BODY COMPOSITION, WATER AND NITROGEN BALANCE IN CALVES INFECTED WITH *COOPERIA PUNCTATA*.

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SUMMARY: Because of the high prevalence of *Cooperia punctata* in Brazilian herds, an experiment was conducted to examine the water, nitrogen balance and body composition changes, in calves infected with that nematode. Four helminth-free males Friesan calves, four-months-old received a daily infection of 20,000-C. *punctata* infective larvae, for a two-week period. Five days before and at the third week after the beginning of infection the calves were housed in metabolic cages for body composition and nutritional studies. They were all injected with 1 MBq of tritiated water per kg body weight. Fecal, urine and blood samples were daily collected during the metabolic observations. Egg counts and PVC examinations were conducted throughout the experiment. The calves were necropsied twenty-four days after the beginning of infection. The mean worm burden at necropsy was 26,643 ± 12,028 *C. punctata*. There were no changes in the PVC parameters. The first eggs were passed out in feces on the 15th day. The study showed a tendency for decrease in the half-life and fractional turnover rate as well as a significant ($p < 0.05$) decrease in the total body water and fat free weight. The infection did not appear to affect adversely the water balance, although the mean fecal output and fecal water excretion were lower in the infected animals. A significantly ($p<0.05$) change in the nitrogen (N) balance was also observed, with lower N intake and reduction in the N retention by infected calves. A significant urinary N loss and decrease in the fecal N excretion affected these changes.

KEY WORDS: *Cooperia punctata*, pathophysiology, water metabolism, body composition, and nitrogen balance.

INTRODUCTION

Cooperia sp. is a common and widely distributed helminth of cattle. In Brazil four species of this genus are present: *C. punctata*, *C. pectinata*, *C. oncophora* and *C. spatulata*. *C. punctata* is the prevalent species throughout the Country (HONER & VIEIRA-BRESSAN, 1992).

An epidemiological study conducted with beef cattle in the Center-West Region showed 75.8% of *Cooperia* spp. among the gastrointestinal nematodes recovered in necropsies; *C. punctata* represented 92% of this genus (BIANCHIN *et alii*, 1990). Similar results were observed in beef cattle of the Southeast (LIMA *et alii*, 1990) and South Region (RASSIER *et alii*, 1990). Notwithstanding the differences of climate and management in those regions, animals submitted to post-mortem examinations were infected by 78.3% and 90% of *Cooperia* spp. respectively, and again the prevalent species was *Cooperia punctata*. VIEIRA-BRESSAN *et alii* (1989) found *C. punctata* as the prevalent helminth in epidemiological studies of dairy cattle in the Southeastern Region. This worm was recovered from all tracer and permanent animals throughout the year with peaks at the end of spring and summer.

In spite of the high incidence of this parasite in cattle, only scarce publications are available on the pathophysiological effects of the bovine infection. The infection by *C. oncophora* causes anorexia, weight loss, leakage of plasma proteins in the intestinal lumen and a significant reduction of nitrogen retention (ARMOUR *et alii*, 1987). Concurrent infections by *C. punctata* and *Haemonchus placei* produced significant protein leakage in the intestinal lumen and hypoalbuminemia in the infected animals, showing that in mixed infections by those parasites *C. punctata* should have an important role in changes of the albumin metabolism (VIEIRA-BRESSAN *et alii*, 1955).

Based on such evidences, the present study was designed to investigate changes in water balance and metabolism, nitrogen balance, and body composition of calves after multiple infection by *C. punctata*.

MATERIALS AND METHODS

Animals: Holstein-Friesan calves, four-months-old, mean body weight of 136 kg at the beginning of experiment, reared helminth-free, fed with powder dry milk (DU Balde Anhanguera) up to the 4th month, and gradually weaned at this age. After this, calves received daily one kg of commercial feed (Ternerina-Purina) and one kg of hay (Coast-cross, *Cynodon dactylon*). Water was given *ad libitum*.

Experimental design: Calves were daily infected for two weeks with 20.000 infective larvae of *Cooperia punctata* given by the oral route.

