

Prevalence of anthelmintic resistance in gastrointestinal nematodes of dairy goats under extensive management conditions in southwestern France

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Abstract

The occurrence of benzimidazole (BZ) and levamisole resistance was investigated in 18 randomly selected dairy goat herds located in southwestern France and characterized by extensive management. On each of the 18 farms, 45 adult goats were randomly allocated into three groups of 15 animals each: an untreated control group, a group that was orally administered fenbendazole (10 mg kg⁻¹ body weight) and a group that received orally a levamisole drench (12 mg kg⁻¹ body weight). Individual faecal egg counts and pooled larval cultures were done 10 days after anthelmintic treatment. Naive lambs were infected with larvae obtained from control and fenbendazole treated groups and were necropsied 35 days after infection for worm recovery. Faecal egg count reductions (FERC) were calculated for fenbendazole and levamisole and, when less than 95 per 100, were considered as indicative of anthelmintic resistance. An *in vitro* egg hatch test (EHT) was conducted with thiabendazole on eggs isolated from pooled faeces of fenbendazole treated goats in nine farms. Faecal egg count reductions indicated the occurrence of benzimidazole resistance in 15 out of 18 farms. Among these farms, nine had EHT values above 0.1 µg thiabendazole ml⁻¹ confirming the benzimidazole resistance status. Levamisole resistance was detected in two farms through FECR. Based on necropsy results, the prevalence of benzimidazole resistance was higher in *Trichostrongylus colubriformis*, medium in *Haemonchus contortus* and lower in *Teladorsagia circumcincta*. In nine farms the benzimidazole resistance was monospecific whereas multispecific resistance was found in the six remaining farms. A negative relationship was found between FECR for fenbendazole and the average number of anthelmintic treatments given per year on the farm. Despite extensive management including a low number of treatments, the prevalence of benzimidazole resistance was very high suggesting that the repeated and sometimes exclusive use of benzimidazole drugs, even at low frequency, is probably the main cause in developing nematode resistance in dairy goat herds. The importance of other factors such as under-dosing or buying animals already carrying resistant nematodes are discussed.

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Introduction

Anthelmintic resistance in nematodes of small ruminants has been reported in all continents and is now recognized as one of the greatest threats to grazing livestock production. Originally the situation was confusing in goats because the poor efficacy of anthelmintics could be due to true resistance or to inadequate dosage of the drug (McKenna, 1984). However, several reports of anthelmintic resistance in goats have been made in Europe (in Cabaret, 2000). The majority of these surveys were conducted on fibre goats, the resistance involved mainly benzimidazole drugs and directed against several species. In France, goats are bred exclusively for milk production and numerous cases of anthelmintic resistance (benzimidazoles) have been reported since 1985 (Kerboeuf & Hubert, 1985; Hubert *et al.*, 1991; Beugnet, 1992; Chartier & Pors, 1994; Cabaret *et al.*, 1995). In contrast, prevalence surveys based on random sampling of farms remained limited to a single study conducted in western France, in which 15 out of 15 surveyed farms harboured benzimidazole resistant worms (Chartier *et al.*, 1998). Intensive management including limited use of pastures and high frequency of anthelmintic treatments were characteristic of the herd management in the latter region. Other important goat production regions present quite different management and worm control schemes, being more extensive and using fewer anthelmintic treatments. One might expect that resistance to the most frequently used anthelmintics in France (benzimidazoles and imidazothiazoles) should be low in such extensive breeding conditions. The aim of the present work is to assess resistance prevalence to benzimidazoles and levamisole in extensive dairy-goat farms in south-western France.

Material and methods

Farms

The dairy goat farms were located in Tarn and Tarn et Garonne departments in the southwestern part of France. This region is characterized by an extensive management of dairy goats due to the use of large areas for grazing or browsing and limited use of commercial concentrates in the indoor diet. The use of anthelmintics is limited to a few anthelmintic treatments per year. Consequently, the level of estimated or measured milk production was rather moderate and ranged between 400 and 750 kg of milk per goat per lactation (table 1).

