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ORIGINAL PAPER

Butox® 7.5 pour on: a deltamethrin treatment of sheep and cattle: pilot study of killing effects on *Culicoides* species (Ceratopogonidae)

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Abstract Topical treatment (at the neck and along the vertebral column) with deltamethrin (Butox® 7.5 pour on) of cattle (30 ml/400-kg body weight) and sheep (10 ml/60-kg body weight) was done to find out, whether the insecticide may reach in a sufficient dosage the legs, which are known to be the main biting site of *Culicoides* specimens that are the vectors of the recently introduced Bluetongue virus in central Europe. At days 7, 14, 21, 28, and 35 after treatment, some hair was cut off from the legs—close to the claws. Freshly (the night before) caught *Culicoides obsoletus* specimens were then exposed for 15, 30, 60, or 120 s to such hair and afterwards transferred to a filter paper within plastic Petri dishes to observe their fate. It turned out that even a short contact of 15 s of the *Culicoides* specimens with deltamethrin-treated hair of cattle or sheep was sufficient to paralyze and kill *Culicoides* specimens within a reasonable short time even when the hair were cut off at day 28 after treatment. While the results obtained in cattle and sheep were rather similar for days 7 and 14 after treatment, the speed of the killing effect of treated hair of cattle on *Culicoides* considerably slowed down beginning from day 21 after treatment. However, all the experiments clearly showed that the insecticide deltamethrin may reach the feet of cattle and may kill *Culicoides* specimens when the product is poured along the vertebral column. Such a treatment may considerably reduce the risk of transmission of the agents of disease. However, in the case of the thick fleece of sheep, the insecticide must be poured directly into the skin to reach full activity.

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Introduction

In the year 2006, central Europe was shocked by the unexpected outbreak of Bluetongue disease (BTD) in cattle and sheep in Belgium, Western Germany, Luxembourg, Netherlands, and northern France becoming even worse in 2007, when the virus reached Denmark, Switzerland, United Kingdom, and even spread out all over Germany until its Eastern border. This virus was known to have its main base in Africa, especially in South Africa, where 20 of the 24 existing serotypes had been described (Lit. c.f. Purse et al. 2005). Only occasionally, countries of southern Europe (along the border to the Mediterranean Sea) had been hit by outbreaks of BTD. In these countries, however, other serotypes of the virus had been described (Mellor and Wittmann 2003) than the serotype 8 that was isolated from the recent German samples by the "Friedrich Löffler Institute" at the Riems Island.

In Africa and Italy, the biting midges (Family Ceratopogonidae) of the species *Culicoides imicola* had been determined as the main vector of the Bluetongue virus (Lit. c.f. Mellor 1990; Mellor and Wittmann 2003; Mellor and Boorman 1995). These midges are very tiny, often less than 1 mm in length, and thus, they are easily hidden from the naked eye. In the past, midges did not show activities as vectors of diseases in central Europe, and therefore, knowledge about their characteristics and their development remained rather poor (Lane and Crosskey 1993; Mehlhorn 2007). As a consequence, none of the available industrial insecticides claims an activity against these

Culicoides species. From our studies, to find the vectors of the recent outbreaks, it turned out that *Culicoides obsoletus* was in both years in 2006 and 2007 the vector of the Bluetongue virus in Germany, while *C. imicola* was never found (Mehlhorn et al. 2007, 2008 unpublished observations). *C. obsoletus* is one of the smallest *Culicoides* species reaching only 0.8 mm in length. However, on the other hand, it is the most common species of Ceratopogonids in Germany with a long-flying activity, reaching at least from March to December. The commercial insecticides for sheep and cattle are based on synthetic pyrethroids and were mainly topically used as pour-on solutions being poured along the vertebral column or as ear-tags providing a protection for about 4–8 weeks against flies. Because the *Culicoides* species have their predominant biting sites along the legs and belly, the question arose whether the products reach these regions in a sufficient amount to kill the *Culicoides* stages (if at all) even 4 weeks after treatment. The present pilot study, thus, had been undertaken in order to investigate the insecticidal activity of deltamethrin-treated hair of cattle and sheep in a follow-up period of up to 35 days after treatment.

