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## **Multiple anthelmintic resistance in *Haemonchus contortus* isolated from South African Boer goats in Switzerland**

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1 **Multiple anthelmintic resistance in a field strain of *Haemonchus contortus* in South**  
2 **African Boer goats in Switzerland.**

3

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12

1 **Abstract**

2 Anthelmintic resistance was suspected in a flock in the canton of Zurich, Switzerland, into  
3 which South African Boer goats were previously imported. A strain of *Haemonchus*  
4 *contortus* was isolated from this herd for further investigations. Twenty sheep were  
5 allocated into one control group and three treatment groups to determine the efficacy of  
6 mebendazole, ivermectin and moxidectin using the faecal egg count reduction test  
7 (FECRT) and a controlled slaughter trial. The faecal egg count reduction percentage  
8 (FECR%) and worm burden reduction were compared using four different mathematical  
9 methods. For both tests this included the use of a maximum likelihood model where the  
10 variance of the egg counts or worm burdens were modelled using the negative binomial  
11 distribution. There was agreement in declaring high resistance levels of the anthelmintics  
12 mebendazole and ivermectin among all methods. This is the first description of resistance  
13 of gastrointestinal nematodes to one of the macrocyclic lactones in small ruminants in  
14 Switzerland. Resistance to moxidectin was suggested by 3 of the 4 mathematical  
15 techniques used for the worm burden reduction. However, with the FECRT only the  
16 negative binomial model suggested the presence of resistance against moxidectin. The  
17 use of the negative binomial distribution in statistical models appears to be more sensitive  
18 in detecting anthelmintic resistance by the FECRT, results that were confirmed by  
19 postmortem examination. The results are discussed in relation to the importance of  
20 detecting low levels of anthelmintic resistance in gastrointestinal nematodes of small  
21 ruminants in order to preserve the efficacy of important anthelmintic therapeutics.

22

23 *Index keywords:* Small ruminants; Macrocyclic lactone and benzimidazole resistance,  
24 Maximum likelihood model.

25

26

## 1 **1. Introduction**

2 The occurrence of anthelmintic resistance (AR) in nematodes of small ruminants has  
3 become a problem of major concern throughout the world (Conder and Campbell, 1995). It  
4 has been demonstrated that under-dosing of anthelmintics may be an important  
5 contributory factor for the development of AR (Conder and Campbell, 1995); (Escudero et  
6 al., 1999). In addition, goats show a different metabolic and pharmacokinetic profile  
7 compared to sheep (Conder and Campbell, 1995) (Escudero et al., 1999) with lower  
8 bioavailability in goats especially after oral administration (Escudero et al., 1999).  
9 Consequently macrocyclic lactone resistance appears to emerge rapidly in this species  
10 and can then spread readily to sheep (Escudero et al., 1999). Therefore particular  
11 attention to AR in goats must be given.

12 Resistance to one or more of the broad spectrum anthelmintics including macrocyclic  
13 lactones has been reported for goat nematodes on numerous occasions from every  
14 continent (Waller, 1997a); (Gopal et al., 1999); (van Wyk et al., 1999); (Hertzberg and  
15 Bauer, 2000); (Zajac Anne and Gipson Terry, 2000); (Terrill Thomas et al., 2001); (Veale,  
16 2002); (Chandrawathani et al., 2003). The reports of AR in Europe mainly concern  
17 resistance against benzimidazoles (BZ), with local reports of levamisole resistance and just  
18 a few cases of ivermectin-resistance (Hertzberg and Bauer, 2000) from Denmark and the  
19 United Kingdom. Imported resistance has been reported twice in Europe. (Himonas and  
20 Papadopoulos, 1994) described a case of BZ resistance in sheep nematodes imported  
21 from the United Kingdom and France into Greece and (Varady et al., 1993) reported a  
22 case of multiple resistant trichostrongyle population in Slovakia imported from New  
23 Zealand with angora goats. This study describes the first case of an ivermectin-resistant  
24 *Haemonchus contortus*-strain in Switzerland, isolated from a flock into which South African  
25 Boer goats had been previously imported, and assesses multiple AR against  
26 mebendazole, ivermectin and moxidectin of the same strain.

