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Field and laboratory studies in a Neotropical population of the spinose ear tick, *Otobius megnini*

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Abstract. One ear of each of five cows on a property close to Dean Funes, province of Córdoba, Argentina, was inspected monthly from December 2004 to November 2006 to determine the presence of *Otobius megnini* (Dugès) and to ascertain its seasonality. Ticks were collected to study the biological parameters of larvae, nymphs and adult ticks. Groups of nymphs were also maintained at three different photoperiods at 25 °C. The abundance of immature stages was greatest during January–April and August–October in the first and second years of the study, respectively. No larvae successfully moulted. Nymphs weighing < 17 mg also failed to moult, but 89% of heavier nymphs moulted into adults. Nymphs moulting to males weighed less (49.5 ± 16.09 mg) than nymphs moulting to females (98.1 ± 34.08 mg). The pre-moult period was similar for nymphs moulting to either sex and significantly longer ($P < 0.01$) for female nymphs maintained at 25 °C compared with nymphs kept at 27 °C. No effect of photoperiod on the pre-moult periods of nymphs was detected. Female ticks produced a mean of 7.0 ± 1.94 egg batches after a preoviposition period of 16.4 ± 8.41 days for the first batch. The mean oviposition period was 61 ± 20.8 days and the duration of oviposition for each batch varied from 1 to 6 days. The mean number of eggs per batch was 93.1 ± 87.53. The minimum incubation period for the first egg batch was 13.6 ± 2.77 days. The total number of eggs laid by each female was 651.6 ± 288.90. Parthenogenesis was not observed. The reproductive efficiency index (REI) (number of eggs laid/weight of female in mg) was 5.5 ± 1.26. Pearson's correlations showed a significant direct relationship between the weight of the female and number of eggs laid ($P < 0.01$) and REI ($P < 0.05$). Several of the biological values presented above for the tick population from the Neotropical zoogeographic region showed marked differences to equivalent values for *O. megnini* populations from the U.S.A. (Nearctic) and India (Oriental). Nevertheless, the only two sequences of 16S rDNA deposited in GenBank from ticks originating in Argentina and allegedly in the U.S.A. indicate that they are conspecific (99.8% agreement). We tentatively consider the biological differences among populations of this tick species to represent adaptations for survival at different conditions.

Key words. *Otobius megnini*, cattle, life cycle, seasonal occurrence, Argentina.

Introduction

Otobius megnini (Dugés), the spinose ear tick, is an argasid thought to be of Nearctic origin that has been able to colonize parts of the Neotropical, Afrotropical, Oriental (Keirans, 1992) and Palearctic regions (Özer & Aydin, 1996). Ticks are usually found deep in the external ear canal of domestic mammals such as cattle, sheep, goats, South American camelids, dogs and horses, but reports of human infestation are also quite frequent, as indicated by Keirans & Pound (2003). In addition to several deleterious effects on domestic animals (Parish, 1949; Bulman & Walker, 1979; Madigan *et al.*, 1995), *O. megnini* can cause otitis in man (Barbará & Dios, 1918), may play a role in the maintenance of the agent of Q fever in nature (Jellison *et al.*,

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1948) and may have been associated with paralysis of a child (Peacock, 1958).

Otobius megnini has a single-host lifecycle consisting of larval stages followed by a variable number of nymphal stages. However, there is disagreement regarding the number of nymphal stages. The parasitic phase may exceed 200 days (Hooker *et al.*, 1912; Jagannath & Lokesh, 1989). The last nymphal stage drops to the ground to moult into the non-parasitic adult stage. Fertilized female *O. megnini* lay several batches of eggs (Parish, 1949). Although *O. megnini* is considered to be adapted to arid and semi-arid conditions (Boero, 1957; Hoogstraal, 1973), it has been reported from fairly humid localities in Argentina and South Africa (Theiler & Salisbury, 1958; Guglielmone *et al.*, 1992).

