaca NY (www.ivis.org), Last updated: 19-Dec-2008; A3427.1208

Flaviviridae

G.R. Carter[1], **D.J. Wise**[2] and **E. F. Flores**[3]

[1]Professor Emeritus of the Department of Medical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA. [2]Department of Biology, Concord University, Athens, West Virginia, USA. [3]Department of Veterinary Preventive Medicine, Federal University of Santa Maria, Santa Maria, RS Brazil.

Table of Contents

This large family consists of enveloped, positive-sense single-stranded RNA viruses. There are three genera, two of which include important veterinary pathogens. One of these, *Flavivirus*, has more than 50 species, many of which are mosquito and tick-borne.

Viral Characteristics

- The Flaviviridae are similar to each other in virion morphology (see Fig. 27.1), genome organization, and replication strategy, but lack serological cross-reactivity across the family.
- The envelope contains at least two viral envelope proteins that are thought to be involved in receptor-mediated endocytosis. However, the target receptor has not yet been identified.
- Once the genome has entered the cytoplasm, it is translated by host ribosomes into a large polyprotein (a polypeptide comprised of several proteins). The polyprotein is then cleaved by viral and host proteases into approximately ten individual proteins.
- The complementary RNA (negative sense) is synthesized by virus non-structural proteins for use as the template for progeny genomes.
- The positive-sense RNA genome is divided into two basic regions: 5'- structural genes - nonstructural genes - 3'. However, the genera differ as far as modification of the genome is concerned: *Flavivirus* have a 5'-cap, but no poly A tail, *Pestivirus* have no 5'-cap but have a poly C tail, and the *Hepacivirus* have no 5'-cap but have either a poly U or polypyrimidine tail.
- A great deal about the replication strategy is currently unknown. This has unfortunately limited vaccine and antiviral drug development.

Figure 27-1. Flaviviridae (40 - 60 nm). Complex virion structure, enveloped, nucleocapsid has icosahedral/polyhedral symmetry. - To view this image in full size go to the IVIS website at www.ivis.org . -

Classification

The Flaviviridae consists of three genera, *Flavivirus*, *Pestivirus*, and *Hepacivirus*. The viruses of these genera causing significant diseases are listed below:

Flavivirus Consists of more than 50 antigenically related viruses. Some are mosquito-borne, some tick-borne and others have not been associated with any arthropod. A number cause disease in animals and humans. Some have been

recovered from bats, marsupials, rodents and birds.

Louping ill virus
West Nile virus
Japanese B encephalitis virus
Wesselsbron virus
St. Louis encephalitis virus: The host is birds. Transmitted by mosquitoes. Causes human encephalitis in the Americas.

Pestivirus Viruses of this genus are not related antigenically to the viruses of the other genera.

Bovine viral diarrhea virus
Swine fever virus
Border disease virus

Hepacivirus

Hepatitis C virus: A major cause of hepatitis in humans.

Flavivirus

Louping Ill
(Ovine encephalomyelitis)

Cause
Louping ill virus. Four subtypes are recognized: Spanish, British, Irish and Turkish.

Occurrence
Louping ill is a tick-borne disease, principally of sheep, that occurs in England, Ireland, and Scotland and in some other European countries. The hosts are sheep and less often, cattle, deer, horses, pigs, wild rodents and humans. Wild rodents, deer, shrews and grouse may be naturally infected without clinical signs of disease.

Transmission
The vector and only reservoir is the tick *Ixodes ricinus*.

Clinical & Pathologic Features
The lymph nodes are initially infected. A viremia follows with spread to other tissues including in some animals the CNS. Louping ill is characterized clinically by a biphasic fever and progressive central nervous system dysfunction. Initial clinical signs are those of high fever and depression of about 24 - 48 hours duration. A second febrile response occurs a few days later accompanied by signs of central nervous system dysfunction including excitability, incoordination, muscular tremors, and paralysis. The mortality rate is high in those animals that develop CNS disease. Some animals recover but display mild neurological signs.

Diagnosis

- Clinical specimens: Fresh and formalin fixed brain tissue.
- A presumptive diagnosis is based on clinical signs and history. Histologic lesions of meningoencephalomyelitis are supportive.
- The virus can be isolated in young mice inoculated intracerebrally and in cell cultures.
- The presence of specific antibodies is diagnostically significant. Serological procedures include ELISA, complement fixation, hemagglutination inhibition (virus agglutinates goose red cells), gel diffusion and indirect immunofluorescence.

