EBLEX FINAL PROJECT REPORT

Project: **Investigating Wormer Failures in Cattle and possible Anthelmintic Resistance**

Duration and Dates: 6 months – 30th June 2009 to 28th February 2010

Aims and Objectives

This main aim of the project was to investigate reports of suspect loss of efficacy (SLOE) reports for wormers using conventional procedures and existing supportive parasitological diagnostic techniques. During the investigations, studies were also conducted to determine the possible presence of Anthelmintic Resistance (AR) in cattle nematodes on UK farms.

As an additional adjunct to the study, a technical manual aimed at veterinarians, advisors has been produced and input provided into the production of advisory leaflets on Beef Parasite Control and Liver Fluke Control.

Background

There are a number of parasitic helminths that may affect cattle health and welfare. Infection with gastrointestinal roundworms may cause weight loss and diarrhoea in calves and loss of production in older cattle. Anthelmintics are widely used both in the treatment and prevention of worm infections of cattle. On the basis of their chemical structure and mode of action, they can be divided into three main broad-spectrum classes, available for use in cattle - Group 1 (Benzimadazole- 1-BZ), Group 2 (Imidazothiazoles - 2-LV), Group 3 (macrocyclic lactones – 3-ML), A fourth group of drugs, the flukicides, include the salicylanilides and substituted phenols, and the sulphonamide, chlorsulon.

All of the anthelmintics available for use in cattle are highly effective against adult and developing larval stages of the common gastrointestinal nematodes but efficacy may vary against arrested larvae present over the autumn and winter months and varies with worm species. There are, therefore, 'dose-limiting species' (e.g, *Cooperia oncophora* is the dose-limiting species for some ML compounds). Individual product activity and persistence against re-infection varies with the active compound, and also with the formulation and method of application. For cattle, there exist a range of methods of applications including pour-ons, injectables and boluses, as well as conventional oral drenches. Given this wide

range of treatments and applications there is always the potential for incorrect application resulting in control failure, which may incorrectly be perceived as AR.

Although widely reported in sheep, AR appears less of a problem in cattle. This may be a reflection of the relative frequency of treatment and also differences in parasite population dynamics between the two hosts. It may also reflect the prolonged survivability of free-living larval stages within the bovine faecal pat, thus ensuring a supply of susceptible worms.

BZ-resistance has been described in *Cooperia* spp., *Haemonchus* spp., *Ostertagia ostertagi* and *Trichostrongylus axei* in Australia, New Zealand, USA, South Africa and parts of Europe. Reports of ML-resistance in cattle nematodes have been less common, but have been described in mainly *Cooperia oncophora*, but also *Haemonchus* spp., *Trichostrongylus longispicularis*, and more recently in *O. ostertagi*, in the USA. There have been only a few reports of multiple anthelmintics resistant cattle nematodes in USA, New Zealand and South America.

Most reports of ML resistance in cattle have been reported in *Cooperia* species following the identification of positive FEC or FECRT after use of pour-on treatments. Poor absorption of pour-on ML anthelmintics and subsequent reduced efficacy against *Cooperia* species, (which are the dose-limiting species for the ML group (Vercruysse and Rew 2002) provides a more likely explanation for positive post-treatment FEC than acquired resistance (McKenna 1995). However, in the longer term, shedding of *Cooperia* spp. eggs during the prepatent period following treatment with topical ML anthelmintics has been shown experimentally to select for AR (Van Zeveren and others 2007) and may lead to increasing AR reports in these species.

Reports of AR in cattle nematodes in the UK are still rare. There has only been one published report of ML resistance in *Cooperia oncophora* in the SW England (Stafford and Coles 1999) plus one report of inefficacy with an ML pour-on in Highland cattle in Scotland (Sargison and others 2009). To what extent these, and other anecdotal reports, are attributable to true AR rather than treatment failure is not always clear. The presence of AR nematodes therefore needs to be clearly differentiated from treatment failures, which may occur for a variety of reasons.

