

## ***Corynebacterium pseudotuberculosis* Infection in Patagonian Huemul (*Hippocamelus bisulcus*)**

Nelly Morales,<sup>1</sup> Dennis Aldridge,<sup>2</sup> Andrea Bahamonde,<sup>2</sup> Julio Cerda,<sup>1</sup> Claudio Araya,<sup>1</sup> Rodrigo Muñoz,<sup>1</sup> María Esther Saldías,<sup>1</sup> Claudio Lecocq,<sup>1</sup> Marcela Fresno,<sup>3</sup> Pedro Abalos,<sup>3</sup> and Patricio Retamal<sup>3,4</sup> <sup>1</sup>Servicio Agrícola y Ganadero, Av. Presidente Bulnes 140, Santiago, Chile; <sup>2</sup>Corporación Nacional Forestal, Av. Presidente Bulnes 285, Santiago, Chile; <sup>3</sup>Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Av Sta Rosa 11735, Santiago, Chile; <sup>4</sup>Corresponding author (email: pretamal@uchile.cl)

**ABSTRACT:** *Corynebacterium pseudotuberculosis* is an intracellular bacteria and the etiologic agent of caseous lymphadenitis in domestic and wildlife species. We report *C. pseudotuberculosis* infection in Patagonian huemul (*Hippocamelus bisulcus*) from the Cerro Castillo National Reserve, Region of Aysen, Chile. Subcutaneous abscesses in the abdominal and pectoral regions from two animals were sampled and bacteriologic isolation was performed. In both cases, we isolated a *C. pseudotuberculosis* strain belonging to the ovine genotype. In addition, one isolate was resistant to ciprofloxacin and streptomycin. We report that *H. bisulcus* is a susceptible species to this bacterium, which is transmitted by direct or indirect contact with domestic sheep (*Ovis aries*) and which represents a potential conservation threat to populations of *H. bisulcus*. Additional research and prevention efforts should be addressed.

**Key words:** Abscess, antibiotic resistance, Chile, *Corynebacterium pseudotuberculosis*, Patagonian huemul.

*Corynebacterium pseudotuberculosis* is a cosmopolitan agent that causes caseous lymphadenitis (CLA) disease in domestic sheep (*Ovis aries*) and goats (*Capra aegagrus hircus*). Additionally, the bacterium produces abscesses in horses (*Equus caballus*), ulcerative cutaneous granulomas in cattle (*Bos taurus*) (Baird and Fontaine 2007), and lesions in other domestic and wildlife species (Tarello and Theneyan 2008; Colom-Cadena et al. 2014) including cervids from North America and Europe (Kelly et al. 2012; Matos et al. 2015). In Chile, CLA is an endemic disease and could generate important economic losses in domestic animals (Tadich et al. 2005; Baird and Fontaine 2007), although ecologic or health effects in wildlife are unknown.

The Patagonian huemul (*Hippocamelus bisulcus*) is an endemic, midsized deer of the

southern Andes of Chile and Argentina found in stepped and irregular foothills at elevations up to 1,300 m in the Andes Mountains. The huemul is considered an endangered species with fragmented populations and declining numbers, listed in the *IUCN Red List of Threatened Species* (2016). Although no sound population figures exist, it is estimated that there may be 2,000 individuals (Vila et al. 2006). Here we report the detection of a *C. pseudotuberculosis* strain from Patagonian huemul in the Region of Aysen, Chile.

During October 2015, a free-ranging, adult, male huemul living in the Cerro Castillo National Reserve (46°01'58''S, 71°58'37''W), Chilean Patagonia, was observed with a lateral subcutaneous abscess in the abdominal body region (Case 1). Its capture was performed under supervision and following recommendations of the Chilean authority. Once anesthetized with medetomidine (0.09 mg/kg) and ketamine (2 mg/kg), samples were obtained for antibiotic testing. The abscess was drained and then cleaned with sterile NaCl 0.9% solution. The animal was in a healthy condition without any other signs of disease. As a precaution, it was transported in a cage to a closed area within the park and maintained under veterinary care until healing. After 2 wk, the animal was released into the same Reserve. Later, during February 2016, a second healthy, free-ranging, adult male was observed within the same Reserve, 8 km away from the first case (45°58'46''S, 71°56'01''W), with a subcutaneous abscess in the left side of its pectoral area (Case 2). The capture and sampling of the abscess was performed as mentioned for Case 1. However, this animal was released in

TABLE 1. Antimicrobial resistance of two *Corynebacterium pseudotuberculosis* isolates from Patagonian huemul (*Hippocamelus bisulcus*) as determined by the disk diffusion method.