Materials and methods

Treatment

Six cattle (400-kg body weight) and seven sheep were treated *lege arte* with Butox® 7.5 pour on (a registered trade mark of Intervet bv, Boxmeer, Netherlands).

Cattle: 30 ml of the original solution was distributed into the neck and along the vertebral column.
Sheep: 2 ml of the original solution was poured into the head, while 2×4 ml was dropped directly into the skin at both outer sides of the belly at the upper part of the legs.

Controls

One sheep and one cattle remained untreated as negative controls. Positive controls were done when exposing beetles and other insects (mosquitoes, flies) to Butox® 7.5 pour on treated hair of sheep and cattle.

Culicoides specimens

The night before the experiments, *Culicoides* specimens (Fig. 1)—mainly *C. obsoletus* —were caught alive using a trap with ultraviolet light. They were transported in a dark bag—covered with aluminum foil—to avoid cold and loss of humidity.

Fig. 1 Light micrograph of females of *Culicoides* species after tests with deltamethrin-treated hair of cattle

Experiments

On days 7, 14, 21, 28, and 35 (the latter test was done only in cattle) after treatment of the animals, some hair was cut off from the legs of the animals, placed into a plastic bag, and was immediately transported to the Institute. There the midges (*Culicoides* specimens; n=4–10 per test) and control insects (n=3–10; e.g., beetles, mosquitoes) were mingled with the treated or untreated hair for 15 s, 30 s, 1 min, or 2 min.

After the exposition period, the exposed insects were taken out, placed into a filter paper within a plastic Petri dish, and observed for the next hours.

Results

Controls

Culicoides specimens, when mingled for 15 s up to 2 min with hair of untreated cattle or sheep, were found always alive after 24 h. *Aedes* and *Culex* mosquitoes, when mingled with the hair of sheep that was treated 21 days before, died within 2.5–11 h depending on the length of the exposition period. However, beetles of the genera *Stegobium* and *Gibbium* survived in all cases for at least 24 h when they had been exposed for the same periods to the treated hair of sheep at day 21 post-treatment (p.t.). When using carnivorous beetles as controls to see the effects of treated hair of cattle, the results below were obtained.

While on day 7, a short contact of only 15 s allow 1/3–1/5 of the beetles to survive for at least 72 h, a contact of 2 min killed them all within 60 h. At later dates after the treatment, the number of surviving beetles constantly increased, reaching 100% starting from day 21 p.t. Mosquitoes and bugs, however, were more sensible

Table 1 Effects of deltamethrin-treated hair from legs of cattle on *Culicoides* species

Exposition period	7 days after treatment	14 days after treatment	21 days after treatment	28 days after treatment	35 days after treatment
15 s	Death of *Culicoides* within 60 min	Death of *Culicoides* within 68–75 min	Death of *Culicoides* within 160–190 min	Death of *Culicoides* within 7.5–9.5 h	2/3 or even all specimens were alive after 24 h
30 s	Death within 38–40 min	Death within 28–50 min	Death within 45–150 min	Death within 7–8.5 h	2/3 or even all specimens were alive after 24 h
1 min	Death within 30–35 min	Death within 24–32 min	Death within 55–90 min	Death within 6 h	Only 1/4 of *Culicoides* died within 24 h
2 min	Death within 20 min	Death within 22–28 min	Death within 40–70 min	Death within 5–6 h	1/3 of *Culicoides* died within 24 h

because they died (e.g., at day 7 p.t.) within 3 h after contact for 1 min with treated hair.

Effects of treated hair from cattle

Culicoides specimens became paralyzed, trembled, or showed reduced motility when coming into contact with treated hair—independently whether it was treated 7 or 28 days before. This is an indication that there is a quick reaction following the contact with the insecticide.

