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Comparing feeding and reproductive parameters of *Amblyomma parvum* tick populations (Acari: Ixodidae) from Brazil and Argentina on various host species

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ABSTRACT

Amblyomma parvum is a Neotropical tick that is widely spread and a potential vector of pathogens, including Rickettsiae. Genetic differences are remarkable between *A. parvum* populations from Brazil and Argentina. In this work, feeding and reproduction parameters of *A. parvum* ticks from these two populations were compared on some key host species to evaluate possible differences in host suitability between them. On the whole parameters of these tick populations were similar when fed on the same host and varied similarly on different host species. Still, bovines were more suitable host for Argentinian larvae than for Brazilian cohorts. It was observed that guinea pigs were the best host *A. parvum* immatures from both origins, as depicted from higher recovery rate of larvae and heavier engorged nymph weights. Canids and bovids were host species most suitable to adults of both tick populations as shown by the highest number of larvae produced by adult females that engorged on these hosts. Taken together, results showed that in spite of the genetic divergence, *A. parvum* from Argentina and Brazil have similar biological performance on various host species.

1. Introduction

Amblyomma parvum is a Neotropical tick species ranging from southern Mexico to northern Argentina (Guglielmone et al., 2003; Nava et al., 2008a). It parasitizes domestic animals, wildlife and even man (Guglielmone et al., 1991; Nava et al., 2006a). Moreover, it is a potential vector of pathogens such as *Ehrlichia chaffensis* (Tomassone et al., 2008), a *Rickettsia* species with unknown pathogenicity and *Coxiella burnetii* (Pacheco et al., 2007, 2013).

In Brazil, this tick has been found mainly on wild animals such as deer, anteaters and carnivores (Pereira et al., 2000; Martins et al., 2004; Labruna et al., 2005). Among the few reports on domestic animals, Szabó et al. (2007) recorded *A. parvum* parasitizing buffaloes, dogs and horses in the Brazilian savannah, the Cerrado, as well as human biting. The authors considered *A. parvum* infestations of men and domestic animals occasional and linked to high environmental infestation. At the same time, life cycle of *A. parvum* in Argentina was shown to depend in part on domestic animals, mainly cattle and goats as hosts for adult stages of ticks, and Caviidae rodents, as *Galea musteoides*, for the immature forms (Nava et al., 2006b).

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Curiously, the comparison of the mitochondrial 16S ribosomal DNA (rDNA) gene sequences of *A. parvum* specimens from Brazil and Argentina, displayed a divergence (3.0–3.7%) which suggests these populations represent different tick species (Nava et al., 2008a). At the same time, how the biological performance of these two tick populations on various host species differs is unknown. Specifically, it is unclear if Brazilian ticks feed on domestic animals as regularly as the Argentinian *A. parvum* populations. This issue is important because the Brazilian *A. parvum* could become a pest due to anthropic environmental changes that establish a bridge and potential pathogen flow between wild animals, domestic animals and ultimately humans.

Thus, the objective of this study was to compare the biological parameters of Argentinian and Brazilian *A. parvum* ticks fed on various animal species and to evaluate the suitability of each host for ticks of each origin.

2. Materials and methods

2.1. Hosts

Cattle (*Bos taurus*), dogs (*Canis familiaris*), rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*) were used as hosts for *A. parvum* ticks. Dogs and cattle were chosen due to reports of natural infestations with *A. parvum* of both hosts in Argentina and dogs in Brazil (Nava et al., 2006b Nava et al., 2008b; Szabó et al., 2007), rabbits because they are usual hosts for ticks in laboratory colonies and guinea pigs because of the importance of the Caviidae rodents in the cycle of *A. parvum* immature ticks in Argentina (Nava et al., 2006b). All hosts were from both genders and adults, except for cattle, which were younger (10–20 days of age) to allow easier handling. Cattle (Holstein-Friesian calves) were from the herd of the Federal University of Uberlândia, rabbits (New Zealand) and guinea pigs (English type) were purchased from commercial breeders, healthy mongrel dogs were provided by the Zoonosis Control Center of the city of Uberlândia. For the experiments dogs were vaccinated. After experiments dogs were spayed and donated. Rabbits and guinea pigs were tick-bite naïve at the beginning of experiments whereas bovines were previously exposed to *Rhipicephalus microplus* infestations. Dog previous exposure to ticks was uncertain but *Rhipicephalus sanguineus* infestations are very common in the city (Szabó et al., 2010). Animals were not treated for ticks before experiments.

