



A SURVEY OF ANTHELMINTIC EFFICACY IN DAIRY GOAT FARMS IN SOUTH-EAST FRANCE

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ABSTRACT

Goat's milk production is an important activity in France. Gastrointestinal nematodes (GIN) remain a main threat for grazing goats, usually controlled using anthelmintic drugs (AH). However, 1) eprinomectin (EPN) is the sole molecule yet available without withdrawal time for milk in France; 2) nematodes AH resistances increase dramatically. First, a survey was performed on 13 farms in the Auvergne-Rhône-Alpes (AURA) region to evaluate the efficacy of 2 AH authorized for dairy goats: a benzimidazole (BZ) and a macrocyclic lactone (EPN pour-on). Fecal Egg Count Reduction Tests (FECRT) were carried out using 3 groups of 10 goats per farm: a control group, one treated with BZ (oxfendazole) and one treated with EPN. The results indicate a resistance to BZ in 11 out of 13 farms (84.6%) and a lack of efficacy for EPN pour-on in at least 10 farms (76.9%). Secondly, EPN was given orally or subcutaneously in 4 farms of the previous trial showing a lack of efficacy to EPN pour-on. A resistance to EPN was confirmed in 3 out of 4 farms. Although based on a few farms, these results confirm the high prevalence of resistance of GIN to BZ in dairy goat farms. They also highlight a high level of resistance to EPN and the lack of efficacy of EPN when used pour-on. Given the level of resistance to marketed drugs, there is an urgent need to explore alternative options to AH to prepare integrated, sustainable control of GIN.

1. Introduction

Gastrointestinal nematode (GIN) infections of livestock remain worldwide a major health issue in grazing farming systems, especially in small ruminants. These infections cause reduced feed intake, impaired digestion and absorption of nutrients and major changes in the metabolism of sheep or goats (Hoste et al., 1997). These pathophysiological disorders of the main digestive processes result in production losses of meat, milk, and wool. Moreover, in severe cases, the animal is unable to immunologically regulate the worm infections, clinical signs (anemia, diarrhea) and sometimes deaths of sheep or goats can occur. Therefore, infections caused by GIN remain one of the major problems for efficient sheep and goat breeding and production in temperate and tropical areas, especially when the blood feeding abomasal parasite *Haemonchus contortus* is involved.

For more than 50 years, the control of GIN has relied mostly on repeated administration of synthetic anthelmintic drugs (AH) based on strategic and/or tactic treatments. However, several limitations in the use of AH have now been identified. The use of AH must be monitored to prevent drug residues in meat and milk products marketed for human consumption. In addition, for some molecule groups (= AH classes) and some specific galenic formulations (e.g. pour-on), the presence of long-term residues in feces has also been related to environmental consequences on the pasture micro fauna (Lumaret and Errouissi, 2002; Lumaret et al, 2012). As a result, regulations to limit the use of AH are applied in the labelling of commercial drugs to reduce the residues and to protect both the consumers and the environment. In some ruminant systems (e.g., dairy small ruminants in Europe), these regulations can severely limit the use of chemical treatments, either by the interdiction of some specific molecules when ewes or goats are producing milk or by

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imposing several days of withdrawal for milk. These restrictions represent main criteria for farmers in the choice of the molecules to treat lactating dairy goats or ewes. Finally, the long-term and intensive use of AH to control GIN has led to the exponential development of resistance to the different classes of commercialized AH. This has been identified for the main genera and species of the GIN inhabiting the digestive tract. The phenomenon of anthelmintic resistance (AR) is present and expanding worldwide including the rise of multi-resistant isolates. In Europe, several surveys have been performed in the different ruminants' systems (see reviews by Geurden et al., 2014; Rose Vineer et al., 2020). It is crucial to continue at identifying the AR status in different small ruminant production systems and regions and to analyze the epidemiological factors explaining the rise of AH resistance. There are evidence to suggest that this combination of such anthropic and biological factors may increase the selection of AR to molecules with no withdrawal time in GIN populations.

