VESICULAR STOMATITIS VIRUS

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SUMMARY

Etiology

- Vesicular stomatitis virus (VSV) is an enveloped RNA virus belonging to the genus *Vesiculovirus* in the family *Rhabdoviridae*.
- Two distinct serotypes are currently classified, VSV New Jersey virus (VSV-NJ) and VSV Indiana virus (VSV-IND), which is further divided into three subtypes: classical (IND-1), Cocal virus (IND-2), and Alagoas virus (IND-3).
- In the United States, distinct strains of VSV-NJ appear during each epidemic, and individual strains have demonstrated specific host predilections.

Cleaning and Disinfection

- VSV is inactivated by sunlight, intense irradiation with ultraviolet light or heat (56˚C for 30 minutes), but the virus can survive for long periods at low temperatures.
- The virus is also reportedly susceptible to chlorine dioxide, formalin (1%), 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, 2% sodium carbonate, 4% sodium hydroxide, 2% iodophore disinfectants, formaldehyde, ether, and other organic solvents.

Epidemiology

- Cattle, horses, and swine are most severely affected by VSV, but the virus can also be found in buffalo, sheep, goats, and camelids. White-tailed deer are thought to be a wild host.
- Humans in direct contact with infected animals or tissues can be infected with VSV; however, disease is mild and self-limiting.
- VSV is the most common vesicular disease of livestock in the Americas and was first isolated in 1925, although VSV has been reported since the 1800s. Mexico, Central America, and northern South America continue to experience endemic cycles of VSV (VSV-NJ and IND-1), while infections are reported less frequently in northern Mexico and the United States. The last reported incidence of VSV-NJ infection in domestic swine in the United States was in 1968. Naturally occurring VSV-IND infection in swine has never been reported in the United States.
- In endemic areas, outbreaks of VSV occur in warmer months and along waterways. VSV is also capable of overwintering in countries such as the United States.
- Morbidity rates vary widely, but can be high (up to 90%) in some herds. Adult animals are most affected. Death due to VSV is uncommon.

Transmission

- VSV is transmitted through direct contact and by insect vectors. Sand flies (*Phlebotomus* and *Lutzomyia* species), black flies (*Simuliidae* family), and mosquitoes (*Aedes* species) are confirmed carriers of some strains of VSV.

Infection in Swine/Pathogenesis

- Lameness caused by foot lesions may be the first clinical sign in swine; as vesicular lesions develop, they can be found on the muzzle, lips, tongue, coronary bands and teats. Reluctance to eat and weight loss can occur due to vesicle-related discomfort.
- Clinical signs caused by VSV cannot be distinguished from other vesicular diseases of swine.

Diagnosis

- National reference laboratories often identify VSV by complement fixation (CF) assays, electron microscopy, and virus isolation, as VSV is easily propagated in cell culture.
- Enzyme-linked immunosorbent assays (ELISAs) are available for detection of antigen and antibody. The indirect sandwich ELISA (IS-ELISA) is considered an inexpensive, rapid test.
- One-step multiplex reverse-transcription polymerase chain reaction (RT-PCR) assays are increasingly used for rapid, simultaneous diagnosis of VSV and other clinically similar vesicular diseases.

Immunity

- The immune response to VSV is variable. A carrier state is not known to exist in swine.
- Inactivated VSV vaccines are available in Ecuador and Venezuela and have been tested in the United States and Colombia using aluminum hydroxide or oil adjuvants. A recombinant VSV vaccine has also been tested in swine but is not commercially available.

Prevention and Control

- Contact with insects should be eliminated through indoor housing and screening; insect repellants can also be used in swine. Insect populations can be controlled with insecticides.
- Biosecurity measures should be in place to prevent virus spread via infected animals, personnel, or other fomites.
- Infections of VSV should be reported to the appropriate authorities within the United States, and 14 day quarantines must be followed to prevent further spread of the disease.

