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# Viral Diseases of New World Camelids

Sanjay Kapil, DVM, PhD<sup>a, \*</sup>, Teresa Yeary, PhD<sup>b</sup>, James F. Evermann, PhD<sup>c</sup>

## **KEYWORDS**

Alpaca 
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Breeding and raising New World camelids (NWC) in North America has become popular among hobby and alternative livestock farmers over the past 25 years. Llama farms and llama populations appeared as separate enumeration items for the first time in the USDA Census of Agriculture of 2002 with a total of 16,887 llama farms and 144,782 llamas inventoried.<sup>1</sup> According to the 2007 census, 8708 alpaca farms and 121,904 alpacas and 26,060 llama farms and 122,680 llamas were inventoried. Response to this census of agricultural operations is voluntary, so it is likely that the number of llamas was underreported and other species of NWC are not itemized in the census. In 2003, the USDA Animal and Plant Health Inspection Service (USDA-APHIS) asked camelid owners who are also members of the United States Animal Health Association to form a stakeholder group, the Camelid ID Working Group (CWG), to address permanent identification of camelids in the United States National Animal Identification System. According to the CWG 2006 report to USDA,<sup>2</sup> approximately 240,000 alpacas and llamas were enrolled by the four registries operating in the United States as compared with 7,500 animals on register in 1986. The CWG currently estimates that the total United States camelid population may be closer to 300,000-325,000, because not all animals are registered. Clearly, the increased popularity and population of NWC in the United States requires the development of a broader base of knowledge of the health and disease parameters for these animals by the veterinary livestock practitioner who encounters clients maintaining NWC as individual pets or herds.

The family Camelidae is divided between two tribes,<sup>3</sup> *Camelina*, the Old World camels (OWC), and *Lamini*, the New World camels, which diverged from their common ancestor about 25 million years ago based on molecular clock analysis of their

\* Corresponding author.

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<sup>&</sup>lt;sup>a</sup> Department of Veterinary Pathobiology, Oklahoma Animal Disease Diagnostic Laboratory, Center for Veterinary Health Sciences, Farm and Ridge Road, Stillwater, OK 74078, USA

<sup>&</sup>lt;sup>b</sup> Center for Veterinary Biologics, Veterinary Services Animal and Plant Health Inspection Service, United States Department of Agriculture, Ames, IA, USA

<sup>&</sup>lt;sup>c</sup> Department of Veterinary Clinical Sciences, Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, USA

E-mail address: sanjay.kapil@okstate.edu (S. Kapil).

complete mitochondrial genomes.<sup>4</sup> Old World camelids consist of two species, the two-humped Bacterian camel (Camelus bactrianus) and the one-humped dromedary camel (C. dromedarius), both large animals indigenous to Asia and Africa. Native to South America, the smaller NWC are divided among four species: guanaco (Lama guanicoe), Ilama (L. glama), alpaca (L. pacos) and vicuna (L. vicugna). Camelids, swine, and ruminants are members of the order Artiodactyla. Disease susceptibility to viral pathogens is controlled by the innate immune system and acquired immune systems. Phylogenetic studies of inflammatory cytokine IL-1, IL-1 $\alpha$  and IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-13, and TNF- $\alpha$  genetic sequences indicate that these NWC cytokines are more closely related to those of pig, cattle, sheep, and horses than of humans, dogs, cats, and rat.<sup>5,6</sup> These inflammatory cytokines are known to play important roles in outcomes of infectious diseases,<sup>7</sup> thus it is not surprising that many of the viral diseases that affect camelids are related to bovine, equine, ovine, and swine viruses. Vertebrate antibody structure typically consists of two large heavy chains and two small light chains. However, camelid antibodies lack light chains<sup>8</sup> yet they have extensive and diverse antigen-binding capacity in the absence of H-L combinatorial diversity.<sup>9</sup> The effect of this unusual antibody structure has yet to be determined.

The true extent of viral infections that can lead to overt disease in camelids is becoming better known as the veterinary community assesses the scientific literature,<sup>10</sup> which has increased greatly over the past 10 years as more research is conducted. Our knowledge base regarding viral infections of NWC and their economic impact has been increased primarily due to the contribution of information on the part of owners, the independent llama and alpaca registries, and veterinarians and diagnostic laboratories that are involved in camelid health treatment and diagnosis. Earlier reports on viral infections of camelids produced two main classifications: those causing disease and those not causing disease (nonpathogenic viral infections).<sup>11</sup> The result has been a priority list of major viral infections and those of minor significance to the health of camelids (Box 1). Reviews<sup>12,13</sup> have also listed some of the major viral infections affecting camelids. Although the classification was useful, it is important to recognize that these reviews tended to reflect the status of some viral infections on a regional basis, which established a bias against a more global understanding of the real and potential effects of viral infections upon camelids, and thus limited the extent of diagnostic surveillance worldwide. Another approach to classifying the causes of overt disease would be to look at the viral infections that occur within the camelid species (alpacas, llamas, dromedaries) and those that can be acquired from other animal species via interspecies spread (Fig. 1).<sup>14</sup> This type of approach uses risk as a criterion for assisting in infection control and disease diagnosis.

