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Research Article

Experimental Infections in Cattle and Sheep with *Haemonchus contortus* Resistant or Susceptible to Benzimidazole Treatments

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Abstract

In order to evaluate the potential risk of *Haemonchus contortus* resistant to benzimidazole (BZ) treatments to pass from sheep to cattle a series of experiments were carried out. Two field isolates of *H. contortus* resistant (Ayacucho) or susceptible (Cedive) to BZ treatments were reproduced in lambs and involved in the study. Two groups of 4 calves each and two groups of 4 lambs each were infected with larvae (L3) of each isolate respectively. The number of eggs in faeces (EPG), worm count and ratio of worm establishment (RWE) were determined. The number of red blood cells, packed cell volume and haemoglobin concentration was assessed. The EPG levels were higher for Ayacucho isolate which reached to a peak of 7,168 and 780 at day 42 post inoculation (PI) in lambs and calves respectively. The RWE at day 45 PI was 28.8 % and 7.16% for Ayacucho isolate in lambs and calves respectively while for Cedive isolate was 36.6% and 0.04% respectively. At day 90 PI the RWE was of 1.94% and 0.69% in lambs for Ayacucho and Cedive isolate respectively, whereas no worms were recovered from calves. The haematological parameters in lambs and calves did not show significant differences between isolates. The present studies demonstrate that some populations of *H. contortus* may complete life cycle in calves and remain established for at least a 45 days period PI. This finding should be strongly considered when farm production involves mixed or alternate sheep/cattle grazing and backgrounds of anthelmintic resistance.

ABBREVIATIONS

BZ: Benzimidazole; EPG: Eggs per Gram; RWE: Ratio of Worm Establishment; PI: Post Inoculation; FECRT: Faecal Egg Count Reduction Test

INTRODUCTION

In small ruminants the stomach worm *Haemonchus contortus* produces a severe anaemia due to its blood sucking habits [1]. In Argentina, this nematode may affect grazing animals from the beginning of the summer onwards up to mid-autumn [2] reducing live weight gains and wool production being also the cause of lambs mortality when larval herbage infectivity is very high [3]. The clinical course of haemonchosis is usually influenced by immunological host level, which in terms, may regulate worm burdens and the pathogenicity of the infection in grazing animals [4].

In practice, the control of haemonchosis in sheep has been mainly performed by intensive use of anthelmintics throughout warmer periods which in terms, may account for the development of anthelmintic resistance. In Argentina, a clinical haemonchosis in calves due to *H. contortus* resistant to benzimidazole (BZ) treatments which belong to an alternate sheep-cattle grazing has been reported [5]. Recently studies have also demonstrated that resistant sheep worms such as *Trichostrongylus axei* and *H. contortus* were also found in cattle which shared pastures [6,7].

The aim of these experiments was to evaluate the potential risk for the transmission from sheep to cattle of *H. contortus* resistant to BZ treatments.

MATERIALS AND METHODS

Haemonchus contortus isolates for the studies

Two field isolates of *H. contortus* belong to sheep naturally infected and named as Ayacucho and Cedive were used for the studies. Species level was confirmed throughout morphometric measures of adult male worms [8]. Susceptibility of each isolate to BZ treatments was established through a faecal egg count reduction test (FECRT) [9] which indicates that Ayacucho isolate was resistant to BZ whereas Cedive isolate showed to be susceptible to BZ treatments as it is shown in Table (1).

Preparation of experimental inoculums

The isolates of *H. contortus* were both reproduced in sheep to obtain the infective larvae (L3) for inoculums. For this purpose two free-worm lambs were each inoculated by oral via with 10,000 L3 of *H. contortus* Ayacucho isolate and other two lambs with the Cedive isolate. After 3 weeks from infections the faeces from each animal were daily collected and cultured in plastic bags under controlled conditions in the laboratory. After 2 weeks of cultivation the infective larvae were recovered by a Baermann technique. Infective larvae were suspended in distilled water, counted and four containers with 30,000 L3 each and other four with 9,000 L3 each of Ayacucho or Cedive isolate were prepared. The inoculums were maintained at 4°C until use.

Experimental infections in sheep and cattle

Ten Corriedale castrated and weaned lambs of 30 kg in average and 10 Holstein male calves of 4-5 months old weighing 105 kg in average free of parasitic infections were used for the study. The animals were transferred to concrete floored pens and fed dry grass free of trichostrongyles infective larvae; fresh water was supplied ad-libitum.

Two comparable groups of 4 calves each (C1 and C2) and two groups of 4 lambs each (L1 and L2) were formed. The animals of C1 and C2 were each infected orally with 30,000 L3 of Ayacucho or Cedive isolate respectively. The lambs of L1 and L2 received each an inoculum of 9,000 L3 of Ayacucho or Cedive isolate respectively. Two animals remained as non-inoculated control for each animal species (C3 and L3). The experimental design is shown in Table (2).

Parasitological studies

Faecal samples from Day 0 up to Day + 90 post infections (PI)

Table 1: Backgrounds of each isolate of *Haemonchus contortus*.

