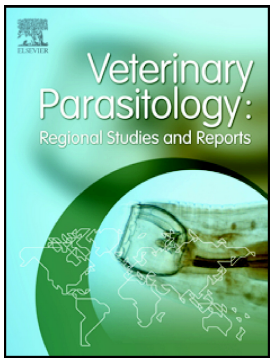

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Case reports

Exotic tick detected in Argentina on a tourist returning from South Africa

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Abstract

The aim of this study was to report the finding of a nymph attached to an Argentinean tourist returning from South Africa. The nymph specimen was morphologically analysed, submitted to DNA extraction and amplifying the 16S rRNA mitochondrial gene. Additionally, the nymph DNA was screened for *Rickettsia, Ehrlichia* and *Anaplasma* infection. The nymph was determined to belong to *Amblyomma marmoreum* species complex. No specific diagnosis was achieved because the comparative descriptions of species in this complex contain important discordances,

and the DNA sequence obtained in the present study is positioned within the same clade with sequences of *A. marmoreum* see above, but the genetic divergence with them (4.96 and 5.76%) indicate that they belong to different species. No DNA of the Rickettsiales order bacterial was detected in the *A. marmoreum* species complex nymph.

Keywords

Amblyomma marmoreum species complex, South Africa, Tourist host, Argentina.

1- Introduction

The main tourist destination in Southern Africa is South Africa, with more than eight million international tourist arrivals per year (Fernandez Ruiz, 2015). The Wildlife tourism increases the probability of contact between animals and humans, environmental change, and human contact with disease vectors (Hall, 2019). Hard ticks are one of the largest important bacterial, viral and protozoan diseases arthropod vectors (Sonenshine et al., 2002), and human parasitism by these ticks is a common event worldwide.

Approximately 88 species of hard ticks are known in Southern Africa as defined in Horak et al. (2018), with 11 belonging to the genus *Amblyomma* (Horak et al., 2018). Although the main cause of export of African *Amblyomma* tick species to American continent is due to the reptile trade (Gonzalez-Acuña et al., 2005; Burridge, 2011), there are also records of parasitism in tourists who had travelled to South Africa (Burridge et al., 2002). This study reports a case of tick parasitism on an Argentinean tourist returning from South Africa, with additional analysis on tick-borne pathogens.

2- Case Presentation

On 14 April 2018, a family resident of Buenos Aires, Argentina made a holiday trip to South Africa and returned to Argentina on April 26, 2018. The trip itinerary was: 1) of the 14 to 19 April, 2018 Cape Town (33°55´S 18°25´ E, 92 m.a.s.l.); 2) of the 19 to 24 April, 2018 Kruger National Park (23°59´S 31°33´E, 305 m.a.s.l.); 3) of the 24 to 25 April Johannesburg (26°12´S 28°02´E, 1758 m.a.s.l.); 4) 25 April 2018 Sao Pablo, Brazil and 26 April 2018 Buenos Aires. This last day, a six-year-old daughter observed a new ¨freckle¨ on her leg, for which she made a medical consultation. On clinical inspection, an engorged tick was observed surrounded by a small erythema area on the affected skin (Figure 1a). The tick was removed manually, exposing the bite area on the skin, and prescribed antibiotic therapy for five days. The tick was deposited in ethanol 96º and sent to the Laboratorio de Inmunología y Parasitología, Estación Experimental Agropecuaria Rafaela, Instituto Nacional de Tecnología Agropecuaria (INTA E.E.A. Rafaela) for its taxonomic determination. Five days after the tick removed, the skin healed without systemic symptoms. The tick specimen was morphologically analysed in comparison with descriptions of South African ticks (Theiler and Salisbury, 1959). Them, it was submitted to DNA extraction using the DNeasy Tissue Kit (Qiagen, Inc., Chatsworth, CA, U.S.A.) and DNA was amplifying an 400-bp fragment of the tick 16S rRNA mitochondrial gene (Mangold et al., 1998). The sequence obtained was edited and compared with those sequences deposited in GenBank. Phylogenetic analyses of the 16S rRNA sequences were performed using the maximum likelihood (ML) method and for construct the tree, the best fitting substitution model was ML model test implemented in MEGA X (Kumar et al., 2018). Sequences of *Hyalomma dromedarii* Koch, 1844*, Hyalomma glabrum* Delpy, 1949*, Hyalomma rufipes* Koch, 1844 and

Hyalomma truncatum Koch, 1844 (GenBank accession numbers. L34306, KU130432, KU130465 and KU130478) were chosen as outgroups.

