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ORIGINAL PAPER

Pilot study on deltamethrin treatment (Butox® 7.5, Versatrine®) of cattle and sheep against midges (*Culicoides* species, Ceratopogonidae)

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Abstract Deltamethrin (Butox® 7.5, Versatrine®)-treated hair of cattle and sheep were brought into contact for 15, 30, 60, or 120 s with freshly caught *Culicoides* specimens, which are the vectors of the Bluetongue virus. The hair was clipped off from the treated animal just above the claws—a region which is one of the predominant biting sites of the midges. Hair obtained on day 7, 14, 21, 28, or 35 after treatment were mingled with the *Culicoides* specimens for the given contact periods. After separation of the midge from the hair and placing them onto white filter paper in a petri dish, their fate was followed for the next hours by microscopic inspection. It was found that deltamethrin (in both formulations Butox®, Versatrine®) reaches for 35 days in such sufficient amounts in the hair of the legs to kill attacking specimens of *Culicoides* in reasonable short periods after very short contacts. The observed speed of kill and the deleting effects were so quick that the midges very probably would not bite before their death.

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Introduction

Bluetongue disease (BTD) is a viral disease, which harms considerably farm ruminants with high mortality rates in cattle and especially in sheep, while wild ruminants become infected, serve as virus reservoirs, but show only rarely severe symptoms of disease (Purse et al. 2005; Conraths et al. 2007; Conte et al. 2007). In 2006, the Bluetongue virus (BTV) was introduced into Western Europe (Belgium, the Netherlands, and West Germany), from where it spread in 2007 practically over most regions of Central Europe, reaching England, Denmark, the Eastern of Germany, and Switzerland, and—coming from the North—spreads to the South of France (Mehlhorn et al. 2007; Thiry et al. 2008; Saegerman et al. 2008); since this important disease was underestimated in its importance as epidemic in Europe—Africa is its main home—the BTD stroke was severe in the European network of rearing cattle and sheep, reaching high mortality rates (3–4% in cattle, up to 40% in sheep). At the time when the virus was shown first—it was defined as serotype 8 out of 24 possible ones—a functional vaccine was not available. Therefore, protection methods were needed to avoid transmission of the virus from one animal to the other. From transmission experiments and epidemiological studies in South Africa and in Southern Europe, it was known that the main vector belonged to the midges (Family Ceratopogonidae, genus *Culicoides*).

Most important was the species *Culicoides imicola*, which occurs in Africa and at the northern rim of the Mediterranean Sea. Since other species of *Culicoides* in Central Europe had been considered as rather unimportant in the transmission of agents of diseases, the entomologists of the European countries did not spend too much time in research on the life cycle of *Culicoides* species. Thus, when it was shown

Fig. 1 Scanning electron micrograph of the head and the bloodsucking mouthparts of a female *C. obsoletus*×85

that in Europe most common species (*Culicoides obsoletus*) act as vector of the BTV (Mehlhorn et al. 2007), the life cycle of this species was insufficiently known. Other species such as *Culicoides pulicaris* (Mehlhorn et al. 2008c) or *Culicoides dewulfi* are apparently less frequently involved (Meiswinkel et al. 2007). With respect to the neglected knowledge on *Culicoides* specimens, the pharmaceutical companies had only a few data on the efficacy of their different insecticides against *Culicoides* specimens since their products had been developed to protect ruminants against flies, mosquitoes, simuliids, and/or tabanids. Therefore, at the beginning, tests were lacking, which might have proven that the different insecticides, which were often applied at the backs of the animals, may reach the main biting sites (feet and belly) of the ruminants and are able to kill the midges at all. Pilot studies on the efficacy of deltamethrin, cyfluthrin, and permethrin then showed that these compounds are active on midges (Mehlhorn et al. 2008a, b; Schmahl et al. 2008). Efficacy, however, always depends on the formulation of the compound. Therefore, the present study was undertaken to determine whether the *Culicoides* specimens are sensitive to deltamethrin in the formulations of the products Butox® 7.5 and Versatrine® (Fig. 1).

Thus, the aim of the study was to compare the efficacy of both products when *Culicoides* specimens come into contact with hair of cattle that had been treated for 7, 14, 21, 28, or 35 days before. This study was needed since it must be clarified whether the products in this formulation of deltamethrin reach the hair of feet in sufficient amounts when they are applied onto the hair along the back line. The products must arrive in sufficient amounts at the feet and along the belly since there are the predominant biting sites of the very tiny (only 0.8–3 mm long) specimens of *C. obsoletus*, *C. pulicaris*, and *C. dewulfi*, the proven vectors of Bluetongue in Europe (Mehlhorn et al. 2007, 2008a, b, c; Meiswinkel et al. 2007).

Materials and methods

Cattle

One group of three young cattle of about 400 kg bodyweight were treated *lege arte* by application (pour on) of 30 ml of the product Butox® 7.5 along the backside of the animals. Butox® 7.5 contains 7.5 g deltamethrin per liter of the ready-to-use solution and is a registered trademark of Intervet Netherlands. Another group was treated with 10 ml of the product Versatrine®, which is a registered trademark of Schering-Plough Vétérinaire (France). It contains 1 g deltamethrin per 100 ml excipient.

