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Case Report

The Control of Trichostrongyle Infections in Grazing Cattle of Argentina in a Context of Multiple Anthelmintic Resistance

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Abstract

A field program to control multiple anthelmintic resistant worm infections in cattle of Argentina is described. In 2003 around 140 calves died as a consequence of high worm burdens despite the administration of monthly alternate treatments with ivermectin (IVM) or fenbendazole (FBZ). A fecal egg count reduction test (FECRT) showed a clinical efficacy of 73%, 49.1% and 90.4% for IVM, FBZ and levamisole (LVM) respectively. An efficacy controlled test (ECT) showed that efficacy of IVM was 76.1% and 22.6% against *Haemonchus spp.* and *Cooperia spp.* respectively; for FBZ efficacy was: 0 % *Ostertagia spp.*; 28.3% *Haemonchus spp.* and 24.2% *Cooperia spp.*

A rational control program based on epidemiological surveillance was established since 2004 up to 2012 based on nematode egg excretion and speciation of worms in coprocultures. The LVM was used when necessary.

After ten years the results of a FECRT indicated a clinical efficacy of 79% for IVM (*Cooperia spp.* resistant), 89.4% for FBZ (*Ostertagia spp.* resistant) and 95.8% for LVM. The ECT showed that efficacy of IVM was ≥99% against *Haemonchus spp.* and *Ostertagia spp.* and 56% against *Cooperia spp.* The FBZ showed an efficacy of 100% against *Haemonchus spp.* and *Cooperia spp,* and 74% against *Ostertagia spp.* These findings demonstrate that resistance to IVM and FBZ persisted for *Cooperia spp.* and *Ostertagia spp.* respectively despite both compounds had not been used throughout a ten year period.

The control programme successfully stopped mortality, minimized subclinical losses and reduced the number of treatments.

ABBREVIATIONS

IVM: Ivermectina; FBZ: Fenbendazole; LVM: Levamisole; FECRT: Fecal Egg Count Reduction Test; ECT: Efficacy Controlled Test

INTRODUCTION

The phenomenon of anthelmintic resistance may be one of the major problems to overcome [1] in set-stocked ruminant where pastures are naturally contaminated with trichostrongyle infective larvae which in term minimize productivity of animals [2].

The anthelmintic resistance in cattle of Argentina was firstly described in 2000 [3]. The resistance of *Cooperia spp.* to macrocyclic lactones (ML) has been broadly reported [4] being also involved other intestinal nematode such as *Nematodirus spp.* [5].

Clinical symptoms in cattle harbouring *Cooperia* burdens resistant to ML treatments are usually undetectable (Anziani & Fiel, 2015)[6] and a relative lower pathogenicity [7] of this worm may account for it; however, the phenomenon become evident, i.e. weight losses, diarrea, when *Ostertagia ostertagia* is mainly involved in the infection [8].

Several cases of multiple resistance to ML and BZM treatments have been described in grazing cattle worldwide being *Cooperia* *spp*. mainly involved and *Haemonchus spp* in a less extension [9-12].

However, reports on field experiments aimed at controlling trichostrongyle infections in grazing cattle in a context of anthelmintic resistance are very scarce in the literature [14,15]; the reports shown that reversion of resistance to different compounds appears to be still ungettable.

The results of a program based on epidemiological surveillance to control nematode infections in grazing cattle in a context of multiple resistances to anthelmintics and the evolution of resistance levels through a period of 10 years are described in this report.

MATERIALS AND METHODS

Beef farm characterization

The beef farm has a surface of 1200 ha and it is located at the Central Region of Argentina province of Córdoba and divided in paddocks of 40-60 ha each which are used for agricultural and livestock production. Beef production is carried out every year with 4500 weaned calves belonging to breeding farms of the subtropical region of Argentina (Corrientes province) which graze on improved pastures from late summer up to mid spring and then transferred to a feedlot system to reach proper live weight for slaughtering. Stocking rate is around 6 animals/ha and 1173 kg/ha is the annual meat production.

The control of worm infections in the last three years has been based on alternate IVM – FBZ monthly treatments through the autumn, winter and first half of spring.

Description of a clinical case of parasitic gastroenteritis

In June 2013 a progressive weight loss, weakness, rough coat, anorexia and diarrhea were observed in a large proportion of the herd and few weeks later 140 calves died as a consequence of high trichostrongyle burdens; the diagnostic was confirmed by necropsy and nematode egg excretion analysis [16]. Anthelmintic resistance to IVM and FBZ was suspected and a treatment with LVM (Ripercol ®) to all calves was indicated. The mortality stopped and the high efficacy of LVM was then demonstrated through a new sampling and analysis of feces at 10 days post treatment.

