



## Monitoring the efficacy of ivermectin and albendazole against gastro intestinal nematodes of cattle in Northern Europe

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### ABSTRACT

Faecal egg count reduction tests (FECRT) using ivermectin (IVM) and benzimidazole (BZ) were conducted to investigate the prevalence of anthelmintic resistance in gastro-intestinal nematodes on cattle farms in Germany, Belgium and Sweden in 2006 and 2007. Based on sufficient numbers of eggs prior to the study, between 3 and 10 farms per country were selected. 10–15 animals were randomly selected per farm and subcutaneously treated with 0.2 mg IVM/kg bodyweight (Ivomec<sup>®</sup>, Merial). Faecal samples were collected individually from every animal on day 0 (treatment), day 7 (Belgium & Sweden) or 14 (Germany), and day 21 (Germany, Belgium and Sweden). Faecal egg counts (FEC) were performed at each sampling occasion to estimate the eggs per gram of faeces (EPG) and the reduction of eggs after treatment. The FECRT using IVM in 2006 revealed mean reduction of egg counts between 69–100% on day 7/14 (95% confidence interval (CI) 19–102) and 35–96% (95% CI 0–102) on day 21. Farms with a suggested problem of anthelmintic resistance have been re-visited in 2007 and except for one case all results obtained in 2006 were confirmed in 2007. Larvae obtained from faecal cultures were identified using microscopic identification keys or genus-specific real time PCR. *Cooperia oncophora* was the predominant species detected after treatment, but *Ostertagia ostertagi* was found in samples on 3 farms in Germany and 3 farms in Sweden post-treatment.

In 2007 additionally a FECRT using benzimidazoles was conducted in Germany and Sweden. In Germany oral Valbazen<sup>®</sup> (albendazole, 10%, Pfizer) was used at a concentration of 7.5 mg albendazole/kg bodyweight; in Sweden Valbazen Vet<sup>®</sup> (albendazole, 10%, Orion Pharma) at a dose of 8 mg/kg was used. For benzimidazoles an efficacy of 100% was obtained on all tested farms in both countries. This is the first report of a multinational anthelmintic efficacy investigation in cattle in Europe. The results suggest that testing of anthelmintic efficacy should be performed more intensively due to possible insufficient efficacy of current drugs.

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### 1. Introduction

Gastrointestinal nematodes of livestock cause serious economic losses, especially where intensive grazing management is practised. During the past twenty years

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most of the livestock industries in Europe have relied heavily on the effective chemically based parasite control, moving from the class of benzimidazoles to the macrocyclic lactone anthelmintics in most countries. In Sweden the drugs of choice still were benzimidazole products until recently. Anthelmintic resistance (AR) against all classes of commercially available anthelmintics is an emerging problem in many parts of the world, particularly in the sheep and goat industry. Several surveys indicate widespread resistance to one or more of the broad spectrum anthelmintics (Kaplan, 2004) in most livestock species. AR in cattle parasites is not reported as frequently as in the small ruminant industry. However, an increasing number of cases of cattle nematode resistance have been reported mostly in New Zealand and South America and occasionally in other countries e.g. the UK (Anziani et al., 2004; Anziani et al., 2001; Coles, 2002; Fiel et al., 2001; Jackson et al., 2006; Mejía et al., 2003; Soutello et al., 2007; Suarez and Cristel, 2007; Waghorn et al., 2006). The apparent lack of data regarding AR in cattle for other countries is probably due to various reasons. In comparison to the sheep industries, different management systems are used in the cattle industry; including less frequent treatment, different grazing schemes and larger percentage of worms in refugia (Coles, 2002). Furthermore the detection of AR in the field is hindered by the absence of resistance surveys undertaken and the lack of sensitivity of the currently available detection methods. While discriminating doses for thiabendazole in the egg hatch test have been established for sheep gastrointestinal nematodes, no such data is available for cattle nematodes at present. A similar situation is found regarding macrocyclic lactone (ML) resistance detection. Currently the faecal egg count reduction test (FECRT) remains the only proven way of detecting AR in the field. So far only *Cooperia oncophora* was reported to be a rising problem detected in field surveys. The fact that this parasite is considerably less pathogenic than *Ostertagia ostertagi*, possibly contributes to less interest in studies aiming on the detection of anthelmintic resistance in cattle nematodes.

