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The natural hosts for larvae and nymphs of *Amblyomma neumanni* and *Amblyomma parvum* (Acari: Ixodidae)

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Abstract Based on the hypothesis that birds and rodents are important hosts for subadults of the Neotropical *Amblyomma neumanni* and *Amblyomma parvum* ticks, a survey of these type of hosts was carried out from July 2004 to March 2006, in Quilino (*A. parvum*) and Dean Funes (*A. neumanni*), Córdoba province, Argentina. Additionally, monthly tick counts were performed on cattle and goats with occasional tick search in other domestic hosts. Records of questing height of subadult ticks on vegetation were also carried out monthly. Rodents (n = 123) and birds (n = 122) captured in Dean Funes showed no infestation with *A. neumanni*. Apart of few nymphs found on horses, all larvae and nymphs of *A. neumanni* were on cattle with a larval prevalence and mean number of 22.2%, and 7.7 ± 22.52, respectively, and a prevalence of nymphs of 47.8% with a mean of 7.9 ± 18.49. The average questing height of larvae and nymphs of *A. neumanni* was 23.5 ± 17.1 cm and 30.7 ± 26.7 cm, respectively. A total of 138 rodents and 130 birds were captured in Quilino but the Caviidae rodent *Galea musteloides* carried 99.3% of larvae and 99.8% of nymphs of *A. parvum*, and no immature stages were detected on cattle, goat or vegetation. Tick counts on *G. musteloides* (n = 74) showed a prevalence of 42% and a mean number of 9.9 ± 24.83 for larvae, while nymphal infestation had a prevalence of 56.5% and a mean of 8.7 ± 11.31. Cattle appear to be suitable hosts to sustain the complete cycle of *A. neumanni* in nature (adult ticks infest cattle too) and questing height of subadults indicates that they are expecting to feed on medium and large-sized mammals, such as cattle and other ungulates. At least in the study site, *G. musteloides* is the principal host for the survival strategy of *A. parvum* subadults; adult ticks are common on cattle and goats. These hosts are introduced in the Neotropics but *A. neumanni* was able to develop a surrogate cycle independent of native hosts while *A. parvum* still depends on probably primeval hosts to sustain their larvae and nymphs.

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Introduction

The adults of *Amblyomma neumanni* Ribaga, 1902 are usually found on domestic mammals, especially cattle (Guglielmone et al. 1990b; Estrada-Peña et al. 2005), while most records of adults of *Amblyomma parvum* Aragão, 1908, are from cattle and goats (Guglielmone et al. 1990a). Human infestation with these tick species is also common (Guglielmone et al. 1991; Nava et al. 2006b). *Amblyomma neumanni* is an experimental vector of *Anaplasma marginale* to cattle (Gaido et al. 1995) but the role of *A. parvum* as vector of animal diseases is unknown. Both species are established in the Neotropics. *Amblyomma neumanni* is essentially an Argentinean tick, with a *bona fide* record from Colombia and uncertain records from Uruguay (Guglielmone et al. 2003a; Estrada-Peña et al. 2005) whereas *A. parvum* is widely established from southern Mexico to Argentina (Guglielmone et al. 2003a). The current infestation pattern of adults of these tick species on domestic mammals introduced to the Neotropical Zoogeographic Region constitutes a surrogate host–parasite relationship as defined by Hoogstraal and Aeschlimann (1982), that replaced the primeval host–tick relationship.

Many ixodid ticks are characterized by a three-host life cycle, with larvae and nymphs engorging on small to medium-sized hosts and adults on larger species (Oliver 1989). This natural pattern is recognized for Neotropical species of *Amblyomma* such as *A. aureolatum*, *A. longirostre*, *A. ovale*, *A. tigrinum* and *A. triste*, among others (Jones et al. 1972; Arzua et al. 2003; Guglielmone et al. 2003b; González-Acuña et al. 2004; Nava et al. 2006a, in press). Nevertheless, most records of larvae and nymphs of *A. neumanni* are usually from large domestic mammals as cattle and horses (Estrada-Peña et al. 2005). Moreover, Guglielmone et al. (1990a) presented preliminary evidence that cattle can currently sustain the natural cycle of *A. neumanni*. There are few records of *A. parvum* larvae and nymphs and speculation about the hosts able to sustain the cycle of this tick species is uncertain. We hypothesized that rodents and birds may play a crucial role in the maintenance of subadults of *A. parvum* in nature and contribute also to the life cycle of *A. neumanni*. Therefore, an investigation was conducted to recognize the natural hosts of larvae and nymphs; the questing position of subadults of these ticks was also studied as the information may be useful to design control strategies to avoid infestations on domestic animals and man.

