Lack of efficacy of topical administration of eprinomectin against gastrointestinal nematode in a French dairy sheep farm: A case of underexposure of worms

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CASE REPORT

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Lack of efficacy of topical administration of eprinomectin against gastrointestinal nematode in a French dairy sheep farm: A case of underexposure of worms

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Abstract

Resistance to eprinomectin was suspected in a dairy sheep farm in southwestern France. The efficacy of topical and injection formulations of eprinomectin against gastrointestinal nematodes (GINs) was compared using a faecal egg count reduction test. GIN species were identified by real-time PCR, and eprinomectin concentrations were measured in serum by High-Performance Liquid Chromatography 2 and 5 days after treatment. Efficacies were 99.6% and 86.1% for injection and topical formulations, respectively. Before treatment, the three species *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* were identified in both groups. After treatment, *H. contortus* and *T. colubriformis* were identified in the topical group only. Two days after treatment, eprinomectin concentrations were above 2 ng/ml in the injection group and under this value in the topical group, suggesting underexposure of GIN to eprinomectin in this latter group. High levels of exposure to eprinomectin are important to avoid loss of efficacy in the field.

BACKGROUND

The control of gastrointestinal nematode (GIN) infection in small ruminants essentially involves chemical control due to the low cost and treatment convenience for the farmers. However, risky practices, such as frequent and systematic use of the same anthelmintic family, may promote the emergence of resistance to GINs. For decades, benzimidazoles have been widely used, but the resistance of GINs to this family is now well established in many countries including France.[1–5] Consequently, small ruminant farmers have switched to the macrocyclic lactone (ML) family; but recently, resistance to ivermectin has been reported for the first time on French meat sheep farms.[6,7]

In small ruminants, both formulations of eprinomectin have recently been registered in Europe for the control of GIN and lungworms. High levels of efficacy against GIN in small ruminants have been reported for topical[8–10] and injectable[11–13] formulations of eprinomectin, while their dosing is quite different: the pour-on (PO) formulation is dosed at 1.0 mg/kg per kg bodyweight (BW), and the injectable (SC) formulation is dosed at 0.2 mg/kg BW.

One of the main dangers for the small ruminant dairy industry is the resistance of GIN to eprinomectin, of which a first French case was recently published on a dairy goat farm.[14] Not all treatment failures are due to GIN resistance but can also be due to low exposure of the animal to this drug due to subtherapeutic dosage or impaired absorption.

In this study, we investigated the reason for the lack of efficacy following topical application of eprinomectin in dairy sheep.

CASE PRESENTATION

This case involved a blond-faced Manech dairy sheep breed farm located in the Pyrénées-Atlantiques *département* in southwestern France. This flock, composed of 300 dairy ewes and 60 replacement ewes lambs, grazes 10 months per year. To control GIN infections, the farmer uses on average two treatments per year, one with eprinomectin during lactation and another one during the dry period with ivermectin. In March 2019, the practitioner noticed, during a visit requested by the farmer, signs of anaemia in about 10% of the lactating

ewes determined by FAMACHA criteria[15] and an average body condition scores around 2.5[16] despite an anthelminthic treatment performed by the farmer in February 2019.

Following this observation, the practitioner proposed to evaluate the efficacy of eprinomectin by comparing PO and subcutaneous (SC) routes of administration.

INVESTIGATIONS

A faecal egg count reduction test (FECRT) was performed as described by Coles et al.[17] At the end of March 2019, ewes were randomly assigned to three groups of 12 animals: an untreated group (control group), an eprinomectin-treated group with the PO formulation (EPRINEX Multi, Boeringher Ingelheim, 1 mg/kg BW) and an eprinomectin-treated group with the SC formulation (EPRECIS, CEVA Santé Animale; 0.2 mg/kg BW). Treatments were carried out by the practitioner, who was careful to part the wool and deposit the product on the skin of the animal for the PO formulation. Ewes were dosed at the rate recommended by the manufacturer for 80 kg, which was heavier than the heaviest animal in the group: the average weight of blond-faced Manech ewes is around 60 kg. The identification number of the ewes was taken, and they were marked with coloured spray paint to ensure their quick identification in the flock for blood and faeces sampling. Faecal samples were collected individually to determine the GIN egg excretion. The number of eggs per gram (EPG) of faeces was determined by using the modified McMaster method,[18] with a sensitivity of 15 EPG. Animals excreting less than 150 EPG were excluded from the study. On day 14, individual faecal samples were collected again, and the percentage of reduction was calculated according to the formula:[17]

