

West Nile Virus Considerations for Llama & Alpaca Breeders

Robert J. Callan, DVM, MS, PhD, DACVIM
Department of Clinical Sciences
Colorado State University

INTRODUCTION

West Nile Virus (WNV) was first identified in the United States in 1999. Since that time, the virus has spread throughout the continental United States.^{1,2} The virus is maintained in nature through an enzootic cycle between mosquitoes and birds. Both birds and mosquitoes play important roles as reservoirs in maintaining the virus in given geographic locations. Spread throughout the U.S. is primarily associated with normal migration patterns of infected birds. Because of the enzootic infection cycle in mosquitoes and birds, WNV is here to stay.

WNV can be transmitted to many other species of animals primarily through the bite of infected mosquitoes.³⁻⁵ Some types of ticks can become infected, however ticks are unlikely to play a significant role in transmission to mammals.^{6,7} The major species affected by WNV are birds, horses and humans. In the last several years, infection and disease in llamas and alpacas has become an increasing concern.^{8,9} In addition, WNV infection is reported in cattle, domestic sheep, goats, big horn sheep, mule deer, black bears, pigs, dogs, cats, several rodent species, some reptiles and amphibians. Most infections in mammals do not result in clinical disease. Infected mammals generally do not develop sufficient viral infection in the blood (viremia) to amplify and transmit the virus to uninfected mosquitoes. Therefore, mammals are generally considered to be dead-end hosts for WNV.

Following transmission by an infected mosquito, the virus enters the blood where there is a short period of viremia. In most animals, spread of infection is limited to peripheral tissues resulting in mild signs of discomfort and lethargy. However, in a small percentage of animals WNV enters and infects nerve cells in the central nervous system resulting in varying degrees of neurological signs. Both humoral (antibody) and cytotoxic immune responses are involved in the resolution of WNV infection. Neutralizing antibody responses are directed toward proteins on the surface of the virus and help clear and prevent future infection. At this time, prevention of viremia and CNS dissemination by neutralizing antibody appears to be the primary immunological factor in preventing clinical disease.

INFECTION IN CAMELIDS

Camelids appear to be less susceptible to clinical disease following WNV infection than horses, birds, or humans.^{3,8,9} While there is no specific data available, the reported frequency of confirmed and suspected clinical cases appears to be higher in alpacas than llamas. Colorado experienced several cases of WNV encephalitis in alpacas during the 2003 mosquito season. During that time, 4 cases were treated at the CSU Veterinary Teaching Hospital. In addition, WNV encephalitis was confirmed by the Colorado State Veterinary Diagnostic Laboratory in 8 of

17 alpacas that died of neurological signs between July and October 2003. The first Colorado case (human) of WNV in 2003 was in early June. The first alpaca case of WNV seen at CSU in 2003 was in mid August and the last confirmed case at CSU was near the end of September. Serological surveys of llamas and alpacas in the Front Range of Colorado demonstrated WNV infection rates as high as 80% in specific locations during the 2003 season. Both the number and severity of reported cases in llamas and alpacas appeared to be lower throughout Colorado during the 2004 season. This was also observed in horses and may be due to effective vaccination and/or development of natural immunity following the heavy infection rates observed in 2003.

Based on information from other veterinarians and producers around the country, Colorado appeared to experience a higher number of clinical cases than expected in alpacas during 2003. This may have been due to generally high mosquito populations observed that year. Another theory is that differences in the predominant mosquito species contributed to a higher infection rate compared to that observed in the eastern United States. *Culex tarsalis* mosquitoes were the predominant mosquito species in Colorado during 2003. This species is much less common in areas east of Colorado. *Culex tarsalis* is an abundant mosquito species in agricultural areas of Colorado and other western states and is a particularly effective vector for WNV. It is possible that infection rates in alpacas were higher in Colorado due to the presence of this mosquito species.

Most WNV infections in llamas and alpacas do not result in serious illness. Limited data suggest that about 10% of infected animals may develop clinical signs of neurological disease. The clinical signs observed in alpacas include:

- Lethargy
- Inappetence
- Ataxia (stumbling uncoordinated movements)
- Weakness
- Head and Neck Tremors
- Muscle Fasciculations
- Opisthotonus (extending the neck up and over the back)
- Convulsions or Involuntary Paddling
- Recumbency

Head and neck tremors were one of the most characteristic early signs that we observed and were considered distinct from the clinical signs observed with other neurological diseases of llamas and alpacas. Our clinical experience suggests that the severity of disease can be divided into three categories. Animals that are mildly affected generally remain standing and have a good appetite but demonstrate lethargy, weakness, mild ataxia, and may have head and neck tremors. The disease generally runs its course over a period of 5 to 14 days. Mildly affected animals generally show a complete recovery.

