

Strategic treatments with systemic biocides to control *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in northwestern Argentina

Tratamientos estratégicos con biocidas sistémicos para el control de *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) en el noroeste de Argentina

Nava, S¹; Mangold, A.J¹; Canevari, J²; Morel, N³; Guglielmo, A.A¹.

¹Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela and Consejo Nacional de Investigaciones Científicas y Técnicas, CC 22, CP 2300 Rafaela, Santa Fe, Argentina. ² Instituto de Investigación Animal del Chaco Semiárido (IIACS, INTA), Leales, Tucumán, Argentina. ³Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Este de Santiago del Estero, Quimili, Santiago del Estero, Argentina.

SUMMARY

This work was performed to evaluate two different schemes of treatment with systemic biocides to control *Rhipicephalus microplus* in northwestern Argentina. Treatments were applied from late winter to late spring to act on the small 1st spring generation of *R. microplus*, precluding the rise of the larger generations of autumn. In the scheme 1, calves were treated with fluazuron on days 0 and 49, and with fipronil on day 105. In the scheme 2, calves were treated with ivermectin 3.15%, fluazuron and fipronil on days 0, 28 and 83, respectively. The number of ticks observed on control group was significantly higher than the number of ticks in the treated groups. The percentage reduction in numbers of ticks with the control scheme 1 was more than 90% during practically all study period. The treatment applied in the scheme 2 provided values of reduction higher than 90% in September, October, December, January and February, but not in November (87.49%) and March (88.78%). The results showed that both treatments schemes achieved an acceptable control level, but complementary studies are needed to determine if the *R. microplus* ticks detected in early autumn could increase in time to high levels.

Key words: (ticks), (cattle), (control), (systemic biocides), (Argentina).

Correspondencia *e-mail*: Santiago Nava nava.santiago@inta.gob.ar.

Recibido: 25-08-2014

Aceptado: 10-12-2014

RESUMEN

En este trabajo se evaluaron dos esquemas de tratamientos con biocidas sistémicos para el control de *Rhipicephalus microplus* en el noroeste de Argentina. Los tratamientos se aplicaron desde fines del invierno a fines de la primavera para limitar el surgimiento de la generación con mayor abundancia de garrapatas en el otoño del siguiente año. En el esquema 1, los terneros fueron tratados con fluazurón los días 0 y 49, y con fipronil el día 105. En el esquema 2 los terneros fueron tratados con ivermectina 3,15%, fluazurón y fipronil los días 0, 28 y 83, respectivamente. El número de garrapatas sobre el grupo control fue significativamente más alto que en los grupos tratados. El porcentaje de reducción de la carga parasitaria en el esquema 1 fue superior al 90% durante casi todo el período de estudio, y en el esquema 2 los valores fueron superiores al 90% en septiembre, octubre, diciembre, enero y febrero, pero no en noviembre (87,49%) y marzo (88,78%). Los resultados indican que los dos esquemas de tratamientos proveen aceptables niveles de control, pero se necesitan estudios adicionales para determinar si son suficiente para suprimir el riesgo del surgimiento de un pico mayor de abundancia de *R. microplus* a mediados o fines del otoño.

Palabras clave: (garrapatas), (bovinos), (control), (biocidas sistémicos), (Argentina).

INTRODUCTION

The cattle tick *Rhipicephalus (Boophilus) microplus* is one of the most important livestock pests in tropical and subtropical areas of the world. Economic losses due to *R. (B.) microplus* are related to depression on weight gain and milk production, hide damage, mortality, morbidity, control costs and tick-transmitted haemoparasites²⁴. Although *R. (B.) microplus* was usually considered to be distributed in America, Africa, south-eastern Asia, Australia and New Caledonia, the reinstatement of *Rhipicephalus (Boophilus) australis* by Estrada-Peña *et al.* (2012)⁹ has showed that *R. (B.) microplus* is present in America, Africa and south-eastern Asia, while populations of *R. (B.) australis* are found in Australia, New Caledonia and also in south-eastern Asia. Therefore, the knowledge generated in Australia on ecology and control of *R. (B.) microplus* usually extrapolated to American or African conditions should be considered cautiously, indicating the need of new studies about these topics in countries of these two continents.

