Arch Med Vet **46, 45-52 (2014)**

ORIGINAL ARTICLE

Paratuberculosis or avian tuberculosis in red deer with chronic diarrhea?[#]

Paratuberculosis o tuberculosis aviar en ciervo rojo con diarrea crónica?

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SUMMARY

Mycobacterium avium subsp. *paratuberculosis* (*Map*) is the etiological agent of paratuberculosis. In Chile, information about *Map* isolation from both domestic ruminant and wildlife species has been accumulating, but it has extended to other species. In Chile, deer farming activity has been focused in hunting and meat production. No paratuberculosis surveillance has been reported for these source herds. In the present study, chronic diarrhea and poor body condition in some animals belonging to a deer farm was informed. Four adult affected hinds were euthanized under suspicion of paratuberculosis. In all animals macroscopic and microscopic lesions consistent with paratuberculosis were observed. *Map*-positive culture results from faecal and tissue samples, complemented with positive-PCR-results from fixed tissues are informed. Evidence has been found on cattle and deer populations being connected since molecular characterization of the *Map* isolated from deer lack of variation between the cattle control strain. The latter could indicate that both species share the same bacteria, suggesting there is interspecies transmission. Complementary diagnostic methods were accurate to diagnose paratuberculosis and to differentiate the clinical deer case from other mycobacterial infection. The epidemiological findings suggest that the infection in the deer farm could be transmitted from a cattle herd, making this the first reported case of paratuberculosis in deer farm in Chile.

Key words: deer, histopathology, paratuberculosis, PCR.

RESUMEN

Mycobacterium avium subsp. *paratuberculosis* (*Map*) es el agente etiológico de la patuberculosis. En Chile, antecedentes del aislamiento de *Map* tanto en rumiantes domésticos como en especies de vida silvestre se han ido acumulando a través del tiempo, sin embargo, esto tiene que estar afectando también a otras especies. En Chile, la crianza de ciervos se ha focalizado en dos objetivos: cotos de caza y producción de carnes exóticas, no existiendo un programa de vigilancia ni siendo reportada esta enfermedad en rebaños de este tipo. Este trabajo reporta la presencia de diarrea crónica y pobre condición corporal en algunos animales pertenecientes a una granja de ciervos donde se sospechó la presencia de paratuberculosis. Cuatro ciervos adultos fueron eutanaziados bajo esta sospecha. Todos los animales presentaron lesiones macro y microscópicas compatibles con paratuberculosis. Resultados positivos al cultivo bacteriológico a partir de heces y muestras de tejido, además de PCR a partir de tejido fijado evidencian la infección. De acuerdo a los resultados de caracterización molecular de las cepas aisladas de los ciervos que no muestran variación entre cepas control de bovino, se evidencia que ambas poblaciones estarían conectadas. Complementariamente, los métodos diagnósticos utilizados fueron muy precisos para diagnosticar paratuberculosis y para diferenciar casos clínicos en ciervos de otras micobacteriosis. Las características epidemiológicas descritas en este estudio sugieren que la infección presente en la granja de ciervos, pudo tener su origen en un rebaño bovino. Finalmente, los hallazgos anteriormente mencionados representan el primer reporte de paratuberculosis en ciervos de granja en Chile.

Palabras clave: ciervos, histopatología, paratuberculosis, PCR.

INTRODUCTION

Paratuberculosis is a chronic wasting enteritis of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). The disease is characterised by granulomatous enteritis, diarrhea, loss of body weight and death. It can be found worldwide and mainly affects domestic ruminants, being responsible for significant economic losses of livestock production (Lombard 2011). In addition, some authors consider *Map* to be a potential zoonotic agent, as it has been associated with Crohn's disease in humans (Chiodini *et al* 2012).

In Chile, *Map* infection has been reported in cattle (Grinbergs and Caorsi 1958), sheep (Zamora *et al* 1975), goats (Kruze *et al* 2006), guanacos (*Lama guanicoe*) (Salgado *et al* 2009) and hares (Salgado *et al* 2011[b]). Due to the close relation between wild and domestic animals in some areas, it is suggested that other wild and captive animal populations could be infected (Salgado *et al* 2011[a])

Infection by *Map* has also been described in wild and captive deer populations in many countries (de Lisle *et al*

Accepted: 22.08.2013.

[#] This study was supported by the project DID S-2011-04, Dirección de Investigación y Desarrollo, Universidad Austral de Chile.