At present, isolated cases of resistance in cattle nematodes in Europe have only been reported for the United Kingdom (UK) (Coles, 2004; Coles et al., 2001; Stafford et al., 2007), while no recent reports on drug efficacy in cattle have been published for other European countries. Thus the current status of anthelmintic efficacy in cattle nematodes remains largely unknown. The results presented in this article are obtained from FECRT carried out in Belgium, Germany and Sweden in 2006 and 2007, using ivermectin (IVM) (2006 & 2007) and benzimidazoles (BZ) (2007).

## 2. Material and methods

To investigate the efficacy of IVM, 11 (Germany), 7 (Belgium) and 6 (Sweden) farms were included in the FECRT study in 2006. All German farms are located in the central North of Germany, the Belgium farms in East Flanders and 6 Swedish farms are in Central Sweden, around Uppsala. In Germany, all calves were Holstein

Frisians, in Belgium either Holstein Frisian or Belgium Blue cross breed and in Sweden mostly Swedish Red with some Holstein Frisians. The infection level of each farm was determined by faecal egg counts from 10–20 randomly chosen first-season grazing calves prior to the beginning of the study. In the German and Swedish laboratories faecal egg counts were determined using a modified McMaster method (Whitlock et al., 1980) with sensitivity between 33.3 and 50 eggs per gram faeces (EPG) (Germany and Sweden, respectively) since these were the established procedures. In Belgium the FECPAK technology ([www.fecpak.com](http://www.fecpak.com)) with a sensitivity of 10 EPG was available and established at the start of the project. During the process of the study this method became also available for the other two laboratories and was thus compared with the modified McMaster methods. Both Sweden and Germany did not reveal any statistically significant differences regarding the number of eggs in individual egg counts as well as the number of positive animals identified per farm ( $p > 0.05$ , data not shown). Accordingly, Germany and Sweden decided to use only the McMaster method, as the FECPAK method is more labour and time intensive.

All calves were individually identified by their ear tags, weighed or measured with a girth tape and treated subcutaneously on day 0 with 0.2 mg IVM/kg bodyweight (Ivomec<sup>®</sup>, Merial). For the duration of the trial the selected calves were grazed on pasture with the rest of the herd—there were no untreated controls in the experiment. Further faecal samples were collected rectally on day 0, 7 or 14, and 21 to determine individual egg counts and to establish the individual percentage of faecal egg count reduction (FECR) by the FECRT. The mean EPGs and % FECR per farm and sampling occasion from all three countries were analysed with the Tukey Test (all pairwise multiple comparison).

Several average FECRT protocols are widely used but except for Coles (Coles et al., 1992) they do not offer a confidence interval. Statistical analysis of the data of the present study was performed to assess the significance of the group mean egg count reductions using the newly developed program “BootStreat” (Cabaret and Antoine, 2008), based on bootstrapping methods. BootStreat intends to calculate the efficacy of the treatment and to provide confidence intervals based on re-sampling-bootstraps (Sprent, 1989). The efficacy data usually do not follow a Gaussian distribution and confidence intervals cannot be calculated; bootstrap is one method for evaluating confidence intervals on non-Gaussian distributions. Mean egg count reduction and lower and upper confidence limits were calculated with BootStreat using a before/after treatment evaluation of the FEC and a re-sampling number of 2000. Sprent, 1989 considered that 100–1000 re-samplings were a minimum for the evaluation of confidence intervals; preliminary trials with FECRT indicated that 2000 re-samplings were sufficient to stabilize the estimations of variability. Similar to the proposal of Coles (Coles et al., 1992) resistance was declared if the mean percentage of EPG reduction was less than 95% and the lower confidence limit was below 90%. Larval cultures of EPG positive samples pre- and post-

treatment were taken to identify species present. Individual animal samples were used in Germany and Belgium and due to limited capacities pooled samples were used in Sweden for coprocultures. Microscopic identification of species was performed using third-stage larvae (Belgium and Sweden). In Germany, genomic DNA of pooled larvae from the samples was isolated and used for molecular species identification in a genus-specific real time PCR assay (von Samson-Himmelstjerna et al., 2002). In Hannover, this method is the established procedure for larval identification in order to determine which genus is present, while in the other two laboratories this method is not available at current.

