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Regional report

The presence of *Borrelia theileri* in Argentina

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ABSTRACT

The presence of *Borrelia theileri* in Argentina is confirmed after recording the spirochete from a bovine in northern Argentina. The analysis of sequences of the flagellin gene (*fla*) and length of *Borrelia* spp. specimens on thick blood films shows that the local isolate clusters within a well-supported clade with *B. theileri* isolates from different geographical origins, confirming the presence of *B. theileri* in Argentina. The mean length of 30 specimens of *B. theileri* was 12.89 μm (standard deviation 2.88 μm, range 9.35–20.16 μm). The only known vector of *Borrelia theileri* in northern Argentina is the cattle tick *Rhipicephalus microplus*, therefore *Borrelia* infection should be regarded as a potential complication of other cattle tick-borne diseases such as babesiosis, especially on cattle introduced from areas free of *R. microplus*. The possibility of serologic cross-reaction with *B. theileri* must not be minimized in studies of other spirochaetes in the *R. microplus* infested region of Argentina.

1. Introduction

Borrelia theileri is a hard tick-borne spirochaete, transmitted by *Rhipicephalus* (*Boophilus*) spp. ticks to domestic ruminants and equids in tropical and subtropical regions of the world (Uilenberg, 1995). This spirochaete belongs to the relapsing fever group of borreliae (Barbour, 2014) being the causative agent of bovine borreliosis, a mild febrile disease of cattle ocasionally causing haemoglobinuria and anemia (Callow, 1967).

In Argentina a spirochaete compatible with *B. theileri* has been described infecting females of *Rhipicephalus microplus* ticks (Guglielmone et al., 1987) and cattle residing at the endemic area of *R. microplus* in the north of the country (Nájera, 1949; Ibáñez and Laffont, 1959; Hadani et al., 1985), but doubts arose regarding it specific identity. In this study we report the identification of *B. theileri* based on molecular and morphological evidence in Argentina, and discuss possible implications of this record.

2. Materials and methods

2.1. Animals, sample collection and microscopy procedures

Ten 12–24 months old Braford heifers were sampled in context of a *R. microplus* control trial conducted in facilities of the Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Colonia Benítez (INTA EEA Colonia Benítez), located at Colonia Benítez (27°18′S, 58°56′ W), Chaco Province, Argentina. Thick blood films were prepared as described by Mahoney and Saal (1961), dried in an oven at 100 °C for a period of 15 min, and stained with 10% Giemsa in water buffered to pH 7.2 for 30 min. Each blood film was examined under oil immersion with 100× objective and 10× oculars for 15 min. During thick blood film examination one of the heifers was detected as infected with a spirochaete and was further sampled in order to identify the spirochaete by mean of DNA sequencing. Five ml of blood was collected through jugular venipuncture, deposited in a tube containing 500 μl of sodium citrate as an anticoagulant and refrigerated immediately at 4 °C, until arrival at the laboratory.

As complementary descriptive information, length of *Borrelia* specimens was determined using Micrometrics SE Premium® (v2.8) software from images taken with a Micrometrics® camera (590CU) calibrated by mean of a stage micrometer.

2.2. DNA extraction

DNA from infected bovine blood was extracted using the standard phenol/chloroform/isoamyl alcohol method. Briefly, erythrocyte