Before the infection, for five days and after the third week of infection, also for five days, animals were kept in metabolic cages. They were submitted to metabolic studies with tritiated water (TOH) and nutritional studies such as the nitrogen balance. During these periods, samples of blood, feces and urine were daily collected; the intake of food and water, as well as the output of feces and urine were measured.

Animals were weighed once a week, blood and fecal samples were taken for hematological and parasitic studies.

Calves were necropsied twenty-four days after the beginning of infection, to assess the helminth burdens.

Parasitic techniques: Strains of *C. punctata* from field isolates have been maintained since 1990 in worm-free calves in the "Departamento de Parasitologia do Instituto de Ciências Biomédicas da Universidade de São Paulo" and the "Biotério de Grandes Animais-ICB/USP". Infective larvae obtained from larval cultures, not older than 15 days were kept at room temperature up to the moment of being used. Calves were daily infected with 20,000 L_3 for two weeks. Fecal egg counts were carried out once a week, by means of a modified Mac Master technique (WHITLOCK, 1948). At necropsy, the small intestine was removed, opened along its entire length and its contents collected in a bucket. The mucosa was rinsed twice in water. After this, the intestine was immersed in a bath of sodium chloride solution 0.85% at 42°C for 12 hours to allow the detachment of nematodes. The total collected washings were made up to a known volume of water and two 10% aliquots were taken for later examination. All recovered *C. punctata* specimens of one these aliquots were counted.

Radioisotope techniques: Radioisotope studies using tritiated water (TOH) were done twice for five days periods. The first study was conducted before the experimental infection; the second started three weeks after the patent period. A stock solution of tritiated water containing 70 MBq ml^{-1}, was prepared. Each calf was injected through a catheter inserted in the jugular vein with 1 MBq kg^{-1} of TOH diluted in sodium chloride 0.85% solution. Plasma samples were collected 2, 3, 4, 5 and 6 h. after injection. This was followed by daily collections from Day 1 to Day 5.

Radioisotope techniques, total body water (TBW) tritium half-life (T1/2) and turnover determinations were calculated by dilution, using plasma values extrapolated by the disappearing rate of TOH in plasma, according to VIEIRA-BRESSAN *et alii* (1992).

The composition of body solids was calculated according to a regressive equation with corrections for body water suggested by MEISSNER *et alii*, (1990). This calculation used the previously determined TBW and living body weights recorded by the time TOH was injected.

Feed and water intake; excretion of feces and urine: Calves were put in metabolic cages a week before sample collection for studies of nitrogen and water balance. Special bags for feces retention were attached to each animal. Water, feed and hay were supplied twice a day, after thoroughly weighed. Feces and urine voided were also weighed, pooled and samples of 5% or 10% were stored at -20°C. Plastic containers used for urine collection were previously acidified with sulfuric acid 12N (MARTIN, 1966).

Metabolic water and losses by evaporation of body water were calculated by the indirect method suggested by VERMA *et al.* (1980). The dry matter contents of the daily-pooled samples of hay concentrate feed and feces were estimated after drying at 103° C for 24 h.

Nitrogen determination was directly made by the assay of total nitrogen by using the macro and micro Kjeldahl methods for feces and food and urine samples, respectively (AOAC, 1980).

Statistical Analysis: Data was analyzed by the analysis of variance. Where effects were significant ($p<0.05$), differences between means (± standard deviation) of each observed parameter, before and after infection, were compared with the Tukey test (SAS, 1985).

RESULTS

Clinical observations: Two infected calves showed soft feces on the third week after infection but recovered at the beginning of the fourth week. There was some anorexia after the third week, but no significant differences were observed in the mean weight gains (Table 1) or either in the packed cell volumes (PCV) throughout the study (Figure 1).

Table 1 - Water balance and body composition in calves before and after infection with *Cooperia puctata.*

Treatment	Live Weight (kg)	T 1/2 (hs)	Turnover (ml d^{-1} bw^{-1})	TBW* (%bw)	Fat* (%bw)	Fat free Weight* (%bw)
Infected n=4	141.57 ± 11.12	116.10 ± 6.0	73.31 ± 3.71	50.80 ± 0.97	62.82 ± 0.71	37.19 ± 0.71
No Infected n=4	136.77 ± 8.49	143.13 ± 9.73	86.78 ± 3.64	74.07 ± 2.16	45.78 ± 1.58	54.22 ± 1.58

n= number of calves per group *p<0.05

Parasitic data: Positive fecal egg counts were detected in the infected calves 15 days after infection increasing gradually up to the end of the experiment. The mean adult worm burden recovered from necropsy 24 days after infection was 26,643 ± 12,028 specimens. Most helminths were recovered from the duodenum and jejunum, no immature stages being found.