#### **VESICULAR STOMATITIS VIRUS**

The vesicular stomatitis virus (VSV) agent is classified as a vesiculovirus under family *Rhabdoviridae* and is widely distributed in North and South America.<sup>15</sup> Two serotypes of VSV circulate in the United States: New Jersey (VSV–NJ) and Indiana (VSV–IN). Isolates exhibit a low level of genetic variation and only four distinct genotypes have been detected in the United States and Mexico. VSV commonly infects horses, cattle, goats, sheep, and pigs. Clinical disease is rare in Ilamas. Serologic evidence of VSV infection in alpacas has been reported.<sup>16</sup> Alpaca VSV isolates have been classified as genotype G2<sup>15</sup> and most infections with VSV are subclinical and go unrecognized. Viral infection is localized in the epithelial cells of the mouth. Excess salivation is the most common clinical sign of VSV infection. Fulminant disease is characterized by formation of vesicles in the mouth, tongue, nostrils, feet, and teats. The incubation

# Box 1

Viral infections of alpacas

- Adenovirus
- Equine viral arteritis virus
- Rabies
- Bluetongue virus
- Foot-and-mouth disease virus
- Respiratory syncytial virus
- Bovine viral diarrhea viruses
- Influenza A virus
- Rotavirus
- Contagious ecthyma (orf)
- Papillomavirus
- Vesicular stomatitis virus
- Coronavirus
- Parainfluenza-3 virus
- West Nile virus
- Equine herpesvirus-1 (equine rhinopneumonitis)

*Data from* Barrington GM, Allen AJ, Parish SM, et al. Biosecurity and biocontainment in alpaca operations. Sm Rum Res 2006;61:217–26; and Wernery U, Kaaden OR. Foot-and-mouth disease in camelids: a review. Vet J 2004;168(2):134–42.

period of VSV is about 2–8 days. Death is rare but animals may refuse to eat. Mild antiseptic mouthwashes may be applied to prevent secondary infections. Little-to-no immunity develops against VSV and no vaccine is currently available to protect against this disease. Other differential viral diseases to consider in diagnosis are rabies, orf (contagious ecthyma), and foot-and-mouth disease, a serious foreign animal disease. Because VSV is a reportable disease in the United States, if you suspect VSV, contact your state's USDA–APHIS area veterinarian-in-charge, who will arrange to collect appropriate samples to be sent to the National Veterinary Services Laboratory in Ames, Iowa. Samples to be submitted include serum for VSV-serum neutralization testing for both serotypes. Vesicular fluid and epithelium scrapings are also collected for virus isolation and PCR. If VSV is detected and confirmed, the animals will be placed on quarantine for 30 days after the symptoms disappear. This is a seasonal disease with more cases happening in summer and fewer in winter.<sup>17</sup> Epidemiologic maps indicate that viral outbreaks occur in populations located along rivers.

#### FOOT-AND-MOUTH DISEASE VIRUS

Foot-and-mouth disease (FMD) is caused by an apthovirus belonging to family *Picornaviridae*. It is an RNA virus that has seven immunologically distinct serotypes and subtypes.<sup>18</sup> Susceptible species include cattle, sheep, goats, pigs, bison, and deer. New World camelids appear to less susceptible to FMD virus.<sup>19</sup> Foot-and-mouth disease is a major barrier for trade around the world because it can cause serious morbidity and mortality in livestock.<sup>13</sup> The role of New World camelids in FMD epidemiology is controversial. Depending on the type of virus contracted, individual

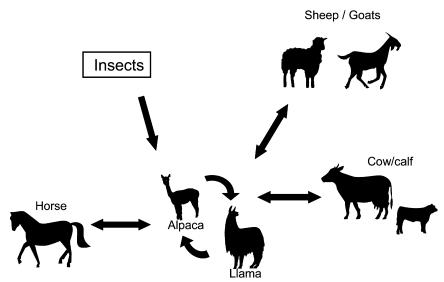


Fig. 1. Dynamics of interspecies spread of viruses between closely and distantly related species. Viral spread may also be facilitated through insects serving as vectors.

responses vary. Based on an experimental study after exposure to FMD strain A24, llamas developed vesicular lesions on their extremities.<sup>20</sup> In this study, animals did not excrete virus in esopharyngeal fluid beyond 8 days.<sup>13</sup> Therefore, it is likely that camelids play a minor role in transmission of FMD virus, because they do not become carriers of the virus. Although not performed commonly, llama embryo transfer presents a low risk for transmission of FMD.<sup>21</sup>

# MUCOCUTANEOUS FIBROPAPILLOMAS

Papillomaviruses are small, nonenveloped DNA viruses that infect the skin of animals. These viruses replicate exclusively in skin and mucosal surfaces, are resistant to disinfectants and stable in the environment. Camelid mucocutaneous fibropapillomas were reported on the lips, nose, nares and cheeks in two llamas and three alpacas, all 6 years of age.<sup>22</sup> Lesions on the face appeared as hyperkeratotic gray nodular tumors. To confirm a diagnosis of papillomavirus, fresh skin biopsies can be submitted to a diagnostic laboratory for pan-papillomavirus PCR. Based on a partial sequence of a conserved sequence of the E1 gene of papillomavirus, the camelid papillomavirus has a 73% homology with bovine papillomavirus-1 and 64% homology with canine papillomaviruses; however, it has not yet been demonstrated to be useful in diagnosing camelid fibropapilloma cases. Electron microscopy can also be used to diagnose papillomavirus infections.

