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Detrimental effect of cypermethrin treated nets on *Culicoides* populations (Diptera; Ceratopogonidae) and non-targeted fauna in livestock farms

R. Del Río[a,*], C. Barceló[a], J. Lucientes[b], M.A. Miranda[a]

[a] Laboratory of Zoology, University of the Balearic Islands (UIB), Cra. Valldemossa Km. 7.5, Palma de Mallorca, Spain
[b] Department of Animal Pathology, Faculty of Veterinary Science, University of Zaragoza, Zaragoza, Spain

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ABSTRACT

Bluetongue (BT) is an important disease of ruminants which exhibits its most severe clinical signs on cattle and especially on certain breeds of sheep. The known vectors of BT are small insects of the genus *Culicoides* (Diptera; Ceratopogonidae). Two species from this genus – *Culicoides imicola* and *Culicoides obsoletus* – play the major role in the transmission of the disease in Europe. Several prophylactic methods are used to avoid transmission; however, an easy and cost-effective preventive technique would be very useful for the control of the *Culicoides* populations near the animals. In the present study, the insecticide effect of cypermethrin treated nets on a *Culicoides* population was evaluated. A polyethylene net sprayed with 1 L cypermethrin solution (1%) surrounding a UV light suction trap was placed at a cattle farm in Majorca (Balearic Islands). Collections of *Culicoides* and other fauna from the trap and floor around the net were compared with a control. Results showed no significant differences in the collection of *Culicoides* midges between the insecticide-treated net and the control. However, significant differences were observed in the collection of the non-target fauna between the treated net and the control, indicating that the dose used in the present trial was enough to kill most of the arthropods that contacted the net. The reasons for these equivocal findings and means to improve this technique for the control of *Culicoides* midges are discussed.

1. Introduction

Bluetongue (BT) is a viral disease of ruminants that exhibits its more severe clinical signs when naive sheep population, and to some extend cattle, are infected with a BT virus (BTV) from endemic areas. BT disease was recorded for the first time in South Africa in late XVIII century (Spreull, 1905) and, even if occasional outbreaks occurred in Europe, did not spread and established in the next continent until the end of the following century. One of the possible reasons of this spread to other regions is the modification of the environmental conditions during the last decades (Purse et al., 2005; Guis et al., 2012) which allows the BTV vectors to breed and survive in farther north regions at the same time that the virus cycle is enhanced (Mullens et al., 1995). The females of some species of the genus *Culicoides* (Diptera; Ceratopogonidae) act as efficient vectors of the BTV. In the southern regions of Europe, such as Spain and Portugal, *Culicoides imicola* Kieffer, 1913 and the Obsoletus complex play an important role in the transmission of the disease (Baylis, 2002; Mellor and Wittmann, 2002) while, in northern Europe, the main

* Corresponding author. Tel.: +34 971 173352; fax: +34 971 173184.
E-mail address: ricardo.delrio@uib.es (R. Del Río).

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vector of BTV is thought to belong only to the Obsoletus complex (Carpenter et al., 2009).

The most efficient method to protect susceptible animals from BT disease is vaccination. However, the versatility of the BTV (Roy and Noad, 2006; Schwartz-Cornil et al., 2008) renders vaccination as an impractical preventive measure in regions where the virus goes undetected during at least two years (WOAH, 2010). A most economic approach for the prevention of the diseases transmitted by *Culicoides* midges is the use of insecticides. Some pyrethroids have demonstrated their effectiveness in laboratory conditions (Floore, 1985; Venail et al., 2011). However, few studies have been conducted in field conditions and limited results were obtained (Venail et al., 2011; Del Río, 2012). The use of insecticide treated nets on stables or animal transport vehicles is an innovative step in the control of BT disease and its potential as an effective preventive measure is currently under study.

Our aim in the present study was to evaluate the effect of cypermethrin treated nets on field conditions. For this, polyethylene nets were manually impregnated with the insecticide and placed in a cattle farm near the animals to assess their impact on the *Culicoides* population. The cost-effectiveness and ease of this technique qualifies the insecticide treated nets as good candidates for vector control and their associated diseases.

2. Materials and methods

The present trial was conducted at a cattle farm named *Ca's Boter* (39° 30′ N; 3° 7′ E) located in Majorca (Balearic Islands, Spain), where the presence of BT vectors were previously detected. Collection of insects was performed with UV light-suction traps (Onderstepoort model; OVI-ARC, Onderstepoort, South Africa) during 16 non consecutive nights, starting on September 19th and ending on October 26th 2012.