Methods

Farm Sampling Protocols

Steps were taken to establish a means of investigating farms with suspected anthelmintic treatment failures, or increased problems with PGE in cattle. Initial contact was made with the veterinary pharmaceutical industry through the National Office of Animal Health (NOAH) Antiparasitics Working Group (AWG). It was agreed that the major companies, would participate in this project and that they would refer suspected treatment failure investigations, reported under the suspected adverse reports scheme (SARS), through FERA. Contact was also made with the British Cattle Veterinary Association (BCVA) informing them of the project and the availability of the laboratory support facilities for investigating potential SARS.

To ensure consistency in the approaches taken for investigating treatment failures and potential AR problems, guidelines and sampling protocols specific to UK farming practices were reviewed and guidelines for collection and submission of appropriate data and samples were produced (Appendix I).

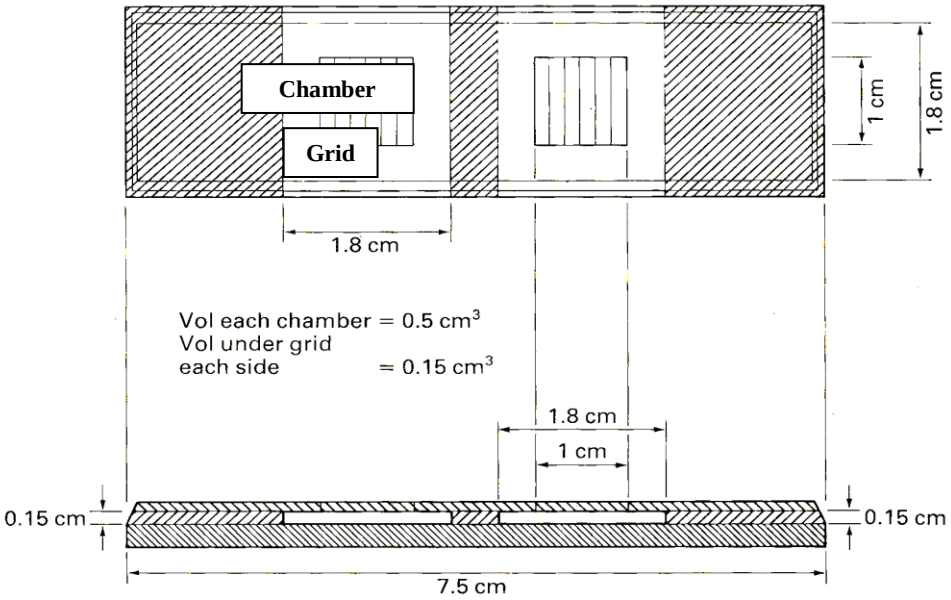
It was decided that for on farm investigations, the submitting pharmaceutical company veterinarian would, where possible, submit faecal samples initially for a routine faecal egg count (FEC). Thereafter, if a positive FEC was present a Wormer Test (WT) would be used to determine both treatment efficacy, and the possible presence of anthelmintic resistance.

Using the preliminary results generated from the study, as well as previously published data and expert knowledge, guidelines for worm control in cattle aimed at limiting the development of AR were to be produced, as part of a separate initiative.

Faecal Egg Counts (FEC)

Counts were performed on individual or bulked faecal samples using a modified McMaster Method (MAFF 1986) and performed in accordance with the FERA standard operating procedure (SOP) WEMH_PT_018. By screening 4.5 grams of either individual or bulked faeces, and examining the total volume of solution in both McMaster chambers (rather than just the grids – see Figure 1) the sensitivity of the test was increased from <50epg to <10epg.

Figure 1. McMaster Counting Chamber (counting both grid areas provides a sensitivity of <50 epg; for both chambers a sensitivity of <10epg)



Larval Culture

Third stage larvae were obtained by culture in dishes at 27°C for 7-10 days, cleaned and recovered using a Baermann apparatus, and examined microscopically by killing with a few drops of Lugol's iodine (MAFF 1986). Larval identification was based on morphological features of the 3rd stage infective larvae (Taylor et al. 2007). Both procedures were performed in accordance with the FERA standard operating procedure (SOP) WEMH_PT_006.