Antimicrobial	µg/ disk	Results	
		Case 1	Case 2
Amikacin	30	Susceptible	Susceptible
Ampicillin	10	Susceptible	Susceptible
Azithromycin	15	Susceptible	Susceptible
Chloramphenicol	30	Susceptible	Susceptible
Cefadroxil	30	Susceptible	Susceptible
Ciprofloxacin	5	Resistant	Susceptible
Gentamicin	10	Susceptible	Susceptible
Ceftriaxone	30	Susceptible	Susceptible
Cefotaxime	30	Susceptible	Susceptible
Erythromycin	15	Susceptible	Susceptible
Ceftiofur	30	Susceptible	Susceptible
Enrofloxacin	5	Susceptible	Susceptible
Kanamycin	30	Susceptible	Susceptible
Penicillin G	10	Susceptible	Susceptible
Streptomycin	10	Resistant	Susceptible
Sulfisoxazole	300	Susceptible	Susceptible
Trimethoprim/ sulfamethoxazol	1.25/23.75	Susceptible	Susceptible
Tetracycline	30	Susceptible	Susceptible

the same place immediately after recovery from anesthesia.

Because coinfection with other biologic agents has been reported (Muller et al. 2011; Matos et al. 2015), several diagnostic procedures were performed with the samples, although unique findings were related to the *C. pseudotuberculosis* infection (see Supplementary Table). Skin biopsies showed many neutrophils with abundant necrotic debris and Gram-positive bacilli. Abscesses contained a soft and pasty exudate resembling CLA lesions observed in goats.

For bacterial isolation, abscesses were sampled using sterile swabs and Cary-Blair transport medium (Copan Transystem®, Brescia, Italy). At the lab, swabs were seeded onto trypticase soy agar (Difco®, Sparks, Maryland, USA), plates supplemented with 5% (v/v) sheep blood, and then incubated at 37 C for 48 h. Suspicious Gram-positive bacteria were confirmed by biochemical characterization

(API Coryne, Biomerieux®, Lyon, France). The cyclic AMP (cAMP) and cAMP-inhibition tests and the PCR assay for phospholipase D gene detection were made following protocols previously reported (Dorella et al. 2006; Pacheco et al. 2007; Retamal et al. 2011). Amplification and purification of the hypervariable *rpoB* gene segment was performed as previously described (Retamal et al. 2011), and automated sequencing was developed at the Faculty of Sciences, University of Chile, Santiago. The disk diffusion method of the Clinical and Laboratory Standards Institute criteria (2013) was followed to determine antimicrobial susceptibility (Table 1).

These assays confirmed the detection of the *C. pseudotuberculosis* bacterium in abscesses of *H. bisulcus*. The sequencing of the *rpoB* hypervariable segment (GenBank accession no. KY560450) revealed a complete nucleotide identity to the ovine genotype (GenBank accession no. HQ401568), and phenotypic resistance for the antimicrobials ciprofloxacin and streptomycin was detected in one isolate (Table 1). These are drugs that are frequently used in domestic animals in Chile.

The presence of *C. pseudotuberculosis* has been detected in slaughtered sheep from the Region of Aysen, with the highest detection rate (11.8%) in adults (Tadich et al. 2005). The risk for infection of wildlife inhabiting the Cerro Castillo National Reserve is probably the herds of domestic sheep located in the vicinity (within 5 km) of its borders. There are 69 herds with around 6,300 animals, most of which (70%) are ewes.

Microbiologic and epidemiologic evidence support the conclusion that sheep are the most probable source of *C. pseudotuberculosis* infection for free-ranging deer living in the Reserve. This contact between infected sheep and huemul could have occurred indirectly through pus-contaminated fomites, in which bacteria can survive for weeks or months (Dorella et al. 2006; Baird and Fontaine 2007). The rupture of contaminated abscesses releases a high number of bacteria which contaminate the environment and particularly the plants of the deciduous forest where these animals feed. Once infection was

established in some individual huemul, transmission and dissemination of bacteria in this population may also have occurred by direct contact. Nevertheless, other predisposing factors could also be at work, as the geographic relationship between the wild and domestic species started almost 100 yr ago, yet the disease had not been previously reported for *H. bisulcus*. On the other hand, the natural course of untreated animals in the wild is unknown.

Whatever the transmission route, the exposure to a pathogen from domestic animals occupying their natural habitat is a real threat for *H. bisulcus* which, in addition to poaching and attacks from feral dogs (Vila et al. 2006; Briceño et al. 2013), constitutes a conservation challenge that should be addressed in the future by competent institutions and researchers. During 2015 and 2016, 40 huemul specimens were seen along 27 km of the Carretera Austral (CH-7) highway crossing the Reserve, 13 (33%) of which had CLA-compatible lesions. Because of the results from our work and from the visual observations, current efforts are being developed at a population level for a better characterization of the disease in Patagonian huemul from the Cerro Castillo National Reserve. Every new abscess in a living or dead specimen should be analyzed at the laboratory to determine the presence of the bacterium, its genotypic and phenotypic traits, and the pathologic consequences for animals.

