

## Multispecies and multiple anthelmintic resistance on cattle nematodes in a farm in Argentina: the beginning of high resistance?

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**Abstract** – There are very few resistance records on cattle nematodes. South American successive records have been increasing rapidly since the year 2000. In Argentina, increasing dissatisfaction based on the exclusive use of macrocyclic lactones has prompted the use of benzimidazoles in the Pampean region. The studied farm is located in the Argentina humid Pampas and had apparently poor results after anthelmintic treatments. Evaluation of resistance was firstly based on faecal egg reduction after treatment in November 2001 and June 2002 and was complemented with worm counts obtained by necropsy in June 2002. The study reports that the reduction of faecal egg excretion after benzimidazole (reduction 31–79%) or ivermectin treatments (76–97%) in November 2001 was insufficient and these poor results were confirmed in June 2002 (benzimidazoles (65–89%) and ivermectin (47–77%)). Several methods for evaluating faecal egg count reduction were performed and yielded different results. A new method based on the estimated faecal egg counts (corrected from the initial faecal egg counts and animal type using a general linear model) was used and gave higher flexibility in the interpretation of putative resistance to anthelmintics. From necropsy results, it could be concluded that *Cooperia oncophora* was resistant to avermectins and benzimidazoles and that *Cooperia punctata*, *Ostertagia ostertagi* and *Haemonchus placei* were resistant to benzimidazoles. This case of multispecies and multidrug resistance is probably not unique and could reflect the emergence of resistances in Argentina. This emergence is probably due to the intensive use of anthelmintics, the absence of refugia, and the frequent circulation of infected cattle.

**nematode / cattle / resistance / anthelmintic / Argentina**

### 1. INTRODUCTION

Resistance to anthelmintics is a widely described phenomenon in ruminants. Some

nematode species and some ruminant species are more prone to develop resistance [5]. An index for nematode resistance was built up and the following ranking of

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the host was: goats > sheep > horses > cattle. A similar ranking of nematode resistance was: *Haemonchus contortus* > *Teladorsagia circumcincta* > *Trichostrongylus* sp. > *Oesophagostomum* sp. > *Cooperia* sp., among which only *Cooperia* was recorded mainly in cattle. The resistance in small ruminants has mainly been directed against benzimidazoles (43% of cases) and then against levamisole or macrocyclic lactones (23% each). The apparent lack of anthelmintic resistant nematodes in cattle is probably due to the management systems used with most cattle and the lack of surveys for resistance [6]. With extensive beef grazing or with beef suckler herds a large percentage of the worms are in refugia (not exposed to anthelmintics) [26] and few anthelmintic treatments are used in most parts of the world. These conditions do not favour the appearance of resistance [23]. During the last 15 years in South America, technologies for gastrointestinal control in livestock have been sustained by the large use of broad-spectrum anthelmintics [25]. This is particularly true in the temperate plains of Argentina, where mixed cattle-crop production predominates, management is very intensive, and drugs have been frequently used for many years [9, 10, 22, 25]. Resistance of *Cooperia* to macrocyclic lactones has been recorded in Argentina [1, 2, 13–15, 20] and treatment using benzimidazoles has regained favour among farmers. In some regions of Argentina, such as in the humid Pampas near Buenos-Aires, a loss of efficacy of macrocyclic lactones is a frequent finding in veterinary practice whereas better results are obtained for benzimidazoles (Mejía, unpublished results). We may expect that farm macrocyclic lactone resistant worms will also become resistant to benzimidazoles when the latter is used extensively. One case of such resistance (based on faecal egg counts and necropsies) to both drugs is presented. We tested several computations since the resistance estimated from faecal egg counts is highly dependent on the way to compute reduction [18].

## 2. MATERIALS AND METHODS

### 2.1. Farm location and characteristics

The farm is located in the Argentina humid Pampas (south of the Cordoba province). Four thousands beef calves that are currently grown from the age of 6 to 20 months were studied. All six-month beef calves were obtained from other farms. In the past they were in part crossbred zebus originating from the north of the country; after the appearance of nematode resistance, Angus crossbreeds were bought, by groups of 70 to 400 from different farms from the near regions. The number of treatments per year was 4 on the farm. Macrocyclic lactones were used more than benzimidazoles before 2001 (3 macrocyclic lactones and 1 benzimidazole), but thereafter benzimidazoles were preferred (3.5 benzimidazoles and 0.5 macrocyclic lactones).

