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Strategic control of *Rhipicephalus* (*Boophilus*) *microplus* infestation on beef cattle grazed in *Panicum maximum* grasses in a subtropical semi-arid region of Argentina

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ABSTRACT

The aim of this work was to test the efficacy of strategic control methods of *Rhipicephalus microplus* infestation on beef cattle grazed in *Panicum maximum* grasses in northwestern Argentina. Also, an analysis to discern how the *R. microplus* population was distributed amongst cattle was also performed to determine if partial selective treatment or cull the small proportion of more heavily infested animals are feasible options to control this tick. The strategic scheme of treatments was designed to act on the small 1st generation of *R. microplus* in early spring and prevent in that way the appearance of the annual peak of abundance of *R. microplus* in summer and autumn. Animals of the group 1 were treated with ivermectin 3.15% on day 0 (25th September 2015), with fluazuron on day 32 (27th October 2015) and with fipronil on day 75 (9th December 2015). Animals of group 2 formed the control group. The overall effect of the treatments was positively significant. The number of ticks observed on the control group was significantly higher than the number of ticks observed on the treated group in all post-treatment counts ($P < 0.01$), with the only exception of the count of March. The distribution of parasites among cattle in all counts was adjusted to the negative binomial distribution, but a temporal variation in the tick aggregation levels associated to changes in tick abundance was found. The higher the abundance of *R. microplus*, the lower the aggregation. It was found that the steers (15.8% of the total number of animals evaluated) belonging to the high infestation group accounted for 23.0% of the total ticks. The strategic control method evaluated during this study provides a remarkable overall effect against *R. microplus* because it significantly reduces the tick infestation on cattle with only three applications of acaricides in one-year period. The analyses of tick distribution amongst cattle suggest that partial selective treatment and culling do not represent feasible methods to control *R. microplus* infestation on cattle.

1. Introduction

The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* constitutes an important constraint to cattle production in tropical and subtropical regions worldwide. The economic losses due to the parasitism of *R.* (*B.*) *microplus* ticks and the haemoparasites they transmit are associated with reduced weight gain and milk production, hide damage, mortality, morbidity, control costs and facilitates the occurrence of screwworm myiasis in cattle (Späth et al., 1994; Reck et al., 2014b). The wide use of chemical tickticides also represents a major problem resulting in multidrug resistance to most commercially available acaricides and the accumulation of chemical residues in meat or milk (George et al., 2008; Nari Henrioud, 2011; Guerrero et al., 2012; Reck et al., 2014a; Klafke et al., 2017).

The advantages of using methods to control cattle ticks that minimize the number of applications of chemical acaricides are linked to the reduction of the frequency and number of treatments and, hence, reducing control costs. In this sense, strategic methods of tick control tested by Nava et al. (2014, 2015) in subtropical subhumid regions of northwestern Argentina appear to be feasible in regions where the dynamics of this tick species is characterized by three annual generations with an increase in abundance from mid- or late spring to autumn and then a decrease towards winter and early spring (Nava et al., 2013, 2015; Canevari et al.,

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2017; Mastropaolo et al., 2017). These methods are based on the application of no more than three chemical treatments per year from late winter to late spring to act on the small cohorts of *R.* (*B.*) *microplus* and prevent a rise in the larger generations in summer and autumn (Nava et al., 2014, 2015). The ultimate objective of the strategic control of ticks is to provide acceptable levels of control while minimizing the number of annual applications of acaricides.

