In vitro tests to establish LC$_{50}$ and discriminating concentrations for fipronil against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) and their standardization

E. Castro-Janer a,b,*, L. Rifran a, J. Piaggio c, A. Gil c, R.J. Miller d, T.T.S. Schumaker b

a Department of Parasitology, Faculty of Veterinary, UDELAR, Uruguay
b Department of Parasitology, Instituto de Biociências, USP, Brazil
c Department of Bioestatistic, Faculty of Veterinary, UDELAR, Uruguay
d USDA-ARS Cattle Fever Tick Research Laboratory, Edinburg, TX, USA

A R T I C L E   I N F O

Article history:
Received 24 October 2008
Received in revised form 2 January 2009
Accepted 6 February 2009

Keywords: *Rhipicephalus (Boophilus) microplus* Fipronil Acaricide Resistance tests

A B S T R A C T

Laboratory test was carried out on larvae and adults of the cattle tick, *Rhipicephalus (Boophilus) microplus*, to determine fipronil toxicity. Adult immersion test (AIT, *N* = 26), larval immersion test (LIT, *N* = 71) and larval packet test (LPT, *N* = 41) were standardized using susceptible strain (Mozo). Dose–response curves were compared with a fipronil resistant strain. Four variables were analyzed from AIT results: mortality, weight of eggs on day 7 and on day 14, index of fertility, and index of fecundity. For larval test, dose mortality curves were analyzed. In spite of the high LC$_{50}$ variability, all variables determined for AIT were appropriate to discriminate both strains. AIT and LIT had more sensitivity than LPT, with larger resistance factors. It was used two times LC$_{99.9}$ as discriminating doses (DCs) following FAO suggestion. For mortality by AIT, LIT and LPT the DCs were estimated: 4.98 ppm, 7.64 ppm and 2365.8 ppm, respectively, for Mozo strain. DCs mortality values estimated for resistant strain by AIT, LIT and LPT were: 6.96 $\times$ 10$^5$ ppm, 343.26 ppm and 5.7 $\times$ 10$^3$ ppm, respectively and their respective resistant factors were: 202.4, 5.36 and 1.52. Protocols for AIT, LIT and LPT have been presented in this paper.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The southern cattle tick, *Rhipicephalus (Boophilus) microplus* (Canestrini), is one of the most damaging pests that attack bovines in tropical and subtropical regions of the world. These ticks cause severe economic losses feeding on host blood and through the transmission of disease-causing agents (Thullner, 1997). In subtropical and tropical countries of South America, *R. microplus* transmits *Babesia bigemina*, *B. bovis* and *Anaplasma marginale*. The economical world losses were estimated in $7 billion (FAO, 2004), $1 billion for Latin-American (FAO, 2004) and $32.5 million/year USD for Uruguay (MGAP, 2005). These losses can be minimized with acaricide treatment, but this has contributed to the development of resistance which is, at least in different parts of the world, the main technical problem for pest and vector control programs in livestock and public health (Shidrawi, 1990). For example, resistance has been detected in *R. microplus* for almost every chemical that is registered for use against it and it stands as number 6th in the world for the number of different acaricides it is resistant to compared to all other arthropods (Whalon et al., 2008).

In Uruguay, tick control has been legislated since the 1940s relying mainly on acaricide use to include organophosphates (OP), synthetic pyrethroids (SP), amidines
(AM), macrocyclic lactones (ML), fluazuron (IGR) and phenylpyrazole (fipronil) (MGAP, 2005). During this time, the success of sanitary campaign has not been uniform due an inadequate use of acaricides and the absence of diagnostic tests to measure acaricide efficacy. Recently, resistance testing has been completed that has diagnosed resistance to OP, SP and fipronil in Uruguay by stable trials (Cuore et al., 2007).