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1993, Power *et al* 1993, Fawcett *et al* 1995, Manning *et al* 1998, Godfroid *et al* 2000, Paolicchi *et al* 2001, Alvarez *et al* 2005, Machackova-Kopecna *et al* 2005, van Kooten *et al* 2006, Mackintosh *et al* 2007). Since 1980, paratuberculosis has been an emergent problem in deer farming with important underestimated losses, mainly due to outbreaks of the clinical disease (Mackintosh *et al* 2002).

In Chile there are approximately 6,000 introduced deer largely distributed in the Southern regions (INE 2007[1]). Most animals have been imported from Argentina, New Zealand and Europe (Paolicchi *et al* 2001, Marco *et al* 2002, de Lisle *et al* 2003, Mackintosh *et al* 2004), where paratuberculosis have been reported in deer in high prevalence (Mereb *et al* 1994, de Lisle *et al* 2003) and are used for hunting and meat production.

In deer, as in cattle, sheep and goats, paratuberculosis produces a long term enteritis that finally leads to loss of body condition and death in severely affected animals (Machackova-Kopecna *et al* 2005). Paratuberculosis diagnosis in deer is based on *Map* detection in faeces, postmortem and histopathological examination of tissues (specially the lower part of the jejunum, ileum, ileocecal valve and its associated lymph nodes) (Clarke 1997). Histopathological assessment includes different grading systems and disease classification, such as paucibacillary and multibacillary, already described for several species with paratuberculosis (Carrigan and Seaman 1990, Clarke and Little 1996, Perez *et al* 1996, Gonzalez *et al* 2005, Balseiro *et al* 2008, Clark *et al* 2010). However, the histopathological observation of lesions is insufficient to confirm the disease, especially due to the similarity of lesions caused by *Map* and *M. avium* subsp. *avium (Maa)*. Co-infection with *Map* and *Maa*, which causes clinical disease and eventually death, complicates and challenges the bacteriological diagnosis of mycobacterial infections in deer (Godfroid *et al* 2005). Besides, just as in the case of avian tuberculosis, lesions found at slaughter in deer subclinically infected with paratuberculosis may cause problems because of their gross and histopathological similarity to lesions of tuberculosis (Campbell 1995).

The aim of the present study was to confirm *Map* infection and differentiate a clinical case from other mycobacterial infections, in farmed deer of Southern Chile.

MATERIAL AND METHODS

ANIMAL POPULATION

Animals belonged to a hunting and breeding deer farm located in the Región de Los Lagos, Southern Chile. The herd was comprised of 180 red deer (*Cervus elaphus*) and 200 fallow deer *(Dama dama*). An interesting epidemiological feature was that the pastures used for the deer had been previously used to raise dairy cattle with an estimated individual prevalence of 15%, with 3-5 clinical cases per year. This herd was comprised of 800 cattle grazing about 700 hectares and it was located in close proximity to the deer herd.

ANIMAL SAMPLING

Four adult hinds, with evident poor body condition, showed liquid and greenish diarrhea (without blood or fibrin) and faeces adhered to their tail and hocks. Paratuberculosis was suspected and the animals were humanely euthanized.

NECROPSY AND SAMPLES

Complete necropsy was performed on each animal and gross lesions were recorded focusing on intestine, lymph nodes and liver. Samples were taken for histopathology, bacteriological culture and PCR. Different sections of small and large intestine, lymph nodes, liver, abomasum, heart, spleen, kidneys and lungs were removed and fixed in 10% buffered formalin for histopathology.

HISTOPATHOLOGY

Sections of 5 mm were stained with Hematoxilin and Eosin (HE) and Ziehl Neelsen (ZN) (at least 3 sections per organ). Lesions were described and graded from 0 to 3 regarding cellular infiltration (Carrigan and Seaman 1990). Also, samples were graded from 0 to 3 depending on the amount of acid fast bacteria (AFB) observed in the tissues, and then classified as multibacillary or paucibacillary (Clarke and Little 1996).

BACTERIOLOGY

Bacterial cultures. Faecal and tissue samples (ileum and ileocecal lymph node) were used. Two grams of each sample were homogenised and decontaminated with hexadecilpiridinium (HPC) 0.9% overnight, and were centrifuged at 3500 x g for 20 min. Pellets were suspended in 1 mL of antibiotic solution (vancomicyn 100mg/ml, nalidixic acid 100mg/ml and amphotericim B 50mg/ml) overnight and an aliquot of 150 ml was inoculated in four slants of Herrold's medium (HEYM), three with mycobactin J and one without it. Cultures were incubated at 37°C up to 5 months. All mycobactin-dependant colonies resembling *Map* were submitted to molecular confirmation.