Two months later a fecal egg count reduction test [17] was performed. The test evaluated the clinical efficacy of IVM, FBZ and LVM treatments and results are shown in (Table 1). Two animals of each treatment group were also necropsied to determine the absolute efficacy (E.C.T.) for each test compound [18]. Results are shown in (Table 2) and revealed that *Haemonchus* and *Cooperia* showed to be resistant to IVM whereas *Haemonchus, Cooperia and Ostertagia* to FBZ. The absolute efficacy of LVM against Ostertagia ostertagi was 65% which is comparable to previous reports [19,20].

A rational program to control resistant nematode infections

Since 2004 onwards up to 2012 a rational program to control nematode infections in set- stocked calves naturally exposed to resistant worms was carried out. The program aimed at: 1) minimize economic losses and 2) follow the evolution and dynamics of the anthelmintic resistant phenomenon in the worm population.

To prevent of economic losses due to parasitic infections two pre-established treatments were administered: the first (LVM) at the arrival of calves to grazing pastures in late summer and the other one (IVM) in late spring against arrested larvae (L4) of *O. Ostertagi* [21]. Out of these two pre-established treatments a control program based on epidemiological surveillance was carried out. To determine the opportunity of treatment to calves fecal samples were monthly collected from ending summer to next spring [22]. The levels of nematode egg excretion (e.p.g.) were established and worm species in coprocultures were identified [23]. Treatments to animals with LVM were indicated when e.p.g. levels were higher than 300 or if lower, when coprocultures revealed the predominance of high pathogenic worm species, i.e. *O. ostertagi.* This procedure allowed enlarging the interval between treatments as well as minimizing the use of LVM to preserve its efficacy as far as possible.

The dynamic and evolution of worm resistant levels to IVM and FBZ were monitored annually throughout a FECRT and an ECT on grazing calves exposed naturally to trichostrongyle infections.

The FECRT involved 40 calves which were selected for e.p.g. levels (> 200), comparable live weight and clinical conditions. Were divided into four groups of 10 calves each and treated at day 0 as follows:

Group 1: Ivermectin 1%, S.C., 0.2 mg/ k.b.w.

Table 1: Fecal egg count reduction test (FECRT) performed in September 2003.

IVM		FBZ		LVM		Control (untreated)	
e.p.g.		e.p.g.		e.p.g.		e.p.g.	
Day 0	Day +15	Day 0	Day +15	Day 0	Day +15	Day 0	Day +15
265	**60**	**255**	**113**	**269**	**21.3**	**330**	**222**
FECR (%)	**73.0**		**49.1**		**90.4**		----
Coproculture	*Haem 48%* *Coop 52%*		*Haem 49%* *Oste 18%* *Coop 33%*		*Oste 97%* *Coop 3%*		*Haem 54%* *Oste 11%* *Coop 34%* *Nema 1%*

Haem: Haemonchus; Oste: Ostertagia; Coop: Cooperia; Nema: Nematodirus

Table 2: Worm counts in calves treated with IVM, FBZ or LVM and absolute efficacy (CET) of each test compound (September 2003).

	IVM		FBZ		LVM		Untreated Control	
Identification	Nº 12	Nº 15	Nº 21	Nº 25	Nº 38	Nº 41	Nº 50	Nº 55
Abomasum	*Haemonchus* 700	*Haemonchus* 1500	*Ostertagia* 31.400 *Haemonchus* 4.300	*Ostertagia* 97.200 *Haemonchus* 2.300	*Ostertagia* 3.500	*Ostertagia* 7.800	*Ostertagia* 15.600 *Haemonchus* 5.500	*Ostertagia* 16.600 *Haemonchus* 3.700
Small Intestine	*Cooperia* 3.700	*Cooperia* 1.100	*Cooperia* 1.300	*Cooperia* 3.400	---------	---------	*Cooperia* 3.500 *Nematodirus* 500	*Cooperia* 2.700
Average	*Haemonchus* 1.100 *Cooperia* 2.400		*Ostertagia* 64.300 *Haemonchus* 3.300 *Cooperia* 2.350		*Ostertagia* 5.650		*Ostertagia* 16.100 *Haemonchus* 4.600 *Cooperia* 3.100 *Nematodirus* 250	
Efficacy	***Haemonchus* 76.1%** ***Cooperia* 22.6%**		***Ostertagia* 0 %** ***Haemonchus* 28.3%** ***Cooperia* 24.2%**		***Ostertagia* 64.9%**			

Worm identification: *Haemonchus contortus, Ostertagia ostertagi, Cooperia oncophora y pectinata, Nematodiirus helvetianus*

Group 2: Fenbendazole 10%, oral via, 7.5 mg/k.b.w.

Group 3: Levamisole 7.5%, S.C., 7.5 mg/k.b.w.