For the success of any pest management strategy, it is necessary to use a test that is practical, quick, economical, and reliable to detect the presence of a resistance phenotype present in target populations. In vitro bioassays are relatively simple to perform, have a low cost, and have few requirements for specialized equipment (Scott, 1995). They are based on the nonparasitic stage (larvae and engorged females). The most frequently used techniques to detect resistance in the southern cattle tick are: the AIT (adult immersion test) (Whitnall and Bradford, 1947; Hitchcock, 1953; Drummond et al., 1973), LPT (larval packet test) (Stone and Haydock, 1962), adopted by FAO (Food and Agriculture Organization, 1971), and LIT (larval immersion test) (Shaw, 1966). The AIT uses engorged females which are immersed in technical or commercial acaricides and are based on rate of oviposition between females of two groups, treated and control. The eggs are analyzed by weight and viability. Additionally, direct mortality can be taken into consideration by comparing females that oviposit or not (Benavides et al., 1999). In this case, the results will be obtained in 1–2 weeks in contrast with percentage of hatching (5–6 weeks). Although there are many AIT techniques published, the Drummond protocol is used for most of the laboratory testing whereas the test that is used the most in the field evaluation of resistance uses a modified adult immersion technique. This modified technique incorporates a discriminatory concentration (DC) to difference tick resistant from susceptible strains in a field. But the main limitation is the number of engorged females required to obtain reliable results (Jonsson et al., 2007). Larval tests are a better alternative to the AIT because the high number of specimens used in the experiment greatly increases the power of the statistical analysis over adult immersion tests. For this reason, it is possible to accurately and repeatably test a range of concentrations of several acaricides, if necessary. The difficulty with the LPT is that test results can only be obtained 5–6 weeks after engorged females are collected. Currently, the FAO Working Group on Parasite Resistance recommends only the use of LPT and the Drummond test for the evaluation of acaricide resistance (FAO, 2004).

Fipronil, is a phenylpirazolic insecticide. Initially, it was used to control crop pests and other insects of public health importance (Colliot et al., 1992). Later, it was used for pet ectoparasites (ticks and fleas). In the 1990s, fipronil began to be used for the control of cattle ticks and Haematobia irritans (Guglielmone et al., 2000; Alberti et al., 2001). Fipronil kills arthropods by contact and ingestion (Medina et al., 2003) most likely through interaction with GABA receptors which are important for the propagation of nerve impulses within the nervous system.

Until now, no tests have been developed or validated for fipronil resistance in the cattle tick. Therefore, to confirm fipronil resistance in R. microplus it has been necessary to do stall trials involving treatment of experimentally infested cattle with formulated product. For this reason, the objective of the present study was to develop an adequate in vitro bioassay technique for the diagnosis of fipronil resistance in cattle ticks and to establish DCs for AIT, LIT and LPT technique.

2. Materials and methods

To standardize each method, the repetions were made in the same laboratory conditions and adjustable volume micropipettes (Acura 835®. Socrux ISBA S.A.) were used. Micropipette imprecision (CV) was <0.5% for 1000 μl volume micropipette and <1% for 50 μl volume micropipette.

2.1. Acaricides

Technical grade (95.3%) fipronil (Agromen Chemicals Co., LTD, HangZhou, China, Lote ZF300) was used in this study.

2.2. Ticks

The Mozo strain was used as susceptible reference strain. It is maintained in laboratory conditions in the Parasitology Department of the División de Laboratorios Veterinarios “Miguel C. Rubino” (MGAP, Uruguay) since May 1973 (Cardozo et al., 1984). Laboratory rearing conditions were as follows; engorged females were collected from experimental infested calves, washed with water and dried with a paper towel. Twenty to thirty engorged females were randomly selected for fecundity measurement and others 80–100 for AIT. Females selected for the fecundity measurement were stuck dorsal surface down on double sided tape on the lid of a plastic petri dish. Later, they were incubated at 27–28°C, 80–85% RH. The eggs from days 3 to 5 were pooled and used for the LPT testing. Larvae for LPT and LIT were obtained 6 weeks after the collection of adult females.

The fipronil resistant strain was originally collected in Uruguay from a population of ticks that could not be controlled with fipronil after 13 applications over an 8-year duration. The resistance was described by Cuore et al. (2007) using stable trials.