Gaps in Preparedness

- Improved VSV surveillance in Mexico may help predict the virus' periodic entry into the United States. The series of events leading to an outbreak in the United States in any given year is still not well understood.
- To increase our understanding of VSV, areas requiring study include transmission cycles (and the role of insect vectors), virus survival, and identification of reservoir hosts, as well as continued genetic analysis of recent isolates.

OVERVIEW

Vesicular stomatitis virus (VSV) is a non-segmented, enveloped RNA virus with a distinct bullet-shaped virion. Currently found only in the Americas, VSV causes a zoonotic vesicular disease of livestock that is grossly indistinguishable from foot-and-mouth disease (FMD), vesicular exanthema of swine (VES), swine vesicular disease (SVD), and Seneca Valley virus (SVV). Two genetically distinct serotypes exist, VSV New Jersey virus (VSV-NJ) and VSV Indiana virus (VSV-IND), each further divided into subtypes based on geographic origin. Both serotypes are pathogenic in domestic livestock, although VSV-IND has not been associated with clinical disease in swine. Two subtypes of VSV-IND, Cocal virus (IND-2) and Alagoas virus (IND-3), have been isolated to South America. Endemic cycles of VSV occur in equatorial regions of Central and South America, with certain strains occasionally migrating north to initiate seasonal epidemics in the United States. Individual strains of the virus are considered to be genetically stable over time within their distinct geographic and ecological niches.

Due to the mild, self-limiting nature of the disease and unlikely international spread through trade of animals, VSV has been de-listed by the World Organization for Animal Health (OIE) as a reportable animal disease. Cattle and horses are most commonly affected, with occasional clinical infection in swine and small ruminants. Subclinical infections are also common, and anti-VSV antibodies have been identified in white-tailed deer and a variety of other wildlife species. Characteristic lesions include blanched, raised, or broken vesicles localized around the oral mucosa, snout, coronary bands, and/or teats. Ulcerations, erosions, and fever may also be observed. Clinical disease in swine typically manifests as lameness resulting from foot lesions, while excessive salivation is often seen in horses and cattle. Transmission occurs largely via arthropod vectors, although the virus can be spread by contact with affected animals or fomites during outbreaks. Sand flies and black flies are known carriers capable of spreading the virus after contact with vesicles or infected flies, irrespective of feeding behavior. Viremia does not occur in livestock, thus there are no reports of infection by blood or semen. Humans are infrequently infected with VSV and can exhibit flu-like symptoms and vesicular lesions within one to two days of exposure.

For economic purposes, rapid diagnosis is crucial to differentiate VSV from FMD and other vesicular diseases. National reference laboratories often identify VSV by complement fixation (CF) assays, electron microscopy, and virus isolation, as VSV is easily propagated in cell culture. Several enzyme-linked immunosorbent assays (ELISAs) are also available, and one-step multiplex reverse-transcription polymerase chain reaction (RT-PCR) assays are increasingly used for rapid, simultaneous diagnosis of VSV and other clinically similar vesicular diseases. Control and prevention of VSV is centered on control of insect vectors and biosecurity measures to protect human handlers and uninfected animals. Infections of VSV should be reported to the appropriate authorities within the United States, and 14 day quarantines must be followed to prevent further spread of the disease.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics
Vesicular stomatitis virus (VSV) is an enveloped RNA virus with a large bullet-shaped virion, belonging to the genus *Vesiculovirus* in the family *Rhabdoviridae*. It is the most common vesicular disease of livestock in the Americas and was first isolated in 1925, although VSV has been reported since the 1800s.