# **PESTIVIRUS INFECTIONS**

Speculation about potential effects of bovine viral diarrhea (BVD) virus infection in NWC were initially viewed with skepticism about the disease potential of the virus,<sup>23</sup> because early evidence centered on serologic studies and sporadic reports of BVD viral infections being associated with respiratory disease, enteric disease, and

occasional abortions.<sup>23</sup> A pestivirus, BVD has two biotypes, cytopathic and noncytopathic, based on production of cytopathic effects in cell culture, and is further classified as two major genotypes, Type I and II; both have been reported in NWC. Bovine viral diarrhea virus has now been shown to be involved in NWC cases of diarrhea, ill thrift, reproductive losses, respiratory disease, and disseminated disease<sup>23-25</sup> and it is capable of causing multisystem effects in NWC in much the same manner as BVD viral infections in cattle, sheep, and goats.<sup>25</sup> The serologic incidence of BVDV in NWC has been reported to be from about 4.4%<sup>24</sup> to as high as 53%.<sup>23</sup> One of the major effects of BVD viruses upon NWC is infection of naïve animals during pregnancy, resulting in abortion or establishing an immunotolerance in the developing fetus via congenital infection. The hallmark of the congenital infection is the formation of a cria that is persistently infected (PI), in much the same way that has been described for BVD virus PIs in cattle. The literature has been gracious in regards to documenting the occurrence of BVD PI crias, and, of major importance, biosecurity measures to minimize contact of pregnant BVD-naïve alpacas/llamas with BVD virus-infected herd mates (including commingling with cattle, sheep, and goats).<sup>23</sup> Much of the clinical interpretation and pathogenesis of BVDV in NWC is derived from studies in cattle. Production of PI in cattle is a characteristic of BVDV. Recently, PI has been reported in alpacas in North America and Europe.<sup>25–29</sup> Because the gestation period is much longer in NWC than in cattle, the period of susceptibility of the camelid fetus for induction of PI has not been accurately determined. BVDV has been isolated from a stillborn alpaca fetus,<sup>30</sup> however, the number of BVDV-induced abortions is still rare in NWC. The virus has been isolated from crias that fail to gain body weight.<sup>27</sup> Ocular and nasal infections have also been reported.<sup>28</sup>

Detection of BVDV in NWC is diagnostically challenging.<sup>31</sup> Most BVDV-detection assays used for cattle, such as virus isolation, serum-neutralization, and RT–PCR, can be used in NWC. However, the commercial antigen-capture ELISA test can give false positive results, because NWC serum samples cause high background reactions. Diagnosis of BVD viral infections in NWC follows strategies similar to cattle, including virus isolation from key tissues (lymph nodes, whole blood, fetal tissues, and placenta), PCR from whole blood, and immunohistochemistry (IHC) on formalin-fixed tissues. The availability of BVD virus PI-specific assays for cattle has not yet been validated for NWC. These assays use ear notch elutes or serum from suspected animals that are tested for BVD viral antigen by an ELISA or antigen-capture ELISA (ACE). Studies conducted on naturally infected BVD virus PI crias have indicated that the serum may be suitable for testing. Until further validation studies are conducted, screening NWC by BVD viral-specific PCR on whole blood will detect active infection/viraemia (either acute, transient infection, or a PI), and serology on serum will detect neutralizing antibody indicating prior infection and probable immunity.

A recent nationwide survey reflected the extent of BVD viral infection in the United States.<sup>32</sup> Of the 63 herds tested, 25% had BVD seropositive crias and 6% had evidence of BVD virus PI crias. Seropositivity to BVD virus was linked to the feeding of supplemental bovine colostrum containing BVD viral antibodies. The study emphasized the need to establish biosecurity measures that prevent transporting female alpacas with crias at side to other farms for breeding. If the cria is persistently infected with BVD, the probability of BVD virus infection to susceptible (naïve) females in other herds is a high risk. Experimental inoculation of alpacas vaccinated with a killed BVD viral vaccine resulted in seroconversion by all the animals and protection against a challenge infection with a field strain of alpaca BVD virus type 1b (S. Byers and colleagues, personal communication, 2008). Although licensed vaccines are available for controlling BVD viral infections in cattle, none are labeled for use in NWC. Use of

BVDV vaccines for NWC is not recommended, because the disease is not a wide-spread problem in camelids.

## ADENOVIRUS-ASSOCIATED PNEUMONIA

Adenoviruses are double-stranded DNA viruses that have been associated with a variety of clinical conditions, though infection may present only as subclinical disease. Four cases of adenovirus infections in llamas have been reported.<sup>33</sup> Bronchopneumonia involving a large portion of the lung in a 5-month-old llama was described. Mucopurulent nasal discharge and lethargy was displayed by the second llama. Both llamas had fibrinous pleuritis and serosanguinous pleural exudates. The third llama had chronic wasting and respiratory alkalosis. Llamas 3 and 4 both had fibrinous peritonitis and pleuritis. Histologic examination of liver showed large intranuclear inclusion bodies compatible with adenovirus infection. Virus was detected by fluorescence antibody testing in the lung parenchyma of all four animals using a porcine adenovirus conjugate.

