SHORT COMMUNICATION

Mycobacterium avium subsp. *paratuberculosis* (MAP) infection in the endangered huemul deer (*Hippocamelus bisulcus*) in Patagonia

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ABSTRACT. In a huemul (*Hippocamelus bisulcus*) population sympatric with cattle, we found evidence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection. Three huemul faecal pellet samples and two cows pats were collected and cultured for MAP presence. DNA was then extracted for PCR analysis of all signal-positive cultures. To assess whether MAP isolates obtained from huemul faeces were associated with typical MAP isolated from livestock, positive confirmed culture samples were sub-typed using a combination of five Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Analysis and one Short Sequence Repeat analysis markers. All faecal samples from both species were MAP positive. One huemul presented a different bacteria profile genotype not described before, suggesting that huemul and cattle in Patagonia could carry a unique MAP strain.

Key words: Patagonia, huemul, cattle, spillover, paratuberculosis, typing.

INTRODUCTION

The International Union for the Conservation of Nature estimates that over 24% of the world's extant mammals are currently threatened with extinction, yet infectious diseases have only been listed as a major threat for a small fraction (1.1%)[1]. It is likely that diseases are underrepresented as a contributing threat to wildlife extinction, especially given that less than half (39%) of critically endangered and endangered artiodactyls, carnivores, and primates from the 2006 IUCN Red List have any published records of pathogens from their wild populations (Pedersen *et al* 2007). Recently, evidence linking huemul (*Hippocamelus bisulcus*), an endemic and endangered deer species from southern Chile and Argentina (Corti *et al* 2010), with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection has been reported (Salgado *et al* 2017). MAP is the causative agent of Johne's disease, infection mostly affecting domestic ruminants, but it has been shown to pose a threat also to wildlife (Manning and Collins 2001, Salgado *et al* 2015). However, the pathogen has also been isolated from non-ruminants species and even from humans, associating it with Crohn's disease (Chiodini *et al* 2012).

Although huemul inhabits remote areas with limited contact with domestic animals/livestock and exists at low population density, the presence of pathogens like MAP may be interpreted as an indicator of spill-over infections from domestic animals. Then, to improve our knowledge about this current issue in huemul, a cross-sectional study was conducted on few huemul and cattle co-inhabiting the same area to inquire possible epidemiological interactions at Torres del Paine National Park in Chilean Patagonia (figure 1).

MATERIAL AND METHODS

The sampling was carried out in November 2016, at the end of the southern hemisphere spring. Three huemul faecal pellet samples, each containing from six to 10 droppings, and two cows pats were collected (Figure 1). The sampling selection criteria was that faecal material from both species was no more than one-day old, estimated according to the appearance of deer pellets and the characteristics of cow pats (Lehmkuhl *et al* 1994). To collect the faeces, we used sterile latex gloves avoiding soil contamination, and the samples were individually kept in sealed plastic bags previously labelled. Samples were stored one week under a refrigeration temperature of 5 ºC until laboratory analyses. Faecal samples were processed in a liquid culture system (BACTEC-MGIT 960, Sparks, Maryland 21152, USA) for MAP presence according to the manufacturer's protocol, and then DNA was extracted from all signal-positive cultures. Real-time IS*900* PCR was performed for MAP detection verification to confirm positive samples (Salgado *et al* 2014). The MAP confirmation consisted of a PCR system targeting the insertion element IS*900*. The PCR total reaction was 20 μl, from which 5 μl was DNA template, 10 μl were 23 TaqMan Universal MasterMix (Roche Applied Science,

[1]   IUCN, International Union for the Conservation of Nature. 2018. IUCN red list of threatened species. Available at http://www.redlist.org
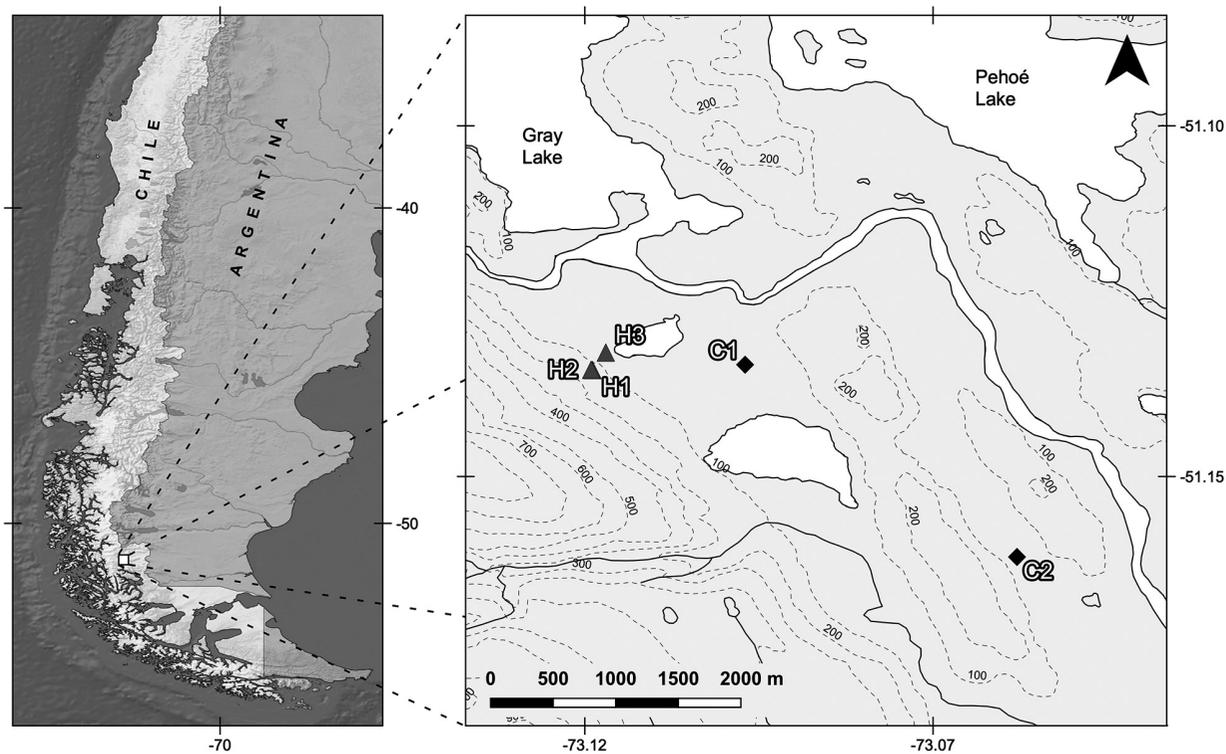
Figure 1. Geographic location of the study area and collection sites at Torres del Paine National Park in Chilean Patagonia. Black dots indicate environmental huemul (H) and cow (C) faecal samples.