Group 4: Untreated control

Feces samples were collected from animals at day +14 p.t. for e.p.g. counts and coprocultures; the clinical efficacy (FECRT (%) for each test compound was established and worm species identified in coprocultures.

To obtain a more precise data on the absolute efficacy of each test compound an ECT was also performed in grazing calves every two years; for this purpose eight animals were selected at random and divided in four groups of two animals each which were assigned to each treatment group. Animals were necropsied at day +14 p.t. for worm counts, speciation and determination of absolute efficacy of each test compound [31].

RESULTS AND DISCUSSION

The parasite control program based on epidemiological surveillance throughout the monthly monitoring of e.p.g. levels and identification of worm species involved in the infection shown to be able to stop mortality, minimize subclinical losses and to diminish the number of treatments. The number of treatments with LVM was drastically lowered varying between three (2004) and one (2005-2012) with the exception of 2009 where no treatments were indicated (Table 3). No clinical signs linked to nematode infections were observed, even in those years where rains enhance the translation of infective larvae from dung to grass on offer. Epidemiological differences between years, i.e. pattern of egg excretion and herbage infectivity, reinforces the importance of a proper diagnostic to determine the opportunity of treatments (Steffan et al., 2013) [24]; thus, determination of e.p.g. levels in growing calves may constitute an useful diagnostic tool to advice anthelmintic treatments and so, to prevent successfully subclinical or clinical losses [25,26].

The FECRT performed in 2004 and 2005 showed an improving of the efficacy of IVM (73 to 89.9%) and FBZ (49.1 to 95%) in terms of reduction of nematode egg excretion which suggest a reversion of the phenomenon of resistance after use LVM as a replacement to both compounds during two consecutive years (Table 4). This is in consonance with [27] who also found that resistance may arise as soon as the compound is re-administered.

The clinical efficacy of IVM across different years varied in a rank of 70-80% being *Cooperia spp.* the single worm recovered in coprocultures of treated animals; this worm species has also been mentioned as dose-limiting for IVM [28].

On the other hand FBZ showed to reach an efficacy between 80-90% which was sustained along the years and nearly to 90% suggested by [29] as the lower limit of efficacy to determine the resistance to any anthelmintic; although *Cooperia spp.* and *Haemonchus spp.* were recovered in coprocultures, *Ostertagia spp.* was the single worm recovered from year 5 onwards of the program. This particular worm has also been mentioned as dose-limiting for FBZ [30].

The different biotic capability of worm species established in the gastrointestinal tract of cattle may be a strong conditioning for the time of the year to perform the FECRT in grazing animals [10]; this may suggest that winter should be the proper season to carried out a FECRT [31] since egg excretion of *Cooperia spp*. and *Haemonchus spp*. is usually lowered by the effects of immunity [21] and colder environment conditions respectively [25,21].

The clinical efficacy of LVM was higher than 90% along the years and is nowadays the anthelmintic to which no resistance has still been reported in cattle of Argentina (Caracostantógolo [8,32].

The worm counts in necropsied animals also shown that absolute efficacy of IVM against *Haemonchus placei* increased from an initial 75% to >95% since 2005 onwards (Table 5) whereas the efficacy against *Cooperia oncophora* and *punctata* was around 50% along the years and lower than efficacy levels shown by FECRT (70-80%). The absolute efficacy of FBZ confirmed the results against *Haemonchus contortus* y *Cooperia oncophora y punctata* demonstrated by the FECRT; however, the absolute efficacy on *Ostertagia ostertagi* increased from 0 to 79%

Table 3: Average number of nematode egg excretion (e.p.g.) and opportunities of treatments with LVM.

	Apr	May	Jun	Jul	Aug	Sep	Oct
Year	**E.p.g.**	**E.p.g.**	**E.p.g.**	**E.p.g.**	**E.p.g.**	**E.p.g.**	**E.p.g.**
2004	(310) LVM	(8)	(292) LVM	(17)	(331) LVM	(0)	(10)
2005	(34)	(110)	(318) LVM	(19)	(124)	(254) LVM	(0)
2006	(114)	(310) LVM	(0)	(10)	(70)	(110)	(60)
2007	(190)	(290) LVM	(22)	(70)	(132)	(113)	(90)
2008	(292) LVM	(0)	(80)	(117)	(90)	(52)	(10)
2009	(40)	(150)	(130)	(184)	(200)	(96)	(34)
2010	(147)	(420) LVM	(0)	(78)	(140)	(120)	(110)
2011	(102)	(370) LVM	(6)	(60)	(90)	(110)	(60)
2012	(20)	(151)	(404) LVM	(10)	(82)	(110)	(90)

Table 4: Clinical efficacy (FECRT %) of IVM, FBZ and LVM and worms identified in coprocultures.

