FOOD/FARMED ANIMALS

Reduced efficacy of ivermectin against *Ostertagia* in a Dutch cattle herd

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SUMMARY

A pilot study on reduced ivermectin efficacy against Ostertagia ostertagi following the detection of a high number of strongyle-type eggs in a 1.5-year-old bull during the first part of the pasturing period in 2015 was conducted. This finding was remarkable because of the pasturing history, treatment history and time after turnout (June). The study involved one beef cattle herd and followed as far as possible the World Association for the Advancement of Veterinary Parasitology guidelines regarding the faecal egg count reduction test. We observed poor ivermectin efficacy (65%) compared with the expected efficacy of >95%. This also has consequences for practitioners, who should ensure the correct application of anthelmintics and perform repeated faecal examinations following the use of parasitical agents for persistent problems. Our finding also should have consequences for the pharmaceutical industry: the administration of registered anthelmintics via the most efficacious route is preferable.

BACKGROUND

Anthelmintics are widely used in animal management, and this has resulted in serious resistance-related problems in horses and sheep (Matthews 2014, Rose and others 2015, Van den Brom and others 2015). Currently, intestinal parasites only exhibit resistance to benzimidazoles (Holzhauer and others 2014). To our knowledge, reduced efficacy of ivermectin against *Ostertagia ostertagi* is unknown in cattle in the Netherlands, but when present it has serious consequences for Western-European practitioners. The emergence of resistance necessitates more frequent assessment of the efficacy of anthelmintics using the faecal egg count reduction test (FECRT). This is especially important when the clinical effect of an anthelmintic appears insufficient.

CASE PRESENTATION

In June 2015, an uncastrated fattening bull aged 1.5 years was sent to the Dutch Animal Health Service (GD Deventer, Deventer, The Netherlands) for a necropsy. The bull had lived in a herd with 12 others ranging in age from 400 to 600 days and had been purchased on 16 April 2015 from a neighbouring farmer. All the bulls were treated by the farmer with a pour-on ivermectin solution (Noromectin, 0.5 mg/kg bodyweight (BW); Norbrook Laboratories, Newry, Northern Ireland) and were pastured immediately after treatment. In the previous year, the bulls had also been pastured and had been treated with a pour-on eprinomectin solution (Eprinex, 0.5 mg/kg BW; Merial Belgium N.V., Diegem, Belgium) at the time of housing (October 2014). Following pathological examination, the decision was made to perform a faecal examination because of the poor condition of the animal (although no adult worm burden was observed by the pathologist), and a remarkably high number of strongyle-type eggs (>1000 eggs per gram of faeces (epg)) were detected using a modified McMaster technique (MAFF(Ministry of Agriculture Fisheries and Food) 1986). This was considered noteworthy because of the pasturing history, bull's age and history of treatment, although uncastrated males are reportedly more prone to nematode infections, as well as higher epg values, than castrated bulls or female ruminants (Barger 1993). To rule out underdosing by the farmer and investigate the possibility of reduced ivermectin efficacy against gastrointestinal nematodes (GIN), additional treatment under supervision of the first author, combined with the examination of faecal samples, was conducted in this pilot study.

Before the start of the pilot study, its design and measures to promote animal welfare were approved by the animal ethics representatives of the GD.

TREATMENT

The pilot study was performed as a case–control study in the period June–July 2015 and consisted of different components (see Fig 1).

After the collection of rectal faecal samples, the epg values and culture of larvae for six bulls aged 400–600 days grazing in the same pasture as the afflicted bull were determined. Four animals were treated and two remained untreated as controls. Fourteen days later, all animals were individually sampled again and the two untreated animals were then treated for the first time. The faecal samples were examined individually at the GD using the modified McMaster method with a detection limit of 50 strongyle-type epg. The rectal faecal samples were mixed with peat moss to create favourable conditions for the development of larvae in terms of aeration and moisture and incubated at 27°C±1°C (Roberts and O'Sullivan technique, MAFF(Ministry of Agriculture Fisheries and Food). 1986). The aeration and moisture of the samples were checked daily. Cultures were harvested after 7 days by filling the pot with lukewarm water and inverting it on a Petri dish; the samples were then replenished with water and left standing for 8 hours to allow the larvae to migrate from the pot to the water in

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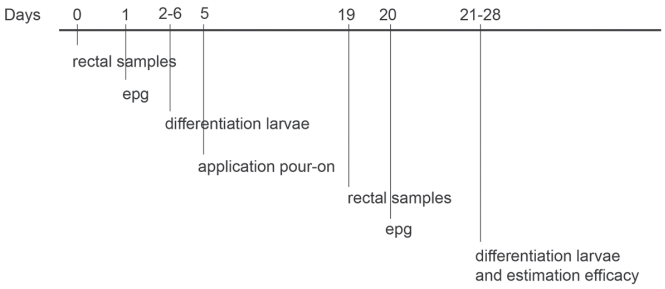

FIG 1: Layout of the pilot study to investigate a possible reduced ivermectin efficacy after pour-on application (epg, eggs per gram of faeces).

the Petri dish. The larvae (mainly L3 stages) were harvested and differentiated under a microscope (magnification ×10; Borgsteede and Hendriks 1974).