Materials and methods

The study was carried out from July 2004 to March 2006 in two localities of Córdoba province, Argentina: Dean Funes (30°22′ S 64°22′ W) for *A. neumanni* and Quilino (30°12′ S 64°32′ W) for *A. parvum*. Dean Funes is located in the Chaqueño Serrano District (CSD) and Quilino in the Western Chaqueño District (WCD), both belonging to the phytogeographical Chaco Domain according to the definition given by Cabrera (1976). The CSD, where *A. neumanni* prevails (Guglielmone and

Viñabal 1994), has a warm climate with summer rainfall (400–800 mm annually), and the vegetation is composed of xerophylic forest and patches of hard grasses. *Amblyomma parvum* is established in the WCD (Guglielmone and Viñabal 1994) characterized by an annual rainfall of 400–700 mm concentrated form November to March, and the vegetation consists almost exclusively of xerophylic forest. The two localities present human disturbance, characterized mainly by partial deforestation, livestock grazing and introduced tropical pastures such as *Panicum maximum*. Properties selected for the study have relevant areas where part of the original forest has been preserved.

Rodents were captured monthly in two-days trapping session using two linear grids each one with 50 Sherman live-trap-type (24 cm in length, 9 cm in height and 8 cm in width) set at 5 m intervals baited with food pellets and seeds, and one trapping linear grids with 50 Tomahawk live-trap-type (32 cm in length, 10 cm in height and 10 cm in width) set at 5 m intervals baited with carrot. Birds were also captured monthly with trap cages, identified, examined for ticks and released. A limited number of birds not prone to be trapped were shot.

The small-sized rodents (Cricetidae and Muridae) are recognized vectors of lethal disease to humans (Mills et al. 1991; Calderón et al. 1999); therefore these rodents were killed and manipulated following biosecurity rules detailed in Mills et al. (1995) and examined for ticks later in the laboratory. The medium-sized rodents (Caviidae) trapped were checked for ticks, identified and marked by a cutting the hair on their backs and released. To prevent underestimated tick counts, Caviidae recaptured the second day of each session were released without examination. Other wild vertebrates that were captured occasionally during the samplings were also examined for the presence of ticks.

Tick counts were also monthly performed (08:00 to 09:00 am) on one side of five cows in Dean Funes, and five cows and 5–10 goats in Quilino, taken at random in the same properties where birds and rodents were caught. Additionally, dogs and horses were searched for subadult ticks if available.

The activity of subadult *A. neumanni* and *A. parvum* was measured with the method described by Schulze et al. (1997). To this aim two transects of 500 m were walked every month by an operator bearing a white overall. Larvae and nymphs were removed from the overall every 50 m and deposited in 96% ethanol after recording the questing height of each specimen. This technique was selected because the type of vegetation in the sampling sites precluded the use of dragging or flagging techniques. Besides, this method of survey by walking is appropriate for the study of tick-questing height because it allow collection of ticks from any part of the vegetation (Schulze et al. 1997).

Small rodents were identified with the collaboration of U.F.J Pardiñas (Centro Nacional Patagónico CONICET, Puerto Madryn, Argentina), the Caviidae following Redford and Eisenberg (1992) and Díaz (2000); taxonomic classification was made according to Wilson and Reeder (2005). The birds were identified following Narosky and Yzurieta (2003) and the taxonomic classification was made according to Dickinson (2003). *Amblyomma neumanni* and *A. parvum* ticks were identified following the descriptions of Estrada-Peña et al. (2005) and Guglielmone et al. (1990a), and were also compared with known laboratory reared material housed in the INTA Rafaela Tick Collection. Additionally, to obtain specimens more suitable for identification, engorged larvae and nymphs were maintained in the laboratory at 27°C ± 1 and 80–85% relative humidity until moulting.

The prevalence of infestation, with mean and standard deviation (SD), were obtained for each type of infested hosts. The index of discrepancy (*D*) (Poulin 1993) was estimated to evaluate the degree of aggregation of parasites on their hosts, where 0 constitutes null aggregation (all hosts with equal level of infestation) and 1 complete aggregation (all members of a parasite population on one individual host). Because several species of birds were represented by just one or two individuals (Table 1), they were grouped for tick analysis according to their use of space following the guild classification detailed in Nava et al. (2006a) that divided different species of birds considering their feeding habits into three categories: non-ground feeding birds, birds prone to feed in open areas and birds prone to feed in forested areas.

Results

Larvae and nymphs of *A. neumanni* and *A. parvum* were found in Dean Funes (WSD) and Quilino (WCD), respectively.

A total of 123 rodents belonging to seven species and three families, and 122 birds belonging to 27 species and 16 families, were captured in Dean Funes as detailed in Table 1. Additionally, 18 reptiles (eight *Liolaemus* sp., five *Tupinambis teguixin*, three *Bothrops alternatus* and two *Lystrophis pulcher*), four amphibians (*Bufo* sp.) and one mammal, the Dasypodidae *Chaetophractus villosus*, were examined. None of these hosts was infested with *A. neumanni* subadults but several birds and rodents were parasitized with larvae and nymphs of *Amblyomma tigrinum*, and one male of *A. pseudoconcolor* was collected on *C. villosus*. These tick species were identified according to Estrada-Peña et al. (2005) and Jones et al. (1972), respectively.

