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Pathophysiological observations on calves concurrently infected with *Cooperia punctata* and *Haemonchus placei*

(Observações fisiopatológicas em bezerros infectados concomitantemente com *Cooperia punctata* e *Haemonchus placei*)

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SUMMARY

Friesian calves given an oral daily concurrent infection of 18,000 *Cooperia punctata* and 2,000 *Haemonchus placei* infective larvae over a 14-day period, were injected with ^{125}I-albumin 23 days after the beginning of infection to study the albumin metabolism alterations. The mean burdens at necropsy were 42,275 ± 20,598 for *C.punctata* and 6,075 ± 1,407 for *H.placei*. The infected calves lost weight during the experimental period and showed a significant decrease of ^{125}I-albumin half-life ($P<0.05$). The catabolic rate, faecal clearance of albumin and distribution of albumin from the extravascular to the intravascular pool, were not statistically significant. In the infected animals the changes were associated with hypoalbuminaemia and a slight increase in the plasma volume. Despite of these alterations the clinical signs were mild.

KEY WORDS: *Cooperia punctata, Haemonchus placei*, calves, radiotracers.

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RESUMO

Bezerros da raça Holandesa preta e branca receberam diariamente, via oral, 18.000 larvas infectantes de *C. punctata* e 2.000 *H. placei* por um período de 14 dias. Vinte e três dias após o início da infecção, os bezerros foram injetados com ^{125}I-albumina para realização do estudo do metabolismo da albumina. O número médio de vermes recuperados na necrópsia foi de 42.275 ± 20.598 para *C. punctata* e 6.075 ± 1.407 para *H. placei*. Os bezerros infectados perderam peso durante o período experimental, e apresentaram diminuição significativa na meia vida biológica da ^{125}I-albumina ($P<0,05$). A taxa de catabolismo e do "clearance" fecal da albumina e a distribuição da albumina do "pool" extra para o intravascular não foram estatisticamente significativos. Os animais infectados apresentaram hipoalbuminemia e um ligeiro aumento no volume plasmático. Apesar destas alterações, os sinais clínicos foram amenos.

PALAVRAS-CHAVE: *Cooperia punctata*, *Haemonchus placei*, bezerros, radiotraçadores.

INTRODUCTION

Cooperia spp and *Haemonchus placei* are the most common helminth parasites of young cattle in all important grazing regions of Brazil (Honer & Vieira Bressan, 1992).

Cooperia spp infection is usually associated with highly pathogenic parasites such as *Haemonchus placei, Trichostrongylus* spp., *Ostertagia ostertagi* and *Oesophagostomum radiatum*. It was observed that in massive doses, this genus can cause weight loss, loss of plasma protein into the gut and clinical enteritis (Armour et al.,1987; Coop et al., 1979). More specifically with *C.punctata* , Bailey (1949) and Alicata & Lynd (1961) reported intermittent or continued diarrhea, progressive emaciation, reduced feed consumption and weight loss in severe by infected animals. According to Urquhart et al. (1987) *C.punctata* and *C.pectinata* are more pathogenic since they penetrate the epithelial surface of the small intestine leading to atrophy and reduction of the area of absorption.

Studies on the pathophysiology of *H.placei* infection in calves have been carried out (Gennari et al., 1991). In this study, despite the low worm establishment and mild clinical signs, the infected calves showed a significant reduction in the PCV values and weight gains. The albumin metabolism showed alterations in pool distribution and increase in the plasma volume. In addition, the plasma iron turnover rates and percentage utilization of ^{59}Fe by red cells were significantlly higher in infected calves.

Vieira Bressan et al. (1989) in a three year epidemiological trial using Friesian tracer calves in a important dairy region of São Paulo State - Brazil, found that *C. punctata* and *H. placei* are the most prevalent, with the former was present all over the year and latter mainly during the spring and summer. At this concomitant infection period a relative proportion around 9:1 (*C.punctata : H.placei*) was present. Based on these observations, the present experiment was conducted to provide information on the pathophysiologycal alterations in calves concurrently infected with *C.punctata* and *H.placei,* in a level of infection similar to that observed under natural grazing conditions.