1           The faecal egg count reduction test (FECRT) is still the most commonly used  
2 technique for the detection of AR, but different methods to calculate and interpret the  
3 results of the faecal egg count reduction percentage (FEGR%) have been used and these  
4 can influence the decision taken on the continued use of an anthelmintic on a farm (Maingi  
5 et al., 1996). The FECRT still needs to be refined or alternative and more specific methods  
6 of detecting AR established. The use of parametric statistical techniques to analyse data  
7 from parasite infections is inappropriate, because such data rarely, if ever, follow a normal  
8 distribution (Barger, 1985), even in experimental infections. In order to overcome this  
9 problem, log transformation of the data before analysis has been recommended. However,  
10 for a direct comparison between different laboratories the same method of transforming  
11 the data has to be applied (Dash et al., 1988). The currently used method is to apply the  
12 transformation  $y = \log(\text{egg count} + 1)$ . Nevertheless, even this transformation often fails to  
13 normalise the distribution, especially when it is highly skewed (Wilson and Grenfell, 1997).  
14 In addition, type 1 errors are likely to be common and type 2 errors increased when using  
15 a log-transformed method (Wilson et al., 1996).

16           The World Association for the Advancement of Veterinary Parasitology (WAAVP)  
17 advises the controlled slaughter test as the most reliable method for evaluating  
18 anthelmintic activity in ruminants (Wood et al., 1995). This paper investigates the use of a  
19 maximum likelihood model, based on the negative binomial distribution, to calculate  
20 FEGR% and worm burden reduction in a controlled slaughter trial and compares this with  
21 other recommended methods. Parasite burdens in hosts and their associated faecal egg  
22 counts are nearly always highly aggregated, and the negative binomial distribution is the  
23 most frequently used distribution to model this aggregation. However, with such an  
24 aggregated distribution, there is always a higher probability that the sample mean will  
25 underestimate the true population mean then over estimate it (Pacala and Dobson, 1988)  
26 and this probability increases the greater the aggregation within the host population. By

1 explicitly modelling the worm burdens and faecal egg counts as a negative binomial  
2 distribution, the results of this study suggest that maximum likelihood techniques are more  
3 sensitive in detection of possible resistance. Furthermore, they can indicate the presence  
4 of resistant parasites using FECRT that would otherwise have only been confirmed in a  
5 slaughter trial.

6

## 7 **2. Materials and methods**

### 8 *2.1. Parasite strain*

9 An isolate was obtained from a flock into which South African Boer goats were previously  
10 imported. The goats, living on a farm in the canton of Zurich, Switzerland, had clinical  
11 evidence of anthelmintic resistance. Faecal samples were obtained from these animals,  
12 cultured and infective larvae obtained. A previously helminth-free sheep was infected with  
13 these larvae and faeces collected when the infection became patent. These faeces were  
14 cultured for the differentiation of third stage larvae (L3) and to obtain infective larvae for  
15 subsequent experimental infection.

16

### 17 *2.2. Experimental design*

18 Twenty 5 to 6-month-old white alpine mountain sheep of mixed sex with a mean body  
19 weight (BW) of 32.6 kg were reared under conditions that minimized helminth infections.  
20 Before experimental infection, animals were treated with pyrantel tartrate (25 mg kg<sup>-1</sup> BW)  
21 to eliminate any possible infections with gastrointestinal nematodes. Each sheep was  
22 subsequently infected orally with 15000 freshly harvested infective *H. contortus* L3 of the  
23 isolate obtained from the goats described above. At twenty-two days post infection, faecal  
24 egg counts confirmed the presence of a patent infection (Table 1) using a modified  
25 McMaster technique according to Schmidt (1971). The lower detection limit in this method  
26 is 50 EPG. Animals were randomly allocated into one control group (CON) and three

1 treatment groups, with 5 animals in each group, held in separate pens. At this time,  
2 mebendazole (MEB, Ovitelmin®, Janssen Pharmaceuticals, 20 mg kg<sup>-1</sup> BW administered  
3 orally), ivermectin (IVM, Ivomec®, Merial, 0.2 mg kg<sup>-1</sup> BW administered subcutaneously)  
4 and moxidectin (MOX, Cydectin®, Wyeth-AHP Switzerland, 0.2 mg kg<sup>-1</sup> BW administered  
5 orally) in dosages according to the manufacturer's recommendation and based on  
6 individual BW were administered to each of the three treatment groups (day 0). FECs were  
7 repeated on all sheep on day 4 and on the day of slaughtering (day 6). The worm burden  
8 of each sheep was determined after slaughter by separate collection of small intestines  
9 and abomasa. Aliquots of 1/10 were taken for adult worm counts and identification.