## **ROTAVIRUS ENTERITIS**

Rotavirus (RV) has been documented as a cause of diarrhea in South American camelids.<sup>34</sup> Based on serum antibody prevalence, RV is quite common in South American camelids (77%-98%).<sup>35</sup> In one report, group A rotavirus was isolated from two newborn guanacos from farms 700 km apart that had previously reported severe outbreaks of diarrheal disease during calving. The presence of RV was confirmed by electron microscopy. Circulating antibodies against RV were not detected in either animal, indicating a failure of passive transfer of maternal antibodies. Coronavirus infection was not detected in the two young guanacos from either farm, based on bovine coronavirus antigen and serology testing; however, bacterial infections due to Escherichia coli and Salmonella were detected. Both guanacos were captured, hand raised, and fed with bovine milk. Based on electrophoresis, both farms experienced a distinctive long dsRNA electropherotype of RV that differed from each other and also from bovine RV isolates. Both rotaviruses were genotype G8; however, the bovine P types of the RV were P[1] and P[14].<sup>35</sup> A subsequent serologic survey conducted on the two farms indicated a high (95%) prevalence of antibody titers in the herds.36

# CORONAVIRUS

Coronavirus is the most common viral cause of enteritis in young crias and adult camelids.<sup>32,37,38</sup> The diarrhea due to coronavirus in camelids can be severe and require intensive therapy. In one report, alpaca coronavirus was involved in 42% of the cases of diarrhea. The virus was detected throughout the year.<sup>37</sup> Alpaca coronavirus is closely related to antigenic group 2 bovine coronavirus.<sup>39</sup> In a recent case report,<sup>38</sup> an adult 4-year-old alpaca experienced weight loss, nutritional stress, and foul smelling, watery diarrhea. Antigenic group 2 coronavirus related to bovine coronavirus was detected by RT–PCR and IHC.<sup>38</sup> Diagnosis of coronaviral diarrhea can be achieved by electron microscopy or by PCR techniques. It has been speculated that the association of coronaviral diarrhea in alpacas may be an indication of a stress (nutritional, immunologic, and so forth), and may be a stress indicator (James Evermann, personal communication, 2009).<sup>38,39</sup> There is a preliminary report that acute respiratory syndrome (ARS), a recently described disease (also known by the colloquial term "snots" in the alpaca industry), is caused by antigenic group I coronavirus.<sup>40</sup> The disease is associated with mild-to-severe respiratory presentations and, on postmortem examination, interstitial pneumonia is observed with lymphocytic infiltrates, thus indicating a viral cause. Koch's postulates have not been fulfilled; however, based on serology, the animals from herds with a reported history of ARS have been found to have increased antibody titers to the group I coronavirus.<sup>40</sup>

#### **EQUINE HERPESVIRUS-1**

Herpesviruses are stable, double-stranded DNA viruses that cause respiratory disease, abortions, ocular infections, and nervous system diseases. Most herpesviruses are highly host-specific, although there are rare reports of transmission of herpesvirus infections to other species after close contact.41 The occurrence of disease following infection of NWC by equine herpesvirus-1 (EHV-1) was a major turning point in studies of infections capable of causing clinical symptoms in alpacas and llamas. Before this report,<sup>41</sup> serologic data had indicated that NWC were susceptible to infection as determined by specific antibodies, but apparently resistant to many equine and bovine viruses. An outbreak of blindness and encephalitis due to infection with EHV-1 occurred in a group of 100 camelids on an exotic animal farm in New York State, one year after their import from Chile.<sup>41</sup> Four animals developed neurologic disease. Unlike EHV-1 infection of horses, which causes a systemic infection, the pathogenesis of EHV-1 in NWC involved infection of the nasal mucosa, followed by spread to the olfactory nerve, optic nerve, and then CNS. The blindness in these animals was characterized by dilated unresponsive pupils that were confirmed by fundoscopic examination and there was evidence of vitritis, retinal vasculitis, retinitis, chorioretinitis, and optic neuritis in 21 alpacas and one llama. All affected animals were seropositive for antibodies to EHV-1. Virus isolated from brain and eye tissues was experimentally tested for virulence by intranasal infection of three llamas.<sup>42</sup> Two animals developed severe neurologic disease and one developed mild disease. Lesions developed in brain and retina after experimental infection and the virus was recovered from thalamus of one animal.<sup>42</sup> Vaccines are available for prevention of EHV-1 in horses only, but, as a rule, they are not to be used off-label to protect NWC unless the animals are in close proximity to horses. Only killed vaccines should be considered in the NWC until further safety-efficacy studies are conducted with modified live virus vaccines.

#### **RABIES VIRUS**

Rabies is an important zoonotic disease that is introduced mostly from wildlife to domestic animals and humans.<sup>43</sup> Reports of rabies virus infections in camelids are rare, but spread of rabies from alpaca to alpaca can occur.<sup>12,44</sup>

Rabies viral infection in camelids is primarily spread by feral dogs, although the red fox, skunks, and bats have also been reported as transmitters. In dromedary camels there are two forms of rabies: "raging fury" and the more rare "silent fury." Incubation ranges from as short as 3 weeks up to 6 months. During the final paralytic stage, dromedaries will be recumbent and attempt to yawn continuously (very characteristic). In NWC, rabies results in an aggressive form and, rarely, the paralytic form. The major symptoms are self-mutilation and attacks upon humans and penmates. It has also been noted that NWC cannot spit due to the paralysis of pharynx. Diagnosis of rabies must be handled by a veterinarian and is conducted by standard methods on impression smears of fresh brain. Prevention of rabies can be achieved by barrier vaccination of animals in close proximity to camelids, such as dogs and cats. Killed vaccines have

been successfully used in both OWC and NWC (Melanie Boileau, personal communication, 2009).

