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

### **RUMIN-D-23-419R1**

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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 in 13 farms of Auvergne-Rhône-Alpes (AURA) region to evaluate the efficacy of 2 AH authorized for dairy goats: a benzimidazole (BZ) and an avermectin (EPN pour-on). Fecal Egg Count Reduction Tests (FECRT) were carried out including 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 has been given orally or subcutaneously in 4 farms showing a lack of efficacy to EPN pour-on. A resistance to EPN was confirmed in 3 out of 4 farms. Although based on 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.

**KEY WORDS:** Gastrointestinal Nematode, Anthelmintic resistance, Benzimidazole, Eprinomectin, Dairy Goat production

### **INTRODUCTION**

Gastrointestinal nematode (GIN) infections of livestock remain worldwide a main 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 corresponding to strong

dysbalances between the host response and the worm burdens, 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 suckling 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 schemes of treatments. However, several limits in the use of AH have now been identified. **They are first associated with the objective to limit the presence of drug residues in food borne products (meat/milk).** In addition, for some AH families and some specific galenic formulations, the presence of long-term residues in feces have also been related to environmental consequences on the pasture micro fauna (Lumaret et al., 2002, 2012). As a result, regulative rules to limit the use of the 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 (eg, dairy small ruminants in Europe), these regulations can severely limit the use of chemical treatments, including either by the interdiction of some specific AH families and/or molecules when ewes or goats are producing milk or by imposing several days of withdrawal for milk. These restrictions represent a strong impetus for the farmers in the choice of the molecules to treat lactating animals. Finally, the long-term and intensive use of AH to control GIN has led to the exponential development of resistances to the different families of commercialized AH. This has been identified for the main genera and species of the GIN developing in the digestive tract. The phenomenon of AH resistance is present and expanding worldwide including by the rise of multi-resistant isolates. Although 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 remains important to continue to identify the situation of AH resistances in different small ruminants systems and territories and to analyse the factors explaining the possible rise of AH resistance.

To this respect, the conditions of use of AH in dairy goats and ewes in France represent to some extent a possible model to illustrate the possible synergies between severe restrictions in the use of molecules which drive the choice towards molecules with no withdrawal time for milk (anthropic factors) and the adaptation of worm populations to the chemical molecules as a factor of selection for the development of resistance to molecules (biological factors).

In France, for several decades, the control of GIN in grazing, dairy goats have relied principally on the use of **a few molecules (fenbendazole, oxfendazole) of benzimidazoles ( BZs)** with no withdrawal time explaining the early rise of resistances to these molecules in goats (Kerboeuf et al., 1988). So, the BZ resistance 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).

The legal rules to use these BZ molecules in France have changed in 2014 imposing from 4 to 14 days of withdrawal time to commercialize milk for human consumption in all ruminant species (Hoste et al., 2014). Therefore, **eprinomectin (EPN) (EPRINEX ND)** remained the sole molecule with no withdrawal time for dairy cattle. It appeared also a main option in dairy small ruminants when used according to the cascade principles (Fresnay, 2004). More recently, **2 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 (EPRECIIS ND, available in Nov 2020)(<https://www.ircp.anmv.anses.fr>).**

Consequently, for almost 10 years, EPN has been increasingly favored by the farmers to treat the grazing dairy small ruminants, and it was often the unique 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 situation can lead to the rapid development of anthelmintic resistance of GIN to EPN.

However, only few surveys on the rise and diffusion of resistance to EPN and other macrocyclic lactones in worm populations were performed in France. Moreover, these surveys were far more frequent 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 flocks of dairy goats in the South- East of France (Region Auvergne-Rhone-Alpes). **In a second step, we aimed at clearly distinguishing a lack of efficacy due to the mode of administration of EPN (Pour-on) and the related variability of results vs true resistance to the molecule of EPN by using it orally or subcutaneously.**

## **MATERIALS AND METHODS**

### **• Study area and farm selection**

The survey was restricted to the Auvergne-Rhône-Alpes (AURA) region, in the centre and Southeast of France including the Massif Central and the Alpes mountains and the Rhône valley. The region AURA (approximately 70 000 km<sup>2</sup>) is one of the main areas of dairy goats' production in France. The survey was performed during 2 successive winter breaks in the grazing seasons (2020/2021 and 2021/2022), from the end of November to end of February, when goats were out of lactation and maintained indoors, with no access to the pastures.

