First report of multiple resistance to eprinomectin and benzimidazole in Haemonchus contortus on a dairy goat farm in France

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Research paper

First report of multiple resistance to eprinomectin and benzimidazole in *Haemonchus contortus* on a dairy goat farm in France.

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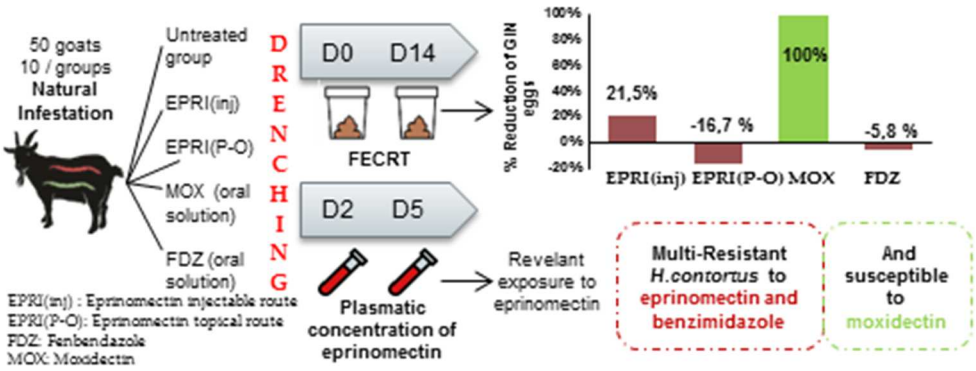
Abstract

Pour-on eprinomectin was recently registered for lactating small ruminants. Given the high prevalence of benzimidazole resistance in gastrointestinal nematodes in dairy goats, many farmers use eprinomectin exclusively to treat their animals. On a French dairy goat farm, a veterinary practitioner noted a poor response to two types of eprinomectin treatment (pour-on application and injectable formulation). Therefore, we evaluated the efficacy of both formulations of eprinomectin, as well as moxidectin and fenbendazole, using the fecal egg count reduction test (FECRT) according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines. Nematode species were identified at days 0 and post-treatment days 14 after bulk larval cultures, by morphology and real-time PCR. Plasma concentrations of eprinomectin were analyzed by high-performance liquid chromatography (HPLC) at post-treatment days 2 and 5 in the eprinomectin-treated groups. Egg count reductions were poor in animals treated with topical (-16.7%; 95% CI:[-237; 59]) or subcutaneous (21.5%; 95% CI:[-126; 73]) eprinomectin, and with fenbendazole (-5.8%; 95% CI:[-205; 63]). *Haemonchus contortus* was the main species identified by morphology and by real-time PCR before and after treatment. The plasma concentrations of eprinomectin were determined in all eprinomectin-treated animals and were above 2 ng/ml at post-treatment day 2, indicating that the lack of effect was not due to low exposure of the worms to the drug. Interestingly, moxidectin remained effective in all infected animals. This is the first report of multiple resistance to eprinomectin and benzimidazole in *H. contortus* on a French dairy goat farm with moxidectin as a relevant alternative.

Keys-word: Goat, Multiple anthelmintic resistance, Benzimidazole, Eprinomectin, *Haemonchus contortus*

Graphical Abstract:



Highlights

- First report of resistance to eprinomectin and benzimidazole on a French goat farm
- On this farm, *Haemonchus contortus* is the multiple-resistant species
- The lack of efficacy of eprinomectin is not due to a low exposure of the worms to the drug
- Moxidectin retained its full effectiveness on this *Haemonchus contortus* population

1. Introduction

Gastrointestinal nematode (GIN) infection is one of the main health threats for grazing dairy goats. Three GIN species predominate in the digestive helminthofauna of goats in temperate countries of Western Europe: *Teladorsagia circumcincta*, *H. contortus* and *Trichostrongylus colubriformis*. All these species cause growth retardation and milk production losses; however, only *H. contortus* infections can lead to high mortality rates in kids as well as in adult dairy goats. *H. contortus* proliferates particularly when climatic conditions are optimal for the development of the free-living stages on pastures (wet and warm periods) and when flock-management practices favor infection (permanent use of small pastures close to the farm). To regulate infection, control measures are thus essential and depend heavily on anthelmintics (AHs) [1]. Since the 1960s, benzimidazole which has a zero-day milk withdrawal period has been used extensively, but given the very high prevalence of benzimidazole resistance in France, the use of this family of AHs is no longer relevant on many dairy sheep or goat farms [2–4]. Levamisole is not approved for use in dairy goats and closantel is tolerated only at the beginning of the dry period, due to the persistence of the drug in milk and milk products [5]. Oral moxidectin is a macrocyclic lactone approved for use in dairy goats and can be used as an alternative to benzimidazole with a short milk withdrawal period (5 days). However, GIN resistance to moxidectin has begun to appear in France, mainly on meat sheep farms [6]. On dairy goat farms, milk is used to make cheese and the goats lactate from 9 to 10 months per year, so the milk residue issue is of major concern and farmers favor the drugs with the shortest milk withdrawal period when treating their animals.