DNA EXTRACTION-PURIFICATION

Real-time IS*900* PCR using the ROCHE system (Roche, Indianapolis, IN) as described previously (Salgado *et al* 2011[b]) was used. The ROCHE system reports crossing-point (Cp) values.

[1] INE, Instituto Nacional de Estadísticas Chile. www.ine.cl

MOLECULAR CONFIRMATION

The Real-time PCR system consisted in a multiplex PCR, where the targets were the insertion elements IS*900* and IS*901* sequences of *Map* and *Maa*, respectively. The primers and probes were designed with the ProbeFinder program version 2.45 (ROCHE). The PCR mix for each reaction consisted of 9.5 µl water (Sigma-Aldrich), 0.5 µl (1 µM) probe, 0.5 µl (2 µM) of each primer (sense and antisense) and 4 µl LightCycler® TaqMan® Master kit. Five microliters of the DNA template were added to the mix. The reactions were run in the LightCycler 2.0 ROCHE system under the following standard conditions: one cycle to 95°C for 10 min and 45 cycles with two steps of 95°C for 10 s, 60°C for 30 s and 72°C for 1 s. and cooling was at 40°C for 30 s. Negative and positive DNA controls (*Mycobacterium avium* subsp. *paratuberculosis* ATCC 19698 and *Mycobacterium avium* subsp. *avium* ATCC 4440) were included.

TYPING

To distinguish subtypes of *Map*, deer isolates were assayed by IS*1311* PCR-REA at the Biochemistry and Microbiology Department, Universidad Austral de Chile according to the protocol described by (Marsh *et al* 1999). The previously described primers M56 (59- GCGTGAGGCTCTGTGGTGAA-39) and M94 (59-CAGCGATCGTCGACAGTGTG-39) were used to amplify a region of the IS*1311* insertion sequence (Whittington *et al* 1998, Marsh *et al* 1999). Briefly, a reaction volume of 50 ml containing 5 ml of the DNA sample, 4 ml of each primer (10 pmol/ml), 22.6 ml of water, 4 ml dNTPs (10mM), 5 ml MgCl2 (25 mM), 5 ml PCR buffer II (103), and 0.4 ml Taq Gold polymerase (5 U/ml) was used. The PCR procedure was performed under the following conditions: one cycle of denaturation at 94 ºC for 3 min followed by 37 cycles of denaturation at 94 ºC for 30 sec, annealing at 62 ºC for 15 sec, and extension at 72 ºC for 1 min. Polymerase chain reaction results were assessed by electrophoresis in 2% agarose gels stained with ethidium bromide. The IS*1311* PCR products were gel-purified using the Qiaquick Gel extraction kit (Qiagen GMBH, Hilden, Germany). Restriction endonuclease analysis was prepared by adding 4 - 12 ml of purified PCR product, 2 U of *Hinf*I restriction endonuclease (Promega Corp., Madison, Wisconsin, USA), 1.6 ml of buffer (supplied with restriction endonuclease), and made up to 16 ml with sterile purified water. Restriction digests were incubated for 2 hr at 37 C and were assessed by electrophoresis in 4% agarose gels stained with ethidium bromide.

To complement the typing information and to obtain more discriminatory power, DNA extracts from the subset of *Map* isolates run in REA-PCR, and confirmed by IS*900* real-time PCR, were subjected to MIRU-VNTR typing. MIRU-VTNR typing was performed using 5 loci identified as polymorphic for *Map* K10 and called MIRU-VNTR 292, X3, 25, 47 and 3 (from locus 1 to locus 5) (Thibault *et al* 2007). Primers designed to target flanking regions of the MIRU-VNTRs and the conditions of the PCR amplification was carried out as described by Thibault *et al* (2007). Briefly, PCR mixture for each MIRU-VNTR locus comprised 5 µl of template DNA, 1.5 mM of magnesium chloride, 1 µl of dimethyl sulfoxide (Sigma, St. Louis, Mo) (except for locus 2), 1 µM of each primer, 50 µM of dATP, dCTP, dGTP, and dTTP and 1.25 U of Platinum Taq (Invitrogen Ltd., Paisley, UK) in a final volume of 25 µl. Reactions were carried out using a GeneAmp 9600 PCR system (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: 1 cycle of 5 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C, and 1 cycle of 7 min at 72°C. To detect differences in repeat numbers, the PCR products were analyzed by electrophoresis using 1.5% agarose gels. Repeat numbers (alleles) were determined according to amplified fragment sizes using Gel Doc 2000 (Biorad, Herefordshire, UK) and Quantity One 4.2.1.software (Biorad) for fragment size calculation.