# EASTERN EQUINE ENCEPHALITIS

Eastern equine encephalitis (EEE) virus is an Alphavirus that belongs to the family Togaviridae. It is transmitted seasonally by mosquitoes to horses and humans, who are dead-end hosts for EEE. There is one retrospective case report with eight alpacas and one llama.<sup>45</sup> Central nervous diseases can be difficult to diagnose in camelids due to their stoic nature, but clinical signs may include lethargy, twitching of head and neck, opisthotonus, seizures, vestibular signs, and ataxia. Like other viral encephalitides, EEE induces an increased protein concentration and mononuclear pleocytosis. The mortality rate in these cases was close to 90%. The virus was localized in the brain tissue. The lesions were not detected in the spinal cord. The diagnostic tools include virus isolation, RT–PCR on brain tissues, and IHC on brain tissue. Serology can be used as a supportive tool. A killed EEE vaccine is under evaluation for camelids.<sup>46</sup> Camelids are also susceptible to Western and Venezuelan equine encephalitis viruses.

# WEST NILE VIRUS

West Nile virus (WNV) is a mosquito-borne Flavivirus that is indigenous to Africa, Asia, Europe, and Australia. It is transmitted to horses and humans by mosquitoes that have fed on birds that carry the virus. Since 1999, NWV has spread progressively across the United States, so that it is now regarded as an endemic infection. Camelids are relatively genetically resistant to WNV; the attack rate (clinical symptoms) is low compared with the overall infection rate. Primary clinical symptoms of WNV infection are ataxia, hyperesthesia, and recumbency. There are three reports of WNV encephalitis in alpacas ranging in age from 3 months to 7 years.<sup>46–48</sup> The clinical signs reported include fever, lethargy, depression, anorexia, encephalitis, head tremors, ataxia, stiff gait, altered mental status, and hyperesthesia. Treatment with hyperimmune camelid serum with WNV-neutralizing antibodies can provide protection in the early stage of the MNV infection.<sup>49</sup> A neutralization assay is available for antemortem diagnosis. RT-PCR can be performed on brain tissues to confirm the pathologic diagnosis, as can IHC on selected fixed brain and spinal cord tissues. There is one report that a licensed equine WNV vaccine is safe for use in camelids; however, the efficacy of the vaccine has not been determined.49

# **BLUETONGUE VIRUS**

Bluetongue virus (BTV) is a double-stranded segmented RNA virus belonging to the family Reoviridae, genus *Orbivirus*, that has the ability to evolve and spread to new geographic areas due to Culicoides vector transmission.<sup>50</sup> There are 24 serotypes of BTV and it has recently emerged in parts of Europe. There are a few reports of serum antibodies against BTV in camelids from South America. The susceptibility of camelids may be affected by the virulence of the infecting BTV. In one experimental infection with bluetongue serotype 10, both inoculated llamas remained clinically normal, but became seropositive after 1–2 weeks.<sup>51</sup> There is one published report of lethal bluetongue infection in a 5-year-old female alpaca with a cria.<sup>52</sup> Before death, the alpaca developed respiratory symptoms with coughing and hiccup-like breathing. On postmortem examination, the adult alpaca had erosions and ulcers on the tongue, palate, and buccal mucosa, and severe congestion, interstitial pneumonia, and alveolar edema in the lung. Mild hypertrophy of type II pneumocytes was observed by

histopathology. The cria remained healthy. A competitive ELISA for detection of bluetongue antibodies has been published.<sup>51</sup> The main impact of BTV seropositivity in camelids may be the effect upon animal trade.<sup>50</sup>

#### **OTHER VIRAL INFECTIONS**

A few other viral infections have been reported in camelids; however, evidence for disease due to these viruses is limited. A yearling llama that had a 6-month history of weight loss, lameness, and opportunistic infections tested positive for retrovirus reverse transcriptase in macrophages and lymph node.<sup>53</sup> Consistent with retroviral infection, a complete blood count showed neutropenia and lymphopenia and the animal had generalized lymphoid hypoplasia and plasma cell depletion. The llama also developed Pneumocystis carinii infection of lungs. Suspected equine arteritis virus (EAV) abortion was reported in a herd of alpacas, but the diagnosis was not confirmed.54 The virus was detected by RT-PCR in fetal tissues collected from a female alpaca that aborted in her last trimester of gestation.<sup>54</sup> All five animals in the herd had antibodies to EAV. There is a preliminary report of isolation of bovine herpesvirus in a llama with bronchopneumonia.<sup>55</sup> Pasteurella haemolytica was isolated from the respiratory tract of the llama. Infectious bovine rhinotracheitis (IBR) herpesvirus was confirmed by virus isolation, yet the animal was negative for IBR antibodies by serum neutralization test; this lack of antibody response may have been to due to acute death.

#### BIOSECURITY

As our knowledge regarding infectious diseases of camelids has increased, it has become apparent that institution of external and internal biosecurity measures must become a standard by both producers and the veterinarians that serve them. According to a recent review,<sup>56</sup> biosecurity can be defined as those efforts designed to prevent the introduction and subsequent spread of a disease in a population of animals. These efforts can be further divided into *external* measures and *internal* measures (Table 1). External measures are those steps that are directed at preventing introduction of new diseases into a group; whereas internal measures are aimed at minimizing the spread and subsequent disease in populations where an infection is already established or endemic. To be valid, the minimum biosecurity plan should address (1) the means of isolating new animals introduced into an existing population of animals; (2) the regulation of animal movement and on-the-farm worker and equipment traffic; and (3) the practical design and implementation of cleaning and disinfection procedures that are specifically directed at reducing the pathogen load within the population of animals.

