Original papers

New association between *Amblyomma parvitarsum* (Acari: Ixodidae) and the endemic lizard *Liolaemus eleodori* (Iguania: Liolaemidae) in Argentina

Gabriel N. Castillo^{1,2,3}, Juan C. Acosta^{1,2}, Melina Rodríguez-Muńoz^{1,2,3}, Tomás Martínez^{1,2,3}, Cynthia González-Rivas⁴, Santiago Nava⁵

¹Departamento de Biología, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de San Juan. Av. Ignacio de la Roza 590, 5402, San Juan, Argentina

²Gabinete de Investigación Diversidad y Biología de Vertebrados del Árido (DIBIOVA). Universidad Nacional de San Juan. Av. Ignacio de la Roza 590, 5402, San Juan, Argentina

³Becario de CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas). Av. Ignacio de la Roza 590, 5402, San Juan, Argentina

⁴Centro de Investigaciones de la Geósfera y la Biosfera (UNSJ-CONICET), Interacciones Biológicas del Desierto (INTERBIODES), San Juan, Argentina

⁵Laboratorio de Parasitología e Inmunología Instituto Nacional de Tecnología Agropecuaria Estación Experimental Agropecuaria, Rafaela, Santa Fe, Argentina

Corresponding Author: Gabriel N. Castillo; e-mail: nataliocastillo@gmail.com

ABSTRACT: Records of lizard parasitizing ticks in Argentina are scarce and incomplete. We recorded a new population of *Liolaemus eleodori*, a lizard endemic to Argentina, parasitized by the hard tick *Amblyomma parvitarsum*. Hence, the objective of this study is to report this new association and enhance knowledge about its parasitism on reptiles of Argentina. To do this, we researched ectoparasitic loads. We determined different ecological predictors: intensity, mean intensity and prevalence. Our results provide the first data on the parasitism of nymphal stages on this lizard. We found a tick prevalence of 91.6% and a mean intensity of 2.45. Considering sexes, mean intensity was higher in females than in males. The gular region and lower ventral abdomen were the highest parasitized anatomical regions. This new record of the host-parasite association suggests that this relationship is not accidental, with this lizard species being central to the tick's life cycle. Probably, the microhabitat of this lizard species is essential for the tick to reach high prevalence. Our study represents an important contribution and provides additional information on parasitism in lizards of Argentina.

Key words: Cordillera, ectoparasites, lizards, San Juan, ticks

Introduction

There are about 140 tick species belonging to the genus *Amblyomma* all over the world. They are characterized by having a three-host cycle. Moreover, ticks of this genus are the most numerous in the Southern Cone of America [1].

Amblyomma parvitarsum is a tick species whose larval and nymphal stages are found in lizards of the genus Liolaemus [1–5]. Although adult stages were recorded on different host species, they are usual parasites of South American camelids (Lama glama,

L. guanicoe, Vicugna vicugna) [1,2,6].

The biogeographic distribution of *A. parvitar-sum* encompasses the regions of Atacama, Monte, Prepuna, Puna (South American Transition Zone); Central Patagonia, Coquimbo (Andean Region) [1]. Its distribution in South America is restricted to Argentina, Bolivia, Chile and Peru [1]. This tick species was found to be infected (in both nymphal and adult stages) with a high prevalence with *Rickettsia parkeri* strain parvitarsum [3,7,8].

Liolaemus eleodori is a species endemic to San Guillermo National Park and Biosphere Reserve, San

28 G.N. Castillo et al.

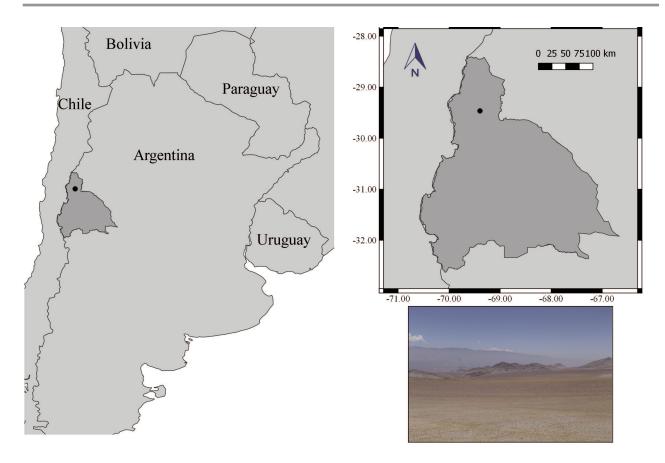


Fig. 1. Study area, San Guillermo Provincial Reserve, northernmost part of Iglesia district, Llano del Fierro area, San Juan Province, Argentina

Juan province, Argentina [9]. It occurs approximately above 3500 m elevation; phytogeographically, it corresponds to Puna and inhabits lowland areas. It is currently categorized as a non-threatened species [10]. This is a viviparous species that gives birth to four offspring in the month of December [11], and feeds on an omnivorous diet composed of arthropods and plant material [12].