MATERIALS AND METHODS

Seven 4-month-old Friesian male calves reared indoors under worm-free conditions, were divided into one group of three animals that were maintained free of parasites and one group of four calves, that were concurrently dosed, orally, with 18,000 third stage infective larvae (L_3) of *C.punctata* and 2,000 L_3 *H.placei*, daily, for two weeks.

Twice a day the calves were fed on hay (1000g) and a commercial complete ration (1000g).

Twenty three days after the beginning of the infection, the animals from both groups were placed in metabolic cages and injected with bovine albumin* labelled with ^{125}I**. The radioactivity of plasma, urine and faeces were measured and changes in plasma albumin were pools and catabolic rate of albumin were determined.

Two weeks after injection, the animals were killed and the worm burdens recorded.

One calf was infected with a pure *C.punctata* culture and another calf with a *H.placei* culture and they were used as donnors. From the faeces of these animals the infective larvae were cultured and the larvae were suspended in water and administered, orally, in the form of a drench. Faecal egg counts were carried out by the modified McMaster method of Gordon & Whitlock (Whitlock, 1948).

At post-mortem examination the abomasum and small intestine were removed intact. The abomasum was turned inside-out, the small intestine opened and the contents collected in a graduated bucket. The surface of the mucosa was washed with tap water and added to the respective buckets. The abomasum and small intestine were then placed in a water bath at 42°C with 0.1N NaOH, for 12h to release the worms from the mucosa. The total content was sedimented and fixed with formol in a 10% proportion. Ten per cent homogenized samples from each organ content was collected and also fixed with formol. The worms present in the total mucosal digest were counted. From the contents, the total number of each genus recovered was determined by multiplying the number found in 1% aliquots by the dilution factor.

* Sigma, USA
** Amersham, UK

During the experimental period, the calves were dosed orally with 10ml of 0.75% potassium iodide to saturate the thyroid and ensure the excretion of ^{125}I released by catabolism of ^{125}I-albumin.

A solution of 2% bovine serum albumin was labelled with ^{125}I as described by McFarlane (1958).

Each calf received 18.5 MBq of ^{125}I-albumin by intrajugular catheter. Before and after the injections the syringes were individually weighed and the weight of each injected material noted. A 1.0 ml syringe was also filled with ^{125}I-albumin, and weighed for preparation of the standard. Several aliquots of this solution were put in counting vials and diluted to 3.0ml 0.01N NaOH. One ml of plasma sample, from each day, was also diluted to 3.0ml 0.01N NaOH. Three samples of 3.0ml of urine and five 3.0g samples of faeces were prepared daily and they were all counted in a gamma spectrometer (Packard cobra-auto gamma) with the standards, at the termination of the study.

Collection of plasma, faecal and urine samples, and calculation of pathophysiological parameters were as described by GENNARI et al. (1991).

The data were analysed using a standard analysis of variance and Tukey's test was used to compare the means and significance was considered where $P<0.05$ (USER'S...,1985). Variations around the mean were expressed as the standard error (SE).

RESULTS

The mean burdens at necropsy was 42,275 $\pm$ 20,598 for *C.punctata* and 6,075 $\pm$ 1,407 for *H.placei* (Table 1). The variation in the bodyweight during the experimental period from infected and

uninfected animals are shown in Table 1. The infected calves lost a mean of 4.83 ± 1.39 kg and the clean calves gained 3.88 ± 1.99 kg. This difference was statistically significant.

Two infected calves, showed softening of faeces at the fourth week after the start of inoculum. The control group remained clinically normal throughout the experiment. Neither the infected nor the control animals showed depression of appetite during the experimental period.