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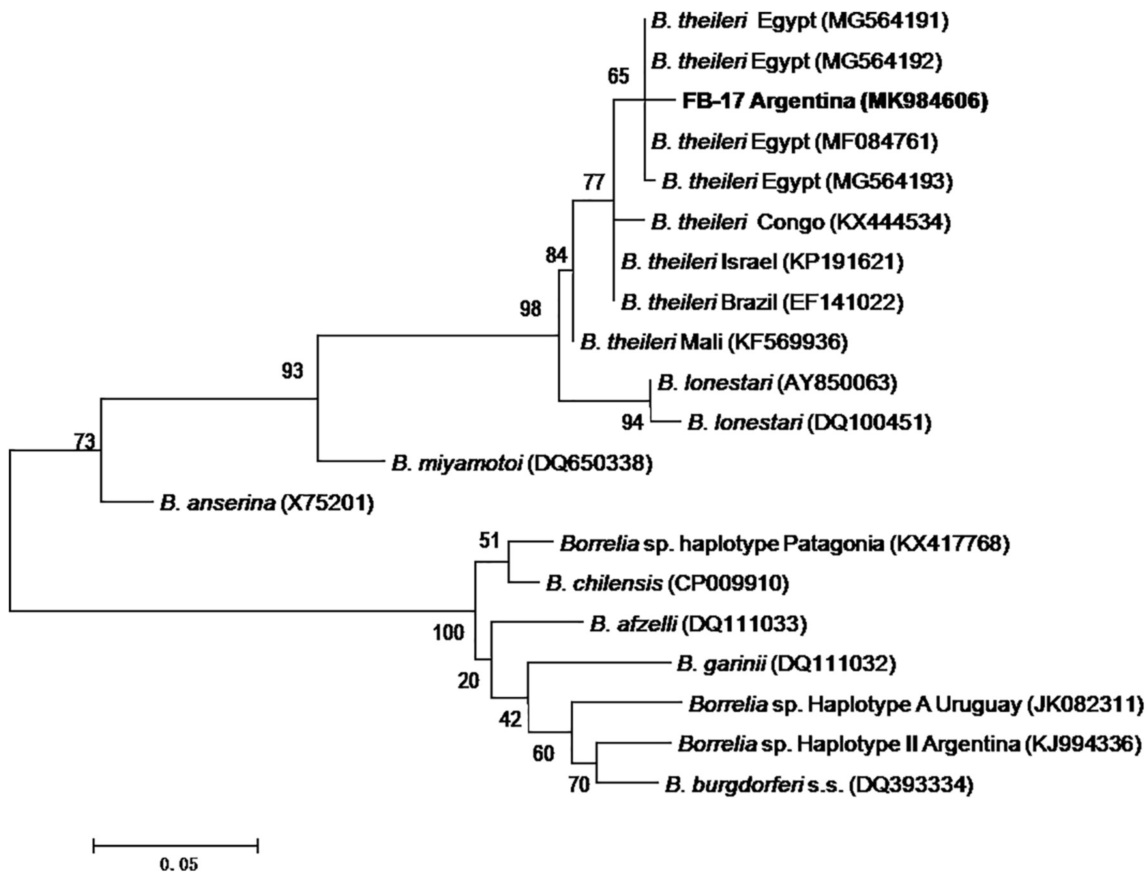

Fig. 1. Maximum-likelihood tree constructed from *Borrelia* fla partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets.

suspensions were lysed with erythrocyte lysis buffer (0.14 M NH_4Cl, 0.17 M Tris–HCl) at room temperature for 30 min and then pelleted. The hemoglobin was washed off using distilled water by centrifugation at 14,000 ×*g* for 15 min, and pellets were lysed at 58 °C for 1 h in lysis buffer (0.05 M Tris–HCl pH 8.0, 0.1 M EDTA, 0.1 M NaCl, 2% SDS) with 160 µg of proteinase K (Invitrogen®). gDNA was extracted with 1 vol of phenol/chloroform/isoamyl alcohol (Invitrogen®), precipitated with ice-cold isopropyl alcohol and washed once with 75% ice-cold ethanol. Pellets were suspended in 50 µl ultra-pure water and were kept at −20 °C until use.

2.3. PCR amplification of Borrelia DNA

Nested PCR was done targeting the flagellin gene (*fla*) of *Borrelia* spp. with the primers Fla. LL (5′-ACA TAT TCA GAT GCA GAC AGA GGT- 3′) and FLA RL (5′-GCA ATC ATA GCC ATT GCA GAT TGT-3′) for the first reaction and with the primers Fla. LS (5′-AAC AGC TGA AGA GCT TGG AAT G-3′) and FLA RS (5′-CTT TGA TCA CTT TC ATT CTA ATA GC-3′) (Barbour et al., 1996) for the nested reaction. DNA of *Borrelia burgdorferi* sensu stricto was employed as positive control. The positive sample (ca. 330 bp) was purified and afterwards sequenced using the appropriate primers.