$$\text{Efficacy} = 100 \times (1 - \text{arithmetic mean EPG of the treated group at day 14/arithmetic mean EPG of the control group at day 14})$$

Other calculation methods, taking into account the D0 of each group, estimate the same efficacies. Therefore, we have only used the guidelines formula.

According to the guidelines,[17] anthelminthic resistance is confirmed when the percentage of reduction is less than 95% and the lower 95% confidence interval is less than to 90%.

On day 0 (D0) and day 14 (D14), composite larval cultures were made for each group to identify GIN species. Each animal contributed more or less equally (4–5 g of faeces), but for some animals, the quantity of faeces was not sufficient, and such animals contributed poorly to the composite larval cultures (0–3 g of faeces). These animals were few in number (less than five in total in the study), which should not have had a major impact on the larval culture results.

Larvae were collected by filling the beaker with tap water and inverting it on a Petri dish at room temperature (± 25°C) in a volume of 40–45 ml of tap water[19] before centrifugation (10 minutes at 3500 rpm) to concentrate the larvae to a final larval suspension volume of 5 ml. These suspensions were stored at 4°C until identification. The identification of GIN species was performed using real-time PCR assays.[20] Briefly, 500 μl of homogenised larval suspension allowed the extraction and purification of genomic DNA with the DNeasy PowerSoil kit (QIAGEN). The primers and probes used and the PCR process are described in Milhes et al.[20] and can specifically detect and quantify *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* larvae.

Blood samples were collected from the two eprinomectin-treated groups at day 2 (D2) and day 5 (D5) after treatment. Individual plasma eprinomectin concentrations were determined using the High-Performance Liquid Chromatography (HPLC) method,[21] with a limit of quantification of 0.07 ng/ml and interassay coefficients of variation below 5%.

LEARNING POINTS/TAKE-HOME MESSAGES

- Pour-on administration of eprinomectin gives plasma concentrations below the minimum required plasma concentrations, which leads to underdosing and failure of treatment.
- The eprinomectin Pour-on formulation is less effective than the injectable formulation, while using a five times higher dosage represents an increased environmental risk.
- The lack of efficacy of anthelmintics is not always synonymous with gastrointestinal nematode (GIN) resistance. It may also originate from an insufficient exposure of worms to the drug.
- In a sustainable approach to GIN control, it is advisable to use the formulation with the best bioavailability and the least ecotoxicity.

OWNER'S PERSPECTIVE

In the presented case, eprinomectin is still effective. During the lactation period, this sheep breeder will be invited to perform eprinomectin treatments with an injectable formulation, and the dosage will be based on the weight of the heaviest animal in the treated group. Outside the lactation period, the farmer can use another class of drugs to treat his animals, such as closantel, authorised in France for dairy ewes at the beginning of the dry period to avoid exclusive use of avermectins in his flock.

OUTCOME AND FOLLOW-UP

The results of FECRT are reported in Table 1. Eprinomectin PO showed an efficacy of 86.1% (79.1–93.1), while the eprinomectin injection showed an efficacy of 99.6% (98.2–101).