Progress in crop production technology in Central Argentina has displaced the cattle industry towards the north of the country (Paruelo et al., 2005), where one of the grass species extensively used in grazing systems is *Panicum maximum* (Brizuela and Cangiano, 2011); however trials to evaluate the efficacy of strategic control methods of *R.* (*B.*) *microplus* on cattle were not performed under this grazing systems. Therefore, the aim of this work was to test the efficacy of the strategic control method against *R.* (*B.*) *microplus* in a semi-arid region of Argentina where *P. maximum* grasses is widely used in cattle production. Besides differences in the type of grass, the current study differs also from the previous works of Nava et al. (2014, 2015) in another two relevant factors: climate of the study area and timing of the first treatment (see details in materials and methods). Differences in type of grass and climate are important because they have influence on the non-parasitic phase *R.* (*B.*) *microplus*, which determines the abundance of tick larvae in the pastures, and the other factor, timing of the first treatment, will differentially affect the population dynamic of the parasite and consequently the treatment efficacy. These three factors potentially imply a different defy to treatment. Finally, an analysis to discern how the *R.* (*B.*) *microplus* population was distributed amongst cattle across the study period was also performed in order to determine if partial selective treatment, defined as the application of an acaricide to the most infested portion of the herd (Molento et al., 2013), or cull the small proportion of more heavily infested animals, as proposed in Wilkinson (1962) and Sutherst and Utech (1981), might be feasible options of tick control under the conditions of the present work.

2. Materials and methods

The study was carried out in Quimilí (27°38′S, 62°25′W), Santiago del Estero Province, Argentina. This area belongs to the Dry Chaco ecoregion as defined by Burkart et al. (1999) and the climate is typified as subtropical semi-arid with an annual rainfall of approximately 600 mm concentrated from October to March (spring–summer). The study site is representative of the agroecological conditions of northwestern Argentina where extensive cattle breeding systems involving a little more than three million bovines (SENASA, Argentina: URL: http://www.senasa.gob.ar/serie-historica-existencias-bovinas-por-categoria-y-departamento-2008-2016) are mostly developed in areas characterized by partial deforestation and introduction of non-native grasses for livestock forage.

The trial to control *R.* (*B.*) *microplus* was performed from September 2015 (early spring) to June 2016 (early winter). Cattle used in the trial were kept in tick infested paddocks entirely covered by pastures of *P. maximum* var. *Gatton panic*. Braford steers naturally infested with *R.* (*B.*) *microplus* were divided on day 0 into two homogeneous groups of 20 animals each according to the level of *R.* (*B.*) *microplus* infestation (Kruskal-Wallis test, $P > 0.05$). They were kept in two paddocks of 20 ha each. Animals of the group I were treated with a commercial injectable (subcutaneous) formulation of ivermectin 3.15% (IVOMEC GOLD®, Merial Argentina S.A.) at a rate of 1 ml/50 kg of body weight on day 0 (25th September 2015), with 1 ml/10 kg of a pour-on formulation of fluazuron (ACATAK®, Novartis Argentina S.A.) on day 32 (27th October 2015), and with 1 ml/10 kg of a commercial pour-on formulation of fipronil (ECTOLINE®, Merial Argentina S.A.) on day 75 (9th December 2015). Animals of the group II formed the control group. Application dosages of each drug were determined following the manufacturer's protocol. This scheme of treatment was designed to act on the small cohorts of *R.* (*B.*) *microplus* in early spring and prevent in that way the appearance of the annual peak of abundance of *R.* (*B.*) *microplus* in autumn explained before.

Counts of *R.* (*B.*) *microplus* females (4.5–8.0 mm long) were monthly performed on one side of each steer. Counts were made during the last week of each month. The number of ticks collected on steers was multiplied by two for statistical analyses. Prevalence (number of hosts infested/number of hosts examined), mean number of ticks (number of ticks/number of hosts examined, including both infested and non-infested hosts) and median with first and third quartiles (1Q–3Q) were calculated. Shapiro-Wilk's test was applied to test the normality of the data prior to statistical analysis, which revealed significant deviations from the normal distribution. Thus, the statistical significances of the differences in the distribution of *R.* (*B.*) *microplus* numbers between the two groups were determined by using the non-parametric Mann-Whitney test. Differences were considered significant at $P < 0.01$. The corrected efficacy percentage was calculated with the modified Abbot's formula by using the mean number of ticks (Henderson and Tilton, 1955). The corrected efficacy percentage was calculated only when the counts in the treated groups were significantly lower than the count in the control group.