2.4. Sequence analysis

The received sequences were edited using BioEdit SequenceAlignment Editor (Hall, 1999) with manual edition whenever it was necessary and aligned with the program Clustal W (Thompson et al., 1994). Phylogenetic analysis was performed with the maximum-likelihood (ML) method. The best-fitting substitution model was determined with the Bayesian Information Criterion using the ML model test implemented in MEGA 5 (Tamura et al., 2011). A tree based on *Borrelia* fla partial sequences was generated with the Tamura 3-parameter model by using a discrete gamma-distribution (+G). Support for the topologies was tested by bootstrapping over 1000 replications. The number of variable nucleotide positions between sequences of fla were used to calculate pairwise estimates of percent sequence divergence among sequences of *Borrelia* spp. found in different areas of the world. Gaps were excluded in the pairwise distance estimation. Genospecies from the *Borrelia burgdorferi* sensu lato complex were used as outgroup.

3. Results and discussion

The phylogenetic tree constructed with *fla* sequences is showed in Fig. 1. Phylogenetically, the isolate from Argentina (GenBank accession number MK984606) clustered within a well-supported clade with *B. theileri* isolates from different geographical origins (Egypt, Congo, Israel, Brazil, Mali), confirming the presence of *B. theileri* in Argentina. The genetic divergence of *fla* sequences within this group ranged from 0.9 to 3.0%.

The mean length of 30 specimens of *B. theileri* was 12.89 µm (standard deviation 2.88 µm, range 9.35–20.16 µm). Mean length was similar to that reported by Callow (1967) for *Borrelia* sp. from South Africa (13. 20 µm) and Australia (12.10 µm). The length range was similar to that described previously by Ibáñez and Laffont (1959) who founded a range of 8.5–22.5 for *Borrelia* sp. from Argentina. Both, range and mean length were superior to those reported for *Borrelia* sp. in thick blood films of bovines in north Argentina by Hadani et al. (1985) (8.4–11.9 and 10.30 µm, respectively). However, the magnitude of these differences should not be overestimated because they fall within the original range described by Laveran (1903). Furthermore, *Borrelia* spp. cell length would not be a specie specific regular feature, being instead related to growing conditions of the spirochaete (Barbour and Hayes, 1986).

Although having low pathogenicity itself, anemia following *B. theileri* infection cannot be ruled-out as a potential complication when occurs concomitantly with other tick-borne diseases of cattle such as babesiosis (Kiptoon et al., 1979; Sharma et al., 2000), since *B. theileri* prepatent period partially overlaps with those of *Babesia bovis* and *Babesia bigemina* (Callow and Hoyte, 1961; Hadani et al., 1985). Simultaneous patency of borreliosis-babesiosis could occur especially on cattle introduced from areas free of *R. microplus*, hence lacking of specific immunity against these tick-borne pathogens. Furthermore, the low spirochetemia characteristic of *B. theileri* infection could hamper the detection of this spirochaete in thin blood films (Matton and Van Melckebeke, 1990).

Serologic cross-reactivity between the zoonotic genospecies belonging to the *Borrelia burgdoferi* s.*L. complex* and *B. theileri* was recorded using indirect fluorescent antibody test (Rogers et al., 1999) and it was suspected in a crude antigen-based indirect ELISA test (Corrêa et al., 2012). Anti-*B. theileri* antibodies may be highly disseminated in cattle within the area where *R. microplus* is distributed in South America. Therefore, studies of *B. burgdoferi* s.l. sero-prevalence in cattle in this region should be carefully interpreted.