Experimental infestation of cattle occurred in the Experimental Glória Farm, that of dogs in the experimental kennels from the Veterinary Teaching Hospital and rabbits and guinea pigs were infested in the Ixodologia Laboratory, all from the Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil. With the exception of cattle, all hosts used restriction collars to avoid grooming.

2.2. Parasites

A. parvum ticks used in experimental infestations were from colonies of distinct populations, one from Araguapaz, Goiás, Brazil, and the other from El Tunal, Salta Province, Argentina. Ticks from these localities were shown to represent populations from Argentina and Brazil with high divergence of the mitochondrial 16S ribosomal DNA gene sequences as described earlier (Nava et al., 2008a). This divergence was confirmed with samples from the tick colonies used in this work (data no shown). To lessen interference of tick laboratory breeding on tick biology, colonies were established specifically for the experiments herein reported and for all experiments parasites ranged from third to sixth laboratory generation, and were, approximately, fifteen days old. Tick colonies were held at 27 °C, 85% of humidity, at daily photoperiod of 12 h light–12 h dark and fed on rabbits as described by Szabó et al. (1995).

2.3. Experimental infestations

All experimental infestations occurred in summer (December/2011 to March/2012) and inside feeding chambers glued to the shaved back of hosts as described before (Szabó et al., 1995). Six feeding chambers were glued to each dog ($n = 5$), cattle ($n = 5$), and rabbit ($n = 5$). For each host, each of the six chambers held ticks from one stage (adult, nymphal, or larval) from either Argentinian or Brazilian origin; thus, each host was infected simultaneously with all stages and both tick populations. Chambers on all hosts except guinea pigs, held five adult male and female pairs, 20 nymphs, and larvae from 20 mg of egg mass (see below). Since guinea pigs are smaller hosts and known to host immature ticks in nature, only two chambers were glued to one host. Thus, each guinea pig was infested with either larvae or nymphs from both origins (Brazil and Argentina) that were separated by chambers. Six guinea pigs were used for each tick stage.

2.4. Parameters analyzed

The biological and reproductive parameters of the ticks and the number of eggs per 20 mg of egg mass in each animal species were calculated as described by Olegário et al. (2011). Tick larvae are tiny and fragile if separated from cohorts and counting individually may affect their viability. Thus larvae numbers released were considered from egg mass samples with 20 mg but with at least 95% of hatching. To estimate the number of larvae present in 20 mg of egg mass, ten samples of such mass from each of Argentinian and Brazilian females fed on rabbits were counted on the twentieth day of oviposition and was considered to have, respectively, a mean number 390 and 368 eggs thereafter.

2.5. Data analysis

To compare the suitability of the various host species to each tick origin, mean number of ticks produced by each host species was calculated as described before (Olegário et al., 2011). Tick numbers produced were determined using means of tick biological parameters from this work and assuming that one, two or all tick stages fed sequentially on the same host species. For this purpose, tick yield was used to express de percentage of ticks that successfully engorged in relation to those released into each feeding chamber, and molting rate the percentage of ticks that molted successfully from those that engorged. For convenience (to avoid fractioned numbers bellow 1) mean number of nymphs and adults obtained from previous tick stages was calculated assuming the feeding on the host of, respectively, 100 larvae and 10 nymphs. Tick numbers were calculated as follows:

Mean number of nymphs obtained from 100 larvae: 100 × mean larval yield × mean larval molting rate;
Mean number of unfed adults obtained from 10 nymphs: 10 × mean nymphal yield × mean nymphal molting rate;
Mean number of unfed adults obtained from 100 larvae: 100 × mean larval yield × mean larval molting rate × mean nymphal yield × mean nymphal molting rate;
Mean number of larvae produced by one engorged female: 1 × mean adult tick yield × mean egg mass weight × mean number of *A. parvum* eggs/mg × mean larvae hatching rate;
Mean number of unfed adult ticks obtained from one engorged female: 1 × mean adult tick yield × mean egg mass weight × mean number of A. parvum eggs/mg × mean larvae hatching rate × mean larval yield × mean larval molting rate × mean nymphal yield × mean nymphal molting rate;

Data of biological parameters from both Brazilian and Argentinian ticks were submitted to one-way analysis of variance and means were compared by Tukey test. Samples of 20 mg of eggs mass from females from both origins were compared by the Mann Whitney test or Student *t* test. GraphPad Prism® program version 5.0 was used for analysis and significance level was set at $p < 0.05$.

2.6. Ethics

All experiments were submitted and approved by the Animal Experimentation Ethics Committee of the Federal University of Uberlândia (process number 097/2011).

3. Results

Overall, *A. parvum* tick populations from Argentina and Brazil displayed similar biological parameters on the same host. Still, a few differences in tick biology could be detected between ticks from both origins. Although significant differences were observed in feeding, molting, pre-oviposition and incubation periods (data not shown), they did not interfere with overall tick biological performance (tick numbers produced in each generation). Details of all parameters are available on Tables S1–S5 (Supplementary Data). Main differences are shown in Figs. 1–5.

Larva parameters (Fig. 1): Larva yield of Brazilian ticks on guinea pigs was higher in relation to all others irrespective of tick origin or host ($P < 0.05$). Argentinian larva yield on this host was lower ($P < 0.05$) but still higher in relation to those from canids and bovides ($P < 0.05$). At the same time Argentinian larval yield on cattle was the double in relation to Brazilian cohorts from the same host ($P < 0.05$) and similar to those from rabbits. Molting rate of Brazilian

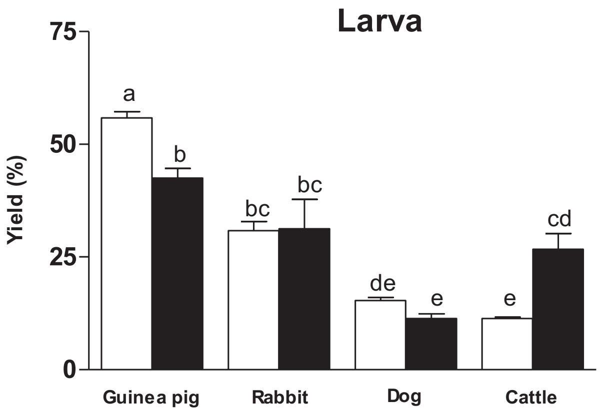

Fig. 1. Tick yield of *Amblyomma parvum* larvae from Brazil (white boxes) and Argentina (black boxes) when fed on guinea pig, rabbit, dog or cattle, Uberlândia, 2012. Data expressed as mean and standard deviation. Different letters indicate a significant difference ($p < 0.05$).