In the nineties, the macrocyclic lactone eprinomectin was developed and approved for use against a broad range of endoparasites (pulmonary and gastro-intestinal nematodes) and ectoparasites (mange, lice and warble fly) in cattle of all ages [7]. Because of its interestingly low partitioning in milk [8], it is the only endectocide approved for use during lactation with a zero-day milk withdrawal period. Until 2016, eprinomectin was available for dairy cattle as a 0.5 mg/kg topical formulation ("pour-on"), but is now also marketed for subcutaneous administration at 0.2 mg/kg for use in cattle only. Since 2016, pour-on eprinomectin is also marketed for dairy sheep and goats with a zero-day milk withdrawal period [9, 10]. However, the pour-on formulation has several disadvantages such as low plasma levels and high individual variability, making the therapeutic outcome uncertain. In order to improve the efficacy of eprinomectin, the dose was increased from 0.5 mg/kg to 1 mg/kg in sheep and goats [9, 11]. In addition, on farms, pour-on eprinomectin is sometimes given orally to dairy

sheep and goats to improve its efficacy [12]. In parallel, the pharmacokinetics and efficacy of a subcutaneous formulation of eprinomectin (0.2 mg/kg) was evaluated in sheep [13] and goats [14, 15] and displayed high efficacy against GIN. However, this formulation is not yet approved for small ruminants, and if used "off-label" then a mandatory milk withdrawal period of seven days is required. Nevertheless, eprinomectin is now considered as the main AH drug in dairy sheep and goats in France and is widely used during the lactation period.

It is well established that the frequent use of the same AH on a farm favors the selection of resistant GIN populations. Increasing numbers of cases of multiple resistance in GIN of small ruminants are being documented in Europe [16] and France [6, 17]; however, data regarding eprinomectin resistance in GIN populations are still scarce, and no cases of resistance have been reported in France. In Brazil, some eprinomectin-resistant populations of *Cooperia* spp., *Haemonchus* spp., *Oesophagostomum* spp., *Ostertagia* spp. and *Trichostrongylus* spp. have been reported in cattle [18]. Eprinomectin-resistant *H. contortus* populations have been recorded in goats from Switzerland and Southern Germany [19, 20]. In northern Italy [21], eprinomectin resistance was suspected on one farm but was not confirmed at a later date. It is important to note that since plasma concentrations of the drug were not reported in these studies, we cannot exclude that the loss of efficacy was due to low exposure of the worms to the drug, rather than true worm resistance.

In the present study, we report the first case of multiple resistance to eprinomectin and benzimidazole in *H. contortus* on a dairy goat farm in southwestern France. This study was performed according to the WAAVP guidelines [22]. Multiple resistant species were identified both by the morphology of infective larvae [23] and using molecular tools [24]. Plasma concentrations of eprinomectin were measured to determine the level of exposure of the worms to the AH in the treated animals. Finally, the factors that may be involved in the selection of resistant GIN populations on this farm are discussed and recommendations for dairy goat farmers are provided.

2. Materials and methods

2.1 Farm history

This study was performed on a farm comprising 70 adult dairy goats of the Alpine breed, located in the French Pyrénées-Atlantiques *département* (a *département* is a French administrative and territorial unit) in southwestern France. This flock was first established in

2012 with the purchase of 30 one-month-old kids from several different flocks. The kids were not infected with gastro-intestinal nematodes because they were born and kept indoors until the date of purchase. Each year since 2012, all does undergo artificial insemination (AI) in September, and non-pregnant does after AI are mated with bucks in October.

The kids, born in February or March, remain indoors until aged 1 year. Lactating goats are kept indoors from September to March but are allowed to graze the rest of the year. More precisely, a first pasture of 4 ha is grazed two to three hours per day from March to June. From July to September, a second pasture of 1 ha is grazed. As grass availability is not sufficient to cover the nutritional needs of the lactating animals, they are given concentrates two times per day.

