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Failure of ivermectin treatment in *Haemonchus contortus* infected-Swedish sheep flocks

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ABSTRACT

Control of gastrointestinal nematodes of veterinary importance in Swedish sheep flocks is primarily based on recurring strategic anthelmintic treatments after detection of strongyle eggs in faeces samples. This study reports reduced efficacy of ivermectin (IVM) against *Haemonchus contortus* in naturally infected Swedish sheep flocks. Faecal egg count reduction tests (FECRT) and examinations of *H. contortus*-specific DNA with qPCR on larval cultures were applied to samples from 11 sheep flocks (A–K) in south-eastern Sweden between 2013 and 2014. Four of these flocks (D, E, J and K) had been in direct contact with flock H, where IVM treatment failure was first observed in October 2013, some years after the introduction of imported dairy sheep. In flock H, the resistance status to IVM was also confirmed by a larval developmental test. IVM concentrations 15–20 times higher than for susceptible strains of *H. contortus* were required to kill the larvae. In addition, faeces samples were obtained from 37 other Swedish sheep farms where the treatment response to IVM was screened initially in six animals using FEC and qPCR 7–10 days after administration of IVM. Six farms where the majority was identified with this pre-screening test (B, C, F, G, I and K), were also investigated in more detail with FECRT as described above after the animals had been allocated to groups and treated orally or injected with a minimum of 0.2 mg IVM, 0.2 mg doramectin (farm F) or 0.2 mg moxidectin per kg body weight (farm A and B). Four flocks (farm A, D, G and I) were also treated with 4.8 mg albendazole and/or 7.5 mg levamisole per kg body weight. Pre-treatment faeces samples were collected from 15 animals on the same day as deworming. Post-treatment samples were collected 7–10 days later, whenever possible from 10 animals per group with the highest pre-treatment egg counts. Based on FECRT results, IVM efficacy to *H. contortus* was reduced on six farms (C, D, E, G, H and I) out of 11 farms studied with FECRT. This is the first report of IVM treatment failure in *H. contortus*-infected sheep in Sweden.

1. Introduction

Anthelmintics are still the most widely used method for treatment and control of gastrointestinal nematodes (GIN) in sheep throughout the world. However, after decades of intensive use of dewormers, anthelmintic resistance (AR) is now widespread in veterinary practise and sometimes threatens the possibilities to control GIN infections. Within the macrocyclic lactones (ML), during recent decades AR to ivermectin (IVM) has become a common global problem in the highly pathogenic species *Haemonchus contortus*, especially in the southern hemisphere (e.g. Echevarria et al., 1996; Van Wyk et al., 1999; Besier and Love, 2003; Chandrawathani et al., 2003; Sutherland et al., 2008).

In Europe, AR in GIN of sheep was mainly associated with benzimidazoles (BZ) until the 1990s (for a review see Papadopoulos et al., 2012). However, some years after the introduction of ML in the 1980s reports began to emerge of IVM resistance in European sheep flocks, although these sometimes involved GIN other than *H. contortus* (e.g. Sargison et al., 2001; Álvarez-Sanchez et al., 2006; Bartley et al., 2006; Cernanská et al., 2006; Borgsteede et al., 2007; Domke et al., 2012; Geurden et al., 2014; Keane et al., 2014; Peña-Espinoza et al., 2014). Until recently, the resistance situation was different in Sweden. In a nationwide faecal egg count reduction test (FECRT) survey based on data from 90 sheep flocks on 45 farms conducted in 2006 and 2007, there was only evidence of BZ resistance in two flocks (Höglund et al., 2009). Unlike in some other European countries, this resistance was exclusively associated with *H. contortus*.

In contrast to BZ anthelmintics, ML exert their effect by binding to glutamate-gated chloride (Glu-Cl) channels expressed on nematode

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neurones and pharyngeal muscle cells. In the model nematode *Caenorhabditis elegans*, it has been shown that genes affecting parallel genetic pathways mediating drug uptake and sensitivity, as defined by the family of GluCl genes, are involved in IVM resistance (Dent et al., 2000). The precise mechanism causing IVM resistance in *H. contortus* is only partly understood, but in one study intensive treatment with IVM resulted in selection of the gene for the alpha-subunit of the glutamate channel in *H. contortus* (Blackhall et al., 1998). It was also demonstrated recently that IVM-resistant *H. contortus* is enriched in a *dyf-7* haplotype gene which is involved in the development of amphid sensory neurons, which in turn affects permeability to the drug (Urdaneta-Marquez et al., 2014). Furthermore, IVM resistance in *H. contortus* is conferred by overexpression of permeability-glycoproteins (P-gp), which have the ability to transport the drug from the cytosol across cell membranes of the worms. In a study by Xu et al. (1998), the levels of P-gp mRNA were higher in IVM-treated *H. contortus* than in an unselected isolate. Despite this progress, validated molecular markers for IVM-resistance are currently lacking for nematodes of veterinary interest. However, clinical resistance can be detected more easily by a faecal egg count reduction test (FECRT), where AR is commonly declared when a mean percentage faecal egg reduction of less than 95% is observed 7–10 days after anthelmintic treatment (McKenna, 1994).

