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Strategic applications of long-acting acaricides against *Rhipicephalus* (*Boophilus*) *microplus* in northwestern Argentina, with an analysis of tick distribution among cattle

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ABSTRACT

Strategic applications of long-acting acaricides for the control of *Rhipicephalus* (*Boophilus*) *microplus* in northwestern Argentina were evaluated for one year. In addition, tick distribution among cattle was analyzed to evaluate if partial selective treatment or culling the small proportion of most heavily infested animals were feasible options to control *R.* (*B.*) *microplus*. Two different treatments schemes based on two applications of fluazuron and one application of 3.15% ivermectin were performed. Treatments were made in late winter and spring so as to act on the small 1st spring generation of *R.* (*B.*) *microplus*, in order to preclude the rise of the larger autumn generation. The overall treatment effect was positively significant in both schemes. The number of ticks observed in the control group was significantly higher than in the treated groups on all post-treatment counts. Group 2 exhibited more than 80% of efficacy almost throughout the study period, whereas Group 1 exhibited an efficacy percentage higher than 80% in September, October, December, February, April and May, but not in November (73.4%), January (58.3%), March (45.2%) or June (53.4%). Absolute control was observed in Group 2 in the counts of September and October, and in Group 1 in the count of February. The control strategies evaluated in this work provide an acceptable control level with only three applications of acaricides; at the same time, they prevent the occurrence of the autumn peak of tick burdens, which is characteristic of *R.* (*B.*) *microplus* in northwestern Argentina. Tick distribution was markedly aggregated in all counts. Although ticks were not distributed evenly among calves, the individual composition of the most heavily infested group was not consistent throughout the study period. In addition, the level of aggregation varied with tick abundance. These results suggest that applying acaricides to a portion of the herd or culling the most infested individuals at a given moment of the year may not be proper methods to achieve a significant control of *R.* (*B.*) *microplus* in northwestern Argentina.

1. Introduction

Rhipicephalus (*Boophilus*) *microplus* is the major tick pest of cattle in tropical and subtropical areas worldwide (Guglielmone et al., 2003; Jongejan and Uilenberg, 2004).

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Parasitism by *R.* (*B.*) *microplus* ticks and the haemoparasites they transmit are important constraints for cattle production because it induces depression on weight gain and milk production, hide damage, mortality, morbidity and control costs (Spath et al., 1994). In addition, the association between high levels of *R.* (*B.*) *microplus* infestation and occurrence of screwworm myiasis in cattle was demonstrated (Reck et al., 2014b).

The use of chemical acaricides for the control of *R.* (*B.*) *microplus* has resulted in increasing problems of multidrug resistance (Frisch, 1999; Guerrero et al., 2012). Resistance to practically all of the commercially available acaricides has been demonstrated in South America (Martins and Furlong, 2001; Guglielmone et al., 2006; Castro-Janer et al., 2010a,b, 2011; Mendes et al., 2011; Guerrero et al., 2012; Lovis et al., 2013; Reck et al., 2014a). In Argentina, populations of *R.* (*B.*) *microplus* were found to be resistant to arsenics, pyrethroids, organophosphates and formamidines (Grillo Torrado and Pérez Arrieta, 1977; Mangold et al., 2004; Guglielmone et al., 2006; Cutullé et al., 2013). Sustainable control of cattle ticks with acaricides is also problematic due to the accumulation of chemical residues in meat or milk and the contamination by release of chemical compounds to the environment (George et al., 2008; Nari Henrioud, 2011). These constraints may be mitigated by adopting strategic applications of acaricides in order to minimize the number of treatments with chemical compounds.