1.2 Strain Variability
Two distinct serotypes are currently classified, VSV New Jersey virus (VSV-NJ) and VSV Indiana virus (VSV-IND).[1,7] Both are pathogenic in domestic livestock,[1] yet they are thought to have diverged from each other over a million years ago.[8] Differences at the nucleic acid level vary between 30–70%, with changes spread throughout the genome.[9] The VSV-IND serogroup is further divided into three subtypes: classical (IND-1), Cocal virus (IND-2), and Alagoas virus (IND-3).[7] Both VSV-NJ and IND-1 can be subdivided into four distinct genotypes based on the *G* gene, each lineage correlating with a different geographic origin.[8] Despite the extensive genetic diversity of VSV between endemic regions consisting of varied ecological niches, genetic sequences within a single region are stable over time.[9] The classical IND-1 serotype is the type species for the genus and a model for scientific research.[8]

Epidemiologic data collected during outbreaks in the United States has indicated that distinct strains of VSV-NJ appear during each epidemic, and individual strains have demonstrated specific host predilections. Similarly, attempts to produce disease experimentally have resulted in decreased virulence across species lines.[10]

2. Cleaning and Disinfection

2.1 Survival
Sunlight will inactivate VSV, but the virus is able to survive long periods of time at low temperatures. It is stable at a pH range from 4–10.[7] The virus can been recovered from contaminated saliva on pails or food buckets for 3–4 days, and from the exterior of inoculated plants for up to 24 hours after surface inoculation.[1] Endemic regions overlap with tropical climates, while epidemic regions are typically arid-semiarid with a temperature range between 15 and 20˚C.

2.2 Disinfection
Chlorine dioxide, formalin (1%), 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, 2% sodium carbonate, 4% sodium hydroxide, and 2% iodophore disinfectants are all effective against VSV. The virus is also sensitive to formaldehyde, ether, and other organic solvents.[7] Alternatively, it can be inactivated by exposure to 56˚C for 30 minutes or intense irradiation with UV light.[1]

3. Epidemiology

3.1 Species Affected
Formerly an OIE-listed terrestrial animal disease in these species, VSV can be found in cattle, buffalo, sheep, goats, camelids, equids, and swine.[14] Natural disease in sheep and goats is rare, but experimental infection is possible.[15] Cattle, horses, and swine are most severely affected, and the clinical resemblance to vesicular diseases such as foot-and-mouth disease (FMD) carries an associated economic impact in the cattle and swine industries.[8] Anti-VSV antibodies in the absence of observable clinical disease have also been found in a variety of wild animals.[1] White-tailed deer, in particular, are thought to be a wild host.[14]

3.2 Zoonotic Potential
Humans infrequently become infected with VSV from handling affected animals. They can exhibit flu-like symptoms and vesicular lesions within one to two days of exposure.[1] The incubation period in humans is usually three to four days, and a human death from VSV has never been reported.[4] The extremely low seroprevalence of VSV in humans, combined with the safety, stability, and replication capability of highly attenuated recombinant VSVs, also makes it a valuable vaccine vector for more serious human diseases, such as Ebola.[16]

3.3 Geographic Distribution
Confirmed VSV infection is limited to the Americas, although the disease has been described in South Africa in the late 19th century and in France in the early 20th century.[7] The virus is thought to have originated in equatorial America and spread north during two separate colonizing events.[8] Mexico, Central America, and northern South America continue to experience endemic cycles of VSV.[1] Specifically, VSV-NJ and IND-1 are endemic in southern Mexico, Central America, Venezuela, Colombia, Ecuador, and Peru. Less frequent infection with VSV-NJ and IND-1 has been reported in northern Mexico and the United States. The current known range of the IND-2 subtype is limited to Argentina and Brazil, and Brazil has reported the only known isolation of IND-3.[7]

In endemic areas, outbreaks show a seasonal association with the transition between wet and dry seasons.[11] Outbreaks of VSV typically occur in warmer months and along waterways.[2] It has been shown that epidemic strains of VSV are capable of overwintering in the United States for up to three years, and at some point they will eventually die out. Phylogenetic analyses propose that the discrete lineages responsible for outbreaks in the southwestern United States originate in endemic areas, then migrate north.[11,12] Epidemics in the United States often begin in late spring to early summer, progressing northward before coming to an end in late fall.[13]

3.4 Morbidity and Mortality
Rates of morbidity can exhibit a wide variation between 5 and 70%, potentially reaching 90% in a given herd. Higher mortality is observed with VSV-NJ infection in swine, although overall mortality is very low. Clinical signs are most commonly observed in 10–15% of animals, and most will recover in about two weeks. Disease is more commonly observed in adult animals.[7]

4. Transmission
Transmission can occur by direct contact via transcutaneous or transmucosal routes and also by insect vectors. Sand flies (*Phlebotomus* and *Lutzomyia* species), black flies (*Simuliidae* family), and mosquitoes (*Aedes* species) are confirmed carriers of some VSVs. Where the virus is endemic, a stable, long-term cycle of transmission occurs between sandflies and hosts with subclinical infection.

