Antibodies to Pathogenic Livestock Viruses in a Wild Vicuña (*Vicugna vicugna*) Population in the Argentinean Andean Altiplano

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ABSTRACT: Serum samples from 128 wild vicuñas (*Vicugna vicugna*) were tested for antibodies (Ab) to rotavirus (RV), bovine parainfluenza virus 3 (BPIV-3), bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV-1), foot-and-mouth disease virus (FMDV), bluetongue virus (BTV), equine herpesvirus-1 (EHV-1), and influenza A virus equine (EIV). Samples were collected in Cieneguillas Province of Jujuy, in northern Argentina. Feces from 44 vicuñas were also collected to investigate RV shedding. Llamas (*Lama glama*) and domestic cattle (*Bos taurus*) from the area studied also were tested for antibodies to these viruses. Antibodies against RV (100%) and BPIV-3 (37%) were detected in the vicuñas sampled. No RV antigen was detected in any of the fecal samples tested. One vicuña was positive for Ab to BHV-1 (0.8%) and another for BVDV-1 (0.8%). The Ab prevalences detected in llamas were: 100% (16/16) for RV, 47% (8/17) for BPIV-3, 17.6% (3/17) for BHV-1, and 5.9% (1/17) for BVDV-1. However, domestic cattle had high antibody prevalences for RV and BPIV-3, 100% (13/13) and 73% (11/15), respectively, but were negative for Ab to BHV-1 and BVDV. No antibodies against FMDV, BTV, EHV-1, or EIV were detected in wild vicuñas or domestic species. Because no data of viral circulation on wild vicuñas are available, this report represents the first evidence of viral infection in wild vicuñas from the Argentinean Andean Puna.

Key words: Antibodies, Argentinean Puna, prevalence, serology, viral disease, wild vicuñas.

There are four species of South American camelids; two are domestic, including the llama (*Lama glama*) and the alpaca (*Lama pacos*), and two are wild including the guanaco (*Lama guanicoe*) and the vicuña (*Vicugna vicugna*). South American camelids have a wide distribution along the Andes, but vicuñas are restricted to the Andean Puna (between 3,200 to 4,700 m elevation) of Perú, Bolivia, Chile, and Argentina, and they have one of the finest fibers in the world (Wheeler and Hoces, 1997). The systematic killing of vicuñas to harvest their fleece caused a severe decline in their population, almost reaching extinction in the middle of the twentieth century. After 20 yr of effective protection laws, the number of vicuñas of some Argentinean populations has increased (Laker et al., 2006), and they are currently classified under Appendix II of the Convention on International Trade in Endangered Species (CITES), which allows the harvesting of fiber from live shorn animals. Camelids are susceptible to infectious agents that cause disease in domestic animals. Previous studies have reported serologic evidence of exposure to rotavirus (RV), bovine parainfluenza-3 virus (BPIV-3), bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV), foot-and-mouth disease virus (FMDV), bluetongue virus (BTV), bovine enterovirus (BEV), bovine adenovirus (Badv III), influenza A virus (IAV), and equine herpesvirus-1 (EHV-1) in domestic South American camelids, most from animals without clinical presentation of disease (Fowler, 1998; Mattson, 1994; Saraiva, 2004; Cabello et al., 2006; Celedón et al., 2006; Evermann, 2006; Parreño and Marcoppido, 2006). Viral antigens were detected in domestic came-

lids in the presence of clinical signs of disease for BVDV, adenovirus, EHV-1, West Nile virus (Mattson and Baker, 1994; Mattson et al., 2006; Parreño and Marcoppido, 2006; Foster et al., 2007), and equine arteritis virus (EAV) (Weber et al., 2006). Studies carried out on wild South American camelids under captive conditions have reported 95% antibody (Ab) prevalence to RV in guanacos from the Argentinean Patagonia region and the first isolation and characterization of RV in guanaco juveniles with acute diarrhea (Parreño et al., 2001, 2004). Serologic surveys conducted in free-ranging guanacos and vicuñas did not detect Ab against BHV-1, BVDV, BPIV-3, BTV, FMDV, bovine respiratory syncytial virus (BRSV), or EHV-1 (Karesh et al., 1998; Celedón et al., 2001). In domestic cattle herds from Jujuy, previous studies have reported high antibody prevalence to RV (100%) and BEV (>90%), moderate antibody prevalence to BVDV (between 30% and 70%), and a low antibody prevalence to BHV-1 (between 4% and 40%) and Badv III (9.5%; Puntel et al., 1999). Because no data for viral circulation on wild vicuñas were available, the purpose of this study was to investigate the presence of antibodies against several livestock-affecting viruses in wild vicuña populations of the Argentinean Andean Altiplano.