Studies of host-parasite associations are fundamental to gain knowledge about the activity of parasites and their level of host specificity, as well as to help clarify their life cycle [13].

Because of the poor knowledge about parasitism in this lizard species endemic to the Argentine Puna, the objective is to determine the parasitic ecological predictors of prevalence and mean intensity of *A. parvitarsum*, and provide the first record of nymphal stages for this lizard species.

Materials and Methods

Samplings were conducted in February 2018, in San Guillermo Provincial Reserve, located on the northernmost part of Iglesias district, an area called Llano del Fierro (-29.44586; -69.41617), San Juan

province, Argentina (Fig. 1 and 2). This area is embedded in the Frontal Cordillera, composed of Ordovician material and outcrops of Devonian marine deposits [14].

The area is of great biological interest, with Monte elements dominating at levels below 2500 m elevation, and with Puna and High Andean elements being predominant between 3000 m and the vegetation border at 4200–4500 m [14]. Samplings were taken in the region corresponding to the Puna province, where the dominant vegetation is composed of low and mid-height shrubland of *Ephedra breana* and *Lycium tenuispinosum*, cactus plants like *Maihuenopsis glomerata* and *Lobivia formosa*, and the grasses *Stipa ichu* and *Aristida mendozana* isolated in the lower layers [15].

Collections were made by random survey of shrubs and areas bare of vegetation [16]. Captures were made with noose poles. A total of 12 adult individuals, 6 males and 6 females, were captured and checked for infestation.

In the field, specimens were examined on their dorsal and ventral surfaces. Ectoparasites were manually removed from the host and stored in 70% alcohol. We recorded presence of larval and



Fig. 2. Sampling area. Llano del Fierro, San Guillermo Biosphere Reserve, San Juan, Argentina.

Table 1. Anatomical regions of *Liolaemus eleodori* most frequently parasitized by larval stages of *A. parvitarsum* in San Guillermo Biosphere Reserve, San Juan, Argentina

Intensity	Anatomical region
6	Ventral gular region
8	Lower ventral abdomen
1	Left claw
2	Axillary region of anterior right forelimb
1	Left femur
1	Commissure
6	Eardrum
2	Medial dorsal region

nymphal stages of the tick *A. parvitarsum*, which were identified following the descriptions of Nava et al. [1] and Estrada-Peña et al. [17] and by comparison with reference material housed in the tick collection of the Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Santa Fe province (Argentina).

The following parasitic indicators were determined, following Bush et al. [18]; Prevalence: Number of infested hosts divided by number of hosts examined (expressed in percentages),

Intensity: Number of parasites affecting the host, Mean intensity: Total number of parasites of a particular species found in a sample divided by the number of infested hosts.

All specimens are deposited in the parasitological collection of the Biology Department, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de San Juan (UNSJPar 248-249).

Results

We recorded a total of 27 A. parvitarsum specimens parasitizing 12 individuals of L. eleodori (Fig. 3). We removed a total of 4 specimens in nymphal stage and 23 specimens in larval stage. Overall, the tick A. parvitarsum showed an infestation prevalence of 91.6%. Mean intensity was 2.45 ticks per lizard. Considering sexes, prevalence was 100% in females and 83% in males. Mean intensity in females was 3.33, and in males it was 1.4 ticks per lizard. The most frequently parasitized region of the body were the gular region and lower ventral abdomen (Table 1). Four nymphs were obtained, two located in a male's eardrum region and two in a female's gular region.

30 G.N. Castillo et al.



Fig. 3. Adult male specimen of *Liolaemus eleodori* from San Guillermo, San Juan, Argentina

Discussion

Knowledge of parasitic ticks occurring on reptiles of Argentina is incomplete [1,4,19]. Our results correspond to the second record of an association between *L. eleodori*, a lizard of the family Liolaemidae, whose population is confined to a portion of the Frontal Cordillera, and *A. parvitarsum*, a tick of the family Ixodidae. This new association represents a new population with high prevalence of parasitism.