Isolate	Farm Location	Farm Management	Stocking rate	Susceptibility to BZ treatment
Ayacucho	Southeast of Buenos Aires Province	sheep-cattle alternate grazing	medium/high	Resistant FECRT: 64.9 %
Cedive	Northeast of Buenos Aires Province	sheep-cattle mixed grazing	low	Susceptible FECRT: 100 %

Abbreviations: FECRT: Faecal Egg Count Reduction Test; BZ: Benzimidazole

Table 2: Experimental groups of animals and the number of *H. contortus* larvae (L3) resistant or susceptible to benzimidazole inoculated to calves and lambs.

Experimental Group	(n)	Animal	Ayacucho Isolate L3 inoculated/ an.	Cedive Isolate L3 inoculated/ an.
C1	4	cattle	30,000	-
C2	4	cattle	-	30,000
C3	2	cattle	Non Inoculated Control	
L1	4	sheep	9,000	-
L2	4	sheep	-	9,000
L3	2	sheep	Non Inoculated Control	

Table 3: Mean group number of *H. contortus* (adult worms) in the abomasum of calves and lambs and ratio of worm establishment (RWE).

Experimental Group	Mean L3 inoculated	45 days post inoculation		90 days post inoculation	
		Mean Adult worms	RWE (%)	Mean Adult worms	RWE (%)
C1	30,000	2,150	**7.16**	0	**0**
C2	30,000	12.5	**0.04**	0	**0**
L1	9,000	2,600	**28.88**	175	**1.94**
L2	9,000	3,300	**36.66**	62.5	**0.69**

were collected manually at weekly intervals. The number of eggs per gram of faeces (EPG) was determined throughout a modified McMaster technique [10].

Two animals from each experimental group were necropsied at day + 45 and + 90 PI respectively following the guidelines proposed by the Comité de Bienestar Animal (087/02), Facultad de CienciasVeterinarias-UNCPBA (http://www.vet.unicen.edu.ar/index.php/facultad/comite-bienestar-animal). The number of worms established in abomasa of each animal was recorded [1] and the ratio of worm establishment (RWE) for each isolate in sheep and cattle was determined throughout this formula:

RWE (%) = (adult worm mean counts / mean number of infective larvae inoculated) *100.

Haematological studies

Blood samples from the jugular vein of each experimental animal were collected at fortnightly intervals to determine the effects of *Haemonchus* infections on some blood parameters. The number of red cells (number of red cells x 10^{12}/L) and packed cell volume (%) were assessed according to standard procedures [11] and haemoglobin concentrations (g/dL) were determined throughout the cyanmethaemoglobin method (Wiener®Hemoglowiener).

Statistics analysis

The analysis of variance (ANOVA) throughout the PROC MIXED SAS 9.2 software for haematological data was used.

RESULTS AND DISCUSSION

Parasitological studies

Infections in sheep: The levels of egg excretion in the faeces of lambs are shown in Figure (1). Eggs of *H. contortus* started to pass in the faeces of lambs at day 21 PI reaching to a mean peak of 7,168 EPG and 5,410 EPG at day 42 PI for Ayacucho and Cedive isolates respectively. From day 49 PI onwards the levels of egg excretion decreased for both isolates.

Infections in cattle: The levels of egg excretion in the faeces of calves are shown in Figure (2). The eggs of *H. contortus* were detected in the faeces of calves at day 21 PI. The Ayacucho isolate showed an average of 780 EPG at day 42 PI. From day 49 PI onwards a decreasing level of egg excretion was recorded. The Cedive isolate presented low egg counts throughout the study with a maximum of 40 EPG at day 35 PI.

Adult worms in sheep and cattle: The number of adult worms from calves and lambs and the RWE for each isolate are shown in Table (3).

The mean worm number in lambs at day 45 PI was 2,600 and 3,300 for Ayacucho and Cedive isolate being of 28.88 % and 36.66 % the RWE respectively. At day 90 PI the mean number of worms was 175 and 62.5 for Ayacucho and Cedive isolate being of 1.94 % and 0.69% the RWE respectively.

The calves infected with Ayacucho isolate showed the higher counts of worms at day 45 PI with a mean number of 2,150 which

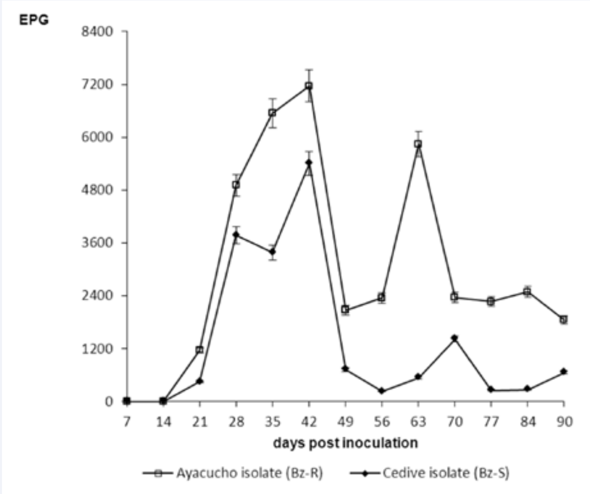

Figure 1 Mean group number of EPG in the faces of lambs inoculated with 9,000 H. contortus infective larvae.