On day 15 of the experiment, three of the infected calves, showed eggs in the faeces and on day 18, all were positive. The infected calves showed a second peak after day 33 and counts continue to rise until the ending of experiment.

The PCV of the infected and control groups are shown in Fig. 1. Over the entire experiment the haematocrit values remained constant in the control clean animal and the infected calves showed a similar pattern with a slight increase and decrease on days 24 and 31 respectively.

Mean plasma albumin concentration at the day of ^{125}I-albumin injection is shown in Table 1. There was no statistical difference between the infected and control groups, with 2.5 ± 0.12 g 100 ml^{-1} and 3.07 ± 0.22 g 100 ml^{-1} respectively, however the infected calves were hypoalbuminaemic.

At post mortem on day 37, the abomasa of infected calves showed oedema and reddish mucosal surface over the entire organ. The external side of the small intestine of two of the infected animals was covered by a gelatinous layer. *C.punctata* were mainly located in the jejunum, which showed a greyish exudate, thickened wall and congestion of the mesenteric vessels.

The plasma volume, intravascular and extravascular pool of albumin

based on the half-life (T1/2) of ^{125}I-albumin together with catabolic rate and faecal clearance measurements, in the infected and control calves are shown in Table 2.

The measurements were done during days 23 to 37 after the start of the infection. The infected calves showed a significant decrease ($p<0.05$) of ^{125}I-albumin half-life (20.0 $\pm$ 1.22 days) when compared with controls (23.7 $\pm$ 0.67 days). An increase in the catabolic rate of albumin was also present in the infected group with 9.68 $\pm$ 0.43% against 8.37 $\pm$ 0.49% for the controls. Consequently, the mean faecal clearance was higher in the infected animals, 155.49 $\pm$ 22.0 ml day^{-1}, when compared with the controls, 88.47 $\pm$ 14.8 ml day^{-1}. There was a tendency that the distribution of albumin from the extravascular to the intravascular pool (EA/IA), was higher in the control animals (1.85 $\pm$ 0.03) when compared with the infected (1.73 $\pm$ 0.05), however these differences were not statistically significant ($p>0.05$). The same tendency was observed in the plasma volume values were slightly higher in the infected animals, 37.03 $\pm$ 2.4 ml kg^{-1}, when compared with the controls, 31.79 $\pm$ 1.4 ml kg^{-1} ($p>0.05$).

DISCUSSION

The results showed that a concurrent infection with *C.punctata* and *H.placei* at a proportion of 9:1, which is often encountered under natural grazing condictions in some regions of Brazil, is responsible for pathophysiological changes in young Friesian calves. Despite the very high variations found in worm burdens among the infected animals and the mild clinical signs, all of them lost weight and showed metabolic alterations, that certainly can have a production effect on growing calves.

The clinical signs, despite of having been mild, showed a relationship with number of infective larvae established. Peak egg production showed that maximum number of worms reached maturity, for *C.punctata* and *H. placei*, respectively, at the third and the fifth week from the start of infection. When diarrhea was observed it coincided with the peak of *Cooperia* egg output. The PCV values, despite of small variations, also increased with the rise of *Cooperia* egg counts and softening of faeces. After patency of *H.placei* there was a slight decrease in the values and at this time, the bloodsucking activity of *H.placei* was certainly involved. It was much less severe than expected, however, for the number of worms established and the differences between the pathogenicity of these two genera conferred a compensatory response on the PCV values.

At necropsy, carried out five weeks after beginning of infection, the macroscopic lesions due *C.punctata* were present mainly in jejunum. Bailey (1949) observed that the lesions caused by the same specie of *Cooperia* were largely confined to the duodenum, but in his experiment calves were killed at different periods througth the experiment. With *C.oncophora* there was a change in the distribution of this worm over the small intestine with the evolution of the infection (Armour et al, 1987). The dynamics of *C.punctata* population during an infection period have not yet been studied and it is possible that the same changes can also be present.