Wormer Tests (WT)

"Wormer Tests" provide a quick indication of anthelmintics efficacy based on laboratory testing of faecal samples taken from 10 cattle post treatment. The time of post treatment sampling depends on the anthelmintic used: **7 days after LV, 10-14 after BZ and 14-16 days after an ML treatment.** In practice, this means sampling after 7 days for LV, or 14 days post treatment, for BZ and ML products. The test is merely an indicator of anthelmintic inefficacy and not necessarily anthelmintic resistance per se, as many other factors can influence test results. The utility of this test is improved if faecal samples from 10 cattle in the treated group are collected and submitted on the day of dosing to provide a rough estimate of the reduction in FEC achieved. FEC were conducted on pre- and post-treatment samples as described for routine FEC.

A survey of parasite control methods on beef farms in SW England, showed that topical treatment (predominantly using ML pour-ons) was the most common method of anthelmintic administration (Barton and others 2006). The other main route of application of MLs is by injection. All veterinarians submitting samples for WT were therefore asked to provide information on methods of application, and whether calibration of the delivery device occurred. An additional requirement was that information should be provided as to whether treated animals were weighed on the sample submission form (Appendix II).

Results

A total of 19 farms participated in the study. More than one submission was submitted from 10 of the farms, either for repeat testing of FEC or to check for efficacy of treatment by WT. Despite clear and written instructions, some submitting vets sent in either < 10 samples or in some cases, insufficient faecal material from individual animals. Fifteen of the routine FEC submissions were <100 epg indicating low levels of infection. The other 7 submissions for routine screening had FECs ranging between 100 and 480epg. The latter sample was from an individual animal only and is not a representative group mean. Results are presented in Table 1.

On three of the farms with epg > 100epg, a WT was subsequently conducted. On two other farms, WTs were conducted where the FEC was >100 epg on the date of treatment. On three farms post-treatment tests were conducted where there were no samples submitted for pre-treatment FEC so only post-treatment FEC results were obtained.

Of the six WTs conducted, only two farm had an efficacy of <95% post-treatment. On these farms, the animals were weighed, and then treated by pour-on using a calibrated applicator. The treatments reduced the FEC by 91% on one of the farms, and by only 17% (from 118 to 98 epg) on the other with 100% of the surviving eggs identified as *Cooperia* spp. on larval culture. On the 4 other farms, treatments were 96% - 100% effective (Table 1).

Only 5 of the 14 farms who treated, indicated that they routinely weighed animals prior to treatment, and 9 farms calibrated either their syringe or pour-on applicator. All farms in the study routinely used ML products either by injection (4) or pour-on (9) (Table 1).

Table 1. Farm Results of FEC (given as eggs per gram of faeces), Larval Differentiation, WT and indication of equipment calibration and weighing of cattle at time of treatment

Farm	Sample Type	EPG	Larval Diff Results					WT % Redn	Route	Prior Treatment	Weighed	Equip Calib'n
			% Oster	% Trich	% Coop	% Haem	% Others					
1	Routine	15	NC	NC	NC	NC	NC					
	Routine	0	NC	NC	NC	NC	NC					
	Routine	0	NC	NC	NC	NC	NC					
2	Routine	120	42	9	49	0	0					
	Routine	170	57	1	42	0	0					
3	Routine	3	NC	NC	NC	NC	NC					
	Routine	100	100	0	0	0	0					
	Routine	231	78	0	22	0	0					
	Routine	1	NC	NC	NC	NC	NC					
4	Routine	67	NC	NC	NC	NC	NC					
	Routine	60	NC	NC	NC	NC	NC					
	Routine	30	NC	NC	NC	NC	NC					
5	Routine	35	NC	NC	NC	NC	NC					
7	Routine	60	NC	NC	NC	NC	NC					
8	Routine	90	NC	NC	NC	NC	NC		Pour on	5 wks	No	No
11	Routine	25	NC	NC	NC	NC	NC					
13	Routine	480	2	94	4	0	0		Pour-on	2 days*	No	No
	Routine	10	NC	NC	NC	NC	NC			4 days*		
6	Routine	100	NC	NC	NC	NC	NC					
	Routine	120	NC	NC	NC	NC	NC					
	WT 0	118	NC	NC	NC	NC	NC		Pour on		Yes	Yes
	WT 14	98	0	0	100	0	0	17				
9	Routine	60	NC	NC	NC	NC	NC		Injection	~10wks	No	No
	WT 0	54	NC	NC	NC	NC	NC	NC	Injection		No	No
	WT 14	2	NC	NC	NC	NC	NC	96.3				
	WT 28	45	NC	NC	NC	NC	NC					
	WT 42	150	NC	NC	NC	NC	NC					
10	Routine	40	NC	NC	NC	NC	NC					
	WT 0	50	100	0	0	0	0		Injection		No	Yes
	WT 14	0	NC	NC	NC	NC	NC	100				
	WT 0	25	18	0	82	0	0		Pour on		No	Yes
	WT 14	0	NC	NC	NC	NC	NC	100				
12	WT 0	506	16	0	84	0	0		Injection		Yes	Yes
	WT 14	2	NC	NC	NC	NC	NC	99.6				
	WT 28	5	0	0	100	0	0					
	WT 42	20	5	0	95	0	0					
	WT 58	113	94	0	6	0	0					
14	WT 0	110	88	0	12	0	0		Pour on		Yes	Yes
	WT 14	10	0	0	100	0	0	91				
15	WT 0	90	32	0	68	0	0		Pour on		No	Yes
16	WT 14	50	40	24	20	16	0		?		Yes	?
17	WT 14	5	NC	NC	NC	NC	NC		Pour on		No	Yes
18	WT 14	27	63	0	31	0	6		Pour on		No	Yes
19	WT 14	10	58	2	30	10	0		Pour on		Yes	Yes