FIXED TISSUE CONFIRMATION PRESENCE/ABSENCE OF *MAP* AND *MAA*

After histopathological and bacteriological results, paraffin blocks of the four animals containing intestine, liver and lymph nodes were selected for Real Time PCR, in order to confirm or discard coinfection of *Map* and *Maa*. To prevent cross contamination on the samples for PCR, the microtome and each paraffin block were disinfected with a chlorine solution 10% and then with ethanol 100% between cuts and before inserting a new one into the microtome. Two 5 mm sections were cut and transferred to a sterile 1.5 mL tube for paraffin removal, as described by Miller *et al* (1997). After the deparafinization process of the paraffin-embedded specimen, the samples were subjected to the above explained protocols for DNA extraction and Real-Time PCR confirmation.

RESULTS

NECROPSY

Poor body condition and greenish diarrhea was observed externally in all animals (figure 1A). As a first approach, the intestinal wall was thickened and firm. The intestinal mucosa was corrugated and proliferative. Additionally, enlarged mesenteric lymph nodes and dilation of mesenteric lymphatic vessels was observed in all animals (figure 1B). Furthermore, mesenteric lymphatic vessel calcification was evident in two animals (figure 1C). There were no other organs with macroscopic lesions.

HISTOPATHOLOGY

Two animals presented the multibacillary form of disease and the other two presented the paucibacillary form.