10

### 11 *2.3. Parasitological investigations*

12 An egg hatch assay (EHA) was performed with nematode eggs isolated from pooled faecal  
13 samples from the goats. Eggs with an LD50 value in excess of 0.1 µg thiabendazole  
14 (TBZ) ml<sup>-1</sup> are indicative of BZ resistance in accordance with the method described in the  
15 WAAVP guidelines for detection of AR (Coles et al., 1992).

16

### 17 *2.4. Data analysis*

18 Data from the EHA were analysed to determine the LD50, i.e. concentrations of TBZ  
19 required to inhibit 50% of eggs from hatching. The percentage of eggs failing to hatch at  
20 each concentration of TBZ, after correction for natural mortality, were transformed to logits  
21 and used to generate dose response lines for the isolate using a logit program. From  
22 these, the LD50 values were calculated. Resistance is declared if the LD50 is above 0.1  
23 µg TBZ ml<sup>-1</sup> (Coles et al., 1992).

24 Data from the FECRT were entered on a spreadsheet (Microsoft Excel, Microsoft Corp.  
25 WA) and the FECR% and the 95% confidence intervals for the reduction were calculated

1 according by various methods. The WAAVP recommendations for detection of  
2 anthelmintic resistance (Coles et al., 1992) recommend that the FECR% is calculated as:

3

$$4 \quad \text{FECR}\% = 100 \times \left(1 - \frac{X_t}{X_c}\right) \quad (1)$$

5

6 where  $X_t$  and  $X_c$  are the arithmetic mean EPG in the treated ( $t$ ) and non-treated control ( $c$ )  
7 groups. Resistance is considered to be present if the FECR is less than 95% and the lower  
8 95% confidence limit for the reduction is less than 90%. If only one of these conditions are  
9 met, resistance is suspected.

10 (Presidente, 1985) and (Dash et al., 1988) recommend that the FECR% reduction is  
11 calculated as:

12

$$13 \quad \text{FECR}\% = 100 \times \left(1 - \frac{X_{t2}}{X_{t1}} \times \frac{X_{c1}}{X_{c2}}\right) \quad (2)$$

14

15 where ( $X_c$ ) and ( $X_t$ ) are the geometric mean (Presidente, 1985) or arithmetic mean (Dash  
16 et al., 1988) EPG for control ( $c$ ) and treated ( $t$ ) groups before ( $_1$ ) and after ( $_2$ ) treatments  
17 respectively. According to (Dash et al., 1988) resistance is declared if the FECR% is less  
18 than 80%, while (Presidente, 1985) stated that treatment with modern broad-spectrum  
19 anthelmintics should result in a FECR% of > 95%.

20 In the controlled slaughter test, according to the WAAVP recommendations (Wood et al.,  
21 1995), the percent efficacy (%E) of an anthelmintic treatment against a given parasite  
22 species in each treatment group is:

23

$$24 \quad \%E = \left(\frac{X_{cg} - X_{tg}}{X_{cg}}\right) \times 100, \quad (3)$$

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where  $X_{tg}$  is the geometric mean of the treatment group and  $X_{cg}$  the geometric mean of the control group. A compound should be declared effective only when effectiveness stands at 90% or above (using pooled data, when appropriate) and there is a statistically significant difference in parasite numbers between control and treated animals.

The percent efficacy (%E) was additionally calculated with the same formula (1) utilised by (Coles et al., 1992) for the FECR%, as suggested by (Hong et al., 1996) and (Watson et al., 1996), and with a method utilised by (Borgsteede et al., 1996):

$$\%E = 100 - \left( \frac{X_t \times 100}{X_c} \right), \tag{4}$$

using arithmetic means. A value below which resistance has to be considered is not given. For both, calculation of FECR% and %E, a maximum likelihood model was also adopted. Parasite abundance in infected animals usually follows an aggregated distribution in naturally infected animals (Wilson, 1996). In many instances, due, for examples, to variations in innate resistance, even artificially infected animals have a non-uniform distribution. Therefore the mean and variance of the parasite burden found at necropsy and the parasite faecal eggs counts from each group were compared. Evidence of aggregation, defined as the variance being greater than the mean, was confirmed in all groups. The frequency distributions of the parasite burdens and faecal egg counts were consequently modelled as a negative binomial distribution with the two parameters of arithmetic mean and negative binomial constant  $k$ . Thus the probability of the number of parasites  $s$  for each observation  $o_i$  given the arithmetic mean  $M$  and negative binomial constant  $k$  is given as:

1 
$$\Pr\{o_i = s\} = \frac{\Gamma(k + s)}{\Gamma(k)s!} \left(\frac{M}{k + M}\right)^s \left(\frac{k}{k + M}\right)^k \quad (5)$$

2

3 where  $\Gamma$  is the gamma function.