Prevention

- Inactivated tissue culture vaccines are used in areas where the virus is endemic. The colostrum of vaccinated ewes protects lambs for a year.
- Animals are dipped, sprayed, etc. in an effort to control ticks.

Public Health Significance
This virus can cause severe encephalomyelitis in humans. Humans may be infected by the bite of infected ticks, aerosol or contact with infected carcasses. Few cases have been attributed to ticks and infected animals; most have occurred in laboratory workers.

West Nile Virus Infection

Cause
West Nile virus. It belongs in the Japanese encephalitis serogroup.

Occurrence
West Nile virus infection (WNVI) occurs in countries of Asia, Africa, Europe and North America. The virus was introduced to the USA in 1999 and by 2004 it had spread throughout the continental USA. There were >4000 human cases in 2002, with 274 deaths, mainly in people over 60 years of age. Less than 1% of those infected are symptomatic. The number of cases will probably decrease as the infection becomes endemic.
As human cases occurred there were many reports of the disease affecting horses in the USA. More than 15,000 cases were reported in 2002 and somewhat less in 2003. Older horses are more susceptible.

In 1997 in Israel a neuroparalytic disease of young geese was attributed to WNV. Geese appear to be the only natural host among domestic avian species.

Transmission
It is estimated that at least 58 mosquito species can carry the virus. *Culex pipiens* is considered the most important for maintaining the virus. Upwards of 300 bird species harbor the virus. House sparrows are thought to be the most important in dissemination. They are readily infected and have high levels of virus. Although some birds such as crows may die of infection house sparrows don't.

Clinical & Pathologic Features
The incubation period in horses is 7 - 14 days. Signs may appear suddenly or gradually; they may include incoordination, dragging hooves, buckling at knees, difficulty eating and drinking, stumbling, muscle weakness, dullness, somnolence, paralysis, inability to rise. Some horses may have a mild infection with low fever, muscle trembling and evidence of a less severe disease.
Many horses that recover from the disease have residual abnormalities such as irregularities of gait, behavioral changes and neurological deficits six months after diagnosis. If horses don't recover sufficiently they may have to be euthanized. The mortality rate in unvaccinated horses developing clinical disease is ~33%. Horses surviving infection are considered immune for life.
The lesions in horses are very similar to those of eastern equine encephalitis (EEE). There is perivascular lymphocyte cuffs, gliosis and neuronal degeneration. What is distinctive with West Nile infection is a poliomyelitis affecting the gray matter of the spinal cord. The brainstem and midbrain are also affected but not usually the cerebral cortex. In EEE the whole brain is affected with the cerebrum most affected.

Diagnosis
- Clinical specimens: Whole blood collected during the febrile stage and brain tissue from horses that have died. Acute and convalescent sera.
- A presumptive diagnosis is often based on clinical signs and the microscopic brain lesions referred to above.
- A definitive diagnosis can be obtained by demonstrating a significant increase in specific antibody between acute and convalescent sera.
- The virus can be propagated on the chorioallantoic membrane, where it produces plaques, and in various cell cultures.
- A presumptive diagnosis can be made on the basis of clinical signs and results of a single serum sample, if those results are positive and the horse has not been vaccinated.

Treatment
- Steroids to reduce inflammation. General supportive care.
- An equine product containing specific viral antibody is being used in treatment although its value is questionable.

Prevention
- Vaccination is recommended. A DNA vaccine (first of its kind licensed in the USA) is available for the prevention of the equine disease. One inactivated vaccine is given in two doses three to six weeks apart. A recombinant vaccine using a canary poxvirus vector is also available. A chimeric vaccine (live, attenuated yellow fever virus with genes from WNV) has been approved for use in the US. A one dose DNA vaccine (the first of its kind licensed in the US) is claimed to prevent viremia in WNV infection. Some practitioners vaccinate semi-annually where mosquitoes are present year-round.
- Strict mosquito control greatly reduces the chances of exposure. Keep horses indoors from dusk to dawn when insects are most active; keep lights off during the evening and keep stable areas clear of birds and poultry.

