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Efficacy of alphacypermethrin applied to cattle and sheep against the biting midge *Culicoides nubeculosus*

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ABSTRACT

The recent emergence of bluetongue virus (BTV) in northern Europe, has led to an urgent need to identify methods to control the *Culicoides* biting midges that transmit it. Here, an *in vitro* assay was used to assess the effects of the proprietary pyrethroid insecticide alphacypermethrin applied to cattle and sheep (Dysect Cattle Pour-On, Dysect Sheep Pour-On; Ford Dodge Animal Health) against the biting midge *Culicoides nubeculosus* (Meigen) (Diptera: Ceratopogonidae). Hair or wool was collected from the back, belly and legs of animals immediately prior to treatment and 7, 14, 21, 28 and 35 days after treatment, and also from untreated controls. In the laboratory assay groups of 10 adult females *C. nubeculosus* were exposed to 0.5 g of hair or wool for 3 min. In all cases, no mortality was observed in the pre-treatment sample or the untreated controls. In the post-treatment samples, for both cattle and sheep mortality was close to 100% 7 days after treatment. For cattle, treatment effect persisted for up to 21 days post-treatment, following which the mortality rate following exposure to hair samples declined. In contrast, for sheep, mortality levels declined more slowly, and approximately 50% mortality was still observed 35 days after treatment. There was no significant difference in the kill rate for wool collected from the back, belly or legs of either sheep or cattle. The results demonstrate the potential for pour-on insecticide treatment to offer a degree of mitigation to livestock against onward transmission from infected animals—and in particular demonstrate that sufficient compound is able to reach the lower legs to kill in contact midges. The practical issues associated with achieving adequate protection are discussed.

1. Introduction

Culicoides biting midges (Diptera: Ceratopogonidae) include some of the smallest haematophagous insects known, with wing-length rarely exceeding 2 mm and over 1500 species described worldwide to date (Mellor et al., 1998). The females of certain species of this genera can occur in huge numbers where conditions are suitable and can be a serious source of irritation and annoyance to livestock. This behaviour is a causal link to an immediate-type hypersensitivity reaction which produces an intensely pruritic skin disease of horses, known colloquially as sweet-itch or Queensland-itch. However, of greatest veterinary importance is the ability of *Culicoides* to act as vectors of a range of disease pathogens particularly the arboviruses responsible for the diseases bluetongue, African horse sickness and bovine ephemeral fever.

Bluetongue virus (BTV) (Orbivirus: Reoviridae), an arboviral pathogen of ruminants, exists as a number of distinct serotypes, 24 of which have been recognized to

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date. The virus is the aetiological agent of the disease bluetongue (BT). In susceptible sheep it can cause fever, enteritis, upper respiratory tract infection, ulceration of the tongue and lameness. The disease can cause very high mortality, in excess of 25%, and morbidity in excess of 75%. Bluetongue occurs generally in Africa, the Middle East, Asia, Australia and parts of North America. However, in 2006 a sub-Saharan strain of BTV serotype 8 (BTV-8) was detected in the Netherlands some 900 km north of any previously recorded outbreak of BTV (ISID, 2006). In 2007 it rapidly spread through Germany, Belgium France, Denmark, Switzerland and Luxembourg—entering the UK in August 2007. Its persistence between years was related to an, as yet, undefined overwintering mechanism for which several theories have been postulated (Wilson et al., 2008).

In northern Europe, positive incrimination of the primary vectors of BTV has proved difficult. To date our knowledge relies primarily upon the association of species with livestock farms, however many of the suspected vectors have also been shown to be capable to replicating virus, and hence potentially acting as vectors of disease (see Carpenter et al., 2008a, 2006 for review). Beyond the northern Palaearctic, many of these species have also been implicated in outbreaks in the southern Mediterranean.