In France, for several decades, the control of GIN in grazing, dairy goats has relied principally on the use of a few molecules of the BZ class (i.e. fenbendazole, oxfendazole). These drugs previously required no withdrawal time. This may explain the early rise of AR to BZ class in dairy goats (Kerboeuf et al., 1988). Thereafter, AR to BZs has been observed in a wide range of French territories dedicated to dairy goats (Cabaret et al., 1995; Chartier et al., 2001, Paraud et al., 2010). Regulations regarding withdrawal times for BZ molecules used in France have changed in 2014 imposing then from 4 to 14 days of withdrawal time (Hoste et al., 2014).

Thereafter, eprinomectin (EPN) (EPRINEX ND) remaining the sole molecule with no withdrawal time for dairy cattle, appeared as a popular option in dairy small ruminants when used following the cascade principles (Fresnay, 2004). More recently, two new commercial presentations based on EPN have been specifically developed and registered for goats and ewes with no withdrawal time for milk: first as a pour-on formulation (EPRINEX MULTI ND, Boehringer, launched in 2016) then, an injectable (SC) formulation (EPRECIS ND, available since November 2020) (<https://www.ircp.anmv.anses.fr>).

Therefore, for almost 10 years, EPN has been increasingly favored by farmers to treat the grazing dairy small ruminants, and it was often the sole molecule used by farmers. In addition, up to 2020, EPN was only available as a pour-on formulation which has a poor bioavailability (Rostang et al., 2020). Such a situation can accelerate AR to EPN in GINs populations. However, only a few surveys on the rise and distribution of AR to EPN in worm populations have been performed in France. Moreover, these surveys were mostly performed in sheep (Bordes et al., 2022; Cazajous et al., 2018; Jouffroy et al., 2023) than in goats (Paraud et al., 2010).

The first objective of the current study was to assess the efficacy of BZ and pour-on EPN in the treatment of GIN parasitism in dairy goats in the South-East of France (Region Auvergne-Rhône-Alpes). The second objective was to determine if any lack of efficacy of EPN identified after the results of the first trial, was due to the mode of administration rather than to AR. This was done in a trial 2 by comparing results when EPN was administered as a pour-on vs oral and/or injectable administration.

2. Materials and methods

2.1. Study area and farm selection

The survey was restricted to the Auvergne-Rhône-Alpes (AURA) region, in the center and South-East of France including the Central Massif, the Alps mountains and the Rhône valley. The region AURA (approximately 70,000 km²) is one of the main areas of dairy goats' production in France.

The survey was performed during two consecutive winter breaks in the grazing seasons (years 2020/2021 and 2021/2022), from the end of November to end of February, when goats were non lactating and kept indoors, with no access to the pastures.

Farms were selected by a veterinarian organisation based on voluntary adhesion by couples of farmers and veterinarian surgeons. The level of GIN infection was assessed at the end of the grazing season (end of October/November) relying on Fecal Egg Count (FEC) performed on pooled samples from 10 goats, including primiparous and multiparous. We performed parasite egg count using a modified McMaster protocol proposed by Raynaud et al. (1970) with a solution of zinc sulphate (ZnSO₄, density = 1.36). Briefly, we mixed in equal proportion feces from 3 to 5 individuals, and mixed 5 g with 70 mL of zinc sulphate (1/15 dilution), (theoretical sensitivity of 50 eggs per gram (epg)). Only farms with a mean value > 250 epg were included in the study.

2.2. Evaluation of the efficacy of benzimidazoles and eprinomectin

In each farm, three groups of 10–12 animals were randomly selected; although respecting the primiparous/multiparous proportion of the herd as follows: a control group (C) which remained untreated; a benzimidazole group (BZ) treated by drenching with oxfendazole (Oxfenil ND, 10 mg/kg Virbac), and a third group (EPN) treated with EPN pour-on (EPRINEX MULTI ND, 1 mg/kg, Boehringer). Drugs were administered following the Marketing Authorization Application (MAA). The dosage per goat was calculated after individual weighing of the animals. The protocol was applied at any time of the day, depending on the availability of the farmer and of the veterinarian practitioner.

The survey was performed according to the WAAVP guidelines to evaluate the efficacy of anthelmintic drugs based on the fecal egg count reduction test (FECRT) (Coles et al., 2006).