Besides the negative effects of ticks on cattle, acaricide resistance currently represent

a relevant problem for tick control^{10,12}. Cases of resistance of *R. (B.) microplus* to practically all available anti-parasiticides were reported in America¹². In Argentina, arsenic¹⁰, pyrethroid^{3, 17, 20}, organophosphate¹¹ and formamidine⁵ resistance were diagnosed in populations of *R. (B.) microplus*. Considering that pyrethroids and formamidines are the acaricides most often used in Argentina¹⁸, alternative control schemes employing other chemical compounds as systemic biocides should be tested to mitigate this increasing problem. Also, these schemes should be designed in such way to minimize the number of treatments with an alternation of antiparasitic compounds.

In northwestern Argentina, the population dynamics of *R. (B.) microplus* is characterized by a major peak of abundance in autumn (and eventual minor peaks in late spring), followed by a decreasing of abundance towards winter and early spring due to the unfavourable climatic conditions for the development of the free-living stages^{13, 14, 15}. This information on the population dynamics of ticks is useful to design ecologically based methods of control^{22, 23}. The strategic applications of chemical acaricides

not only increases the efficacy of the treatment with a lower number of applications, but also delays the emergence of resistance, decreases the accumulation of chemical residues in meat or milk and reduces the contamination by release of chemical compounds to the environment. Therefore, the aim of this work is to evaluate two different schemes of treatment with acaricides to control of *R. (B.) microplus* in northwestern Argentina, which consist in the strategic application of systemic biocides based on ecological criteria.

MATERIALS AND METHODS

The trial was conducted at the Instituto de Investigación Animal del Chaco Semiárido (IIACS, INTA), located at Leales (27°11'S 65°14' W), Tucumán Province, Argentina. This site belongs to the Chaco Phytogeographic as defined by Cabrera (1994)². Forty-five Braford calves nine months of age and naturally infested with *R. (B.) microplus* were maintained from July 2012 to March 2013 on a pasture composed mainly of *Cynodon dactylon*, *Chloris gayana* and *Setaria geniculata*. On day 0 the calves were divided into three homogeneous groups of 15 animals each according to the level of *R. (B.) microplus* infestation (Kruskal-Wallis test, $P > 0.05$). The pasture was divided into three paddocks of 10 ha each by an electric fence in order to maintain the three groups separately, and all calves were fed with corn silage and expeller soybean meals.

Individuals of the group 1 were treated with a commercial pour-on formulation of fluazuron (ACATAK®, Novartis Argentina S.A.) applied on the dorsal midline at a rate of 1 ml/10 kg of body weight on days 0 (29th August 2012) and 49 (17th October 2012), and with an additional application of a commercial pour-on formulation of fipronil (ECTOLINE®, Merial Argentina S.A.) at a rate of 1 ml/10 kg of body weight on day 105 (12th December 2012). Animals of the group 2 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 (29th August 2012), with 1 ml/10 kg of a pour-on formulation of fluazuron on day 28 (26th September 2012), and with 1 ml/10 kg of a commercial pour-on formulation of fipronil on day 83 (20th November 2012). The calves of the group 3 formed the not treated control group. The dosages to which the drugs have been applied were determined following the manufacturer's label. Ivermectin (IVOMEC GOLD®, Merial Argentina S.A.) and fluazuron (ACATAK®, Novartis Argentina S.A.) provided extended protection against *R. (B.) microplus* for 23 and 42 days, respectively. In both schemes, the treatments began in late winter, when the populations of *R. (B.) microplus* in the northwest of Argentina were at very low abundance^{14,15}. Thus, they were designed to act on the small 1st spring generation of *R. (B.) microplus*, precluding the rise of the larger generations of autumn. The application of fipronil in December (group 1) and November (group 2) was performed to prevent a significant re-infestation with ticks that have escaped treatment with ivermectin and fluazuron.