Water metabolism and body composition: Table 1 shows the measurements of TOH throughout the pre-infection period (not infected/Day –5 through Day 0) and after the beginning of infection (Infected/ Day +20 through Day +24). The mean of TBW expressed in a body weight percent shows a significant decrease in the infected calves when compared with the pre-infected period. Consequently, the body weight free from fat was reduced and an apparent increase of fat as a component of body weight was observed in those animals ($p<0.05$). The turnover rate and half-life of tritium apparently showed some decrease after infection, but were not statistically significant.

Water balance: Data of total water intake before or after infection did not show significant differences. However, the mean daily excretion of water in feces significantly decreased after infection being compensated by an apparent increase of urine output within the same period. The mean daily excretion of feces decreased after infection ($p<0.05$). There were no significant differences between the ingestion and excretion of dry matter in each one of the periods under study. The mean values are shown in Table 2.

Table 2 - Mean daily intake and excretion of water and dry matter and faecal excretion in calves before and after infection with *C. punctata.*

Treatment	Water intake (l d^{-1})	Water Excretion (l d^{-1}) urine	feces	total	Dry matter (kg d^{-1}) intake	excretion	Excretion of feces*
Infected n=4	9.21 ± 0.22	3.74 ± 0.76	3.19 ± 0.06	6.93 ± 0.81	3.85 ± 0.01	0.93 ± 0.01	4.13 ± 0.07
No Infected n=4	9.40 ± 0.43	2.67 ± 0.63	3.67 ± 0.19	6.35 ± 0.48	3.84 ± 0.02	0.98 ± 0.04	4.65 ± 0.22

n= number of calves per group *p<0.05

Nitrogen balance: Results of the daily means of input and output of nitrogen (N) in the two periods are shown in Table 3. In the post infection period there was a decrease in the N intake ($p<0.05$). The infected calves also showed a decrease of fecal N ($p<0.001$) and increase of N in urine ($p<0.05$).

Table 3 - Mean dailyN balance in calves before and after infection with *C. punctata.*

Treatment	N intake* (g d^{-1})	N excretion (g d^{-1}) feces*	urine*	total	Balance* (g d^{-1})
Infected n=4	87.71 ± 0.16	11.69 ± 0.19	28.00 ± 3.07	39.70 ± 3.17	48.01 ± 3.2
No Infected n=4	91.94 ± 0.44	15.00 ± 0.14	21.34 ± 0.99	36.34 ± 1.07	55.59 ± 0.73

n= number of calves per group *p<0.05

Consequently, the N retention was significantly reduced in the infected calves when compared with that observed in the pre-infection period.

DISCUSSION

Physiopathologic changes produced in calves by single infections of *C. punctata* were previously unpublished. In the present study the same animal is used as its own control, observations being done before and after the experimental infection.

Results showed that infections caused by *C. punctata*, helminth to which are frequently exposed young calves under field conditions throughout the year in virtually all livestock regions of Brazil are responsible by impaired development and future low productivity.

Clinical signs of infection by intestinal nematodes such as those produced by *C. punctata* like soft feces observed at the beginning of the patent period were also noticed at the beginning of that period (COOP *et alii*, 1979) or later (ARMOUR *et alii*, 1987) with serial infections by *C. oncophora*. These signs were concurrent with the increase of fecal egg counts and physiopathological changes occurring in the post-infection period keeping a close correlation with the individual worm burdens and egg counts. The pre-patent period confirmed the previous observations made on *C. punctata* (ALICATA & LYND, 1961). The lack of changes in the PCV does not agree with observations made on donor animals harboring the same level of a single infection by *C. punctata*. In these animals, the increase of PCV was concurrent with the peak of egg counts, softening of feces or diarrhea (VIEIRA-BRESSAN, personal communication); these differences are probably due to the type of infection or to the condition of animals. Notwithstanding, they are in agreement with the observations made with multiple infections by *C. oncophora* (ARMOUR *et alii*, 1987).

