

Rhipicephalus (Boophilus) microplus Ticks can Complete their Life Cycle on the Water Buffalo (*Bubalus bubalis*)

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Abstract: *Rhipicephalus (Boophilus) microplus* is considered one of the most important ectoparasites of cattle worldwide. Due to the increase in the number of water buffaloes (*Bubalus bubalis*) in *R. microplus*-infested areas, this study was designed to determine whether these ruminants are able to sustain the complete tick life cycle. To this aim, a seven-month old water buffalo of the Mediterranean breed and a Holstein bovine of the same age, both tick-naïve, were infested with *R. microplus* tick larvae, and the parasitic and non-parasitic tick stages were analyzed and compared. The studied parameters include the number of recovered engorged females, the time points at which the first and last engorged females fell to the ground; the pre-oviposition duration, the percentage of hatching and the reproductive efficiency index. No statistically significant differences were found between the buffalo and the bovine in all parameters measured. It was concluded that the water buffalo can act as a suitable reservoir for *R. microplus* ticks. These results should be taken into account when implementing tick control and eradication campaigns in water buffalo grazing lands.

Keywords: *Boophilus microplus*, cattle tick, tick epidemiology, *Bubalus bubalis*, tick egg.

INTRODUCTION

Rhipicephalus (Boophilus) microplus is considered to be the most widespread tick parasite of livestock. Only in Central and South America, about 175 million cattle are exposed to this tick, which corresponds to approximately 70% of the cattle raised in this area [1]. The negative impact of ticks on cattle production is due to both direct and indirect effects. The first include irritation, weight loss, and damage of leather. Among the second, the most important are the transmission of tick-borne pathogens and myiasis at the point of bite [2-3]. Acaricides are the primary means used to control tick infestations. However, the misuse of some of these products, such as pyrethroids and organophosphates, has led to the evolution of resistant tick populations [4]. This factor, together with potential tick reservoirs in wild and/or domestic animals, can hamper the success of tick-eradication campaigns. In fact, *R. microplus* has been shown to parasitize the white-tailed deer (*Odocoileus virginianus*) in the United States [5] and the cervids *Cervus rufus* ("guazú pita") and *C. simplicornis* ("guazú birá") in Argentina [6].

In *R. microplus*-endemic areas of South America, water buffaloes (*Bubalus bubalis*) are increasingly popular as alternative to cattle both for meat and milk production. The first water buffaloes in America were introduced in the French Guyanas from Indochina in

1859. Brazil received the first buffaloes in 1890, and now, their numbers surpass 3.5 million in that country [7]. In Argentina, they were first imported in 1976, currently reaching 80,000 head [7]. Buffaloes present a number of advantages over bovines in tropical and subtropical cattle-raising fields, including better adaptation to warm temperatures, floodable lands and poor pastures [8], and a higher resistance to different infectious agents [9].

Bovines and buffaloes often share the same grazing areas, and are thus exposed to the same ecto and endoparasites. Since buffaloes are generally regarded as resilient to most infections, sanitary control of these animals is less stringent than for bovines in the same regions. *R. microplus* ticks have been observed parasitizing water buffaloes in the Northeastern province of Corrientes, Argentina [10-11]. In the frontier region of Peshawar, Pakistan, water buffaloes were found to be parasitized by ticks of the genera *Boophilus* sp., *Hyalomma* sp. and *Rhipicephalus* sp., with percentages of parasitized animals of 53%, 31% and 24%, respectively [12]. Also, Kakar and Kakarsulemankhel reported the presence of *Boophilus* sp. ticks on water buffaloes of the Pakistani Quetta City region (6.9 % parasitized animals) [13].

Additionally, in a study carried out in Argentina, indirect evidence of infestation of water buffaloes with *R. microplus* was obtained by the detection of DNA of the tick-transmitted hemoparasites *Babesia bovis* and *B. bigemina*, as well as of antibodies against a *B. bovis* surface protein in bubaline blood and serum, respectively [14].

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Given these observations, the present investigation was designed to analyze whether *R. microplus* ticks can complete their life cycle on water buffaloes, and whether they constitute a tick reservoir of epidemiological relevance.

