CASE REPORT

Evidence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection in huemul deer (*Hippocamelus bisulcus*) in patagonian fjords

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ABSTRACT. In the Chilean coastal Patagonia, fourteen wild deer huemul faecal pellet samples were collected and cultured for *Mycobacterium avium* subsp. *paratuberculosis* detection. Six samples were positive, but only one was able to show a molecular type similar to the most common strain reported for cattle in Chile.

Key words: Patagonia, huemul, paratuberculosis, remoteness.

RESUMEN. En el Parque Nacional Bernardo O'Higgins de la Patagonia chilena se recolectaron catorce muestras de heces de huemul para detección de *Mycobacterium avium* subsp. *paratuberculosis*. Seis de las muestras resultaron positivas, pero solo una mostró un patrón molecular similar al patrón más común de cepas aisladas de bovinos en Chile.

Palabras clave: Patagonia, huemul, paratuberculosis, aislamiento geográfico.

Mycobacterium avium subsp. *paratuberculosis* (MAP) is a worldwide reported, obligate intracellular bacterial pathogen, the causative agent of Johne's disease, a long-lasting, usually fatal granulomatous enteritis of domestic and wild ruminants. It is estimated that up to 80% of Chilean cattle herds are MAP infected (Kruze *et al* 2013) and reports from wildlife in Chile have been increasing in the last decade, especially in surrounding farming areas (Salgado *et al* 2015).

Special attention should be paid to wild ruminants living in remote areas such as huemul deer (*Hippocamelus bisulcus*), since deer are more (highly) sensitive to MAP infection than other ruminants (Mackintosh *et al* 2004). This deer is endemic from southern Chile and Argentina and currently the most endangered Neotropical deer[1]. Huemul populations are highly fragmented, with estimated population size <2,000 individuals (Corti *et al* 2010). The largest remnant huemul populations are mostly located in remote mountainous ecosystems in the Chilean coastal Patagonia. Despite their small populations and remoteness, huemul appear to have an important contact with both managed and feral cattle populations (Corti *et al* 2013). While it is unknown whether diseases are affecting huemul persistence, there is suggesting evidence that in some huemul populations the survival of offspring is low, due

to a high percentage of unknown mortality causes (Corti *et al* 2010). Under this context, we proposed to investigate evidence of MAP infection in huemul as evidence of anthropogenic pressures from livestock in a remote area of Chile, where this pathogen has not yet been reported.

Fourteen huemul faecal pellet samples, each consisting from six to ten droppings, were collected in Bernardo O'Higgins National Park (figure 1). The sampling selection criteria were that faecal material was no more than one day old, estimated according to the pellets' appearance and characteristics (Lehmkuhl *et al* 1994). Faecal samples were collected using sterile latex gloves, avoiding soil contamination, and individually deposited in a sealed plastic bag previously labeled. Samples were kept one week under refrigeration temperature (5 ºC) until laboratory analyses. Faecal samples were processed in a liquid culture system (BACTEC-MGIT 960) for MAP presence according to the manufacturer's protocol, and then followed by real-time PCR for confirmation of positive samples (Salgado *et al* 2015). Cross contamination of samples was avoided through strict lab protocols, so all assays included positive and negative controls. To assess whether MAP isolates obtained from huemul faeces were associated with typical MAP isolated retrieved 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 two Short Sequence Repeat analysis (SSR 2 and 8) markers (Amonsin *et al* 2004). The combination of MIRU-VNTR and SSR allele repeats formed a unique profile, then compared to MAP sub-types obtained from a large survey of the dairy sector in Chile (Verdugo *et al* 2016).

Six out of 14 faecal samples gave positive MGIT signal. All of them were identified through an IS*900* PCR as MAP. Only one out of the six positive culture isolates, only one was subtyped by molecular techniques.