Japanese Encephalitis Virus Infection
The cause is the Japanese encephalitis virus (*Flavivirus*) a member of the Japanese encephalitis serogroup. The virus infects birds mainly but also bats and is present widely in Asia. It is transmitted by culicine mosquitoes.
The infection in humans is usually mild although there are some severe cases. The disease in horses is clinically similar to EEE, WEE, VEE, and Borna disease, but the mortality rate is relatively low (0 - 10%). Domestic pigs may also be affected.
The virus can be isolated employing cell cultures of ovine origin and the yolk sac of chicken embryos.
Vaccines are used in China and Japan.

Wesselsbron Disease
This disease is caused by Wesselbron virus (*Flavovirus*) a member of the yellow fever group and affects sheep, cattle and humans. It occurs widely in Africa and has been reported from Thailand. It is transmitted by mosquitoes.
The disease in sheep is characterized by high fever and death in newborn lambs and abortion in pregnant ewes. Infection of pregnant cattle may result in abortions and congenital anomalies of the central nervous system. Flu-like symptoms are noted in human beings.
The virus can be isolated from liver and spleen employing cell cultures of ovine origin and the yolk sac of chicken embryos.
Modified live virus vaccines are used in areas where the virus is endemic. Mosquito control reduces exposure.

Pestivirus
Bovine Viral Diarrhea
(Mucosal disease)

Cause
Bovine viral diarrhea virus, BVDV. There are two biotypes: cytopathic (cp) and non-cytopathic (ncp). The classic virus is ncp; cp isolates are generated by mutations or genome rearrangements in the original/parental ncp strain. Most (>95%) of the field isolates are ncp. Genotype refers to genetic/antigenic characteristics: two major genetic/antigenic types exist: BVDV-1 and BVDV-2 (genotypes). BVDV-1 may exist as ncp (majority) or ncp (minority) and as BVDV-2 also. Thus biotype and genotype are independent traits.

Occurrence
Bovine virus diarrhea occurs frequently in the primary host cattle worldwide. Sheep, goats, pigs, water buffaloes and wild ruminants are also susceptible.

Transmission
The virus may be present in various secretions and semen. Spread is by direct and indirect contact. The mode of infection is by ingestion and inhalation. Transplacental infections are frequent and result in serious consequences for the embryo/fetus. Bulls may be persistently infected and the virus in semen is spread by coitus an artificial insemination.

Clinical & Pathologic Features
There are two biotypes of BVD virus, one that causes cytopathic effects in cell cultures (cpBVDV) and one that does not (ncpBVDV). Most field isolates are ncp, considered the "true or classic BVDV". CpBVDVs are derived from ncpBVDV by mutations and only identified in special situations (see below).
Infection of seronegative cattle with ncpBVDV may result in a variety of clinical manifestations, ranging from inapparent infections to gastroenteric, respiratory and hemorrhagic syndromes to the severe acute BVD and the fatal mucosal disease (MD). Although originally isolated from cases of gastroenteric disease and named, as such BVD, BVDV is essentially a virus affecting the reproductive system. Infection of pregnant cows is often associated with reproductive losses, including early or late embryonic deaths, abortion or mummification, malformations, stillbirth and the birth of weak, unthrifty calves. Fetuses infected between 40 and 120 days of gestation with ncp isolates may survive the infection and be born as immunotolerant, persistently infected (PI) calves.

Most persistently infected animals die of mucosal disease (MD) within the first 6 - 24 months of age; cp and ncp BVDV biotypes are usually isolated from sick animals. This severe and uncommon disease is often referred to as "mucosal disease". Leukopenia, anorexia, loss of condition, and pyrexia are common signs. Temperatures range from 104 to 106°F and most animals develop a severe diarrhea with the feces containing mucus and streaks of blood. Salivation and a thick, stringy nasal discharge may be evident and shallow erosions of the oral and nasal mucosa are frequent. The skin of the muzzle may be eroded and crusty. Cytopathic and noncytopathic viruses isolated from cases of MD are antigenically identical and constitute what has been called a "virus pair". Molecular analyses of - cp-ncp pairs indicate that the cp counterpart originated from the original ncp by mutation, genome rearrangement or recombination in the viral genome. Frequent necropsy findings are erosions and ulcerations of the upper respiratory and digestive tracts; characteristic ulcers are seen in the esophagus and involving Peyer's patches. The virus has an affinity for lymphoid tissues, resulting in suppression of the cell-mediated immune response.
In pregnant cows in early gestation the fetus may be infected resulting in fetal death and resorption of the fetus leading to infertility and repeat breeding. Abortions may occur during the first and second trimester, but fetuses infected during the third trimester usually suffer no ill effects because of the fetal immune response. Fetuses that survive infections during early gestation may be born with congenital anomalies such as ocular defects, alopecia, arthrogryposis, and cerebellar hypoplasia.
Persistent infections frequently result if fetuses are infected between days 40 and 120 of gestation. Some of the resulting calves appear healthy while others are "poor doers". They may have respiratory and enteric disease with secondary bacterial infections because of an impaired immune system. Persistently infected cows may breed satisfactorily but infect their fetuses transplacentally.
Persistently and non-persistently infected bulls shed virus in their semen, which may be transmitted by coitus or artificial insemination. Persistently infected cattle are a continuing source of infection in a herd and a large percentage of the herd may be seropositive.