Lactating goats were treated regularly with anthelmintics from 2012 to 2017: twice a year with eprinomectin (EPRINEX Multi®, Boeringher Ingelheim, 1 mg/kg of bodyweight (BW)) in June and August. A third treatment was administered every year in December (SUPAVERM®, Elanco France, with closantel, 10 mg/kg BW and mebendazole, 15 mg/kg BW) to control gastro-intestinal nematodes and liver fluke.

In 2017, the farm experienced an outbreak of enterotoxemia which was finally contained by vaccination. In summer 2018, lactating goats showed weakness, facial edema and pale ocular mucosae associated with a high mortality rate (17%, whatever the age of adult goats). The flock was treated in June 2018 with EPRINEX Multi® (1 mg/kg BW), but no improvement in the goats' health was observed. Further fecal examinations were performed on July 20th and showed substantial levels of GIN egg excretions (composite fecal egg count of 1,650 eggs per gram (EPG)). The goats were immediately treated with EPRECIS® (CEVA Santé Animale, 0.2 mg/kg BW subcutaneously). However, a second round of fecal examinations fourteen days later did not show any reduction in GIN egg excretion (composite fecal egg count of 2,500 EPG).

At housing (beginning of October), another series of analyses showed high egg excretions in five out of 10 lactating goats (from 1,900 to 11,450 EPG) and poor hematological values (packed cell volumes below 20% in five animals). Subcutaneous eprinomectin (EPRECIS® 0.2 mg/kg BW) was again administered to these five high egg-shedding goats with poor reduction values again 14 days later (reduction rate: 63%). A fecal egg count reduction test following eprinomectin treatment (both topical and injectable routes) was therefore performed

147 in November 2018. At the request of both the vet practitioner and goat farmer, the efficacies
148 of moxidectin and fenbendazole were also tested on this farm.

149 2.2 Study design, efficacy calculation and evaluation of anthelmintic resistance

150 According to the guidelines of the WAAVP [22], the FECRT was performed on this flock.
151 Firstly, in November 2018, fecal samples were collected for the entire adult flock (70 goats)
152 to measure individual GIN egg excretion. Animals were ranked according to their pre-
153 treatment Fecal Egg Count (FEC). The modified-Mc-Master method [25], with a sensitivity of
154 15 EPG, was used to determine the individual fecal egg counts. Based on these results, the
155 50 goats with the highest FECs were allocated to five well-balanced groups of ten animals
156 according to their FEC and age. One group remained untreated (control group), while the
157 other four groups were treated with commercially available anthelmintics: injectable
158 eprinomectin (Inj-EPRI) (EPRECIS®, CEVA Santé Animale, 0.2 mg/kg BW subcutaneously),
159 topical eprinomectin (pour-on EPRI) (EPRINEX Multi®, Boeringher Ingelheim, 1 mg/kg BW,
160 along the dorsal line from the withers to the tail head), oral moxidectin (MOX) (CYDECTINE
161 0.1%®, ZOETIS, 0.2 mg/kg BW), and oral fenbendazole (FBZ) (PANACUR®, MSD Animal
162 Health, 5 mg/kg BW). Treatment was initiated (day 0 (D0)) as soon as the FEC results were
163 known (24 hours post-sampling) by the vet practitioner. Young animals (born in 2017)
164 received doses equivalent to 70 kg BW, whereas adult goats received doses equivalent to 80
165 to 100 kg BW. Based on the goats' body condition scores at the time of the study, these body
166 weights were clearly overestimated. At day 14 (D14), individual fecal samples were collected
167 again. The animals remained indoors during the whole test.

168 To calculate the percentage of reduction, we used the formula:

169 Efficacy = 100 x (1 – arithmetic mean EPG of the treated group at day 14 / arithmetic mean
170 EPG of the control group at day 14).

171 WAAVP guidelines state that anthelminthic resistance (AR) occurs when the percentage of
172 reduction in egg counts is less than 95% and when the 95% confidence interval (CI) is less
173 than 90%. If only one of the two criteria was met, the finding should be recorded as suspected
174 AR (SAR) [22].