Eighteen farms were randomly selected from practice files of local veterinarians, eight being 'cheese-making' farms and the remaining ten milking-dairy farms. The size of flocks ranged between 40 and 300 adult goats (table 1). Does more than 1 year old grazed approximately 10 months a year on permanent, sown pastures and in forest. All farms were visited in August 1997 before the start of the survey in October 1997 in order to check the level of nematode egg output, to stop any anthelmintic treatments on the animals and to fill in a questionnaire about the type, the dose rate and the number of anthelmintic treatments given during the previous years.

Treatment and sampling

In October 1997, each farm was visited twice at intervals of 10 days in order to avoid any misinterpretation of the faecal egg count reduction test with levamisole (Grimshaw *et al.*, 1996). The general procedure to detect anthelmintic resistance in nematodes was that recommended by the World Association for the Advancement of the Veterinary Parasitology (WAAVP) (Coles *et al.*, 1992). On each of the 18 goat farms, 45 female adult (≥ 2 years old) goats were selected and randomly distributed into three groups of 15. After an estimate of the weight of the heaviest animal of the group (Coles *et al.*, 1992), the following three treatments were assigned on day 0 using the specific dose rates defined for goats for benzimidazoles (Bogan *et al.*, 1987) and levamisole (Coles *et al.*, 1989): (i) untreated control; (ii) fenbendazole (a benzimidazole compound) given orally at 10 mg kg^{-1} body weight; (iii) levamisole given orally at 12 mg kg^{-1} body weight. These two drugs were chosen as they represent two of the three available large spectrum anthelmintic families. The third family (ivermectin and milbemycin) was not included in this survey because the use of ivermectin has been very limited in dairy goats (dry period) and because of the concern of ivermectin resistance in small ruminants, at least in Europe, seems very unlikely at the moment. As levamisole has a one day milk withdrawal period, most of the goats were not lactating, i.e. in the dry period, when treated with levamisole.

Table 1. Characteristics of dairy goat farms relative to flock size, milk production and number of yearly anthelmintic treatments in 18 dairy goat farms (1997).

Farm no.	Flock size	Milk production per goat per lactation*	No. of anthelmintic treatments per goat per year		
			Total	BZ	Others
1	160	600	4	4	0
2	120	700	2	2	0
3	40	750	3	3	0
4	80	400	3	2	1
5	85	450	4	4	0
6	120	550	2	2	0
7	100	?	2	2	0
8	60	600	0	0	0
9	80	500	0	0	0
10	150	500	6	6	0
11	75	600	2	2	0
12	240	500	4	3	1
13	150	500	2	2	0
14	80	550	3	2	1
15	95	550	2	1	1
16	100	650	2	2	0
17	300	600	1	1	0
18	40	500	3	3	0

* Estimated.

Faecal egg count reduction test (FECRT)

On day 10 after treatment, a faecal sample was taken from the rectum of each goat. A modified McMaster technique with a sensitivity of 50 eggs per gram of faeces (EPG) was performed for faecal egg counting. The mean faecal egg count for each treatment group (EPGt) was calculated and compared with that of the control group (EPGc) using the formula for determining the faecal egg count reduction (FECR): $FECR = [1 - EPGt/EPGc] \times 100$ (Coles *et al.*, 1992). Resistance has been considered when the FECR was less than 95% and when the lower limit of the 95% confidence interval was less than 90%. Coprocultures were performed with pooled faeces on a group basis (control, fenbendazole or levamisole groups) for groups giving sufficient numbers of infective larvae (FECR \leq 80%). Identification was conducted to assess the following genera: *Teladorsagia*/*Trichostrongylus*, *Haemonchus* and *Oesophagostomum*. To obtain more accurate information on the helminth fauna involved in benzimidazole resistance, approximately 2000 infective larvae obtained in coprocultures were given to 3-month-old naive lambs kept indoors, one lamb being used for larvae of each group (control and fenbendazole treated groups). Thirty five days post infection, lambs were necropsied and their digestive tract processed for worm recovery following standard procedures. Only the three commonest nematode species (*Teladorsagia circumcincta*, *Haemonchus contortus*, *Trichostrongylus colubriformis*) were used in estimating worm burdens.