On Day zero (D0), individual fecal samples were obtained from each goat to measure FEC and the goats of groups BZ and EPN were treated according to the protocols previously described. Each goat was individually identified. The EPN group was isolated during 14 days from the flock to avoid the other groups to be exposed to EPN via licking behavior. On Day 14 (D14), individual fecal samples were collected from the same goats in each group to perform post-treatment FEC. Based on the results of the first trial by using the pour-on administration of EPN, a follow-up protocol was designed in a trial 2 to confirm or not resistance to EPN, after an observed lack of efficacy and hence suspected AR resistance of the pour-on EPN formulation in trial 1. This main question aims in order to ensure the control of GINs in dairy goats farms in France and to prevent the development of AH resistance to EPN that has been previously determined and supported by data obtained in controlled conditions (Badie et al., 2015).

Therefore, a second trial was performed in 4 farms where a low efficacy of pour-on EPN was detected. The goats, still non lactating, were assigned in 2 groups (balanced if possible, according to the mean values of EPG on day 14): a control group (Group 1) and a treated group (Group 2) which was composed, in 3 farms (Farm 1, 5 and 10), by goats treated off-labelled orally with the pour-on formulation of EPN (EPRINEX MULTI ND, 1 mg/kg, Boehringer) and in one farm (Farm 2), by using a sub cutaneous EPN formulation (EPRECIS ND at 0,2 mg/kg BW). The goats composing the 2 groups of trial 2 were selected primarily from either the control groups or the BZ treated groups of trial 1. The time between the two trials was greater than 5 weeks. FECRT were performed as in trial 1. All individual FEC were performed using a modified McMaster procedure (Raynaud et al., 1970) as described before, but by using a salt solution (NaCl, s.g. = 1.20).

2.3. Determination of treatment efficacy

For each farm, mean epg and 90% confidence intervals were calculated using a negative binomial regression (eggs counted in McMaster slide being the response variable, and group and date the explanatory variables). The percentage of reduction was estimated using the estimated means according to the formula: $FECR = 100 \times (1 - [T2/T1][C1/C2])$, where T1 and T2 were pre- and post-treatment geometric means of

the epg in treated groups respectively, and C1 and C2 were pre- and post-treatment geometric means of the epg in control groups respectively. Confidence intervals (90%) were calculated using the regression estimators in Stata (StataCorp).

Results were interpreted according to WAAVP guidelines (Kaplan et al., 2023). We considered an expected efficacy of 99% and a grey zone of 90–99%. GIN in a farm were considered as susceptible when the lower 90% CI is greater than or equal to the lower limit of the grey zone and the upper 90% CI is greater than or equal to the upper limit of the grey zone. They were considered as resistant when the lower 90% CI is less than the lower limit of the grey zone. In all other situation, results are inconclusive on the efficacy of the drug.

2.4. Larval cultures and identification of gastrointestinal nematode genera

Larval cultures were performed on each sampling date based on pooled individual fecal samples per group (Control, BZ or EPN). Each animal within a group contributed approximately in a similar quantity to the pooled larval culture (4–5 g of feces). The pooled feces per group were incubated for 12 days at 23 °C and humidified regularly. Third stage larvae (L3) were recovered according to the Baermann method, stored at 4 °C in approximately 50 mL of tap water for each group and the main classes of genera were identified within a maximum delay of 4 weeks.

On D0, two larval cultures were performed per farm based on pooled feces, without distinguishing the 3 groups. The values reported on D0 are thus the means of the 2 results. On day 14, the larval cultures were performed per treatment group (control, BZ or EPN pour-on) as previously described.

Morphological identification of the larvae was performed according to the criteria of Van Wyk and Mayhew (2013) making difference between larvae of 4 main groups of genera: *Teladorsagia/Trichostrongylus*, *Haemonchus* sp., *Cooperia* sp. and *Oesophagostomum* sp.

3. Results

3.1. Efficacy of benzimidazoles and eprinomectin pour-on

The survey involved 13 dairy goat farms from 8 out of the 12 subdivisions (Departments) that comprise the AURA Region during the winters of 2020–2021 and 2021–2022. The main results of FECRT in the different farms, by drug classes (namely BZ and EPN pour-on), are presented in Tables 1 and 2.

For BZ, a lack of efficacy was detected in 84.6% (n=11/13) farms. For EPN pour-on, a lack of efficacy of treatment was observed in 76.9% (n= 10/13) farms. Moreover, results were inconclusive for one farm (Farm 4) because the lower 90% CI is below 90% (88%).