Two blue shading nets, made from inert polyethylene fibers (fiber wideness: 1 mm, gap between fibers: 2 mm), were used to analyze the effect produced by a cypermethrin barrier on the *Culicoides* population. Nets were assembled on a round metallic mesh 1.5 m high and 1 m wide covering also its upper part. A hanger placed inside this structure was used to hold a battery powered (12 V) UV light trap. One of the nets was impregnated with 1 l of cypermethrin solution 1% with a compressed-air hand-pump sprayer and was re-sprayed with the same dose once a week or after heavy rain. The other net was assembled in the same way and used as a control. A piece of white polyethylene fabric (2 m × 2 m) was placed at the base of each structure and aspirated the morning after every sampling with a customized battery powered hand vacuum cleaner to analyze the killing effect of the insecticide around the net on the *Culicoides* population and the non targeted fauna. Control and treated nets were placed outside the stables but near the cattle, at an open area with little vegetation around, separated about 30 m from each other, avoiding interference between the lights of the traps. Traps were operated from dusk till dawn (08:00 pm to 07:00 am) and the survival of trapped midges was enhanced by placing a folded cellulose paper inside the collection container of the traps sheltering the insects from the air current of the fan trap. After each sampling night, the *Culicoides* midges and the non-target fauna were collected from the traps and the floor early in the morning and transported to the laboratory in a thermal isolation container for classification. The locations of the structures with the nets were shifted between them after each sampling night to avoid possible biased collections due to the 'site effect'.

Dead and alive *Culicoides* were separated in laboratory and, during the last 13 samplings, all live midges collected in the traps (control and treatment) were split in groups of ≈25 individuals and maintained in WHO test tubes (WHO, 1981). Mortality after 24 h between midges collected from the treatment and the control traps was compared. Morphological identification of *Culicoides* was conducted in laboratory according to their wing pattern, and the gonotrophic status of the females was discriminated according Dyce (1969) criteria in nulliparous, blood-fed, parous and gravid specimens.

2.1. Statistical analysis

ANOVA and independent variables *T* tests were performed to estimate differences in the collections and in the mortality rates of the groups using IBM SPSS Statistics 19 Software. Analysis of the mortality rates after 24 h were based on the cumulative data of the different WHO chambers (each with ≈25 *Culicoides*) obtained from each trap session. A general lineal model with repeated measures was conducted to analyze possible differences in the mortality rate due to the effect of the trap session. Significant differences were considered for $P < 0.05$.

3. Results

A total of 4009 *Culicoides* midges belonging to at least eleven species were collected, namely: *Culicoides brunicans* Edwards, 1939 (0.05%), *Culicoides cataneii* Clastrier, 1957 (8.9%), *Culicoides circumscriptus* Kieffer, 1918 (56.0%), *C. imicola* Kieffer, 1913 (11.6%), *Culicoides jumineri* Callot & Kremer, 1969 (7.4%), *Culicoides longipennis/sahariensis* (2.6%), *Culicoides newsteadi* Austen, 1921 (4.1%), *Culicoides paolae* Boorman, 1966 (6.6%), *Culicoides puncticollis* Becker, 1903 (2.5%), *Culicoides univittatus* Vimmer, 1932 (0.1%) and the Obsoletus complex (0.8%) (Table 1). Of these, 228 (5.7%) were male, 94 (2.3%) intersex specimens of *C. circumscriptus* and 3687 (92.0%) female. Female included 256 (6.4%) nulliparous, 319 (8.0%) parous, 2870 (71.6%) gravid, 10 (0.2%) engorged specimens and 232 (5.8%) midges without abdomen. The most abundant species was *C. circumscriptus* followed by *C. imicola*. The Obsoletus complex represented <1% of the total catch (Table 1). Females of *C. circumscriptus* were collected mostly in gravid condition and, due to its abundance in the collections, were responsible for the large proportion of gravid females observed.

The control trap captured 1915 *Culicoides* midges, 667 were collected alive and 1248 dead. The trap surrounded by the treated net captured 2076 *Culicoides midges*, 656 of them were collected alive while 1420 dead. The percentage *Culicoides* midges collected was 47.8% for the control trap and 51.8% for the treatment trap and not statistical