MATERIALS AND METHODS

Tick Infestation of a Water Buffalo and a Bovine

A male water buffalo of the Mediterranean breed, that was seven months of age, and a male Holstein heifer of the same age, both from tick-free regions of Argentina, were maintained under controlled conditions in separate open-air corrals at the Agricultural Experimental Station (EEA) of Mercedes, National Institute of Agricultural Technology (INTA), Argentina. Each animal was infested with 10^4 15 day-old *R. microplus* tick larvae on their withers (day 0). Larvae were obtained from a hemoparasite-free tick colony at EEA-Mercedes, INTA.

Infestation with larvae was carried out as described by FAO [15], using a girdle that held two plastic vials, each of which had a hole in the bottom. Larvae were allowed to escape through these holes and to freely distribute on the animals. The experiment was carried out in summer. Temperatures oscillated between 17.6 °C and 32.7 °C and relative humidity varied from 64.5 to 74% during the course of the study. At day 18, all engorged females present on the left side and measuring between 4.5 and 8 mm in length were counted in both animals. Additionally, the time points when the first and the last engorged females detached and fell to the ground were recorded.

Tick Life Cycle Parameters

Ten engorged females that had just detached from the bovine corral and the same number from the buffalo corral were collected separately and transported to the laboratory. All ticks were washed for one minute with distilled water, individually weighed, placed in separate Petri dishes, and incubated at 27 °C \pm 1°C and 80 – 85% relative humidity. Ticks were observed daily to record the following parameters [16]: (i) the period elapsed between the detachment of the last engorged female and the oviposition (pre-oviposition time); (ii) the period during which the engorged female deposited eggs (oviposition time); (iii) the period between the beginning of oviposition and the eclosion of the first larvae (incubation time); (iv) the number of eggs deposited by each female; (v) the

number of tick larvae that were borne from each female; (vi) the percentage of hatched larvae with respect to the total number of eggs deposited by each female (% eclosion); (vii) the number of eggs per mg of female weight (Reproductive Efficiency Index, REI). The values for each measured parameter corresponding to each female were recorded separately and means \pm SD were subjected to statistical analysis using a one-way analysis of variance (ANOVA), and differences between means were determined by Tukey's method.

RESULTS

After 18 days post-infestation of a heifer and a water buffalo infested with 10,000 *R. microplus* larvae, all engorged tick females were counted on the left side of each animal. The obtained values were multiplied by 2 (to take into account both sides) and then again by 2 (considering a female/male ratio of 1:1). The resulting values were 540 for the buffalo and 1200 for the bovine, i.e. 5.4 % and 12 % of the initial larvae remained on each animal, respectively. Thus, the degree of resistance to infestation in each case, which corresponds to the reciprocal of these values, was 94.6% for the buffalo and 88% for the bovine. Although these results correspond to single individuals and, thus, are not suitable for statistics analysis, they suggest that the buffalo had a tendency for a higher resistance to tick infestation than was observed in the bovine host. Interestingly, a local inflammatory reaction was frequently observed in the buffalo skin at sites where a large number of ticks had attached (Figure 1). This type of reaction was not observed in the bovine skin.

The mean weight values of engorged females, on the other hand, were similar and not significantly different between the bovine and the buffalo (0.251 ± 0.120 and 0.268 ± 0.40 g, respectively).

In both cases, the first engorged female detached at day 21. However, the last engorged female detached at 35 days in the case of the bovine, and 31, in the case of the buffalo. These results are in line with those on the resistance to infestation, again showing a tendency for the buffalo to be a slightly less suitable tick host than the bovine.

Engorged females collected from the bovine and the buffalo were allowed to oviposit under controlled conditions of temperature and humidity. The timing of this process, as well as the number of deposited eggs and born larvae are shown in Table 1.



Figure 1: Buffalo skin of the chest region infested with *R. microplus* ticks. The arrows point at adult female ticks in early stage of engorgement (a); nymphs (b); and inflammatory reactions in the skin (c).