Moderately affected animals demonstrate the same signs but will show more profound weakness and become recumbent. They will generally be able to maintain themselves in a cushed position and will continue eating and drinking as long as food and water is kept nearby.

Sometimes they will lie in a lateral position but can return to a cushed position with assistance. These animals have a more prolonged recovery that may take up to a month or more for complete resolution of signs. We have observed a persistent paralytic syndrome similar to what is seen in some human patients in one animal. This animal returned to a near normal status after about one year.

Severely affected animals develop a very rapid onset of clinical signs that progress to lateral recumbency and convulsions. While survival in the mild and moderate cases is very high, severely affected animals are generally either euthanized or die of respiratory arrest in spite of treatment.

TREATMENT

Treatment for animals with suspected WNV encephalitis should focus on providing basic support for the animal. Appropriate nursing care including soft, clean bedding, available food and water should be provided. It is believed that efforts should be made to mitigate the CNS inflammatory process, however, there is no information regarding specific efficacy or contraindications of steroids (i.e. dexamethasone, prednisolone), non-steroidal anti-inflammatory drugs (i.e. Banamine), or DMSO in treating camelids with disease caused by WNV. Oral vitamin E (up to 10 IU/kg) has also been utilized for its anti-oxidant properties in treating central nervous system infections.

Human WNV patients describe severe muscle pain (myalgia) and muscle spasms during clinical disease. Specific treatments to relieve pain and provide muscle relaxation are important considerations in treating human cases of WNV. Non-steroidal anti-inflammatory drugs are used to provide systemic pain relief in mild cases. Opioid analgesics are utilized in more severe cases. Diazepam or other long acting diazepam derivatives are helpful in controlling muscle spasms in humans.

WNV positive plasma or specific WNV antibody has been advocated for treating horses and humans. Studies in mice demonstrate that passively acquired WNV antibody has prophylactic and therapeutic benefit.¹⁰ Limited data on cases at CSU suggest that serum antibodies to WNV are already increased by the time clinical disease is apparent. Thus, treatment with additional antibody may have limited clinical benefit. It is possible that the high WNV titers observed at presentation may be a consequence of the stage of disease in patients admitted to the CSU VTH. These observations suggest that if therapeutic WNV antibody is going to be administered, it should be administered as early as possible in the course of disease in order to prevent progression to more severe clinical disease. WNV positive plasma is commercially available for camelids (Triple J Farms, Bellingham, WA).

DIAGNOSIS

A presumptive diagnosis of WNV encephalomyelitis in a camelid patient requires compatible clinical signs, cerebrospinal fluid (CSF) analysis, and exclusion of other causes. There are no specific changes in bloodwork (CBC

or serum chemistry) that confirm a diagnosis of WNV encephalitis although 3 of 4 cases observed at CSU showed decreased numbers of lymphocytes in the blood at the time of clinical presentation. Elevated protein concentration and/or numbers of lymphocytes in cerebrospinal fluid are supportive of a diagnosis of WNV infection.

Current or previous infection with WNV can be confirmed by detection of serum antibody titer using a microtiter serum neutralization assay (Cornell Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY or Oregon State University Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Corvallis, OR) or a plaque reduction assay (National Veterinary Services Laboratory, Ames, IA). A four-fold or greater rise in antibody titers that occurs following observed clinical signs of disease is considered supportive of a diagnosis. Paired serum from suspect animals should be obtained early in the course of disease and again 10-21 days later. In the cases that we have observed at CSU, WNV antibody titers can be elevated at the time of clinical presentation but still increased markedly after recovery. CSF antibody titers were lower than the serum titers at presentation but increased over time. Postmortem diagnosis of WNV encephalomyelitis requires the presence of compatible gross and/or histological lesions, as well as demonstration of virus within nervous tissue by virus isolation, reverse transcriptase polymerase chain reaction (RT-PCR), and/or immunohistochemistry (IHC).¹¹⁻¹³

VACCINATION

As with other vector borne infections, WNV disease is best prevented by control of the vector, in this case mosquitoes, and stimulating immunity in susceptible animals. With most contagious diseases, establishing 70% to 80% herd immunity will successfully limit the spread of clinical disease through a herd. However, in the case of WNV, individual immunity is much more important than population immunity because the reservoir hosts are birds and mosquitoes. Because of this, a susceptible non-immune animal is just as likely to become infected whether 0% or 99% of herd mates are immune. Therefore, when looking at WNV vaccination programs it is important ensure that **EVERY** animal has sufficient immunity during the critical period of seasonal exposure.