In 2007, farms where a reduced efficacy was obtained in 2006 were re-visited in Belgium (3), Sweden (5) and Germany (2). In addition 2 farms, where in 2006 an IVM FECR efficacy >95% was recorded plus 2 new farms were investigated in Germany in 2007. The two farms (No. 1 and 9) where on day 21 still an IVM efficacy >95% was recorded were included in order to establish larval cultures from coprocultures of susceptible populations, which can be used as susceptible reference isolates in *in vitro*-assays. The two new German farms (11 and 12) were included upon requests of the farmers since both reported problems with the efficacy of the anthelmintic used previously. The remaining farms tested in 2006 were unfortunately not available for re-testing in 2007.

In Germany and Sweden efficacy of BZ was additionally investigated in 2007 on 10 and 2 farms, respectively. Between 10 and 20 animals were individually identified per farm by their ear tags and EPGs pre-treatment were established using a modified McMaster method (sensi-

tivity 33.3–50 eggs per gram). On day 0 the animals were individually treated with an oral albendazole formulation (Valbazen<sup>®</sup>, 10% albendazole, Pfizer), at a dosage of 7.5 mg/kg bodyweight in Germany and 8 mg/kg bodyweight in Sweden (albendazole, 10%, Orion Pharma). Faecal samples were taken on day 7 and 14 post-treatment.

### 3. Results

#### 3.1. Larval cultures

Faecal larval cultures were used to determine the different trichostrongyles present pre- and post-treatment. In 2006 the predominant species on all farms where the FECRT was performed (20) prior to treatment was *C. oncophora*. Smaller numbers of *O. ostertagi* were found on 7 out of 8 farms in Germany, all 7 farms in Belgium and all 5 farms in Sweden. Post-treatment *C. oncophora* was present on 17 out of 20 farms on day 7/14 and on all 20 farms on day 21. *O. ostertagi* was present on 3 out of 8 German farms, none of the Belgian farms, and on 2 out of 5 farms in Sweden on day 7/14 (Table 1).

In 2007 the predominant species prior to treatment was again *C. oncophora* on all farms tested with IVM, with a smaller number of *O. ostertagi* also found on all farms in Germany and Belgium and on 2 out of 4 farms in Sweden. Post-treatment, *C. oncophora* was still present on all farms with a positive post-treatment EPG in all countries. There were no farms with *O. ostertagi* in Germany and Belgium post-treatment, but all 4 tested farms in Sweden still had this species (Table 2).

**Table 1**

Results of the faecal egg count reduction test (FECRT) in Belgium, Sweden and Germany conducted with ivermectin (IVM) in 2006. The 95% confidence intervals for the FECR are given in brackets. Numbers in bold highlight farms where an IVM efficacy <95% FECR was obtained.

2006 No./Country	EPG <sup>a</sup> day 0			CC <sup>b</sup> day 0		FECR (IVM) in %		Presence of <i>Ostertagia</i>		Presence of <i>Cooperia</i>	
	mean	min	max	C. <sup>c</sup>	O. <sup>d</sup>	Day 7/14	Day 21	Day 7	Day 21	Day 7	Day 21
1/Belgium	35	10	90	x	x	100	58 (0–91)	–	–		x
3/Belgium	60	10	130	x	x	100	67 (9–95)	–	–		x
4/Belgium	215	50	650	x	x	97 (91–102)	93 (84–100)	–	–	x	x
5/Belgium	162	10	470	x	x	96 (91–100)	95 (88–101)	–	–	x	x
6/Belgium	112	10	320	x	x	74 (19–99)	73 (8–99)	–	–	x	x
7/Belgium	50	10	320	x	x	97 (93–100)	95 (86–100)	–	–	x	x
8/Belgium	40	10	100	x	x	95 (86–100)	90 (76–99)	–	–	x	x
1/Sweden	597	100	2250	x	x	99 (97–102)	96 (92–102)	–	–	x	x
2/Sweden	333	100	1000	x	x	83 (44–100)	47 (0–92)	x	x	x	x
3/Sweden	1250	700	3450	x	x	98 (95–101)	88 (67–98)	–	–	x	x
4/Sweden	1975	300	4600	x	x	95 (82–102)	94 (87–101)	x	x	x	x
5/Sweden	280	100	850	x	x	95 (90–101)	79 (59–99)	–	–	x	x
1/Germany	185	100	533	x	x	96 (91–100)	95 (88–98)	–	–	x	x
2/Germany	950	600	2200	x	x	90 (83–98)	84 (72–95)	x	x	x	x
3/Germany	137	67	333	x	x	97 (90–100)	96 (86–98)	–	–	x	x
5/Germany	40	33	100	x	x	87 (68–99)	81 (66–94)	–	–	x	x
6/Germany	176	100	266	x	x	98 (96–100)	94 (88–100)	x	–	x	x
7/Germany	146	67	266	x	x	91 (82–98)	93 (83–99)	x	–	x	x
8/Germany	58	33	100	x	x	69 (34–82)	35 (0–66)	–	–	x	x
9/Germany	107	67	167	x	–	100	96 (89–100)	–	–		x

<sup>a</sup> EPG: eggs per gram faeces.