To assess the tick burdens, counts of *R. (B.) microplus* females (4.5-8.0 mm long) were monthly performed on one side of the calves. The number of ticks collected on calves was duplicated for the statistical analysis. Mean and median with first and third quartiles (1Q – 3Q) were calculated. Because Shapiro-Wilk's test²⁶ showed significant departure from normality, 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. A value of $P < 0.01$ was considered significant. A modified Abbot's formula¹⁹ was used to calculate the percentage reduction in numbers of ticks in the treated groups. This formula was applied only when the counts in the treated groups were significantly lower than the count in the control group.

RESULTS

Values of mean number and median of ticks on the treated and control groups and the percentage reduction are showed in Table 1. The number of ticks observed on control group was significantly higher than the number of ticks recovered from the treated groups ($P < 0.01$). The differences between the two treated groups were not significant, with the exception of the count of November, where the number of ticks in group 2 was higher than the number of ticks in group 1 (Group 1: mean 1.33, median 0; Group 2: mean 4.93, median: 4). The percentage reduction in numbers of ticks with the control scheme applied on group 1 was more than 90% during practically all study period, however in the March count the percent reduction was 80.32% (Table 1). The treatment applied on group 2 provided values of reduction higher than 90% in September, October, December, January and February, but not in November (87.49%) and March (88.78%) (Table 1). Absolute control (mean: 0) was only reached in the counts of October and December for group 1, and in the counts of September and December for group 2 (Table 1).

DISCUSSION

There was a significant overall treatment effect in both schemes (Table 1; Fig. 1). The percentage reduction in treated groups was always higher than 80%. Though the mean number of ticks at the final period of the trial in early autumn (count of 26 March 2013) were similar (10.86 in group 1 and 6.93 in group 2) to the infestation level in the day 0, the statistical differences with the control group were significant. In fact, the mean number of ticks in the control group in March was 59.6. According to the resolution 27/1999 of the Servicio Nacional de Sanidad y Calidad Agroalimentaria of Argentina (<http://www.senasa.gov.ar/contenido.php?to=n&in=1043&io=4588>), the eradication of *R. (B.) microplus* is not obligatory in northwestern Argentina. In cases like this, the control strategies proposed in this study appear to be appropriate, because with only three

applications of acaricides the mean number of ticks per calve during spring and summer was not superior to 1.33 and 4.93 in groups 1 and 2, respectively (see Table 1). Additionally, the two schemes of treatment applied in this study minimized the exposition of ticks to antiparasitic compounds with a fast knock-down effect (e.g. amitraz, pyrethroids), preserving their use for when it is strictly necessary, for example, in movements of cattle from a tick infested area to tick free areas.

Even though these results indicate that both treatments schemes achieved an acceptable control level, they are not enough to preclude the existence of *R. (B.) microplus* ticks in the pastures in March, which could generate the major peak of late autumn known to occur in northwestern Argentina^{13,14,15}. Therefore, the control schemes tested in this work were suitable for controlling *R. (B.) microplus* during spring and summer but they did not preclude the risk of appearance of the major peak of *R. (B.) microplus* in autumn. In order to complement the results of this work, further studies are necessary to determine if the *R. (B.) microplus* ticks detected in early autumn (see Fig. 1) on the treated animals could in time increase to high levels.

The therapeutic effect of ivermectin 3.15%, fluazuron and fipronil against *R. (B.) microplus* in America was evaluated in previous studies^{1, 4, 7, 8, 16, 21, 25}. Although in these works the three acaricides provided high control values of *R. (B.) microplus*, there was a post-treatment period in which the drugs did not have a significant effect on adult ticks. For example, studies on the control of *R. (B.) microplus* carried out in the USA by Davey and George (2002)⁶ demonstrated that ivermectin was less effective against females when they were in the final stages of engorgement, and Davey et al. (2010)⁸ found a shorter persistent efficacy of ivermectin 3.15 (IVOMEK GOLD®, Merial Inc. Mexico) than what was claimed in the label. Therefore, a small proportion of the tick population remains off treatment, and this fact could explain, assuming absence of resistance, the small peak of *R. (B.) microplus* females detected in the treated groups

Table 1. 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 group 2) and control (group 3) calves. The percent reduction (PR) in numbers of ticks in the treated groups in relation to group 3 is also showed. NA: not applicable.