There are no WNV vaccines licensed for use in camelids in the United States. Two commercial vaccines are currently licensed for use in horses and have been used on a limited basis in camelids. One is a killed virus vaccine (Innovator, Fort Dodge Animal Health) and the other is a recombinant canarypoxvirus DNA vaccine (Recombitek, Merial). There are no experimental challenge systems that reproduce neurological signs following WNV infection in horses so efficacy is determined based on seroconversion and the reduction or prevention of measurable viremia. The killed WNV vaccine can induce neutralizing antibody in horses within 14 days following the second dose of vaccine. Following WNV challenge 12 months after the second dose of vaccine, transient viremia was detected in 1 of 19 vaccinated horses and 9 of 11 control horses.¹⁴

The canarypox virus DNA vaccine incorporates WNV membrane and envelope protein genes into the canarypox virus DNA and expresses these proteins following administration.¹⁵ The method of antigen expression with the canarypox virus vaccine would suggest stimulation of both humoral antibody responses and cytotoxic T-cell responses believed to be important in both the prevention and clearance of WNV infection. The canarypox virus DNA vaccine prevented detectable viremia in 100% of the vaccinated horses two weeks after receiving 2 doses of vaccine while viremia was detected in 8 of 10 control horses. At 12 months, viremia was detected in only 1 of 10 vaccinated horses and 5 of 6 control horses, demonstrating duration of immunity. In addition, a single dose of vaccine confers some amount of immunity as only 1 of 9 horses vaccinated with a single dose developed viremia at 26 days post vaccination.

The killed equine WNV vaccine (Innovator, Fort Dodge Animal Health) has been evaluated in llamas and alpacas.¹⁶ No adverse reactions were observed when administered to adult llamas and alpacas, including pregnant females. Three intramuscular doses administered 30 days apart were required to stimulate an antibody response in >90% of vaccinated llamas and alpacas. While it is presumed that effective humoral immunity can decrease the incidence and severity of neurological disease caused by WNV in camelids, efficacy data following virulent challenge is not currently available.

Numerous llamas and alpacas were vaccinated with the killed equine WNV vaccine in Colorado in 2003 and 2004. Anaphylactic-like reactions were observed in two alpacas from Colorado that were vaccinated in 2003 with this vaccine. While both animals survived, this observation highlights the potential for adverse reactions when administering any vaccine to any animal. Because of the importance of monitoring adverse vaccine reactions, owners and veterinarians are strongly encouraged to report any adverse vaccine reactions to both the vaccine manufacturer and the USDA Center for Veterinary Biologics by visiting the Adverse Event Reporting web site at <http://www.aphis.usda.gov/vs/cvb/ic/adverseeventreport.htm> or by calling 800-752-6255.

We evaluated the safety of the canarypox virus vector WNV vaccine in over 100 alpacas and 40 llamas in the Spring of 2004. Two doses of vaccine were administered intramuscularly three weeks apart. No adverse reactions were observed in any of the animals. Injection site reactions were rare and consisted of mild swelling without evidence of pain. Thirty five pregnant alpacas were vaccinated and there was no evidence of pregnancy complications. Evaluation of humoral antibody response with this vaccine is in progress. Based on extrapolation from efficacy studies in horses, this vaccine should have a comparable efficacy to the commercially available killed vaccine.

Extralabel vaccination of camelids may be considered as a prophylactic management tool given the economic value and the apparent incidence of clinical disease in these

animals. While there is significant latitude in developing any vaccination program, the following guidelines are recommended.

- **Non-Vaccinated Adult Animals**
 - Fort Dodge Innovator Vaccine: Administer 3 intramuscular doses at 3-5 week intervals.
Or
Merial Recombitek Vaccine: Administer 2 intramuscular doses 3 weeks apart.
 - Begin vaccination at least 6 weeks before the start of mosquito season so that the animals will have antibody titers prior to high WNV activity.
 - As with any vaccine, inflammatory responses induced by vaccination can adversely affect pregnancy. If possible, avoid vaccinating breeding females within 30 days of breeding or within 30 days of parturition.

- **Previously Vaccinated Adults, Adults With Confirmed WNV Titers, or Unvaccinated Adults in an Endemic Area**
 - Administer a single booster vaccination at least 2 weeks before the start of mosquito season.
 - As with any vaccine, inflammatory responses induced by vaccination can adversely affect pregnancy. If possible, avoid vaccinating breeding females within 30 days of breeding or within 30 days of parturition.
 - At this time, there is no indication that animals will need an additional booster in the middle of the WNV season. However, some owners and veterinarians may want to consider an additional booster mid summer to provide higher immunity during the later WNV months.

