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Short Communication

Identification of *Brucella ovis* exclusive genes in field isolates from Argentina

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ABSTRACT

Brucellosis caused by *Brucella ovis* is one of the most important infectious diseases of sheep. The aim of this study was to determine the presence of genes both inside and outside the specific *B. ovis* pathogenicity island 1 (BOPI-1) in a large collection of field isolates of *B. ovis* and other *Brucella* spp. from Argentina. The BOV_A0500 gene from *B. ovis* BOPI-1 was identified in all 104 *B. ovis* isolates studied. The BOPI-1 complete sequence was found to be conserved in 10 *B. ovis* strains from the collection, for which whole genome sequencing was performed. The BOV_0198 gene, which is outside BOPI-1 and considered exclusive to *B. ovis*, showed 90–100% identity with genomic regions of *B. ovis*, *B. melitensis*, *B. abortus*, *B. canis*, *B. suis*, *B. microti*, *B. ceti* and *B. pinnipedialis*. The results demonstrate that BOPI-1 is the only exclusive genetic region of *B. ovis* and marine *Brucella* spp. and that it is highly conserved in *B. ovis* field isolates from Argentina.

Brucellosis in sheep can be caused by *Brucella ovis* and *Brucella melitensis*. However, in Patagonia, Argentina, it is exclusively caused by *B. ovis* (Robles et al., 1998). This disease causes ram infertility (Robles et al., 1998) and is one of the most important infectious diseases of sheep (Blasco, 1990). Patagonia, the most important sheep-breeding region in Argentina, is a vast area that includes five provinces and two thirds of the country's 12 million sheep (Cardellino and Mueller, 2014). In Patagonia, 66.2% of sheep farms have at least one infected ram, and the general prevalence is 5.8% (C.A. Robles, personal communication).

Diagnosis of brucellosis by molecular methods has been studied in the last two decades (Yu and Nielsen, 2010). Tsolis et al. (2009) described *B. ovis* exclusive genes, which could be useful to design specific molecular diagnostic tools to allow the differentiation of *B. ovis* from other bacteria. Some of these genes, such as BOV_A0500, are located in the specific *B. ovis* pathogenicity island 1 (BOPI-1), whereas others, such as BOV_0198, are located outside this island. BOPI-1 has been the target of PCR designs to improve the diagnosis of sheep brucellosis caused by *B. ovis* (Xavier et al., 2010; Moustacas et al., 2013). However, information about the presence of these genes in *B. ovis* field-isolated strains is scarce (Tsolis et al., 2009; Costa et al., 2012). Thus, this study aimed to determine the presence of genes inside and outside BOPI-1 in a large collection of isolates of *B. ovis* and other *Brucella* spp. from Argentina.

The presence of the BOV_A0500 gene from BOPI-1 and the BOV_0198 gene was assessed in 118 *Brucella* spp. field strains (see Appendix: Supplementary Table S1). DNA was extracted using the Accuprep Genomic DNA Extraction Kit (Bioneer). DNA from *B. abortus*, *B. melitensis*, REV-1, S19 and RB51 and water were used as negative controls. To amplify a section of BOV_A0500, primers previously described by Tsolis et al. (2009) were used (forward: 5′-TGGTA TCTTCAGCCGTTCCAAG-3′ and reverse: 5′-ATCTTTGCCCGTTCAGTCG-3′). To amplify a section of BOV_0198, the primers forward 5′-AGA TCACCTTCAATCACGTC-3′ and reverse 5′-GGGCATAGGTGATGTTTTC-3′ were designed using Oligoperfect Designer (Life Technologies). The PCR reaction mixture consisted of 200 μM of each dNTP, 0.5 μM of each primer, 1.25 U of Taq DNA polymerase and 100 ng of DNA in a final volume of 25 μL. The amplification parameters were: 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, with a final step of extension at 72 °C for 5 min.

The 104 *B. ovis* field isolates showed positive PCR results for BOV_A0500 from BOPI-1. This is in agreement with Tsolis et al. (2009), who studied the presence of genes from BOPI-1 in 17 *B. ovis* strains. The gene BOV_0198, which does not belong to BOPI-1, was also found in the 104 *B. ovis* field isolates analysed here (See Appendix: Supplementary Table S1).

To evaluate the level of identity of the complete BOPI-1 in local isolates from Argentina, the genome of 10 *B. ovis* field isolates from the National Institute for Agricultural Technology (INTA) Bariloche (Argentina) was sequenced at Brucella II initiative, Broad Institute[1] (see Accession numbers in Appendix: Supplementary Table S1). When the complete BOPI-1 sequence from *B. ovis*

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[1] See: https://olive.broadinstitute.org/projects/brucella Accessed December 4, 2015.

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1090-0233/

Table 1
Identity of *Brucella ovis* "exclusive" genes in other *Brucella* spp.

	Genes from *B. ovis* 25840											
	0104	0198	0224	0529	0721	0770	A0269	A0295	A0500	A0536	A0925	A1070
B. ovis 25840	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
B. abortus 9-941	99%	99%	99%	99%	99%	90%	100%	99%	–	99%	98%[a]	100%
B. melitensis 16M	99%	99%	99%	99%	99%	90%	100%	100%	–	99%	97%[a]	100%
B. suis 1330	99%	99%	99%	99%	99%	90%	100%	99%	–	99%	100%[a]	100%
B. canis ATCC23365	99%	99%	99%	99%	99%	90%	100%	99%	–	99%	100%[a]	100%
B. ceti TE10759-12	100%	99%	99%	99%	99%	90%	100%	99%	–	99%	98%[a]	100%
B. suis ATCC23445	99%	99%	99%	99%	99%	90%	100%	99%	–	99%	100%[a]	99%
B. abortus 2308	99%	99%	99%	99%	99%	90%	100%	99%	–	99%	98%[a]	100%
B. abortus S19	99%	99%	99%	99%	99%	90%	100%	99%	–	99%	100%[a]	100%
B. melitensis ATCC23457	99%	99%	99%	99%	99%	90%	100%	100%	–	99%	97%[a]	100%
B. microti CCM4915	100%	99%	99%	99%	99%	90%	100%	99%	–	99%	100%[a]	100%
B. pinnipedialis B2/94	100%	99%	99%	99%	99%	90%	100%	99%	99%	99%	100%[a]	100%

[a] Low score and query coverage.