Farms were selected by the veterinarian based on voluntary adhesion and an evaluation of the level of GIN infection at the end of the grazing season (end of October / November) was performed relying on Faecal Egg Count (FEC) performed on pooled samples from 10 goats, including primiparous and multiparous goats. We performed parasite **egg count** using a modified McMaster protocol proposed by Raynaud et al. (1970) with a solution of zinc sulphate (ZnSO<sub>4</sub>, density = 1.36). Briefly, we mixed in equal proportion feces from 3-5 individuals, and mixed 5g with 70 mL of zinc sulphate (1/15 dilution), **(theoretical sensitivity of 50 eggs per gram)**. Only farms with a mean value > 250 epg were included in the study.

### **• Evaluation of the efficacy of benzimidazoles and eprinomectin**

Within each farm, 3 groups of 10 – 12 animals, when possible, were composed by respecting the proportion of primiparous and multiparous goats according to their proportion in the farm: a control group (C) which remained untreated; a 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, **1mg/kg**, Boehringer). Drugs were administered following **the Marketing Authorization (MA).The doses per goat were 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 surgeon.**

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 group EPN was separated (isolated) for 14 days from the rest of the flock to avoid any contact with the other groups and possible transmission of EPN via licking behaviour. On Day 14 (D14), individual fecal samples were collected from the same goats composing each group to perform FEC after treatment.

**Based on the results of this first trial using the pour-on administration.of EPN, a follow-up protocol was designed in a second step to confirm or not resistance to EPN after a suspicion of lack of efficacy**

due to the “pour-on” formulation. This main question aims in order to secure the control of GINs in dairy goats farms in France and to prevent the development of AH resistance to EPN has been previously evoked and supported by data obtained in controlled conditions (Badie et al., 2015)

Therefore, a second step protocol was designed in 4 farms where an apparent low efficacy of pour-on EPN was detected. The goats, still out of lactation, were distributed in 2 groups (balanced if possible, according to the mean values of EPG on day 14): a control group (Group 1) and a group 2 which was composed, in 3 farms (Farm 1, 5 and 10), by goats treated with off labelled oral use of the pour-on formulation of EPN (EPRINEX MULTI ND, 1mg/kg, Boehringer) and in one farm (Farm 2), by using a recently launched sub cutaneous EPN formulation (EPRECIS ND at 0,2 mg/kg BW). The goats composing the 2 experimental groups of this second step were mainly obtained from the previous control groups and BZ treated groups in step 1. The delay between the first day of the step 1 (first treatment with Bz and/or pour-on EPN) and the start of this step 2 (Treatment with EPN orally or SC) was over 5 weeks. FECRT were performed according to the same way as for the first trial. All individual FEC were measured using a modified McMaster procedure (Raynaud et al, 1970) as described before, but by using a salt solution (NaCl, s.g. = 1.20).

- **Calculation of the efficacy of drugs**

For each farm, mean epg and 90% confidence intervals were calculated using a negative binomial regression (eggs counted in Mac Master cell 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 upper 90 % CI is less than the upper limit of the grey zone. In all other situation, results are inconclusive on the efficacy of the drug.

- **Larval cultures and identification of gastrointestinal nematode species**

Larval cultures were performed on each date based on pooled individual fecal samples per group (Control, BZ or EPN) for both step 3 and step 4. Each animal within a group contributed approximately in a similar quantity to the pooled larval culture (4 to 5 g of feces). The feces 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, one larval culture was performed in duplicates for each farm based on pooled feces. The results correspond to the mean of the 2 lectures. On day 14, the larval cultures were performed per treatment group (control, BZ or EPN pour on).

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..

## RESULTS

### Efficacy of benzimidazoles and eprinomectin pour-on

The survey involved 13 dairy goats' farms from 8 out of the 12 subdivisions (*Departments*) which composed the AuRA Region during the winters 2020-2021 and 2021-2022. The main results of FECRT in the different farms, and thus to evaluate the efficacy of the 2 families of AH drugs (namely BZ and EPN pour-on), are summarised in Tables 1 and 2.

For BZ, a lack of efficacy was detected in 84.6% (n=11/13) farms. For EPN pour-on, the 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%).

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, a full efficacy for both AH families was suspected for one single farm (Farm 4) although strictly speaking EPN CI was just below the limit (88%).

### Lack of efficacy of eprinomectin pour-on vs confirmed resistance to eprinomectin

To confirm the suspicion of resistance to EPN based on previous results identifying a lack of efficacy to EPN pour-on, we used EPN per os or injectable. However, this follow up survey could only be implemented in 4 farms out of the 10 where a lack of efficacy to EPN pour-on was detected.

The lack of efficacy of EPN when used per os or SC was confirmed in 3 out of the 4 farms (Table 3 and 4). On the other hand, in farm 1, a full efficacy was identified when EPN was applied per os whereas a lack of efficacy was suspected when the same drug was used as a pour-on formulation.