Apart from 15 nymphs and 22 larvae found on five of 14 horses examined, larvae and nymphs of *A. neumanni* where found only on cattle and vegetation. Larval tick counts on cattle showed a prevalence of 22.2% and a mean number and SD of larvae on one side of the body of 7.7 ± 22.52, $D = 0.892$. The data for nymphs showed a prevalence of 47.8%, mean = 7.9 ± 18.49, $D = 0.796$. The average questing height of larvae ($n = 993$) was 23.5 ± 17.1 cm (range: 7–55 cm), and 30.7 ± 26.7 cm (range: 8–70 cm) for nymphs ($n = 1{,}173$). The difference between the questing height of larvae and nymphs was not significant (Mann–Whitney *U*-test, $P = 0.73$). Infestations with immature stages of *Otobius megnini* were also detected on cattle and *A. neumanni* adults were found on cattle, horses and dogs, and *A. tigrinum* adults on dogs.

A total of 138 rodents belonging to three species and two families, and 130 birds belonging to 24 species and 14 families were trapped in Quilino (Table 1). Additionally, 21 reptiles (13 *Liolaemus* sp., three *Tupinambis teguixin*, two *Bothrops neuwiedii*, two *Crotalus durissus* and one *Lystrophis pulcher*), three amphibians (*Bufo* sp.), one marsupial (*Thylamys elegans*), one armadillo (*C. villosus*) and one carnivore (*Conepatus chinga*) were examined. As in Dean Funes several birds and rodents were infested with larvae and nymphs of *A. tigrinum*, *C. villosus* with one female of *A. pseudoconcolor*, and *C. chinga* was infested with a nymph of *Amblyomma* sp.

No larvae or nymphs of *A. parvum* were detected on cattle (nymphs of *O. megnini* were detected on two cows), goats, other domestic hosts and vegetation. Apart from one larva on a *Sicalis flaveola* (prevalence = 25.0%, mean = 0.25 ± 0.500), one on a *Saltatricula multicolor* (prevalence = 3.5%, mean = 0.03 ± 0.186), two larvae on

Table 1 Number and species of rodents and birds captured and examined for ticks in Dean Funes and Quilino, where *Amblyomma neumanni* and *A. parvum* are established, respectively. Birds are grouped according to feeding habits

Hosts	*n* Dean Funes	*n* Quilino
Birds		
(1) Non-ground birds		
Accipitridae		
Accipiter striatus	2	0
Emberizidae		
Poospiza torquata	2	4
Picidae		
Picoides mixtus	0	1
Psittacidae		
Myiopsitta monachus	10	11
Thraupidae		
Thraupis bonariensis	0	2
Tyrannidae		
Empidonomus aurantioatrocristatus	0	5
Elaenia albiceps	1	0
Pitangus sulphuratus	1	0
Mimidae		
Mimus saturninus	1	0
(2) Ground birds in open areas		
Columbidae		
Columba maculosa	1	0
Columbina picui	13	8
Zenaida auriculata	5	6
Emberizidae		
Sicalis flaveola	3	4
Sicalis luteola	3	1
Icteridae		
Molothrus bonariensis	26	5
Tyrannidae		
Macheotornis rixosa	2	0
(3) Ground birds in forested areas		
Cardinalidae		
Saltator aurantiirostris[a]	3	8
Cuculidae		
Guira guira[a]	5	4
Emberizidae		
Aimophila strigiceps	1	3
Coryphospingus cucullatus	9	10
Lophospingus pusillus	10	7
Saltatricula multicolor	4	29
Zonotrichia capensis	1	2
Furnariidae		
Asthenes sp.	2	1
Coryphistera allaudina	7	1
Furnarius cristatus	0	2
Furnarius rufus	0	4
Pseudoseisura lophotes	2	9
Polioptilidae		
Polioptila dumicola	3	2
Rhinocryptidae		
Rhinocrypta lanceolata	0	1
Troglodytidae		
Troglodytes aedon	2	0

Table 1 continued

Hosts	*n* Dean Funes	*n* Quilino
Tynamidae		
Eudromia elegans	1	0
Nothura maculosa	2	0
Rodents		
Caviidae		
Galea musteloides	31	74
Cricetidae		
Akodon dolores	32	8
Calomys venustus	18	0
Calomys sp.[b]	17	0
Graomys sp.[c]	18	56
Necromys benefactus	5	0
Muridae		
Rattus rattus	2	0