The observation period and time of infection were not sufficient to produce changes in weight gains. The small body weight increase detected in the post-infection period was attributed to normal growth.

The significant reduction of body water and the not significant decrease of the half-life of tritium (27 h.) in the post-infection period apparently affected the water turnover.

Animals have lost water in proportion to their live body weights. Consequently, when evaluating body composition, this reduction resulted in the increase of fat proportion related to body weight (with water) and a decrease of body weight free of fat (without water). However, what really occurred was a concentration of fat caused by the body water loss and the reduction of body weight free of fat (without water).

Although the water ingestion did not increase after infection, there was some little effect on the amount of water excreted in feces. This quantity was small because of the reduced excretion of feces within this period. A compensatory 30% increase in the output of urine maintained the water balance.

The lessening of feed intake which occurred on the third week of infection by *C. punctata* has also been observed after infections with *C. pectinata* (BREMMER, 1982) and *C. oncophora* (ARMOUR *et alii*, 1987). The biological mechanism of this anorexia is not yet known.

Although the digestibility coefficient of dry matter did not change between the periods, the smaller quantity of N ingested after the third week of infection leads to a significant reduction of N retention because of the increased output of N through the urine. Similar changes were observed in calves infected with *C. oncophora*, but this occurred between the fourth and eight weeks after infection (ARMOUR *et alii* 1987).

The smaller excretion of N in feces suggests a good protein digestibility, whereas the greater output of N through the urine denotes that catabolism has increased but not in such degree to cause N unbalance. The reduction in N balance means that less protein is being used to build up the muscular tissues because of the increased catabolism; there is, therefore, more availability of protein.

Apparently, this study suggests that *C. punctata* is more pathogenic to cattle than *C. oncophora*. Some remarkable changes of the analyzed parameters were detected after the ingestion of a weekly small number of infective larvae. This changes occurred at a lessen degree even when a larger number of *C. oncophora* larvae were used (COOP *et alii*, 1979; ARMOUR *et alii*, 1987).

As demonstrated by VIEIRA-BRESSAN *et alii* (1995), these metabolic changes are enhanced in mixed infections of *C. punctata* and *H. placei* particularly if it is considered that the effects are rather multiplicative than aditive.

RESUMO

Devido a alta prevalência de *Cooperia punctata* em rebanhos brasileiros, um experimento foi conduzido com finalidade de avaliar as alterações nos balanços hídricos e de nitrogênio, bem como na composição corporal de bezerros infectados com este nematóide. Quatro bezerros machos holandeses foram criados livre de parasitas e, aos quatro meses de idade foram inoculados com 20.000 larvas infectantes de *C. punctata* por dia durante duas semanas. Cinco dias antes da infecção, e três semanas após, os bezerros foram alojados em gaiolas metabólicas e submetidos a estudos nutricionais e de composição corporal. Todos os animais receberam uma injeção de 1 Mbq de água tritiada por kg de peso vivo. Amostras de fezes, sangue e urina foram coletadas diariamente durante o período experimental. Contagens de ovos por grama de fezes (opg) e acompanhamento do hematócrito foram realizados durante o experimento. Os animais foram necropsiados vinte e quatro dias após a infecção. A carga parasitária média à necropsia foi de 26,643 ± 12,028 adultos por animal. Não observou-se alterações no hematócrito. Os primeiros ovos foram encontrados nas fezes ao décimo quinto dia. O experimento demonstrou uma tendência de diminuição na meia-vida do trício e no turnover da água, juntamente com um significante ($p<0,05$) decréscimo na água corporal total e no peso corpóreo desengordurado. A infecção aparentemente não afetou de modo adverso o balanço hídrico, contudo a excreção fecal média e a excreção fecal de água foram menores nos animais infectados. Também foi observada uma alteração significante ($p<0,05$) no balanço de nitrogênio (N), com uma menor ingestão e redução na retenção de N pelos animais infectados. Estas alterações foram influenciadas por uma perda

significante de N por via urinária e um decréscimo na excreção fecal de N.

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(Received 4 June 1996, Accepted 20 July 1996)