### 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). Results were obtained on 12 farms since data of larval cultures were not available for the farm 7. In addition, after some efficient treatments leading to low and/or nil EPG, there were no larvae available for identification.

Overall, the dominant genera found were identified from the group *Teladorsagia/Trichostrongylus* and to *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 group of treatment did not allow any statistical analyses. However, a general stability in the proportion of larvae between the control and the 2 treated groups was observed in the majority of farms. Main changes in the percentage of larvae, when compared to control values on D0 and D14, were observed only after EPN pour-on in farm 3 and 10 with an increased proportion of *Haemonchus* larvae and after BZ treatment in farm 8 (increased proportion of larvae of the group *Teladorsagia/Trichostrongylus*) and farm 12 (increased proportion of *Haemonchus* larvae)

For the last step of the study (EPN per os or subcutaneous in 4 farms) , the results of larval cultures were limited to 2 farms. In farm 5, the composition of larvae of different genera remained stable. In contrast, in farm 10, a rise in the percentage of *Haemonchus* sp. larvae was observed.

## DISCUSSION

This survey is the first one in French dairy goats' flocks aiming at assessing **concomitantly** the efficacy of BZ and EPN on GIN populations. The 13 farms participating to the survey were distributed in different territories of the region AURA and can be seen as representative to the different systems of goats' production in the Region and the epidemiological conditions of GIN infections, because of a wide range of climatic and agronomic conditions (O Connor et al., 2006).

In regard of the lack of efficacy of BZ, which can be assimilated to AH resistance, several surveys have been performed previously in different areas of France, namely Centre, Poitou Charentes, or Midi Pyrénées (Kerboeuf et al., 1988, Chartier et al., 1998; Chartier et al., 2001). However, information **was** missing for the South-East of France although the Auvergne-Rhone-Alpes region remains one the main site of dairy goat production with outdoors breeding. In contrast, surveys in France focusing on the possible lack of efficacy of **pour-on EPN with no withdrawal** time have been far less frequent. They are more recent and mainly focussing 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 concerned only a single observation on one farm when our survey included 13 farms.**

The survey has been performed during winters 2020/21 and 2021/22 when goats are dry and usually maintained indoors. We experienced difficulties to recruit farms for several reasons: 1) all the farmers couldn't separate efficiently the EPN group; 2) because 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) we experienced hot and dry seasons that globally lead to low parasite **infections** and to reject several tested farms; 4) due to COVID, some trials 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 these last recommendations for the statistical interpretation of results. We included a control group and samplings at D0 to take into account of possible differences of excretion between the groups and the natural variation of egg excretion with time (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 analyse 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., 2023)

For BZ drenches, a lack of efficacy can be assimilated to an **AH** resistance to the used molecule and a suspicion of resistance to all molecules of the BZ family. The prevalence rate (85%) observed in our study agrees with those obtained from previous surveys in French dairy goats' farms in other areas/territories which indicated similar and increasing values with time of prevalence for BZ resistances (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 (Rostang et al, 2020), we then evaluated the efficacy of EPN by oral or subcutaneous route in a limited number (4) of farms. Resistance was confirmed in 3 out of 4 farms. Although based on a limited number of farms, the current results suggest a noticeable prevalence of lack of efficacy of EPN in dairy goat farms in AURA. These results tend to confirm recent data obtained in dairy ewe farms in France (Jouffroy et al., 2023) or in goats' and sheep' farms in Switzerland (Murri et al., 2014, Scheuerle et al., 2009).

By facilitating the administration of AH, the development of topical (pour-on) formulation of macrocyclic lactones has represented a main advance for the control of GIN by chemical treatment in cattle. Moreover, the launch of topical EPN with no withdrawal time for milk has represented a **second** main improvements to control GIN in cattle with avermectins. However, beside these 2 mains advantages, further studies have identified some defaults by using the pour-on formulations, some of them being suspected to be potential factors favouring the rise of resistance to EPN in worm populations in ruminants. **Overall, for any EPN pour on formulation in different ruminants species, previous pharmacological studies and results have led to questions on the variable effects of this drug when used pour on and how they can be identified as possible confounding factors affecting the interpretation of FECRT to measure an apparent lack of efficacy of EPN vs a confirmed status of AH resistance ( Rostang et al., 2020; Bouy et al., 2021, Morgan et al., 2022, Badie et al; 2015).**

**These questions related to the use of topical EPN have been identified as pregnant issues in dairy goats' farming because favouring the risk of development of EPN resistance in GINs populations (see review by Lespine et al., 2012).**

**The use of topical EPN in dairy goats have shown individual variations in anthelmintic efficacy with consequences in regard of the efficacy (Badie et al; 2015; Bouy et al, 2021) As a consequence, the use of pour-on formulation of EPN per os has been expanding in dairy goat farms in France to secure the efficacy of eprinomectin. This is acceptable on farm when respecting the frame of the Cascade principle.**