#### DIAGNOSIS AND VACCINES FOR CAMELID VIRUSES

Although the diagnosis of camelid viral diseases is still relatively new, the observation that there is considerable interspecies spread of viruses (see **Fig. 1**) among camelids and members of the equine and bovine species allows for veterinarians in search of diagnostic capabilities to locate reputable diagnostic laboratories to assist in disease diagnosis and biosecurity surveillance. **Table 2** lists those infectious agents that may be encountered. The table is divided into subclinical infections, where a biosecurity profile would be of interest in a presale/purchase, preshipment for regulatory purposes, and prebreeding. The profile usually includes serology testing for *Brucella abortus*, BTV, BVDV, and Johne's disease serology, BVDV PI detection, and

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#### Table 1

## Examples of external and internal biosecurity

External Biosecurity	Internal Biosecurity			
	Host Animal	Infections Agent	Environmental	
Isolation of new animals	Animal density	Virulence factors	High population density	
Quarantine facilities/ procedures Infection testing (detecting carrier animals) Preventive measures	Nursing behavior Failure of passive transfer Nutritional deficiencies (diet) Optimum body condition Unlimited access to clean water	Survival of agent in environment outside the host Persistence of agent inside host–carrier Size of pathogen load	Temperature, humidity, ventilation Housing (barns versus pastures) Physical environment (bedding, animal exposure, cleaning/	
Good hygiene	Immune competence: neonatal, pregnant, etc.	Solo pathogens versus concurrent–synergistic opportunistic pathogens	disinfection) Transportation/ handling	

Data from Barrington GM, Allen AJ, Parish SM, et al. Biosecurity and biocontainment in alpaca operations. Sm Rum Res 2006;61:2177–26.

Subclinical Biosecurity Profiles	Clinical Disease	Antemortem Specimens	Postmortem Specimens
	Respiratory	BVDV serology (RTT)	Fixed/fresh lung H, <sup>d</sup> V <sup>e</sup>
Brucella abortus serology (RTT <sup>a</sup> )		PI-3 serology (RTT)	
BTV serology (RTT)		RSV serology (RTT)	
BVDV Serology (RTT)		IBR serology (RTT)	
BVDV PI (PTT <sup>b</sup> )	Reproduction	BVD serology (RTT)	Fixed/fresh fetal tissues H,V
Johne's disease serology (RTT)		IBR serology (RTT)	
Coronavirus shedding (fecal $\rightarrow$ EM <sup>c</sup> )		EHV-1 serology (RTT)	
Coronavirus serology (RTT)	Neurologic	EHV-1 serology (RTT)	Fixed/fresh brain H,V <sup>f</sup>
	-	WNV serology (RTT)	
		Rabies	
	Skin	CE/orf (scab $\rightarrow$ EM)	Biopsy H
		VSV serology (RTT) swab $\rightarrow$ V	
		BTV serology (RTT)	
		PCR/VI (PTT)	

<sup>a</sup> Red top tube (ie, clot tube, for serum harvest).
<sup>b</sup> Purple top tube (ie, contains EDTA; for whole blood collection).
<sup>c</sup> Electron microscopy.
<sup>d</sup> Histopathology.
<sup>e</sup> Virology (PCR/virus isolation).
<sup>f</sup> Contact lab before sending.

coronaviral shedding (electron microscopy) or serology. These biosecurity profiles may vary from region to region and it is best to contact the laboratory for specifics on proper samples, form of shipment, and laboratory fees. The other section of **Table 2** lists those diseases that may be suspected and the samples that can be collected either antemortem or postmortem. This section of the table is further divided into where the predominant disease symptoms are manifested, such as respiratory, reproduction, neurologic, or skin. It is best to contact the laboratory before collecting specimens to determine the best samples to collect for maximizing diagnostic results.

#### DIAGNOSIS OF CAMELID VIRAL DISEASES IS OFFERED AT THE FOLLOWING:

- New York Animal Health, Cornell University, Ithaca, New York; http://diaglab.vet. cornell.edu
- Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma; http:// www.cvhs.okstate.edu
- Oregon State University Diagnostic Laboratory, Corvallis, Oregon; http:// oregonstate.edu/vetmed/vdl/vdl.htm
- Washington State University, Pullman, Washington; http://www.vetmed.wsu. edu/depts\_waddl

Other veterinary diagnostic laboratories that handle bovine and equine samples are also capable of handling camelid viral diagnosis. No reagents specific for camelid virus diagnosis are available but one established camelid cell line is available through the American Type Culture Collection, namely, the Dubca cell line (ATCC CRL-2276),<sup>57</sup> which was established from a 2-month-old dromedary fetus and immortalized by transformation with SV40.

Camelid species are relatively healthy animals and infectious diseases are rarely reported in the United States. Vaccines administered to camelids in this country are strictly off-label; thus, killed vaccines are preferred for all camelids, especially pregnant animals. Killed rabies virus vaccines have been used in camelids (Melanie Boileau, personal communication, 2009). Equine herpesvirus-1 vaccines have been administered to camelids that may be in contact with horses.<sup>58</sup> Because the practice of camelid medicine is a relatively new field in North America, it important to seek out the advice and information of seasoned colleagues who have experience treating camelids for viral diseases that are common in your region.

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## REFERENCES

 USDA Census of Agriculture. U.S. national level data. Table 32. Other Animals and animal products - inventory and number sold, vol. 1. Available at: http:// www.agcensus.usda.gov/Publications/2002/Volume\_1\_Chapter\_1\_US/index.asp. Accessed April 7, 2009.