It is evident that AR can be induced through misuse and as a consequence of underdosing in combination with frequent overuse of any anthelmintic. According to independent laboratory studies, IVM resistance in *H. contortus* can be induced by exposure to increasing doses of IVM within three generations (Coles et al., 2005) or in up to 22 generations (Ranjan et al., 2002). However, there are also examples of IVM-resistant nematodes being introduced to new countries along with animal movements. For example, in the former Czechoslovakia, multiple AR was detected in GIN of goats imported from New Zealand (Varady et al., 1993). Similarly, multiple AR has been confirmed in a controlled test on a Swiss farm to which South African Boer goats had previously been imported (Schnyder et al., 2005). A study in the Netherlands also found that resistant parasites were moved with animals to different farms within the country (Borgsteede et al., 2007).

In this work, we describe for the first time IVM failure of ivermectin treatment or clinical resistance in *H. contortus* in a Swedish sheep flock to which imports of infected dairy sheep are strongly suspected to have introduced an IVM-resistant strain of this highly pathogenic parasite.

2. Materials and methods

2.1. Inclusion criteria of study farms

This study was initiated as a result of observations of poor performance and positive despite IVM treatment on a sheep farm (H) on the northern part of the island of Gotland in the Baltic Sea off Sweden's east coast (Fig. 1). This flock, consisting of Gotlandic Pelt and East Friesian Dairy Sheep, was established decades ago. However, in 2008 East Friesian Dairy Sheep originating from the Netherlands and imported via Finland were introduced on the farm. It is unclear whether quarantine was applied before their introduction. In addition, we investigated sheep from four (farms D, E, J and K) out of six flocks which had been in direct contact with the animals on farm H were included. Of the other two farms, one (B) had imported animals from the same source as farm H, while farm C had imported animals from Switzerland. In addition, four other farms (A, F, G, and I) were investigated. All of these except farm A were investigated with FECRT after a pre-screening test (see below).

2.2. Parasitological investigations

2.2.1. Treatment controls

Nematode faecal egg counts (FEC) were performed on faeces samples from groups of lambs or ewes between 2013 and 2014. In the treatment control (TC) flocks, faeces were collected from the rectum of six sheep prior to oral treatment with 0.2 mg/kg Noromectin® (0.8 mg/mL oral susp. N-vet/Norbrook Laboratories). The samples were pooled three by three and investigated using a modified McMaster method to determine the number of strongyle nematode eggs in 3 g faeces, with a minimum diagnostic sensitivity of 50 eggs per gramme

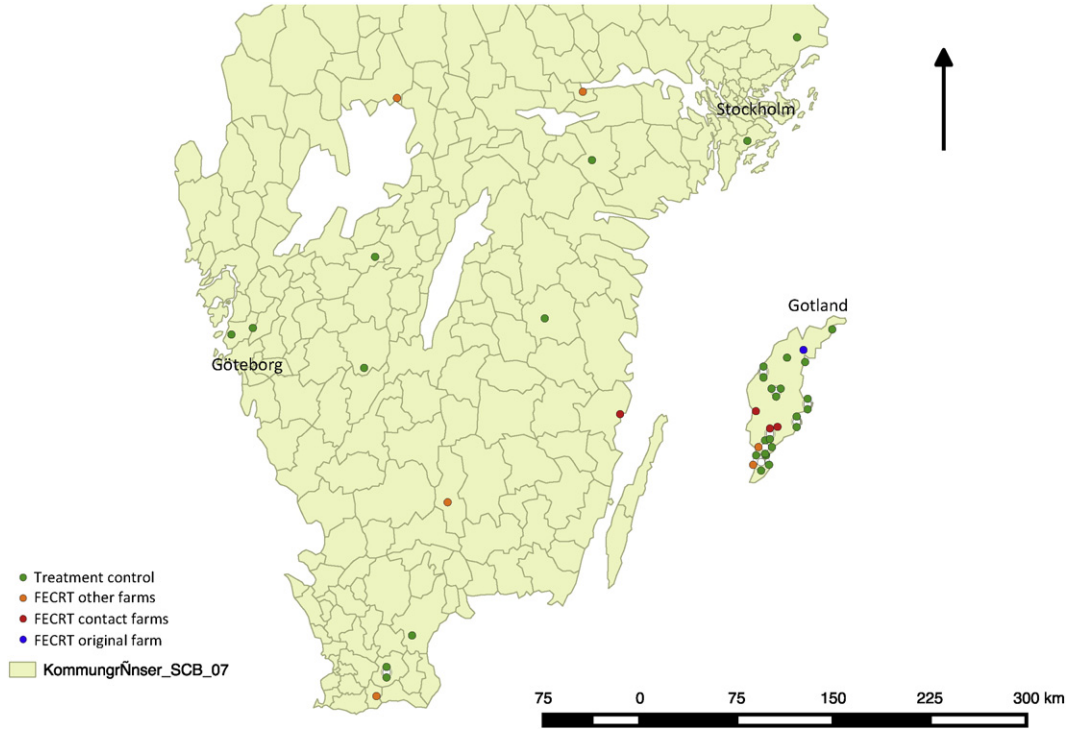

Fig. 1. Map of southern Sweden showing where the location of the sheep farms included in this study samples came from.