Information on the population dynamics of a tick is useful to design strategic methods of control that minimize the number of acaricide applications. In northwestern Argentina, population dynamics of *R.* (*B.*) *microplus* is characterized by a major peak of abundance in autumn, decreasing towards winter and early spring, when climatic conditions become unfavourable for the free-living stages (Guglielmone et al., 1981, 1990a; Guglielmone and Nava, 2013). Accordingly, treating cattle in late winter and spring to suppress the small 1st -spring generation of *R.* (*B.*) *microplus* should preclude the development of the larger autumn generation. For that reason, the aim of this work was to evaluate the strategic applications of long-acting acaricides for the control of *R.* (*B.*) *microplus* in northwestern Argentina for one year. Additionally, levels of aggregation and individual variation in tick abundance among hosts were analyzed to evaluate if a partial selective treatment, i.e., applying an acaricide to the most heavily infested portion of the herd (Molento et al., 2013), or cull the small proportion of most heavily infested animals, as proposed in Wilkinson (1962) and Sutherst and Utech (1981), are feasible options to control *R.* (*B.*) *microplus* in northwestern Argentina.

2. Materials and methods

2.1. Study area

All trials were conducted at the Instituto de Investigación Animal del Chaco Semiárido (IIACS, INTA), located in Leales (27°11′S 65°14′W), Tucumán, Argentina. The site belongs to the Chaco Phytogeograpic Province sensu Cabrera (1994), and the climate is subtropical subhumid with an average annual rainfall of 900 mm concentrated from October to March. A tick infested pasture composed mainly of *Cynodon dactylon, Chloris gayana* and *Setaria geniculata* was divided into 10-ha paddocks with electric fencing to maintain separate groups of calves. Animals were fed corn silage and expeller soybean meals throughout the study period. Water access was ad libitum.

2.2. Strategic applications of long acting acaricides

This trial was performed from August 2013 to June 2014. Braford calves nine months of age naturally infested with *R.* (*B.*) *microplus* were divided into three homogeneous groups on day 0. Each group comprised 15 animals of similar level of *R.* (*B.*) *microplus* infestation (Kruskal-Wallis test, $P > 0.05$). Group 1 calves were treated with a commercial pour-on formulation of fluazuron (ACATAK®, Novartis Argentina S.A) at a rate of 1 ml/10 kg of body weight on day 0 (21st August 2013) and 49 (9th October 2013), and with an application of a commercial injectable (subcutaneous) 3.15% ivermectin formulation (BAGOMECTINA®, Biogénesis Bagó S.A., Argentina) at a rate of 1 ml/50 kg of body weight on day 98 (27th November 2013). Group 2 calves were treated with a commercial injectable (subcutaneous) 3.15% ivermectin formulation (IVOMEC GOLD®, Merial Argentina S.A.) at a rate of 1 ml/50 kg of body weight on day 0 (21st August 2013), and with two applications of 1 ml/10 kg of a pour-on formulation of fluazuron (ACATAK®, Novartis Argentina S.A) on days 35 (25th September 2013) and 84 (13th November 2013). Group 3 animals were the control group, where the number of individuals was 14 for quantitative analyses because one calf was lost. Application dosages of each drug were determined following the manufacturer's protocol. Both treatment schemes were designed to prevent the annual peak of abundance of *R.* (*B.*) *microplus* in autumn.

Females of *R.* (*B.*) *microplus* (4.5–8.0 mm long) were counted monthly on one side of the calves. The number of ticks collected on calves 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. Data were subjected to the Shapiro-Wilk's test of normality prior to statistical analysis. Because the test revealed significant deviations from the normal distribution, statistically significant differences in the distributions of *R.* (*B.*) *microplus* numbers among the three groups were determined by using the non-parametric Kruskal-Wallis test with a Dunn post-hoc test (Zar, 1999). Differences were considered significant at $P < 0.01$. The corrected efficacy percentage was calculated with the modified Abbot's formula using the mean number of ticks (Henderson and Tilton, 1955). The efficacy percentage was calculated with the arithmetic means because in presence of aggregation the efficacy estimated from the arithmetic mean has provided unbiased results, whereas geometric means often yield skewed outputs (McKenna, 1998; Dobson et al., 2009). The corrected efficacy percentage was calculated only when the counts in the treated groups were significantly lower than the count in the control group.