- **Neonates**
 - Fort Dodge Innovator Vaccine: Administer 3 intramuscular doses at 3-5 week intervals.
Or
Merial Recombitek Vaccine: Administer 2 intramuscular doses 3 weeks apart.
 - Strategically vaccinate prior to the onset of WNV season when possible.
 - If the dam was vaccinated or has a confirmed WNV titer, start vaccination after 3 months of age or later depending on the season.
 - If the dam was not vaccinated and/or has a negative WNV titer, start vaccination at 1 month of age or later depending on the season.

Based on limited epidemiological information, a full primary vaccination series is recommended in herds located in areas that have not yet observed significant WNV activity. This would primarily include Oregon and areas of Washington. Single annual booster vaccination for adults is recommended in previously vaccinated animals. A single booster vaccination may also be sufficient in animals located in areas that have seen at least two years of WNV activity since a significant proportion of the animals will already have been exposed to natural infections. All animals less than one year of age should get a full primary vaccination series.

The economics of vaccination are also important considerations for llama and alpaca owners. In general, vaccination of animals is economically warranted if the cost of vaccination is less than the potential savings attributed to disease prevention through vaccination. It is very difficult to make general recommendations relative to the economic merits of vaccination in a specific herd and it is suggested that you discuss this with your veterinarian. The economics of vaccination will depend on the risk of infection in your area (WNV history, seasonal weather, types and numbers of mosquitoes, use of other mosquito control measures, etc.), previous exposure or vaccination, veterinary treatment costs, and vaccination costs.

A simplified economic model can be used to estimate the break even value of vaccination for an individual animal. For this example we will use the assumptions that 50% of susceptible animals will be infected, 10% of those infected will become ill, and 50% of those that become ill will die. Average veterinary and treatment costs are estimated to approach \$500 per animal showing disease. If your animals are in a previously unexposed and unvaccinated herd (100% susceptible animals), it is estimated that utilizing an initial vaccination protocol is economically warranted if the individual animal's value exceeds \$2000. If your animals are in a previously exposed or vaccinated herd (20% susceptible animals), then vaccination with a single booster is economically warranted if the individual animal's value exceeds \$3800. There is a great deal of latitude between these ranges but they provide a good starting point for making herd and individual animal decisions relative to the economic merits of WNV vaccination. You should work closely with your veterinarian to determine if WNV vaccination is appropriate for your herd

ADDITIONAL PREVENTION

Additional prevention of WNV infection should be directed at controlling mosquito vectors and their contact with animals. Since WNV disease is partly dependent on the total viral load and viral load is dependant on mosquito exposure, it becomes apparent that limiting the opportunity for mosquitoes to feed will help decrease the risk of disease. Excellent information on mosquito control can be found at <http://www.fightthebitecolorado.com> and <http://westnilevirus.nbii.gov/mosquitoes.html>. Areas of standing water should be drained if possible to minimize mosquito breeding areas. Water troughs should be emptied and refilled at least once weekly. Mosquito Dunks are a donut-shaped biological pest control product containing a bacterium, *Bacillus thuringiensis israelensis* (Bti), which infects and kills mosquito larvae in small bodies of water. These larvicidal products are nontoxic to animals including fish, birds, and mammals and can be placed in water troughs or small ponds to kill mosquito larvae. There are other granular Bti products (i.e. Vectoban) that can be spread on fields or low lying wet areas that serve as mosquito breeding grounds.

Mosquito contact with animals can be minimized by application of mosquito repellants. DEET (N,N-diethyl-m-toluamide) is one of the most effective mosquito and tick repellants available.¹⁷ DEET can be obtained in several

concentrations ranging from 5% to nearly 100%. The different concentrations determine the duration of repellent activity from a single application with 5% DEET providing approximately 1.5 hours of protection and 25% DEET providing nearly 5 hours of protection. Concentrations greater than 25% do not provide significantly more protection. Other basic husbandry practices that can limit mosquito exposure include housing animals indoors at dusk and dawn and the use of fans to limit indoor invasion and feeding by mosquitoes.

SUMMARY

WNV has become an important disease in the camelid industry. Since this virus is now endemic in the mosquito and bird populations of the continental United States, we will need to continue to take precautions to protect ourselves and our animals from infection. While most infections are mild, occasionally severe neurological disease can occur and these are sometimes fatal. Current equine WNV vaccines appear to be safe in alpacas and llamas. While these vaccines provide effective protection in horses, it is not known how much protection these vaccines provide for llamas and alpacas. Additional prevention should be directed at minimizing mosquito breeding areas and limiting exposure of your animals to mosquitoes.

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