An additional analysis was conducted to assess the distribution of *R.* (*B.*) *microplus* abundance among cattle. Twenty Braford steers were allowed to range freely in a 20 ha paddock of *P. maximum* from September 2015 to June 2016 (corresponding to the control group of the experiment of strategic control above-described). This group was submitted only to palliative treatment with deltamethrine 1% (BUTOX® POUR-ON, Intervet Argentina S.A.) at a rate of 1 ml/10 kg of body weight, once during the trial period, on day 158 (2nd March 2016), in order to avoid severe infestation drawbacks. Infestation with *R.* (*B.*) *microplus* ticks was quantified monthly. The prevalence, mean abundance, median (M), variance to mean ratio and the index of discrepancy (*D*) (Poulin, 1993, 2007) were calculated to examine the distribution of parasites within the cattle hosts by using the program Quantitative Parasitology 3.0 (Rózsa et al., 2000). *D* quantifies aggregation as the departure between the observed parasite distribution and a perfectly uniform distribution, it can be employed to compare distributions that vary in prevalence or mean number of parasites per host (Poulin, 1993, 2007). In *D*, 0 means null aggregation (all hosts with equal level of infestation) and 1 means complete aggregation (all members of a parasite population on one individual host). All these parameters were analyzed jointly with data obtained by the authors and presented in previous studies (Nava et al., 2015; Canevari et al., 2017), in order to evaluate the relationship between parasites abundance and aggregation (stocking rate in all studies was 1 animal per hectare).

To analyze individual variation in tick infestation levels through time, the steers were monthly divided into three groups taking into account the percentiles of infestation 33% and 66%. In this way, each animal was classified monthly in one of the following groups: a) low infestation (<percentile 33%), b) medium infestation (percentile 33% to percentile 66%) and c) high infestation (>percentile 66%). Then, considering the monthly classification obtained with this criteria, the steers were classified into three groups as follows: a) low infestation (at least 6 months with low infestation classification), b) high infestation (at least 6 months with high infestation classification), c) medium infestation (rest of the animals). The level of infestation among the animals in the three groups was evaluated using a generalized lineal model of repeated measures using a Poisson distribution as link function for the tick load. Statisti-

Table 1
Prevalence, mean, median (M) and first and third quartiles (1Q–3Q) of *Rhipicephalus* (*Boophilus*) *microplus* females 4.5–8.0 mm long of the treated (group 1) and control (group 2) steers. The efficacy percentage (EP) in numbers of ticks in the group1 in relation to group 2 is also shown. NA: not applicable. Animals of the group 1 were treated with ivermectin 3.15% on day 0 (26th September 2015), fluazuron on day 31 (27th October 2015) and fipronil on day 74 (09th December 2015).[a]

Month	P (%)	Group 1			P (%)	Group 2	
		Mean	M (1Q–3Q)	EP (G1–G2)		Mean	M (1Q–3Q)
September	95.0	5.9[a]	4(2–8)	NA	95.0	5.5[a]	4(2–6)
October	5.0	0.2[a]	0(0–0)	99.78	100.0	85.6[b]	77(52–108)
November	10.0	0.2[a]	0(0–0)	94.67	80.0	3.5[b]	2(2–4)
December	0.0	0[a]	0(0–0)	100	100.0	50.8[b]	44(16–70)
January	80.0	6.2[a]	3(2–8)	61.72	94.7	15.1[b]	14(8–22)
February	0.0	0[a]	0(0–0)	100	100.0	238.8[b]	236(154–314)
March	100.0	47.9[a]	46(34–62)	NA	100.0	66[a]	60(34–100)
April	72.2	7.2[a]	6(0–8)	87.19	100.0	52.4[b]	48(30–72)
May	16.7	0.3[a]	0(0–0)	99.71	100.0	97.7[b]	96(66–128)
June	66.7	2.1[a]	2(0–4)	90.58	94.7	20.8[b]	18(8–32)

Mann–Whitney test. Numbers not sharing superscripts are significantly different (P < 0.01).
[a] The control group was submitted to a palliative treatment with deltamethrine 1% on day 158 (2nd March 2016).

Table 2
Distribution of *Rhipicephalus (Boophilus) microplus* females (4.5-8.0 mm long) among cattle. Mean (ME) and median abundance (*M*), first and third quartiles (1Q–3Q), variance to mean ratio (V:M) and discrepancy index (*D*) are shown.