### 2.2. Resistance based on faecal egg counts

The faecal egg counts were performed using a modified McMaster technique (flotation liquid being an NaCl saturated solution). Each egg counted represented 20 eggs/gram of faeces. A first evaluation was done in November 2001. Five groups of 10 calves were used either as the control or were treated with fenbendazole (per os Axilur<sup>®</sup> 7.5 mg/kg bodyweight or intraruminally injected Bendax<sup>®</sup> 7.5 mg/kg b.w.), ivermectin (Ivomec<sup>®</sup> or Rosenbusch<sup>®</sup>, 200 µg/kg b.w., both injected). Axilur<sup>®</sup> and Ivomec<sup>®</sup> are the international brands whereas Bendax<sup>®</sup> and Rosenbusch<sup>®</sup> are generic drugs prepared in Argentina. Treatment was given after 24 h of the diet in order to obtain a maximum efficacy [16]. The faecal egg counts were done on the day of treatment and 12 days later. A second evaluation in June 2002 involved 3 groups (14 to 15 calves each): a control and two treated groups (Axilur<sup>®</sup> and

Ivomec®). The conditions for treatment were as in the first evaluation.

### 2.3. Resistance based on necropsies

In June 2002, six calves were necropsied in order to determine which species were involved in resistance. Two were treated with Axilur®, two with Ivomec®, and two remained as the untreated controls. The necropsies were done according to the technique of Suarez [24], and the 1/10th aliquot was examined. The identification of worms was done according to Suarez [24].

### 2.4. Statistical methods

The methods to evaluate flock faecal egg count reduction (FECR) are numerous and may yield very different figures. We used:

(1) after treatment evaluation in the control and treated hosts:  $FECR = 100 (1 - [T2/C2])$  where T2-treated and C2-control are the arithmetic mean of eggs per gram of faeces (epg), 10 to 14 days after treatment (WAAVP method [7]);

(2) before and after treatment evaluation in treated hosts (without control group):  $FECR = 100 (1 - [T2/T1])$  where T2 is the post treatment and T1 is the pre-treatment arithmetic mean of the epg (in [18]);

(3) Dash formula, before and after treatment evaluation in treated and control hosts:  $FECR = 100 (1 - [T2/T1] \times [C1/C2])$  where T2 and T1 are pre- and post-treatment arithmetic means of the epg in treated groups and C1 and C2 are pre- and post-treatment arithmetic means of the epg in the controls (in [18]);

(4) Presidente formula (in [18]) is based on the Dash formula where the geometric instead of the arithmetic means are used;

(5) A general linear model-GLM (EPG after treatment depending on initial EPG and type of treatment) based on natural logarithm transformed data (to stabilise variance) was also used since it permitted to

unambiguously evaluate the statistical differences between batches, due to the treatment only. GLM allows much greater flexibility than standard analysis of variance-ANOVA or analysis of covariance-ANCOVA procedures by allowing one to freely combine quantitative and qualitative factors. It also handles the balanced and unbalanced ANOVA design by providing automatic adjustments for an unequal cell size (the regression evaluation was used). FECR based on the estimated geometric means using the linear general model was also calculated; it is a new method for estimating FECR. This analysis can be summarised as follows: (i) after-treatment faecal egg counts + 1 were Neperian logarithm transformed; (ii) the factors to be included as the covariable were the initial faecal egg count + 1 Neperian logarithm transformed, month (November or June), type of treatment (generic or standard commercial preparation); (iii) after treatment estimated values from GLM were obtained, back-transformed using exponential function (equivalent to the geometric mean); and finally (iv) the faecal egg count reduction was calculated on the estimated after treatment and control means (means corrected from covariable effects).