Experimental transmission of VSV-NJ from black flies (*Simulium vittatum*) to domestic cattle and swine has also been demonstrated.[7] Probing by infected flies was sufficient to transmit the virus, irrespective of whether a blood meal had been taken.[10] Results have shown that lesions are more severe when black flies are used to inoculate the virus, suggesting a biological component may be influencing the pathology of VSV.[17] Horizontal transmission of VSV-NJ has also been observed between black flies co-feeding on the same animal. This may help to explain the maintenance and spread of the virus in nature, despite the inability to locate an animal reservoir or amplifying host capable of sustained viremia.[18]

The virus is found mainly in epithelial tissues, and there is no evidence of transmission by blood or semen.[4] Though not confirmed, plants and soil are also suspected of harboring the virus.[7]

5. Infection in Swine/Pathogenesis

5.1 Clinical Signs
Lameness and foot lesions are often the first clinical manifestations in swine, while excessive salivation and drooling are common early signs in other species.[4] Blanched, raised, or broken vesicles of varying sizes may be seen upon close observation,[7] specifically on the muzzle, lips, tongue, coronary bands, and occasionally the teats.[10] Discomfort caused by these blister-like lesions can lead to a reluctance to eat and severe weight loss in some animals.[7] Vesicles often rupture within one to two days, with red ulcerative lesions persisting for at least a week.[10] In pigs, these lesions are commonly found on the feet[2] and/or snout and should completely resolve within two weeks.[7]

Clinical signs cannot be reliably distinguished from those caused by FMD virus, vesicular exanthema of swine virus (VESV), swine vesicular disease virus (SVDV), and possibly Seneca Valley virus (SVV). When pigs, cattle, and horses are affected simultaneously, VSV should be suspected (horses are resistant to FMDV). When only cattle and pigs are affected, FMDV should also be suspected; evidence of SVV infection has also been found in both cattle and pigs. SVDV or VESV should be added to differential diagnoses when only pigs are affected.[7]

5.2 Postmortem Lesions
Because mortality is rarely associated with VSV infection and lesions are confined to epithelial surfaces, additional postmortem lesions have not been described.

6. Diagnosis

6.1 Clinical History
Sporadic epidemics of VSV have been observed in the United States, primarily affecting horses and cattle.[10] An outbreak of "sore tongue" in livestock, attributed to the arrival of VSV-NJ, occurred in the United States in the late 1700s. Nearly 100 years later, IND-I is thought to have arrived in this country.[8] A large suspected epidemic in horses occurred in 1862, followed by the first formal report of VSV in 1916. There have been outbreaks in livestock, primarily in the Southwestern United States, in six out of the past ten years.[5] While animals may develop vesicular lesions, subclinical infections in livestock are also common.[17]

While VSV-NJ is not known to naturally occur in domestic swine populations, swine can be susceptible and will develop clinical signs following experimental infection. The last reported incidence of VSV-NJ infection in domestic swine in the United States was in 1968,[1] although VSV may still be endemic in the feral pig population found on Ossabaw Island, Georgia.[7] Naturally occurring VSV-IND infection in swine has never been reported in the United States.[1]

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens
VSV can be easily propagated in cell culture.[17] Baby hamster kidney cells (BHK-21) have been used in growth and amplification of VSV and African green monkey kidney (Vero) cells have been utilized for plaque purification.[19] Cytopathic effect (CPE) can be observed upon inoculation of BHK-21, Vero, and pig kidney IB-RS-2 cell cultures with VSV, distinguishing it from SVDV and FMDV.[15] Embryonated chicken eggs and unweaned mice may also be used to isolate the virus. [4,20]

