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Evidence of Bovine Viral Diarrhea, but Absence of Infectious Bovine Rhinotracheitis and Bovine Brucellosis in the Endangered Huemul Deer (*Hippocamelus bisulcus*) in Chilean Patagonia

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ABSTRACT: We screened 18 endangered Chilean huemul (*Hippocamelus bisulcus*) for antibodies to infectious agents. We detected no antibody to bovine herpesvirus-1 (BHV-1) or *Brucella abortus* (BA); two huemul had antibody to bovine viral diarrhea virus (BVDV). Cattle ($n=35$) had antibody to BVDV and BHV-1 but not BA.

Infectious disease threatens biodiversity, but lack of information on pathogens affecting endangered species thwarts efforts to understand the role that diseases might play in species extinction. Diseases can cause significant mortality in wild ungulates (e.g., bighorn sheep *Ovis canadensis*; Clifford et al., 2009) and reduction of reproductive fitness of infected individuals (Tompkins et al., 2011). These effects are critical when population size is small and reservoir hosts are consistently present.

The huemul (*Hippocamelus bisulcus*), endemic to the southern Andes, is the most endangered Neotropical deer species (<2,000 total). Its population size has been reduced by 99% and its range by 50% over the last 500 yr, and remaining populations are small and highly fragmented (Corti et al., 2011). Although infectious diseases have been proposed as an important factor threatening huemul, no studies of disease impact have been published. Anecdotal information suggests that huemul in Argentina have been impacted by foot and mouth disease with high mortality (Serret, 2001). The frequency of exposure of huemul to pathogens from livestock is still unknown.

We assessed the presence of three important infectious diseases of livestock in southern Chile in a huemul population that has been exposed to sheep and cattle for at least 80 yr. We surveyed for antibodies to bovine herpesvirus-1 (BHV-1; cause of infectious bovine rhinotracheitis), bovine viral diarrhea virus (BVDV), and *Brucella abortus* (BA; cause of bovine brucellosis). We searched for antigens specific to cattle to test possible cattle to deer transmission. In cattle, infection with BHV-1 produces respiratory problems, conjunctivitis, abortions, encephalitis, and generalized systemic infections (Muylkens et al., 2007); BVDV infection in cattle causes diarrhea, respiratory infection, infertility or abortion, and mucosal issues (Lindberg, 2003); *B. abortus* in cattle provokes abortions, placentitis, epididymitis, and orchitis (Neta et al., 2010). Deaths are rare in adult cattle but frequent in fetuses and neonates (Neta et al., 2010). *Brucella* species are also maintained in wildlife reservoirs in Patagonia (e.g., European hares, *Lepus europaeus*; Gyuranecz et al., 2011). Although BHV-1 and BVDV are highly prevalent in cattle from Chilean Patagonia, *B. abortus* has been declared absent (Naranjo et al., 2005).

From 2006 to 2007, we sampled sera from 18 huemul (from a population of approximately 45) and 35 cattle at Lago Cochrane National Reserve (LCNR, 69.25 km^2; 47°12′S, 72°30′W) and adjacent areas, respectively (Corti et al., 2010, 2011). Extensive livestock management, ranging from small farms to medium-size operations, is the most common land use in the surrounding areas (Corti et al., 2011).

Captured huemul were blood sampled through jugular puncture (Corti et al., 2010). Samples were placed in serum

separator tubes (Nipro Medical Corporation, Miami, Florida, USA), and after clot formation, sera were separated by centrifugation in a portable 12 V centrifuge (Mobilespine, Vulcan Technologies, Grandview, Missouri, USA) at 1,100 × G for 15 min, and stored in a freezer at −10 C. Sera were tested by serum neutralization against BHV-1 and BVDV. For BHV-1 antibody testing, sera were exposed to 100 TCID50 (50% tissue culture infective dose) of the reference strains of BHV-1 Los Angeles strain. For BVDV antibody testing, sera were exposed to 100 TCID50 of strain NADL. Samples were tested for BA antibody using the Rose Bengal test and through serum antigen (Sanofi Pasteur, Lyon, France) according to Chilean official animal health requirements (Naranjo et al., 2005).

All huemul samples were negative for antibodies to BHV-1 and BA, but one adult male and one adult female (11.1%) had detectable antibody to BVDV (titers: 128 and ≥1,024, respectively). Four domestic cows (11.4%) had antibody to BVDV (titers: 28), and 28 cows (80%) were antibody-positive for BHV-1. All cows were antibody-negative for BA, consistent with the absence of reported bovine brucellosis in Aysén since 2005 (Naranjo et al., 2005). Serology from cattle was consistent with reported prevalences of BHV-1 and BVDV infection in Aysén (40 and 60% respectively; Chacón and Naranjo, 2009).

Although transmission between huemul and cattle is unknown, BVDV infection is associated with fluid secretions, excretions, and abortions, allowing virus persistence and indirect transmission among species (Lindberg, 2003). Transmission of BHV-1 requires direct contact and large aggregations of animals (Muylkens et al., 2007), which are not part of huemul behavior (Corti et al., 2011). These differences in transmission could explain in part the absence of BHV-1 antibody while BVDV antibody was present.

The finding of BVDV antibody would be the second pestivirus antibody report in native Chilean ungulates and the first for huemul. Pizarro-Lucero et al. (2005) recorded BVDV antibodies in pudu (*Pudu puda*). Because no specific cervid strains of BVDV have been described (Araújo et al., 2010), the possibility of deer as a BVDV source for cattle seems remote (Nielsen et al., 2000). It is more likely that antibody-positive deer were exposed to cattle. Border disease virus (BDV) should also be considered. Prevalence of antibody to BDV in the same area was 26.2% (Ramírez and Martínez, 2006), and BDV is cross-reactive with BVDV (Haas, 1997). In addition, no BVH-1 antigen specific to huemul has been described.

The consequences of these infections are unknown but require attention because of the endangered status of the huemul. Survival of young huemul is <35%, and large proportions of mortalities are of undetermined cause (Corti et al., 2010). Some of these might be attributable to infection with BVDV or BHV-1. No postmortem surveys were conducted; scavengers (e.g., culpeo foxes, *Lycalopex culpaeus*) rapidly consume carcasses, limiting disease impact estimation.

We encourage the determination of the health status of huemul populations, relating it to female fecundity and fertility, and survival of young. Surveys should be conducted in areas where huemul may contact infected livestock to prevent detrimental impact on its populations. Huemul susceptibility to infection with BVDV and other pathogens is unknown. The role of infectious agents in Patagonia requires further investigation to establish the extent to which, if any, these agents have affected huemul. Management tools such as translocation, reintroduction, rehabilitation, and reproduction centers should include appropriate testing to avoid introduction and transmission of infectious agents into wild populations.

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