Keirans & Pound (2003) listed 928 references to *O. megnini* during the period 1868–2000. About 7% of these refer to the Neotropical region and mainly to the presence on different hosts in various localities. Few report seasonality and even fewer contain information on life cycle. To increase our knowledge of this tick in the Neotropics, field and laboratory studies were conducted in the southern Neotropical region to determine if there are differences in life cycle parameters between *O. megnini* from the Neotropics and those from other zoogeographic regions.

Materials and methods

The field study was carried out on a property close to the city of Dean Funes (30°26′S, 64°20′W), province of Córdoba, Argentina. One ear of each of five cows was inspected monthly, from December 2004 to November 2006, between 08.00 h and 09.00 h, to determine the number of larvae and nymphs of *O. megnini* present. Larvae and nymphs were collected on each sampling day. They were weighed and then maintained at 27 °C or 25 °C, and 83–86% relative humidity (RH) to record moulting success, pre-moult period and sex. A *t*-test was used to compare the weights and pre-moult periods of nymphs moulting to males or females. In addition, nymphs were maintained at three different photoperiods (25 °C) of light : dark (LD) 12 : 12 h, ($n = 30$), 14 : 10 h ($n = 24$) and 10 : 14 h ($n = 24$). Analysis of variance was used to test for differences among groups maintained under different photoperiodic regimens.

Ten pairs of male and female ticks that had moulted in the laboratory were maintained together (at 27 °C) until the females had laid their first eggs, after which the males were removed; the time that elapsed between the introduction of the females to males and the laying of the first eggs was considered as the preoviposition period. The oviposition period was recorded and the number of eggs per batch was determined by collecting all eggs from each female in individual vials. Oviposition was considered complete when no new eggs were observed for at least 24 h. Time to first hatch (i.e. minimum incubation period) was recorded for the first batch of eggs deposited by each female by daily observations. The reproductive efficiency index (REI) described by Drummond & Whetstone (1970) (total eggs laid/ weight of female in mg) was also calculated. Pearson's correlation indices (r) were used to detect relationships between the weight of female ticks and number of eggs laid, preoviposition, oviposition and minimum incubation periods, number of batches of eggs and REI.

Ten female ticks were maintained without males for 87 days to determine if parthenogenesis occurred. Afterwards five of these females were placed with five males and the other five females remained alone.

16S r-DNA fragment sequences were obtained from Argentinean *O. megnini* as described by Mangold *et al.* (1998) to be compared with the same sequences from ticks of other origins deposited in GenBank.

Results

The farm where the study was performed is characterized by the presence of an old and poorly maintained concrete dairy barn surrounded by small paddocks with cement waterers (also poorly maintained) to which cattle have unlimited access. These characteristics are not shared with other farms in the area on which the presence of *O. megnini* on cattle is occasional.

The 16S rDNA sequence of the *O. megnini* in this study (GenBank accession no. EF120989) has 99.8% agreement with the only other corresponding sequence deposited in GenBank (L34325). The origin of the specimens from which this sequence was obtained is alleged to be the U.S.A., although this was not explicitly defined by the authors (Black & Piesman, 1994).

The abundance of larvae was higher during April–May and August–October during the first and second years of the study, respectively, whereas the number of nymphs was higher during January–April in the first year of the study, and during July–August in the second year (Fig. 1). The larvae were remarkably abundant during the final phase of the study period.

The mean weight of larvae ($n = 50$) was 1.98 ± 0.29 mg ($\pm$ standard deviation [SD], range 1.8–2.5 mg). No larvae were observed to moult.