Egg hatch test

An egg hatch test (EHT) for the detection of benzimidazole resistance was applied to the eggs recovered from

pooled faeces taken on day 10 after fenbendazole treatment following the method of Beaumont-Schwartz *et al.* (1987). This *in vitro* test was performed to obtain a better quantitative estimate of levels of resistance than the FECR test. According to Maingi *et al.* (1998), there is a reasonably good agreement between FECRT and EHT in detecting benzimidazole resistance although EHT might be more sensitive. When FECR was $>95\%$, there were insufficient eggs available to perform an EHT. Results were expressed as the concentration of thiabendazole in $\mu\text{g ml}^{-1}$ required to prevent 50% of viable eggs hatching (Logarithmic Concentration 50–LC50). Values of LC50 in excess of $0.1 \mu\text{g thiabendazole ml}^{-1}$ are indicative of benzimidazole resistance (Coles *et al.*, 1992). For technical reasons, EHT was not carried out on six farms.

Results

The main characteristics of the farms are presented in table 1. Faecal egg counts, faecal egg count reductions and egg hatch tests for benzimidazoles are presented in table 2. In 15 out of 18 surveyed farms, results indicated that a benzimidazole resistance was present (FECR $<95\%$ and/or LC50 $> 0.1 \mu\text{g thiabendazole ml}^{-1}$). In these farms exhibiting benzimidazole resistance, FECR values ranged from 26 to 94% whereas LC50 values when determined ranged from 0.227 to $0.43 \mu\text{g thiabendazole ml}^{-1}$. Nematode egg output in control untreated goats was very variable in benzimidazole resistant farms, ranging from 269 to 2873 EPG, and did not differ from benzimidazole susceptible farms (mean egg count of 1427 ± 917 EPG vs 1404 ± 1546 EPG respectively). The distribution of nematode genera or species in the 15 benzimidazole resistant and the three benzimidazole

Table 2. Faecal egg counts (EPG), percentages of faecal egg count reduction (FECR), lower limits of the 95% Confidence Interval after treatment with fenbendazole (10 mg kg^{-1} body weight) or levamisole (12 mg kg^{-1} body weight) and egg hatch tests (EHT) for benzimidazole resistance (LC50 in $\mu\text{g thiabendazole ml}^{-1}$) in 18 dairy goat farms.

Farm no.	Control		Fenbendazole			Levamisole		
	EPG	EPG	FECR (%)	95% CI Lower limit	EHT LC50	EPG	FECR (%)	95% CI Lower limit
1	2733	1083	60.4	18.9	0.264	30	98.9	93
2	1913	683	64.3	35.7	0.426	0	100	100
3	1640	813	50.4	21	0.282	3	99.8	98.4
4	2716	1010	62.8	39	n.d.	47	98.3	95.3
5	2060	870	57.8	33.9	0.430	33	98.4	95.8
6	3170	43	98.6	95.8	–	6	99.8	99
7	593	313	47.2	–22.8	n.d.	393	33.7	–64.2
8	753	0	100	100	–	13	98.2	94.8
9	393	130	66.9	43.8	n.d.	17	95.8	83.7
10	1450	1036	28.5	–21.8	n.d.	10	99.3	96.8
11	336	236	29.7	–27.2	n.d.	6	98	91.7
12	833	366	56	–56.6	n.d.	3	99.6	96.3
13	1900	116	93.9	89.6	0.292	10	99.5	98.3
14	2873	1617	43.7	–6.9	0.331	317	89	44.8
15	1063	387	63.6	33.8	0.227	0	100	100
16	637	253	60.2	40.5	0.280	21	96.6	90.7
17	290	0	100	100	–	13	95.4	84
18	269	200	25.7	–44.8	0.235	19	92.9	83.9

n.d., Not done; –, insufficient eggs available to perform EHT.

susceptible farms is summarized in table 2. When considering results of experimental lamb infection from benzimidazole post-treatment coprocultures, *Trichostrongylus colubriformis* was encountered in high numbers (more than 30% of the total worm burden) in 10 out of 15 benzimidazole resistant farms whereas *Haemonchus contortus* was present in seven farms and *Teladorsagia circumcincta* in five farms. In fact, different situations occurred according to the farms. In nine farms a single nematode species was present after fenbendazole treatment (more than 90%): *T. colubriformis* (5/9), *H. contortus* (3/9) and *T. circumcincta* (1/9). In the remaining six farms, two or three resistant nematode species were present (*T. colubriformis*/*T. circumcincta*: 2/6; *T. colubriformis*/*H. contortus*: 2/6; *T. circumcincta*/*H. contortus*: 1/6; *T. colubriformis*/*T. circumcincta*/*H. contortus*: 1/6). In one farm (15), *Oesophagostomum* larvae were present together with *Haemonchus* in post-treatment coprocultures (table 3).