Diagnosis
Clinical specimens: Nasal discharge, feces, blood, blood smears, spleen, kidney, lymph nodes, turbinates, intestine, lung, acute and convalescent sera, and fetal liver and kidney.

- The most convenient way to diagnose BVD infections is by virus isolation in cell culture followed by fluorescent antibody (FA) staining.
- The virus is easily isolated in cell cultures of bovine turbinate or primary bovine cells but since most strains are noncytopathic, the presence of the virus in inoculated cultures is confirmed by FA staining.
- A diagnosis can be inferred by demonstration of a four-fold increase in the level of BVD antibody between acute and convalescent serum samples by virus neutralization.
- Persistently infected animals are identified by the isolation of BVD virus from the serum or by the demonstration of viral infected leukocytes by FA. The latter often can be achieved by the examination of blood smears, but the

isolation and culturing of monocytes (from freshly collected EDTA blood) for several days prior to FA examination increases the chance of accurately identifying infected animals. These cells should be cultured in a medium containing no bovine serum (e.g., horse serum) because bovine serum may be contaminated with non-cytopathic strains of BVD virus.
- Recently, identification of persistently infected (PI) animals has been performed by immunohistochemical procedure (see Chapter 7) on skin biopsies (ear notches). The ear notch system has been of great value in screening for PI animals.
- Ear notches may also be subjected to an ELISA in a recent adaptation of the original method.
- PCR has also been used for detection of PI animals.

Prevention
- Both modified live and killed vaccines are available to aid in the prevention of BVD.
  Modified live vaccines are generally safe, but may induce mucosal disease in animals persistently infected with BVDV. This may occur only if the infecting virus is antigenically very similar to the vaccine virus. Modified live vaccines should not be used in pregnant cows as they can cause fetal infections. Killed virus vaccines with boosters are recommended for pregnant cows.
- Both modified live and killed vaccines have been used with relative success in preventing BVDV-induced disease. A major caveat with these vaccines is their inability to prevent fetal infection and thus PI animals.
- Eradication of BVD from herds is difficult. It involves testing to remove all persistently infected animals from the herd. All cattle to be added to the herd must undergo prior testing.
- In Europe and recently in Southern Brazil, bulk milk testing for BVDV antibodies is used to detect herds with active infection.
- Eradication programs are being carried out in some countries.

Swine Fever
(Hog Cholera)

Cause
Swine fever virus.

Occurrence
Although swine fever still occurs in many countries its incidence has been much reduced in recent years. The eradication program initiated in the United States in 1962 has resulted in complete eradication of the disease. Other countries free of the virus are Canada, Great Britain, New Zealand, Australia, Iceland, and Switzerland. Although the disease still occurs in South America, outbreaks are infrequent to rare.

Transmission
The virus is present in saliva, nasal secretions, feces, blood, and urine. Spread is by direct and indirect contact. Pigs are infected by ingestion or inhalation; birds and hematophagous arthropods may be mechanical vectors. The disease has been spread by consumption of uncooked pork scraps.