We thank Consuelo Foerster (Zoológico Nacional), Mario Alegría Cea (Corporación Nacional Forestal), José Cerna Abarca (Programa de Protección al Huemul), and laboratory personnel from the Region of Aysen for their collaboration during this study. We also thank Cristobal Briceño for a critical review of the manuscript. This work was funded by Servicio Agrícola y Ganadero, Corporación Nacional Forestal, and the Universidad de Chile.

#### SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://dx.doi.org/10.7589/2016-09-213>.

#### LITERATURE CITED

- Baird GJ, Fontaine MC. 2007. *Corynebacterium pseudotuberculosis* and its role in ovine caseous lymphadenitis. *J Comp Pathol* 137:179–210.
- Briceño C, Knapp LA, Silva A, Paredes J, Avendaño I, Vargas A, Sotomayor J, Vila AR. 2013. Detecting an increase in an endangered huemul *Hippocamelus bisulcus* population following removal of cattle and cessation of poaching in coastal Patagonia, Chile. *Oryx* 47:273–279.
- Clinical and Laboratory Standards Institute. 2013. *Performance standards for antimicrobial susceptibility testing; 23rd informational supplement*. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, 205 pp.
- Colom-Cadena A, Velarde R, Salinas J, Borge C, Garcia-Bocanegra I, Serrano E, Glasso D, Bach E, Casas-Diaz E, López-Olvera JR, et al. 2014. Management of a caseous lymphadenitis outbreak in a new Iberian ibex (*Capra pyrenaica*) stock reservoir. *Acta Vet Scand* 56:83–94.
- Dorella FA, Pacheco LG, Oliveira SC, Miyoshi A, Azevedo V. 2006. *Corynebacterium pseudotuberculosis*: Microbiology, biochemical properties, pathogenesis and molecular studies of virulence. *Vet Res* 37:201–218.
- IUCN (International Union for Conservation of Nature). 2016. *The IUCN red list of threatened species*. Version 2016-3. <http://www.iucnredlist.org>. Accessed March 2017.
- Kelly EJ, Rood KA, Skirpstunas R. 2012. Abscesses in captive elk associated with *Corynebacterium pseudotuberculosis*, Utah, USA. *J Wildl Dis* 48:803–805.
- Matos AC, Dias AP, Morais M, Figueira L, Martins MH, Matos M, Pinto ML, Coelho AC. 2015. Granuloma coinfection with *Mycobacterium bovis*, *Mycobacterium avium* subsp. *paratuberculosis* and *Corynebacterium pseudotuberculosis* in five hunted red deer (*Cervus elaphus*) in Portugal. *J Wildl Dis* 51:793–794.
- Müller B, de Klerk-Lorist L, Henton MM, Lane E, Parsons S, van Pittius NCG, Kotze A, van Helden PD, Tanner M. 2011. Mixed infections of *Corynebacterium pseudotuberculosis* and non-tuberculous mycobacteria in South African antelopes presenting with tuberculosis-like lesions *Vet Microbiol* 147:340–345.
- Pacheco LGC, Pena RR, Castro TLP, Dorella FA, Bahia RC, Carminati R, Frota MNL, Olivera SC, Meyer R, Alves FSF, et al. 2007. Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. *J Med Microbiol* 56:480–486.
- Retamal P, Rios M, Cheuquepán F, Abalos P, Pizarro-Lucero J, Borie C, Gutierrez J. 2011. Host associated polymorphisms in the *Corynebacterium pseudotuberculosis rpoB* gene sequence. *Vet Microbiol* 151:400–403.

- Tadich N, Alvarez C, Chacón T, Godoy H. 2005. Linfadenitis caseosa (LAC) en ovinos de la XI Región, Chile. *Arch Med Vet* 37:161–167. [In Spanish.]
- Tarello W, Theneyan M. 2008. *Corynebacterium pseudotuberculosis* and *Corynebacterium renale* isolated from two Arabian oryx (*Oryx leucoryx*). *Vet Rec* 162:862–863.
- Vila AR, López R, Pastore H, Faúndez R, Serret A. 2006. Current distribution and conservation of the huemul (*Hippocamelus bisulcus*) in Argentina and Chile. *Mastozool Neotrop* 13:263–269.

Submitted for publication 14 September 2016.

Accepted 10 January 2017.