4 All the observations were fitted using this likelihood function and the total likelihood was:

5 
$$\prod_{i=1}^n \Pr\{o_i = s\}$$

6 A likelihood profile (Venzon and Moolgavkar, 1988), and hence probability density  
7 distributions of the arithmetic means given the data, were found with the negative binomial  
8 likelihood function by means of the likelihood profile function of the Excel™ (Microsoft  
9 Corp, Redmond WA) PopTools add on (CSIRO Australia). Confidence limits for reductions  
10 in the mean worm burden or faecal egg counts were calculated using Monte-Carlo  
11 methods. Using Crystal Ball software (Decisoneering Corp, Denver CO) random samples  
12 were drawn from the probability distribution of the arithmetic mean, generated by the  
13 likelihood profile, of the control and test groups. For each pair of random samples the  
14 efficacy was calculated using the formula of (Coles et al., 1992). This process was  
15 repeated 10000 times. The lower 2.5 percentile and upper 97.5 percentile of the results of  
16 these 10000 iterations are accurate estimates of the 95% confidence limits of the  
17 anthelmintic efficacy assuming a negative binomial model of parasite burden and faecal  
18 egg counts.

19

### 20 **3. Results**

21 The LD50 values obtained in the EHA from the strain isolated from the goat farm in the  
22 egg hatch test was 0.72  $\mu\text{g TBZ ml}^{-1}$ , demonstrating the presence of BZ resistant  
23 nematodes. Faecal cultures from pooled faecal samples of the South African Boer goats,

1 after culture and L3-infection of a single sheep, had strongylid larvae composed of a mean  
2 larval proportion of 86% *H. contortus*, 8% *Trichostrongylus* sp. and 6% *Ostertagia* sp.

3 The post-treatment faecal cultures obtained from the slaughtered sheep showed a  
4 strongylid larvae composition with almost exclusively *H. contortus*: 100% in the CON and  
5 MOX groups, 100% also in the MEB group with an additionally presence of *Strongyloides*  
6 sp., and 99% *H. contortus* and 1% *Ostertagia* spp. in the IVM group.

7 The results of the FEC and the controlled slaughter test of the control group are shown in  
8 Table 1. Worm burden comprehend only adult worms found in the abomasa. Small  
9 amounts of adult worms were also found in small intestines from sheep of the CON and  
10 the MEB groups. In one sheep from the CON group *T. colubriformis* was found, but all  
11 other isolated adult male worms were identified as *H. contortus*.

12 There was agreement in declaring resistance of *H. contortus* among all four methods of  
13 calculating FECR% and all four methods of calculating %E of the anthelmintics MEB  
14 (Table 2) and IVM (Table 3).

15 The FECR% obtained with MOX (Table 4) on the day of slaughtering showed different  
16 results. Susceptibility was declared according to the WAAVP (Coles et al., 1992) method  
17 (the FECR% is more than 95% and the lower confidence limit for the reduction is more  
18 than 90%) and the method proposed by (Dash et al., 1988) (FECR% more than 80%). The  
19 FECR% calculated according to the method of (Presidente, 1985) is located on the cut-off  
20 level for declaring resistance. Only the FECR% calculated with the use of the maximum  
21 likelihood model showed a suspected resistance as the lower confidence limit was less  
22 than 90%. The results of the controlled slaughter test show high efficacy of MOX  
23 compared to MEB and IVM, but the %E calculated with three methods (Table 4) and the  
24 lower confidence limit calculated with the WAAVP method (Coles et al. 1992) and the  
25 maximum likelihood models (58.1 and 58.5 respectively) indicated resistance to MOX.