Month	ME	*M* (1Q–3Q)	V:M	*D*
September	5.5[ab]	4 (2–6)	3.7	0.39
October	85.6[cd]	77 (52–108)	25.3	0.29
November	3.5[a]	2 (2–4)	3.5	0.47
December	50.8[c]	44 (16–70)	25.9	0.37
January	15.1[b]	14 (8–22)	8.4	0.34
February	239.0[e]	236 (154–314)	48.6	0.24
March	66.0[cd]	60 (34–100)	25.8	0.33
April	52.4[c]	48 (30–72)	18.2	0.30
May	97.7[de]	96 (66–128)	12.7	0.19
June	20.8[b]	18 (8–32)	12.5	0.41

Kruskal–Wallis test with *a posteriori* Dunn's multiple comparison. Numbers with different superscripts are significantly different (*P* < 0.01).

cal analyses were performed using InfoStat software (Universidad Nacional de Córdoba, Argentina). A linear regression analysis was carried out to determine the relationship between tick abundance (expressed as median) and level of aggregation (measured as *D*). Arcsine transformation was applied to normalize the data.

3. Results

The effect of the control scheme against *R.* (*B.*) *microplus* evaluated during this study is shown in Table 1, where data are expressed as prevalence, mean number of ticks, median and efficacy percentage. The overall effect of the treatment was positively significant (Table 1; Fig. 1). The number of ticks observed on control group (Group 2) was significantly higher than the number of ticks observed on the treated group (Group 1) in all post-treatment counts (P < 0.01), with the only exception in the count of March. In this count the lower number of ticks in the treated group than in the control group, failed to reach statistically significance (Table 1).

The efficacy percentage of the control scheme applied on the animals of Group 1 was more than 87% during the study period (Table 1), with the exception of the counts of January (61.72%) and March when the efficacy percentage was not calculated because the non-significant statistical difference between the treated and control groups. Absolute control (mean: 0 in treated group) was only reached in the counts of December and February (Table 1). The mean number of ticks in the group of animals subjected to strategic control was always less than 10, with the aforementioned exception of the count of March (Table 1).

Rhipicephalus (*Boophilus*) *microplus* abundance levels for the control group varied significantly throughout the study (Table 2), four peaks of abundance occurred, among which the February peak was the most important (Fig. 1). The distribution of parasites among cattle in all counts was adjusted to the negative binomial distribution (P > 0.05) (Chi-square test), however aggregation (expressed as *D*) varied throughout the study (Table 2). When aggregation and abundance data from the current work were analyzed jointly with data from previous studies (Nava et al., 2015; Canevari et al., 2017) a significant inverse relationship between the abundance and the degree of aggregation was observed (r: 0.80; r^2: 0.74) (Fig. 2). Thus, the higher the abundance of *R.* (*B.*) *microplus*, the lower the aggregation index, until reaching a threshold value above which an increase in abundance was not necessarily translated into lower levels of aggregation.

Five animals were classified in the low-infestation group over the ten-month sampling, while eleven and three animals were classified in the medium- and high-infestation groups, respectively. The tick load was different (P < 0.001) among the three groups of animals. The group of low-infestation animals had an average tick load throughout the study of 48.48, while the medium and high infestation groups had average loads of 62.25 and 94.80 ticks per animal, respectively. However, the three animals (3/19 = 15.8% of the total number of animals) belonging to the high infestation group accounted for 23.0% of the total ticks found in the 19 animals, while the medium (11/19 = 57.9% of the total number of animals) and low infestation groups (5/19 animals = 26.3% of the total number of animals) accounted for 57.0% and 20.0% of total ticks, respectively.