175 2.3 Identification of gastrointestinal nematode species

176 To identify GIN species in each group, a composite larval culture was made at each date (D0
177 and D14) and for each group. Each animal within a group contributed more or less equally to

the composite larval culture (4 to 5 g of feces) but for some animals, this amount of fecal material was not available. All composite larval cultures were incubated for 12 days at 24 °C ± 1 °C and humidified every two days with tap water. Third stage larvae (L3s) were recovered by filling the beaker with tap water at room temperature (+/- 25 °C) and inverting it on a Petri dish [26]. In a first step, L3s were collected in a volume of 40-45 mL of tap water then centrifuged (10 minutes at 4,500 rpm) to obtain a final suspension of 5 mL. These suspensions were stored at 4 °C until the counting and identification step. Morphological identification of the larvae was performed according to the criteria of Van Wyck and Mayhew [23]. In addition, molecular identification of GIN species was performed using real-time PCR according to Milhes et al. [24]. Briefly, genomic DNA was extracted and purified from 500 µL of homogenized larval suspension using the DNeasy PowerSoil kit (QIAGEN). All experiments were based on real-time PCR assays using TaqMan technology in simplex PCR reactions. The primers and probes used are described in detail in Milhes et al. [24]. Standard curves for larval DNA quantitation were established for each PCR run and for the three species *H. contortus*, *T. circumcincta* and *T. colubriformis.* Additional data are available in Milhes et al. [24].

2.4 Blood sampling and eprinomectin determination

Blood samples were collected on post-treatment days 2 (D2) and 5 (D5) in the two eprinomectin groups. Eprinomectin plasma levels were determined using HPLC according to the previously described method [27]. The quantification limit of the method was 0.07 ng/mL, and inter-assay coefficients of variation were below 5%. The differences in the plasma levels of eprinomectin between the animals treated with the two formulations were examined using a Kruskal-Wallis non parametric test.

3. Results

Table A shows FEC and FECRT values at day 0 and day 14. At day 0, although significant between-individual variation was observed for FEC (for example in the control group: 200 to 10,350 EPG), the level of egg excretion was high in most of the goats, revealing substantial GIN infestation. In each treatment group, mean EPG values (from 1,280 to 2,135 EPG) were consistent the values of the control group (2,135 EPG), and the median values were similar in all groups (from 825 to 1,025 EPG).

At post-treatment day 14, mean EPG values remained high after treatment with FBZ (1,045 EPG), injectable EPRI (775 EPG) and pour-on EPRI (1,153 EPG), and in the same range as the untreated group (987 EPG). The median values for these treatment groups (ranging from 275 to 575 EPG) were slightly lower than for the control group (800 EPG). On the other hand, at day 14, no GIN eggs were observed in any of the individual samples from the MOX group, demonstrating the efficacy of this molecule on this farm.

Very poor reduction of egg excretion were calculated for the FBZ (-5.8%), injectable EPRI (21.5%) and pour-on EPRI (-16.7%) groups. Moreover, the lower limit of the 95% CI was -205%, -126% and -237% respectively in the 3 groups and the upper limit was 63%, 73% and 59%, respectively. According to the criteria of the WAAVP guidelines, AR was therefore demonstrated in these groups.

Tables B and C show the morphological and molecular identification results of L3s, respectively, following fecal culture. At day 0, *H. contortus* was the highly predominant species (93 to 99% of larvae by morphological identification depending on the group), followed by *Trichostrongylus*/*Teladorsagia* species. No larvae of *Oesophagostomum venulosum* or *Chabertia ovina* species were identified morphologically. This result was confirmed by real-time PCR, which demonstrated the predominance of *H. contortus* (87% to 98.6% of larvae). The DNA of *Trichostrongylus* spp. and *T. circumcincta* were detected in small and very small proportions respectively. Regarding morphological identifications at day 14, *H. contortus* was the unique species identified in all GIN-positive groups, including the untreated group. However, in the group treated with MOX, no larvae were observed. No GIN DNA was detected by real-time PCR in the larval culture of the MOX group. All other groups demonstrated a high proportion of *H. contortus* (89.3% to 98.7%) and a small proportion of *Trichostrongylus* spp. (1.3 to 10.7%).