Table 1: Comparison of Reproductive Parameters Measured in Engorged *R. microplus* Female Ticks Infesting a Water Buffalo and a Bovine. All Measurements were Carried Out with 10 Specimens Collected from each Type of Ruminant and are Indicated as Averages ± SD

Parameter	Buffalo-fed ticks (n=10) ^a	Bovine-fed ticks (n=10) ^a
Pre-oviposition (days)	2.9 ± 1.6	3 ± 0
Oviposition (days)	5.9 ± 3.5	6.7 ± 1.4
Incubation (days)	17.7 ± 9.39	23.1 ± 0.74
Nº of eggs	1821.7 ± 1024	2248 ± 272.82
Nº of larvae	1410.1 ± 787.7	1528.6 ± 259.48
% eclosion	78.25 ± 4.78	68.83 ± 13.86
REI	8.45 ± 5.58	8.53 ± 3.96

^aMeans within each row are not significantly different (p<0.5) tested by Tukey’s method.

There were no significant differences between the bovine-fed and the buffalo-fed female ticks in pre-oviposition, oviposition, and incubation periods. The

mean number of eggs deposited by buffalo-fed ticks was lower than that of bovine-fed ticks (1821.7 ± 1024 vs 2248 ± 272.82), but the difference was not

statistically significant. Likewise, the values corresponding to number of larvae, % eclosion, and REI were similar for both host animals, with no significant differences among any of the means. These results clearly showed that *R. microplus* tick larvae fed, grew, molted, mated and oviposited both on the water buffalo as on the bovine used in this experiment. Additionally, the viability of the deposited eggs was similar in both cases.

DISCUSSION

This study tested the capacity of a bovine and a buffalo to act as hosts for *R. microplus* ticks.

The value of resistance to tick infestation obtained for the bovine in this experiment (88 %) is similar to that described by Villar Cleves [17] for a Holstein heifer (85%). The value observed for buffaloes (94.6 %) compares well with those of Brahman cattle and their cross-breeds, which have been reported as 95-99% [17]. The time point when the first engorged female detaches determines the duration of the parasitic phase of the tick. The value obtained for the bovine (21 days) coincides with reports of Ivancovich [6] and Nuñez *et al.* [3], who determined a time of 20 days in spring/summer and 21 to 23 days in winter. In our study, which was performed during summer, the value was identical for both the bovine and the buffalo. The drop of the last engorged female was 4 days-shorter for the buffalo than for the bovine. For the latter, this time point fell well within the time reported previously by Nuñez *et al.* [3] (up to 41 days). Although a statistical analysis of these data was not possible, it could suggest, together with the difference in resistance to infestation observed, that the water buffalo is somewhat less suitable than the bovine as a host for *R. microplus* ticks. A possible explanation could be the thick skin of the buffalo that reduces the ability of these ticks to attach because of their short hypostome, as compared to bovines. Additionally, according to the inflammatory reactions observed in this work, the buffalo immune system appears to be more reactive than that of the bovine to tick saliva allergenic components. Under natural conditions, an additional constraint for tick infestation of buffaloes might be associated to their habit of spending considerable time immersed in water or rolling in the mud, which constitutes a natural means of controlling ectoparasites [7].

Importantly, 5.4 % of the larvae that initially infested the buffalo reached the final stage of their life cycle.

This indicated that at least some of the larvae were able to perforate the thick buffalo skin and obtain the necessary nutrients from its blood that allowed them to survive, molt twice to the stages of nymph and adult, and mate. Fertilized females feed constantly while eggs are maturing, and increase their size several times (engorgement), a process that took place both in the buffalo and the bovine.

No significant differences were observed between the two groups of ticks with respect to pre-oviposition, oviposition, and incubation periods. Also the mean numbers of eggs deposited per female, the mean number of larvae that hatched, and thus the % eclosion, as well as the REI indexes were similar for both groups. All parameters recorded for bovine-fed ticks were close to the values recorded by others [6], strengthening the validity of these observations.

The results of this study point at the water buffalo as an important reservoir for *R. microplus* ticks. This information is highly relevant for epidemiological studies, and should also be taken into account when tick-eradication efforts take place in areas where bovines and water buffaloes are raised in close proximity.

