V INTERNATIONAL SEMINAR OF ANIMAL PARASITOLOGY
World situation of parasite resistance in Veterinary Medicine
V INTERNATIONAL SEMINAR
IN ANIMAL PARASITOLOGY

"WORLD SITUATION OF PARASITE
RESISTANCE IN VETERINARY MEDICINE"


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ADULT IMMERSION TESTS OF ACARICIDE SUSCEPTIBILITY IN AMERICAN AND AUSTRALIAN STRAINS OF *Boophilus microplus*.

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Abstract

Adult immersion tests (AITs) for acaricide susceptibility were conducted in a similar manner in Texas, USA, using the Muñoz strain and in Queensland, Australia, using the N-strain of *B. microplus*. Data were collected on oviposition 7 d after exposure to acaricide and subjected to probit analysis. Most of the data provided a poor fit with the probit analysis model used and there were substantial differences in LC$_{50}$ and LC$_{99}$ between the two strains as tested in the respective laboratories.

1. INTRODUCTION

The standard bioassay recommended by FAO for testing resistance to acaricides in the one-host cattle tick *Boophilus microplus* is the larval packet test (LPT), originally described by Stone and Haydock (1962). Many other tests have been used, however, including the larval immersion test (LIT) of Shaw (1966) and adult immersion tests (AIT) described variously by Whitnall and Bradford (1947), Hitchcock (1953) and Drummond et al. (1973). The LPT is a laborious test, takes between five and six weeks to complete and it has not been adopted in all countries. Neither molecular nor biochemical assays have yet been developed to the point where they are useful for diagnosis of resistance in the field. Interest in the development of standard protocols for AITs has been prompted by the ease with which the test can be conducted, the lack of any special equipment requirements and the fact that the test can be completed within 7 d.

The development of a standard protocol for AITs in the field is dependent on the identification of discriminating doses (DDs), above which no susceptible adults are able to survive and produce eggs. Using a 7 day oviposition protocol for the AIT, DDs have been reported recently for moxidectin, ivermectin, abamectin and doramectin (Sabatini et al., 2001), however, DDs have not been reported for other products.

The purpose of the work described in this paper was to apply a standard AIT methodology to Australian and American strains of *B. microplus* to determine LC$_{50}$ and LC$_{99}$s for coumaphos, cypermethrin, amitraz and moxidectin.
2. MATERIALS AND METHODS

2.1. Acaricides

Commercial acaricides were purchased and stored according to label directions for this study.

2.2. Ticks

In Queensland, N-strain ticks from Yeerongpilly Veterinary Laboratories were used for all the studies. This is the standard, susceptible reference strain used in Australia. In Texas, Muñoz strain was used. Muñoz strain was collected from Zapata County in Texas in 1999, is susceptible to all acaricides tested and has been reared in the laboratory without acaricide selection. Engorged female ticks were washed in water after collection and were stored at 4°C for a maximum of 48 h before being subjected to the bioassay.

2.3. AIT procedure

The FAO modified protocol for the AIT was followed (FAO, In Press). For each acaricide, an initial set of concentrations was prepared by performing ten-fold dilutions, bracketing the DD suggested in the FAO guidelines (FAO, In Press). In Australia, each concentration was tested using three replicates each containing 10 ticks. In the U.S.A., each replicate contained 6 ticks. For each acaricide, after the initial ten-fold dilution series, a DD was approximated by observation of raw data and a second series of dilutions was prepared.

Ten (N-strain) or six (Muñoz strain) clean, healthy, engorged female ticks were placed in 20 ml of water or into 20 ml of one of each dilution of acaricide in a 100 ml plastic container. The ticks were held in the plastic containers, with occasional gentle agitation, at room temperature (approximately 25°C) for 30 min before being removed and gently dried on paper towelling. All ticks from each container were stuck, ventral side up, with double-sided sticky tape in a Petri dish. They were then placed in larger, plastic boxes containing a moistened sponge for 7 d at temperatures between 25 and 30°C. Seven days after immersion, the number of ticks in each treatment that produced eggs was counted.

2.4. Determination of dose-response

For each treatment, the number of ticks tested at each concentration and the number of ticks that did not lay eggs was determined and submitted to POLO-PC for probit analysis and estimates of the LC50 and LC99 and their 95% confidence intervals were obtained.

3. RESULTS

Plots of dose-response curves for N-strain ticks in Queensland and Muñoz-strain ticks in Texas are shown in Figure 1. Table 1 lists the LC50, LC99 for each strain and each acaricide. 95% Confidence intervals for LC50 and LC99 were very wide and in some cases could not be estimated. For each strain and acaricide combination, excepting the N-strain with moxidectin, the chi-square goodness of fit estimation
exceeded the level that would be expected for a good fit of the data with the probit analysis model.

