

I. Introduction

A. Purpose

An after action review (AAR) of the 2004 VSV outbreak was held in 2005. One of the recommendations of the AAR was to develop a Vesicular Stomatitis Working Group (VSWG) composed of Federal employees representing different aspects of the Veterinary Services organization. The role of the VSWG was to address assigned issues related to vesicular stomatitis viruses (VSV) and provide information to the Veterinary Services Deputy Administrator in the form of decision memorandums.

The VSWG addressed the potential for laboratories to provide diagnostic testing of clinically ill horses during a VSV outbreak. In April 2006, the Deputy Administrator signed a decision memorandum that allowed for National Animal Health Laboratory Network (NAHLN) laboratories located in states that had VSV in 2004 and/or 2005 to participate in the testing program. Eligible labs were contacted in November 2006 to determine if they wished to participate. As of March 2007, six NAHLN labs (CO, MT, NM, TX, UT, WY) had trained with the National Veterinary Services Laboratories on the complement fixation (CF) test for detection of VSV antibodies in clinically ill horses. Activation of NAHLN labs for VSV testing in clinically ill equine species occurs once VSV has been identified in the NAHLN lab's state and the lab has been notified by the NAHLN coordinator that they may begin testing. Participating NAHLN labs can be found at: http://www.aphis.usda.gov/animal_health/nahln/

Per the decision memorandum, approved NAHLN labs will not test clinically ill non-equine species. Samples from these species must go to the Foreign Animal Disease Diagnostic Laboratory (FADDL) even after NAHLN labs have been activated. Clinically ill equine species can be tested by a NAHLN lab if the equine is located in the state of the NAHLN lab and if the lab has been activated for testing. Until such time as a NAHLN lab is activated, equine VSV FAD investigations will continue to occur at NVSL-Ames. NAHLN labs can only test sera, so for cases with both sera and virus isolation samples, all samples must be sent to NVSL-Ames.

The purpose of this manual is to provide information about VSV and to provide guidance on the testing program.

B. VSV Description

Vesicular stomatitis is a sporadically occurring viral disease characterized by vesicular lesions. The disease primarily affects cattle, horses, and swine, but may also affect sheep, goats, and camelids. Many species of wild animals, including deer, bobcats, goats, raccoons, and monkeys, are also susceptible, as evidenced by seroconversion. Humans are susceptible to infection and appropriate safety precautions should be taken when handling affected animals or samples from affected animals. The disease is currently limited to North, Central, and South America.

1. Etiologic Agent. Vesicular stomatitis viruses (VSV) are enveloped RNA viruses of the family Rhabdoviridae. Two different serotypes, New Jersey and Indiana-1,

Procedure Manual for Vesicular Stomatitis Viruses (VSV)

have caused vesicular stomatitis in the United States. Indiana-2 and Indiana-3 subtypes are endemic to parts of South America and have not been reported in the United States.

- 2. Clinical Signs.** Vesicular stomatitis viruses can produce vesicles on the tongue, lips, oral and nasal mucosa, teats, prepuce, and epithelium of the coronary band of infected animals. Vesicles may be seen early in the course of the disease. As the disease progresses, lesions observed can include ruptured vesicles and small to large areas of tissue in which the epithelial layer has been sloughed. In severe cases, a significant portion of the epithelial surface of the tongue may be lost. Lesions around the mouth, nose, feet, prepuce, and udder can also develop into scab-like lesions as the denuded tissue begins to heal. Depending on the severity of the disease and the amount of tissue impacted, permanent scarring can occur.

Clinical signs associated with these lesions can include nasal discharge when the nose is involved. When the mouth is involved, animals may demonstrate mild to severe salivation and may stand with their mouth open. Animals with oral lesions may refuse to eat and/or drink due to the discomfort associated with oral intake of fluids and solids, resulting in weight loss.

Coronary band lesions can result in lameness in one or more feet. In severe situations, the hoof may slough or hoof growth may be impacted, potentially permanently impacting locomotion. If animals have painful feet, they may spend more time than normal lying down. They may also be reluctant to rise in order to obtain food and water. Nursing dams may develop lesions on their udder and the nursing offspring may develop lesions in their mouth. The dam may refuse to nurse the offspring when the udder is painful and the offspring may refuse to nurse due to the oral lesions. This disease does not generally cause death; however, morbidity rates are highly variable.

VSV can not be clinically distinguished from other significant vesicular diseases of ruminants (foot and mouth disease) and swine (foot and mouth disease, vesicular exanthema of swine, and swine vesicular disease). Therefore, if non-equine species are involved, these vesicular diseases need to be considered and ruled out by laboratory testing.

- 3. Epidemiology.** Vesicular stomatitis is most likely to occur during warm months in the Southwest, particularly along river ways and in valleys. Recent outbreak years in the United States were 1982, 1995-96, 1997, 1998, 2004-2005, 2005-2006, and 2006.

Transmission of VSV viruses is not fully understood. However, animal-to-animal transmission by transcutaneous or transmucosal contamination and mechanical transmission by arthropods and fomites are likely. Insects also may serve as vectors in the biological transmission of VSV. Biting flies have been shown, both in natural and experimental infections, to be capable of becoming infected and transmitting VSV after an extrinsic incubation period. More specifically, sand flies (*Lutzomyia* spp.) and black flies (*Simulium* spp.) have been identified as important species in endemic and epidemic transmission of VSV, respectively. Grasshoppers have been

shown experimentally to become infected with VSV and transmit virus when consumed by cattle.