Clinical & Pathologic Features
In susceptible swine, the disease is usually acute and characterized by a high temperature, depression, and anorexia. The morbidity is high and the mortality is usually about 90% in fully susceptible pigs.
Neurological signs are not uncommon, and abortions and stillbirths may occur.
Typical hog cholera is frequently complicated with secondary bacterial infections. The two most common are *Pasteurella multocida* and *Salmonella choleraesuis*. Those with secondary infections often have bronchopneumonia and severe enteritis. Leukopenia is common.
The changes observed in affected pigs are related to the strong affinity of the virus for the vascular system. Among the more common lesions are: petechial and ecchymotic hemorrhages involving all the serous surfaces; petechial hemorrhages of the kidney ("turkey egg kidney"), hemorrhagic lymphadenitis ("strawberry" lymph nodes), and the so-called "button" ulcers of the intestinal mucosa.
The most striking microscopic change observed is the accumulation of lymphocytes in the perivascular spaces. Infection of fetuses may result in malformations, such as cerebellar hypoplasia and microencephalopathy.
A less severe chronic form of the disease may be seen that often escapes detection and makes eradication difficult. This may be due to some immunity or to a less virulent strain of virus.

Diagnosis
- Clinical specimens: Kidney, spleen, tonsil, lymph nodes, brain, and blood.
- The diagnosis is based on clinical signs, gross and microscopic lesions, and laboratory tests. The fluorescent antibody (FA) test on frozen sections of spleen, tonsil, and lymph nodes is the simplest and most reliable means of diagnosis.
- The virus can be cultivated in cell cultures of swine origin but grows without discernible CPE. Cell culture coverslips are stained with specific FA to confirm the presence of virus.
- Reverse transcription (RT)-PCR is being used to detect swine fever virus in clinical specimens. This method converts viral RNA to DNA and allows for specific amplification of swine fever virus nucleic acid. This method is rapid and highly sensitive, but has yet to be accepted for routine clinical diagnostic use.
- Virus-specific monoclonal antibodies are used to distinguish the viruses of swine fever, BVD and border disease.

Virus characterization in this manner confirms any antigen detection or virus isolation tests that may be performed.

Prevention

- Countries free of hog cholera have strict importation and quarantine requirements to prevent entry of the disease.
- Live attenuated virus vaccines are used in countries where the virus is endemic. Such vaccines are inappropriate where eradication is being attempted in that the virus may continue to circulate subclinically in vaccinates.
- A marker vaccine (gene-deleted vaccine) has been developed that doesn't express one of the viral glycoproteins. Thus the vaccinated animals can be differentiated from infected (wild type virus) by the lack of an antibody response to the viral glycoprotein. Marker vaccines are not yet widely used in eradication programs.

Border Disease
(Hairy shaker disease)

Cause
Border disease virus. The virus of border disease is antigenically closely related to the viruses of bovine viral diarrhea and hog cholera.

Occurrence
Border disease of sheep was first described from the border region of Wales and England. It occurs in sheep flocks in Great Britain, New Zealand, Australia, and other countries, including the United States and Canada. Kids (goats) are susceptible and the disease has been seen infrequently in calves.

Transmission
The virus is shed in excretions and secretions. Spread is by direct or indirect contact.

Clinical & Pathologic Features
The virus causes no overt clinical signs in adult sheep, but infection of pregnant ewes prior to ~80 days of gestation results in fetal infections with much the same consequences observed in bovine fetuses infected with BVD virus (see above). Fetal resorption, abortion, and the birth of persistently infected animals are common sequelae. Persistently infected lambs often have abnormally hairy birthcoats and congenital tremors resulting from defective myelination of CNS. Persistently infected lambs that are severely affected usually die. Those minimally affected may live and provide a source of infection for other animals.

Diagnosis

- Clinical specimens: Whole blood and serum; affected lambs for necropsy.
- Clinical signs and history are diagnostic.
- The histological finding of nerve fibers with defective myelin sheaths is supportive.
- The virus can be propagated in a variety of cell cultures but without observable cytopathic effects. Viral antigen can be demonstrated in infected cell cultures, blood smears (precolostral blood preferred), and affected tissues (frozen sections) by immunofluorescence.
- Virus neutralization and ELISA can be used on serum samples to determine the degree of flock infection.

Prevention

- Prevention is best accomplished by maintaining closed flocks. Efforts to control the disease involve serologically testing (ELISA, virus neutralization) of all animals and removing those that are positive. Only negative animals are admitted to the flock.
- If eradication is not feasible, breeding animals should be exposed to persistently infected individuals at least two months before breeding.
- An inactivated, adjuvanted vaccine containing BDV and BVDV-1 is used.