2.3. AIT

The protocol described by Drummond et al. (1973) and Benavides et al. (1999) were considered to standardize the AIT with fipronil. The following parameters were measured: female mortality, weight of eggs 7 day and on 14 day after the treatment and percent hatch. Twenty-six and 46 assays were performed for the Mozo and fipronil resistant strain, respectively. A 1% stock solution from technical fipronil was prepared in acetone and held at 4°C for 15 days. To lower the final acetone concentration, two pre-dilutions were prepared on the same day of assay. One ml of stock solution was diluted in 9 ml of acetone, and after 1 ml of this...
solution was diluted in 9 ml of distilled water. The final fipronil concentration was 0.01% in 10% of acetone. For bioassays with the Mozo strain, the following concentrations were used: 3 ppm, 2.5 ppm, 2 ppm, 1.7 ppm, 1.5 ppm, 1.2 ppm, 1 ppm, 0.8 ppm, 0.7 ppm, 0.6 ppm, 0.5 ppm, 0.4 ppm, and 0.2 ppm. The control group was submerged in an acetone solution 10% without acaricide. Bioassays were made with 5–13 dilutions, depending on the number of ticks available. Each concentration was repeated 2–3 times also depending on the number of ticks available at the time of testing. To determine the LC50 for the fipronil resistant strain, replicated serial dilutions were made from 0.1% to 0.01% with a final acetone concentration 50%, and the control group was immersed in an acetone solution 50%. Homogeneous groups in size, weight and vigor of 10 engorged females for dilution and for the control group were done. Each group was weighed and immersed for 1 min in 20 ml of the respective dilution in a 50-ml beaker and gently agitated. Later, ticks were recovered from the dilutions, dried rapidly and stuck dorsal side down on double sided tape on the lid of a plastic petri dish and incubated at 27–28 °C, 80–85% RH. On day 7 and 14, the number of females laying eggs was recorded and eggs were weighed. Treated engorged female ticks were considered live if laying eggs (independently of egg viability) and dead when not laying. At 6 weeks percentage hatch was recorded.

2.4. LPT

The LPT was carried out according to the protocol described by FAO (2004). Fipronil was diluted in a solution that contained one part commercial olive oil and two parts trichloroethylene (Merck) (TCE-OO) and ionol (0.01%) as conservator. The same stock fipronil solution (1% in acetone) described in item 2.3 was used. One ml of this solution was diluted in TCE-OO to make 100 ml. Ten serial dilutions were prepared to cover the entire dose–response range (300 ppm, 150 ppm, 75 ppm, 37.5 ppm, 18.75 ppm, 9.37 ppm, 4.69 ppm, 2.34 ppm, 1.17 ppm and 0.59 ppm) in daylight conditions. Six hundred and seventy microtubes were exposed to the filter paper treated only with the organic solvents and OO, without acaricide. The papers were held at room temperature for 24 h to permit TCE and acetone evaporation and then the papers were formed into packets by folding in half and applying clips to the sides. Approximately, 100 larval ticks (15-day-old) were placed into the packets, sealed with a clip on the open end, and incubated at 27–28 °C and 80–85% RH for 24 h. After incubation, the packets were opened and the number of live and dead larvae recorded. Each dilution was replicated three times and 15 assays were performed for the standardization. Later, the LC50 of the fipronil resistant strain was calculated using the data from 7 assays with 3 replicated for dilution.

2.5. Larval immersion test for fipronil

Modifications to the Shaw (1966) protocol were done making it similar to the LIT used by Benavides and Romero (1999). Fipronil stock solution (1% described in item 2.3) was used. However, Triton X-100 was added (0.04%) to the stock solutions prior to dilution for the test. One ml of fipronil (0.01%) was diluted in 9 ml of a solution that contained 10% acetone technical grade (100%) in distilled water and 0.04% Triton X-100. The final concentrations used were: 5 ppm, 3 ppm, 2.5 ppm, 2 ppm, 1.7 ppm, 1.5 ppm, 1.2 ppm, 1 ppm, 0.8 ppm, 0.6 ppm, 0.4 ppm and 0.2 ppm. As a control the diluent solution without fipronil was used. To determine the LC50 of the fipronil resistant strain, two higher dilutions were included (50 ppm and 100 ppm).