A third group of three adult cattle was treated with 20 ml Versatrine®.

Sheep

Three groups (three animals each) of young sheep of about 60 kg bodyweight were each treated *lege arte* by

Table 1 Effects of Butox®-treated hair of sheep on specimens of *Culicoides* species (n=10)

Time of exposition (s)	Effects on day 7 after treatment	Effects on day 14 after treatment	Effects on day 21 after treatment	Effects on day 28 after treatment	Effects on day 35 after treatment
15	All dead within 55–72 min	All dead within 55–88 min	All dead within 58–94 min	All dead within 90–145 min	5/10 survivors after 6 h
30	All dead within 42–63 min	All dead within 35–65 min	All dead within 58–87 min	All dead within 78–128 min	1 survivor after 6 h
60	All dead within 23–34 min	All dead within 32–50 min	All dead within 53–72 min	All dead within 62–87 min	All dead within 70–130 min
120	All dead within 15–19 min	All dead within 22–38 min	All dead within 30–44 min	All dead within 38–59 min	All dead within 55–112 min

Table 2 Effects of Versatrine® (10 ml)-treated hair of sheep on specimens of *Culicoides* species (n=10)

Time of exposition (s)	Effects on day 7 after treatment	Effects on day 14 after treatment	Effects on day 21 after treatment	Effects on day 28 after treatment	Effects on day 35 after treatment
15	All dead within 55–70 min	All dead within 60–105 min	All dead within 75–112 min	All dead within 63–130 min	All dead within 2–6 h
30	All dead within 40–60 min	All dead within 55–84 min	All dead within 55–98 min	All dead within 60–108 min	All dead within 85 min–4 h
60	All dead within 15–25 s	All dead within 40–100 s	All dead within 30–110 s	All dead within 45 s–5 min	All dead within 21–74 min
120	All dead within 5 s	All dead within 25–75 s	All dead within 22–118 s	All dead within 34–175 s	All dead within 9–32 min

application (pour on) of 10 ml of the product Butox® 7.5 or with 10 and 5 ml Versatrine® directly onto the skin along the backside of the animals.

Seven, 14, 21, 28, and 35 days after treatment, hair was clipped off from the feet of the cattle and sheep (just above the claws), collected in separate, suitable plastic bags, and transported to the institute, where it was mixed with freshly caught midges, which had been caught in the previous night with the aid of an ultraviolet light lamp. Each vial contained at least ten *Culicoides* specimens besides other insects. The trapped insects were incubated with treated hair or with hair of an untreated animal (control). Beetles and true mosquitoes were also brought into contact with treated hair as further (positive) control.

The exposure periods of the insects to hair lasted for 15, 30, 60, or 120 s—a period which was thought to be realistic compared to the field conditions. The insects were thereafter separated from the hair and placed on filter paper inside closed plastic petri dishes, where they were observed at regular intervals (5–10 min) using a stereo microscope (Olympus SZH 10) to record reactions and the time of death after the first contact with treated hair. The observations were listed in protocols.

Results

Negative controls

Specimens of *Culicoides* species remained alive when mingled with untreated hair for 72 h.

Positive controls

The experiments showed that the effects of Butox® and Versatrine® on different insects from different genera varied considerably even in rather closely related species. It was noted that *Culex* mosquitoes were sensitive to deltamethrin, while the two beetles (*Stegobium paniceum* and *Dermestes peruvianum*—two important pests in households) showed a very low sensitivity to the dosage of deltamethrin used in the present test and even did not die when incubated for 72 h with hair that had been treated 21 days before. Mosquitoes turned out to be either sensitive or not. All *Aedes* specimens survived a 5-min contact with treated hair starting from day 21 after treatment. They were completely fit and started immediately sucking blood when released from the petri dishes,

Table 3 Effects of Versatrine® (5 ml)-treated hair of sheep on specimens of *Culicoides* species (n=10)

Time of exposition (s)	Effects on day 7 after treatment	Effects on day 14 after treatment	Effects on day 21 after treatment	Effects on day 28 after treatment	Effects on day 35 after treatment
15	All dead within 1 h	All dead within 84–120 min	All dead within 100–160 min	All dead within 130–200 min	5/10 survivors after 6 h
30	All dead within 55–135 min	All dead within 55–90 min	All dead within 75–135 min	All dead within 90–180 min	All dead within 3–4 h
60	All dead within 25 s	All dead within 84–150 s	All dead within 3.5–10 min	All dead within 8–18 min	All dead within 29–93 min
120	All dead within 10 s	All dead within 35–140 s	All dead within 3–8.5 min	All dead within 5–12 min	All dead within 20–74 min

Table 4 Effects of Butox®-treated hair of cattle on *Culicoides* specimens (n=10)

