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Amblyomma tigrinum (Acari: Ixodidae): New data on hosts and biology of immature stages and on DNA composition

D.H. Aguirre [a,*], A.J. Mangold [b], M.M. Cafrune [a], A.A. Guglielmone [b]

[a] *Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Salta, CC 228, CP 4400 Salta, Argentina*
[b] *Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, CP 2300, Rafaela (Santa Fe), Argentina*

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Abstract

Biological data of immature stages of *Amblyomma tigrinum* were obtained from larvae and nymphs both fed on rats and rabbits. Data from nymphs recovered from a wild rodent (*Galea musteloides*) are also reported. Additional results in DNA composition of males moulted from nymphs fed on laboratory and wild hosts are presented. The ticks were maintained in darkness at 27 ± 1 °C and 83–86% RH. The mean recovery rates were 49.1% and 43.6% with a moulting success of 96.2% and 90.8% in larvae fed on rats and rabbits, respectively. The engorgement weights were almost identical in larvae recovered from both hosts. The mean recovery rates of nymphs were 37.3% and 69.9% in specimens fed on rats and rabbits, respectively. The moulting success was 94.5%, 100% and 98.1% in nymphs fed on rats, rabbits and *G. musteloides*, respectively. Nymphs from all hosts moulting to females were significantly heavier ($P < 0.01$) than those moulting to males despite their range of engorgement weight showed overlap. A higher proportion (≥61.5%) of nymphs from all hosts moulted to females. Present results suggest that members of Rodentia and Lagomorpha are suitable hosts for the immature stages of *A. tigrinum*, contrasting with previous results from Brazilian colonies of this tick. The DNA sequence from ticks fed on *G. musteloides* showed 99.7% identity with that from ticks fed on rabbits and also with the DNA sequence already available (GenBank AY498562) for *A. tigrinum*.

Keywords: *Amblyomma tigrinum*; Immature stages; Biology; Adults; DNA composition

1. Introduction

Amblyomma tigrinum Koch, 1844, a tick found from Venezuela to Argentina (Jones et al., 1972) shows a wide distribution in different geographical areas of the Neotropical region (Guglielmone et al., 2000). Adult stages were found all year round with a peak during summer, parasitizing mainly wild and domestic carnivores (Guglielmone et al., 2000). Dogs constitute most of the host reports of *A. tigrinum* adults from Southern Brazil (Evans et al., 2000) and Argentina (Guglielmone et al., 2000). In contrast,

* Corresponding author. Tel.: +54 387 4902081; fax: +54 387 4902081.
E-mail address: daguirre@correo.inta.gov.ar (D.H. Aguirre).

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few finds of larvae and nymphs of this *Amblyomma* species were done (Jones et al., 1972; Massi Pallarés and Benítez Usher, 1982; Ivancovich and Luciani, 1992; Venzal et al., 2001; González Acuña et al., 2004). Therefore, little is known on the biology of these stages, including their host preferences. Recently, Labruna et al. (2002) tested six hosts (one avian and five mammal species) for the immature stages of *A. tigrinum* and reported biological data of larvae and nymphs collected mainly from the avian host.

This article describes biological parameters of *A. tigrinum* immature stages from specimens fed on two species of mammals in the laboratory and also from nymphs collected from a naturally parasitized wild rodent. Additional results in DNA composition of males moulted from nymphs of laboratory and wild hosts are presented.

2. Material and methods

2.1. Biological data of immatures

The ticks used in this study were derived from the progeny of eight engorged females obtained from dogs of the environs of Salta city (24°47′S, 65°25′W), Argentina, maintained in darkness at 27 ± 1 °C and 83–86% RH for egg laying. Labruna et al. (2002) stated that the mean weight of an egg of *A. tigrinum* is 0.045 mg. Expecting an egg hatching of ≥95%, eggs masses of two groups of four females each were pooled and separated into aliquots of 15 mg (using a Mettler H78AR electrobalance) in order to obtain approximately 300 larvae in each batch to proceed to infestations.

Twelve naïve male adult mammals were used to feed the immature stages from the two groups of the tick. Three wistar rats (*Rattus norvegicus*) and three rabbits (*Oryctolagus cuniculus*) were infested with 300 larvae each, while equal numbers of both rodent species were infested with 49–65 ($n = 354$) nymphs each. Larvae and nymphs were 12–60 and 40–70 days old, respectively, when allowed to feed.