Nymphs weighing < 17 mg did not moult ($n = 36$; mean weight 5.80 ± 3.44 mg, range 2.5–15.0 mg). The situation was different with nymphs weighing > 17 mg because 89.0% (162/182) moulted to adults. Table 1 summarizes weights and pre-moult periods for male and female nymphs maintained at 27 °C and 25 °C. Female nymphs were significantly heavier than male nymphs, but pre-moult periods were not statistically influenced by sex ($P > 0.05$). As expected, pre-moult periods for male and female nymphs were longer at 25 °C than at 27 °C; however, the difference for male nymphs was not statistically significant. Nymphs weighing > 15 mg, but dead before moulting ($n = 20$), had a mean weight of 57.6 ± 30.0 mg (range 20.0–132.0 mg). The weight range indicates that both sexes were represented in these unsuccessful nymphs. The mean moulting times of fed nymphs under different photoperiod conditions were: 10.8 ± 1.2 days (range 8–13 days) for LD 12 : 12 h; 11.35 ± 1.2 days (range 8–14 days) for LD 14 : 10 h, and 10.9 ± 0.9 days (range 9–12 days) for LD 10 : 14 h. Photoperiods did not significantly affect the pre-moult periods of engorged nymphs ($P > 0.05$).

Parthenogenesis was not observed. Females that were paired for 87 days after moulting oviposited successfully but their isolated counterparts did not. Table 2 presents information on

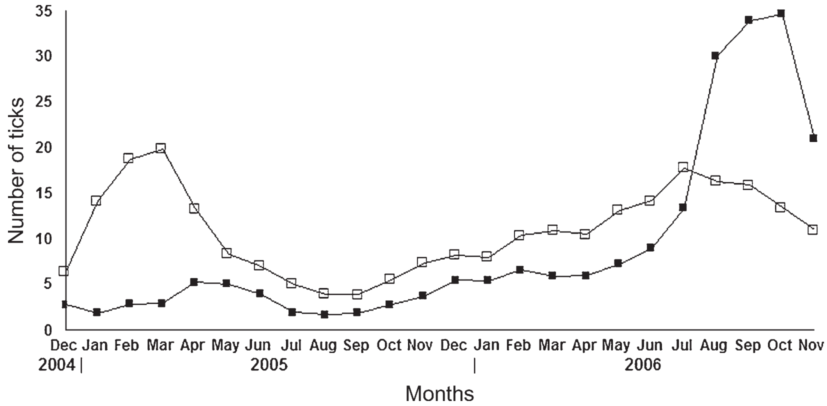

Fig. 1. Monthly moving average numbers of larvae (■) and nymphs (□) of *Otobius megnini* in the ears of cattle.

number of eggs, batches laid, preoviposition, oviposition and minimum incubation periods, and *r* in relation to female weight. The *r* was statistically significant for numbers of eggs laid and REI. The duration of oviposition for each batch was extremely variable: 25 lasted 1 day; eight lasted 2 days; 22 lasted 3 days; 12 lasted 4 days; two lasted 5 days, and one lasted 6 days. The number of eggs laid per batch was also extremely variable, with a mean of 93.1 ± 87.5 eggs (range 7–360 eggs). More than 50% of the total number of eggs were laid in the first three batches.

Discussion

Although *Otobius megnini* is established in the area of the study farm, infestation of cattle is not common. Dreyer *et al.* (1998) found that poorly maintained premises with cracks, crevices and grooves constitute a suitable environment where non-parasitic tick stages can take refuge. These conditions were observed on the study site, where cattle were consistently infested with *O. megnini*, which probably explains the difference between the level of parasitism on the study farm and levels on neighbouring farms.

Larvae and nymphs were found on cattle throughout the study period, but the periods of greatest abundance differed between the 2 years of the study. Guglielmone & Mangold (1986) described the general situation in Argentina, where larvae and nymphs of *O. megnini* were present in the greatest numbers in spring and autumn, respectively, and Medrano & Suárez (1983) found the highest infestation level on cattle in central Argentina to occur during the autumn. Similar discordant results have been reported in South Africa, where Theiler & Salisbury (1958) found no seasonal trend for *O. megnini*, but Dreyer *et al.* (1998) reported the highest infestation during the warmer months of the year. Drummond (1967) found seasonal peaks in different seasons during a 6-year-long study in southwestern Texas (U.S.A.).

