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Diagnosis of amitraz resistance in *Boophilus microplus* in New Caledonia with the modified Larval Packet Test

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Abstract

The tick *Boophilus microplus* represents a serious pathological constraint to livestock production in New Caledonia. Cattle ticks are controlled by chemical application of two acaricides that are currently used in New Caledonia; deltamethrin is used at 46% of the cattle production facilities and amitraz at the remaining 54% premises where resistance to deltamethrin has been identified. In 2003, a modified Larval Packet Test (LPT) was used to conduct a survey for amitraz resistance. Ticks were collected from 29 farms, including farms using deltamethrin ($n = 8$) or amitraz ($n = 21$). Of eighteen different tick populations, sixteen populations were defined susceptible to amitraz and two populations were considered amitraz-resistant. This is the first report of populations of *B. microplus* being resistant to amitraz, using the modified LPT in New Caledonia. A thorough survey of tick susceptibility to amitraz in cattle farms of the country should be conducted to assess the presence of amitraz-resistant populations. The emergence of amitraz resistance so soon after its introduction has some important implications for the strategy and organisation of tick control in New Caledonia, and this paper discusses some of the urgent actions that should be undertaken.

Keywords: *Boophilus microplus*; Modified Larval Packet Test; Amitraz; Resistance; New Caledonia

1. Introduction

The tick *Boophilus microplus* (Canestrini) was introduced into New Caledonia (between 20° and 22° South) in 1942 via importation of animals from Australia (Rageau and Vervent, 1959). This one-host-tick is the principal ectoparasite of Caledonian cattle. Climatic conditions favour *B. microplus* activity all year, and the tick can complete at least four generations per year (Bianchi et al., 2003). Fortunately, the microbial diseases transmitted by *B. microplus* were not imported into New Caledonia along with the tick. However, blood loss and reduction in weight gain resulting from tick feeding represents one of the most important pathological constraints to livestock production in the country (Daynes et al., 1984).

There are about 150,000 cattle belonging to 1200 breeders in New Caledonia (Barré, 2003). The main

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cattle breeds include Limousin, Charolais and Hereford. These breeds, being *Bos Taurus*, are highly susceptible to ticks (Wharton et al., 1973; Pegram et al., 1993; de Castro and Newson, 1993). Tick control in New Caledonia is the responsibility of territorial authorities, and acaricide is freely delivered to cattle producers. The tick control program is based exclusively on the use of chemicals applied on a regular basis every 4–5 weeks (Bianchi et al., 2003). In the past, arsenic (1943–1950), DDT (1947–1973) and diethylethion (1973–1980s) were used for tick control until resistance to these compounds made further use impractical (Brun et al., 1983). Diethylethion was replaced by the synthetic pyrethrinoid (SP) deltamethrin (Butox ND). Six years after introduction, the first cases of deltamethrin resistance were reported by Beugnet and Chardonnet (1995). The organophosphate (OP) chlorpyriphos-ethyl (Dursbel ND) was introduced into New Caledonia in 1994 but replaced in 1996 by amitraz (Taktic ND) for use on deltamethrin-resistant farms. Since that time, only amitraz and deltamethrin are provided by Veterinary Services to producers for the control of *B. microplus* throughout the country. The emergence of tick populations resistant to amitraz was predictable after several years of use.

Currently, suspicion of resistance to deltamethrin in a cattle production facility is confirmed by a bioassay conducted at the Laboratory of Parasitology of IAC at Port-Laguerre. The bioassay technique used in New Caledonia was developed by Stone and Haydock (1962), and was subsequently adopted by the FAO as a standard method to determine the susceptibility of tick populations to acaricides as the Food and Agriculture Organization Larval Packet Test (LPT). This bioassay is based on the observation of larval mortality after placement in a paper packet treated with a known concentration of acaricide. Unfortunately, the LPT technique does not measure the susceptibility of ticks to amitraz because no dose related response is produced (Kemp et al., 1998; Miller et al., 2002). Recently, a modification of the LPT for the determination of amitraz resistance in *B. microplus* was developed at the United States Department of Agriculture, Cattle Fever Tick Research Laboratory (USA) by Miller et al. (2002), and it was decided to conduct a survey for amitraz resistance in New Caledonia using this test.

2. Materials and methods

2.1. Choice of farms

A total of 29 farms were visited including (1) farms where deltamethrin was still used and where animals have never been treated with amitraz ($n = 8$) and (2) farms where amitraz was used regularly (10–12 times a year) for 3–10 years ($n = 21$). Among these farms, one breeder had observed a lack of efficacy of the treatment.

2.2. Collecting of ticks

At each farm, ≈30 engorged females of *B. microplus* were collected with a maximum of five ticks from any one animal. Ticks were transported to the Laboratory of Parasitology of Port-Laguerre and held in a rearing room at 26–27 °C and 80–92% RH for 2 weeks. Twelve to 16-day-old larvae were used for testing.

2.3. Bioassays

The modified-LPT was conducted following the procedures described in Miller et al. (2002). In this test, nylon fabric (Type 2320, Cerex Advanced Fabrics, Pensacola, FL) was used as a substrate. Serial dilutions from a top dose of amitraz (1%) were made using a 2:1 trichloroethylene and oil diluent. Formulated amitraz (Taktic ND 12.5% EC, product of Intervet) was used. Twelve doses, including the control (diluent only) were prepared for each bioassay and each dose had three replicates. A volume of 0.67 ml of each dilution was applied to a piece (7.5 cm × 8.5 cm) of nylon fabric. After 2 h in a fume hood, to allow for the trichloroethylene to evaporate, pockets were made with the treated fabrics. Approximately 100 larvae were placed into each pocket before they were placed in an incubator at 27 °C and 85–92% RH for 24 h. After incubation the live and dead larvae were counted in each packet to determine mortality.

2.4. Statistical analysis

Probit analysis was run on the bioassay results using Polo-PC (Le Ora Software, 1987). The log-probit model estimated by Polo-PC is illustrated by a