Table 1

Fecal egg count (eggs per gram) geometric means (M) and CI90% (lower and upper limit) obtained in each group of the 13 surveyed farms on day 0 (before treatment) and on day 14 after treatment used to characterize the GIN infections and to evaluate the efficacy of BZ and EPN pour-on.

Farm	CONTROL				BENZIMIDAZOLES				EPRINOMECTIN POUR-ON									
	Day 0		Day 14		Day 0		Day 14		Day 0		Day 14							
	M	CI 90%	M	CI 90%	M	CI 90%	M	CI 90%	M	CI 90%	M	CI 90%						
1	790	493	1266	1245	782	1983	480	296	778	1025	642	1636	1075	674	1715	680	423	1093
2	640	468	874	495	359	683	650	476	887	170	115	252	500	363	689	70	42	118
3	715	471	1084	790	522	1196	735	485	1114	430	279	662	715	471	1084	105	62	179
4	108	63	186	237	146	386	150	90	251	4	1	23	188	114	309	12	4	36
5	230	131	403	280	161	485	500	294	850	110	60	203	1067	619	1839	1000	579	1726
6	2045	1205	3473	886	518	1516	1433	861	2385	83	45	155	1758	1058	2921	8	2	30
7	156	91	265	228	138	376	114	60	216	0	0	0	150	85	265	37	17	84
8	521	311	872	437	260	735	459	267	788	214	121	377	932	550	1580	391	227	674
9	532	338	838	732	468	1144	717	467	1100	337	215	529	433	279	673	79	45	138
10	1615	1049	2486	1570	1020	2417	1405	912	2166	1225	793	1891	955	616	1480	485	308	763
11	430	164	1126	400	153	1049	710	273	1847	835	321	2169	25	7	83	360	137	946
12	1245	783	1979	940	589	1500	1005	631	1602	140	81	242	895	561	1429	0	0	0
13	520	311	869	295	173	502	960	581	1585	645	388	1072	720	434	1194	295	173	502

Table 2

Calculation of the efficacy (expressed as % FECRT in bold) and CI90% of benzimidazole (BZ) and eprinomectin (EPN) pour-on. According to the WAAVP guidelines (Kaplan et al., 2023), results indicate treatment failure to either BZ or EPN pour on when the lower 90% CI is less than the lower limit of the grey zone (90–99%). Treatment failure to BZs and to EPN pour on are indicated respectively by the superscript a (for BZs) and/or b (for EPN) for each farm (column 1).

FARM	BZ	Lower CI 90	Upper CI90	EPN	Lower CI 90%	Upper CI90%
1 a,b	-36%	-248%	47%	60%	-3%	84%
2 a,b	66%	34%	93%	82%	61%	92%
3 a,b	47%	-23%	77%	87%	68%	95%
4	99%	91%	100%	97%	88%	99%
5 a,b	82%	44%	94%	23%	-132%	74%
6 a,	87%	60%	96%	99%	95%	100%
7 b	100%	100%	100%	83%	42%	95%
8 a,b	45%	-62%	81%	50%	-43%	83%
9 a,b	66%	17%	86%	87%	66%	95%
10 a,b	10%	-113%	62%	48%	-26%	78%
11 a,b	-26%	-761%	81%	-1448%	-11986%	-98%
12 a	82%	51%	93%	100%	100%	100%
13 a,b	-18%	-231%	58%	28%	-105%	75%

Overall, a lack of efficacy for both classes of drugs (BZ and EPN pour-on) was detected in 76,9% (n=10 /13) farms. On the other hand, on only one farm (Farm 4), a full efficacy for both AH classes was suspected, although the lower CI for EPN (88%) was below the lower expected limit of 90%.

3.2. Treatment failure of EPN pour-on vs confirmed resistance to EPN

To confirm the suspicion of resistance to EPN in farms where a treatment failure of EPN pour on was identified in trial 1, the trial 2 was designed on 4 farms by using EPN either orally (farms 1,5 and 10) or by subcutaneous injection (farm 2). A lack of efficacy of EPN and therefore a suspicion of resistance was confirmed on 3 out of 4 farms retained for trial 2 (Tables 3 and 4). However, on farm 1, EPN was effective when administered orally whereas a lack of efficacy was found when EPN was used as a pour-on formulation in Trial 1 (Table 2). These compared results suggest that the pour on route of administration was the reason for the treatment failure in that specific farms.