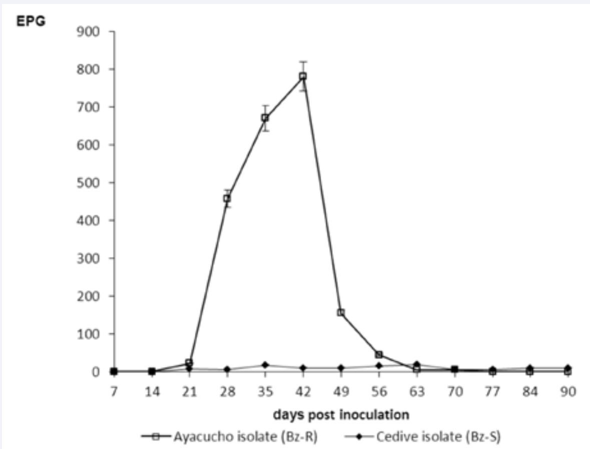

Figure 2 Mean group number of EPG in the faeces of calves inoculated with 30,000 H. contortus infective larvae.

led to 7.16 % of RWE. The number of worms in calves infected with Cedive isolate was very low at day 45 PI being of 0.04% the RWE. No worms were recovered at day 90 PI for any of the isolates.

The resistant *H. contortus* Ayacucho isolate showed to have a significant capability to cycle in cattle which was evidenced throughout both, the high EPG levels in feces and high worm burdens found in abomasums of calves at day 45 PI. This particular isolate belong to an alternate sheep/cattle grazing management through many years which might allowed this adaptation and ability of *H. contortus* to cycle in both host indistinctly.

These findings are in consonance with previous reports which alert that calves may acquire *H. contortus* infections when grazing on sheep pastures [12,13]; this phenomenon has also been described in farms with backgrounds of anthelmintic resistance [7,14].

This particular observation has also been mentioned for populations of *T. axei* resistant to BZ treatments [6] when sheep and cattle share grazing pastures. The lost of host specificity that may show some worm species, i.e. *H. contortus* under certain animal grazing management should be highly considered in a context of a high and progressive phenomenon of anthelmintic resistance. A field case on the transmission of *H. contortus* resistant to BZ treatments from sheep to cattle was already reported in Argentina [5]. It could also be observed in the present studies that susceptible *H. contortus* isolate (Cedive) did not show the capability to complete the life cycle in calves as it was observed throughout the EPG counts and worm burdens. These observations could support the fact that cattle may contribute to increase *H. contortus* populations resistant to BZ treatments in both, animals and refugium when share grazing with sheep.

However, field observations revealed that some isolates of *H. contortus* belong to sheep and resistant to BZ treatments were not able to complete the life cycle in cattle (Guzmán et al. unpublished data) suggesting that such a capability may be mainly linked to a phenomenon of worm adaptation more than to features or genetic changes of worms resistant to BZ treatments. Therefore, it might be argued that capability of *H. contortus* populations -susceptible or resistant to BZ treatments- to infect cattle could be strongly conditioned by management backgrounds such as proportions of sheep/cattle and the rate of stockings in the years before [12].

These observations may also account for sporadic failures seen in some parasite control programs based on worm host specificity [15], suggesting that a mechanisms of adaptation of *H. contortus* to establish in other host than sheep may occur.

Haematological studies

The haematological studies in lambs and calves after infections with larvae of *H. contortus* shown that red blood cell counts, packed cell volume and haemoglobin levels in blood samples were in a normal range of values ($p > 0.05$). The accute anaemia has been described for haemonchosis from two weeks of the infection onwards when worms reach the mature stages in the abomasum [4,16]. However, blood samples from lambs and calves inoculated each with 9,000 and 30,000 infective *H. contortus* larvae respectively shown that numbers of red cells were modified lightly when compared to normal range of values [11]. Variations in the pathogenicity of *H.contortus* isolates used in the present studies could not be observed as well. Probably, the relative low number of infective larvae used for experimental infections in calves and lambs and the high levels of nutrition offered throughout de studies may account for such observations.

CONCLUSION

The results of these studies may contribute to enhance knowledge on the behaviour of *H. contortus* in those farms where cattle and sheep share grazing in mixed or alternate schedules on naturally infected pastures of Argentina. Alternate sheep-cattle grazing to provide safe pastures based on host *Haemonchus* spp. specificity has been frequently advised to farmers [12,15]. However, the fact that some populations of *H. contortus* may complete the parasitic cycle in calves and remain established for at least a 45 days period PI -as it was demonstrated in the present studies- should pay the attention when a sustainable control program is intended; this warning become highly relevant when resistance to BZ treatments is well established in the farm.

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