DETECTION OF DELTAMETHRIN RESISTANCE IN BUFFALO LOUSE, 

HAEMATOPINUS TUBERCULATUS

Nirbhay Kumar Singh*, Manjurul Haque, Jyoti and Harkirat Singh

ABSTRACT

Buffalo sucking lice Haematopinus tuberculatus collected from buffaloes of the Tajpur Dairy Complex, Ludhiana, Punjab, India were tested by in vitro treated surface (contact) bioassay against a range of dilutions of deltamethrin for detection of resistance status. The regression graph of probit mortality of lice was plotted against log values of progressively increasing concentrations of deltamethrin. From the regression equation the LC50, LC95 and LC99.9 values of deltamethrin were calculated as 16.55, 76.66 and 297.18 ppm and the resistance factor was calculated as 23.77. The indiscriminate and extensive usage of these synthetic pyrethroids predominantly deltamethrin particularly in this part of the country for control of ectoparasites resulted in the development of resistance in lice populations. The implication is that effective control for the ectoparasites particularly lice in dairy animals requires proper control management including selection of insecticide along with policy for its rotation to increase its effective life.

Keywords: deltamethrin, Haematopinus tuberculatus, Punjab, resistance

INTRODUCTION

The sucking louse Haematopinus tuberculatus (Burmeister, 1839) Lucas, 1852, is a harmful ectoparasite found on buffalo (Bubalus bubalis) and has been reported from various parts of Asia, Africa, Australia, South America and Europe (Veneziano et al., 2007). The presence and feeding of lice causes irritation, with the animal reacting by rubbing and scratching, resulting in patchy hair loss, sores and untidy appearance. Lice can significantly affect hide and leather quality. Reduced feed intake and weight gain are also common and can have a profound impact on productivity of the dairy animals (FAO, 2004).

Specific treatments for lice control are uncommon throughout the world; however, products aimed at other external parasites (ticks, mites, flies) have had an effect on buffalo lice populations (Levot, 2000). The regularity of some of these treatments in certain areas has probably reduced lice infestation to sub-clinical levels whereas indiscriminate use with incorrect concentrations of insecticides has probably contributed to the development of resistance in these arthropods (FAO, 2004). The synthetic pyrethroids (deltamethrin and cypermethrin) are commercially available in India and at present are the two predominant insecticides used for control of ectoparasites in the country. Large-scale resistance to synthetic pyrethroids has
recently been experimentally validated in Indian isolates of cattle ticks *Rhipicephalus* (*Boophilus*) *microplus* (Sharma *et al*., 2012) and *Hyalomma anatolicum* (Shyma *et al*., 2012). Although dairy farmers have reported treatment inefficiency of these chemicals in field conditions, data on lice resistance to these chemicals are currently not available from the country.

Keeping in view the requirement of preserving the life span of costly insecticides, reluctance of multinationals in funding on insecticide research, and costs involved in generation and marketing of new group of chemicals for the arthropod control, it is becoming essential to develop resistance data for implementation of future arthropod control measures. The current study was undertaken to generate data on the deltamethrin resistance status of *H. tuberculatus* collected from buffaloes of Ludhiana, Punjab, India.

**MATERIALS AND METHODS**

Lice were collected by manual picking from the naturally infested buffaloes of the Tajpur Dairy Complex, Ludhiana, Punjab and were identified under optical and dissection microscopes based on the keys proposed by Chaudhuri and Kumar (1961).

Technical grade 99.3% pure deltamethrin (AccuStandard® Inc. U.S.A) was used to prepare the stock solution in acetone. For the experimental bioassay, different working concentrations of deltamethrin (12.5, 25, 37.5, 50 and 62.5 ppm) were prepared in distilled water from the stock solution and tested against *H. tuberculatus*.

Laboratory *in vitro* treated surface (contact) bioassay (Levot and Hughes, 1990) was adopted with slight modifications. Briefly, freshly prepared working dilutions of deltamethrin were used for layering of clean dry Petri dishes and three replicates were maintained for each concentration. Ten live lice were placed into each Petri dish and incubated (in darkness) at 34°C and 70%-80% relative humidity (RH) for 24 h. The mortality was recorded by counting the number of dead lice which were immobile and showed signs of desiccation.

Dose response data were analyzed by probit regression (Finney, 1962) and the LC$_{50}$, LC$_{95}$ and LC$_{99.9}$ values of deltamethrin were determined by applying regression equation analysis to the probit transformed data of mortality. Resistance factor (RF) was worked out by the quotient between LC$_{99.9}$ of field isolates and LC$_{99.9}$ of susceptible isolate. Due to absence of a reference susceptible isolate of *H. tuberculatus* the LC$_{99.9}$ of susceptible was calculated from recommended commercial concentration (RCC) of deltamethrin. The RCC of an insecticide is twice the LC$_{99.9}$ of susceptible isolates (FAO, 2004) hence, as deltamethrin is used at 25 ppm the LC$_{99.9}$ for susceptible isolate as 12.5 ppm was used in the study.