<sup>b</sup> CC: coprocultures.

<sup>c</sup> C.: *Cooperia* spp.

<sup>d</sup> O.: *Ostertagia* spp.

**Table 2**

Results of the faecal egg count reduction test (FECRT) in Belgium, Sweden and Germany conducted with ivermectin (IVM) in 2007. The 95% confidence intervals for the FECR are given in brackets. Numbers in bold highlight farms where an IVM efficacy <95% FECR was obtained.

2007 No./Country	EPG <sup>a</sup> day 0			CC <sup>b</sup> day 0		FECR (IVM) in%		Presence of <i>Ostertagia</i>		Presence of <i>Cooperia</i>	
	mean	min	max	C. <sup>c</sup>	O. <sup>d</sup>	Day 7/14	Day 21	Day 7	Day 21	Day 7	Day 21
1/Belgium	119	10	300	x	x	81 (52–96)	54 (0–94)	–	–	x	x
3/Belgium	119	20	240	x	x	100	96 (91–101)	–	–	–	x
6/Belgium	203	10	590	x	x	95 (89–100)	86 (74–96)	–	–	x	x
2/Sweden	196	0	500	x	–	84 (67–95)	78 (48–97)	x	x	x	x
3/Sweden	550	0	1100	x	x	92 (79–101)	65 (13–90)	x	–	x	x
4/Sweden	370	0	700	x	x	85 (62–99)	79 (45–98)	x	x	x	x
5/Sweden	221	0	700	x	–	57 (0–93)	66 (0–98)	x	–	x	x
1/Germany	257	100	600	x	x	96 (89–102)	–	–	–	x	–
2/Germany	466	400	633	x	x	93 (81–102)	83 (60–97)	–	–	x	x
5/Germany	100	33	367	x	x	94 (75–101)	80 (39–100)	–	–	x	x
9/Germany	122	100	200	x	x	96 (85–101)	93 (83–99)	–	–	x	x
11/Germany	233	100	300	x	x	97 (92–102)	85 (69–100)	–	–	x	x
12/Germany	112	67	170	x	x	75 (49–97)	70 (41–93)	–	–	x	x

<sup>a</sup> EPG: eggs per gram faeces.

<sup>b</sup> CC: coprocultures.

<sup>c</sup> C.: *Cooperia* spp.

<sup>d</sup> O.: *Ostertagia* spp.

### 3.2. Faecal egg count reduction test (FECRT) IVM

The FECRT results for 2006 and 2007 are presented in [Tables 1 and 2](#), respectively. The mean EPGs per farm for 2006 and 2007 are presented in [Table 3](#).

Presumably due to the unusually hot and dry summer in Western and Central Europe in 2006, egg counts observed were generally very low in Germany and Belgium with a mean of <100 EPG on some farms. In Germany several farms were sampled until October and still no or

very few eggs were found. In Belgium 7 farms were tested in 2006. Of the 11 farms investigated in Germany only 8 farms were included in the trial (mean EPG >33), and in Sweden 1 farm was excluded due to low pre-treatment egg counts (mean EPG <50). The mean EPGs and % FECR per farm and sampling occasion from all three countries have been analysed with the Tuckey Test (all pairwise multiple comparison) and showed significant differences between day 7/14 and 21 concerning the EPG ( $p = 0.025$ ) and the FECR ( $p = 0.005$ ).

The FECR observed in 2006 on day 7/14 after treatment ranged from 69 to 100% (CI 34–100) in Germany, 74 to 100% (CI 19–102) in Belgium and 83 to 99% (CI 44–102) in Sweden. By day 21 the reduction was down to 35–96% (CI 0–100) in Germany, 58–95% (CI 0–101) in Belgium and 47–96% (CI 0–102) in Sweden ([Table 1](#)). These results indicate a reduced efficacy of IVM against gastrointestinal trichostrongyle species on 5 out of 8 farms in Germany, 5 out of 7 in Belgium and on 4 out of 5 farms in Sweden. While the efficacy against *Ostertagia* was 100% in Belgium, in Germany *Ostertagia* was still present on 3 farms on day 14 and only 1 farm on day 21. On the 2 Swedish farms, where *Ostertagia* was present before treatment, IVM failed to completely eliminate this species on day 7 and 21.