Discussion

Whilst the number of farm submissions was lower than anticipated, it should be remembered that this was a reactive study based on reports of treatment failures (SARS), or reported increased problems with PGE in cattle. The low number of submissions therefore suggests that there were few perceived PGE and treatment problems and this was further indicated by the low FEC (<100epg) from 15 of the 19 farms submitting samples. Of the other 7 submissions with FEC >100epg, larval cultures were performed, where possible, to determine genera present. On 8 farms, *Ostertagia* was the dominant genus in coprocultures, whilst *Cooperia* spp. was the dominant genus in 7 coprocultures. Both genera were both present in 13 coprocultures. *Trichostrongylus* spp was the dominant genus in the one animal with an the 480epg on farm 13.

WTs were performed on 5 farms (with 2xWT on Farm 10) and showed >96% efficacy apart from Farm 6 where the reduction in FEC was only 17% (from mean 118 epg to mean 98 epg) and farm 14 where the reduction in FEC was 91% (from mean 110 epg to mean 10 epg). Larval culture on post-treatment faecal samples on both these farms indicated 100% *Cooperia* spp (the dose limiting species). On both farms, treated cattle were weighed and calibration of the pour-on gun was checked. Farm 6 was the only farms where the presence of AR could be suspected. A full FECRT was not possible because the mean epg, remained below the threshold level of 200 epg, as recommended under WAAVP guidelines (Coles et al. 2006).

On all farms except one (Farm 12), the levels of infection, both pre- and post-treatment, were low and if pre-screening had been performed would not have met the inclusion criteria. It should also be mentioned that because of the sensitivity of the FEC method some of FEC results were extremely low (<10epg) and would have been reported as negative (or <50epg) if conducted by a standard McMaster Method at a routine diagnostic laboratory, such as a VLA regional laboratory. As a consequence, the 91% efficacy reported for farm 14, would be 100% be conventional screening methods. This clearly demonstrates how differences in sensitivity of FEC methods can lead to variability in interpretation of treatment efficacy and as a consequence, suspect AR. Where an FECRT can be conducted, under the WAAVP guidelines, similar variation in interpretation can be anticipated depending on the level of sensitivity of the FECs performed. This is clearly an area requiring further research.

Conclusions and Recommendations

This small scale study, combined with evidence of reports to the VMD under SARS, suggest that treatment failures or SLOEs for wormers in cattle, are uncommon. Where they do occur this is often the result of failure to administer the product correctly. The most likely reason is under dosing through under estimation of bodyweight. Only around a third of the farms involved in this study indicated they weighed animal prior to treatment.

An ML product was involved in all reports of SLOEs in the study and these products now dominate the UK market. Pour-on products offer ease of use but also a greater risk of treatment failure. Failure to apply correctly and under the correct conditions, as described in the product literature, is the next likely reason for treatment failure. There appears to be greater efficacy of the same product is given by injection.

