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Short communication

Doramectin resistance in *Haemonchus contortus* on an alpaca farm in Belgium

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ABSTRACT

Parasitism by gastrointestinal nematodes is a health concern in New World Camelids (NWC) worldwide, and anthelmintic treatment is often needed for parasite control. Although anthelmintic resistance has been reported in ruminants worldwide, data in NWC are only scarce. In the present study, a case of suspected doramectin resistance in alpacas was examined. A field efficacy study was conducted for the evaluation of two different dosages of doramectin using a faecal egg count reduction test. A group of 8 alpacas was treated with a subcutaneous injection of doramectin at 0.2 mg/kg bodyweight. Individual faecal samples were collected before treatment and 7 days after treatment. The faecal egg counts indicated a treatment efficacy of only 68%. To determine whether the treatment failure was caused by true anthelmintic resistance or suboptimal dosage in this animal species, a group of 4 alpacas was subsequently treated at 0.3 mg/kg bodyweight. Faecal egg counts 7 days post treatment were reduced by only 41%, indicating that the treatment failure was more likely to be caused by the presence of resistant parasites on this farm. Coprocultures of faecal samples collected after treatment indicated the presence of 98.5% *Haemonchus contortus* and a small percentage of *Cooperia oncophora* (<1.5%). A controlled efficacy trial in sheep, for which the optimal dosage of doramectin is known, was conducted to ensure that this truly was a case of resistant parasites. Infective larvae collected from the faeces of these alpacas were used to infect eight nematode-free lambs. These lambs were assigned to one of two groups based on faecal egg counts post infection. One group was treated with doramectin injectable at 0.2 mg/kg bodyweight, the other group served as a non treated control group. Pharmacokinetics indicated that the doramectin treatment was adequate, yet an efficacy of only 16% was determined on day 7 after treatment. Identification of the larvae after treatment revealed 100% *H. contortus*. On day 7 after treatment, *H. contortus* worm counts were only reduced by 8% in the treated lambs. The results of the present study report for the first time a case of doramectin resistance in alpacas, mainly in *H. contortus*.

1. Introduction

In domesticated New World Camelids (NWC), *i.e.* llamas and alpacas, parasitic gastroenteritis can cause clinical and subclinical problems. In regions where the NWC were imported, animals are often kept under (semi-) intensive grazing conditions, in contrast to more extensive grazing

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management in South America. Furthermore, NWC in new habitats often share pasture with other ruminants, such as sheep, which can significantly increase the infection pressure because pasture contamination is no longer limited to the NWC defecation areas (Leguia, 1991; Hill et al., 1993; Beldomenico et al., 2003). Climatic conditions are often more favourable for the development of gastro-intestinal parasites in temperate climates, and regular anthelmintic treatment may be required (Windsor et al., 1992a,b). However, several papers have previously reported anthelmintic treatment failures in NWC (Windsor et al., 1992b; Gillespie et al., 2010). Some studies attributed these treatment failures to the fact that the 0.2 mg/kg bodyweight (BW) dose is suboptimal for NWC and claim that this species requires a higher dose of macrocyclic lactones (ML) compared to sheep and cattle (Jarvinen et al., 2002; Whitehead, 2008). In contrast, a previous field efficacy study indicated that an ivermectin treatment at a dosage of 0.2 mg/kg BW resulted in a 100% reduction in faecal egg output in llamas as well as in alpacas (Geurden and Van Hemelrijk, 2005). Another potential reason for anthelmintic treatment failures in NWC is the occurrence of resistant parasites. Gastrointestinal nematodes from NWC in regions in which they have been imported appear to be mostly shared with domesticated ruminants such as sheep, goats and cattle (Ballweber, 2009). As multi-drug resistance in nematodes of sheep and goats has been extensively reported, resistance can be expected in NWC grazing the same pastures as well (Kaplan, 2004; Gillespie et al., 2010).

The aim of this study was to examine an alleged case of doramectin resistance on an alpaca farm in Belgium and to investigate whether treatment failure was caused by a suboptimal dosage or the presence of resistant parasites.