Compound	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
IVM	**73.0** *Haem 48% Coop 52%*	**67.0** *Coop 100%*	**89.9** *Coop 100%*	**88.3** *Coop 100%*	**70.9** *Coop 100%*	**71.8** *Coop 100%*	**72.2** *Coop 100%*	**78.3** *Coop 100%*	**67.3** *Coop 100%*	**79** *Coop 100%*
FBZ	**49.1** *Oste 18% Haem 49% Coop 33%*	**81.2** *Oste 44% Coop 56%*	**95.0** *Oste 76% Coop 24%*	**94.5** *Oste 68% Coop 32%*	**88.6** *Oste 82% Coop 18%*	**79.6** *Oste 100%*	**92.6** *Oste 100%*	**93.1** *Oste 100%*	**86.8** *Oste 100%*	**89.4** *Oste 100%*
LVS	**90.4** *Oste 98% Haem 2%*	**95.4** *Oste 100%*	**99.8** NL	**99.7** NL	**100** NL	**98.3** NL	**99.5** NL	**100** NL	**93.7** *Oste 100%*	**95.8** *Oste 100%*
Untreated Control	*Oste 11% Haem 54% Coop 34% Nema 1%*	*Oste 6% Haem 26% Coop 68%*	*Oste 4%, Coop 94%, Oeso 2%.*	*Oste 10%, Trich 42%, Haem 4%, Coop 82%,*	*Oste 23% Haem 15% Coop 62%*	*Oste 19% Haem 22% Coop 51% Oeso 8%*	*Oste 8%, Haem 12%, Coop 72%, Oeso 8%-*	*Oste 8% Haem 14% Coop 72% Oeso 6%*	*Oste 24% Haem 54% Coop 21% Oeso 1%*	*Oste 16% Haem 44% Coop 36% Oeso 4%*

IVM: Ivermectina; **FBZ:** Fenbendazole; **LVS:** Levamisole. **Control:** sin tratamiento
Haem: *Haemonchus;* ***Oste:*** *Ostertagia;* ***Trich:*** *Trichostrongylus;* ***Coop:*** *Cooperia;* ***Oeso:*** *Oesophagostomum;* ***Nema:*** *Nematodirus;* NL: No larvae in coproculture

Table 5: Absolute efficacy (%) of IVM and FBZ estimated throughout a controlled efficacy test (CET).

	2003	2005	2007	2009	2011
IVM	*Haem 76% Coop 23% Ost 100%*	*Haem 96% Coop 33% Ost 99%*	*Haem 99% Coop 54% Ost 100%*	*Coop 50 % Ost 100%*	*Haem 100% Coop 56% Ost 100%*
FBZ	*Haem 8% Ost 0% Coop 24%*	*Haem 96% Ost 79% Coop 86%*	*Haem 100% Ost 60% Coop 99%*	*Ost 75% Coop 98%*	*Haem 100% Ost 74% Coop 100%*

in 2005 -lower than efficacy shown by FECRT (80-90%)- and remained in this percentage throughout the years.

The results of this report show that efficacy of IVM or FBZ on worm resistant populations may be recovered partially if compounds are not used for a certain period of time; this agree with [32] who also demonstrated that *Cooperia spp.* remained resistant to macrocyclic lactones after five years without use of these compounds. Comparable results have been reported by [15] and appear to be that reversion of anthelmintic resistance is unlikely to occur when at least 25% of the worm population contains resistant homozygous genes [33]. It may be then speculated that in the present work a high frequency of resistant alleles could had been established specially in *Cooperia* and *Ostertagia* worm populations which in term might influence reversion –specially- to those compounds with dose-limiting effects.

CONCLUSIONS

The implementation in growing cattle of a rational program based on epidemiological surveillance –monthly monitoring of nematode egg excretion and identification of parasites in coprocultures- to control multiple resistant trichostrongyle infections to IVM and FBZ resulted an efficient diagnostic tool to determine the opportunity of herd drenching.

The rational control program allowed to stop mortality of calves as a consequence of high worm burdens, minimized subclinical losses and diminished drastically the annual number of drenching in grazing cattle.

The FECRT showed to have lower sensibility than ECT to detect anthelmintic resistance, specially, when worms involved in the infection have a relative low egg excretion, i.e. *Ostertagia spp.*

The compound LVM maintained high levels of efficacy after 10 consecutive years of rational use in grazing cattle naturally exposed to worm populations resistant to IVM and FBZ.

A partial recovery of the efficacy of IVM and FBZ against *Cooperia oncophora / punctata* (23 to 56%) and *Ostertagia ostertagi* (0 to 74%) respectively was recorded after a period of ten years in which the use of both compounds was disrupted in the farm.

On the other hand, multiple resistant *Haemonchus placei* become fully susceptible to IVM and FBZ treatments at the end of the study.

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