The species composition of larval suspensions obtained after faecal cultures in the three groups before and after eprinomectin treatment is reported in Table 2. The predominant species in all groups (with over 90%) was *H. contortus* at D0. At D14, the species composition did not change in the control group. No larvae were obtained in the group treated with

TABLE 1 Faecal egg count at day 0 and day 14 (after eprinomectin treatment) and results of the faecal egg count reduction test (FECRT)[17]

Group (*n*)	Eggs per gram		FECRT (%)
	Day 0	Day 14	
Control (12 ewes)	1213 (150–3600)	1363 (150–5500)	–
Eprinomectin pour-on (10 ewes)	1279 (250–3800)	190 (0–300)	86.1 (79.1–93.1)
Eprinomectin injection (11 ewes)	1238 (600–2350)	5 (0–50)	99.6 (98.2–101)

TABLE 2 Gastrointestinal nematode species before and after treatment with eprinomectin based on molecular identification of L3 larvae[20]

	Number of L3 recovered for RT–PCR		Molecular identification of infective larvae					
			Haemonchus contortus (%)		*Teladorsagia circumcincta* (%)		*Trichostrongylus colubriformis* (%)	
Date	D0	D14	D0	D14	D0	D14	D0	D14
Control	11,574	38,730	99.6	93.8	0.4	0.5	0	5.6
Eprinomectin pour-on	16,450	7126	90.7	36	0.2	0.3	9.1	63.7
Eprinomectin injection	2434	0	96.5	0	0.2	0	3.4	0

Abbreviation: RT-PCR, real-time PCR.

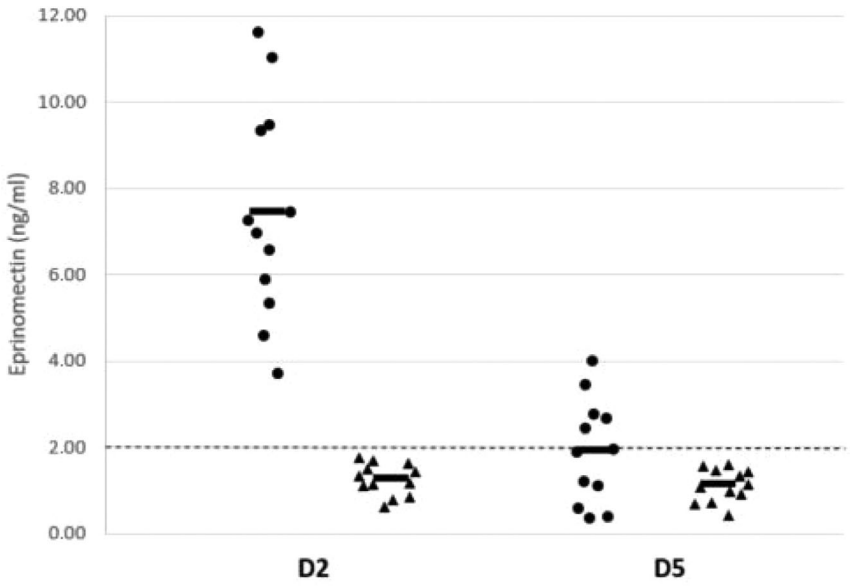

FIGURE 1 Eprinomectin concentrations in plasma at days 2 and 5 post-treatment in ewes treated with (circles) eprinomectin injection (Eprecis ND; 0.2 mg/kg bodyweight [BW]) or (triangles) eprinomectin pour-on (Eprinex Multi ND; 1 mg/kg BW). Days 2 and 5 are individually expressed for each ewe. Dashed lines show the minimal active concentration for full efficacy for macrocyclic lactones,[8,28] estimated to be 2 ng/ml at day 2. The black bar represents the average concentrations in each treatment group and on each date

injection eprinomectin due to the fact that only one animal still excreted GIN eggs at a very low excretion intensity (50 EPG). For the PO-treated group, the proportion of *H. contortus* decreased (36%) and those of *T. colubriformis* increased (63.7%).

The results of individual eprinomectin plasma concentrations at D2 and D5 posttreatment are reported in Figure 1. In the eprinomectin injection group, the mean concentrations were 7.44 ± 2.48 ng/ml at D2 and 1.91 ± 1.20 at D5. In the eprinomectin PO group, the mean concentrations were 1.12 ± 0.38 ng/ml and 1.26 ± 0.37 ng/ml at D2 and D5, respectively. Important individual variations were noticed in the two groups treated with eprinomectin. Despite animals in the eprinomectin PO group being treated with a dosage five times higher than animals in the eprinomectin injection group, this latter group showed the highest eprinomectin plasma concentrations at D2.