Recommendation 1: It is important to promote good worming practice on farms ensuring accurate dosing and correct use of products, particularly for those used as pour-ons.

Where treatment failures do occur, then *Cooperia* spp, as the dose-limiting species for ML products, will predominate in post-treatment FEC. Where WT or FECRT are conducted, it is therefore important to identify the genus/species present in post-treatment FEC. In this respect there needs to be consistency in the application and interpretation of FECs when used in determining efficacy or possible presence of AR. Recommended WAAVP guidelines for determining AR in cattle using the FECRT are not straightforward or applicable to many UK herds.

Recommendation 2: There is a need for UK guidelines and training for veterinarians and advisors for investigating reported treatment failures and suspect AR

Recommendation 3: Further research is required on the standardisation of current laboratory based worm egg counting methods, and methods aimed at determining resistance status. Appropriate statistical and variability methods of analyses should be developed and standardised.

Recommendation 4: The development of novel methods of determining and quantifying resistance genes is a longer term priority.

Recommendation 5: Ongoing monitoring of wormer treatment failures should be continued to provide more accurate assessments of SLOEs in AR in cattle worms. This should also be extended to include other cattle parasites such as liver fluke.

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Appendix I

Faecal Sample Submissions – Cattle Worm Counts and SLOEs (Suspect Loss of Efficacy)

Where a suspected loss of efficacy (SLOE) or treatment failure with an anthelmintic in cattle is suspected.

Standard Worm Faecal Egg Count – Samples Required

1. Take 10 random faecal samples of approximately 10g each (fill each pot) from the batch of animals and submit to the lab using the kit and forms provided **

2. A bulked faecal egg count (FEC) will be performed at the laboratory. If negative no further action required.

3. Where there is a positive count (usually >200epg) and a suspicion of SLOE then a Wormer Test can be considered.

Wormer Test

- This is designed to check anthelmintic efficacy under optimal conditions.
- Animals should be weighed prior to treatment, the volume of dose accurately calculated, measured and administered correctly.
- For pour-on treatments observe any contra-indication claims on the label.
- If the SLOE is reported as a Suspect Adverse Reaction (SAR) it is likely that the manufacturer may be involved.

1. At the time of treatment record all details requested on the form and repeat steps 1 and 2 above.

2. For treatments with macrocyclic lactone (ML) and benzimidazole (BZ) products, repeat steps 1 and 2 at two weeks (14 days) post treatment

3. For treatments with levamisole (LV) products, repeat steps 1 and 2 at one week (7 days) post treatment

For technical support contact Professor Mike Taylor (01904 462679; email mike.taylor@fera.gsi.gov.uk) or Dr Irene Zimmer (01904 462492; email: irene.zimmer@fera.gsi.gov.uk)

** Sample kits can be obtained from:

Dr Barbara Craig or Colin Morgan
Room 14FA03
Food and Environment Research Agency
Sand Hutton
York
YO41 1LZ
Tel: 01904 462583 or 01904 462689
Email: barbara.craig@fera.gsi.gov.uk or colin.morgan@fera.gsi.gov.uk

Appendix II. Faecal Sample Submission Form

Vet/Company Details	**Farm Details**

Date Sampled: _____

Number of Faecal Samples Submitted:
Sheep [Ewes/lambs] ____
Cattle [Adult/Calves]____

Test Requested: Routine FEC ☐ [Bulked/Individual]
Pre-Treatment Test [Bulked ☐ dividual]
Post-Treatment Test [Bulked ☐ dividual]
Larval Differentiation ☐

Date last wormed? _____

Product: _____

Please provide details on:
Animals: Weighed/Not weighed
Treatment Application: Drench/Pour on/Injection/Other
Equipment: Calibrated/Not Calibrated

Additional Information:

Sample ID	Lab ID	Trichostrongyle Count	Others Count	Comments

Laboratory results

Larval Differentiation: [Not Required/To follow/Completed]

Species	%	Comments

FERA Use Only

Date: Received:	

FEC **Pre-Treat [Bulked/Individual]** **Post-Treat Number_____**

Larval Differentiation: Y/N

Invoice Y/N **Date: _____** **Cost: £_____**