Subcutaneous abscess caused by *Trueperella pyogenes* in a Southern pudu (*Pudu puda*)

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ABSTRACT. A case of an abscess on the head of a captive Southern pudu (*Pudu puda*) caused by *Trueperella pyogenes* is described. A diagnosis was done based on bacterial culture and MALDI-TOF MS identification. Traumatic inoculation of the bacterium in the subcutaneous tissue is speculated.

Key words: pudu, abscess, *Trueperella pyogenes*, deer.

The Southern pudu (*Pudu puda*) is a small sized deer species inhabitant of the Valdivian temperate rainforests of Chile and Argentina (Jiménez 2010). Pudus can also live in areas with sufficient understory vegetation cover and food availability, including anthropogenically disturbed habitats (i.e. agricultural and peri-urban landscapes), where they face a continuous risk of interacting with domestic and exotic animals (Jiménez 2010, Silva-Rodríguez and Sieving 2012). The pudu is categorised as “near threatened” by the IUCN (International Union for Conservation of Nature) and populations along its distributional range are declining due to anthropogenic factors, namely habitat loss and fragmentation, poaching, and dog predation (Jiménez 2010, Silva-Rodríguez and Sieving 2012). Currently, infectious diseases are not considered as a conservation threat for pudu populations in Chile, although information about pathogenic agents is still very limited. To the author’s knowledge, there are only two reports of death due to natural infection with pestivirus (Pizarro-Lucero *et al* 2005) and *Mycobacterium avium* subsp *paratuberculosis* (González-Acuña *et al* 2011) in wild individuals.

On December 12, 2018, a farm located in San Pedro de La Paz, Chile (36°53’01”S, 73°03’48”W) requested veterinary services because a female adult pudu presented a swollen right side of the head. Thirty pudus were maintained in two different enclosures of 2000 and 7000 m², respectively, both next to a paddock with exotic deer (*Eudorcas thomsonii*, *Dama dama*, and *Axis axis*). Pudus were fed daily with alfalfa pellets, wheat, grass, and willow branches. On physical examination of the pudu, a 3 cm diameter soft and fluid-filled mass was identified over the angle of the jaw and part of the inferior border of the mandible (figure 1). The animal was sedated with midazolam (Midazolam, Pfizer; 0.5 mg/Kg IM) and ketamine (KET-A-100®, Agrovetmarket; 3 mg/Kg IM). All veterinary procedures were performed using gloves and face masks. After antisepctic preparation of the area, swabs were obtained from the mass and transported to 4 °C to the Laboratorio de Investigación en Agentes Antibacterianos (LIIA), Universidad de Concepción (Concepción, Chile) for further analysis. Once the abscess was drained and cleaned, the animal was treated with systemic antibiotics (enrofloxacin 5%, Baytril®; 1mg/Kg IM) and ketamine (KET-A-100®, Agrovetmarket; 3 mg/Kg IM) for 7 days. After three weeks of treatment, clinical signs were absent in the pudu.

The sample was cultured on plates of tryptone soy agar (Oxoid, UK) supplemented with 5% sheep blood and incubated at 37 °C for 48-72 h. White small and irregular colonies were obtained surrounded by small β-hemolysis rings. Gram stain revealed a short and pleomorphic Gram-positive bacillus that were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, VITEK® MS system, bioMérieux, Chile). The isolate was incubated for 24 h at 37 °C on Columbia blood agar (Valtek® Diagnostics, Chile). One millilitre of matrix solution (MS-CHCA, bioMérieux) was used to inoculate the colony using a pre-loaded equipment protocol for the identification of bacterial strains. Collection and analysis of spectrometry data were performed with software VITEK-MS data acquisition and Myla™ V4,
respectively (VITEK-MS Acquisition Station, bioMérieux). The analysis was done in triplicates and results with an identity ≥ 99% were considered. Antimicrobial susceptibility was determined by disk diffusion following standard procedures using Mueller-Hinton agar plates supplemented with 5% sheep blood (CLSI 2002). Penicillin (10 U), ampicillin (10 µg), cefuroxime (30 µg), ceftiofur (30 µg), erythromycin (15 µg), azithromycin (15 µg), florfenicol (30 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tetracycline (30 µg), rifampicin (5 µg), daptomycin (10 µg), bacitracin (0.04 U), and trimethoprim/sulfamethoxazole (25 µg) were used for analysis.

Results revealed *Trueperella pyogenes* as the agent present in the abscess. The analysis showed susceptibility to all antimicrobials assayed, except for bacitracin. *Trueperella pyogenes* is an opportunistic and ubiquitous Gram-positive bacterium of the mucosal surfaces of livestock and wild animals, including deer (Turner et al 2013). The main route of entry is through abrasions in the skin (Belser et al 2015). This agent was reported to cause septicemia in a pudu under captivity (Twomey et al 2010).

The introduction of exotic species of deer into Chile and Argentina could have a role in *T. pyogenes* infections in native deer. This bacterium is widespread among white-tail deer (*Odocoileus virginianus*) in North America, although rarely causes disease in this species (Turner et al 2013). Exotic deers may act as reservoirs of *T. pyogenes* in this particular farm, favouring the transmission to captive pudues in nearby enclosures. Nonetheless, other deer species were not tested for *T. pyogenes* to determine their infection status. Alternatively, it is also possible that this bacterium is naturally present in the mucosae of pudus, and stress associated with captivity or other events allowed the opportunistic invasion of deeper tissues after injury in the skin or mucous.

The occurrence of domestic ungulates in natural environments in Chile, particularly national parks and reserves, expose native ungulates to *T. pyogenes* transmission. Since the livestock-wildlife interface serves as a mechanism for the maintenance of this pathogen in wild ungulate populations (Turner et al 2013), it is likely that infection with *T. pyogenes* is occurring in Chilean pudus inhabiting anthropogenically disturbed areas, in which domestic ungulates are acting as...
a source of pathogenic organisms for pudus, as previously reported (Pizarro-Lucero et al 2005, Jimenez 2010). Future studies may determine if populations of free-ranging pudus are currently \textit{T. pyogenes} carriers, and if the infection causes disease, as well as the role of livestock and exotic deer in maintaining \textit{T. pyogenes} in natural environments.

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