One ml of each dilution was added to 2.0 ml Eppendorf tubes. A group of approximately 500 tick larvae (±100) were immersed for 10 min. Tubes were agitated vigorously by hand to submerge the larvae, later the tubes with larvae were agitated gently by hand for 10 min. After, the tubes were opened and the contents drained. The larvae were removed from the microvial with a paint brush. The larvae were placed on a filter paper to dry. Approximately, 100 larvae were transferred to a filter packet paper (described previously in the LPT protocol). The packets were held at 27–28 °C and 80–85% RH for 24 h. After 24 h the packets were opened and the number of live and dead larvae recorded.

2.6. Statistical analyses

All statistical analyses were performed with Intercooled Stata 10 program (StataCorp, 2007). Lethal doses (LC50 and LC99.9) for each strain were compared taken into consideration their 95% confidence limits. Significance of this comparison was determined only if one was not included in the confidence interval. If the limits overlap, lethal doses do not differ significantly.

2.6.1. AIT

Two strains (susceptible and fipronil resistant strain) were used to evaluate the test. First, the correlation egg weight to fipronil concentration on day 7 and 14 was determined. The analyses included logarithm transformation dose. To calculate dose–response the following parameters were compared:

(a) Mortality: engorged females that oviposited were considered as live and females that did not oviposit were considered as dead.
(b) Egg weight on day 7 and 14. The LC50 was determined as the dilution that diminish egg weight by half.
(c) Index of fertility (IF) = egg mass weight (g)/females weight (g).
(d) Index of fecundity (IFec) = (IF \times \% de hatching)/100.

LC50 for IF and IFec was determined as the concentration that lowered the IF and IFec by half. Linear regression analyses were run on bioassay results. Taken into consideration all assays mean lethal concentration for each dilution was calculated. As measure of the precision of the bioassays variance was determined. Resistance factors (RF) were only calculated as the coefficient of LC50 of fipronil resistant strain and LC50 of susceptible strain (Mozo), because the LC99.9 values would provide unre-
Fig. 1. Relationship between egg weights on day 7 and 14 for susceptible Mozo (n = 62) and fipronil resistant (n = 305) ticks.

Table 1
Lethal fipronil concentration 50 and 99.9 obtained by AIT for several variables for the susceptible (Mozo) R. microplus strain.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>n</th>
<th>Slope</th>
<th>SE slope</th>
<th>t</th>
<th>R²</th>
<th>LC50 (ppm) (95% CI)</th>
<th>LC99.9 (ppm) (95% CI)</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>26</td>
<td>267</td>
<td>0.79</td>
<td>0.04</td>
<td>17.8</td>
<td>0.55</td>
<td>0.75° (0.72–0.84)</td>
<td>0.04</td>
<td>2.49° (2.35–2.6)</td>
</tr>
<tr>
<td>Egg weight 7 day</td>
<td>23</td>
<td>197</td>
<td>−1.10</td>
<td>0.08</td>
<td>−13.9</td>
<td>0.50</td>
<td>0.62° (0.55–0.67)</td>
<td>0.02</td>
<td>1.38° (1.16–1.56)</td>
</tr>
<tr>
<td>Index of fertility</td>
<td>23</td>
<td>202</td>
<td>−0.31</td>
<td>0.02</td>
<td>−12.7</td>
<td>0.45</td>
<td>0.66° (0.57–0.7)</td>
<td>0.02</td>
<td>1.46° (1.23–1.75)</td>
</tr>
<tr>
<td>Index of fecundity</td>
<td>17</td>
<td>125</td>
<td>−28.56</td>
<td>3.95</td>
<td>−7.2</td>
<td>0.3</td>
<td>0.45° (0.39–0.52)</td>
<td>0.01</td>
<td>0.84° (0.7–1.04)</td>
</tr>
</tbody>
</table>

N: number of assays, n: number of observations, t: test and R²: regression coefficient.