2.3. Analysis of tick distribution among cattle

Two experiments were performed to analyze the distribution of *R.* (*B.*) *microplus* ticks among cattle. Groups of 13 and 14 Braford calves that were not subjected to treatments with acaricides were evaluated in experiments 1 and 2, respectively. Infestation with *R.* (*B.*) *microplus* ticks was quantified monthly from July 2012 to March 2013 in experiment 1, and from August 2013 to June 2014 in experiment 2 (this group corresponds to the control group of the trial described in Section 2.2). The variance to mean ratio and the index of discrepancy (*D*) (Poulin, 1993) were calculated to examine the distribution of parasites within each group of calves by using the program Quantitative Parasitology 3.0 (Rózsa et al., 2000). 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). This index quantifies aggregation as the departure between the observed parasite distribution and a perfectly uniform distribution, and it can be employed to compare distributions that vary in prevalence or mean number of parasites per host (Poulin, 1993, 2007). The number of calves that carried 50% of the total *R.* (*B.*) *microplus* ticks at each count was determined in order to analyze the individual variation in tick infestation levels within a group of calves. Finally, a linear regression analysis was performed to determine the relationship between tick abundance and level of aggregation (measured as *D*). Since mean number of ticks has a binomial distribution, data were arcsine-transformed as suggested by Zar (1999).

3. Results

The effect of the strategic applications of ivermectin and fluazuron on the population abundance of *R.* (*B.*) *microplus* in northwestern Argentina is shown in Table 1, where data are expressed as prevalence, mean number of ticks, median and efficacy percentage. The overall treatment effect was positively significant in both schemes (Table 1; Fig. 1). The number of ticks in the control group (Group 3) was significantly higher than in the treated groups (Groups 1 and 2) in all post-treatment counts ($P < 0.01$) (Table 1). The treated groups (Groups 1 and 2) showed differences in tick infestation, with the number of ticks being significantly higher in Group 1 in some counts (January, March, June) and in Group 2 in others (February, May).

The efficacy percentage of the control scheme applied to Group 2 was more than 80% almost all throughout the study period, except in November, which was 73.5% (Table 1). The treatment performed in Group 1 provided an efficacy percentage higher than 80% in September, October, December, February, April and May, but not in November (73.4%), January (58.3%), March (45.2%) and June (53.4%) (Table 1). Absolute control (mean: 0) was reached for Group 2 in the counts of September and October, and in the Group 1 in the count of February (Table 1).

As expected for parasites, variance-to-mean ratio was by far greater than 1 in all data sets, indicating an aggregated distribution of ticks (Table 2). However, the degree of aggregation, expressed as *D*, varied among counts (Table 2). Linear regression analysis showed a significant inverse relationship between number of ticks and degree of aggregation (r: 0.88; r^2: 0.77). Thus, the higher the abundance of *R.* (*B.*) *microplus*, the lower the aggregation. The percentage of calves that carried 50% of the total *R.* (*B.*) *microplus* ticks on each count ranged from 23 to 30.7% in experiment 1, and from 14.2 to 50% in experiment 2 (Table 2), with group composition changing all throughout the experiment. Approximately 90% of the calves were in the most heavily infested group at some point in experiment 2, whereas 69.2% of the calves were in this group during experiment 1 (Table 2).

4. Discussion

The two treatment schemes evaluated in this work had a significant overall effect on the population abundance of *R.* (*B.*) *microplus* (Table 1; Fig. 1). Differences between control group and the two treated groups were significant throughout the study period. The seasonality exhibited by *R. (B.) microplus* ticks associated with the calves of control group (Fig. 1) is consistent with the pattern of seasonal variation previously described for this tick species in northwestern Argentina (Guglielmone et al., 1981, 1990a). The comparison of seasonal abundance of *R. (B.) microplus* between the control group and the two treated groups (Fig. 1) indicates that the control strategies evaluated in this work are appropriate, because they not only provide an acceptable control level with three applications of acaricides, but also prevent the occurrence of the autumn peak.