The results of the different reactions are summarized in Table 1. However, slight variations in the period until the death of the *Culicoides* specimens occurred in the seven different treated cattle. It is obvious that beginning at day 21 after treatment (p.t.), the speed of the killing activity slows down considerably. However, although at least 5 h (depending on the exposure time) is needed until all *Culicoides* are dead on day 28 p.t., the activity of the insecticide is still present. On the other hand, on day 35 p.t., many *Culicoides* specimens survived for 24 h even after the longest exposition time.

Effects of treated hair from sheep

When coming into contact with treated hair of sheep, all *Culicoides* showed a reduced motility, trembling, or even paralysis, as it was seen in the cattle experiment, too. The results of the experiments are summarized in Table 2. The *Culicoides* species died rather quick, even when they had only rather short contacts with hair cut off 28 days after treatment. The time needed until death at days 21 and 28 p.t. is much shorter compared to that when using treated hair of cattle (Table 1).

Discussion

The results of the experiments clearly showed that the insecticide deltamethrin (Butox® 7.5 pour on), when applied into the hair of cattle and sheep, has killing effects on *Culicoides* specimens, if the latter are brought into contact with hair treated 7, 14, 21, and 28 days before. The slower killing of *Culicoides* with hair of cattle beginning at day 21 after treatment may be explained by the fact that in cattle, the product was exclusively poured onto the backline of the animals, while in the case of the sheep, 4/5 of the amount of the product was poured into the skin at the lateral sides of the belly and only 1/5 into the head. Therefore, the way of the product to the feet might be shorter. Thus, the amount of the product arriving at the feet might be greater at days 21 and 28 p.t. in the case of sheep. In sheep with long, dense hair, it seems very important that the product is obligatorily poured directly onto the skin, otherwise it may not stay as long as in cattle, and perhaps, may not be transported to the feet and to the lower side of the belly.

The present study shows three important features. First, the biting midges of the genus *Culicoides* are sensitive to deltamethrin being poured onto the skin of cattle and sheep, while the positive controls (beetles, mosquitoes, bugs) are

Table 2 Effects of deltamethrin-treated hair from legs of sheep on *Culicoides* species

Exposition period	7 days after treatment	14 days after treatment	21 days after treatment	28 days after treatment
15 s	Death of *Culicoides* within 35–70 min	Death of *Culicoides* within 50–75 min	Death of *Culicoides* within 50–70 min	Death of *Culicoides* within 75–100 min
30 s	Death within 35–45 min	Death within 28–50 min	Death within 32–50 min	Death within 40–80 min
1 min	Death within 25–27 min	Death within 18–33 min	Death within 20–36 min	Death within 25–45 min
2 min	Death within 18–20 min	Death within 17–24 min	Death within 12–36 min	Death within 18–34 min

much less sensitive. Second, the product reaches the feet, which is among the preferred biting sites of *Culicoides* species. Third, the *Culicoides* coming into contact with deltamethrin—even only for a short time—show a reduced motility, trembling, and later eventually, a full paralysis. This would mean for the praxis in the field that the midges after touchdown into the hair stay probably unwillingly longer among the hair and thus are killed more likely. Whether they really start to suck blood could not be observed in the present study, but it is not very probable due to the observed paralytic effect. Furthermore, it could not be observed whether the midges are repelled when approaching the animal because they were given to closely packed-treated hair being cut off for an in vitro experiment to make sure a defined period of contact.

With respect to the potential transmission of the Bluetongue virus, it can be noted that insecticide treatment of ruminants brings advantages because each avoided bite decreases the risk of a transmission, and each killing of a female midge suppresses its possible progeny. Complete prevention of any transmission of viruses by insecticides seems, however, not possible, because the nose, udder, etc. of the host might not be sufficiently protected. Therefore, a double strategy—development of a vaccination and the serial use of insecticides—seems recommendable. Furthermore, the full action of the insecticide is only reached in sheep, if it is poured directly into the skin. This means that the dense sheep hair (fleece) has to be bent until the skin becomes visible.

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