4. Discussion

The strategic control method evaluated during this study provides a remarkable overall effect against *R.* (*B.*) *microplus* because it significantly reduces the tick infestation on cattle (Table 1; Fig. 1). Differences between control group and the treated group were significant along the entire study period, with the exception of March. The values of efficacy percentage were higher than 87% in all counts (March excepted) The results found in this work were similar to those obtained by Nava et al. (2014, 2015), showing that these strategic schemes are applicable to *P. maximum*-based grazing systems in the semi-arid region of northwestern Argentina. The strategic methods tested in this work and by Nava et al. (2014, 2015) are based on three treatments with systemic acaricides from late winter (or early spring) to late spring following by a period of approximately eight months without treatments. The principal difference between the control method evaluated in the current work and those tested by Nava et al. (2014, 2015) also in northwestern Argentina but in different pastures and climatic conditions, is related to the month in which the first treatment was applied. In the control schemes designed by Nava et al. (2014, 2015), the first

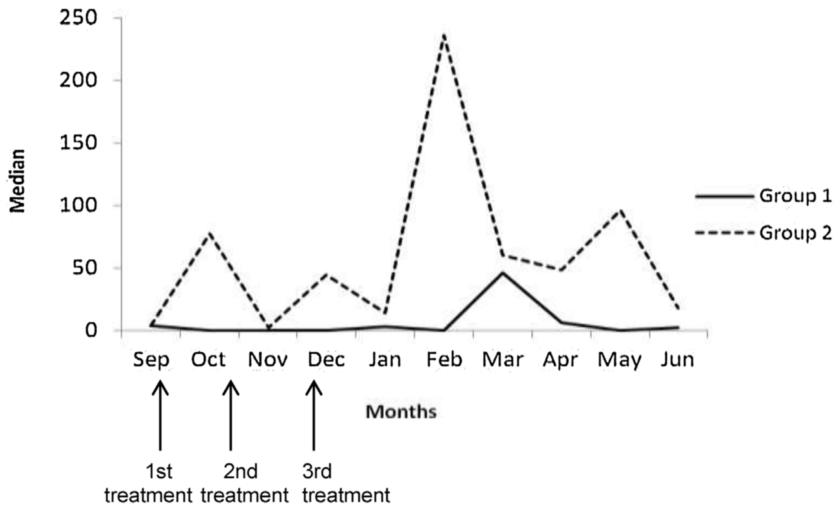

Fig. 1. Median number of *Rhipicephalus* (*Boophilus*) *microplus* females (4.5–8.0 mm long) on cattle. Group 1 was treated with ivermectin 3.15%, fluazuron and fipronil, group 2 remained as control. See details of the treatments in materials and methods.

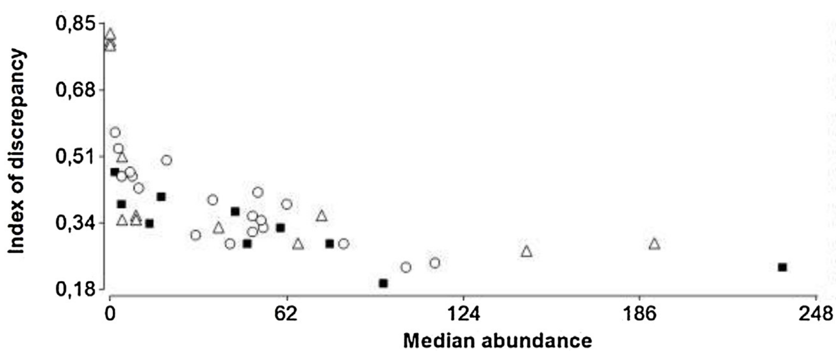

Fig. 2. Degree of aggregation (index of discrepancy) as function of the median of *Rhipicephalus* (*Boophilus*) *microplus* females (4.5–8.0 mm long) infestation on cattle. Data from the current study (black squares), Canevari et al. (2017) (triangles) and Nava et al. (2015) (circles) are shown.

treatments were made in late August, while in the method tested in the current work the first treatment was applied in late September. However, the results were similar, which indicates that the first chemical treatment in these schemes of strategic control can begin either late winter or early spring. This fact expands the temporal spectrum to apply the first treatment. It is important to keep in mind that even though these schemes of ticks control achieved an acceptable control level, they are not aimed to eradicate the tick populations in a given area, but to maintain low levels of tick infestations on cattle compatible with economic production.