3.3. Results of larval cultures

The results obtained on larval culture are summarized in Table 5 (D0 and D14 after treatment with BZ or EPN pour-on) and in Table 6 (treatment with EPN per os or SC). Larval culture data were not available post treatment when the treatment lead to very low number of eggs.

Table 3

Fecal egg count (eggs per gram) geometric means and CI90% obtained in each group of 4 farms before and after treatment with eprinomectin administered orally (farms 1, 5 and 10) or subcutaneously (farm 2).

FARM	CONTROL						EPN					
	Day0			Day 14			Day 0			Day 14		
	Mean	Lower CI 90%	Upper CI90%	Mean	Lower CI 90%	Upper CI90%	Mean	Lower CI 90%	Upper CI90%	Mean	Lower CI 90%	Upper CI90%
1	1315	1024	1266	775	595	1009	1370	1068	1758	10	3	33
2	345	221	874	890	585	1354	413	252	674	94	51	174
10	1428	951	1084	2267	1517	3387	1400	952	2059	355	233	541
5	1000	638	186	665	421	1050	800	509	1258	600	379	950

Table 4

Calculation of the efficacy (%) and CI90% of eprinomectin (EPN) administered orally (farms 1, 5 and 10) or subcutaneously (farm 2). Treatment failure to oral EPN or sub cutaneous EPN, equivalent to resistance to this drug, are indicated by the superscript "a" for each farm (column 1).

FARM	% of REDUCTION			Upper CI90%
	EPN	Lower CI 90%		
1	99%	96%		100%
2 a	91%	76%		97%
10 a	84%	64%		93%
5 a	-13%	-180%		55%

Therefore, for trial 1, results were obtained on 12 farms out of 13 since data of larval cultures were not available for farm 7. For trial 2, data of larval cultures were not available for Farm 1 and 2 in the groups treated with EPN.

Overall, the dominant genera found were identified from the group *Teladorsagia/Trichostrongylus* and *Haemonchus* spp. No larvae identified as belonging to the genus *Cooperia* were observed. Last, larvae of the genus *Oesophagostomum* sp. were identified only in 2 farms in low proportion.

In 3 farms (Farm 9, 11 and 13), the population of larvae identified on D0 was exclusively composed of L3 of the group *Teladorsagia/Trichostrongylus*.

The lack of duplicates in larval cultures per treatment group did not

Table 5

Proportion of the different genera in the different farms pre (Day0) and post treatment (D14) with Benzimidazoles (BZ) or eprinomectin (EPN) pour-on. For each group and farm, from 80 to 130 larvae were used to calculate the percentage (%). NA= Data not available.

Farm number		Pre treatment = Day 0			Post treatment =D14			
		Tel/ Trichos	Haem.	Oesoph.	Tel/ Trichos	Haem.	Oesoph.	
1	Control	88	12	0	Control	68	32	0
					Benzimidazole	74	26	0
					EPN pour-on	84	16	0
2	Control	57	43	0	Control	89	11	0
					Benzimidazole	81	19	0
					EPN pour-on	100	0	0
3	Control	56	33	11	Control	23	75	2
					Benzimidazole	75	25	0
					EPN pour-on	5	98	3
4	Control	67	33	0	Control	78	22	0
					Benzimidazole	No larvae		
					EPN pour-on	No larvae		
5	Control	2	98	0	Control	6	94	0
					Benzimidazole	4	96	0
					EPN pour-on	1	99	0
6	Control	92	7	0	Control	97	3	0
					Benzimidazole	100	0	0
					EPN pour-on	No larvae		
7	Control	NA	NA	NA	NA	NA	NA	NA
		NA	NA	NA	NA	NA	NA	NA
		NA	NA	NA	NA	NA	NA	NA
8	Control	76	24	0	Control	66	34	0
					Benzimidazole	93	7	0
					EPN pour-on	69	30	0
9	Control	100	0	0	Control	100	0	0
					Benzimidazole	99	1	0
					EPN pour-on	98	2	0
10	Control	96	3	1	Control	91	6	3
					Benzimidazole	96	4	0
					EPN pour-on	78	20	2
11	Control	100	0	0	Control	100	0	0
					Benzimidazole	100	0	0
					EPN pour-on	100	0	0
12	Control	38	61	0	Control	77	17	5
					Benzimidazole	1	99	0
					EPN pour-on	No larvae		
13	Control	100	0	0	Control	100	0	0
					Benzimidazole	100	0	0
					EPN pour-on	97	3	0

Tel/ Trichos: *Teladorsagia/Trichostrongylus*, Haem.: *Haemonchus* sp., and Oesoph.: *Oesophagostomum* sp.