The recent emergence of BTV in northern Europe, has led to an urgent need to identify methods to control the disease and the biting midges that spread it (Carpenter et al., 2008b). However, the control of *Culicoides* is difficult because of the extensive and sometimes dispersed larval habitats and the fact that adult flies spend limited time feeding on their hosts in comparison to many other ectoparasite groups. Applications of pyrethroid insecticides have the potential to provide effective and convenient, albeit short-term, local control, by preventing onwards transmission from viraemic animals. Pour-on treatments and impregnated ear tags have been shown to be effective in some studies, however, as yet, relatively little detailed work has been carried out, so that few insecticidal veterinary medicines have authorised claims regarding use against *Culicoides* species. One key issue is the fact that different species of *Culicoides* may have different predilection sites for feeding. For example, *Culicoides imicola* has been reported to feed on the back (Braverman, 1991) while *Culicoides sonorensis* feeds on the belly (Jones and Akey, 1977) and other species may feed on the lower legs. Hence, it is imperative to establish whether commercial pour-on insecticides, which are most commonly applied along the dorsum of ruminant livestock, will give sufficient coverage to protect animals from biting midge at potential sites of *Culicoides* feeding such as the belly and legs.

The aim of this *in vitro* assay therefore was to examine the effects of formulations of the proprietory pyrethroid insecticide alphacypermethrin, applied to sheep and cattle against the biting midge *C. nubeculosus*.

2. Methods

2.1. Animal treatment

Groups of eight calves and eight sheep, matched for age and breed, were selected at random from a commercial herd/flock. The calves were Aberdeen Angus cross, approximately 150 kg in weight and 6 months old. The sheep were Texel crosses, approximately 30 kg bodyweight and 5 months old. Six animals from each group, selected at random, were treated with the registered pyrethroid insecticide alphacypermethrin in accordance with manufacturer's instructions (Dysect Cattle Pour-On, 15 g/L alphacypermethrin and Dysect Sheep Pour-On, 12.5 g/L alphacypermethrin, respectively). For cattle, 10 mL was applied from an applicator gun from the crown of the head down the neck and along the middle of the back of the animal. For the sheep, 40 mL was applied as two half-doses using a T-bar applicator. The first half dose was applied evenly from the neck to the saddle and the second half dose evenly from the saddle to the tail head and around the rump. Two animals in each group remained untreated and acted as controls. All animals were grazed outside during the course of the study (open to prevailing weather conditions) but were brought inside briefly for the duration of each sample collection. The untreated control animals were housed in an area adjacent to the treated animals, but were not allowed to come into physical contact with the treated individuals.

2.2. Hair/wool samples

Hair/wool samples (approximately 2–5 g in weight) were collected from all treated and control animals, from three body sites (back, belly and lower legs), immediately prior to treatment (day 0) and then at 7, 14, 21, 28 and 35 days after treatment. Hair/wool was clipped as close to the skin as possible using scissors. At each collection, the hair/wool from each body position was pooled for the groups of treated or untreated animals. The hair/wool from each body site was then wrapped in aluminium foil, labelled and stored at −20 °C.

2.3. Insect material

Culicoides nubeculosus were obtained from a breeding colony maintained at the Institute for Animal Health, Pirbright, UK. Newly emerged adult females or female pupae were sent to Bristol by post. On arrival, batches of about 400 midges were maintained at a constant 20 °C and 75% r.h., supplied with small balls of cotton wool soaked in sucrose solution. Midges were used in experiments when 3–4 days old.

2.4. Contact assay

Groups of 10 midges were removed, immobilised by light chilling for 1 min. These ten immobilised midges were placed into a 5 cm diameter Petri-dish, and then allowed to recover. Each sample of hair/wool was mixed, after which a sub-sample of approximately 0.5 g was removed at random for each trial. The sub-sample of hair/wool was then introduced into the Petri-dish containing the midges and the Petri-dish gently tilted to bring the midges and hair into contact, so that the midges alighted on and walked over the surface of the hair.

In initial trials to assess the effects of contact time on mortality, midges remained in the Petri-dish with hair/wool