**In regard of the efficacy of pour on EPN against GINs early studies have first shown the need to apply a higher posology in goats (1mg/ kg BW) vs cattle (0.5 mg/ kg BW) (Alvinerie et al., 1999, Chartier et al., 1999) In addition, further studies are also available comparing the efficacy of EPN pour on vs per os. These studies have confirmed a higher and more reproducible efficacy when EPN was used orally (Badie et al, 2015, Silvestre et al., 2007 ).**

**The use of an oral route of a pour on formulation of EPN also questions the risk of possible residues in milk. However, 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 level 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, for our survey, it is worth to underline that although Eprinex multi (ND) is a commercialised 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 statements explain why a **second** step of our survey was designed. The aim was to differentiate a lack of efficacy of pour on EPN vs a suspicion of EPN resistance when this drug was used either off-labelled per os (Badie et al., 2015) or by a sub-cutaneous injection. Because farmers' availability, the number of farms involved in this step 4 was low. The suspicion of resistance to EPN was confirmed for 3 out of 4 farms. Only in one farm, the suspicion of lack of efficacy for pour-on EPN was not confirmed by off-labelled oral administration of EPN indicating a full efficacy.

In regard of the results of larval cultures, as previously mentioned, our results should be considered only as observations because of the lack of replicates per treatment group and thus of possible statistical analyses. Nevertheless, in most farms, the use of EPN pour-on did not seem to induce main changes in the proportion of genera. This stability in genera composition in farms where resistance to BZ was confirmed led to suspect resistances in several GIN genera (*Teladorsagia*; *Trichostrongylus* and *Haemonchus*)

Only in 2 farms, the use of EPN pour on was associated with a rise in the percentage of *Haemonchus* sp. larvae, suggesting a development of resistance to EPN in this species. For farm 3, this was associated with 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 this study 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 eprinomectin-resistant isolates of *H. contortus* in 2 farms of dairy ewes (Jouffroy et al., 2023).

Of main interest are also the prevalence rates of combined lack of efficacy for both BZ and EPN pour-on (69.2 %) based on the first FECRT and the fact that in 3 out of the 4 farms participating in the second FECRT protocol and survey, there was a suspicion of combined resistance to BZ and EPN. Only in one farm, resistance to BZ was observed associated with a maintained EPN efficacy when used off labelled.

This increasing prevalence of multiresistance, including molecules with zero withdrawal time represents a long-term threat for the therapeutic control of GIN in grazing dairy small ruminants in France and probably in the European Union. The management of GIN based exclusively on synthetic AH might become a dead-end therapeutic solution. The GIN parasitic infections can be considered soon as orphan diseases because of the lack of fully efficient anthelmintic treatments. According to Kaplan (2004) and Waller (2006), resistances to anthelmintic and other xenobiotics appears between 10 to 15 years after the commercial launch of novel families. 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 family (AAD) (Kaminsky et al., 2008; Van den Brom et al., 2015).

The repeated and quasi 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, multidisciplinary research examining a range of solutions targeting different stages of the GIN life cycle (Torres Acosta and Hoste, 2008).

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## CONFLICT OF INTEREST

The authors confirm that they do not have any conflict of interest.

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

**Table 2** Calculation of the efficacy (expressed as % FECRT in bold) and CI90% of benzimidazole (BZ) and eprinomectin (EPN) pour-on.

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

**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	<b>1315</b>	1024	1266	<b>775</b>	595	1009	<b>1370</b>	1068	1758	<b>10</b>	3	33
2	<b>345</b>	221	874	<b>890</b>	585	1354	<b>413</b>	252	674	<b>94</b>	51	174
10	<b>1428</b>	951	1084	<b>2267</b>	1517	3387	<b>1400</b>	952	2059	<b>355</b>	233	541
5	<b>1000</b>	638	186	<b>665</b>	421	1050	<b>800</b>	509	1258	<b>600</b>	379	950

**Table 4:** Calculation of the efficacy (%) and CI90% of eprinomectin (EPN) administered orally (farms 1, 5 and 10) or subcutaneously (farm 2)

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

**Table 5** Proportion of the different genera in the different farms before (Day0) and after treatment (D14) with benzimidazole 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		STEP 1 = Day 0			STEP 2=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	<b>No larvae</b>		
					EPN pour on	<b>No larvae</b>		
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	<b>No larvae</b>		
7	Control	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	
					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	<b>No larvae</b>		
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	<b>No Larvae</b>		
2	Control	78	22	0
	EPN SC	<b>No larvae</b>		
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..