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# Field efficacy of four anthelmintics and confirmation of drug-resistant nematodes by controlled efficacy test and pyrosequencing on a sheep and goat farm in Denmark

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ABSTRACT

We describe a case of anthelmintic resistance on one of the largest organic small ruminant farms in Denmark. The flock was established in 2007 by purchase of animals from other Danish farms and had history of clinical parasitism, high mortality of young stock and anthelmintic treatment failure. In October 2011, 40 lambs and 40 kids were selected for a faecal egg count reduction test (FECRT) with fenbendazole (FBZ), ivermectin (IVM), moxidectin (MOX) and levamisole (LEV). Lambs were treated with the recommended sheep dose of each product while kids received the sheep dose of IVM, 1.5× sheep dose of MOX and 2× sheep dose of FBZ and LEV. Untreated lambs and kids were also included and three methods for calculating faecal egg count (FEC) reduction were compared. In a subsequent investigation, a controlled efficacy test (CET) with FBZ and IVM was performed in lambs infected with *Haemonchus contortus* and *Trichostrongylus colubriformis* isolated from adult goats on the farm. Recovered specimens of *H. contortus* were subjected to pyrosequencing for detection of single nucleotide polymorphisms (SNPs) related to benzimidazole (BZ) resistance. During the FECRT, FECs in untreated lambs dropped significantly by 47%. No FEC reduction was detected in untreated kids. After FBZ treatments, FEC reductions in lambs and kids ranged from 15 to 54% and 49–56%, respectively, according to the different calculation methods. Post IVM treatments, FEC reductions in lambs and kids varied between 71–90% and 81–83%, correspondingly. LEV and MOX reduced FECs by 98–100% in both species. In the CET, FBZ reduced *H. contortus* worm counts by 52–56% and no reduction in *T. colubriformis* counts were detected after treatment. IVM eliminated 100% of *H. contortus* and reduced *T. colubriformis* counts by 84–92%, according to different calculation methods. Pyrosequencing of isolated *H. contortus* revealed increased frequencies of the BZ resistance-related SNP in codon 200 of the β-tubulin isotype 1 gene. Frequency of BZ resistance-related SNPs in codons 167 and 198 were very low and did not exceed levels as obtained in the

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susceptible reference isolate. Anthelmintic resistance was confirmed in this recently established organic farm and low field efficacy of FBZ was verified by CET and pyrosequencing. BZ-resistant populations of *H. contortus* and *T. colubriformis* were isolated for the first time in Denmark. Problems with correct dosing of goats, the observed FEC reduction in untreated lambs and the relevance of including a control group in the FECRT are discussed.


## 1. Introduction

In both, conventional and organic herds, preservation of the therapeutic efficacy of anthelmintics is vital for the treatment of heavily parasitized animals (Pomroy, 2006; Waller, 2006). Furthermore, effective anthelmintics are needed for the implementation of integrated parasite control programs which combine non-pharmacological methods with strategic use of drugs (Waller and Thamsborg, 2004). As a result, the monitoring of faecal egg count (FEC), the evaluation of treatment efficacy and the detection of anthelmintic resistance (AR) are becoming increasingly important for health programs of grazing livestock (Kaplan and Vidyashankar, 2012). AR is a problem usually associated with conventional production systems, which are more reliant on the use of antiparasitic drugs, but less in organic systems, where the prophylactic use of anthelmintics is banned (Waller, 2006; Hoste et al., 2014). In the present article we describe the investigations following a suspected case of AR in one of the largest organic sheep and goat dairy farms in Denmark. The objectives of our study were (1) to assess the efficacy of different anthelmintic treatments in animals naturally infected with gastrointestinal nematodes in the study farm, (2) to evaluate different methods for calculation of faecal egg count reduction percentages using untreated and treated lambs and kids and (3) to confirm the presence of suspected drug-resistant nematode strains in a controlled efficacy test. Additionally, benzimidazole (BZ) resistance was investigated using pyrosequencing analysis of the codons 167, 198 and 200 of the β-tubulin isotype 1 gene in nematode populations isolated in the controlled efficacy test.

## 2. Materials and methods

### 2.1. Study farm

The flock was established in 2007 by the purchase of animals from several Danish farms. No quarantine anthelmintic treatments were performed before introduction of new animals into the herd. At the start of the study (October 2011) it was one of the largest organic sheep and goat dairy herds in the country, including a total of 400 East Frisian sheep and 698 Saanen and Danish Landrace goats. Routinely, lambs and kids were weaned in April and managed as one group on common pastures. In early September young stock was moved to clean pastures. From the second grazing season onwards does and ewes grazed in separate groups, although using alternate grazing, on the same pastures. Until 2010, individual young stock with clinical signs of parasitism (<30% of the group per year) were treated subcutaneously (s.c.) with macrocyclic lactones (ML), and affected adults (<10% of the group per year) were treated orally (p.o.) with BZ. In 2011, lambs and kids were weaned in late May. In early July several lambs and kids were observed with anaemia, diarrhoea, depression and suboptimal growth and animals with severe clinical signs (approximately 50% of the group) were treated with moxidectin (MOX, 0.2 mg/kg p.o., Cydectin® 0.1%, Scanvet). Roughly 25% of the animals were treated again with the same drug in mid-August. Clinical signs of parasitism continued in untreated animals and by mid-September the mortality reached approximately 25% in lambs and kids. Five lambs with diarrhoea and depression were submitted to the Large Animal Hospital, University of Copenhagen, and were treated with ivermectin (IVM) (0.2 mg/kg s.c., Ivomec® 1% Vet. injection, Merial Norden A/S), but no clinical recovery was observed. Hence, presence of AR was suspected.

### 2.2. Faecal egg count reduction test (FECRT)

In early October 2011 preliminary FECs of a group of lambs and kids at the study farm revealed a mean egg count of 4570 (±3632) eggs per gram (EPG) of faeces. From this group of animals, aged 4–6 months and not treated with anthelmintics within 4 weeks prior to the study, 40 lambs and 40 kids were selected. A FECRT was conducted following the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992). Individual body weights (BW) were obtained for all the animals using an electronic scale. Lambs and kids were stratified by FEC and randomly allocated to treatment groups ($n = 8$ animals/group, four groups for each species) and control groups ($n = 8$ animals/control group, one for each species). Lambs were treated with the recommended doses of fenbendazole (FBZ; 5 mg/kg p.o., Panacur® oral susp. 10%, MSD Animal Health), IVM (0.2 mg/kg s.c., Ivomec® 10 mg/mL Vet. injektion, Merial Norden A/S), MOX (0.2 mg/kg p.o., Cydectin® mikstur 1 mg/mL, Scan Vet Animal Health A/S) or levamisole (LEV; 7 mg/kg s.c., Levaject® 75 mg/mL, ChemVet DK A/S). At the time of study, none of these anthelmintics were registered for use in goats in Denmark (VIF, 2011). Consequently, different doses were considered and kids were treated with FBZ (10 mg/kg p.o.), IVM (0.2 mg/kg s.c.), MOX (0.3 mg/kg p.o.) or LEV (14 mg/kg s.c.). The recommended dose of IVM for sheep (0.2 mg/kg s.c.) was maintained for kids based on previous experience by the authors of side-effects in goats treated subcutaneously with this drug. Faecal samples were collected rectally at the day of treatment (day 0) and at day 14 post treatment (p.t.) in all groups. After collection faeces was stored in ice-boxes at 5 °C, transported