Individual plasma concentrations of eprinomectin at post-treatment days 2 and 5 are shown in Figure A.1 (injectable eprinomectin, 0.2 mg/kg BW) and Figure A.2 (pour-on eprinomectin, 1 mg/kg BW). For injectable eprinomectin, the mean plasma concentrations were 10.2 ± 3.8 ng/mL and 3.6 ± 1.3 ng/mL at days 2 and 5, respectively. The mean eprinomectin plasma concentrations measured after pour-on administration of the drug were 2.91 ± 0.49 ng/ml and 1.77 ± 0.43 ng/ml at days 2 and 5. As expected, drug concentrations in plasma after pour-on administration were significantly lower than those measured after subcutaneous administration (p-value = 0.0002 and p-value = 0.0028 at days 2 and 5, respectively), while

pour-on formulation contains five times more active drug. In all cases, there were high individual variations of plasma concentrations as observed.

4. Discussion

Our study shows a low fecal egg count reduction (FECR) after treatment with eprinomectin, whatever the route of administration, and with benzimidazole. This clearly indicates the lack of efficacy of these two drugs against *H. contortus* in the flock studied. Interestingly, this *H. contortus* population remained highly sensitive to moxidectin. While there is some degree of cross resistance between the avermectin and moxidectin [6], it is well documented that moxidectin at the recommended dose can still be highly effective in ivermectin-resistant nematode isolates in sheep or goats [28]. A similar pattern was recently reported in a *H. contortus* isolate from a meat sheep farm located in the French Pyrenees [17]. Since the lack of efficacy could be due to suboptimal exposition of the worms to the active compound, we measured the plasma concentrations of eprinomectin in the two eprinomectin-treated groups at days 2 and 5 following drug administration which correspond, respectively, to the peak drug concentration in the plasma and the last elimination phase. The concentrations measured in this study were in the same range as those reported in the literature using similar treatment protocols [15, 29]. As expected, plasma levels were higher at days 2 and 5 in the animals that received subcutaneous eprinomectin compared with pour-on eprinomectin. The animals treated by subcutaneous injection hence displayed higher levels of the drug in their blood, even though they received five times less drug (0.2 mg/kg) than those treated by topical administration (1 mg/kg). These results are in complete agreement with the well-known poor availability when the drug is applied topically when compared with the subcutaneous route.

Nevertheless, most of the eprinomectin concentrations measured in both groups treated with eprinomectin were above 2 ng/mL, which is considered as the minimal active concentration for macrocyclic lactones [9, 30], indicating that the lack of efficacy was not primarily due to suboptimal exposition of worms to the drug.

Therefore, based on drug concentrations, FECRT and the presence of GIN DNA in bulk larval cultures, we conclude that the nematodes infecting the goats on this farm were resistant to eprinomectin. We clearly identified that *H. contortus* was the resistant species. Indeed, before implementing the FECRT on this dairy goat farm, a high mortality rate was reported with anemia (assessed by pale color of ocular mucosae and decreased packed cell volumes) and heavy egg excretions, which are typical symptoms of *H. contortus* infection. This was

confirmed by the morphological and molecular identification of *H. contortus* infective larvae obtained after larval cultures in both eprinomectin-treated groups.

We noted some between-group variations of the number of L3/mL after larval culture. As previously stated, each composite larval culture was made with the same amount of individual fecal samples, but the feces of some animals were absent from the larval cultures due to an insufficient quantity of fecal material.

The identification of very few *T. colubriformis* revealed that multiple resistance appears to be marginal in this species. It is important to note that *H. contortus* was the only eprinomectin-resistant species identified in a previous study [18] and the main eprinomectin-resistant species in another [19]. The primary causes of multiple drug resistance in *H. contortus* isolates are still under debate. The flock described in this study is relatively young. In 2012, the farmer purchased 30 one-month old female kids, which marked the beginning of the farm. At that time, no quarantine or drench was applied to these young kids because they were kept indoors from their birth to their arrival in the flock. Interestingly, no new kids or bucks have been introduced to the flock since 2012. Consequently, multiple resistance to eprinomectin and benzimidazole probably occurred on this farm over a short period (2012 to 2018) and involved many different factors. Firstly, the animals were treated frequently over a short grazing period that did not exceed six months (from March to September). Three AHs were administered routinely to the adult goats: two eprinomectin treatments in June and August and another treatment that combined mebendazole and closantel in December. From 2012 to 2017, pour-on eprinomectin was used off-label, often at 0.5 mg/kg BW (i.e. the half of the recommended dose for goats), with a fixed milk withdrawal time of seven days. Sometimes the product was administered orally (Dumont, personal communication). Badie *et al.* [12] reported that the oral administration of topical eprinomectin was 100% efficient against GIN and that maximum concentrations of eprinomectin residues determined in milk after oral treatment were below the Maximum Residue Limits for goat milk (defined by the European Medecines Agency, 27 July 2018, EMA/CVMP/607398/2017). However, changing the recommended route of administration of an AH drug is not allowed. In 2017 and 2018, the number of eprinomectin treatments was increased from two to three per year due to the low response of treated animals in terms of health and milk production recoveries. This probably accelerated the resistance selection process over the last two years. Most of goats were lean in 2017 and 2018, and the extensive loss of fat reserves may have contributed to diminished drug levels in heavily infected animals [31], leading to sub-therapeutic concentrations of the drug, and favoring the selection of drug resistant worms. Because pour-on eprinomectin is