A notorious variation of tick numbers and prevalence was evident in Group 1 from December to June (last treatment carried out during November). The effect of acaricide treatments on tick population and/or dynamics of tick population itself in the corresponding paddock may have contributed to these variations but we have no information to support any hypothesis. The erratic variation of tick burden on Group 1 resulted also in inconsistent statistical data in relation to Group 2 with two monthly tick counts showing no statistical significant differences between Group 1 and Group 2 (December, April), three counts with significant higher tick numbers in Group 1 than Group 2 (January, March, June) and two counts with lower tick number on Group 1 than Group 2 (February, May). These inconsistencies difficult the comparison of benefits of treatment against ticks of Group 1 in comparison to Group 2, whose efficacies varied from 45% to 100% in Group 1 and from 80% to 98% in Group 2 considering the seven tick counts from December to June. These data appear to show a greater advantage of cattle treatment with ivermectin followed by two applications of fluazuron (Group 2) than the opposite (Group 1). Perhaps, initial treatment with ivermectin followed by an application of fluazuron on day 35 (Group 2) produced a more drastic long-lasting effect on tick population than initial treatments with fluazuron 49 days apart (Group 1); however, additional studies are needed to obtain conclusive evidences about the usefulness of sequential treatments with acaricides with different

Table 1
Prevalence, mean number, 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 group 2) and control (group 3) calves. The efficacy percentage (EP) is also indicated. NA: not applicable. Animals of the group 1 were treated with fluazuron on days 0 (21st August 2013) and 49 (9th October 2013), and with ivermectin 3.15% on day 98 (27th November 2013). Animals of the group 2 were treated with ivermectin 3.15% on day 0 (21st August 2013), and with two applications of fluazuron on days 35 (25th September 2013) and 84 (13th November 2013).

	Group 1				Group 2				Group 3		
	P (%)	Mean	*M* (1Q–3Q)	EP (G1–G3)	*P* (%)	Mean	*M* (1Q–3Q)	EP (G2–G3)	*P* (%)	Mean	*M* (1Q–3Q)
August	86.6	9.4[a]	8 (2–14)	NA	93.3	9.8[a]	10 (4–14)	NA	85.7	9.2[a]	7 (2–14)
September	20.0	0.5[a]	0 (0–0)	90.0	0	0[a]	0 (0–0)	100	64.3	4.7[b]	4 (0–8)
October	20.0	0.4[a]	0 (0–0)	90.1	0	0[a]	0 (0–0)	100	64.3	3.4[b]	3 (0–4)
November	73.3	16.1[a]	9 (0–28)	73.4	53.3	14.8[a]	2 (0–32)	73.5	92.8	57.8[b]	54 (30–82)
December	20.0	1.0[a]	0 (0–0)	98.1	13.3	0.8[a]	0 (0–0)	98.5	100	50.1[b]	53 (18–82)
January	80.0	19.7[a]	17 (6–34)	58.3	53.3	4.5[b]	2 (0–8)	90.8	92.8	45.7[c]	50 (20-68)
February	0	0[a]	0 (0–0)	100	53.3	4.2[b]	2 (0–4)	94.9	92.8	77.5[c]	82 (46–103)
March	93.3	20.8[a]	22 (10–28)	45.2	86.6	4.5[b]	2 (2–4)	88.3	92.8	35.8[c]	42 (19-51)
April	93.3	12.4[a]	11 (4–18)	90.4	93.3	7.8[a]	8 (4–12)	94.0	92.8	120[b]	114 (91–153)
May	100	12.4[a]	9 (4–18)	90.1	100	21.0[b]	18 (14–30)	83.8	92.8	119.8[c]	104 (64–149)
June	93.3	13.7[a]	14 (10–20)	53.4	93.3	6.0[b]	6 (4–6)	80.4	92.8	28.4[c]	30 (22–32)

Kruskal–Wallis test. Numbers not sharing superscripts are significantly different ($P < 0.01$).

mode of action against *R. (B.) microplus*. In any situation, there is no doubt that both treatments were effective to diminish tick populations as shown by a constant tick burden significantly lower than the control group (Group 3), which was preliminary more evident in Group 2 than in Group 1.