DISCUSSION

In this study, the efficacy of two formulations of eprinomectin (injection and topical) was investigated in a dairy sheep farm in southwestern France. The GIN species recovered were mainly *H. contortus* and, in smaller quantities, *T. circumcincta* and *T. colubriformis*. These differences in proportions are not surprising according to the ecology of *H. contortus*. Indeed, the region has a temperate climate with high annual rainfall (over 1500 mm per year), which is well suited to the development of this parasite.[22]

The FECRT results clearly showed a discrepancy between the two formulations: A low efficacy was detected for the PO group, whereas injectable eprinomectin was fully efficient. This result is surprising considering that the concentration of eprinomectin is five times higher in the topical formulation than in the injectable formulation.

These results do not support the hypothesis of GIN resistance to eprinomectin on this farm but rather an underexposure of GIN in the case of topical formulation. If there had been GIN resistance, both formulations should have shown reduced efficacy.[14] Previous studies reported resistance to eprinomectin mainly in one GIN species, in particular, *H. contortus*.[23–25]

To validate the hypothesis of underexposure of GIN to eprinomectin, plasma concentrations at days 2 and 5 were compared between the two treated groups. The concentrations measured showed some differences from those of previous studies.[9,13,14,26] Serum concentration levels were higher at days 2 and 5 in animals treated with injectable eprinomectin, compared to animals treated with the PO formulation; this difference has been shown previously in goats.[14] However, serum eprinomectin concentrations in the PO group in this study were lower than those obtained in the study of Hamel et al.[8] These differences can be explained by biological and physiological differences in the animal used for the test. The animals used in this test were ewes of dairy breed, of various ages, some of them heavily parasitised, at the peak of milk production. In Hamel et al.'s study,[8] the animals used were non-lactating ewes from Merino crosses, 4.5 years old, not pregnant and not parasitised. This has been shown by Rostang et al.[27] that these differences induce important differences in pharmacokinetics.

Previous studies have proposed a minimal active concentration of 2 ng/ml for MLs including eprinomectin.[8,28] In our study, only the injectable eprinomectin group reached these concentrations in all individuals. In contrast, in the PO eprinomectin group, no animal reached this concentration, which may explain the low efficacy in this group. Thus, the low efficacy observed and its impact on the three identified GIN species are likely due to a lack of drug exposure and not to GIN resistance to eprinomectin.

A high frequency of treatment with the same molecule class and underexposure of nematodes to the active molecule are risk factors for the development of resistance.[29,30] Added to other risk factors, such as the introduction of animals carrying resistant nematodes in the flock,[29] transhumance (use of summer grazing pasture common to several flocks) or the systematic and exclusive use of MLs, the long-term efficacy of eprinomectin is affected.

The ecotoxicity of eprinomectin for both aquatic and terrestrial fauna is well known, particularly for dung beetles, which are natural recyclers of faeces on pastures.[31,32] For a good trade-off between efficacy and a minimal risk for the environment, it is necessary to choose the formulation with the best bioavailability, the lowest concentration of the molecule administered to the animals and then the lowest contamination of pastures. As a result, the topical formulation seems inadequate for this issue. It appears that the use of a formulation five times more than the injectable formulation presents not only a lower absorption but also an important release of the product in the environment.

As the emergence of resistance and multiresistance of GINs to eprinomectin in small ruminants is an hot important in the region,[14] it is interesting to investigate cases of suspicion in the field to discriminate between resistance and improper use of the molecule. This investigation will allow the practitioner as well as the farmer to know the cause of the therapeutic failure they may encounter, but it will also support a decision to modify practices that may be at risk regarding the management of GINs during the lactation period.

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CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

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ETHICS STATEMENT

Stool and blood collection and anthelminthic treatments are a part of routine veterinary procedures without any traumatic method. Such procedures are not qualified as animal experimentation involving vertebrates according to French laws, so no specific ethical clearance was required.

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