Larvae and nymphs were released on the entire body of the rats, while both stages were disposed inside cloth capsules (7 × 15 cm) glued to the ears of the rabbits. During the first 24 h post-infestation, rats were kept in individual wire cages small enough to prevent self-grooming; then, they were transferred to larger cages to allow host grooming. These cages were disposed into a dry pan with an adhesive tape attached to the borders to avoid escaping of engorged ticks. During tick feeding the hosts were maintained at an approximately 12:12 photoperiod rendered by natural lighting.

Detached engorged ticks were collected and counted daily at 4.00 p.m. Nymphs were weighed individually while engorged larvae were weighed in groups of ten. Thereafter they were maintained in darkness at 27 ± 1 °C and 83–86% RH and their development was followed daily as described previously (Guglielmone et al., 1991).

Additional data was obtained from nymphs collected from a yellow toothed cavy (*Galea musteloides*) captured in August 2003 from the same site inhabited for the dogs parasitized with the adults stages of *A. tigrinum* that started the colonies of the present work. These nymphs were maintained and followed as those collected from laboratory hosts.

The *t*-test was used to determine differences between the means of the biological data of larvae and nymphs.

2.2. DNA sequences

Male specimens of *A. tigrinum* moulted from nymphs fed on both laboratory host (rabbits) and wild rodent (cavy) were preserved in 96% ethanol and stored at −20 °C until its use to sequence mitochondrial 16S rDNA. Extraction of DNA was carried out from a single male tick of both populations and polymerase chain reaction (PCR) amplification was set-up as described by Mangold et al. (1998). The strands of DNA were assembled using a sequence alignment editor (BioEdit©, 1997–2004 by Tom Hall, Isis Pharmaceuticals, Inc) and analyses were conducted using MEGA version 2.1 (Kumar et al., 2001). The mitochondrial 16S rDNA sequence of *A. tigrinum* available in the GenBank (AY498562) were used for pairwise comparisons.

3. Results

3.1. Biological data of immatures

The recovery rate, moulting success, engorgement weight (EW) and feeding and pre-moult periods of the

Table 1
Biological parameters (mean ± S.D.) of larvae and nymphs of *Amblyomma tigrinum* fed on rats

	Larvae	Nymphs		
		Males and females	Males*	Females
Recovery rate (%)	49.1 ± 29.6	37.3 ± 19.83		
Number of rats infested	3	3		
Moulting success (%)	96.2 ± 1.42	94.5 ± 6.79		
Number of rats infested	3	3		
Engorgement weight (mg)	0.55 ± 0.054		9.5 ± 2.55[a]	11.9 ± 1.26[b]
n	370		23	37
Feeding period (days)	5.0 ± 0.42		6.5 ± 0.26[a]	6.3 ± 0.70[a]
n	442		23	37
Pre-moult period (days)	12.6 ± 0.67		20.8 ± 4.46[a]	18.4 ± 1.86[b]
n	427		23	37

* Numbers not sharing the same letter are significantly different ($P < 0.01$).

larvae and male and female nymphs fed on rats and rabbits are shown in Tables 1 and 2, respectively. Larvae fed on rats showed a higher recovery rate than those fed on rabbits. The mean EW of larvae from rats was slightly higher than larvae from rabbits ($P > 0.05$) and the feeding period shorter ($P < 0.001$). The moulting success of larvae fed on rats was also higher than larvae fed on rabbits and the pre-moult period longer ($P < 0.01$).

In contrast, the recovery rate of nymphs was markedly higher in rabbits than in rats. The mean EW was higher in nymphs from rabbits than in nymphs from rats ($P > 0.05$ for males and $P < 0.01$ for females) and the feeding period longer ($P < 0.001$). The moulting success of nymphs fed on rabbits was also higher than nymphs fed on rats and the pre-moult period longer ($P < 0.01$). The EW ranges of the nymphs moulting to males (3.0–14.4 in rats; 2.6–16.4 in rabbits) showed overlap with the EW ranges of the female nymphs (2.9–18.8 in rats; 5.8–19.3 in rabbits), indicating that this parameter cannot be used to predict the sex with accuracy. A markedly higher proportion of nymphs collected from both hosts moulted to females (61.7% in rats and 64.5% in rabbits).