° Values followed with the same letter are not significantly different (P < 0.05).
was different than that observed in LC99.9 (Fig. 3), where variation was bigger. IFec was the variable with the least dispersion at the LC50 and LC99.9. The LC50 calculated for egg weight as well as IF showed symmetrical distributions.

In the fipronil resistant strain, the LC50 for each variable presented minor dispersion in relation to LC99.9 (Fig. 4). Likewise, the LC50 for IFec was the variable with least dispersion, but, in reference to LC99.9, presented similar

### Table 2

Lethal fipronil concentration 50 and 99.9 obtained by AIT for several variables for fipronil resistant *R. microplus* (*N* = 46) strain.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Slope</th>
<th>SE slope</th>
<th>r</th>
<th>R²</th>
<th>LC50⁺ (ppm) (95% CI)</th>
<th>LC99.9⁺ (ppm) (95% CI)</th>
<th>RF50⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>408</td>
<td>0.11</td>
<td>0.003</td>
<td>35.23</td>
<td>0.75</td>
<td>151.9⁺ (140.2–163)</td>
<td>348013.7⁺ (293606.8–412502.5)</td>
<td>202.4</td>
</tr>
<tr>
<td>Egg weights 7 day</td>
<td>365</td>
<td>−0.15</td>
<td>0.004</td>
<td>34.2</td>
<td>0.76</td>
<td>59.3⁺ (50.4–70.5)</td>
<td>2297.5⁺ (2017.3–2590.5)</td>
<td>96.3</td>
</tr>
<tr>
<td>Index of fertility</td>
<td>365</td>
<td>−0.04</td>
<td>0.0013</td>
<td>−32.7</td>
<td>0.75</td>
<td>66.36⁺ (56.4–78.8)</td>
<td>2391.3⁺ (2099.6–2750.8)</td>
<td>134.9</td>
</tr>
<tr>
<td>Index of fecundity</td>
<td>318</td>
<td>−3.55</td>
<td>0.486</td>
<td>−23.9</td>
<td>0.64</td>
<td>36.7⁺ (21.9–35.2)</td>
<td>506.8⁺ (427.4–588.9)</td>
<td>81.97</td>
</tr>
</tbody>
</table>

n: number of observations, r: test and R²: regression coefficient.
* Values followed with the same letter are not significantly different (P < 0.05).
* RF = resistance factor

n: number of observations, r: test and R²: regression coefficient.

In the fipronil resistant strain, the LC50 for each variable presented minor dispersion in relation to LC99.9 (Fig. 4). Likewise, the LC50 for IFec was the variable with least dispersion, but, in reference to LC99.9, presented similar

---

**Fig. 2.** Comparative activity of fipronil on susceptible and resistant tick strains for dose–response mortality, egg weight, index of fertility and index of fecundity. Dashed lines indicate 95% confidence intervals.

**Fig. 3.** Distribution of LC50 and CL99.9 of fipronil to mortality, egg weight, index of fertility and index of fecundity for Mozo strain.
dispersion for IF and egg weight. Mortality presented the greatest variability in the observed parameters at the LC$_{50}$ and LC$_{99.9}$ estimates.

3.2. LPT

The results of LPT standardization of the Mozo strain are shown in Table 3. High doses of fipronil were required to kill half of susceptible strain in relation with AIT and LIT. The LC$_{50}$ dispersion (Fig. 5) showed similar results when compared to the AIT. When assays were performed simultaneously using the fipronil resistant strain (N = 18), similar slope values were obtained (Table 4). The regression lines were parallel but not equal (Fig. 6). The regression coefficient ($R^2$) was 0.74 for Mozo strain and 0.71 for fipronil resistant strain. Because the CI (95%) for