National reference laboratories in the United States detect VSV by complement fixation (CF), electron microscopy, and virus isolation.[14] Reverse transcription polymerase chain reaction (RT-PCR) [15,20] and indirect sandwich enzyme-linked immunosorbent assays (IS-ELISA) are also effective in confirming

clinical cases. Of these methods, the IS-ELISA is considered to be the least expensive and most rapid test.[15]

A multiplex real time RT-PCR has been developed to detect known VSV isolates from Central and North America. Detection is based on the nucleotide (*N*) gene, which is the most conserved across both serotypes in samples from a wide geographic range. Sensitivity was found to be greater than with virus isolation, but the potential for missing previously unknown lineages also exists.[9] Similarly, several one-step multiplex RT-PCR assays exist for the simultaneous detection of the common vesicular disease of swine.[21-23] One is based on the *L* gene, highly conserved in both serotypes, and is capable of identifying VSV, FMDV, and SVDV.[22] Another, also based on the *L* gene, incorporates a microarray component, capable of serotyping VSV and FMDV, and can also identify SVDV and VESV.[21] All are capable of detecting VSV-NJ and VSV-IND serotypes.[21-23]

New immunochromatography assays may provide even more rapid diagnostic capabilities for on-site testing. A simple immunochromatographic strip test using monoclonal IgG antibodies against the G protein of VSV-IV is able to detect VSV-IND with 91.4% sensitivity and 98.9% specificity compared to RT-PCR, showing no cross-reactivity with FMDV, SVDV, VESV, porcine reproductive and respiratory syndrome virus (PRRSV), and classical swine fever virus (CSFV).[24] Other lateral flow devices for detection of VSV-NJ and IND1 demonstrate a sensitivity and specificity greater than or equal to a reference antigen ELISA.[25]

6.3 Tests to Detect Antibody
Serum antibody against both VS-IND and VS-NJ can be detected by competitive ELISA (cELISA), virus neutralization (VN), CF, and liquid-phase blocking ELISA (LP-ELISA).[15] A fourfold change in antibody titer in paired sera is required for confirmation of infection, due to the prolonged duration of anti-VSV antibody in the blood.[1]

6.4 Samples
6.4.1 Preferred Samples
Antibody testing of serum can be diagnostic, as well as detection of the virus in swabs of lesions, blister fluid, or affected tissue.[2] Fluid from vesicles, skin covering unruptured vesicles or flaps from newly ruptured vesicles, and swabs of ruptured vesicles are considered the best samples for diagnosis.[15,17] If samples from lesions are not available, paired sera taken one to two weeks apart from the same animal may be used for serological assays.[15] Virus can generally be isolated in high concentrations from lesions for up to ten days postinfection.[1]

6.4.2 Oral Fluids
Samples may be collected from mouth lesions if vesicles are occurring there. Esophageal-pharyngeal fluid from cattle and throat swabs from pigs can be used for virus isolation, in the absence of epithelial tissue from vesicular lesions.[15] Nasal and pharyngeal swabs are also useful for early diagnosis with RT-PCR assays, prior to the onset of clinical signs.[22]

7. Immunity

7.1 Post-exposure
Presence of VSV antibodies indicates prior exposure, but does not confirm active infection. Complement fixing antibodies generally persist for less than a year, while those detected by ELISA or VN assays may be detected for years post-infection.[15] As many as 70% of the animals at an affected premises may demonstrate increased antibody titers following exposure, although closer to 10% will generally show clinical signs.[3] Seroconversion can potentially be detected as soon as five days after exposure, and antibody development correlates with decreased detection of viral shedding.[1]

A carrier state in swine is not known to exist, and spread through international trade is unlikely.[4]