Additionally, DNA obtained from the tick was screened for *Rickettsia, Ehrlichia* and *Anaplasma* infection through testing by a multi gen real-time PCR assay amplify a fragment of the 16S rRNA gene (family Anaplasmataceae) and the *gltA* gene (genus *Rickettsia)* following to Monje et al. (2019) and Guedes et al. (2005).

The tick was found to be a nymph of the genus *Amblyomma* with morphological characters similar to the nymphs of the *Amblyomma marmoreum* species complex depicted in Theiler & Salisbury (1959). The *A. marmoreum* species complex includes *Amblyomma falsomarmoreum* Tonelli Rondelli, 1935, *Amblyomma marmoreum* Koch, 1844*, Amblyomma nuttalli* Dönitz, 1909 and *Amblyomma sparsum* Neumann, 1899 (Theiler and Salisbury, 1959). Analysis of the morphology of the nymph (figure 1 b-f) revealed the following characters: scutum wider than long (1.13 mm width, 0.83 mm long); posterolateral margin of scutum somewhat sinuous; cervical grooves, deep, converging anteriorly and then diverging posteriorly; scutum with large and deep punctations evenly distributed, larger and deeper in lateral fields around the eyes; basis capituli dorsally sub-triangular in shape, cornua absent, with posterior margin straight and lateral margins slightly convex. Hypostome spatulate, dental formula 2/2. All coxae about the same size; coxa I with two pointed spurs, the external slightly longer than the internal; coxae II, III and IV with a small triangular spur each. Spiracular plate oval with dorsal prolongation long and narrow.

While the characters showed above are consistent with nymphs from the *A. marmoreum* species complex, the morphological similarity of the nymphs belonging to

this complex precludes a certain specific determination. Furthermore, the comparative descriptions of species in this complex by Theiler & Salisbury (1959) contain discordances between text descriptions and the morphological characters showing in the corresponding figures, being difficult a specific diagnosis beyond *A. marmoreum* group with the information provided by these authors. Alternative morphological diagnosis of nymphs from the *A. marmoreum* complex in Arthur (1975) and Voltzit & Keirans (2003) are not useful to resolve the comparative morphological definition of nymphs from this complex.

In line with the morphological diagnosis, the 16S rRNA sequence obtained (402 bp) from the nymph of *A. marmoreum* species complex found attached to the Argentinean tourist presented a 95.04% of similarity with a sequence *A. marmoreum* from South Africa (Irene, Gauteng Province) (Genbank accession number: KY457515), and 94.29% of similarity with a sequence *A. marmoreum* also from South Africa (Grahamstown, Eastern Cape Province) (Genbank accession number: KY457516) according to BLAST analysis (Basic Local Alignment Tool). The ML phylogenetic tree (Figure 2) shows that these three sequences are phylogenetically related. Since there are currently no sequences of other members from the *A. marmoreum* group such as *A. nuttalli* and *A. sparsum*, the sequence obtained in this study could not be compared with these two-morphologically related species. Therefore, although the DNA sequence obtained is positioned within the same clade with sequences of *A. marmoreum* obtained by Mans et al. (2019), the genetic divergence with them (4.96 and 5.76%) indicate that they belong to different species.

The specimen was deposited as a nymph of *A. marmoreum* species complex in the Tick Collection of INTA E.E.A. Rafaela (accession number INTA-2479). The partial sequence

of the mitochondrial 16S rRNA gene generated in this study for *A. marmoreum* species complex has been deposited in GenBank under the accession number (will be acquired in case of acceptation).

Ehrlichia, Anaplasma and *Rickettsia* bacteria DNA was not amplified in the sample obtained from the engorged *A. marmoreum* species complex nymph.

3- Discussion and Conclusion

The differential diagnosis among the members of the *A. marmoreum* species complex entails a noticeable degree of uncertainty (Guglielmone et al., 2020). All these species, with the exception of *A. falsomarmoreum*, are present in Southern Africa (Tonelli-Rondelli, 1935; Horak et al., 2018;). Nevertheless, a specific diagnosis was not obtained, neither by morphology nor molecular techniques, and we were unable to classify the specimen beyond *A. marmoreum* species complex.

In our study, a nymph of the African *A. marmoreum* species complex parasitizing a tourist in Argentina with a previous trip to South Africa is reported and no obvious sanitary consequences resulted from the tick bite. In Argentina, cases of African tick bite fever have been reported in humans with a history of tick bites and travel to South Africa (Armitano et al., 2018). This put in evidence the potential risk that tourists have when they visit areas where ticks with potential zoonotic role live.