<table>
<thead>
<tr>
<th>Test</th>
<th>n</th>
<th>Slope ± SE</th>
<th>t</th>
<th>$R^2$</th>
<th>LC$_{50}$ (ppm)</th>
<th>Variance</th>
<th>LC$_{99.9}$ (ppm)</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPT</td>
<td>392</td>
<td>17.09 ± 0.62</td>
<td>27.41</td>
<td>0.66</td>
<td>44.8, 35.8–54.4</td>
<td>0.44</td>
<td>1182.9, 688.4–2031.9</td>
<td>17.81</td>
</tr>
<tr>
<td>LIT</td>
<td>693</td>
<td>67.27 ± 1.46</td>
<td>46.02</td>
<td>0.75</td>
<td>1.43, 1.34–1.51</td>
<td>0.02</td>
<td>3.82, 3.53–4.1</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Fig. 4. Distribution of LC$_{50}$ and LC$_{99.9}$ estimation of fipronil to mortality, egg weight, index of fertility and index of fecundity for fipronil resistant strain.

Fig. 5. Distribution of LC$_{50}$ and LC$_{99.9}$ estimation of fipronil to mortality for susceptible strain (Mozo) by larval packet test (LPT) and larval immersion test (LIT).
both the LC$_{50}$ and the LC$_{99.9}$ overlapped, the lethal doses do not differ significantly.

### 3.3. LIT

The results of the LIT standardization of the Mozo strain are shown in Table 3 and Fig. 5. The toxicity of fipronil was higher when the LIT and the AIT technique were used when compared with the LPT. When bioassays were performed simultaneously with the fipronil resistant strain ($N = 12$), very different slopes values were obtained (Table 4). The $R^2$ for Mozo and fipronil resistant strain was 0.78 and 0.77, respectively, indicating a good fit to the statistical model. Significant differences were measured between the LC$_{50}$ from both strains ($P = 0.0001$) ($t = -11.272$, GL = 22). The resistance factor obtained by LIT was larger than that obtained by LPT (Table 4).

### 4. Discussion

The choice of technical fipronil and not commercially formulated product was based in the difficulty to prepare a control solution and the facility to dissolve it. Because commercial products have proprietary ingredients it is difficult to distinguish the mortality due to the active ingredient versus that which could be due to the other components of the formulation (Shaw, 1966). Solubility problems with other commercial products have been reported. Sabatini et al. (2001) detected problems with commercial injectable formulations limiting its use for LPT. In the present study, technical fipronil dissolved well in acetone, facilitating the standardization of the three bioassay techniques used in this study. However, acetone is highly volatile, and we observed that fipronil stock solutions could be maintained refrigerated for 2 weeks without changes in the results of the test for this reason. Likewise, it was not convenient to maintained stock solutions with low fipronil concentrations (0.01%) in acetone 5% or 10% because after a few days, the fipronil began to precipitate. In the present study, ethanol was used because its lower volatility.

For AIT, each variable could be used for resistance diagnosis despite the fact that the degree of LC$_{50}$ dispersion for mortality, egg weight and IF was very large in relation to IFec for Mozo strain. This behavior was expected for mortality because the numbers of ticks for observation unit was low ($n = 10$), but it was not expected in relation to egg weight and IF since they were continuous variables.

---

### Table 4

<table>
<thead>
<tr>
<th>Test</th>
<th>Strain</th>
<th>Slope ± SE</th>
<th>$t$</th>
<th>LC$_{50}$ (ppm) (95% CI)</th>
<th>LC$_{99.9}$ (ppm) (95% CI)</th>
<th>RF$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPT</td>
<td>Mozo</td>
<td>16.93 ± 0.78</td>
<td>21.55</td>
<td>64.39$^a$, 50.4–82.2</td>
<td>2724.69$^c$, 1540.4–4819</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Fipronil resistant</td>
<td>15.54 ± 0.78</td>
<td>20.01</td>
<td>98.15$^c$, 77–125.1</td>
<td>2848.12$^c$, 1533.4–5289.2</td>
<td>1.52</td>
</tr>
<tr>
<td>LIT</td>
<td>Mozo</td>
<td>71.34 ± 3.37</td>
<td>21.15</td>
<td>1.8$^a$, 1.8–1.9</td>
<td>3.83$^a$, 3.5–4.1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Fipronil resistant</td>
<td>20.60 ± 1.02</td>
<td>20.34</td>
<td>9.7$^a$, 7.3–12.8</td>
<td>171.63$^b$, 71.6–409.7</td>
<td>5.36</td>
</tr>
</tbody>
</table>

* Values follow with the same letter no statistically differences for $P < 0.05$.  
$^a$ RF = resistance factor.