In the family Liolaemidae in Argentina, four lizard species of the genus *Liolaemus* have so far been recorded to be parasitized by larval stages of *A. parvitarsum*, *Liolaemus puritamensis*, *Liolaemus eleodori*, *Liolaemus ruibali* and *Liolaemus yalguaraz* [3–5]. The remaining records were obtained in Chile, *Liolaemus jamesi*, *Liolaemus alticolor*, *Liolaemus andinus*, *Liolaemus copiapoensis*, *Liolaemus nigriceps*, *Liolaemus patriciaiturrae* and *Liolaemus pleopholis* [1,2].

The low number of lizard species infested by this tick reported for Argentina is likely due to the poor sampling effort associated with searching for ectoparasites.

To date, most of the published reports have failed to include data on prevalence or intensity. This proves a difficulty at the time of contributing information about the current status of lizard populations in Argentina. Although this measure variable has currently been disregarded, it would be a fundamental contribution, for it has been mentioned that parasitism could negatively alter these populations [20].



Fig. 4. Photograph of an infested *Liolaemus eleodori*, showing the presence of the ectoparasite *Amblyomma parvitarsum* on its ventral areas (see arrows for examples)

Our work reports the first data on parasitic ecological indices for the tick *A. parvitarsum* on a lizard of the genus *Liolaemus*. Results yielding extremely high prevalence, with 91.6% of infestation, have not been reported for any other population of lizards of the genus *Liolaemus* in Argentina.

Our current record, in the present study, corresponds to a new, highly parasitized, lizard population that pertains to San Guillermo Provincial Reserve. Our recent data differ in prevalence (91.6%) and in mean intensity (2.45) from the previous record for San Guillermo National Park, where prevalence was 33% and mean intensity was one (unpublished data).

The gular region and lower ventral abdomen are the most frequently tick-affected anatomical regions in *L. eleodori*. These regions, and the other mentioned anatomical regions where ticks were observed, correspond to ventral and lateral surfaces. These are in intimate contact with the substrate, favoring higher parasitism.

These anatomical regions coincide with those reported for other species of the genus *Liolaemus*. Eardrum openings were the site of infestation for *L. ruibali*. In *L. yalguaraz*, ticks were located at the eardrum opening and in the gular fold area [5].

In the present work, we contribute to parasitic knowledge by providing the first record of nymphal stages of this tick on the lizard species *Liolaemus eleodori*. Nymphal stages have been reported for

two species of lizards, Liolaemus puritamensis (Argentina's Andean region) and L. pleopholis (Chile) [3]. Our record of four nymphs of A. parvitarsum on L. eleodori is the second for a lizard of the genus Liolaemus for Argentina.

The Andean lizard Liolaemus eleodori is frequently found sun basking on bare ground o at the entrance of burrows of the rodent tuco-tuco (Ctenomys sp.) [11,21]. It can be observed that these burrows of Ctenomys sp. are located in the vicinity of the wallowing places of a population of guanacos (Lama guanicoe) and vicunas (Vicugna vicugna). This would probably increase parasitism, because adult stages of A. parvitarsum are found in camelid species [1]. The parasites present in the same place (microhabitat or shelters) as their hosts can be transmitted because of the very frequent use made of the microhabitat [22].

It has been mentioned that the reproductive status of lizards would play a major role in ectoparasitism [23,24]. During the mating season, lizards are more susceptible to having an increased number of ticks [23,24]. According to the "Immunocompetence handicap" hypothesis proposed by Folstad and Karter [25], testosterone stimulates expression of sexual features, therefore diminishing the immune system. This hypothesis holds that an increase in tick parasitism would be associated with a compromised immune system. Thus, this lizard would be investing energy resources in its reproductive activities rather than in its immune system.

At present we lack information about the reproductive cycle of L. eleodori, which does not allow us to make a correlation with the high prevalence of the tick A. parvitarsum. However, data contributed by Cabrera and Monguillot [11] indicates that December would be the egg-laying time for this species. Notwithstanding, this would not be enough to attribute L. eleodori's ectoparasitism to a compromised immune system.

The endemic Andean lizard L. eleodori plays an important role as part of this tick's life cycle by hosting its larval and nymphal stages. Despite there being information on the different stages of this tick for different times of the year, the duration of its life cycle is unknown [1].

As final conclusions; 1) We contribute the first data on prevalence and intensity of a tick in a lizard of the genus Liolaemus for Argentina. 2) We provide the second record of a nymphal stage of A. parvitarsum on a lizard of the genus Liolaemus for Argentina. 3) Microhabitat use appears to be fundamental for the high tick prevalence recorded. 4) Due to such high prevalence, we estimate that this parasitism is not accidental. 5) We point out the main anatomical regions which are most frequently parasitized by larval stages of A. parvitarsum.

Future studies are necessary to understand the process of interaction between parasites and host, as well as the impact this has on populations of this endemic lizard in said protected area.