Table 1. Results of adult immersion test (AIT) for N-strain and Muñoz strain ticks using formulated amitraz, coumaphos, cypermethrin and moxidectin

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>Strain</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>X-square&lt;sup&gt;b&lt;/sup&gt;</th>
<th>d.f.</th>
<th>Slope</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; g/L (95% CI)</th>
<th>LC&lt;sub&gt;99&lt;/sub&gt; g/L (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitraz</td>
<td>N</td>
<td>45</td>
<td>74.7</td>
<td>43</td>
<td>0.94</td>
<td>0.0078 (0.0043-0.013)</td>
<td>2.30 (0.80-12.4)</td>
</tr>
<tr>
<td></td>
<td>Muñoz</td>
<td>21</td>
<td>21.9</td>
<td>19</td>
<td>0.28</td>
<td>0.00029 (0.000041-0.0024)</td>
<td>45200 (155-)</td>
</tr>
<tr>
<td>Coumaphos</td>
<td>N</td>
<td>48</td>
<td>134</td>
<td>46</td>
<td>1.1</td>
<td>0.027 (0.010-0.051)</td>
<td>3.3 (1.01-36.6)</td>
</tr>
<tr>
<td></td>
<td>Muñoz</td>
<td>24</td>
<td>33.3</td>
<td>22</td>
<td>0.67</td>
<td>0.0036 (0.0083-0.012)</td>
<td>10.6 (1.00-2628)</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>N</td>
<td>48</td>
<td>100</td>
<td>46</td>
<td>3.88</td>
<td>0.003 (0.002-0.003)</td>
<td>0.011 (0.008-0.022)</td>
</tr>
<tr>
<td></td>
<td>Muñoz</td>
<td>18</td>
<td>20.7</td>
<td>16</td>
<td>0.54</td>
<td>0.003 (0.001-0.011)</td>
<td>0.079 (0.014-1.58)</td>
</tr>
<tr>
<td>Moxidectin</td>
<td>N</td>
<td>12</td>
<td>8.95</td>
<td>10</td>
<td>0.76</td>
<td>0.026 (0.010-0.064)</td>
<td>29.3 (5.22-560)</td>
</tr>
<tr>
<td></td>
<td>Muñoz</td>
<td>18</td>
<td>41.9</td>
<td>16</td>
<td>0.85</td>
<td>0.000 (0.000-0.000)</td>
<td>0 (0.000-0.0005)</td>
</tr>
</tbody>
</table>

<sup>a</sup>N, number of dose groups  
<sup>b</sup>Goodness of fit  
<sup>c</sup>degrees of freedom
Figure 1. Dose-response curves for N-strain and Muñoz strain *B. microplus* ticks subjected to a modified AIT with aqueous dilutions of commercially formulated acaricides.
4. DISCUSSION AND CONCLUSIONS

This small study indicates that the identification and application of discriminating doses (DDs) to the modified adult immersion test (AIT) for the detection of acaricide resistance in *B. microplus* is likely to be problematic. Only one of the bioassays showed a good fit with the probit analysis model. For all acaricides, the Muñoz strain ticks generated rather flat slopes, none being greater than one. For N-strain ticks, only cypermethrin (3.88) and coumaphos (1.1) had gradients greater than one. Except for cypermethrin and coumaphos, there was considerable divergence between the LC₅₀ and LC₉₀ of American studies on Muñoz and Australian studies using N-strain ticks. This could be due to differences between strains or arising from small variations in bioassay procedures. Regardless of the source of the differences it is not promising for the application of the DD concept.

Estimated LC₅₀s and LC₉₀s for N-strain ticks exposed to moxidectin were much higher (0.026 and 29.3) than those (0.0002 and 0.0007 g/l) reported by Sabatini et al (2001). Except for moxidectin, the DDs estimated by the FAO from doubling the LC₉₀ (FAO in press) of 5 g/l for coumaphos (N-strain LC₉₀ of 3.3 g/l), 0.05 g/l for cypermethrin (N-strain LC₉₀ of 0.01 g/l), 2.5 g/l for amitraz (N-strain LC₉₀ of 2.3 g/l) are more or less in line with LC estimates for N-strain ticks tested in the present study. The suggested DD for moxidectin was 1 g/l, which lies between the estimated N-strain LC₉₀ (29.3 g/l) and the estimated LC₉₀ value of <0.0005 g/l for Muñoz ticks.

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6. REFERENCES