We surveyed free-living vicuñas, llamas, and domestic cattle from Cieneguillas (22°08′S, 65°08′W), a small town on the Province of Jujuy on the Argentinean-Bolivian border of the Andean Puna, for evidence of exposure to viral agents affecting livestock. Wild vicuñas were captured under the Animal Welfare protocols (Gimpel and Bonacic, 2006), during May and November 2003 and November 2004. In total, 128 vicuñas (76 males and 52 females) were sampled and their ages were estimated as juveniles (between 3 mo and 1 yr; $n=20$), subadults (1–2 yr; $n=55$), and adults (>2 yr; $n=53$). Additionally, 17 llamas and 15 mixed-breed domestic cattle that grazed in the area with the vicuñas

were also sampled during November 2003 and November 2004. Blood samples were obtained by venipuncture (Fowler, 1998) with 10-ml silicone-coated tube Vacutainers™ (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA), and after centrifugation, sera were separated and kept at -20 C until assay. Fecal samples from 44 vicuñas (20 juveniles and 24 adults) were collected and screened for group A RV by enzyme-linked immunosorbent assay (ELISA), using reagents and techniques previously described for RV antigen detection (Cornaglia et al.,1989). This technique was adapted for detection of Ab to RV in South American camelids serum samples (Parreño et al., 2001). Briefly, I-801 NCDV Cody P[1]G8 BRV reference strain was used as positive antigen, and Mock-infected MA-104 cells were used as negative control. A commercial peroxidase-labeled polyclonal goat anti-llama immunoglobin G (IgG) (H+L) (Bethyl Laboratory Inc., Montgomery, California, USA) at 1:2,000 dilution was used as conjugate. Antibody titers were expressed as the inverse of the highest dilution with positive signal.

Serum neutralization tests (SN) for BHV-1 and BVDV-1 were carried out following standard methods (OIE, 2008), using reference strains BHV-1 Los Angeles and BVDV-1 Singer (cytopathic biotype 1a), respectively. Antibodies to BPIV-3 were detected by a hemagglutination-inhibition test (HI; Collins et al., 1996) especially adapted for South American camelid sera, using BPIV-3 reference strain ST20. Briefly, potassium periodate-glycerol pretreated sera were diluted from 1/5 to 1/320 in "U"-shape microtiter plates and mixed with an equal volume of BPIV-3 containing 8 hemagglutination units (HU). After 1 hr incubation at room temperature, two volumes of 0.25% guinea-pig erythrocytes suspension in Phosphate Buffered Saline (PBS) were added. Serum Ab titer was expressed as inhibitory hemagglutination units (IHU) at the highest serum dilution showing complete

TABLE 1.   Prevalence of antibodies to viral pathogens in wild vicuñas, llamas, and domestic cattle from Cieneguillas, Jujuy.

Species	Antibody prevalence to viral pathogens[a]			
	RV	BPIV-3[b]	BHV-1	BVDV-1
Wild vicuñas	100% (128/128)	37% (44/119)[c]	0.8% (1/128)	0.8% (1/128)
Llamas	100% (16/16)[c]	47% (8/17)	6% (1/17)	18% (3/17)
Domestic cattle	100% (13/13)[c]	73% (11/15)	0% (0/15)	0% (0/12)[c]

[a] Rotavirus (RV), bovine parainfluenza virus 3 (BPIV-3), bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV-1).

[b] Only the serum samples with antibody titers >80 IHU were considered positive.

[c] Not all the volume of blood was available for testing the Ab against the viral pathogens in all the individuals.

inhibition of the viral hemagglutination activity, multiplied by the 8 HU of the virus used in the test. Antibodies against BTV were detected using a commercial immunodiffusion (BTID) test (Veterinary Diagnostic Technology, Inc., Wheat Ridge, Colorado, USA). A blocking ELISA was used for detection of Ab to FMDV, using the Argentinean isolates O1 Caseros (Mattion et al., 2004). Antibodies against EHV-1 were detected by SN (OIE, 2008), using the Kentucky reference strain. For EIV, an HI test with the Argentinean isolate AR93 (Lai et al., 2001) was carried out using chicken erythrocytes.

For RV and BPIV-3, sera from naturally infected guanacos served as positive controls (Parreño et al., 2001; Marcoppido, unpubl. data). The positive controls for BHV-1 and BVDV-1 were obtained from vaccinated llamas. For FMDV, BTV, EHV-1, and EIV, sera from naturally infected domestic cattle and equines were used as positive controls, respectively.