The results of this work indicate that, although both treatment schemes achieved high control values, they are not enough for an absolute control of *R. (B.) microplus*. Similarly, previous studies evaluating the therapeutic effect of 3.15% ivermectin and fluazuron against *R. (B.) microplus* (Martins et al., 1995; Guglielmone et al., 1998; Vieira et al., 2003; Cuore et al., 2008; Arieta-Román et al., 2010; Davey et al., 2010) found acceptable control levels of *R.* (*B.*) *microplus* with the two acaricides, but failed to reach absolute control. All these evidences clearly show that the strategic applications of long-acting acaricides against *R.* (*B.*) *microplus*, as those proposed in this work, are useful for the pest control but not for its eradication. The control schemes proposed during this study included three acaricide treatments during three months, followed by nine months without treatments. This scheme may not have a strong effect in developing resistance to avermectins or fluazuron; however, this assumption should be evaluated in future studies.

Two independent experiments were performed to assess the distribution of *R.* (*B.*) *microplus* ticks in calves not treated with acaricides. The results were similar in both experiments, with tick distribution being markedly aggregated in all counts (Table 2). This outcome is not unexpected because host–parasite relationship is characterized by a ubiquitous pattern of negative binomial distribution (Wilson et al., 1996; Shaw et al., 1998). Although ticks were not distributed evenly among calves, because a relatively small percentage of the calves carried 50% of the ticks in most counts (a value higher than 40% was observed in only one count; see Table 2), the individual composition of the most heavily infested group was not consistent over the study period (see Table 2). In addition, the level of aggregation varied with the abundance of ticks; the higher abundance of *R.* (*B.*) *microplus* ticks, the greater the dispersion of these parasites among calves. This inverse

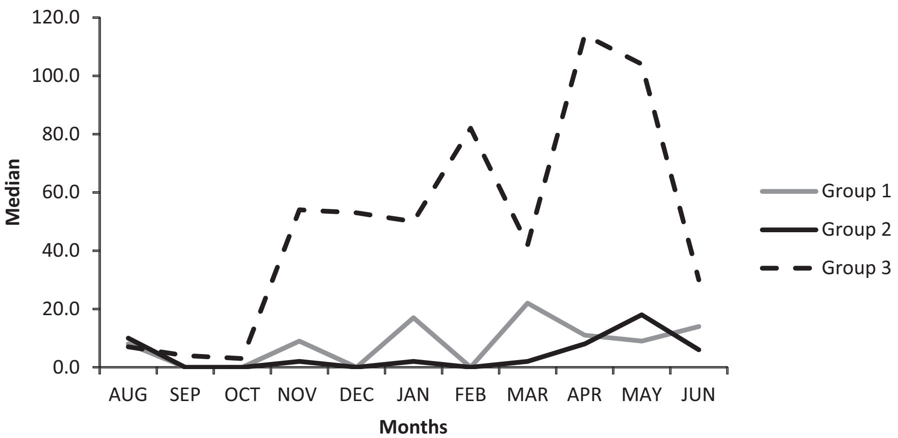

Fig. 1. Median number of *Rhipicepahlus* (*Boophilus*) *microplus* females (4.5–8.0 mm long) on cattle. Groups 1 and 2 were treated with fluazuron and ivermectin 3.15%, and the group 3 remained as control. See details of the treatments in materials and methods.

Table 2
Distribution of *Rhipicephalus* (*Boophilus*) *microplus* ticks among cattle. Median (M) and first and third quartiles (1Q-3Q), variance to mean ratio (V/M), the index of discrepancy (*D*), % of calves in the most infested group carrying 50% of total tick numbers (CMIG) and individual identification of calves in the most infested group (IICMIG), are showed for each count of ticks infesting cattle at Leales, Tucumán Province, Argentina. Experiment 1 was conducted between August 2012 and March 2013, and experiment 2 was carried out between August 2013 and June 2014.