	<u>Group 1</u>			<u>Group 2</u>			<u>Group 3</u>		
	<u>Mean</u>	<u>M (1Q-3Q)</u>	<u>PR (G1-G3)</u>	<u>Mean</u>	<u>M (1Q-3Q)</u>	<u>PR (G2-G3)</u>	<u>Mean</u>	<u>M (1Q-3Q)</u>	<u>PR (G2-G3)</u>
August	11.70 ^a	8 (2-20)	NA	13.21 ^a	8 (2-22)	NA	11.65 ^a	10 (3-20)	
September	0.26 ^a	0 (0-0)	90.11	0 ^a	0 (0-0)	100	3.30 ^b	2 (0-4)	
October	0 ^a	0 (0-0)	100	0.13 ^a	0 (0-0)	98.73	9.23 ^b	8 (3-14)	
November	1.33 ^a	0 (0-0)	96.21	4.93 ^b	4 (2-8)	87.49	34.67 ^c	20 (6-46)	
December	0 ^a	0 (0-0)	100	0 ^a	0 (0-0)	100	66.13 ^b	50 (14-50)	
January	1.26 ^a	0 (0-2)	97.48	2.13 ^a	2 (0-4)	96.23	49.73 ^b	36 (18-72)	
February	0.53 ^a	0 (0-0)	99.37	1.86 ^a	0 (0-2)	98.05	84.13 ^b	62 (36-90)	
March	10.86 ^a	9 (4-16)	80.32	6.93 ^a	4 (2-8)	88.78	59.60 ^b	52 (22-76)	

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

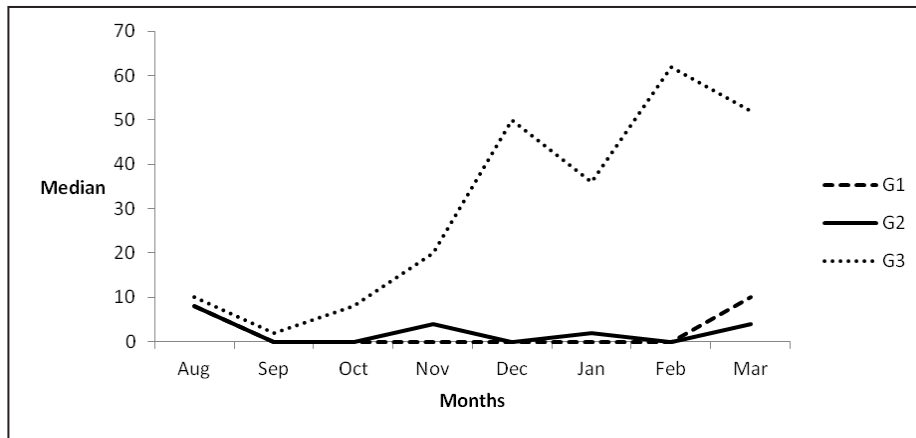


Figure 1. Median number of R. (B.) microplus females (4.5-8.0 mm long) on cattle. Group 1 was treated with flouzuron and fipronil, group 2 was treated with ivermectin 3.15%, flouzuron and fipronil, and the group 3 remained as control. See details of the treatments in materials and methods.

in March. Another ecological phenomenon that might reduce the number of ticks exposed to the effects of the acaricides is a low attachment rate of questing larvae to cattle during the period in which biocides reach an appropriate level of plasma concentration. But this hypothesis appear to be less plausible because the average stocking rate in the paddocks employed in this study was 1.5 UA/ha (this value can be considered high for the livestock production system in northwestern Argentina), which probably determine a high encounter rate of larvae with calves.