Goats were drenched on average 2.8 times a year in the benzimidazole resistant farms vs 1.0 time a year in the benzimidazole susceptible ones (t test, $P < 0.05$). More than 90% of treatments were realized with benzimidazole compounds. The number of anthelmintic treatments given per year was negatively correlated with the percentage of faecal egg count reduction ($r = -0.6$; $P < 0.01$).

Faecal egg count reductions after levamisole treatment are presented in table 2. In most farms FECR values indicated a high efficacy of levamisole except in farms 7 and 14 (33 and 89% respectively). Post-treatment coprocultures showed that *Teladorsagia/Trichostrongylus* was the only larval type in farm 7 and that *Haemonchus* larvae were predominant (86%) in farm 14. No levamisole treatment was realized on all farms during the three previous years.

Discussion

The present survey demonstrated that benzimidazole resistant nematodes occurred in 15 of 18 extensive dairy goat farms in southwestern France. These farms were randomly selected and no previous history of drench failures or concerns in nematode control was recorded through farmer questionnaires. The nematode species involved in benzimidazole resistance were mainly, in decreasing frequency order, *Trichostrongylus colubriformis*, *Haemonchus contortus* and *Teladorsagia circumcincta* in monospecific (60%) or multispecific (40%) infections. These results need to be compared with the previous prevalence survey on anthelmintic resistance in goats performed in western France (Chartier *et al.*, 1998) where all 15 surveyed dairy goat farms exhibited benzimidazole resistant nematodes through the faecal egg count reduction test and the egg hatch assay. Chartier *et al.* (1998) showed that *Teladorsagia/Trichostrongylus* were the predominant genera in seven farms and multispecific resistance (*Teladorsagia/Trichostrongylus* and *Haemonchus*) was encountered in six farms. In the Burgundy area in 1999 (F. Beugnet, personal communication, 2000), all the ten surveyed dairy goat farms showed benzimidazole resistant nematodes through the FECRT. In central France, on three dairy goat flocks, Cabaret *et al.* (1995) also showed that benzimidazole resistance was multispecific and that resistant populations included mainly *Trichostrongylus colubriformis* (50 to 95% of the resistant populations) followed by *Teladorsagia circumcincta*, *H. contortus* and *O. venulosum*.

In the present survey, the number of anthelmintic treatments given per year was rather limited compared to Chartier *et al.* (1998) in western France (2.5 vs 6.5) and this number was higher in the benzimidazole resistant farms compared to benzimidazole susceptible ones (2.8

Table 3. Larval cultures (%) and experimental lamb infections (%) from control and fenbendazole treated goats in 18 dairy goat farms.

Farm no.	Larval cultures (%)			Experimental sheep infection (%)		
	<i>Teladorsagia/Trichostrongylus</i> C/T	<i>Haemonchus</i> C/T	<i>Oesophagostomum</i> C/T	<i>Teladorsagia circumcincta</i> C/T	<i>Trichostrongylus colubriformis</i> C/T	<i>Haemonchus contortus</i> C/T
1	3/8	94/90	3/2	0/3	0/7	100/90
2	38/77	62/23	0/0	4/0	86/99	10/1
3	46/63	50/23	4/14	9/6	77/93	14/1
4	49/91	10/2	41/7	8/19	82/81	10/0
5	93/98	4/0	3/2	17/9	81/91	2/0
6	8/-	84/-	8/-	37/100	0/0	63/0
7	100/100	0/0	0/0	41/34	59/66	0/0
8	8/-	92/-	0/-	n.d.	n.d.	n.d.
9	87/100	10/0	3/0	50/0	43/100	7/0
10	24/45	58/55	18/0	27/48	20/34	53/18
11	11/0	89/100	0/0	0/0	0/0	53/36
12	6/3	94/97	0/0	47/64	0/0	100/100
13	4/35	95/59	1/6	17/93	0/0	83/7
14	6/9	79/84	15/7	3/0	7/70	90/30
15	4/1	50/71	46/28	1/0	0/0	99/100
16	85/82	15/18	0/0	39/7	55/92	6/1
17	48/-	52/-	0/-	n.d.	n.d.	n.d.
18	51/62	41/34	8/4	33/8	34/76	33/16