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REFERENCES

- [1] Montenegro-James S. Prevalence and control of babesiosis in the Americas. Mem Inst Oswaldo Cruz 1992; (Suppl 3); 27-36.
<http://dx.doi.org/10.1590/S0074-02761992000700003>
- [2] Cetra B. La garrapata del bovino. Noticias y comentarios N° 203, INTA Mercedes Corrientes, 1995.
- [3] Nuñez J, Muños Cobeñas M, Moltedo H. *Boophilus microplus* la garrapata común del ganado vacuno. Editorial Hemisferio Sur S.A. Buenos Aires 1997; pp. 3-7
- [4] Guglielmone AA, Castelli M, Mangold A, Aguirre DH, Alcaraz E, Cafrune MM, Cetrá B, Luciani CA, Suárez VH. El uso de acaricidas para el control de *Rhipicephalus (Boophilus) microplus* (Canestrini) (Acari: Ixodidae) en la Argentina. Revista de Investigaciones Agropecuarias, INTA Argentina 2007; pp. 155-167.
- [5] George JE. Wildlife as a constraint to tick eradication of *Boophilus microplus* spp. Animal Production and Health Report 75, chapter 5: The eradication of ticks. Food and

- Agriculture Organization of the United Nations (FAO), México 1987; pp. 146-153.
- [6] Ivancovich JC. Bioecología de la garrapata del Ganado, *Boophilus microplus* (Canestrini, 1888). Revista de Investigaciones Agropecuarias, INTA Argentina 1981; pp. 1-54.
- [7] Crudeli G, Patiño EM, Cedres J, Gonzales Fraga J, Maldonado Vargas P, Raciopi O, Zava M, Pellerano G, (Crudeli et al., eds.) Búfalos en la Argentina. Editorial Moglia, Corrientes, Argentina 2004; pp. 17-53.
- [8] Cadoppi A. Development for the Demand for High Quality Water Buffalo Meat and Elaborated Meat products In Argentina and Germany. Revista Veterinaria 2010; 21(Suppl 1); 268.
- [9] Ottley M. The water Buffalo: New prospects for an underutilized animal. Report of an *Ad Hoc* Panel of the Advisory Committee on Technology Innovation Board on Science and Technology for International Development Commission on International Relations, National Research Council, National Academy Press, Washington D.C., USA 1984; pp. 66-72.
- [10] Benitez D, Sarmiento N, Bevans W. Prevalence of *Rhipicephalus microplus* in a herd of buffalo in Corrientes province. Revista Veterinaria 2010; 21(Suppl 1): 750-52.
- [11] Muños Cobeñas M, Barci D, Popovici A. Ectoparasitosis encontradas en un rodeo de búfalos (*Bubalus bubalis*). Veterinaria Argentina 1987; 38; pp. 724-727.
- [12] Manan A, Khan Z, Ahmad B, Abdullah. Prevalence and identification of Ixodid Tick genera in Frontier region Peshawar. J Agric Biol Sci 2007; 2(Suppl 1); 21-25.
- [13] Kakar MN, Kakarsulemankhel JK. Prevalence of endo (trematodes) and ecto-parasites in cows and buffaloes of Quetta, Pakistan. Pak Veter J 2008; 28(Suppl 1); 34-36.
- [14] Ferreri L, Benitez D, Domínguez M, et al. Water Buffalos as carriers of *Babesia bovis* in Argentina. Ann NY Acad Sci 2008; 1149; 149-51.
<http://dx.doi.org/10.1196/annals.1428.036>
- [15] Food and Agriculture Organization of the United Nations (FAO). The epidemiology of tick-borne diseases: diagnosis and differential diagnosis; The epidemiology of tick-borne diseases: serological diagnostic tests. In: FAO ed. Ticks and tick-borne disease control. A practical field manual. Vol. II, Rome: FAO/UNO, 1984:300-332; 333-372.
- [16] Guglielmone AA, Mangold A, Aguirre DH, Gaido A. The effect of infection by *Babesia* sp. on some biological parameters of engorged females of *Boophilus microplus*. Folia Parasitologica 1989; 36; pp. 1-6.
- [17] Villar Cleves C. Cruzamientos genéticos, una alternativa para controlar la garrapata del ganado. Infoganadero Revista Gráfica, Costa Rica, 2008; pp. 42-44. Available from: <http://www.infoganaderocentroamericano.com>.