Indianapolis, Indiana 46250, USA), 2 µl of each primer (0.2 µM), and 1 µl probe (0.1 µM). The sequence for IS*900* primers, which amplified a 63 nucleotide fragment of the IS*900* gene target, was 5' GACGCGATGATCGAGGAG-39 (L) and 59-GGGCATGCTCAGGATGAT-3' (R). The reactions were run in a Roche LightCycler 2.0 system (Roche Applied Science) under the following standard conditions: one cycle to 95°C for 10 min, 45 cycles with three steps of 95°C for 10 sec, 60°C for 30 sec, 72°C for 1 sec, and a final cooling step at 40°C for 30 sec. Negative and positive (MAP ATCC 19698) PCR controls were included as well as a DNA extraction negative and positive control.

To assess whether MAP isolates obtained from huemul faeces were associated with typical MAP isolated from livestock, positive confirmed culture samples were sub-typed using a combination of five Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Analysis (MIRU-VNTR 292, 25, X3, 7, and 3; Thibault *et al* 2007), and one Short Sequence Repeat analysis markers (SSR 2; Amonsin *et al* 2004). The combination of MIRU-VNTR and SSR allele repeats formed a unique profile that allowed the allele profile to be compared to MAP sub-types obtained from a survey on huemul and other domestic animals in Chile (Salgado *et al* 2017).

RESULTS AND DISCUSSION

All three huemul faecal samples were culture positive for MAP, and the two cow faeces samples were also positive for MAP, both confirmed through PCR (table 1). All DNA

Table 1. Huemul MIRU-VNTR and SSR pattern result and its comparison with local cow strains from Torres del Paine National Park.

	Sample ID	292	x3	25	47	3	MIRU-VNTR*	INMV**	SSR-L2***
HUEMUL	134	4	2	3	3	2	42332	INMV 1	10
HUEMUL	135	4	2	3	3	2	42332	INMV 1	10
HUEMUL	137	3	2	3	3	2	32332	INMV 2	14
COW	138	3	2	3	3	2	32332	INMV 2	14

*MIRU-VNTR: Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Analysis.
**INMV: The INRA Nouzilly MIRU-VNTR profile nomenclature, as defined by Thibault *et al* (2007).
***SSR L2: Short Sequence Repeat analysis markers-Locus 2.

of the isolated strains showed a sufficient quality to consider the results of the molecular characterisation conclusive, except for the strain obtained from one of the cows. The lack of variation between one cow and one huemul MAP strains suggests that cattle and huemul populations are sympatric, as previously stated (Salgado *et al* 2017). This result suggests that both species share the same bacteria, evidencing interspecies transmission as shown elsewhere between wildlife and livestock based on the same typing protocol (Fritsch *et al* 2012). Also, Thibault *et al* (2008) highlighted that MIRU-VNTR typing followed by SSR was the most cost-effective and efficient strategy for strain discrimination.

It has been also hypothesised (Salgado *et al* 2017) that the infection to huemul primarily may be through drinking faecal-contaminated water and secondarily through faecal-oral route since huemul species are browsers, mostly feeding on shrubs, trees, and forbs but rarely on graminoids near the ground (Vila *et al* 2009). However, one specific MIRU-VNTR and SSR genotype found in Torres del Paine National Park, from huemul faecal material, was different from those detected in cattle herds and from genotypes previously informed in Chile (table 1)(Salgado *et al* 2017).

It is highly interesting, within this restricted area, that epidemiologically associated free-ranging cattle and huemul could carry specific MAP genotypes. Therefore large-scale studies are urgently needed to gain deeper knowledge on this important sanitary issue in an endangered species like the huemul.

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