## 3. RESULTS

### 3.1. Resistance based on FECR (Tab. I)

FECR is shown in Table I. The efficacy of fenbendazole and ivermectin ranged respectively from 51 to 79% and 47 to 95% depending on the computation and period of investigation. The computation methods yielded fairly different results (65 to 89% for fenbendazole in June 2002 for example). The GLM analysis showed that there was no significant difference between the generic and original drugs. The efficacy of benzimidazoles in the November experiment was not really high since the statistical difference between the treated and control calves ranged from  $p = 0.08$  (Axilur®) to

**Table I.** Faecal egg count (in egg per gram, EPG) reduction (FECR) in fattening beef calves in November 2001 and June 2002 using a benzimidazole (fenbendazole: 7.5 mg/kg body weight) and a macrocyclic lactone (ivermectine: 200 µg/kg b.w.).

		EPG (Range)		FECR <sup>a</sup> (%)
		Day 0	Day 12	
<b>November 2001</b>				
Fenbendazole				
(n = 10)	Axilur <sup>®</sup>	176 (80–500)	58 (0–240)	61, 67, 67, 79, 51
(n = 10)	Bendax <sup>®b</sup>	166 (80–360)	104 (0–40)	31, 36, 37, 67, 41
Ivermectin				
(n = 10)	Ivomec <sup>®</sup>	156 (80–260)	12 (0–40)	92, 92, 92, 95, 80
(n = 10)	Rosenbusch <sup>®b</sup>	154 (80–260)	20 (0–140)	87, 87, 87, 97, 76
Untreated control (n = 10)		154 (80–260)	150 (0–280)	
<b>June 2002</b>				
Fenbendazole (n = 14)	Axilur <sup>®</sup>	473(220–1280)	64 (0–300)	80, 80, 87, 89, 65
Ivermectin (n = 14)	Ivomec <sup>®</sup>	435 (280–1220)	149 (0–640)	54, 50, 66, 77, 47
Control (n = 15)		476 (240–1340)	324(100–760)	

<sup>a</sup> FECR calculated using five methods (1. Coles et al. 1992 [7]; 2. before and after treatment evaluation, in treated hosts without the control group (in [18]); 3. Dash formula (in [18]); 4. Presidente (in [18]); 5. and a new one based on the means estimated by the least square methods in a general linear model).

<sup>b</sup> Generic drugs prepared in Argentina.

$p = 0.11$  (Bendax<sup>®</sup>). Fenbendazole efficacy was higher in June 2002 than in November 2001. An opposite result was observed for ivermectin. Resistance to ivermectin appeared in 2000 (data not shown) and ivermectin was not used in 2001 and 2002 except for an evaluation on a limited number of cattle.

### 3.2. Resistance as established on necropsy results (Tab. II)

*H. placei* was well controlled with ivermectin but the efficacy of fenbendazole was very low. *O. ostertagi* was removed by ivermectin but fenbendazole was poorly efficient (the Newman-Keuls test showed significant differences ( $p = 0.01$ ) between ivermectin and fenbendazole treated calves; fenbendazole and control

calves harboured similar worm burdens). No significant difference could be shown for *C. oncophora*, which indicated that both treatments were not efficient. For *C. punctata*, significant differences ( $p = 0.01$ ) were evidenced (a significant difference between the ivermectin treated calves versus the control and fenbendazole treated calves): only ivermectin was efficient.

## 4. DISCUSSION

The establishment of resistance is difficult to assess on the sole measure of FECR since it depends strongly on the experimental procedure (with or without controls) and the evaluation of the reduction [18]. The Presidente method yielded the best calculated efficacies (95% and 97% for Ivomec<sup>®</sup> and Rosenbusch<sup>®</sup>

**Table II.** Worm burden obtained necropsies from untreated and treated herds (June 2002).

Strongyle species	Untreated control (2 calves)	Benzimidazole Axilur <sup>®</sup> -oral (2 calves)	Macrocyclic lactone Ivomec <sup>®</sup> -injected (2 calves)
<i>Haemonchus placei</i>	100–100	0–207 (–4) <sup>a</sup>	0–0 (100)
<i>Ostertagia ostertagi</i>	1957–7324	2019–3567 (39)	1–2 (100)
<i>Cooperia oncophora</i>	4032–19264	1704–16614 (21)	5800–5900 (50)
<i>Cooperia punctata</i>	4368–15136	696–6786 (62)	0–0 (100)
<i>Trichostrongylus axei</i>	0–200	0–0 (100)	0–0 (100)
All species reduction (%)		40	78