Average mean egg counts observed in 2007 were higher than in the previous year. In Germany they ranged between 33 and 633, in Belgium from 0 to 590 and in Sweden from <50 to 1100.

The FECR observed in 2007 on day 7/14 after treatment ranged from 75 to 97% (CI 49–102) in Germany, 81 to 100% (CI 52–100) in Belgium and 57 to 92% (CI 0–101) in Sweden. By day 21 the reduction was down to 70–93% (CI 39–100) in Germany, 54–96% (CI 0–101) in Belgium and 65–79% (CI 0–98) in Sweden ([Table 2](#)). The efficacy against *Ostertagia* was 100% in Germany and Belgium, but in Sweden this parasite was found on all farms after treatment. On both newly tested farms in Germany a reduced efficacy of IVM (70–85%) was recorded on day 21. The farms with a

**Table 3**

Mean EPG per farm in the faecal egg count reduction tests (FECRT) in Belgium, Sweden and Germany conducted with ivermectin (IVM) in 2006 and 2007.

No./Country	2006			2007		
	Day 0	Day 7/14	Day 21	Day 0	Day 7/14	Day 21
1/Belgium	35	0	14	119	5	16
3/Belgium	60	0	17	119	0	4
4/Belgium	215	6	15			
5/Belgium	162	6	6			
6/Belgium	112	27	29	203	35	91
7/Belgium	50	0	2			
8/Belgium	40	1	3			
1/Sweden	597	3	20			
2/Sweden	333	47	127	196	29	42
3/Sweden	1250	38	192	550	40	190
4/Sweden	1975	105	110	370	61	72
5/Sweden	280	12	65	221	71	43
1/Germany	185	6	6	257	10	10
2/Germany	950	89	133	280	33	78
3/Germany	137	3	3			
5/Germany	40	3	3	100	4	17
6/Germany	176	3	10			
7/Germany	146	13	17			
8/Germany	58	12	22			
9/Germany	107	0	3	122	4	5
11/Germany				233	6	33
12/Germany				111	28	39

recorded IVM efficacy >95% in 2006 appeared mainly susceptible in 2007. The status of both farms with a reduced efficacy in Germany in 2006 was confirmed in 2007. But in contrast to 2006, there were no *O.ostertagi* found on farm No.2. In Belgium, the reduced IVM efficacy on day 21 was only confirmed for farm No.1. While the IVM efficacy of 1 Belgium farm (No.6) improved significantly in 2007 in contrast to the data obtained in 2006, no reduced IVM efficacy was found on farm No.3 in 2007. In Sweden, the 4 farms with a reduced IVM efficacy in 2006 were re-tested in 2007. In comparison to 2006, all farms showed a reduced IVM efficacy already on day 14 and 21 in 2007, indicating an increasing IVM efficacy problem on these farms.

### 3.3. Faecal egg count reduction test (FECRT) BZ

Parallel to the investigation of IVM, a FECRT using BZ was performed in Germany and Sweden in 2007. In Sweden, the efficacy of BZ was tested on 2 out of 4 farms which were also included in the IVM FECRT study using a second group of animals while in Germany 10 new farms were investigated. The mean pre-treatment EPG in Sweden was around 550 on both farms, in Germany it ranged from 60 to 220. On all farms the FECR was 100% in both countries.

## 4. Discussion

This study provides the first European multinational investigation on the efficacy of anthelmintics in cattle. According to current WAAVP guidelines a reduction of the faecal egg count below 95% plus a lower confidence limit below 90% strongly indicates the presence of AR while the presence of only one of the two parameters suggests an onset of AR (Coles et al., 1992). Here, dosing and application have been performed by the investigators and are therefore not likely to be a reason for the failure of the drug.

The results presented in this survey indicate that the anthelmintic efficacy of IVM may be frequently incomplete in cattle nematodes in Europe. The parasite mainly observed to be associated with insufficient IVM efficacy was *Cooperia* spp. In the survey performed in 2006, *C. oncophora* was found on day 7/14 on 85% of all farms tested, on day 21 on all farms, suggesting, that resistance to IVM may be much more common in Europe than previously expected.