Time of exposition (s)	Effects on day 7 after treatment	Effects on day 14 after treatment	Effects on day 21 after treatment	Effects on day 28 after treatment	Effects on day 35 after treatment
15	All dead within 32–45 min	All dead within 37–68 min	All dead within 65–95 min	All dead within 84–145 min	1 survivor, 9/10 dead after 3 h
30	All dead within 22–37 min	All dead within 31–43 min	All dead within 39–62 min	All dead within 58–79 min	1 survivor, 9/10 dead after 2.5 h
60	All dead within 8–16 min	All dead within 12–21 min	All dead within 18–32 min	All dead within 45–76 min	All dead within 82–107 min
120	All dead within 5–9 min	All dead within 9–14 min	All dead within 15–24 min	All dead within 28–51 min	All dead within 38–66 min

Table 5 Effects of Versatrine® (10 ml)-treated hair of cattle on *Culicoides* specimens (n=10)

Time of exposition (s)	Effects on day 7 after treatment	Effects on day 14 after treatment	Effects on day 21 after treatment	Effects on day 28 after treatment	Effects on day 35 after treatment
15	All dead within 60–70 min	All dead within 80–96 min	All dead within 75–128 min	All dead within 90–260 min	All dead within 135–225 min
30	All dead within 28–35 min	All dead within 33–45 min	All dead within 52–66 min	All dead within 72–94 min	All dead within 92–138 min
60	All dead within 5–40 s	All dead within 55–105 s	All dead within 3.5–5.5 min	All dead within 8–15 min	All dead within 15–24 min
120	All dead within 10 s	All dead within 10–45 s	All dead within 2–4 min	All dead within 4–9 min	All dead within 7–16 min

Table 6 Effects of Versatrine® 20 ml-treated hair on *Culicoides* specimens on day 35 after treatment of three adult cattle

Animal no.	Effects after 15 s	Effects after 30 s	Effects after 60 s	Effects after 120 s
44-154	All dead within 155–260 min	All dead within 120–178 min	All dead within 19–31 min	All dead within 10–18 min
08-676	All dead within 128–295 min	All dead within 105–205 min	All dead within 16–28 min	All dead within 9–18 min
08-675	All dead within 155–286 min	All dead within 130–185 min	All dead within 19–30 min	All dead within 11–18 min

within which they had been kept for 72 h after the experimental contact to treated hair.

On the other hand, *Culex* specimens were sensitive to deltamethrin when exposed for 5 min to treated hair, e.g., five of ten *Culex* died when mingled with Versatrine® hair, while others did not die when mingled with Butox® 7.5 (in both formulations: Butox® 7.5, Versatrine®) on day 35 after treatment of the cattle. However, this sensitivity was much lower than that of midges.

Midges (*Culicoides* species)

The midges are apparently highly sensitive to deltamethrin in both formulations (Butox® 7.5, Versatrine®) since they died even after rather short contacts to hair treated 35 days before (Tables 1, 2, 3, 4, 5, and 6). There were no significant differences between the species of treated animals (sheep or cattle), although the distance from the place of application (back) until the feet is longer in cattle than in sheep. Thus, both formulations reach in sufficient amounts the region of the predominant biting sites of the *Culicoides* species (feet, belly). Nevertheless, there are apparently some slight differences in the speed of killing. This can be concluded from the fact that the midges die earlier when exposed for short contacts to Butox®-treated than to Versatrine®-treated hair (Tables 1, 2, 3, 4, 5, and 6).

This is changed when the exposure period is prolonged. Then, the contacts to Versatrine®-treated hair introduce a quicker reaction than that after Butox® contacts (Tables 1, 2, 3, 4, 5, and 6). But in all cases, the effects are very satisfying until the end of the study on day 35 after the treatment.

Discussion

The results obtained clearly show that deltamethrin when applied as a pour-on solution onto the back of the animals has significant killing effects on the *Culicoides* species, which are known vectors of the Bluetongue virus in Europe. The results of the present study—although collected only from a low number of animals—are congruent to studies with other pyrethroid products (Mehlhorn et al. 2008a, b; Schmahl et al. 2008) showing significant time—and dosage—depending on the reactions of the *Culicoides* specimens. This was not self-evident, as can be concluded from the observations with other insects in the present positive controls, where some insect species (groups) turned out to be sensitive to deltamethrin while others were not (e.g., beetles)—at least in the available dosages. Comparing the efficacy of ear-tags with insecticides, the insecticidal pour-on preparations keep their activity for a longer period (Liebisch and Liebisch 2008; Mehlhorn et al. 2008a, b).

On the other hand, it is not yet finally decided whether the application of the insecticides may protect completely from infections with, e.g., Bluetongue virus or agents of other infectious diseases since several places on a ruminant remain probably less protected, e.g., the udder or the nose of ruminants will offer places with lower amounts of insecticides due to licking or due to daily cleaning before getting milk.

However, even if the protection might not be 100%, any killed female *Culicoides* prevents its possible progeny and hinders the transmission of agents of diseases.

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