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[1] IUCN. 2016. The IUCN Red List of Threatened Species. Version 2015-4. www.iucnredlist.org. Downloaded on 08 June 2016.
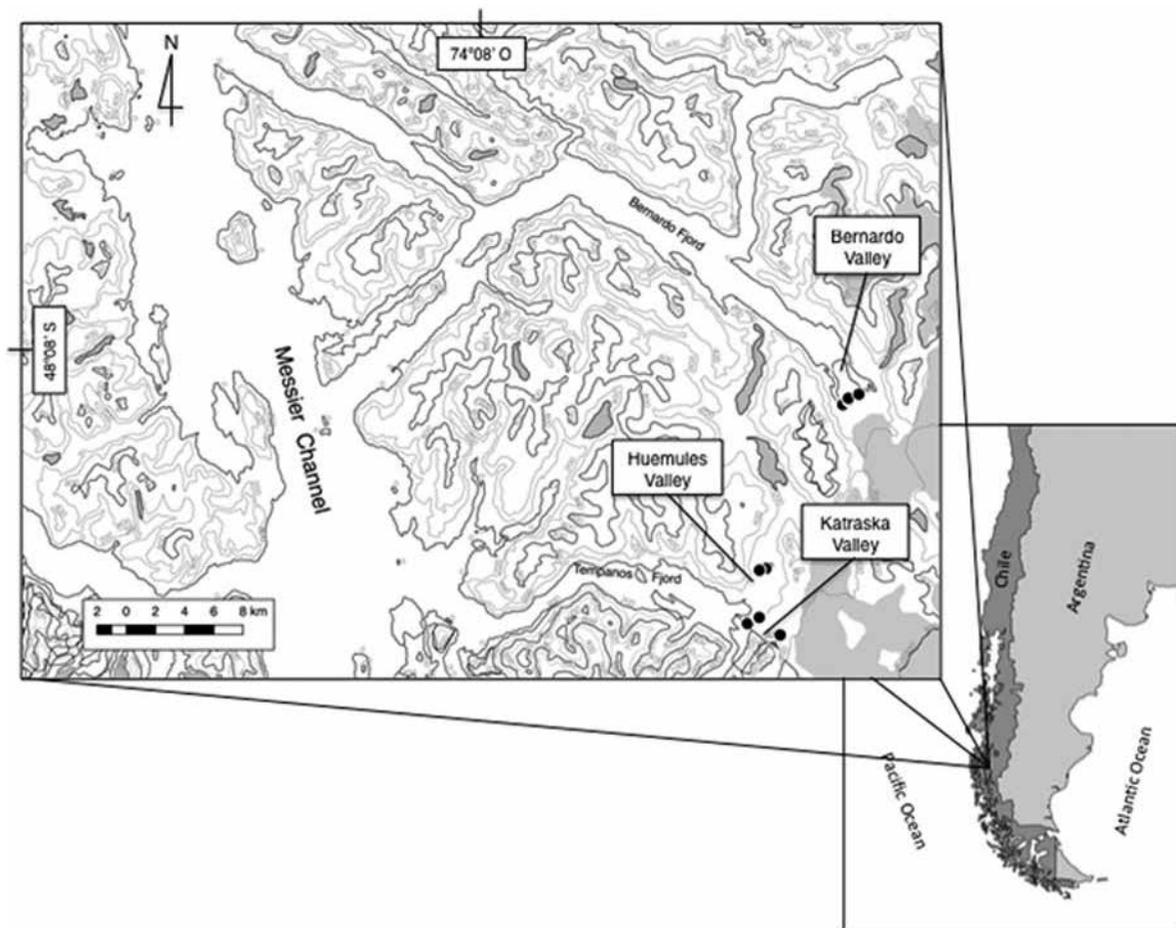
Figure 1. Geographic location of the study area at Chilean coastal Patagonia. Black dots indicate environmental huemul faeces sampling sites at three periglacial valleys of Bernardo O'Higgins National Park in Chilean coastal Patagonia.

The remaining five evidenced high contamination, most likely due to soil microorganisms such as members of the *Bacillus* genus, which made the molecular profile analysis results ambiguous. The analysed isolate showed the same MIRU-VNTR and SSR pattern described as the most common strain reported in cattle from Chile. It showed four repeats for TR292 (locus 1) in contrast to

the three repeats for the second most common (Verdugo *et al* 2016). Four copies of SSR 8 and 11 for SSR were detected (table 1).

This first isolation of MAP, in a remote region with the largest huemul populations in pristine conditions, expands both the distribution boundaries of MAP infection and the diversity of wild species suffering this pathogen infection.

Table 1. Huemul MIRU-VNTR pattern result and its comparison with local *Mycobacterium avium* subsp. *paratuberculosis* strains from different ruminant host species.

ID	locus1 TR292	locus2 TRX3	locus3 TR25	locus4 TR47	locus5 TR3	SSR2	SSR8	Ruminant species
1	4	2	3	3	2	11	4	Cow
2	4	2	3	3	2	11	4	Goat
3	3	2	3	3	2	10	4	Goat
4	3	2	3	3	2	10	4	Red deer
5	4	2	3	3	2	12	4	Cow environmental faecal sample
VB5	4	2	3	3	2	11	4	Huemul

The most plausible hypothesis explaining MAP presence in these deer populations is the transmission through contact with infected cattle, illegally introduced at Témpanos fjord in 1991 (Frid 2001). The evidence confirming that cattle and huemul populations are sympatric is the fact that molecular typing strategies used to characterise MAP isolated from huemul lack of variation with cattle control strains. The latter could be indicative that both species share the same bacteria, evidencing interspecies transmission.

Once MAP is shed in faeces from hosts, it survives for prolonged periods in the environment (Whittington *et al* 2005). While the transmission of MAP is mainly faecal-oral route, the infection to huemul primarily through drinking faecal-contaminated water cannot be ruled out since huemul, like most mid-sized ruminants, are browsers mostly feeding on shrubs, trees, and forbs, but rarely on graminoids near the ground (Vila *et al* 2009). Thus, fecal-contaminated water increases transmission possibilities to browser ruminants because this pathogen can survive for long periods in fresh water (Pickup *et al* 2005). The fact that domestic ruminants were introduced into huemul's protected areas is another example of habitat encroachment generating negative effects for wildlife. Our study reveals an important issue about the impact of diseases on wild endangered populations. Although huemul inhabit remote areas with limited contact with humans and existing at low population densities (Corti *et al* 2010), the presence of pathogens like MAP may be interpreted as an indicator of infections spill over from domestic animals creating a greater animal health risk in areas thought to be pristine.

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