- Camelid Identification Working Group (CWG) status report. Available at: http:// animalid.aphis.usda.gov/nais/naislibrary/documents/plans\_reports/CamelidWG\_ 2006\_Status\_Report.pdf. Accessed April 7, 2009.
- Larson J, Ho J. AWIC Resource series no. 12. Information resources on the South American camelids: Ilamas, alpacas, guanacos, and vicunas 1943–2006. Compiled by: Animal Welfare Information Center, USDA, ARS, NAL, Beltsville, MD. Revised 2006. Available at: http://www.nal.usda.gov/awic/pubs/llama.htm.
- Cui P, Ji R, Ding F, et al. A complete mitochondrial genome sequence of the wild two-humped camel (*Camelus bactrianus ferus*): an evolutionary history of camelidae. BMC Genomics 2007;8:241.
- Odbileg R, Lee SI, Ohashi K. Cloning and sequence analysis of Ilama (*Lama glama*) Th2 (IL-4, IL-10 and IL-13) cytokines. Vet Immunol Immunopathol 2005;104(3–4): 145–53.
- Odbileg R, Konnai S, Ohashi K, et al. Molecular cloning and phylogenetic analysis of inflammatory cytokines of Camelidae (Ilama and camel). J Vet Med Sci 2005;67(9):921–5.
- 7. Murtaugh MP, Baarsch MJ, Zhou Y, et al. Inflammatory cytokines in animal health and disease. Vet Immunol Immunopathol 1996;54(1–4):45–55, Review.
- 8. Hamers-Casterman C, Atarhouch T, Muyldermans S, et al. Naturally occurring antibodies devoid of light chains. Nature 1993;363:446–8.
- 9. Conrath KE, Werney U, Muyldermans S. Emergence and evolution of functional heavy-chain antibodies in Camelidae. Dev Comp Immunol 2003;27:87–103.
- 10. Mattson DE. Update on Ilama medicine. Viral diseases. Vet Clin North Am Food Anim Pract 1994;10(2):345–51.
- 11. Thedford TR, Johnson LW. Infectious diseases of new world camelids (NWC). Vet Clin North Am Food Anim Pract 1989;5(1):145–57.
- Abbas B, Omer OH. Review of infectious diseases of the camel. Vet Bull 2005; 75(8):N1–16.
- 13. Wernery U, Kaaden OR. Foot-and-mouth disease in camelids: a review. Vet J 2004;168(2):134-42.
- Parrish CR, Holmes EC, Morens DM, et al. Cross-species virus transmission and the emergence of new epidemic diseases. Microbiol Mol Biol Rev 2008;72: 457–70.
- Rainwater-Lovett K, Pauszek SJ, Kelley WN, et al. Molecular epidemiology of vesicular stomatitis New Jersey virus from the 2004–2005 US outbreak indicates a common origin with Mexican strains. J Gen Virol 2007;88(7):2042–51.
- 16. Rivera H, Madewell BR, Ameghino E. Serologic survey of viral antibodies in the Peruvian alpaca (*Lama pacos*). Am J Vet Res 1987;48(2):189–91.
- Schmidtmann ET, Tabachnick WJ, Hunt GJ, et al. 1995 epizootic of vesicular stomatitis (New Jersey serotype) in the western United States: an entomologic perspective. J Med Entomol 1999;36(1):1–7.
- Musser JM. A practitioner's primer on foot-and-mouth disease. J Am Vet Med Assoc 2004;224(8):1261–8.
- 19. Fondevila NA, Marcoveccio FJ, Blanco Viera JB, et al. Susceptibility of Ilamas (*Lama glama*) to infection with foot-and-mouth-disease virus. Zentralbl Veterinarmed B 1995;42(10):595–9.
- Lubroth J, Yedloutschnig RJ, Culhane VK, et al. Foot-and-mouth disease virus in the llama (*Lama glama*): diagnosis, transmission, and susceptibility. J Vet Diagn Invest 1990;2(3):197–203.
- 21. Sutmoller P. Risk of disease transmission by Ilama embryos. Rev Sci Tech 1999; 18(3):719–28.