C/T, Control/treated; n.d., not done; -, insufficient eggs available to define larval composition.

vs 1.0). It was, however, impossible to determine whether this higher drenching was the cause or the consequence of resistant nematode occurrence. On the other hand, benzimidazole compounds were the most frequently used drugs and the single class of anthelmintics used on several farms as neither levamisole/pyrantel nor avermectins/milbemycins have been used for at least 3 years. The repeated and sometimes exclusive use of benzimidazole drugs for many years undoubtedly exerts a tremendous selection pressure on nematode populations even though the annual frequency of drenching is low (McKenna, 1984; Sangster, 1999). Reasons for the widespread use of benzimidazoles anthelmintics in dairy goats include: the wide anthelmintic spectrum, safety in use, the number of marketed drugs available in this class, and last but not least, the absence of a milk withholding period that allows the use of benzimidazoles during lactation. In two farms, levamisole resistance in nematodes was suspected. Very little information is available concerning levamisole resistance in goats in Europe except for a case report in France (Chartier & Pors, 1994) and one confirmed case amongst 70 goat farms tested in the UK (Hong *et al.*, 1996). Another survey in Denmark (Maingi *et al.*, 1996) recorded eight suspected cases out of 15 farms but the dosage of levamisole was not adequate and the maximal period of 10 days post-treatment for the FECRT was not applied, thus making the interpretation of results unreliable. In the present survey, the dose and test 10 days after treatment were carefully checked. However, as mentioned by Hong *et al.* (1996) and Maingi *et al.* (1998), even when FECRT is performed adequately, discrepancies with other tests like larval development assays and controlled trials (dosing followed by nematode counts in necropsied lambs) raise doubts on the reality of resistance to levamisole.

Under-dosing is a common feature in goat drenching, as specific dose rates have to be used at least for benzimidazoles and levamisole. The impact of suboptimal dosage on the development of anthelmintic resistance is still a matter of debate. Relative to dose levels, under-dosing promotes or impedes resistance (Smith *et al.*, 1999). Recent investigations have shown that under-dosing benzimidazoles could promote resistance as susceptible heterozygotes were not fully removed at 25% of the usual dosage (A. Silvestre, personal communication, 2000). However, the influence of under-dosing remains somewhat limited and any increase in resistance should be explained by other management factors. Silvestre *et al.* (2000) showed that resistance to benzimidazoles was correlated with breeding management factors such as the number of farms of origin when the flock was created and the area of permanent pastures. Dairy goat farms are relatively isolated as far as the helminth fauna is concerned because once herds are established, no adult infected animals are subsequently purchased (Cabaret & Gasnier, 1994). Thus data from Silvestre *et al.* (2000) suggest that benzimidazole resistant nematodes were introduced with the primary stock of parasite species at the time of establishment of the farm. This situation is different from that prevailing in alpine areas, for example, where goats from different farms usually graze together on common pastures and may

exchange resistant strains of nematode (Hertzberg *et al.*, 2000).

Grazing management and its relationship with anthelmintic control is another important point in the development of resistant nematode populations. The available population of free living helminth stages on pastures when animals are treated determines the rate of selection of resistance (Jackson, 1993) and all strategies combining anthelmintic treatment and provision of clean or safe pastures tend to increase the selection of resistant worms (Sangster, 1999). In the present survey the majority of farmers administered anthelmintic treatment when housing animals at the end of the grazing period in autumn. The duration of the animals' stay indoors during winter varied, according to the farm, from some weeks to several months and this could lead, in some cases, to a substantial reduction in infective larvae at the turnout in the following spring. Although the duration of winter stay has been shown to decrease the diversity of nematode species in farms, no relationship between diversity and levels of anthelmintic resistance has been demonstrated (Silvestre *et al.*, 2000).

The high prevalence of benzimidazole resistance in French grazing dairy goat flocks is of great concern and there is an urgent need to define nematode-control schemes based on better usage of anthelmintics and integrated approaches with non-chemical options.

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