Table 6

Results of larval culture in the 4 farms where the efficacy of eprinomectin (EPN) has been applied orally (per os) or subcutaneously (SC) (farm 2).

		Tel/ Trichos	Haem.	Oesoph.
1	Control	73	27	0
	EPN per os	No Larvae		
2	Control	78	22	0
	EPN SC	No larvae		
5	control	7	93	0
	EPN per os	8	92	0
10	Control	77	23	0
	EPN per os	2	98	0

Tel/ Trichos: *Teladorsagia/Trichostrongylus*, Haem.: *Haemonchus* sp., and Oesoph.: *Oesophagostomum* sp.

allow any statistical analyses. In trial 1, on most farms, the proportion of parasite genera identified by larval culture initially, did not vary post-treatment regardless of AH treatment used (Table 5). This suggests that overall, there was little to no difference in susceptibility of the different genera of GINs to either BZ or EPN. There were a few exceptions. Main changes in the percentage of larvae, when compared to control values on D0 and D14, were observed after administration of i) EPN pour-on in farm 3 and 10 with an increased proportion of *Haemonchus* larvae and ii) after BZ treatment in farm 8 (increased in the proportion of larvae of the group *Teladorsagia/Trichostrongylus*) and farm 12 (increased percentage of *Haemonchus* larvae)

In the trial 2 focusing on EPN (either use orally or subcutaneously in 4 farms), the results of larval cultures were limited to 2 farms. On farm 5, the genus proportion of larvae remained similar between the control and EPN treated groups. In contrast, in farm 10, an increased percentage of *Haemonchus* sp. larvae was observed.

4. Discussion

This survey is the first one in French dairy goats' flocks aiming at assessing concurrently the efficacy of BZ and EPN on GIN populations. The 13 farms included in the survey are located in different areas of the AURA Region, therefore, they can be seen as representative of various dairy goat production systems in this Region of France and of differing epidemiological conditions of GIN infections, explained by a diversity of climatic and agronomic conditions (O'Connor et al., 2006).

The lack of efficacy of BZ can be likely attributed to AR since several previous surveys performed in various areas in France, namely Centre, Poitou Charentes, or Midi Pyrénées have suggested the same conclusions (Kerboeuf et al., 1988, Chartier et al., 1998; Chartier et al., 2001). However, information was missing for the South-East of France including the Auvergne-Rhone-Alpes region where dairy goats are commonly grazed in outdoors conditions. In contrast, surveys in France examining the possible lack of efficacy of pour-on EPN with no withdrawal time have been far fewer. They are more recent and mainly focusing on sheep and/or dairy ewes (Bordes et al., 2022, Cazajous et al., 2018, Jouffroy et al., 2023). For dairy goats, a case report of EPN resistance was recently described (Bordes et al., 2020). However, it is related to a single observation on one farm whereas our survey included 13 farms.

The survey was performed during winters 2020/21 and 2021/22 when goats were non-lactating and usually housed indoors. We experienced difficulties to recruit farms for several reasons: 1) farmers were not able to effectively isolate the EPN group; 2) farm management reasons as end-of-year holidays, samplings and treatment often occurred in January and it was sometimes too close to the start of the lactating and grazing periods; 3) weather conditions in the AURA regions were abnormally hot and dry in 2020/2021 and 2021/2022 contributing to low levels of parasite infections on some farms. These factors explain why some farms were excluded from the study; 4) due to COVID-19 pandemic, some assays in farm have to be stopped prematurely.

Our study was designed following the WAAVP recommendations (Coles et al., 2006; Coles et al., 1992). Recently, new guidelines were published (Kaplan et al., 2023). We used the more recent recommendations for the statistical interpretation of results. A control group was included and samplings at D0 were performed to take into account of variability of FEC between the treatment groups (cf Table 1).