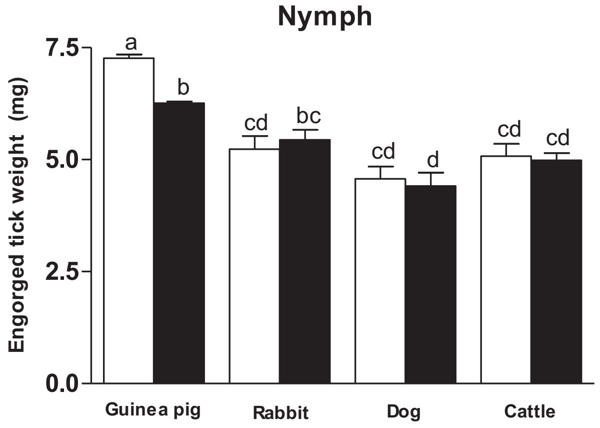

Fig. 2. Engorged weight of *Amblyomma parvum* nymphs from Brazil (white boxes) and Argentina (black boxes) when fed on guinea pig, rabbit, dog or cattle, Uberlândia, 2012. Data expressed as mean and standard deviation. Different letters indicate a significant difference ($p < 0.05$).

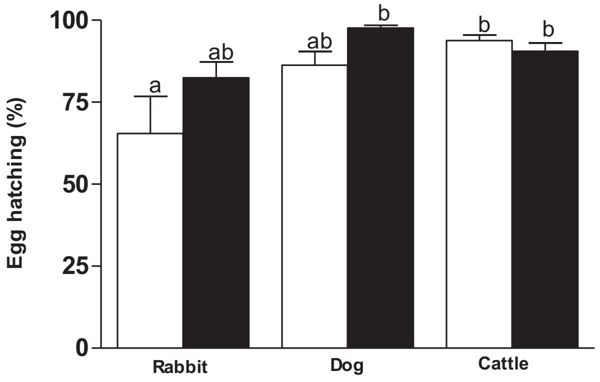

Fig. 3. Egg hatching rate of *Amblyomma parvum* ticks from Brazil (white boxes) and Argentina (black boxes) when fed on rabbit, dog or cattle, Uberlândia, 2012. Data expressed as mean and standard deviation. Different letters indicate a significant difference ($p < 0.05$).

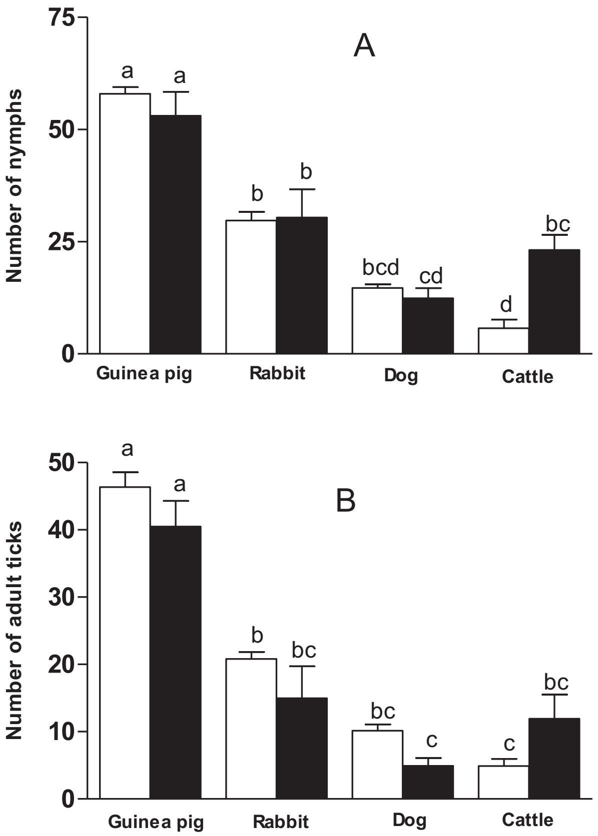

Fig. 4. Mean number of nymphs (A) and adults (B) obtained after an initial infestation with 100 *Amblyomma parvum* larvae from Brazil (white boxes) or Argentina (black boxes) of guinea pigs, rabbits, dogs or cattle, Uberlândia, 2012. Data expressed as mean and standard deviation. Different letters indicate a significant difference ($p < 0.05$).

larvae engorged on cattle was slightly, nonetheless significantly ($P < 0.05$) lower than those engorged on other hosts irrespective of the tick origin (data not shown).