26

#### 1 **4. Discussion**

2 The results of the FECRT carried out and interpreted by four different methods, the LD50  
3 values obtained in the EHA and the results of the worm burden reduction calculated by  
4 four different methods provided evidence of resistance to BZ of the strain isolated from the  
5 South African Boer goats. BZ resistance has already been described in Switzerland  
6 (Hertzberg et al., 2000), with a prevalence of 91% in goats (Meyer, 2001). Resistance to  
7 IVM has been highly suspected by the data from the FECRT (ranging from 39.8% to  
8 55.7% between the four different methods) and confirmed by a worm burden reduction  
9 result of 61%, with agreement between all four methods. The lower confidence limit  
10 calculated according to the WAAVP recommendations (Coles et al., 1992) is higher  
11 compared with the one calculated using the negative binomial model (43.4% and 38.8%  
12 respectively), but there is agreement in declaring resistance. In the case of MOX, there  
13 were disagreements in declaring resistance with the FECRT between the different  
14 methods. With the methods by (Coles et al., 1992) and (Dash et al., 1988) the *H.*  
15 *contortus*-strain isolated from the South African Boer goats is declared susceptible against  
16 MOX, with the (Presidente, 1985) method, obtaining a FECR% of 94.9%, the strain is  
17 suggested to be considered on a level of suspected resistance (McKenna, 1990). In fact, a  
18 lower confidence limit of 81.3% calculated with the maximum likelihood model confirms a  
19 suspect case of resistance. The use of a maximum likelihood model here takes into  
20 account the probability distribution of parasite data and calculates the confidence limits of  
21 the mean accordingly. (Wilson, 1996) demonstrated that the use of models where the error  
22 structure of the data is explicitly defined as a negative binomial distribution is much less  
23 likely to produce type 1 or type 2 statistical errors than other analytical techniques. This  
24 method demonstrates that suspected resistance can be detected with FECs which may  
25 have been missed using the method recommended by the WAAVP. Furthermore, the  
26 possibility of resistance against MOX was confirmed by post-mortem worm counts,

1 demonstrating the value of this analytical method. The sample mean has a higher  
2 probability of underestimating the true mean when parasite burdens are aggregated  
3 (Pacala and Dobson, 1988). Hence the true faecal count reduction has a high probability  
4 of being less the observed reduction, particularly when small sample sizes are used. The  
5 negative binomial model accounts for this and hence gives asymmetric confidence  
6 intervals. The wide confidence interval models the uncertainty inherent in the small sample  
7 sizes. These confidence intervals would narrow if the minimum groups size was increased  
8 to greater numbers than those recommended by the Guidelines for international  
9 harmonisation of anthelmintic efficacy (Vercruyisse et al., 2001).

10 The controlled slaughter test, according to the WAAVP recommendations (Wood et al.,  
11 1995), is the most reliable method for evaluating anthelmintic activity in ruminants, but due  
12 to its costs and complexity not applicable for routine diagnosis. In contrast to the controlled  
13 slaughter trial, FECRT uses few resources, is easily performed and is also applicable in  
14 the evaluation of the performance of any anthelmintic (Maingi et al., 1996). Fortunately  
15 there is good correlation in the relationship between the reduction in worm burden and  
16 FECRT following anthelmintic treatment (McKenna, 1990). However, reductions in faecal  
17 egg counts tend to be greater than the reductions in worm burden (McKenna, 1990). Using  
18 the maximum likelihood model, there was a greater sensitivity in the detection of  
19 resistance in this study using reductions in egg counts than the statistical methods  
20 recommended by the WAAVP (Coles et al., 1992)(Coles et al., 1992). The two criteria to  
21 declare resistance against MOX according to WAAVP (Coles et al., 1992)(Coles et al.,  
22 1992) are met: FECR% is less than 95% and the lower 95% confidence level is less than  
23 90%. The confirmation of suspected MOX-resistance is given by the results of the worm  
24 burden reduction. The method by Coles et al. (1992)(Coles et al., 1992) and the method  
25 using a maximum likelihood model gave very similar results in %E (90.7% both) and in  
26 confidence limits (58.1-98.0% and 58.5-96.7% respectively). Discrepancies in declaring