---

Fig. 6. Dose–response mortality data of susceptible (Mozo) ($n = 206$) and fipronil field resistant larvae ($n = 162$) of *R. microplus* using LPT and LIT.
The IFec variable showed the most data variation in the dose–response curve with a CI 95% very large. Perhaps this was due to the long incubation time. The data was obtained after 5–6 weeks in contrast with mortality and egg weight that were obtained within 1 week. The small amount of deviation in LC50 mortality distribution indicates that it satisfactory to only record oviposition to determine if engorged females are resistant without consideration of egg mass weight or egg viability. The Mozo strain could be considered homogeneous so the LC50 variations in all parameters reflect the natural variation of this strain (Robertson et al., 2007).

The fipronil resistant strain should be considered as heterogeneous. Therefore, the high variation at the LC50 estimate for mortality was expected because both susceptible and resistance genes were present in this strain when tested. The confidence intervals were large after 5–6 weeks in contrast with mortality and egg weight was due to the long incubation time. The data was obtained within 1 week. The small amount of deviation in LC50 mortality distribution indicates that it necessary to weigh eggs and determine percentage of hatching. Additionally, the advantage of recording mortality at 7 days, avoids the use of more complex equipment for the incubation of eggs.

Fast data attainment is of great practical importance when it is necessary to decide about acaricide rotation in a particular area where ticks maybe resistant to the current acaricide in use. However, a great number of engorged females with uniform size are needed to execute an accurate test and this is not often the case. However, when few engorged females are collected, a larval test can be used in place of the AIT.

The fact that fipronil used in the LPT showed less toxic than when used in the LIT (Table 3), agrees with data obtained by Shaw (1966). Likewise, White et al. (2004) encountered greater toxicity using larval immersion test by means microassay than LPT, due to larger product tick contact. LIT more efficiently discriminated between the resistant and susceptible strain. This is valuable in that fipronil resistance is more likely to be detected sooner than when the LIT is used rather than when the LPT is used.

According to Jonsson et al. (2007), low slopes values for AIT could be a problem to define DCs for the diagnosis of resistance, particularly if RFs are low. In the present study, the slope values were low, however, RF values were high. The high RF value for mortality calculated for the fipronil resistant strain is in agreement with the very low percentage of efficacy (18%) determined by stable trials (Cuore et al., 2007). Our data show that it is possible to use the AIT as a diagnostic for the detection of fipronil resistance.

Either one of the bioassays developed in this study can be used for the diagnosis of resistance in R. microplus to fipronil. The LIT showed the best performance in this study. However, the AIT could be improved by using ethanol as solvent and testing more ticks per concentration in the DC, and more LPT assays need to be completed with more strains to verify the discriminate power of resistance for fipronil. For more reliable results, it is desirable to use a susceptible strain along with the unknown strain (at less than one dilution). More studies will be required to associate the field acaricide efficacy with RF value. These results establish an accurate and representative DC for the detection of fipronil resistance in field collected R. microplus.

Acknowledgements

We thank Maria Angélica Solari and Ulises Cuore from the Department of Parasitology-DILAVE (Uruguay) for providing us with Mozo strain and Armando Nari for the critical reviewing. We also appreciate the assistance with the project of Rosario Silveira and Rosmary Domínguez from the College of Veterinary Medicine (Uruguay) and Guilherme Klaufke from the Institute of Biosciences, University of Sao Paulo (Brazil).

This project was done with financial support of INIA-FPTA No. 243 (Uruguay) and FAPESP (Brazil).

References


StataCorp., 2007. Stata Statistical Software: Release 10. StataCorp LP, College Station, TX.