Most of larvae and nymphs released on the rats were attached to the head and the neck as shows in Fig. 1.

Table 2
Biological parameters (mean ± S.D.) of larvae and nymphs of *Amblyomma tigrinum* fed on rabbits

	Larvae	Nymphs		
		Males and females	Males*	Females
Recovery rate (%)	43.6 ± 4.67	69.9 ± 18.24		
Number of rabbits infested	3	3		
Moulting success (%)	90.8 ± 7.97	100		
Number of rabbits infested	3	3		
Engorgement weight (mg)	0.54 ± 0.049		10.6 ± 0.99[a]	13.9 ± 1.69[b]
n	360		45	82
Feeding period (days)	5.4 ± 0.39		8.3 ± 1.22[a]	7.7 ± 1.54[a]
n	393		45	82
Pre-moult period (days)	11.5 ± 1.49		22.9 ± 2.95[a]	21.3 ± 3.40[b]
n	355		45	82

* Numbers not sharing the same letter are significantly different ($P < 0.001$).

Fig. 1. Wistar rat experimentally infested with nymphs of *Amblyomma tigrinum*.

A total of 53 nymphs were recovered from the infested *G. musteloides*. The mean EW (±S.D.) was 9.7 (±3.06) mg for the nymphs moulting to males and 13.1 (±3.77) mg for the nymphs moulting to females ($P < 0.01$). The moulting success was of 98.1% in mean (±S.D.) pre-moulting periods almost identical for both sexes (15.0 ± 0.72 and 14.8 ± 0.61 days for males and females, respectively; $P > 0.05$). These periods were significantly shorter ($P < 0.001$) than the corresponding periods for nymphs fed on the laboratory hosts. As in these latter hosts, a markedly higher percentage of nymphs (61.5%) moulted to females. No other stage or species of ticks were found on this wild cavy.

3.2. DNA sequences

The sequence of mitochondrial 16S rDNA fragment obtained from the specimen collected on laboratory host (GenBank Accesion Number AY836004) was identical to the GenBank AY498562 sequence (Estrada-Peña et al., 2005) and both showed 99.7% identity with the sequence of mitochondrial 16S rDNA fragment obtained from the specimen collected on wild host (AY836005), which showed the insertion of one nucleotide (timina) in position 231 of alignment.

4. Discussion

Members of Rodentia and Lagomorpha appear to be suitable hosts for larvae and nymphs of *A. tigrinum*, as showed by the results of artificial infestations with both immature stages on rats and rabbits and natural infestation with nymphs on a wild rodent. This situation is remarkably different with that reported by Labruna et al. (2002), who using similar holding and infestation methods (at least for rats) obtained maximum recovery and moulting rates of 3.1% and 60.0% for Brazilian immature stages released on rats and rabbits, respectively.

Host records for larvae and nymphs of *A. tigrinum* were scarce until recently (Venzal et al., 2001; González Acuña et al., 2004). Only four nymphs have been found parasitizing a dog in Argentina (Ivancovich and Luciani, 1992). Nymphs were also found on domestic and wild Canidae from Venezuela and Paraguay (Jones et al., 1972; Massi Pallarés and Benítez Usher, 1982). In Brazil only one nymph was reported on a partridge (Evans et al., 2000) despite Labruna et al. (2002) pointed the notation of Aragao (1936) ‘‘that nymphs of *A. tigrinum* (referred to as *A. maculatum*) were frequently found on quails (*Nothura maculosa*) and partridges (*Rhynchotus rufescens*)’’. Recently, Venzal et al. (2001) reported the presence of larvae of *A. tigrinum* on *Drymornis bridgesii* birds from Uruguay and González Acuña et al. (2004) found both immature stages of this tick on three species of wild birds (*Zenaida auriculata*, *Callipepla californica* y *Nothoprocta perdicaria*) from Chile. These findings suggest that birds are important for the life cycle of *A. tigrinum*.