active for only a relatively short time (around 15 days) compared with the grazing period [32, 33], goat farmers are advised to treat their animals several times during the grazing period. In the present study, the whole lactating adult flock was treated with eprinomectin each time, without prior FEC assessment. Therefore, almost no refugia were maintained for susceptible worms. Finally, the use of very small pastures (3 ha for 70 adult goats) inevitably led to overgrazing and heavy contamination, and hence repeated treatment. When combined, these factors created an ideal environment for development of multiple drug resistance.

In this context, it is important to propose appropriate recommendations for goat farmers in order to minimize the impact of resistant GINs on animals, reduce the population of resistant GINs and avoid the selecting of drug-resistant worms. Zero-grazing for a period of at least one year (to let multiple-resistant *H. contortus* infective larvae completely disappear from the pastures) was proposed as an option. The producer refused this option as his business image was based on grazing animals on pasture. Obviously, eprinomectin and benzimidazole cannot be now used on this farm. Since moxidectin inhibited egg excretion in the flock, it still represents a relevant alternative. But it should be used with parsimony to avoid rapid selection of resistance. Closantel was not tested in this study but should be considered as a possibility, and its efficacy on this *H. contortus* isolate should be tested as soon as possible. The recommended treatment scheme could be the alternation of oral moxidectin during the lactation period (five days of milk withdrawal) and closantel at the beginning of the dry period. However, to avoid the selection of moxidectin resistance, treatment should be targeted and selective after FECs performed on a representative number of animals of the flock. Hoste *et al.* [1] demonstrated that leaving a part of the flock without treatment during the grazing period is a possible option. Epidemiological observations identified the categories of host populations at risk (high milk producers and young adult goats). Targeted selective treatments on this basis were shown to be relevant in dairy goat farms as they did not compromise the annual amount of milk production on the farm and because this strategy reduces the amount of endectocide residues in feces which affect the non-target fauna [34]. Pasture management including frequent tillage and ray grass seeding could be useful to decrease the contamination of grazed areas with infective larvae between two grazing seasons.

The presence of a *H. contortus* isolate with multiple resistance to eprinomectin and benzimidazole is alarming in southwestern France. In this region, there are more than 2,500 dairy sheep farms (representing more than 500,000 dairy ewes) and rely exclusively on eprinomectin during lactation. Eprinomectin resistance is probably currently being selected on

many of these farms, but data are not yet available. However, we cannot exclude the risk of multiple-resistant *H. contortus* dissemination [35] by wild animals (in particular roe deer) that could transport resistant worms from one farm to another. This threat should be taken into account and regular evaluations of anthelmintic efficacy should be proposed to sheep and goat farmers.

5. Conclusion

The multiple-resistant *H. contortus* isolate described in this study highlights the alarming situation regarding the control of gastrointestinal nematodes on livestock farms, mainly due to the low number of anthelmintic alternatives currently available for this farmer and the high pathogenicity of *H. contortus*. It is therefore important to establish a new therapeutic scheme to prevent the further spread of resistance. Strategies including maintaining refugia, alternation of molecules and targeted selective treatment have to be put into practice and appropriate GIN control recommendations should be communicated to all farmers and veterinarians in this region.

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Conflict of interest

The authors declare that there were no conflicts of interest at any point of time.

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Table A: Fecal egg counts (mean, SD, median, minimum and maximum values) at day 0 and day 14 after treatment, number of positive animals at day 14 and results of fecal egg count reduction tests FECRT (% of reduction and 95% confidence interval, CI) within each group.

Table B: Morphological identification of infective larvae obtained from bulk cultures in each group at day 0 and day14 post-treatment.

Table C: Gastrointestinal nematode species in the different groups at day 0 and day 14 based on the molecular identification of L3 larvae.