Nymph parameters (Fig. 2): On the whole nymphs engorged on guinea pigs were the heaviest and Brazilian ticks on this host were heavier than those from Argentina ($P < 0.05$). Molting rates of nymphs engorged on cattle from both tick populations were slightly but significantly ($P < 0.05$) lower in relation to ticks from other hosts (data not shown).

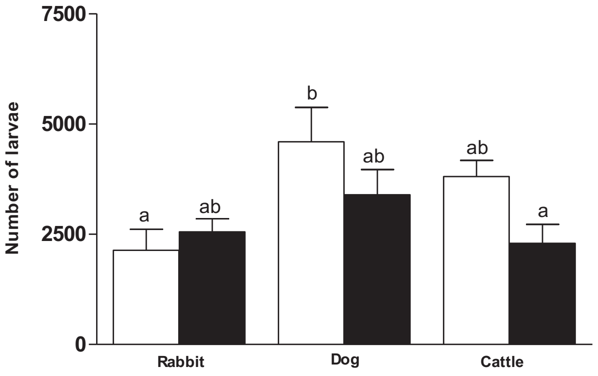

Fig. 5. Mean number of Brazilian and Argentinian *A. parvum* larvae produced by one engorged female on each host species, Uberlândia, 2012. Data expressed as mean and standard deviation. Different letters indicate a significant difference ($p < 0.05$).

Adult and reproductive parameters (Fig. 3): No significant difference was detected on most of the feeding and reproductive parameters of adult ticks between populations from Brazil and Argentina. Egg hatching rate of Brazilian ticks from rabbits was lower in relation to those of Argentinian ticks fed on dogs ($P < 0.05$) and both tick populations fed on cattle ($P < 0.05$).

Host suitability for immatures (number of ticks produced): Irrespective of the origin of the tick, differences in biological performance varied greatly among ticks fed on different hosts and such differences were, in many instances, stage specific (Fig. 4). Thus, guinea pigs were the most suitable hosts for *A. parvum* larvae of both populations as depicted by the higher number of nymphs obtained from larvae fed on this host species in relation to all other hosts ($P < 0.05$) (Fig. 4A). Noticeably, fewer nymphs from the Brazilian population were obtained from larvae fed on bovines if compared to the Argentinian population ($P < 0.05$).

A. parvum nymphs had a more uniform development on the various host species and although guinea pigs provided higher and canids and bovids lower number of adults from nymphs, differences were not significant (data not shown). Consequently the highest adult number ($P < 0.05$) obtained on guinea pigs assuming that both larvae and nymphs fed on this host (Fig. 4B) was related to the highest number of larvae rather than nymphs obtained from this host species.

Host suitability for adults (Fig. 5): Best adult tick performance, measured by mean number of larvae obtained from one engorged female *A. parvum* tick, was achieved by females from the Brazilian population on dogs. Number of larvae produced from this group was significantly greater than from Brazilian ticks fed on rabbits ($P < 0.05$) and Argentinian ticks fed on cattle ($P < 0.05$).

Overall suitability of host species: Mean number of unfed adult ticks obtained from one engorged female assuming that the same host species was used to feed immatures and adults was highly variable and tick numbers obtained from various host species by both tick populations did not differ significantly (data not shown).

4. Discussion

It is increasingly evident, that some tick species with wide geographic distribution are indeed a cluster of species with similar morphology but with different biological, ecological and pathogen-transmission capacities (Szabó et al., 2005; Labruna et al., 2009, 2011; Mastropaolo et al., 2011).

R. sanguineus sensu stricto, for example, is considered the tick with the widest distribution in the world (Pegram et al., 1987) but associated with different tick-borne diseases in different regions. In the Mediterranean area it is the main vector of the human Mediterranean spotted fever agent, *Rickettsia conorii*, but it is only a minor vector for Rocky Mountain spotted fever in the Americas. The lack of overlap between tick and disease distribution may be explained, in part by, to a range of differing tick populations or cryptic species not yet detected. In fact, it is now known that *R. sanguineus* s.s ticks in the Neotropical Region are represented by, at least, two populations, and possibly two species (Szabó et al., 2005; Moraes-Filho et al., 2011; Nava et al., 2012).