1 resistance can influence the decision taken on the continued use of an anthelmintic on a  
2 farm. (Maingi et al., 1996) compared four different methods to calculate FECR% and  
3 stated that use of any of the four methods is likely to influence the decision taken  
4 concerning the resistance status on a farm when the level of AR is low. In this study, all  
5 test and methods used to check the resistance status agreed in declaring a high level of  
6 BZ and IVM resistance, while there were discrepancies in declaring resistance for MOX.  
7 The only method that suggested MOX resistance by the FECRT, involving a control group  
8 and therefore consistent with the International Anthelmintic Efficacy Guidelines  
9 (Vercruysse et al., 2001), was by modelling the FECs using a negative binomial model.  
10 Some statistical packages can now be used in which the error variance can be expressed  
11 explicitly. However, in this paper the analysis was undertaken in Excel with the relevant  
12 formulae (eg equation 5) written into individual cells to give the likelihood of the data, given  
13 the model and the total likelihood maximised using the solver add on. With the increasing  
14 sophistication of computer hardware and software, such methods should be more widely  
15 used.

16 The presence of multiple AR including resistance against IVM has been described recently  
17 from different continents: from Malaysia (Chandrawathani et al., 2003) in sheep and goats,  
18 from the USA (Zajac Anne and Gipson Terry, 2000) and from Kenya in goats (Mwamachi  
19 et al., 1995).

20 Anthelmintic dosages for goats have been the source of much discussion because of the  
21 different metabolism of goats compared to sheep ((Escudero et al., 1999)). However, there  
22 is agreement that goats require higher doses of anthelmintics (Conder and Campbell,  
23 1995). Higher dosages for anthelmintics registered for goats should be mentioned in the  
24 package instructions.

25 There is even greater controversy about the use of MOX for goats. MOX provided a high  
26 degree of protection against reinfection with *H. contortus* and *T. circumcincta*, but no

1 useful effect against *T. colubriformis* (Torres-Acosta and Jacobs, 1999). Beyond it, is MOX  
2 not approved for use in goats (Baynes et al., 2000) and in New Zealand there is a  
3 contraindication statement on the MOX label for use in goats (Cobb and Murphy, 1995).  
4 Despite there being statement on the label that MOX is contraindicated in goats, farmers in  
5 New Zealand are reported to use MOX “off-label” in the face of IVM resistance (Watson et  
6 al., 1996). Leathwick (1995) reported an instance where MOX failed to control an IVM  
7 resistant strain of *Ostertagia* species in goats. Before use of MOX, where IVM resistance  
8 is suspected, efficacy needs to be established. Finally, Escudero et al. (1999)  
9 recommended pharmacokinetic data for goats to be produced to avoid extrapolating  
10 dosages from other species. Low doses can lead to ineffective therapeutic levels and  
11 therefore increase chances of development of resistant parasites. In this study, the  
12 resistant *H. contortus* strain obtained from South African Boer goats was transmitted to  
13 sheep . The results support the observations of Watson et al. (1996): an *Ostertagia* spp.  
14 strain with IVM-MOX side resistance isolated from Boer goats was transmitted to sheep.  
15 The conclusion was that where IVM resistance is present, the development of MOX  
16 resistance is expected to ensue.

17 Caution should be exercised when importing livestock from countries with widespread  
18 development of multiple AR (Waller, 1997b). Varady et al. (1993) and Himonas and  
19 Papadopoulos (1994) already reported from AR imported with transfer of life stock in  
20 Europe. Resistance to any component of the macrocyclic lactones has never been found  
21 before in Switzerland. This is the first description of a *H. contortus* strain resistant against  
22 exponents of the group of the macrocyclic lactones (IVM and MOX) in Switzerland. There  
23 is no history of excessive use of IVM in the investigated flock, nor of any use of MOX.  
24 Imported South African Boer goats are therefore the likely origin of the resistant parasites  
25 reported in this study. Varady et al. (1993) recommended the introduction of legislation to

1 control this problem. Likewise, Himonas and Papadopoulos (1994) suggested that animals  
2 originating from countries with AR should at least be investigated before export/import.

3

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6 contortus strain for their collaboration. We would also like to thank Prof. P. Deplazes of the  
7 University of Zurich for fruitful discussions and Dr. med. vet. M. Ebeid for his help in  
8 sample collection.

9

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1 **Table 1**

2 Faecal egg counts (FEC) and post mortem worm burdens recorded in the control group  
3 infected with a suspected anthelmintic resistant strain of *H. contortus*. FECs were recorded  
4 22 days post infection (day 0) and 6 days later (day 6) on the day of slaughtering.