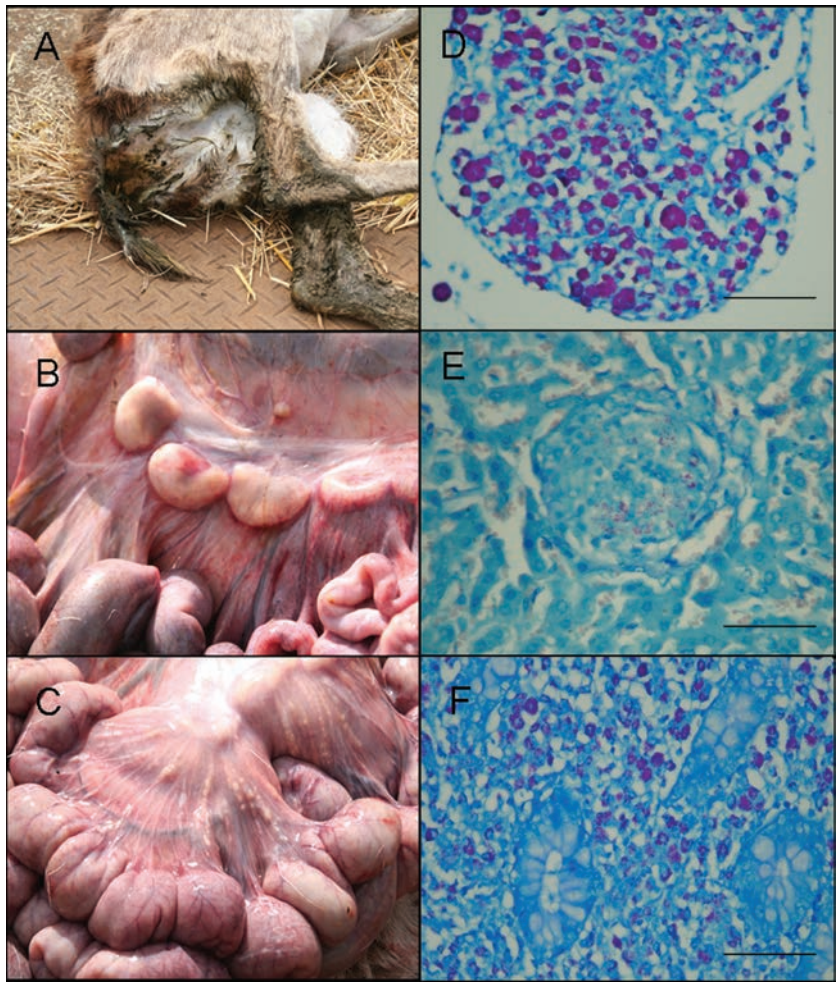

Figure 1. Poor body condition and greenish diarrhea (A), Enlarged and activation of mesenteric lymph nodes (B), Calcification of the mesenteric lymphatic vessels (C), Intense macrophage infiltration with many acid-fast bacteria in the ileum mucosa (D), Granuloma with acid-fast bacteria in liver (E), Diffuse macrophage infiltrate in caecum with acid fast bacteria in corion (F) (Ziehl-Neelsen. 40X. Bar = 100 µm.)

Mala condición corporal y diarrea verdosa (A), Nódulos linfáticos mesentéricos aumentados de tamaño y activos (B), Calcificación de vasos linfáticos mesentéricos (C), Mucosa del ileon con intenso infiltrado macrofágico con múltiples bacterias ácido alcohol resistentes (D). Granuloma hepático con bacterias ácido alcohol resistentes (E), Ciego con infiltado macrofágico difuso y bacterias ácido alcohol resistentes en corion (F). (Ziehl-Neelsen. 40X. Bar = 100 µm.)

In the multibacillary form, Langhan's giant cells were observed containing numerous AFB. Paucibacillary lesions tended to an increased numbers of giant cells and smaller macrophages rather than the multibacillary form. Paucibacillary forms of the disease were more evident in deer with mild disease, while multibacillary forms were more evident in deer with the severe lesions. Ileum was the intestinal segment that was mostly affected in both forms.

In the multibacillary form, one case showed ileum with severe diffuse histiocytic infiltration in the lamina propria (graded 3) with abundant AFB (graded 3) (figure 1D). In the submucosal layer, small amounts of AFB were observed, including mild macrophage inflammatory infiltrate. The serosa of this portion presented moderate histiocytic infiltration with a moderate amount of AFB associated to blood and lymphatic vessels (graded 2). In the mesenteric lymph nodes, multifocal granulomas and diffuse histiocytic infiltration with giant cells (graded 3) and necrosis were observed; lymphoid follicles and medullar sinuses were invaded with mononuclear inflammatory infiltrate. Moderate amounts of AFB were associated with macrophages and giant cells (graded 2). In liver, multifocal granulomas (figure 1E) and focal macrophagical inflammatory infiltrate were observed (graded 2). Perivascular macrophagical infiltration was also associated with the periportal area, with moderate AFB (graded 2). Some focal necrotic areas were present, surrounded by histiocytic infiltration without AFB.

Also, in the multibacillary form the large intestine was affected. Caecum presented mononuclear infiltration

and abundant macrophages in the corion (graded 3) and abundant AFB (graded 3) (figure 1F). In the submucosal layer, infiltration was moderate and mild dilatation of lymphatic vessels was observed. Acid fast bacteria were present inside the macrophages (graded 1). Deeper layers, such as muscularis and serosa, besides the related connective tissue, were also affected with inflammatory mononuclear infiltration (grading 2). In the serosa, AFB were associated with perivascular areas (graded 1). There were no lesions or AFB observed in abomasum, heart, spleen, kidneys and lungs (graded 0).

In the two paucibacillary cases, mixed inflammatory infiltrate in ileum was present (grade 3), and lymphoid activation besides eosinofilic infiltratation in medullar sinuses of lymph nodes was observed (grade 2). No AFB was observed in ileum, mesenteric lymph nodes or in the hepatic lymph nodes. However, in one animal mild histiocytic infiltration and small granuloma with few AFB (grade 1) were present in liver. The main lesion in this case was a moderate mononuclear infiltration with macrophages in ileum and periportal fibrosis in liver (graded 2). Results are summarized in table 1.

Incidental findings, such as eosinophilic infiltration in ileum and mesenteric lymph node with abscess, were observed. Protozoa as coccidia in ileum and sarcosporidium in heart were present.

CULTURE

Overall, from just two animals *Map* isolation (ileum, lymph nodes and faeces) was informed, although all animals showed gross lesions consistent with paratuberculosis.