The parasitic cycle of this argasid is extremely variable, ranging from 2 to 4 months (Hoogstraal, 1985), and from 118 to 207 days (Jagannath & Lokesh, 1989), although Hooker *et al.* (1912) stated that the parasitic period varied from 31 to 209 days. The lifespan of adult ticks may extend to almost 500 days (Parish, 1949) and the period of oviposition is also relatively long (Table 3). The motile, free-living stages of *O. megnini* are able to find refuge that partly protects them from climatic hazards (Dreyer *et al.*, 1998) and the current study shows that photoperiod does not appear to influence the pre-moult period of nymphs. The combination of these biological characteristics probably explains the presence of parasitic ticks all year round. However, the factors governing dissimilar seasonal patterns among years remain unknown.

No larvae collected from cattle moulted, which is probably a result of the manipulation required to remove these specimens from the ears of cattle. Wanchinga & Barker (1986) determined that few larvae extracted from the ears of cattle moulted in the laboratory, contrasting with high levels of moulting success for naturally detached larvae. Naturally detached larvae had a mean weight of 8.3 mg, considerably higher than the corresponding weight of larvae extracted from cattle ears in this study.

Boero (1957) reported *O. megnini* to have three nymphal instars, but most authors have shown only two nymphal instars (Brumpt, 1936; Cooley & Kohls, 1944; Loomis, 1961; Hoogstraal, 1985; Jagganath & Lokesh, 1989). By contrast, Wanchinga & Barker (1986) found one nymphal stage. Hooker *et al.* (1912) and Parish (1949) did not mention the presence of a second nymphal stage in *O. megnini.* In the current study there was no evidence that *O. megnini* has more than one nymphal instar. This important divergence on a crucial point in the lifecycle may indicate that the number of nymphal stages varies among populations.

Table 1. Means ± standard deviations and ranges for weight (in mg) and pre-moult periods (in days) at 27 °C and 25 °C, and *P*-values (*t*-test) for nymphs of *Otobius megnini* moulting to males or females.

Male nymphs	Female nymphs	*P*-value
Weight, mg		
$n = 59$	$n = 103$	
49.5 ± 16.09 (17.0–84.0)	98.1 ± 34.08 (43.9–190.0)	< 0.001
Pre-moult period at 27 °C		
$n = 29$	$n = 53$	
10.3 ± 1.67 (8–15)	10.5 ± 1.44 (8–13)	> 0.05
Pre-moult period at 25 °C		
$n = 30$	$n = 50$	
10.8 ± 1.48 (8–14)	11.4 ± 1.14 (9–14)	> 0.05

Mean values for nymphs of the same sex maintained at 27 °C or 25 °C are significantly different ($P < 0.05$).

Table 2. Means ± standard deviations (SDs) and ranges of weight, preoviposition*, oviposition and minimum incubation periods (days), number of eggs, number of batches and reproductive efficiency index (REI)† for 10 females of *Otobius megnini* maintained at 27 °C; *r* indicates Pearson's correlation coefficient for the corresponding parameter in relation to weight.

	Mean	SD	Range	*r*
Weight, mg	114.7	27.41	81–164	
Preoviposition period, days	16.4	8.41	7–29	0.141 ($P > 0.05$)
Oviposition period, days	61	20.80	30–88	0.062 ($P > 0.05$)
Minimum incubation period, days‡	13.6	2.77	9–17	–0.329 ($P > 0.05$)
Number of eggs	651.6	288.90	398–1187	0.931 ($P < 0.001$)
Number of batches	7.0	1.94	4–10	0.044 ($P > 0.05$)
REI	5.5	1.26	3.9–7.5	0.708 ($P < 0.05$)

*Time between a female being deposited in a vial with a male and the first eggs being laid.
†REI = total number of eggs/weight of females (mg) (Drummond & Whetstone, 1970).
‡Evaluated for the first batch of eggs laid for 10 females.