The radioisotopic measurements revealed significant alterations in the albumin metabolism. All infected calves were hipoalbuminaemic. Rowe et al (1982) observed that the protein lost into the abomasum of *H.contortus* infected sheep is, in part, reabsorbed by the small intestine. In a monoespecific moderate *H.placei* infection (Genari et al, 1991), the calves did not show a decrease of albumin concentration, despite the fact that animals harboured a similar worm population to those recorded in the present study. The faecal clearance of ^{125}I-albumin was 43% higher in the infected group, suggesting that in this concurrent infection, the lesions in both, abomasum and small intestine, greatly enhanced the losses of

albumin. This leakage was the cause of the reduction of ^{125}I-albumin half-life and of the increase of 13.5% in catabolic rate of albumin.

The slight increase in the total plasma volume found in the infected calves, probably is an indication of some alterations in water metabolism caused by the mixed infection.

This study permits the conclusion that, in combination, *C.punctata* and *H.placei* produced additive alterations in calf albumin metabolism, despite the absence of acute clinical signs.

TABLE 1 Plasma albumin (g 100 ml^{-1}), weight gain (kg) and worm burdens in calves concurrently infected with *Cooperia punctata* and *Haemonchus placei* and in uninfected controls (mean ± SE)

Experimental Group	Plasma Albumin (g ml^{-1})	Weight Gain (kg)	Worm Burdens	
			C. punctata	*H. placei*
Infected n=4	2.50 ± 0.12	-4.83 ± 1.39	42,275 ± 20,598	6,075 ± 1,407
Control n=3	3.07 ± 0.22	3.88 ± 1.99	-	-

n = number of calves per group

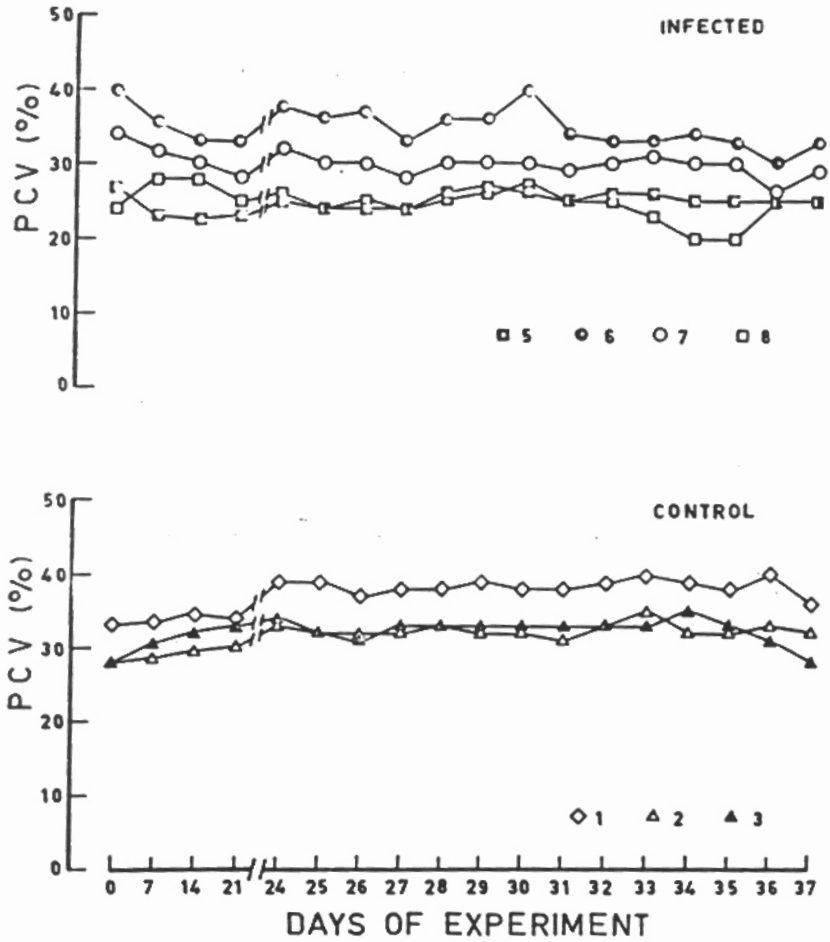

Figure 1 - Packed cell volume (%) values per calf concurrently infected with *C. punctata* and *H. placei* and the respective controls during the experimental period.