Count numbers	M (1Q–3Q)	V/M	*D*	CMIG	IICMIG
Experiment 1					
1 (August 2012)	10 (3–20)	8.4	0.43	23.0	536, 648, 844
2 (September 2012)	2 (0–4)	4	0.57	23.0	648, 660, 701
3 (October 2012)	8 (3–14)	8.2	0.46	23.0	536, 648, 810
4 (November 2012)	20 (6–46)	29.2	0.50	23.0	536, 810, 844
5 (December 2012)	50 (14–50)	29.4	0.32	30.7	565, 618, 660, 670
6 (January 2013)	36 (18–72)	35.6	0.40	23.0	648, 670, 844
7 (February 2013)	62 (36–90)	77.8	0.39	23.0	536, 648, 670
8 (March 2013)	52 (22–76)	49.2	0.42	30.7	536, 565, 648, 670
Experiment 2					
1 (August 2013)	7 (2–14)	9.1	0.47	21.4	352, 375, 386
2 (September 2013)	4 (0–8)	3.9	0.46	28.5	352, 357, 366, 374
3 (October 2013)	3 (0–4)	5.4	0.53	14.2	361, 393
4 (November 2013)	54 (30–82)	26.2	0.33	28.5	357, 367, 375, 393
5 (December 2013)	53 (18–82)	25.2	0.35	28.5	352, 357, 367, 393
6 (January 2014)	50 (20–68)	23.16	0.36	50.0	235, 356, 359, 366, 367, 386, 388
7 (February 2014)	82 (46–103)	28.5	0.29	28.5	357, 366, 386, 393
8 (March 2014)	42 (19–51)	11.8	0.29	28.5	357, 366, 367, 393
9 (April 2014)	114 (91–153)	29.2	0.24	35.7	352, 359, 367, 386, 393
10 (May 2014)	104 (64–149)	50.5	0.23	28.5	359, 367, 386, 393
11 (June 2014)	30 (22–32)	7.8	0.31	35.7	357, 359, 375, 388, 393

relationship between parasite loads and aggregation has been well documented (Poulin, 1993; Eppert et al., 2002; Poulin, 2007). For this reason, a constant level of aggregation of *R.* (*B.*) *microplus* ticks over a given period of time cannot be expected in an area where the abundance of this tick is characterized by a marked seasonality.

Partial selective treatment and culling were proposed as reliable tools to control cattle ticks (Wilkinson, 1962; Sutherst and Utech, 1981; Molento et al., 2013). Nevertheless, the lack of consistency in the individual composition of the most heavily infested group and the temporal variation in the aggregation levels are constraints for selective treatments of the cattle most prone to *R.* (*B.*) *microplus* infestation in northwestern Argentina. Similar results were previously reported for *R.* (*B.*) *microplus* and *Amblyomma variegatum* infestation in several bovine biotypes (Guglielmone et al., 1990b, 1992a,b; Naves et al., 1998). A caveat to this conclusion is our uncertainty about how the tick population would develop after elimination or treatment of the most infested animals. However, Martins et al. (2002) and Paim et al. (2011) did not find a significant advantage of selective treatments of cattle for the control of *R.* (*B.*) *microplus* in trials performed in southern Brazil. Martins et al. (2002) concluded that selective treatments did not confer an adequate protection either to the treated or the untreated animals, due to the presence of larvae in the pasture, derived from untreated engorged female ticks, reinfesting both the treated and untreated cattle. Furthermore, differences in individual tick burdens can be explained by patchiness in the spatial distribution of questing tick density and not by intrinsic differences in individual susceptibility (Calabrese et al., 2011). Therefore, management strategies which assume that variation in observed tick burdens are solely due to host-related factors may be ineffective.

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