The principal caveat of this strategic method to control *R.* (*B.*) *microplus* is the low efficacy reached in late summer (count of March). According with data obtained in previous studies on the life cycle of *R.* (*B.*) *microplus*, which were also performed in semi-arid areas of northwestern Argentina (Nava et al., 2013; Mastropaolo et al., 2017), the ticks that infest cattle in late summer may have originated from females detached from their hosts between late December and late January. In this sense, low levels of infestations were observed during the count of January (Table 1). Therefore, it is probable that ticks infesting cattle in late March were originated from females detached from cattle in January. Considering once more the results showed by Nava et al. (2013) and Mastropaolo et al. (2017), the ticks observed on cattle in January may have originated from females detached from cattle during November and in the first half of December. During this period infestation should have been controlled with the residual effectiveness of fluazuron (ACATAK®, Novartis Argentina S.A.) (41 days according to SENASA, Argentina: URL: http://www.senasa.gov.ar/prensa/DNSA/Garrapata_bovinos_aprobados/grrptaprob.html) and with the application of fipronil, but it is probable that some ticks escaped the treatment with both drugs. Keeping in mind that resistance of *R.* (*B.*) *microplus* to fluazuron and fipronil was already diagnosed in countries such as Brazil and Uruguay (Castro-Janer et al., 2010; Reck et al., 2014a; Klafke et al., 2017), it is necessary to monitor resistance of *R.* (*B.*) *microplus* to fluazuron and fipronil in northern Argentina. Also, an additional factor that may have contributed to diminish the difference between the groups in late March is the application on the control group of a palliative treatment with deltamethrine 1% on the 2nd of March.

The dynamics of the *R.* (*B.*) *microplus* infestation on cattle observed in the control group during this study coincides, in general terms, with the pattern of seasonal variation previously described for this tick species in northwestern Argentina, where it is characterized by an increase in abundance from mid- or late spring to autumn and then a decrease towards winter and early spring (Guglielmone et al., 1981; Nava et al., 2015; Canevari et al., 2017). However, in the current work, the highest peak of abundance was observed in mid-summer (count of February), while the maximum infestation level of *R.* (*B.*) *microplus* on cattle recorded by Guglielmone et al. (1981), Nava et al. (2015) and Canevari et al. (2017) occurred in autumn. Probably this difference is related to short-term variations in the microclimatic conditions governing the eclosion rate of eggs and larval survival during late spring and summer, because the micro-climatic events that occur in these two periods directly influence the abundance of ticks in summer and autumn, respectively. Mastropaolo et al. (2017) have shown the strong effect of short-terms variations in the microclimatic conditions on the development periods of the non-parasitic stages of *R.* (*B.*) *microplus* in northern Argentina. According to the data on the non-parasitic phase of *R.* (*B.*) *microplus* in northern Argentina given in Canevari et al. (2017) and Mastropaolo et al. (2017), it could be hypothesized that high values of temperatures and saturation deficit occurring in mid and late summer could have negatively affected the eclosion rate of eggs and the larval survival, thus decreasing the abundance of ticks in autumn.

Two alternative methods to control cattle ticks with a minor use of chemical acaricides are the partial selective treatment and culling (Wilkinson, 1962; Sutherst and Utech, 1981; Molento et al., 2013), but the efficacy of these methods depends on the structure of the tick distribution amongst cattle. In the analysis performed in this work, a temporal variation in the tick aggregation levels associated to changes in tick abundance was found. The higher the abundance of *R.* (*B.*) *microplus*, the lower was the aggregation. This inverse relationship between abundance and aggregation shows that a constant level of aggregation of *R.* (*B.*) *microplus* cannot be expected across a given period of time in northern Argentina, where the abundance of this tick is characterized by a marked seasonality. This fact represents a constraint for the use of partial selective treatment and culling because the distribution of ticks amongst cattle varies over time with change in tick abundance. Furthermore, it was found that the steers (15.8% of the total number of animals evaluated) belonging to the high infestation group accounted for a relatively smaller percentage of ticks (23.0% of the total ticks). All these results suggest that partial selective treatment and culling do not represent feasible methods to control *R.* (*B.*) *microplus* infestation on cattle, which confirms the conclusion reached by Guglielmone et al. (1990, 1992), Martins et al. (2002), Paim et al. (2011) and Nava et al. (2015) in studies carried out in northern Argentina and southern Brazil.

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