In controlled registration trials the efficacy of injectable IVM (0.2 mg) was given as a >99% reduction for *Ostertagia* spp. and between 95 and 98% reduction for *Cooperia* spp. (Benz et al., 1989). Since *C. oncophora* is known to be the dose-limiting species for MLs (Vercruyse and Rew, 2002) resistance to this species could be expected to occur first. Noteworthy, in this context is that at least in experimental studies it was shown that repeated sub-therapeutic treatments with MLs can lead to resistant nematode populations of sheep (Ranjan et al., 2002) and cattle (Molento et al., 1999; Van Zeveren et al., 2007). The fact, that mainly *Cooperia* spp. appear after treatment could be a reason why resistance to the drug is rarely recognized by the farmers, due to the generally lowed pathogenicity of this parasite. However, a high number of parasites remaining

after treatment can also result in economic losses or clinical parasitism. Usually *Cooperia* spp. occurs as a mixed infection together with *Ostertagia* spp., increasing the reciprocal effect of both parasites. If clinical signs of *Cooperia* spp. are not associated with an infection with *Ostertagia* spp., the predominant clinical signs are diarrhoea, decreased appetite and a loss in weight gain. However, in New Zealand, where most of the cases regarding resistance to MLs have been reported, there are still no case reports of clinical parasitism due to this species (Jackson et al., 2006).

In addition to the occurrence of *C. oncophora*, the more pathogenic species *O. ostertagi* was observed to be present after treatment with IVM in 2006 on some farms in Germany and Sweden. While these findings could not be confirmed in Germany a year later, this species was present after IVM treatment on all 4 Swedish farms in 2007. In Belgium, the efficacy of IVM against *O. ostertagi* was 100% in both years. Despite the fact that IVM resistance can be experimentally induced in *O. ostertagi* (Van Zeveren et al., 2007) there are, in contrast to *Cooperia*, still very few reports of suspected IVM-resistant *Ostertagia* in the field. The basic population biology of these bovine nematodes is likely to play an important role in this context (Coles, 2002). Under normal field conditions, *Cooperia* produces more eggs compared to *Ostertagia* in cattle (Anderson, 2000; Michel, 1969; Vercauteren et al., 2004). *Cooperia* adults that survive the treatment have not only a large advantage over the susceptible worms but their contribution to the next generation is considerably greater compared to resistant *Ostertagia*. Furthermore, the limited sensitivity of the FECRT in combination with a higher egg output of *Cooperia* can perhaps underestimate the drug efficacy against *Ostertagia*. In addition, the IVM concentration that *Ostertagia* experiences in the abomasal mucosa is higher than in the intestine, the target site of *C. oncophora* (Lifschitz et al., 2000). This may be one reason why *Cooperia* is the dose-limiting species. It was therefore expected that *Cooperia* would be the first genus showing ML resistance in cattle (Coles, 2002).

For all participating countries this is the first report of suspected resistance of cattle nematodes to IVM. Resistance of *Cooperia* to IVM has been widely documented in New Zealand (Jackson et al., 2006; Waghorn et al., 2006) and South America (Mejía et al., 2003; Soutello et al., 2007; Suarez and Cristel, 2007). However, since there is no linear relationship between FEC and worm counts in *Cooperia*; it might be that lower worm numbers after treatment were partially compensated by higher egg production of females (Kanobana et al., 2001). *Ostertagia* spp. resistant to MLs have also been reported in New Zealand, but further investigations to confirm these findings have failed so far (Waghorn et al., 2006). This is additionally the first report of presumably IVM-resistant *O. ostertagi* in Europe. Clearly, further investigations such as controlled efficacy experiment including egg and worm counts with the isolated field resistant population are needed to confirm these results.

Globally, in comparison to the situation regarding MLs, resistance of cattle nematodes to BZs is less frequent (Anziani et al., 2004; Jackson et al., 2006; Suarez and Cristel, 2007). Accordingly, in the present study BZs remained effective against both predominating species of cattle