Acknowledgements

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patas (Acari: ixodidae), de importancia sanitaria en Argentina". We thank Lucila Lasry for collected tick and Fernando Sebastian Flores for assistance with imagens.

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Legends to Figures

Figure 1. A nymph of *A. marmoreum* species complex collected parasitizing an Argentinian tourist with prior trip to South Africa: (a) The tick nymph biting the tourist´s leg, four days after discovery. (b) Scutum, dorsal view, (c) Capitulum, ventral view, (d) Coxa I, (e) Spiracular plates, (f) Tarsi IV.

Figure 2. Maximum-likelihood tree based on partial 16S rRNA gene sequences of tick species of the *Amblyomma* genus that have been reported parasitizing humans in Argentina and Southern Africa [Substitution model: Generalised time-reversible model with Gamma distribution (GTR+G)]. Numbers near nodes represent bootstrap support (1000 replicates). GenBank accession numbers are indicated in brackets. Sequence obtained in this study indicated in bold letters.

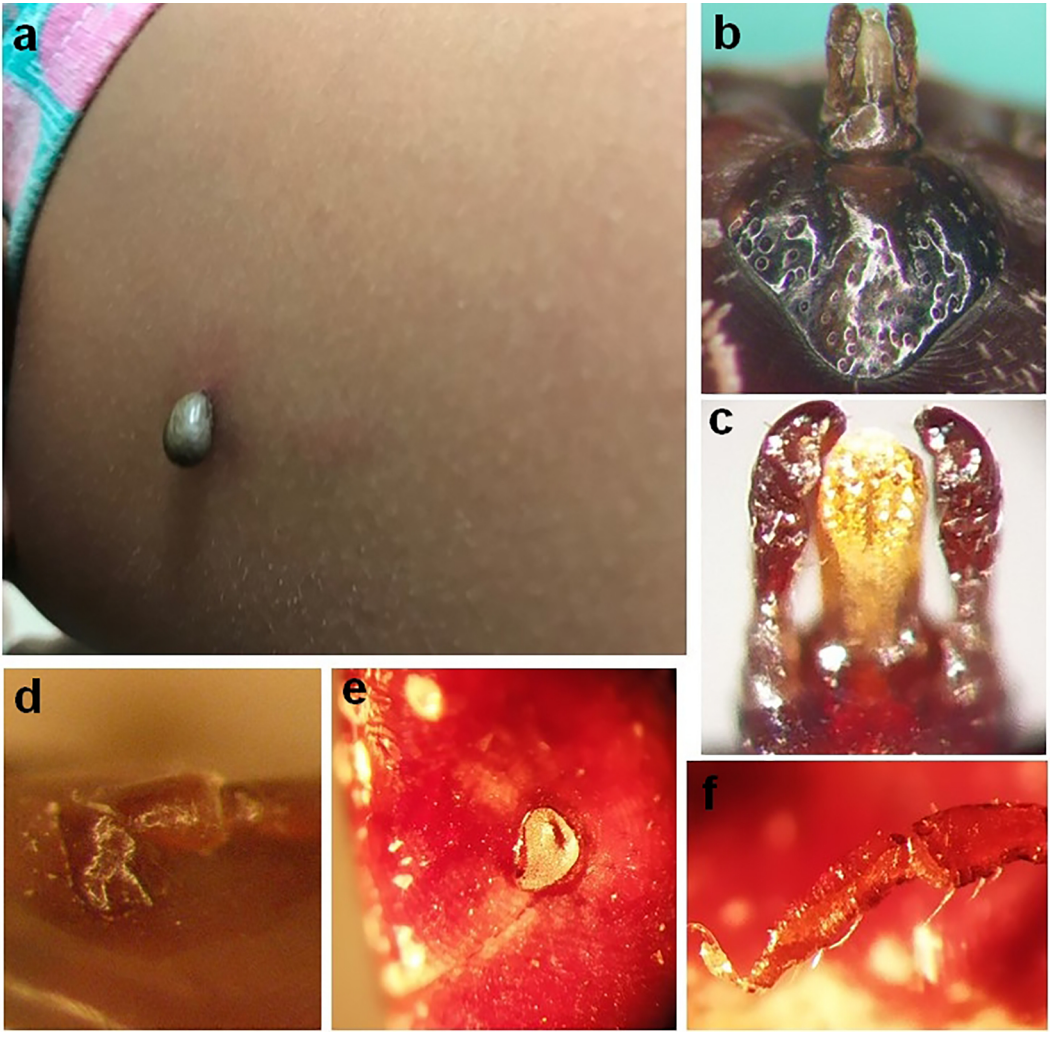
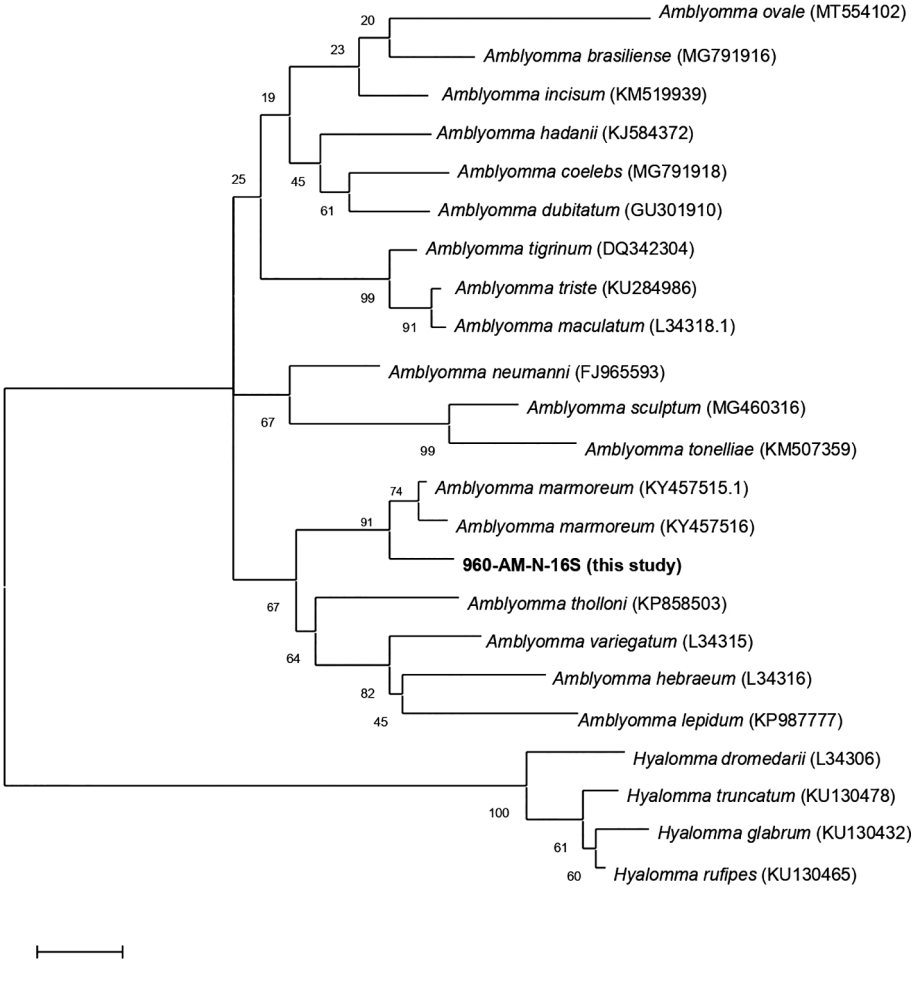


Figure 1


Amblyomma ovale (MT554102)
Amblyomma brasiliense (MG791916)
Amblyomma incisum (KM519939)
Amblyomma hadanii (KJ584372)
Amblyomma coelebs (MG791918)
Amblyomma dubitatum (GU301910)
Amblyomma tigrinum (DQ342304)
Amblyomma triste (KU284986)
Amblyomma maculatum (L34318.1)
Amblyomma neumanni (FJ965593)
Amblyomma sculptum (MG460316)
Amblyomma tonelliae (KM507359)
Amblyomma marmoreum (KY457515.1)
Amblyomma marmoreum (KY457516)
960-AM-N-16S (this study)
Amblyomma tholloni (KP858503)
Amblyomma variegatum (L34315)
Amblyomma hebraeum (L34316)
Amblyomma lepidum (KP987777)
Hyalomma dromedarii (L34306)
Hyalomma truncatum (KU130478)
Hyalomma glabrum (KU130432)
Hyalomma rufipes (KU130465)
20
23
19
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99
74
91
67
64
82
45
100
61
60
0.050

Figure 2