MOLECULAR TYPING

All colonies resembling *Map* molecularly characterised by REA-PCR were positive for IS*1311* and corresponded to the cattle type (C-type) strain (figure 2)

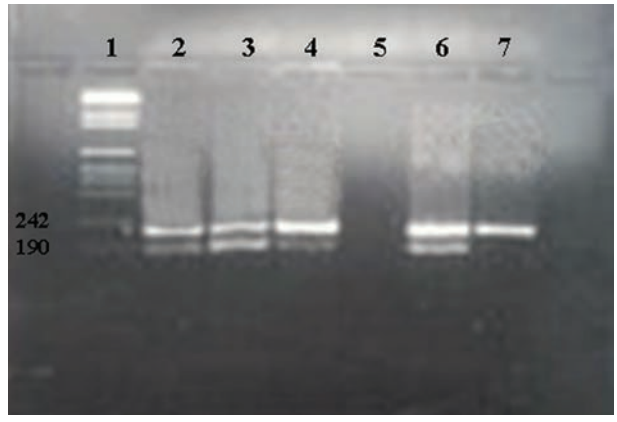

Figure 2. IS*1311* polymerase chain reaction-restriction endonuclease analysis electrophoresis with *Hinf*I (PCR-REA) on 2% agarose gel. Lane 1: Kb DNA size marker. Lane 2 and 3: C type pattern from deer isolates obtained in the present study. Lane 4: and 6: C type cattle strain and lane 7 shows an S pattern from a chilean ovine isolate.

Reacción de polimerasa en cadena IS3111 y análisis de endonucleasa de restricción con *Hinf*I (PCR-REA) en gel de agarosa al 2% . Línea 1: Marcador de peso molecular. Línea 2 y 3: Patron tipo C provenientes de cepa aislada de uno de los ciervos del presente estudio. Línea 4 y 6: Patrón tipo C proveniente de una cepa aislada de bovino y Línea 7 muestra un patrón tipo S provenientes de una cepa aislada de un ovino chileno.

Table 1. Performed tests in four hinds with clinical signs and lesions resembling paratuberculosis.
Pruebas realizadas en los 4 ciervos con signos clínicos y lesiones compatibles con paratuberculosis.

		Animals															
		1				2				3				4			
Test		SI	LI	MLN	Liver	SI	LI	MLN	Liver	SI	LI	MNL	Liver	SI	LI	MNL	Liver
Culture	HEYM	+	np	+	np	-	np	-	np	+	np	+	np	-	np	-	np
Histopathology	H&E/*	3	3	3	2	3	0	2	0	3	3	3	2	3	0	2	1
	ZN/AFB	3	3	2	2	0	0	0	0	3	3	2	2	0	0	0	1
Real Time PCR	*Map* (*IS*900)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Maa (*IS*901)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

SI: Small Intestine, LI: Large Intestine, MLN: Mesenteric Lymph Node, np: not performed
H&E* grade of inflammation

The two isolates tested belonged to the same MIRU-VNTR type, and showed 4 repeats for TR292 (locus1) in contrast to the 3 repeats typical of the reference strain K10 (table 1). The number of repeats found in the remaining loci was identical to that of K10 strain (table 2).

FIXED TISSUE CONFIRMATION PRESENCE/ABSENCE OF *MAP* AND *MAA*

Animals with lesions but culture negative were suspected of *Maa* infection. Four animals were retested by Real Time PCR on fixed tissue to confirm or discard *Map* and *Maa* co-infection, all of them were PCR positive to *Map* and negative to *Maa* (table 2).

DISCUSSION

Due to similarities in the clinical signs and histopathology of the infection produced by *Maa* and *Map* (Mackintosh *et al* 1997, Mackintosh *et al* 2004), a diagnostic strategy was proposed in the present study to troubleshoot it. First of all, clinical signs and lesions observed in deer with the multibacillary form of the disease match the lesion descriptions for cattle and small ruminants in multibacillary cases (Buergelt *et al* 1978, Clarke 1997, Huda and Jensen 2003, Gonzalez *et al* 2005, Mackintosh *et al* 2007). However, lesions in the large intestine with diffuse inflammatory infiltration and AFB was observed as well as multifocal granulomas in liver, probably due to more susceptibility of the deer or may be a more pathogenic *Map* strain. The present study showed that in the multibacillary cases there were multifocal disseminated liver granulomas, with moderated intracellular AFB in the macrophages. This feature is commonly described in paratuberculosis cases in deer, but with scarce mycobacteria inside the macrophages (Del Pozo *et al* 2013). In the paucibacillary cases, neither hepatic lesions nor AFB were observed, probably due to the small amount of mycobacterium which remained in small intestine or lymph nodes, failing to reach the liver. On the other hand, in the study cases diffuse inflammatory infiltration in ileum was observed, instead of multifocal granulomas, which has been the main lesion described due to *Map* infection in deer (Mackintosh *et al* 2004, Mackintosh *et al* 2007, Balseiro *et al* 2008, Clark *et al* 2010). The latter may be due to the participation of several factors that could influence the immune response such as stage of infection, immunological status, age, animal susceptibility, infecting strain or breed (de Lisle *et al* 1993, Perez *et al* 1996, Clarke 1997, Mackintosh *et al* 2007, Balseiro *et al* 2008). In our opinion, that infection pressure, strain type and epidemiological story of the herd are the most important factors in the severity of the lesions.