nematodes in the investigated countries. The FECRT using BZs on 10 and 2 farms investigated in Germany and Sweden, respectively, resulted in 100% efficacy against *C. oncophora* and *O. ostertagi*. One reason for the difference to the findings regarding IVM is possibly related to the frequent use of MLs since their introduction to the market in the early 80s in many countries due to the broad endectocidic spectrum and ease of use. On the other hand, the use of MLs in cattle in Sweden is and has always been limited. Although there is an increasing amount of cattle producers that have started to use the doramectin pour on formulation, only  $\approx 50\%$  of the conventional farmers use anthelmintics and in general only the first season grazers are treated. For a long time Swedish farmers relied heavily on slow and intermittent release devices e.g. morantel (Paratect<sup>®</sup>) or oxfendazole (Systamex<sup>®</sup>). Of the small percentage of farms using BZs in Germany the majority uses long acting bolus products in first season grazing calves only. Due to the unexpected results regarding the use of BZ, the 10 farmers participating in the BZ FECRT in Germany have been asked which anthelmintic was used in the past. Four reported the use of IVM, one that of BZ formulations and on five of the investigated farms no anthelmintics have been used for the first season grazing calves. The German farms investigated in this survey used IVM (30%), oral BZ formulations (10%) or no anthelmintics (60%) at all. Both Swedish farmers from farms where BZ was tested stated that they didn't use any anthelmintics in the previous year, however, the FECRT results obtained on these farms indicated resistance against IVM.

The results presented suggest that the full extent of the problem of AR in cattle nematodes is probably underestimated. Concerning worm control in cattle, the MLs in particular dominate the anthelmintic market in most European countries. This suggests, that a considerable number of products are used which do not remove the worm burden. Therefore it is of concern that on approximately one third of the investigated farms a reduced efficacy of IVM was found, with FECR of less than 70% in some animals.

Measuring AR with the FECRT, however, could lead to some misinterpretation of the current situation. Numerous factors can contribute to treatment-unrelated variability in FECR data, like over-dispersed distribution of worms in the host populations, non-uniform distribution of egg in faecal samples and inappropriate drug application procedures (Vidyashankar et al., 2007). Here, dosing and application have been performed by the investigators and are therefore not likely to be a reason for the failure of the drug. In regards to IVM, the reference product IVOMECS<sup>®</sup> (Merial) was used and for the BZs, only original drugs from leading manufacturers were used. Furthermore, the FECRT only measures effects on egg production of mature worms, and the output of eggs does not sufficiently correlate with the actual worm burden (Eysker and Ploeger, 2000). Furthermore the interpretation of FEC on cattle is difficult due to mixed populations of adult and immature worms of different species and varying egg production over time (Jackson et al., 2006). Certain characteristics of the FEC method used, namely the sensitivity and accuracy, may also have a potential effect on the outcome of FECRT. Here

we did not find a significant difference between the two methods applied in this investigation. In addition, the impact of density dependent egg output on the use of the FECRT for detecting AR resistance has recently been assessed in *Ancylostoma* (Kotze A.C. and Kopp S.R., CSIRO Livestock Industries, Australia, personal communication). They concluded that at least some adult females that survive a drug treatment will most likely increase their egg output significantly compared to their pre-treatment levels, resulting in an underestimation of the actual drug efficacy when the FECRT is used. In cattle parasites the misinterpretation of faecal egg counts due to the density dependent egg output was mentioned by Michel (Michel, 1969) and Brunsdon (Brunsdon, 1971). Therefore it would be useful to take these considerations into account when evaluating the efficacy of a drug. In order to prove the resistance of a field population, controlled efficacy tests would be necessary. An experimental infection of calves with an isolated field IVM-resistant *Cooperia* population, including a control group could confirm the suspected results and egg and worm counts could probably also be used to investigate the potential effects on density depending egg release in bovine nematode species. But these kind of experiments are time consuming and highly costly. Within the framework of this study, such investigations were not possible.

Additional tests such as the egg hatch test, larval development test or the larval migration inhibition test for the reliable detection of AR in field populations are available but rarely used in the field at present. For the egg hatch test, discriminating doses have been established for thiabendazole, so that this test can be used to evaluate the resistance status of field populations to BZs. But at least for the MLs the two *in vitro* methods, the larval development test and the larval migration inhibition test still need to be proved to correlate with the FECRT. The use of other *in vitro* methods for the detection of ML resistance in field populations is currently under investigation within an EU framework project (unpubl. data).

Further studies are required to determine the extent of AR in European cattle farms. The present findings highlight that it is important that farmers are encouraged to determine if the anthelmintic group used on their property is still fully effective. In 2008 within the ongoing PARASOL-project a larger evaluation of the efficacy of anthelmintics on a larger number of farms is planned for 2008 in Germany, Belgium, Sweden and the UK.

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