[a] Non-typical ground bird. Frequently found also on upper vegetation

[b] *Calomys cf. C. laucha-C. musculinus*

[c] Theiler and Blanco (1996) stated that the karyomorph $2n = 42$ from Córdoba is a separate sibling species from *G. griseoflavus* complex ($2n = 38–36$)

Graomys sp. (prevalence = 11.1%, mean = 0.11 ± 0.323), and one nymph detected on a *S. multicolor* (prevalence = 3.5 %, mean = 0.03 ± 0.186), all specimens of *A. parvum* subadults were found infesting *Galea musteloides*. This Caviidae carried 99.3% and 99.8% of total larvae and nymphs of *A. parvum*. Larval tick counts on *G. musteloides* showed a prevalence of 42%, a mean number and SD number per host of 9.9 ± 24.83, $D = 0.841$, while the data for nymphal infestation showed a prevalence of 56.5%, and a mean number and SD of 8.7 ± 11.31, $D = 0.654$.

Discussion

Indeed, *A. neumanni* subadults are not dependant on wild birds or rodents for their survival, and the infestation with larvae and nymphs of cattle found in this study allow to conclude that cattle are suitable hosts that can sustain the complete cycle of *A. neumanni* in nature. In fact, questing heights of larvae and nymphs of this species indicate that they are expecting to feed on other hosts than small to medium-sized rodents or terrestrial birds, and that medium and large-sized mammals are those that mainly pickup ticks from the vegetation. Artiodactyls of the families Cervidae, Camelidae and Tayassuidae were well represented in South America since the Pleistocene (Menégaz and Ortiz Jaureguizar 1995) but currently cattle represent the most important mass of large mammals in the region, and the only wild artiodactyls established in the study site are the Cervidae *Mazama guazoupira* and the Tayassuidae *Pecari tajacu* (Morando and Polop 1997), both with greatly reduced populations. It is most probable that one or more of species of large mammals from those families were the original hosts for *A. neumanni* and that all stages of this tick adapted to feed on cattle when the primeval fauna of wild large mammals started to be displaced by the introduced livestock 400 years ago. All evidences indicate that the surrogate cycle of *A. neumanni* is a successful one and, as proposed by

Guglielmone et al. (1990b), this species can actually survive with the presence of cattle only in the environment.

Perhaps, the only evidence against the above statement is the low prevalence of larvae of *A. neumanni*, and the high values of SD and *D* that indicate a strongly skewed distribution with high infestation of few hosts. Although this type of host–parasite relationship is characteristic of parasitism (Poulin 1993), dependence on a few hosts also constitutes a weak link for parasite survival. However, that difference may be due to seasonal distribution of larvae (an objective beyond this study) with high numbers of larvae parasitizing cattle during seasonal peaks. Further studies on the ecology of this tick should further explain *A. neumanni* larva–cattle relationship. In general terms, the above statement also applies to nymphs of *A. neumanni* on cattle and larvae and nymphs of *A. parvum* on *G. musteloides*, although in these cases the prevalence was substantially higher than the prevalence of *A. neumanni* larvae on cattle.

The strategy of *A. parvum* for feeding larvae and nymphs is different to the strategy of *A. neumanni. Amblyomma parvum* appears to depend on the presence of the only medium-sized rodent established at the study site, the Caviidae *G. musteloides*, and in a much lesser extent on small rodents and terrestrial birds. Nevertheless, performance of a given parasite population on its sympatric host population must be compared with that on several allopatric host population to account for variation in suitability among different species of this type of hosts (Kaltz and Shykoff 1998). This is especially relevant for the case of *A. parvum* because its range exceeds that of Caviidae, a family consisting on 12 South American species (Wilson and Reeder 2005). Consequently, additional research on the potentials hosts for larvae and nymphs of *A. parvum* in other areas need to be known, and it is necessary to know which are their hosts in the northern areas of the Neotropical Zoogeographic Region where *A. parvum* is also established. Neither larvae nor nymphs of *A. parvum* were found on cattle, goats and vegetation, probably because most subadults ticks are awaiting hosts on the ground or in the hosts burrows.

The role of rodents of the family Caviidae as hosts for subadults of Neotropical South American tick species has been largely overlooked. However, recent investigations showed that this type of host is relevant to the feeding of nymphs of *A. tigrinum* (Nava et al. 2006a). The current study shows their important contribution to the cycle of *A. parvum*. Probably rodents of this family are also involved in the cycle of other South American ticks species; therefore, it seems of scientific value to further investigate the role of this type of host for tick ecology.

Finally, this study show different strategies of *A. neumanni* and *A. parvum* to survive under the pressure of losing primeval hosts. The actual life cycle of *A. neumanni* is a surrogate cycle for all stages while *A. parvum* still depends on autochthonous hosts to sustain larvae and nymphs.

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