In this report *Map*-positive cultures plus the PCR IS*901* negative results in all animals, clearly show that the histopathological findings are due to *Map* infection only. However, some authors have described *Maa-Map* co-infection with indistinguishable clinical and pathological lesions (Mackintosh *et al* 2004, Machackova-Kopecna *et al* 2005, Glawischnig *et al* 2006). The present results show a reliable technique that combined with tools such as culture, help to obtain a correct diagnosis. Additionally, the results obtained from formalin fixed paraffin embedded tissues allowed performing retrospective studies to determine coinfection, even when fresh feaces or tissues were not available for culture.

The occurrence of *Map*-infected deer found in this area suggests a potential transmission of *Map* from livestock through the faecal-oral route. Since a clinically affected cow may shed over 10^8 bacteria per gram of faeces (Whittington *et al* 2000), and an infective dose is considered to be 10^6, the likelihood of transmission to free-ranging immature ruminants, sharing pasture directly or indirectly with infected cattle is high. Epidemiological evidence that should be addressed is the fact that the deer herd is located in the proximity of a dairy farm that has more than 800 lactating cows and both farms belongs to the same owner. Furthermore, biosecurity management measures were violated, because the same workers participate in both farms and herds, vehicles transit without any disinfection from cattle to deer farm. Additionally, other evidence that confirms that cattle and deer populations are connected is the fact that molecular typing strategies used to characterize *Map* isolated from deer lack of variation between cattle control strain. The latter could be indicative that both species shares the same bacteria

Table 2. MIRU-VNTR results in two deer isolates and control strain.
Resultados de MIRU-VNTR en dos de las cepas aisladas de ciervo y cepa control.

Strain	Number of repeats								
	locus1 TR292	locus2 TRX3	locus3 TR25	locus4 TR47	locus5 TR3	locus6 TR7	locus7 TR10	locus8 TR32	Host
1	4	2	3	3	2	2	2	2	deer
2	4	2	3	3	2	2	2	2	deer
K10	3	2	3	3	2	2	2	2	ATCC control

and by this contribute with a piece of evidence to think of interspecies transmission.

We expected this dairy cattle herd to be infected with *Map*, since currently between 50 to 87% of dairy herds in Southern Chile are considered infected (Kruze *et al* 2013) and no control programs have been implemented neither at a national nor local level. The chance that *Map* remains in the environment and become a potential risk for susceptible host is high due to its ability to survive for long periods outside the hosts, enabling it to persist and spread in grasslands, and withstand a periodic lack of suitable hosts. The time required to eradicate the organism from the environment is unknown. It has been suggested that at least 6 months to a year is required to render pastures safe after being grazed by infected cattle (Lovell *et al* 1944). In this case, it could be concluded that pasture might be a source of infection, because they had been recently used to raise dairy cattle probably affected by paratuberculosis.

This first report of farmed deer affected by paratuberculosis in Chile is a starting point to determine the appropriate diagnostic tools for its recognition and differentiation between *Map* infection, *Maa* infection and co-infection with both subespecies. Although differentiation of avian tuberculosis and paratuberculosis is technically challenging, multiple diagnostic tools used together, plus detailed epidemiological study are advisable in order to reach a precise diagnosis of captive deer.

Furthermore, the study shows the effectiveness of the bacteria to infect susceptible populations, even when the animals are not in direct contact, reaffirming the bacteria's ability to remain and survive on soil. The studied deer herd becomes now a new *Map* infection focus to other domestic and wild animals in their surroundings. Finally, it is important to address the zoonotic potential of these bacteria, considering that it this farm produces venison for human consumption.

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