Fisher's exact test was used to compare Ab titers in relation to the capture season, sex, and age of vicuñas. Differences in Ab titers between groups were evaluated by general analysis of variance (ANOVA) followed by Bonferroni post-ANOVA test (Statistix 8, Analytical Software, Tallahassee, Florida, USA). Significance was established at $P<0.05$.

All animals sampled had serum Ab against RV (Table 1). The mean Ab titers were 4,032 (range 16 to 16,384) in vicuñas, 362 in llamas (range 4 to 16,384), and 121 in domestic cattle (range 16 to 1,024). However, RV shedding could not be detected by ELISA in any of the wild vicuñas sampled, including the 20 juveniles. Comparison of Ab titers in vicuñas according to age revealed statistically significant differences (Table 2). Analyzing the total wild vicuñas sampled, the highest Ab titer to RV was detected in the subadult vicuñas, while the lowest was detected in the juveniles ($P=0.0156$). When comparing the mean Ab titers to RV between captures in 2003, the juveniles showed lower titers than subadult and adult animals in November ($P=0.0182$), while the juveniles captured in May had very high titers, similar to those of the older categories (Table 2). No statistically significant difference in Ab titers to RV was observed between sexes.

Average antibody prevalence to BPIV-3 was 37% for vicuñas, 47% for llamas, and 73.3% for domestic cattle (Table 1). The Ab titers for BPIV-3 ranged from 160 to 1,280 for vicuñas, 80 to 2,560 for llamas, and 80 to 320 for domestic cattle. The Ab prevalence to BPIV-3 recorded in vicuñas during November 2003 was significantly higher than that recorded in November 2004 (Table 2). No significant differences were observed between the age classes of vicuñas, while a significantly higher antibody prevalence to BPIV-3 was detected in females (50%; 23/46) than in males (28.76%; 21/73) ($P=0.0313$; data not shown).

Only one subadult male vicuña and a male llama were seropositive to BHV-1

TABLE 2.   Geometric mean (GM) antibody (Ab) titers and antibody prevalence for rotavirus (RV) and bovine parainfluenza virus 3 (BPIV-3) in relation to the date of capture and age of the vicuñas sampled.

Capture	Age	n	RV GM Ab titer[b]	RV Ab prevalence[c]	BPIV-3[a] GM Ab titer[b]	BPIV-3[a] Ab prevalence[c]
May 2003	Adult	13	1,745[B]	100%	89	23% (3/13)
(n=28)	Subadult	11	6,781[A]		141	55% (6/11)
	Juvenile	4	5,793[AB]		80	25% (1/4)
			P=0.0250		P=0.2359	35.71%[AB]
Nov 2003	Adult	21	897[AB]	100%	120	53% (10/19)
(n=50)	Subadult	17	1,670[A]		147	63% (10/16)
	Juvenile	12	323[B]		103	36% (4/11)
			P=0.0182		P=0.5769	52.17%[A]
Nov 2004	Adult	19	5,098	100%	94	35% (6/17)
(n=50)	Subadult	27	1,996		69	17% (4/24)
	Juvenile	4	1,024		57	0% (0/4)
			P=0.0556		P=0.2095	22.2%[B]
Total wild	Adult	53	1,969[AB]	100%	102	39% (19/49)
vicuñas	Subadult	55	2,413[A]		102	39% (20/51)
	Juvenile	20	724[B]		86	26% (5/19)
			P=0.0156		P=0.6923	

[a] Only the serum samples with Ab titers >80 IHU were considered positive.

[b] Different letters (A, B, and AB) in the same column indicate significant differences in mean titers between animals of different categories within a capture (one-way ANOVA, Bonferroni P<0.05).

[c] Total of seropositive animals/total of animals tested (% positive).