- 22. Schulman FY, Krafft AE, Janczewski T, et al. Camelid mucoutaneous fibropapillomas: clinicopathologic findings and association with papillomavirus. Vet Pathol 2003;40(1):103–7.
- 23. Evermann JF. Pestiviral infection of Ilamas and alpacas. Small Ruminant Research 2006;61:201–6.
- 24. Belknap EB, Collins JK, Larsen RS, et al. Bovine viral diarrhea virus in new world camelids. J Vet Diagn Invest 2000;12(6):568–70.
- 25. Byers SR, Snekvik KR, Righter DJ, et al. Disseminated bovine viral diarrhea virus in persistently infected alpacas (*Vicugna pacos*). J Vet Diagn Invest 2009;21:145–8.
- 26. Foster AP, Houlihan M, Higgins RJ, et al. BVD virus in a British alpaca. Vet Rec 2005;156(22):718–9.
- 27. Foster AP, Houlihan MG, Holmes JP, et al. Bovine viral diarrhoea virus infection of alpacas (*Vicugna pacos*) in the UK. Vet Rec 2007;161(3):94–9.
- 28. Mattson DE, Baker RJ, Catania JE, et al. Persistent infection with bovine viral diarrhea virus in an alpaca. J Am Vet Med Assoc 2006;228(11):1762–5.
- 29. Carman S, Carr N, DeLay J, et al. Bovine viral diarrhea virus in alpaca: abortion and persistent infection. J Vet Diagn Invest 2005;17:589–93.
- 30. Goyal SM, Bouljihad M, Haugerud S, et al. Isolation of bovine viral diarrhea virus from an alpaca. J Vet Diagn Invest 2002;14(6):523–5.
- Evermann JF, Berry ES, Baszler TV, et al. Diagnostic approaches for the detection of bovine viral diarrhea (BVD) virus and related pestiviruses. J Vet Diagn Invest 1993;5:265–9.
- 32. Topliff CL, Smith DR, Clowser SL, et al. Prevalence of bovine viral diarrhea virus infections in alpacas in the United States. J Am Vet Med Assoc 2009;234:519–29.
- 33. Galbreath EJ, Holland RE, Trapp AL, et al. Adenovirus-associated pneumonia and hepatitis in four Ilamas. J Am Vet Med Assoc 1994;204(3):424–6.
- 34. Whitehead CE, Anderson DE. Neonatal diarrhea in Ilamas and alpacas. Small Ruminant Research 2006;61:207–15.
- 35. Parreño V, Bok K, Fernandez F, et al. Molecular characterization of the first isolation of rotavirus in guanacos (*Lama guanicoe*). Arch Virol 2004;149(12):2465–71.
- Parreño V, Constantini V, Cheetham S, et al. First isolation of rotavirus associated with neonatal diarrhoea in guanacos (*Lama guanicoe*) in the Argentinean Patagonia region. J Vet Med B Infect Dis Vet Public Health 2001;48(9):713–20.
- Cebra CK, Mattson DE, Baker RJ, et al. Potential pathogens in feces from unweaned llamas and alpacas with diarrhea. J Am Vet Med Assoc 2003; 223(12):1806–8.
- Genova SG, Streeter RN, Simpson KM, et al. Detection of an antigenic group 2 coronavirus in an adult alpaca with enteritis. Clin Vaccine Immunol 2008;15: 1629–32.
- 39. Jin L, Cebra CK, Baker RJ, et al. Analysis of the genome sequence of an alpaca coronavirus. Virology 2007;365(1):198–203.
- 40. Crosley, B, Mock R, Barr B, et al. Diagnostic investigation of acute respiratory syndrome in alpacas. Proceedings AAVLD, 51st Annual Conference, Greensboro, NC, October 2008:71.
- Rebhun WC, Jenkins DH, Riis RC, et al. An epizootic of blindness and encephalitis associated with a herpesvirus indistinguishable from equine herpesvirus I in a herd of alpacas and Ilamas. J Am Vet Med Assoc 1988;192(7):953–6.
- 42. House JA, Gregg DA, Lubroth J, et al. Experimental equine herpesvirus-1 infection in Ilamas (*Lama glama*). J Vet Diagn Invest 1991;3(2):137–43.
- 43. Krebs JW, Mandel EJ, Swerdlow DL, et al. Rabies surveillance in the United States during 2004. J Am Vet Med Assoc 2005;227:1912–25.

- 44. Wernery U, Kaaden OR. Infectious diseases of camelids, 2nd edition. Blackwell Publ, Vienna (Austria), p. 163–236.
- 45. Nolen-Walston R, Bedenice D, Rodriguez C, et al. Eastern equine encephalitis in 9 South American camelids. J Vet Intern Med 2007;21(4):846–52.
- 46. Dunkel B, Del Piero F, Wotman KL, et al. Encephalomyelitis from West Nile flavivirus in 3 alpacas. J Vet Intern Med 2004;18(3):365–7.
- 47. Kutzler MA, Bildfell RJ, Gardner-Graff KK, et al. West Nile virus infection in two alpacas. J Am Vet Med Assoc 2004;225(6):921–4, 880.
- 48. Yaeger M, Yoon KJ, Schwartz K, et al. West Nile virus meningoencephalitis in a Suri alpaca and Suffolk ewe. J Vet Diagn Invest 2004;16(1):64–6.
- 49. Kutzler MA, Baker RJ, Mattson DE. Humoral response to West Nile virus vaccination in alpacas and Ilamas. J Am Vet Med Assoc 2004;225(3):414–6.
- 50. MacLachlan NJ, Osburn BI. Impact of bluetongue virus infection on the international movement and trade of ruminants. J Am Vet Med Assoc 2006;228:1346–9.
- Afshar A, Heckert RA, Dulac GC, et al. Application of a competitive ELISA for the detection of bluetongue virus antibodies in Ilamas and wild ruminants. J Wildl Dis 1995;31(3):327–30.
- 52. Henrich M, Reinacher M, Hamann HP. Lethal bluetongue virus infection in an alpaca. Vet Rec 2007;161(22):764.
- Underwood WJ, Morin DE, Mirsky ML, et al. Comment on: apparent retrovirusinduced immunosuppression in a yearling Ilama. J Am Vet Med Assoc 1992; 201(9):1318.
- 54. Weber H, Beckmann K, Haas L. [Case report: equine arteritis virus (EAV) as the cause of abortion in alpacas?]. Dtsch Tierarztl Wochenschr 2006;113(4):162–3 [Article in German].
- 55. Williams JR, Evermann JF, Beede RF, et al. Association of bovine herpesvirus type 1 in a llama with bronchopneumonia. J Vet Diagn Invest 1991;3(3):258–60.
- 56. Barrington GM, Allen AJ, Parish SM, et al. Biosecurity and biocontainment in alpaca operations. Small Ruminant Research 2006;61:217–26.
- 57. Klopries M, Wernery U, Kaaden OR. Characterization of the camel skin cell line Dubca. Br Vet J 1995;151(5):555–65.
- Anon. Vaccination guidelines for small ruminants (sheep, goats, llamas, domestic deer, and wapiti). Council on biologic and therapeutic agents and the American association of small ruminant practitioners. J Am Vet Med Assoc 2004;224:414–6.