Nevertheless, studies in progress in Argentina show that Sigmodontinae and Caviidae rodents are frequently found infested with larvae and nymphs of this tick species (S. Nava, personal communication). In this sense, Jones et al. (1972) recorded 21 nymphs of *A. tigrinum* on a Sigmodontinae rodent, *Oligoryzomys fulvescens*, from Venezuela. Present results on laboratory hosts sustain the hypothesis of Guglielmone et al. (2000) that ‘‘perhaps rodents play a role in feeding nymphs (of *A. tigrinum*)’’. Probably the same is truth for the larval stages of the tick. Moreover, Estrada-Peña et al. (1993) described the nymphs of *A. tigrinum* working with specimens from moulted larvae fed on rats. The severe natural infestation with nymphs here reported on *G. musteloides* also reinforces the above pointed hypothesis. Obviously, more studies are needed to clarify the host rank and preferences for the immature stages of *A. tigrinum*.

The mean EW of nymphs feeding on rats (9.5 mg and 11.9 mg for male and female nymphs) and rabbits (10.6 mg and 13.9 mg for male and female nymphs)

were substantially higher than the corresponding weights reported by Labruna et al. (2002) for Brazilian nymphs fed on chicken (4.7 mg and 6.5 mg for male and female nymphs, respectively). If EW is a proper indicator of adaptation it may also indicate that rodents and lagomorphs are suitable hosts for the nymphs of *A. tigrinum*. Actual data on recovery rates also shows that rats were more appropriate hosts for larvae than rabbits despite the infestation procedure in the latter host made it unable for self-grooming. In contrast, rabbits appear to be more suitable hosts for the nymphs (not considering the differences in the infestation methods). This reflects the difficulties to interpret laboratory findings on natural cycles since rabbits are not natural hosts for *A. tigrinum*. Moreover, natural Neotropical Lagomorpha are not widespread in extended areas of Argentina were *A. tigrinum* is established (Redford and Eisenberg, 1992). Therefore, Lagomorpha are probably of small importance for the life cycle of this tick species in the nature.

As in most ixodids, female nymphs were significantly heavier than male nymphs. However, sexes cannot be separated with accuracy by using this parameter due to overlap in the EW. In a sense, *A. tigrinum* is similar to other local members of the genus such as *A. cajennense* (Guglielmone et al., 1992) and *A. neumanni* (Aguirre et al., 1999) but different to another species such as *A. parvum* (Guglielmone et al., 1991) and *A. pseudoparvum* (Mangold and Guglielmone, 1993). The feeding period of *A. tigrinum* larvae showed to be shorter than the corresponding nymphal period. This pattern is common for ixodids, like other representatives of the genus, *A. parvum* and *A. pseudoparvum* (Guglielmone et al., 1991; Mangold and Guglielmone, 1993) but different to another ones, *A. cajennense* and *A. neumanni* (Guglielmone et al., 1992; Aguirre et al., 1999).

The higher proportion of engorged nymphs from all host species that moulted to females (63.2%) is of interest. This result differs also from Labruna et al. (2002) who reported a sex ratio (males to females) of 1.37: 1 from 216 moulted nymphs of *A. tigrinum* fed mainly on chickens. Guglielmone et al. (2000) found in the nature a high percentage of females (68.1%) amongst adult specimens of *A. tigrinum*, and speculated that this bias was bound to the easier detection of the usually bigger females. However, this proportion is similar to that from our work, suggesting another explanation for the biased sex ratio of specimens recorded from nature. On the other hand, similar female percentages have been previously reported from moulting nymphs of *A. parvum* (65.1%) and *A. neumanni* (58.2%) amongst others aboriginal species from the genus (Guglielmone et al., 1991; Aguirre et al., 1999).

No information was found about sites of attachment of the different stages of *A. tigrinum*. However, empirical observations from the authors showed that adult ticks were frequently fixed on the head and the neck of Carnivora. Present results suggest a similar pattern of attachment for the immature stages feeding on Rodentia (rats and *G. musteloides*)

The DNA sequences showed that both Argentinean populations belong to *A. tigrinum* and the same is considered true for the Brazilian ticks used by Labruna et al. (2002) for their laboratory study. Therefore, it was unexpected to find remarkable biological differences between these relative geographically close colonies. These differences may be bound to ecological adaptations or a distorted picture from laboratory studies. In any situation it would be of great value to carry out research under natural conditions of Argentina and Brazil to further understand differences in the ecology of *A. tigrinum* from distinct habitats.

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