There have been several reports on the lifecycle of *O. megnini*, but comparisons are difficult because the temperatures at which the ticks were maintained varied. For purposes of comparison, we used the studies of Wanchinga & Barker (1986) in the U.S.A. (Nearctic region), where ticks fed on cattle and were kept at 26 °C, and Jagannath & Lokesh (1989) in India (Oriental region), where ticks originating from horses were maintained on rabbits and kept at 28 °C. The results are presented in Table 3 with notes that refer to other studies when relevant.

The female : male ratio was substantially higher in the Argentinean population of *O. megnini* than in the U.S. population. The mean weight of nymphs moulting into males was significantly lower than that of nymphs moulting into females in both the American and Argentinean populations. Nevertheless, weight is not a useful predictor for sex because of the overlap between male and female nymph weights in the Argentinean population (equivalent information was not provided for the U.S. population). Rates of moulting success of nymphs from Argentina, India and the U.S.A. were similarly high, although pre-moulting and preoviposition periods differed. In the present study the preoviposition period was considered to be the time between the placing of a female with a male and the first egg being laid, which included pre-copulation time. However, it is not clear if this period was defined similarly in the other studies. Egg incubation periods were quite similar in the American and Argentinean tick populations (1.2 days of difference) and considerably lower than the period recorded for the Indian population. The mean number of eggs laid per female in the Neotropical populations (651.6) was intermediate between those recorded in the American and Indian populations, but all of them were substantially lower than the median egg number (1444) registered by Parish (1949) for another American population of *O. megnini*. Wanchinga & Barker (1986) reported that the number of eggs laid was directly related to female weight, a finding that reflected the observations of the current study, in which we found a direct relationship between female weight and REI, although the mean REI of Argentinean ticks was low compared with the corresponding value in the U.S.A. population.

Indeed, there are several remarkable biological differences among *O. megnini* populations. Nevertheless, the two sequences

Table 3. Data from biological studies on *Otobius megnini* of different origins. Means ± standard deviations and ranges are given when available.

	Wanchinga & Barker (1986)	Jagannath & Lokesh (1989)	Present study
Origin	U.S.A.	India	Argentina
Maintenance temperature, °C	26	28	27
Sex ratio, F : M	1.3 : 1	No data	1.7 : 1
Nymphal engorgement weight, mg	78.3 ± 53.2	No data	77.8 ± 37.7, 20–190
Pre-moulting period, days	7.5 ± 1.6	14–42	10.4 ± 1.5, 8–15
Moulting success, %	98.1 ± 1.9	85.3	92.4
Preoviposition period, days	5.4 ± 2.5*	20.6 ± 0.79, 16–26	16.4 ± 8.41, 7–29
Oviposition period, days	71.2 ± 13.5, 58–85	32 ± 2.68, 21–51	61 ± 20.8, 30–88
Minimum incubation period, days	12.4 ± 2.5	21.0 ± ??¶, 19–23	13.6 ± 2.77, 9–17
Total number of eggs†	789.9 ± 199.5	476.3 ± 54.91, 241–863	651.6 ± 288.9, 398–1187
Number of egg batches‡	No data	No data	7.0 ± 1.94, 4–10
REI§	9.6 ± 2.08	No data	5.5 ± 1.26, 3.9–7.5

*It is not clear if this period is equivalent to that in the Argentinean study where the preoviposition period was considered as the time between a female being placed with a male and the first egg being laid.
†Parish (1949) reported a mean of 1444 eggs per female.
‡Parish (1949) registered a mean of 5.8 batches of eggs (range 2–11).
§REI = total number of eggs/weight of females (mg) (Drummond & Whetstone, 1970).
¶Value was not indicated by the authors (Jagannath & Lokesh, 1989).

for 16S rDNA from *O. megnini* originating from Argentina and allegedly originating from the U.S.A. suggest that they are conspecific. Therefore, we tentatively consider the biological differences among populations of this tick species as representative of adaptations for survival under different conditions. Apart from this general reasoning, aspects of the biology of *O. megnini*, such as factors governing seasonality and the numbers of nymphal instars, remain obscure. Further investigations are needed to properly understand the biology of *O. megnini*.

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