ATCC25840 reference strain was compared with the 10 completely sequenced isolates from our collection using the BioEdit Sequence Alignment Editor (Hall, 1999), 99% identity was found (data not shown). These results confirm a high level of conservation of BOPI-1 in *B. ovis*, as previously described by Tsolis et al. (2009) using strains from the USA.

We also evaluated the presence of the BOV_A0500 and BOV_0198 genes in other *Brucella* spp. As expected, we could not amplify BOV_A0500 in *B. melitensis*, *B. abortus*, REV-1, RB51 and S19 (Fig. 1a). In agreement with this, Hinic et al. (2008) demonstrated that BOV_A0504 was present in *B. ovis* ATCC25840 but absent in 64 strains of other *Brucella* spp. Additionally, Xavier et al. (2010) found that 12 genes from BOPI-1 are absent in other bacterial species that could cause epididymitis in rams.

Surprisingly, the BOV_0198 gene, considered exclusive to *B. ovis*, was also amplified in *B. melitensis*, *B. abortus*, REV-1, RB51 and S19 strains (Fig. 1b). To evaluate the specificity of these fragments, the PCR products from BOV_0198 were sequenced at the Biotechnology Institute of INTA Castelar (Argentina) and compared with the BOV_0198 gene from *B. ovis* ATCC25840, using the online tool Clustal Omega[2], showing an identity of 99% (Appendix: Supplementary Fig. S1). To confirm these results, the BOV_0198 gene from *B. ovis* ATCC25840 was compared with all the *Brucella* spp. available genomes using the BLAST program. The results showed that the BOV_0198 gene shares 99% identity at the nucleotide level to genomic regions from other *Brucella* spp. (Table 1). In light of this unexpected result, we then analysed the other 10 *B. ovis* exclusive genes outside BOPI-1 described by Tsolis et al. (2009). Each gene was compared with *Brucella* spp. genomes. Eight of the 10 genes were found to share 99–100% identity with other *Brucella* spp. genomic sections at the nucleotide level, whereas BOV_0770 shared 90% identity and BOV_A0925 97–100% identity, but with low scores and query coverage (Table 1). The same analysis was performed with 22 genes from BOPI-1, excluding mobile genetic elements and pseudogenes. Results showed that they do not share identity to any region of the *Brucella* spp. genomes, except for *B. pinnipedialis* and one strain of *B. ceti* (*B. ceti* str. Cudo). Therefore, genes outside BOPI-1 should not be used as *B. ovis* species-specific genetic markers.

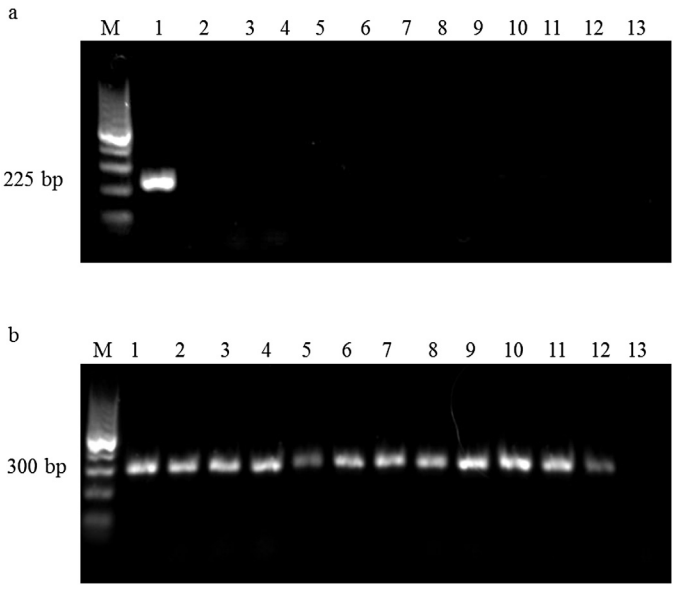

Fig. 1. Presence of the BOV_A0500 (a) and BOV_0198 (b) genes in *Brucella* spp. Agarose gel showing the PCR products of BOV_A0500 in *Brucella* spp. isolates. The numbers in brackets correspond to the strains listed in Appendix: Supplementary Table S1. Lanes. M: 100 bp molecular marker; 1: *B. ovis* (field isolate 60); 2, 3 and 4: *B. melitensis* (field isolates 126, 127, 128); 5, 6 and 7: REV-1 (commercial vaccine strains 28, 30, field isolate 121); 8 and 9: *B. abortus* (field isolates 129, 130); 10 and 11: RB51 (commercial vaccine strains 27, 29); 12: S19 (commercial vaccine strain 132); 13: non-template control.

In summary, the present study demonstrates that the BOV_A0500 gene from BOPI-1 is a good target for specific diagnosis of *B. ovis* infection by PCR, while the BOV_0198 gene is not. These results also determined that the BOPI-1 genomic island is highly conserved in the 10 sequenced *B. ovis* field isolates from Patagonia and represents the only exclusive genetic region of *B. ovis* reported until now.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2015.12.005.

[2] See: http://www.ebi.ac.uk/Tools/msa/clustalo/

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