The 4 successive versions of the WAAVP guidelines recommended to use arithmetic means of egg counts to calculate reduction index following treatments. However, in this study, we used negative binomial regressions to analyze raw count data and calculate confidence intervals. In fact, the negative binomial distribution, like the Poisson distribution, fits better count data than a normal distribution since it is truncated on the left side (no negative values are expected) and assumes data are discrete. The corollary is that statistical estimates correspond to geometric means (log-transformed estimates) (Dohoo et al., 2003).

For BZ drenches, a lack of efficacy can be attributed to an AH resistance to oxfendazole and therefore, a suspected resistance to all BZ class. The farm-level prevalence of BZ resistance (85%) found in this study is similar to findings of previous surveys on dairy goats' farms elsewhere in France (Kerboeuf et al., 1988, Chartier et al., 1998; Chartier et al., 2001).

A lack of efficacy with pour-on EPN was observed in 77% (10/13) of the surveyed farms. Due to the poor bioavailability of the topical route applied (Rostang et al., 2020), we then evaluated the efficacy of EPN by oral or subcutaneous route. Although based on a limited number of farms, the results suggest a lack of efficacy of EPN in 3 out of 4 dairy goat farms in the AURA Region. These results tend to confirm recent data obtained in dairy ewe farms in France (Jouffroy et al., 2023) or in goat and sheep farms in Switzerland (Murri et al., 2014, Scheuerle et al., 2009).

The pour-on application of macrocyclic lactones has greatly increased the ease of their administration and thus advanced for the chemical control of GIN. Moreover, the licensing of topical EPN with no milk withdrawal requirement time has further advantaged the use of avermectins in cattle. However, beside these main advantages, further studies have identified some risks to using the pour-on formulations, including by possibly increasing the risk of development of AR to EPN in worm populations in ruminants. Overall, results from studies on the use of EPN pour in different ruminants species have described variable efficacy. This might be explained by AR. However, it may also be due to underdosing because of poor absorption of the drug when administered topically, as shown by previous pharmacological studies and results (Rostang et al., 2020; Bouy et al., 2021, Morgan et al., 2022, Badie et al.; 2015, Lespine et al., 2012). These questions on the origin of variability of the efficacy of EPN pour-on affect the interpretation of results of FECRT.

The issues related to the use of topical EPN and the variability in calculated efficacy have been identified as pregnant issues in dairy goat farms because identified as possible factor increasing the risk of development of EPN resistance in GINs (Lespine et al., 2012, Badie et al.; 2015; Bouy et al., 2021) As a consequence, the use per os of pour-on formulation of EPN has been an increasingly common practice on French dairy goat farms in order to improve the efficacy of EPN.

Such an off label use of a drug is admitted on farm in EU and UK, when respecting the Cascade principle. Overall, the main objective of the Cascade principle is to provide a legal frame to use off licensed chemical drugs to treat some diseases in some small niches of livestock production (e.g. dairy goats and ewes) while preserving the health of the consumers by maintaining exposition to drug residues under the Minimal Limit of Residues. The conditions of application of this Cascade principle have been well identified and defined in the website (EUR-Lex - 5, 2015).

In regard of the efficacy of pour-on EPN against GINs, pharmacological studies have compared the efficacy of EPN when used pour-on vs per os. These studies have confirmed a higher and more reproducible efficacy when EPN was used orally (Chartier et al., 1999; Badie et al.,

2015, Silvestre et al., 2007).

The use of an oral route of an EPN pour-on formulation, according to the Cascade principle also inquiry the risk of possible residues in milk. Several scientific arguments have been acquired explaining that this risk is limited. First, previous pharmacokinetics data have been obtained (Badie et al., 2015) indicating that the oral use of pour-on EPN has limited consequences on the concentration of milk residues which remained lower than the Maximum Residue Limit. Secondly, the off license use of any drug means that it has to be performed according to the Cascade principles, under the supervision of veterinarians and by respecting more severe constraints, in particular longer withdrawal times for the use of milk in human consumption. (Fresnay, 2004, 20220314_faq_rmv-ram_2022-03-18_15-21-47_364.xlsx (anses.fr).

Last, it is worth to underline that although Eprinex multi (ND) is a commercialized drug firstly designed for the pour-on route, its vehicle, namely N-hydroxybutyltoluene, is similar to two other commercial formulations of macrocyclic lactones proposed for oral route, namely ivermectin (Oramec ND), and moxidectin (Cydectin ND) with available information on the pharmacology, efficacy and safety addressed in the RCP.