Figure A: Eprinomectin concentrations in plasma at day 2 and day 5 post-treatment in goats treated (1) with injectable eprinomectin (Eprecis ND; 0.2 mg/kg BW), or (2) pour-on eprinomectin (Eprinex Multi ND; 1 mg/kg BW). Day 2 (blue) and day 5 (red).

497 Table A:

	Day 0 (fecal egg count)				Day 14 (fecal egg count)					**Reduction** % [95% CI]
Group	Mean	SD	Median	[Min – Max]	Mean	SD	Median	[Min - Max]	Nb of positive goats	
Control	2,225	3,120	1,025	[200-10350]	987	930	800	[100-3000]	8/10	-
Injectable eprinomectin	2,135	2,353	975	[150-7500]	775	971	275	[0-2650]	9/10	**21.5%** [-126; +73]
Pour-on eprinomectin	1,765	2,031	900	[100-6350]	1,153	1,709	400	[0-5250]	9/10	**-16.7%** [-237; +59]
Oral moxidectin	1,565	1,752	875	[100-5650]	0	-	-	-	0/10	**100% -**
Oral fenbendazole	1,280	1,246	825	[100-3500]	1,045	1,116	575	[50-2850]	10/10	**-5.8%** [-205; +63]

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499 Table B:

Group	Date	Total number of L3/ mL estimated by counting larvae in 80 microliters	Morphological identification of infective larvae (according to Van Wyk and Mayhew [23])		
			Number of *H. contortus* larvae	Number of *Teladorsagia/ Trichostrongylus* larvae	Number of *Oesophagostomum/ Chabertia* larvae
Control	D0	5,400	99	1	0
Injectable eprinomectin	D0	3,250	95	4	0
Pour-on eprinomectin	D0	2,625	93	5	0
Oral moxidectin	D0	2,850	97	2	0
Fenbendazole	D0	4,425	97	3	0
Control	D14	337	43	0	0
Injectable eprinomectin	D14	760	100	0	0
Pour-on eprinomectin	D14	5,000	100	0	0
Oral moxidectin	D14	0	0	0	0
Fenbendazole	D14	337	47	0	0

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501 Table C:

Group	Date	Total number of L3/ mL estimated by Real-Time PCR	Molecular identification of infective larvae (Real-time PCR according to Milhes et al. [24])		
			% of *H. contortus* larvae	% of *Teladorsagia circumcincta* larvae	% of *Trichostrongylus* spp. larvae
Control	D0	29,486	96.7	0.1	3.2
Injectable eprinomectin	D0	12,594	91.9	0.2	7.9
Pour-on eprinomectin	D0	7,764	98.6	0.6	0.8
Oral moxidectin	D0	11,466	87	0.8	12.2
Fenbendazole	D0	15,696	91	0.4	8.6
Control	D14	1,388	89.3	0	10.7
Injectable eprinomectin	D14	6,214	98.7	0	1.3
Pour-on eprinomectin	D14	35,882	97.4	0	2.6
Oral moxidectin	D14	0	0	0	0
Fenbendazole	D14	728	97	0	3