7.2 Vaccines

Inactivated VSV vaccines are available in Ecuador and Venezuela[14] and have been tested in the United States and Colombia using aluminum hydroxide or oil adjuvants.[15] Through 2014, the use of a vaccine to control or prevent VSV in livestock in the United States was prohibited.[14]

A recombinant VSV has shown efficacy as a vaccine in swine, although it is not commercially available. Recent data has shown that the matrix (M) protein, important in virus assembly and budding, plays an important role in pathogenicity in swine. A live-attenuated VSV with a triple amino acid mutation of the M protein did not produce vesicular lesions upon initial inoculation and was able to stimulate a protective immune response in all challenged pigs. Neutralizing antibody titers were determined up to 28 days post-inoculation, when pigs were then challenged with a wild type VSV. Antibody titers began to decline at three weeks post-infection, thus further research is needed to accurately determine the duration of protective immunity.[19]

7.3 Cross-protection

The two serotypes of VSV, VSV-NJ and VSV-IND, generate distinct neutralizing antibodies, despite the similar pathology they cause.[13] These serotype-specific antibodies will develop within eight days of infection.[15]

8. Prevention and Control

Control measures for VSV in swine, as indicated by the OIE, have previously included general surveillance, precautions at international borders, control of movement inside the country, treatment of infected individuals for the purpose of controlling infection, and control of insect vectors that spread the virus.[14] Animals should be housed indoors, if possible, during peak insect feeding hours, and insect repellants, such as permethrin, are available for use in swine.[1] Pastured animals are affected more frequently, and insecticide sprays, insecticide treated eartags, and elimination of insect breeding areas are recommended, especially once a diagnosis has been made on a farm.[2] Biosecurity measures should also be in place to prevent spread of the virus by infected animals, fomites, and farm personnel. The virus can remain viable in contaminated saliva for three to four days, so care should be taken to prevent spread via shared water and feed buckets.[1]

During past outbreaks in the United States, infected premises have been subject to 21-day quarantines after all lesions have healed,[2,3] but the period has now been shortened to 14 days from clinical onset of the last affected animal.[5] Swine and ruminant samples from animals displaying vesicular lesions are tested by the Foreign Animal Disease Diagnostic Laboratory (FADDL). Equine samples can be tested by National Veterinary Services Laboratories (NVSL) in Ames, IA.[3]

Care must be taken to prevent exposure in the laboratory when working with live virus, handling diagnostic specimens, and coming in contact with animals suspected of VSV infection.[1] Personal protective equipment should be used when handling affected animals.[2]

Preliminary research has explored the antiviral activity of doxycycline against VSV in human cell cultures, suggesting the potential for future treatment of viral infection.[26] Antiviral activity of the porcine *MX2* gene found in some breeds is also being investigated to explore the possibility of breeding VSV-resistant swine.

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code

The 2015 OIE Terrestrial Animal Health Code (http://www.oie.int/international-standard-setting/terrestrial-code/access-online/) does not cover VSV. There are no longer any recommendations for importation of cattle, swine, or horses from countries or zones infected with VSV.

10. Gaps in Preparedness

The improvement of a VSV surveillance system in Mexico and ability to predict the introduction of southern endemic strains into the United States would serve to better prepare us for the inevitable arrival of the virus and take preventive measures to limit the extent of any future outbreaks. Improved understanding of transmission cycles and the knowledge of which lineages are able to spread north can direct further research into the factors that favor transmission of these particular lineages over others.[11] Continuing genetic analysis of isolates responsible for outbreaks can assist in identification of virulence factors and understanding of host predilection across different lineages.[10]

The series of events leading to an outbreak in the United States in any given year is still not well understood. Many factors – water sources, comingling of populations, insect vectors – have the potential to affect the magnitude and scope of transmission of VSV once it arrives here. Many unanswered questions remain about the effects of local climate, environment, and management practices on the spread of VSV in different species.[13] Further elucidation of the role of insect vectors, survival of the disease in the environment, and identification of reservoir hosts could add further clarity to our understanding of the disease.

ACKNOWLEDGEMENTS

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