Recently it was shown that genetic divergence between *A. parvum* ticks from Argentina and Brazil is high enough for them to be considered different species (Nava et al., 2008a). Such divergence could indicate differing preference for hosts as well as vectoring capacity. However cross-breeding studies with these two tick populations showed that descendants are fertile (Nava, unpublished data). Moreover, data from our work reinforced previous laboratory and field observations on *A. parvum* parazitising an array of host species (Nava et al., 2008a; Olegário et al., 2011) irrespective of the tick population, either Argentinian or Brazilian. Here guinea pigs were the best host for *A. parvum* immatures regardless of the origin, as depicted from higher recovery rate of larvae and heavier engorged nymph weights. It shall be emphasized that heavier nymphs molt to bigger adults and that potentially originate heavier engorged females and egg masses. Furthermore dogs and bovines in our work were shown to be the host species most suitable to adults of Brazilian and Argentinian ticks as shown by the highest number of larvae produced by adult females engorged on this hosts. These data is also correlated with previous observations; *A. parvum* is a tick found on wild canids (Labruna et al., 2005) and domestic dogs (Szabó et al., 2007) in Brazil and Argentina (Nava et al., 2008a) and cattle in Argentina (Nava et al., 2008a).

Even though overall comparable biological performance was observed, a few significant differences between ticks from Brazil and Argentina were also detected. For example engorged Brazilian nymphs weighed more than those from Argentina if fed on a laboratory Caviidae. Moreover, according to our data analysis criteria, bovines were more suitable for Argentinian larvae than for Brazilian cohorts. On a broader analysis however, it should be noted that these significant differences were within a small range and cannot account for meaningful effect at a population level. Biological advantages provided by slightly higher yield or molting rate of ticks on a more suitable host species, for example, could be overcome by the higher density of a less suitable host. Thus, the present study rather displayed that ticks from Argentina and Brazil have overall similar features when fed on the same host species. Furthermore it is clear from previously mentioned field data and results herein presented that this tick species has a wide host range but with adults exhibiting better biological performance on larger mammals and immatures on rodents, particularly Caviidae.

On the whole these data suggest that host questing behavior and ecological requirements, rather than specificity for hosts, are fundamental to determine the distribution and host infestations of *A. parvum*. In this regard, Klompen et al. (1996) suggested that tick–host association patterns may be explained as artifacts of biogeography and ecological specificity rather than host specificity, and a recent meta-analysis of host specificity of Neotropical hard ticks, reinforced such assumption (Nava and Guglielmone, 2013). Nonetheless some care with this assumption should be taken. It was also shown that within a specific ecosystem, some degree of host specialization may be attained by ticks and be linked to some minor genetic differences (McCoy et al., 2001). Thus introduction of a new and abundant host species in the ecological niche of *A. parvum*, as is the case of goats and bovines in Argentina, might account for a shift in the genetic background of tick populations as well. In a more extreme example a surrogate life cycle on bovines, non-Neotropical host as described before for another Neotropical tick in Argentina, *Amblyomma neumanni* (Nava et al., 2006b). Anyhow a closer follow up of *A. parvum*–host relationships both in Argentina and Brazil is mandatory as these tick populations exhibit a remarkable host plasticity, may harbor pathogenic microorganisms, and are now submitted to selective pressure that has altered over a short period of time. In this regard, systematic and careful examination of ticks on cattle in Brazil in regions with *A. parvum* populations should be performed as already done in Argentina (Guglielmone and Hadani, 1982; Nava et al., 2008a).

Conflicts of interest

Authors declared no conflict of interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetpar.2013.06.018.

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