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Ethical statement

The authors assert that all procedures contributing to this work comply with the ethical standards of International Guiding Principles for Biomedical Research Involving Animals.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

Barbour, A.G., 2014. Phylogeny of a relapsing fever *Borrelia* species transmitted by the hard tick *Ixodes scapularis*. Inf. Gen. Evol. 27, 551–558.
Barbour, A.G., Hayes, S.F., 1986. Biology of *Borrelia* species. Microb. Rev. 50 (4), 381.
Barbour, A.G., Maupin, G.O., Teltow, G.J., Carter, C.J., Piesman, J., 1996. Identification of an uncultivable *Borrelia* species in the hard tick *Amblyomma americanum*: possible agent of a Lyme disease-like illness. J. Infect. Dis. 173 (2), 403–409.
Callow, L.L., 1967. Observations on tick-transmitted spirochaetes of cattle in Australia and South Africa. Brit. Vet. J. 123 (11), 492–497.
Callow, L.L., Hoyte, H.M.D., 1961. (1961). Transmission experiments using Babesia bigemina, Theileria mutans, Borrelia sp. and the cattle tick, Boophilus microplus. Australian -Veterinary Journal 37 (10), 381–390.
Corrêa, F.D.N., Teixeira, R.C., Oliveira, C.M.C., Barbosa, J.D., Fonseca, A.H.D., 2012. Detection of anti-*Borrelia burgdorferi* antibodies in buffaloes (*Bubalus bubalis*) in the state of Pará, Brazil. Rev. Bras. Paras. Vet. 21 (3), 338–341.
Guglielmone, A.A., Aguirre, D.H., Mangold, A.J., Gaido, A.B., 1987. *Borrelia* sp. en *Boophilus microplus*, la garrapata común del ganado vacuno, en Tucumán (Argentina). Vet. Arg. 4, 248–249.
Hadani, A., Guglielmone, A.A., Bermúdez, A.C., Mangold, A.J., de Haan, L., Vanzini, V., Luciani, C.A., 1985. Detección de espiroquetas del gónero *Borrelia* en bovinos de la provincia de Salta, Argentina. Rev. Med. Vet. (Buenos Aires) 66, 292–296.
Hall, T.A., 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.
Ibáñez, E.A., Laffont, H.E., 1959. Hallazgo de parasitos del genero borrelia en bovinos. Fac. Agric. Ganad. Ind. Afines Univer. Nac. Nord, Corrientes, Argentina.
Kiptoon, J.C., Maribel, J.M., Kamau, L.J., 1979. Bovine borreliosis in Kenya: *Borrelia theileri* associated with bovine anaemia. Kenya Vet. 3, 11–12.
Laveran, A., 1903. Sur la spirillose des bovidés. CR Acad. Sci. Paris 136, 939–941.
Mahoney, D.F., Saal, J.R., 1961. Bovine babesiosis: thick blood films for the detection of parasitaemia. Aust. Vet. J. 37 (2), 44–47.
Matton, P., Van Melckebeke, H., 1990. Bovine borreliosis: comparison of simple methods for detection of the spirochaete in the blood. Trop. An. Health Prod. 22 (3), 147–152.
Nájera, L.E., 1949. Hallazgo de "*Borrelia theileri*" (Laveran, 1903) Nájera, 1949 en bovinos de Argentina. Soc. Arg. Patol. Epidemiol. Enf. Transm. 1, 5–11.
Rogers, A.B., Smith, R.D., Kakoma, I., 1999. Serologic cross-reactivity of antibodies against *Borrelia theileri*, *Borrelia burgdorferi*, and *Borrelia coriaceae* in cattle. Am. J. Vet. Res. 60 (6), 694–697.
Sharma, S.P., Amanfu, W., Losho, T.C., 2000. Bovine borreliosis in Botswana. Onderstepoort J. Vet. Res. 67 (3), 221–223.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28 (10), 2731–2739.
Thompson, J.D., Higgins, D., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Uilenberg, G., 1995. International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health. Vet. Paras. 57 (1–3), 19–41.