<sup>a</sup> Number of worms (efficacy in %). Efficacy =  $100 \times (\text{number of worms in the untreated controls} - \text{number of worms in the treated calves}) / (\text{number of worms in the untreated controls})$ .

respectively) and thus ivermectin could be considered as non resistant in the first trial (see Tab. I). The method we proposed using a reduction based on a least square means (evaluated from a general linear model geometric means) indicated that in all cases, efficacy was low and hence resistance to benzimidazole and ivermectin existed in the worm community. The least square estimation in relation to the treatment was corrected from the initial faecal egg count. This method is flexible and could possibly incorporate other sources of variations such as the type of animal or type of drenching (per os, pour on, etc.). The reduction of worm burden was low for both drugs (see Tab. II) but was not in the same magnitude as established on the FECR (40 vs. 87% and 78 vs. 66% for benzimidazole and ivermectin, respectively). The differences in evaluation is due to the fact that in the estimated geometric mean methods, differences in efficacy are only due to the main factor (treatment) corrected for initial egg output. The discrepancy was particularly important for the benzimidazole treated group, possibly due to the ovicidal activity of these drugs. This means that FECR probably overscores the actual resistance for benzimidazoles. A similar phenomenon has been reported in New-Zealand when using the ivermectin treatment on *Cooperia*: FECR indicated a reduction of 44%

whereas no worm reduction was demonstrated [8]. The species involved in resistance (*Cooperia* or *Ostertagia*) may also play a role on the efficacy evaluations: *Cooperia* sp. lay much more eggs than *O. ostertagi* and FECR evaluations are probably more efficient for the former species. All considered, a conclusion on the resistance to both drugs could be drawn in our case.

Resistance in cattle nematodes has remained limited in most countries [6], except in New-Zealand [3, 12, 17, 19], Argentina and Brazil. Selective pressure in both South American countries is high due to numerous treatments, absence of refugia, intense immigration of new animals with possibly resistant helminths. Resistance to ivermectin has been widely documented in *Cooperia* in New-Zealand (it could concern 89% of the farms [18]), South America (Argentina: [1, 2, 13–15, 20], and possibly [22]; Brazil: [11, 21], (20% of the farms), although it has not always been assessed at the nematode species level. Resistance to benzimidazoles is less frequent [21, 27, 28] and may concern *Ostertagia* or *Haemonchus*. Resistance to levamisole has been rarely described [4, 27]. In our case, multispecies resistance to benzimidazoles (*Haemonchus placei*, *Ostertagia ostertagi*, *Cooperia oncophora*, *Cooperia punctata*) was associated with

*Cooperia oncophora* resistance to avermectins. *Haemonchus placei* resistance to benzimidazoles has already been recorded by Pinheiro and Echevarria in Brazil [21]: the efficacy of oxfendazole and albendazole ranges from 60 to 80%. Such a resistance has already been suspected in the subarid Pampas [22]: farms with a history of frequent benzimidazole treatments have a higher percentage of *H. placei* in the worm community and it is thought to be introduced from northern tropical areas of Argentina into the Pampas. Conversely *O. ostertagi* resistance is probably a locally acquired resistance.

Farmers in the affected farms in the humid Pampas are dissatisfied with the use of ivermectin, and they changed from 4–5 ivermectin treatments to only one, needed for control of ectoparasites. At the same time they replaced other scheduled treatments with benzimidazoles (data from 10 farms: Mejía, unpublished). The farmers are not eager to switch to levamisole (since it is poorly efficient on inhibited larvae of *O. ostertagi*) and intend to use benzimidazoles more frequently in the future as shown from the above data. If the situation recorded on the studied farm is representative of other farms, one may question the switch to benzimidazole use and consider: (i) the use of levamisole when *Cooperia* sp. are the most prevalent, (ii) possibly consider the simultaneous use of levamisole and ivermectin (at periods when ectoparasites are a problem), levamisole and benzimidazole (when adult *O. ostertagi* is the main problem), (iii) ivermectin when inhibited *O. ostertagi* are present. The difficulty to choose an anthelmintic, the necessity to associate several drugs will be a current issue in the future and should not be the only mean to control internal parasites. The use of safe pastures and the use of treatments when needed on a weight gain or faecal egg count basis is a necessity. The application of such a programme should be integrated in the different farming managements existing in the humid Pampas.

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