Acknowledgements

We thank the Sub-secretary of the Environment of San Juan province for the permits provided, the technical team and rangers of San Guillermo Biosphere Reserve of San Juan. This work is part of the agreement between the Biology Department (San Juan University) and the Sub-secretary of the Environment of the Government of San Juan.

Animals welfare: All specimens were reviewed and returned to their natural environment. All applicable national, and/or institutional guidelines for the care and use of animals were followed.

Conflicts of interest: The authors declare that they have no competing interests.

References

- [1] Nava S., Venzal J.M., González-Acuńa D., Martins T.F., Guglielmone A.A. 2017. Ticks of the Southern Cone of America: diagnosis, distribution, and hosts with taxonomy, ecology and sanitary importance. Academic Press, London, United Kingdom.
- [2] Muńoz-Leal S., González-Acuńa D., Beltrán-Saavedra L.F., Limachi J.M., Guglielmone A.A. 2014. Amblyomma parvitarsum (Acari: Ixodidae): localities, hosts and host-parasite ecology. Experimental and Applied Acarology 62: 91-104. doi:10.1007/s10493-013-9725-3_
- [3] Muńoz-Leal S., Tarragona E.L., Martins T.F., Martín C.M., Burgos-Gallardo F., Nava S., Labruna M.B., González-Acuńa D. 2016. Liolaemus lizards (Squamata: Liolaemidae) as hosts for the nymph of Amblyomma parvitarsum (Acari: Ixodidae), with notes on Rickettsia infection. Experimental and Applied Acarology 70: 253-259. https://doi.org/10.1007/s1049
- [4] Castillo G.N., González-Rivas C.J., Villavicencio H.J., Acosta J.C., Nava S. 2015. Primer registro de infestación en un reptil por larvas de Amblyomma parvitarsum (Acari: Ixodidae) en Argentina. Cuadernos de Herpetología 29: 91-93 (in Spanish). http://www.scielo.org.ar/pdf/cherp/v29n1/v29n1a10.pdf
- [5] Castillo G., Nava S., Pizarro J., Acosta J.C.,

32 G.N. Castillo et al.

González-Rivas C.J. 2017. *Amblyomma parvitarsum* (Acari: Ixodidae) parasitizing two species of lizards of the genus *Liolaemus* (Iguania: Liolaemidae) in Mendoza, Argentina. *Revista Argentina de Parasitología* 6: 21-23.

- http://www.revargparasitologia.com.ar/pdf/RevArg Parasitol_Vol6_Castillo.pdf
- [6] Guglielmone A.A., Vińabal A.E. 1994. Claves morfológicas dicotómicas e información ecológica para la identificación de garrapatas del genero Amblyomma Koch, 1844 de la Argentina. Revista de Investigaciones Agropecuarias 25: 39-67 (in Spanish).
- [7] Ogrzewalska M., Nieri-Bastos F.A., Marcili A., Nava S., Gonzalez-Acuńa D., Muńoz-Leal S., Ruiz-Arrondo I., Venzal J.M., Mangold A. Labruna M.B. 2016. A novel spotted fever group *Rickettsia* infecting *Amblyomma parvitarsum* in highlands of Argentina and Chile. *Ticks and Tick-borne Diseases* 7: 439-442. https://doi.org/10.1016/j.ttbdis.2016.01.003
- [8] Nieri-Bastos F.A, Marcili A., De Sousa R., Paddock C., Labruna M.B. 2018. Phylogenetic evidence for the existence of multiple strains of *Rickettsia parkeri* in the New World. *Applied and Environmental Microbiology* 84(8). e02872-17. https://doi.org/10.1128/AEM.02872-17
- [9] Acosta J.C., Villavicencio H.J., Marinero J.A. 2007. Anfibios y Reptiles. Biodiversidad, Bio-Ecologia y Especies de valor especial para monitoreo. In: Diversidad biológica y cultural de los Altos Andes Centrales de la Argentina-Linea de Base de la Reserva de Biosfera San Guillermo-San Juan. (Ed. E. Martinez-Carretero). Editorial Fundación Universidad Nacional de San Juan, San Juan, Argentina: 167-179 (in Spanish).
- [10] Abdala C.S., Acosta J.L., Acosta J.C., Blanca B.A., Arias F., Avila, L.J., Blanco M.G., Bonino M., Boretto J. M., Brancatelli G., Breitman M.F., Cabrera M.R., Cairo S., Corbalan V., Hernando A., Ibarguengoytia N.R., Kacoliris F., Laspiur A., Montero R., Morando M., Pelegrin N., Perez C.H.F., Quinteros A.S., Semhan R.V., Tedesco M.E., Vega L., Zalba S.M. 2012. Categorización del estado de conservación de las lagartijas y anfisbenas de la República Argentina. *Cuadernos de Herpetología* 26: 215-248 (in Spanish).http://www.scielo.org.ar/pdf/cherp/v26s1/v26s1a03.pdf_
- [11] Cabrera R.A., Monguillot J. 2007. *Liolaemus eleodori* (San Guillermo's lizard): Reproduction. *Herpetological Bulletin* 101: 34-35.
- [12] Astudillo G.V., Acosta J.C., Villavicencio H.J., Córdoba M.A. 2015. Ecología trófica y dimorfismo sexual del lagarto endémico *Liolaemus eleodori* (Iguania: Liolaemidae) del Parque Nacional San Guillermo, San Juan, Argentina. *Cuadernos de Herpetología* 29: 27-39. http://www.scielo.org.ar /pdf/cherp/v29n1/v29n1a02.pdf
- [13] Sá-Hungaro I.J., Luz H.R., Silva H.R., Famadas