2. Materials and methods

2.1. Farm management

The case started with a 2-year old alpaca stallion displaying symptoms of hypersalivation, anorexia and weight loss. High faecal egg counts (>1000 EPG) were observed, even after two treatments with doramectin at a dose rate of 0.3 and 0.6 mg/kg BW. The sick stallion originated from an alpaca breeding farm in Belgium, with approximately 45 animals. Four groups of approximately 10 animals graze separate pastures of about 0.3 ha and faeces are removed from pasture on a weekly basis. The farm history indicated that the alpaca stallions frequently grazed pastures where horses, calves and sheep had grazed before. During the present grazing season, the stallions had shared a pasture with a sheep flock for a couple of months. These sheep are dewormed 4 times a year, using a rotation system in which fenbendazole (Panacur® oral suspension 2.5%, Intervet at 5 mg/kg BW), ivermectin (Ivomec®, Merial at 0.2 mg/kg BW subcutaneously) and doramectin (Dectomax®, Pfizer A.H. at 0.2 mg/kg BW subcutaneously) are used. The farmer regularly imports alpacas from New Zealand. These animals receive a doramectin treatment (Dectomax®, Pfizer A.H., dose unknown) before shipment. Upon arrival, the animals are put in quarantine for 1 week. In Belgium, the flock is treated 4 times a year, alternately using fenbendazole (Panacur® oral suspension 10%, Intervet at 20 mg/kg BW), ivermectin (Ivomec®, Merial at 0.3 mg/kg BW subcutaneously) and doramectin (Dectomax®, Pfizer A.H. at 0.3 mg/kg BW subcutaneously). Mares get an additional treatment after parturition.

2.2. Faecal egg count reduction tests in alpacas

To investigate the alleged doramectin resistance, two modified faecal egg count reduction tests (FECRTs) were performed. In the first trial, the FECRT was performed on a group of 8 adult alpacas (5 stallions and 3 mares), using the prescribed dosage of doramectin. The animals were weighed using a cattle weighing scale with a calibration of 1 kg and treated subcutaneously with doramectin at a dose rate of 0.2 mg/kg BW. Individual faecal samples were taken prior to treatment and 7 days after treatment. The faecal egg output was determined using a modified McMaster technique (Thienpont et al., 1986) with a sensitivity of 50 eggs per gram faeces (EPG). In a subsequent trial, the FECRT was performed using 3 stallions and 1 mare. The 4 animals were treated at a dose rate of 0.3 mg/kg BW, as this dosage is often recommended in NWC. The egg counts were performed as described above. A wash out period of 4 weeks between the first and the second trial was respected and the egg excretion prior to the second treatment was above 250 EPG in all animals. The percentage reduction in faecal egg output was calculated based on arithmetic mean egg counts using the following formula:

$$\text{FECRT}\,[1](\%) = 100 \times \left(1 - \frac{\text{arithmetic mean (FEC after treatment)}}{\text{arithmetic mean (FEC before treatment)}}\right)$$

95% confidence intervals (95% CI) were calculated using a bootstrap analysis based on 10,000 iterations (R version 2.4.1, The R Foundation for Statistical Computing).

In all experiments coprocultures were made of pooled faeces before and after treatment. Third stage larvae were collected by the Baermann technique and 200 larvae were differentiated from each pooled sample (van Wyk et al., 2004).

2.3. Faecal egg count reduction test and controlled efficacy study in sheep

As the optimal treatment dosage of doramectin is known in sheep, we used this species to confirm the alleged case of resistance in the alpacas. A FECRT and a controlled efficacy study were performed in sheep, using infective larvae recovered from doramectin treated alpacas. Seven days after treatment faecal samples were collected for 3 consecutive days from the egg excreting alpacas and incubated for 14 days at 25 °C until a sufficient amount of third stage larvae (L3) were harvested to infect one helminth-naïve lamb (3500 L3 larvae). From 16 days post infection, faecal samples were collected from this lamb and cultured *in vitro* to obtain new infective larvae.

Using the infective L3 larvae harvested from this coproculture, 8 lambs of both sexes were infected. These animals were about 9 months old and had never grazed before. Each lamb was orally infected with 25,000 L3 larvae. On day 21, 22 and 24 after infection, individual faecal egg counts were