ACKNOWLEDGEMENTS

We thank Fernando García Posse and Oscar Warnke and for help during field work. This work was supported by INTA, Asociación Cooperadora INTA Rafaela, Asociación Cooperadora INTA Leales, CONICET (PIP 132) and the International Cooperation Project MINCYT-CAPEP (BR/11/08).

REFERENCES

1. Arieta-Román, R.J.; Rodríguez-Vivas, R.I.; Rosado-Aguilar, J.A.; Ramírez-Cruz, G.T.; Basto-Estrella, G. Persistencia de la eficacia de dos lactonas macrocíclicas contra infestaciones naturales de *Rhipicephalus* (*Boophilus*) *microplus* en bovinos del trópico mexicano. *Rev Mex Cienc Pecu.* 2010; 1: 59-67.
2. Cabrera, A.L. Enciclopedia Argentina de Agricultura y Jardinería. Fascículo 1. Regiones fitogeográficas argentinas. Tomo II. Editorial ACME, Buenos Aires, 1994.
3. Caracostantogolo, J.; Muñoz Cobefías, M.E.; Eddi, C.; Ambrústolo, R.R.; Bulman, G.M.; Marangunich, L. Primera determinación en la República Argentina de una población de *Boophilus microplus* (Can.) resistente al piretroide sintético alfacipermetrina caracterizada mediante pruebas preliminares. *Vet Arg* 1996; 13: 575-582
4. Cuore, U.; Cardozo, H.; Trelles, A.; Nari, A.; Solari, M.A. Características de los garrapatacidas utilizados en Uruguay. Eficacia y poder residual. *Veterinaria (Montevideo)*. 2008; 43: 13-24.
5. Cutullé, C.; Lovis, L.; D'Agostino, B.I.; Balbiani, G.G.; Morici, G.; Citroni, D.; Reggi, J.; Caracostantogolo, J.L. *In vitro* diagnosis of the first case of amitraz resistance in *Rhipicephalus microplus* in Santo Tomé (Corrientes), Argentina. *Vet Parasitol.* 2013; 192: 296-300.
6. Davey, R.B.; George, J.E. Efficacy of macrocyclic lactone endectocides against *Boophilus microplus* (Acari: Ixodidae) infested cattle using different pour-on application treatments regimens. *J Med Entomol.* 2002; 39: 763-769.