- K.M. 2018. A new association between *Ornithodoros saraivai* (Acari, Argasidae) and a species of rock frog in the genus *Thoropa* (Amphibia, Cycloramphidae) in Brazil. *International Journal of Acarology* 1-5. https://doi.org/10.1080/01647954.2018.1466912
- [14] Martínez-Carretero E. 2007. Marco contextual. In: Diversidad biológica y cultural de los Altos Andes Centrales de la Argentina: Línea de base de la Reserva de la Biosfera San Guillermo, San Juan. (Ed. E. Martínez-Carretero). Editorial Universidad Nacional de San Juan, San Juan, Argentina: 115-152 (in Spanish).
- [15] Cabrera A.L., Willink A. 1973. Biogeografía de América latina. Serie de Biología, Monografía 13. The General Secretariat of the Organization of American States, Washington (in Spanish).
- [16] Tellería J.L. 1986. Manual para el censo de los vertebrados terrestres. Editorial Raíces, España (in Spanish).
- [17] Estrada-Peña A., Venzal J.M., Mangold A.J, Cafrune M.M., Guglielmone A.A. 2005. The Amblyomma maculatum Koch, 1844 (Acari: Ixodidae: Amblyomminae) tick group: diagnostic characters, description of the larva of A. parvitarsum Neumann, 1901, 16S rDNA sequences, distribution and hosts. Systematic Parasitology 60: 99-112. https://doi.org/10.1007/s11230-004-1382-9
- [18] Bush A.O., Lafferty K.D., Lotz J.M., Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83: 575-583. doi:10.2307/3284227
- [19] Debárbora V.N., Acosta J.L., Mangold A.J. 2015. Primer asociación parásito hospedador de Amblyomma argentinae Neumann, 1904 (Acari: Ixodidae) con tres especies de saurios del Chaco Semiárido de Argentina. Revista Argentina de Parasitología 3: 6-7 (in Spanish).
- [20] Esch G.W., Gibbons J.W., Bourque J.E. 1975. An analysis of the relationship between stress and parasitism. *The American Midland Naturalist* 93: 339-353.
 - https://www.jstor.org/stable/2424167
- [21] Acosta J.C., Blanco G.M., Gómez- Alés R., Acosta R., Piaggio Kokot L., Victorica A.E., Villavicencio H.J., Fava G.A. 2017. Los Reptiles de San Juan. Editorial Brujas, Córdoba (in Spanish).
- [22] Leu S.T., Kappeler P.M., Bull C.M. 2010. Refuge sharing network predicts ectoparasite load in a lizard. *Behavioral Ecology and Sociobiology* 64: 1495-1503. https://doi.org/10.1007/s00265-010-0964-6
- [23] Salvador A., Veiga J.P., Martin J., Lopez P., Abelenda M., Puerta M. 1996. The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behavioral Ecology* 7: 145-150. doi:10.1093/beheco/7.2.145_
- [24] Olsson M., Wapstra E., Madsen T., Silverin B. 2000. Testosterone, ticks and travels: a test of the

immunocompetence-handicap hypothesis in freeranging male sand lizards. *Proceedings of the Royal Society of London, Series B: Biological Sciences* 267: 2339-2343. doi:10.1098/rspb.2000.1289

[25] Folstad I., Karter A.J. 1992. Parasites, bright males, and the immunocompetence handicap. *The American*

Naturalist 139: 603-622. https://www.jstor.org/stable/2462500

Received 04 October 2018 Accepted 11 January 2019