These previous statements explain why a second trial was undertaken in our survey. The aim was to differentiate a lack of efficacy of pour-on EPN vs a confirmed suspicion of EPN resistance when this drug was used either off-labelled orally (Badie et al., 2015) or by sub-cutaneous injection. Due to farmers availability, the number of farms involved in this second trial was low. Treatment failure and thus the suspicion of resistance to EPN was confirmed in 3 out of 4 farms. Only in Farm 1, a lack of efficacy of pour-on EPN was not confirmed by an off-labelled oral administration of EPN indicating a lack of resistance.

In regard of the results of larval cultures, as previously indicated, our results should be considered only as observations because of the lack of duplicates per experimental group and thus of possible statistical analyses. Nevertheless, it was observed that on most farms, the proportion of genera did not change post treatment. This consistency in genera composition in farms where resistance to BZ was confirmed led to the conclusion that BZ resistances was equally present in GIN genera (*Teladorsagia*; *Trichostrongylus* and *Haemonchus*)

Only in 2 farms, the use of EPN pour-on was associated with an increased percentage of *Haemonchus* sp. larvae, suggesting a development of resistance to EPN in this particular genus.

For farm 3, this was detected in trial 1 after the use of EPN pour-on. For the farm 10, a similar switch in the proportion of larvae of different genera was found after use of EPN either pour-on or per os. In few recent studies based on a limited number of farms in dairy ewes in France, suspicion of resistance to EPN pour-on have yet been identified (Jouffroy et al.; 2023, Bordes et al., 2022). One of these studies has shown an increased proportion of *T. colubriformis* after treatment with EPN pour-on (Bordes et al., 2022). The second one concluded to cases of EPN-resistant isolates of *H. contortus* in 2 farms of dairy ewes (Jouffroy et al., 2023).

One main results of trial 1 in our survey is the high prevalence (69.2% = 9 out of 13 farms) of failure of drug efficacy found for both BZ and EPN pour-on, as indicated by the FECRT results. These results suggest multiresistance to both BZ and EPN. Although relying on a limited number of farms, this conclusion is supported by results of the trial 2. They indicate that in 3 out of 4 farms, multiple class resistances were confirmed. Such high prevalence of resistance to multiple classes of AH, especially concerning drugs with zero withdrawal time in dairy small ruminants, represents a main threat for the future control of GIN in EU countries where outdoors, grazing dairy small ruminants remained a main economical production.

The management of GIN relying exclusively on synthetic AH might become a dead-end therapeutic solution. The GIN parasitic infections can be considered soon as so-called “orphan diseases” because of the lack of highly effective AH treatments. According to Kaplan (2004) and Waller (2006), resistance to anthelmintic and/or other xenobiotics

appears between 10 and 15 years after the commercial launch of novel molecules. For the control of GIN in sheep, this general concept has been illustrated by the rapid occurrence of resistant strains to monepantel, the first molecule of the novel AH molecule family (AAD) (Kaminsky et al., 2008; Van den Brom et al., 2015).

The repeated and almost exclusive use of AH to control GIN is considered nowadays as a non-sustainable mode of control of GIN (Waller, 2006). Considering this previous statement, a general agreement is nowadays emerging to promote, develop and implement alternative or complementary strategies to synthetic AH (Charlier et al., 2022; Morgan et al., 2019). The concept of a more sustainable integrated approach by combining several solutions corresponding to different principles to control GIN is nowadays supported by innovative, multi-disciplinary research examining a range of solutions targeting different stages of the GIN life cycle (Morgan et al, 2018; Torres Acosta and Hoste, 2008).

CRedit authorship contribution statement

Jacques Devos: Writing – review & editing, Writing – original draft, Conceptualization; **Gilles Bourgoin:** Writing – review & editing; **Philippe Thorey:** Conceptualization; **Tanguy Marcotty:** Formal analysis; **Slimania Benabed:** Resources; **Osmite Berlus:** Methodology, Formal analysis; **Lea Masson:** Methodology; **Eric Pardo:** Writing – review & editing; **Hervé Hoste:** Writing – original draft, Visualization, Conceptualization.

Declaration of Competing Interest

We confirm that we do not have any conflicts of interest.

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