7. Davey, R.B.; Ahrens, E.H.; George, J.E.; Hunter, III J.S.; Jeannin, P. Therapeutic and persistent efficacy of fipronil against *Boophilus microplus* (Acari: Ixodidae) on cattle. *Vet Parasitol.* 1998; 74: 261-176.
8. Davey, R.B.; Pound, J.M.; Miller, J.A.; Klavons, J.A. Therapeutic and persistent efficacy of a long-acting (LA) formulation of ivermectin against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) and sera concentration through time in treated cattle. *Vet Parasitol.* 2010; 169: 149-156.
9. Estrada-Peña, A.; Venzal, J.M.; Nava, S.; Mangold, A.J.; Guglielmone, A.A.; Labruna, M.B.; de la Fuente, J. Reinstatement of *Rhipicephalus (Boophilus) australis* (Acari: Ixodidae) with redescription of the adult and larval stages. *J Med Entomol.* 2012; 49: 794-802.
10. George, J.E.; Pound, J.M.; Davey, R.B. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. *Parasitology* 2004; 129: 353-366.
11. Grillo Torrado, J.M.; Pérez Arrieta, A. Nuevo tipo de fósforo-resistencia en la garrapata común del Ganado bovino (*Boophilus microplus*) en la República Argentina. *Rev Med Vet.* 1977; 58: 101-105
12. Guerrero, F.D.; Lovis, L.; Martins, J.R. Acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus*. *Rev Bras Parasitol Vet.* 2012; 21: 1-6.
13. Guglielmone, A.A. The level of infestation with the vector of cattle babesiosis in Argentina. *Mem Inst Oswaldo Cruz* 1992; 87: Suppl. III, 133-137.
14. Guglielmone, A.A.; Hadani, A.; Mangold, A.J.; De Haan, L.; Bermudez, A. Garrapatas (Ixodoidea-Ixodidae) del Ganado bovino en la provincia de Salta: especies y carga en 5 zonas ecológicas. *Rev Med Vet.* 1981; 62: 194-205.
15. Guglielmone, A.A.; Mangold, A.J.; Aguirre, D.H.; Gaido, A.B. Ecological aspects of four species of ticks found on cattle, in Salta, Northwest Argentina. *Vet Parasitol.* 1990; 35: 93-101.
16. Guglielmone, A.A.; Volpogni, M.M.; Anziani, O.S.; Hereu, C. Evaluación del fluazurón (Acatak®) para el control de poblaciones naturales de *Boophilus microplus* (Acari: Ixodidae) en Santiago del Estero, Argentina. *Vet Arg.* 1998; 15: 710-714.
17. Guglielmone, A.A.; Mangold, A.J.; Castelli, M.; Suárez, V.H.; Aguirre, D.H.; Alcaraz, E.; Cafrune, M.M.; Cetrá, B.; Fader, O.W.; Luciani, C.A.; Medus, P.D.; Nava, S. Toxicidad *in vitro* de la cipermetrina para *Rhipicephalus (Boophilus) microplus* (Can.) y del diazinón para *Haematobia irritans* (L.) en la Argentina. *Rev Inv Agrop* 2006; 35: 31-41.
18. Guglielmone, A.A.; Castelli, M.E.; Mangold, A.J.; Aguirre, D.H.; Alcaraz, E.; Cafrune, M.M.; Cetrá, B.; Luciani, C.A.; Suárez, V.H. El uso de acaricidas para el control de *Rhipicephalus (Boophilus) microplus* (Canestrini) (Acari: Ixodidae) en la Argentina. *Rev Inv Agrop* 2007; 36: 155-167.
19. Henderson, C.F.; Tilton, E.W. Tests with acaricides against the brow wheat mite. *J Econ Entomol.* 1955; 48:157-161.
20. Mangold, A.J.; Castelli, M.E.; Nava, S.; Aguirre, D.H.; Guglielmone, A.A. Poblaciones de la garrapata *Rhipicephalus (Boophilus) microplus* resistentes a los piretroides en Córdoba y Salta, Argentina. *Rev FAVE.* 2004; 3: 55-59.
21. Martins, J.R.; Correa, B.L.; Ceresér, V.H. Ação carrapaticida do fluazurón, formulação "pour on", sobre o carrapato bovino *Boophilus microplus*. *Pesq Agrop Gaúcha.* 1995; 1: 257-261.
22. Martins, J.R.; Evans, D.E.; Ceresér, V.H.; Correa, C.L. Partial strategic tick control a herd of European breed cattle in the state of Rio Grande do Sul, southern Brazil. *Exp Appl Acarol.* 2002; 27: 241-251.
23. Norton, G.A.; Sutherst, R.W.; Maywald, G.F. A framework for integrating control methods against the cattle tick, *Boophilus microplus* in Australia. *J Appl Ecol.* 1983; 20: 489-505.
24. Spath, E.J.A.; Guglielmone, A.A.; Signorini, A.R.; Mangold, A.J. Estimación de las pérdidas económicas directas producidas por la garrapata *Boophilus microplus* y las enfermedades asociadas en la Argentina. 1^{ra} parte. *Therios* 1994; 23: 341-360.
25. Vieira, M.I.B.; Leite, R.C.; Sacco, A.M.S.; Silva, J.G.C. Estratégias de controle do carrapato *Boophilus*

microplus (Canestrini, 1887) e influencia na estabilidade enzoótica da babesiose bovina. Rev Bras Parasitol. Vet. 2003; 12: 139-144.

26. Zar, J.H. Biostatistical analysis. 4th ed., Prentice-Hall, New Jersey, 1999.