Control group (n=5)	FEC		worm burden
	day 0	day 6	day 6
Arithmetic mean	1400	4600	3632
Variance	437500	3921250	529170
95% negative binomial CL	931-2245	3030-7470	2995-4461
Geometric mean	1282	4194	3570
95% CL of geometric mean	852-1930	2691-6535	2964-4299

5 CL = confidence limits.

6

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8

1 **Table 2**

2 Faecal egg counts (FEC) and post mortem worm burdens recorded in the mebendazole  
 3 treated group infected with a suspected anthelmintic resistant strain of *H. contortus*. FECs  
 4 were recorded 22 days post infection (day 0) and 6 days later (day 6) on the day of  
 5 slaughtering. Percent faecal egg count reduction (FECR%) and percent worm burden  
 6 reduction efficacy (%E) were calculated according to four methods on the 5 sheep.

7

Mebendazole group (n=5)	FEC		worm burden
	day 0	day 6	day 6
Arithmetic mean	1022	1800	1642
Variance	2256670	2393750	224020
95% negative binomial CL	412-3749	973-3901	1286-2142
Geometric mean	535	1428	1596
95% CL of the geometric mean	196-1456	762-2674	1273-2000
FECR% and %E (Coles et al., 1992)		60.9	54.8
95% CL (Coles et al., 1992)		-13.6-86.5	33.3-69.4
FECR% (Dash et al., 1988)		46.4	
FECR% (Presidente, 1985)		18.4	
%E (Wood et al., 1995)			55.3
%E (Borgsteede et al., 1996)			54.8
FECR% and %E (maximum likelihood)		60.8	54.7
95% CL (maximum likelihood)		8.9-81.8	29.8-70.2

8 CL = confidence limits.

9

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11

1 **Table 3**

2  
 3 Faecal egg counts (FEC) and post mortem worm burdens recorded in the ivermectin  
 4 treated group infected with a suspected anthelmintic resistant strain of *H. contortus*. FECs  
 5 were recorded 22 days post infection (day 0) and 6 days later (day 6) on the day of  
 6 slaughtering. Percent faecal egg count reduction (FECR%) and percent worm burden  
 7 reduction efficacy (%E) were calculated according to four methods on the 5 sheep.

8

Ivermectin group (n=5)	FEC		worm burden
	day 0	day 6	day 6
Arithmetic mean	1700	2770	1430
Variance	1081250	4959500	136550
95% negative binomial CL	1083-2886	1506-5947	1133-1840
Geometric mean	1523	2209	1394
95% CL of geometric mean	999-2322	1166-4186	1118-1737
FECR% and %E (Coles et al., 1992)		39.8	60.6
95% CL (Coles et al., 1992)		65.6-78.1	43.4-72.6
FECR% (Dash et al., 1988)		50.4	
FECR% (Presidente, 1985)		55.7	
%E (Wood et al., 1995)			61.0
%E (Borgsteede et al., 1996)			60.6
FECR% and %E (maximum likelihood)		39.8	60.5
95% CL (maximum likelihood)		-36.1-71.1	38.8-73.9

9 CL: confidence limits.

10

11

1 **Table 4**

2 Faecal egg counts (FEC) and post mortem worm burdens recorded in the moxidectin  
 3 treated group infected with a suspected anthelmintic resistant strain of *H. contortus*. FECs  
 4 were recorded 22 days post infection (day 0) and 6 days later (day 6) on the day of  
 5 slaughtering. Percent faecal egg count reduction (FECR%) and percent worm burden  
 6 reduction efficacy (%E) were calculated according to four methods on the 5 sheep.

7

Moxidectin group (n=5)	FEC		worm burden
	day 0	day 6	day 6
Arithmetic mean	360	110	336
Variance	154250	11750	204730
95% negative binomial CL	171-960	31-898	123-1486
Geometric mean	250	42	147
95% CL of geometric mean	114-548	6-294	38-573
FECR% and %E (Coles et al., 1992)		97.6	90.7
95% CL <sup>c</sup> (Coles et al., 1992)		92.1-99.3	58.1-98.0
FECR% (Dash et al., 1988)		90.7	
FECR% (Presidente, 1985)		94.9	
%E (Wood et al., 1995)			95.9
%E (Borgsteede et al., 1996)			90.7
FECR% and %E (maximum likelihood)		97.6	90.7
95% CL <sup>c</sup> (maximum likelihood)		81.3-99.3	58.5-96.7

8 CL= confidence limits.

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