(SN Ab titer of 1.8 in both), while an adult male vicuña and three llamas (two males and one female) had detectable antibodies against BVDV-1 (Table 1). Mean Ab titers of positive animals of each species are depicted in Figure 1. None of the seropositive animals sampled had clinical signs of diseases, and all were in good physical
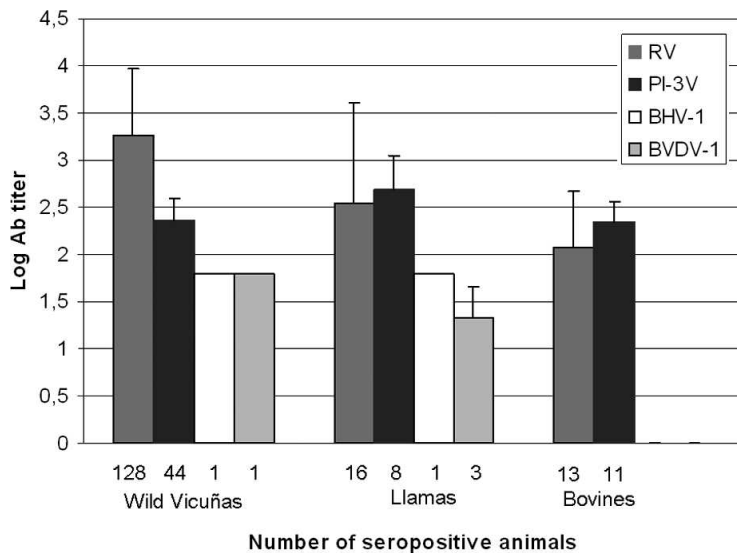

FIGURE 1.   Average antibody titers of seropositive vicuñas, llamas, and domestic cattle for each virus studied. Error bars represent standard deviation.

condition. No Ab to BHV-1 or BVDV-1 was detected in the domestic cattle sampled. No animals seropositive for FMDV, BTV, EHV-1, and EIV were detected in any species.

Using indirect evidence, we have demonstrated the asymptomatic circulation of RV and BPIV-3 in wild vicuñas, llamas, and domestic cattle from the Argentinean Puna. Similar prevalences of RV Ab have been found in a survey carried out in llamas from different provinces of Argentina, including Jujuy (Puntel et al., 1999). The prevalence for BPIV-3 detected in this study is consistent with the rates previously reported (Mattson and Baker, 1994; Cabello et al., 2006; Parreño and Marcoppido, 2006). These results highlight the importance of avoiding captive breeding programs in vicuñas due to the high possibility of a massive outbreak of these indigenous diseases. The stressful stimuli imposed by captivity have detrimental effects, including a decrease in the immune competence of animals, which affects the survival of the individuals when faced with pathogens (Texeira et al., 2007).

Antibody prevalences for BHV-1 and BVDV-1 in the vicuñas and llamas studied were very low. This finding agrees with the low prevalence reported in other studies in llamas and wild South American camelids from Argentina, Perú, and the USA (Parreño and Marcoppido, 2006). In this report, antibodies against FMDV, BTV, EHV-1, and EIV were not detected in wild vicuñas or domestic animals. Similar results have been reported in free-ranging guanacos (Karesh et al., 1998). Our results agree with other studies that did not detect seropositive llamas to FMDV in Argentina (Puntel et al., 1999) and support the hypothesis that camelids play a minor role in the transmission of the virus (Fondevilá et al., 1995; Saraiva, 2004; Wernery and Kaaden, 2004). The lack of detection of Ab to BTVs in the animals sampled agrees with previous reports in domestic and wild South American came-lids and domestic cattle from the northern end of Argentina (Puntel et al., 1999). Because BTVs are transmitted by *Culicoides* spp., the absence of detectable Ab in the animals tested could be due to the absence of competent vectors in the Argentinean Puna around the time of sampling (Lager, 2004). No Ab against EHV-1 or EIV was found in the animals sampled. Despite the lack of published data on diseases of equids in the Argentinean Puna, the collaborating farmers in this study did not allow the sampling of donkeys.

Although virus isolation was not performed in this study, serologic results allow the conclusion that all species sampled had been exposed to RV and BPIV-3. In addition, of the animals sampled, only wild and domestic South American camelids had been in contact with a viral agent antigenically related to BHV-1 and BVDV-1. The serologic tests used in this study were those standardly used for domestic cattle (OIE, 2008), but they have not been validated for camelids, and thus conclusions should be interpreted with caution. Because of the small sample sizes of llamas and domestic cattle and the low antibody prevalence in this study, no conclusions concerning the risk of interspecies transmission could be drawn. A larger survey over longer periods of time is needed to better elucidate potential trends or relationships.

This study suggests that populations of free-ranging vicuñas from the Argentine Altiplano are relatively disease-free but susceptible to common livestock diseases. The present study represents the first survey for viral agents in wild vicuñas in Argentina and demonstrates the need for additional work in order to better understand the dynamics of viral infections in wild vicuñas and the potential role of these species in the epidemiology of livestock diseases. Knowledge about infectious agents affecting South American camelids is crucial when initiating appropriate systems for sustainable manage-

ment of domestic llamas and conservation of populations of wild vicuñas.

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