However, during outbreaks researchers have found other species of biting and non-biting insects are frequently either infected or contaminated with VSV. Virus isolates have been made from mosquitoes, biting midges (*Culicoides* spp.), and muscoid flies (*Musca domestica* - the house fly and *Musca autumnalis* - the face fly). The role of other biting flies, such as horse flies and stable flies, and other non-biting flies, such as blowflies, has yet to be established.

Based on experimental studies, infected animals can develop lesions and clinical signs within one day of infection. Virus is generally shed for several days followed by seroconversion. Depending on the severity of the lesions, healing times can be quite extensive.

- 4. Economic Impact.** Vesicular stomatitis can have variable economic impacts in a country depending on the severity of the disease, frequency of occurrence, number and type of species affected, distribution of the disease in a country, and management practices. National and international trade restrictions can result in significant economic impacts due to a lack of ability or limited ability to transport animals and animal products and due to increased testing requirements for movement. Significant economic impacts can result when activities such as sales and shows have to be cancelled due to the proximity of infections to these venues.

Economic impacts in production animals include, but are not limited to, reduced or lost production in dairy animals, reduced rate of gain in ruminants and swine, increased costs associated with veterinary activities such as exams, diagnostics, and medical treatments, increased costs associated with insecticide use, increased labor costs associated with medical treatment and application of insecticides, and the culling of affected animals. The economic impact in the equine companion animal is similar to production animals.

II. Sampling and Submitting Specimens

A. Specimens to collect (all species)

- Vesicular fluid - place in screw-cap tube. If a screw-cap tube is not available, place in a plastic serum tube (“snapcap”) and tape or parafilm the lid to prevent leakage. **DO NOT** submit in a syringe.
- Vesicular tissue (epithelium) - place in screw-cap tube with a **maximum of 3 mls** of tris-buffered tryptose broth (TBTB).
- Sterile swabs - (dacron or cotton) of affected area placed in a screw-cap tube with a **maximum of 3 mls** of TBTB.
- Serum - at least 2 mls, preferably separated from the clot.

B. Additional specimens to collect for ruminants and swine

- Esophageal-pharyngeal (OP) fluid - should be collected in TBTB (50:50) in a wide-mouth screw cap tube.
- Whole blood - 10 ml (both heparin and EDTA)
- Complete set of tissues - especially lymph nodes from dead animals.

C. Mode of shipment

- If at all possible, ship tissues to the laboratory for arrival within 24 hours of collection - ship with frozen ice packs without freezing the specimen.
- If it is not possible to ship the tissues for arrival within 24 hours - refrigerate specimens (DO NOT freeze) and ship as soon as possible by overnight service with frozen ice packs.

D. Cases involving only horses or symptomatic horses with asymptomatic ruminants or swine

- If asymptomatic ruminants and/or swine are on the same premises as symptomatic horses, surveillance samples can be collected from the ruminants and/or swine and submitted with the horse samples to NVSL, Ames.
- If samples are to arrive on the weekend, call the Diagnostic Virology Laboratory at 515-663-7551 prior to shipment and ask for someone in the BP Section.
- Ship samples to:

National Veterinary Services Laboratories
1800 Dayton Avenue
Ames, IA 50010

E. Cases involving ruminants and/or swine

- If ruminants and/or swine have vesicular lesions, samples from all VSV susceptible species including horses must be submitted to FADDL.
- If samples are submitted during the week, notify FADDL with the airbill number and date of shipping in order to arrange for morning package pick up. If notification was not done in advance, samples will be delivered late in the day. This will delay the sample processing and test results. Day phone numbers for FADDL are 631-323-3256 or 3206.
- If samples are submitted on the weekend or after hours, call FADDL cell phone numbers Samia Metwally (631-375-5314) or Barry Latney (631-871-3112).
- Ship sample by FedEx to:

USDA, APHIS, VS, FADDL
40550 RT 25
Orient Point 11957

- On the FedEx form, write on the line under this address to hold at:

579 Edwards Avenue
Calverton, NY 11933

F. Cases involving ONLY HORSES and NAHLN lab activated

- If a state is positive for VSV and the state has a NAHLN lab that has been activated for testing, equine **SERUM** samples from clinical horses located in that state may be submitted to the NAHLN lab.
- VSV outbreak samples tested in a NAHLN lab should be clearly identified by FAD number using indelible marker and maintained at -20C or colder temperatures for archival purposes. NVSL will make arrangements for shipment of these samples to NVSL from the NAHLN lab.
- If virus isolation samples (swabs, tissue, vesicular fluid) are collected in addition to serum samples, both the sera and virus isolation samples must be forwarded to the Diagnostic Virology Laboratory at NVSL in Ames, IA (see above).
- Contact the laboratory prior to shipping if samples are being shipped for arrival on the weekend to confirm samples can be received.

****NOTE:** If symptomatic ruminants or swine are present on a premises, all samples, including those collected from equine must be submitted to FADDL regardless of the NAHLN activation status of the laboratory (see above).

G. Submission Paperwork

- Samples submitted for VSV FAD testing should be submitted on a VS Form 10-4 (http://www.aphis.usda.gov/animal_health/lab_info_services/forms_publications.shtml)
- This form should be used for submission to NVSL, FADDL, and NAHLN labs activated to test clinically ill horses.
- If samples are being forwarded to NVSL or FADDL from another lab, include a copy of the paperwork submitted to the lab.