**RESULTS AND DISCUSSION**

Lice collected from buffaloes of the Tajpur Dairy Complex, Ludhiana, Punjab were identified as *Haematopinus tuberculatus*. Data on the effects of various concentration of deltamethrin on *H. tuberculatus* are presented in Table 1. The results of the *in vitro* study revealed that maximum louse responses in terms of mortality were observed within 1-3 h of exposure. After 6 h of exposure, almost no concentration-dependent change in mortality was seen following contact with deltamethrin. The incubation time is important and an optimum 16 h exposure is recommended, despite highest
mortality within 2 - 4 h (Levot, 2000). The lice were incubated in darkness at 34°C and 70 to 80% RH to reduce the stress caused by environmental conditions as stressed lice may be affected by lower concentrations and give false susceptibility readings (Levot and Hughes, 1990).

The mortality of lice was increased with increasing concentrations of deltamethrin and maximum mortality of 96.67% was recorded at 62.5 ppm (Table 1). It was observed that exposure of lice to the concentration at which deltamethrin is being widely used (25 ppm) could only achieve 60% mortality and even the much higher concentration of 62.5 ppm failed to produce 100% mortality, thus indicating development of resistance against deltamethrin. About 7% of the lice died in the control

Table 1. Effect of different concentrations of deltamethrin on *H. tuberculatus*.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Number of lice died</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N 30 min 1 h 3 h 6 h 12 h 24 h</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>30 0 2 8 2 2 0</td>
<td>46.67</td>
</tr>
<tr>
<td>25</td>
<td>30 0 4 8 5 1 0</td>
<td>60.0</td>
</tr>
<tr>
<td>37.5</td>
<td>30 0 6 12 4 0 0</td>
<td>73.33</td>
</tr>
<tr>
<td>50</td>
<td>30 1 5 13 6 0 0</td>
<td>83.33</td>
</tr>
<tr>
<td>62.5</td>
<td>30 2 3 18 5 0 0</td>
<td>96.67</td>
</tr>
<tr>
<td>Control</td>
<td>30 0 0 0 1 1 0</td>
<td>6.67</td>
</tr>
</tbody>
</table>

Figure 1. Dose mortality curve of *H. tuberculatus* against deltamethrin.
group and this may be due to suffocation or some other reason unknown to us. In bioassay, technical grade deltamethrin was selected over commercial formulation because commercial products are prepared with many proprietary ingredients and it is difficult to assess the responses due to active ingredients (Shaw, 1966).

The regression graph of probit mortality of lice plotted against log values of progressively increasing concentrations of deltamethrin is shown in Figure 1. The dotted lines in the regression curve represented the 95% confidence limits. The slope of mortality was $2.464\pm0.6783$ whereas the value of goodness of fit ($R^2$) was 0.8148. From the regression equation the LC$_{50}$, LC$_{95}$ and LC$_{99.9}$ values of deltamethrin were calculated as 16.55, 76.66 and 297.18 ppm and the RF was 23.77. It should be noted, however, that without a reference population of pyrethroid naive $H. tuberculatus$ it is not possible to unequivocally prove that the lice tested have developed resistance. Reference populations unfortunately could not be obtained for this study due to the scarcity of untreated animals.

However, there is relatively little comparable published data on the efficacy of deltamethrin against buffalo lice. Previous studies have reported pyrethroid resistance in Australian field populations of the sheep body louse, $Bovicola (Damalinia)$ ovis (Johnson et al., 1992; Levot et al., 1995; Jazayeri, 2004). In similar studies, four populations of $B. ovis$ indicated possible resistance to deltamethrin from the United Kingdom (Bates, 2001).

The development of resistance against deltamethrin in lice from Ludhiana, Punjab may be attributed to the fact that farmers adopted frequent treatment of animals with available insecticides without maintaining an optimum dose regime for the control of ectoparasites particularly ticks. Spray (deltamethrin and cypermethrin) and injection (ivermectin) were mainly used for application of insecticides while pour-on (flumethrin) is used by nearly 15% of the farm owners from Ludhiana (Sharma et al., 2012). This indiscriminate and extensive usage of these synthetic pyrethroids particularly deltamethrin resulted in the development of resistance in lice populations. Also, the higher reproductive rate of lice that have heritable resistance factors and the resulting increase in the proportion of the population of lice that carry genes for these factors results in the establishment of resistance in the population (FAO, 2004). Further, development of resistance in populations of lice may be rapid for several other reasons: lice are host-specific obligate parasites and there is relatively little immigration from different populations. Hence any resistant genotypes quickly replace the susceptible under the selection pressure imposed by insecticide (Ellse et al., 2012).

There are two means of pyrethroid resistance development in lice, either through single point mutations in the gene coding for the drug target protein (Lee et al., 2000) or by upregulation of metabolic, monooxygenase enzymes (Scott, 1999). Therefore, future studies directed against detection of the mechanism of the resistance would be of immense help in development and implementation of strategies for effective control for the ectoparasites particularly lice in dairy animals.

ACKNOWLEDGEMENT

Authors are thankful to Director of Research, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana for providing facilities to carry out the research work.
REFERENCES


Levot, G.W. and P.B. Hughes. 1990. Laboratory studies on resistance to cypermethrin in

Buffalo Bulletin (June 2015) Vol.34 No.2


Veneziano, V., M. Santaniello, S. Carbone,