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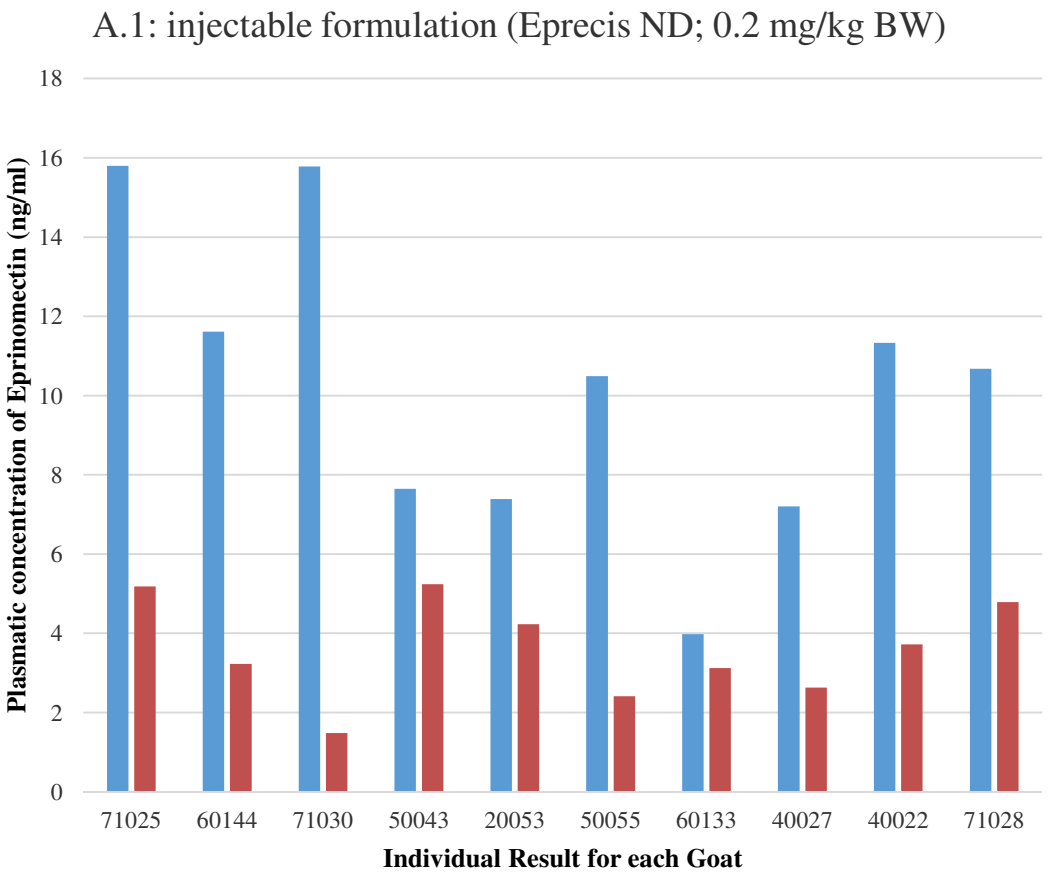
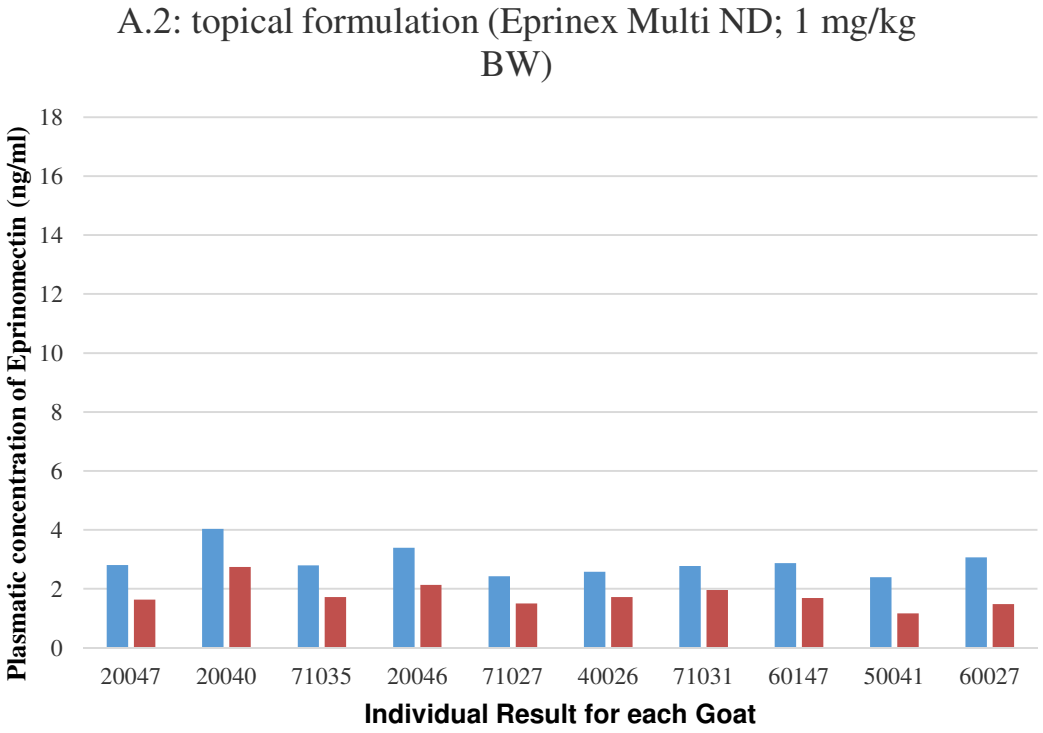
503 Figure A.1:



504

505 Figure A.2



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