The FECRT was used to evaluate anthelmintic efficacy as described by Coles and others (1992). This method compares the faecal egg counts just before anthelmintic treatment with those 14 days after treatment using the following formula:

—FECR=100 × (1 − EPGpost/EPGpre),

where EPGpre and EPGpost represent the faecal egg counts before and after treatment, respectively. Usually the arithmetic means from at least 10 animals are used but fewer animals can be used if necessary. The values obtained were compared with the expected efficacy of ≥95% (Coles and others 1992).

OUTCOME AND FOLLOW-UP

The World Association for the Advancement of Veterinary Parasitology guidelines define anthelmintic resistance for small ruminants as present in vivo if:

1. the percentage reduction in egg count is, depending on the anthelmintic, <95%; and
2. the 95% confidence level is <95% (Coles and others 1992).

If only one of these two criteria is met, resistance is suspected, although this definition of anthelmintic resistance applies to small ruminants. No clear definitions of resistance are currently available for parasitic nematode infections in cattle or explanations on how to perform and interpret FECRT results in this species.

The mean faecal egg counts in the case and control groups on day 14 were 102 and 288 eggs, respectively, which equate to an average FECR of 65% via the equation (100 × (1 − 102/288)) and may indicate reduced ivermectin efficacy (Table 1). However, the number of sampled and treated animals (six) was suboptimal; it was impossible to test more animals because of the size of the bulls and physical condition of the farmer. In the pretreatment samples, the strongyle larvae were all determined to be *O ostertagi*, whereas the day 14 samples showed 99.9% *O ostertagi* and a single *Strongyloides* larva.

DISCUSSION

To our knowledge, this is the first time that a pour-on ivermectin solution has shown an efficacy of <95% against *O ostertagi* in the Netherlands. Our results are consistent with those of a Swedish study that described a limited effect (88% efficacy as determined by a FECRT) after administration of topical macrocyclic lactones (ML) against GIN (Areskog and others 2013).

The set-up of our pilot study did not allow discrimination between reduced efficacy of the active ML ingredient and reduced efficacy due to the method of application. The efficacy of pour-on applications depends in part on the activity of the products when they are licked off the back by the animal, and this differs from other methods of administration such as direct oral application. The risk of partial efficacy in these circumstances was documented in a French study, in which pour-on application was suggested to result in under-dosage (Bousquet-Mélou and others 2011). In that herd, self-grooming was inhibited during housing, where the bulls were kept in stalls and tied to a post. Evidence supporting the influence of the mode of application was provided by a study conducted in New Zealand, in which different formulations of moxidectin were compared in terms of FECRT results for their effects on GIN in cattle on 14 commercial farms (Leathwick and Miller 2013). The reduction in faecal egg count was significantly greater after treatment with oral moxidectin than following treatment using the same product as injectable or pour-on formulations.

Unfortunately, because of the physical condition of the farmer and age and character of the bulls, a repeat experiment using a different application route for the ML tested in this study herd was not possible. Considering the location of the adult nematodes, oral administration of anthelmintics is the preferred application route (more effective), but oral anthelmintics are unavailable in the Netherlands.

In 2005, the efficacy of a pour-on moxidectin solution was evaluated on a Mexican ranch and 100% efficacy was estimated (Maritorena-Diez and others 2005). Nevertheless, ivermectin resistance has been demonstrated in South America (Orpin 2010), and the efficacy of ML against other GIN was also recently examined in European studies (Rehbein and others 2012, Geurden and others 2015, Borges and others 2015). Contradictory results in these European studies mean that vigilance is necessary. The largest study to investigate the

TABLE 1: Presentation of the epg before and 14 days after treatment with ivermectin pour-on, the weights of the bulls in kilograms and the FECRT

	epg Day 0	Weight of bulls in kg	ml applied Noromectin® at day 0	ml applied Noromectin® at day 14	epg Day 14	FECRT	Mean	CI
Case	17	346	35		0	100		
	33	495	50		11	67	48	4 to 91
	33	392	40		28	15		
	400	342	35		367	9		
Control	83	396		40	506	–6		
							22	–34 to –78
	100	346		35	50	50		

epg,eggs per gram of faeces; FECRT, faecal egg count reduction test.

existence of anthelmintic resistance in Europe was performed by Geurden and others (2015) and, in line with the results of this pilot study, a decreased efficacy was found on at least half of the farms involved. This is of serious concern and warrants urgent investigation by practitioners, farmers, scientists and the pharmaceutical industry. Provision of information to the end users of anthelmintics, as for other drugs, is advisable. Veterinarians should monitor drug efficacy systematically.

Contributors This pilot-study has been performed with contributions of all authors (MH: clinical work, prep manuscript; CH: laboratorial work and manuscript; DD: upset study, statistical work and manuscript).

Competing interests None declared.

Ethics approval The study was approved by the internal GD Ethical Committee for Clinical studies

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Notes