Table 2 - Albumin metabolism in calves after infection with *Cooperia punctata* and *Haemonchus placei* and the respective clean controls (mean ± SE).

Experimental Group	VP (ml/kg)	IA (g/kg)	EA (g/kg)	EA/IA	Faecal Clearance (ml/day)	^{125}I T½ (days)	Catabolic Rate (%)
Infected n=4	37.03 ± 2.4	0.98 ± 0.1	1.58 ± 0.05	1.73 ± 0.05	155.49 ± 22.0	20.0 ± 1.22	9.68 ± 0.43
Control n=3	31.79 ± 1.4	0.92 ± 0.05	1.83 ± 0.23	1.85 ± 0.03	88.47 ± 14.8	23.7 ± 0.67	8.37 ± 0.49

n = number of calves per group
VP = plasma volume
IA = intravascular pool of albumin
EA = extravascular pool of albumin
T½ = half-life of ^{125}I

REFERENCES

ALICATA, J. E. & LYND, F. T. Growth rate and other signs of infection in calves experimentally infected with *Cooperia punctata*. *Am. J. V. R.*, v.22, p.704-706, 1961.

ARMOUR, J., BAIRDEN, K.; HOLMES, P. H.et al. Pathophysiological and parasitological studies on *Cooperia oncophora* infections in calves. *Res. Vet. Sci.*, v.42, p.373-381, 1987.

BAILEY, W. S. Studies on calves experimentally infected *Cooperia punctata* (v. Linstow, 1907) Ransom, 1907. *Am. J. Vet. Res.*, v.10, p.119-129, 1949.

COOP, R. L., SYKES, A. R., ANGUS, K. W. The pathogenicity of daily intakes of *Cooperia oncophora* larvae in growing calves. *Vet. Parasitol.*, v.5, p.261-269, 1979.

GENNARI, S. M., VIEIRA BRESSAN, M. C. R., ROGERO, J. R., et al. Pathophysiology of *Haemonchus placei* infection in calves. *Vet. Parasitol.*, v.38, p.163-172. 1991.

HONER, M. R. VIEIRA-BRESSAN, M. C. R. Nematódeos de bovinos no Brasil - O estado da pesquisa, 1991. *Rev. Bras. Parasitol. Vet.*, v.1, p.67-79, 1992.

McFARLANE, A. S. Efficient trace labelling of protein with iodine. *Nature*, v.182, p.53, 1958.

ROWE, J. B.; ABBOTT, E. M.; DARGIE, J. D. et al. The effect of Haemonchosis and blood loss into the abomasum on N digestion in sheep. *Proc. Nut. Soc.*, v.41, p.74A, 1982.

USER'S guide statistics. 5 ed. Cary N. C.: SAS Institute Inc. 1985.

URQUHART, G. M., ARMOUR, J., DUNCAN, J. L. et al. *Veterinary Parasitology*. Essex England. Longman Scientific & Technical, 1987, p. 286.

VIEIRA BRESSAN, M. C. R.; GENNARI, S. M., BIONDI, P. et al. Epidemiologia de nematóides gastrintestinais de bovinos leiteiros no Vale do Paraíba Paulista (Observações Preliminares). In: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 6, Bagé: *Anais*...Bagé, 1989. p.24. Resumo.

WHITLOCK, H. V. Some modifications of the McMaster helminth egg-counting technique and apparatus. *J. Council Scientif. Ind. Res.*, v.21, p.177-180, 1948.