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Pathophysiology of *Haemonchus placei* infection in calves

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ABSTRACT

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This study was conducted to investigate the pathophysiology of *Haemonchus placei* infection in Friesian calves. Seven calves were divided into two groups, three uninfected calves (control group) and four infected animals. The latter group were infected orally with 500 *H. placei* larvae kg^{-1} body weight. Five weeks after infection they were all housed in metabolic crates and injected with ^{125}I-bovine albumin, ^{51}Cr-red cells and ^{59}Fe-transferrin, to study albumin metabolism, erythrokinetics and ferrokinetics. The results showed that there was a significant reduction in the mean haematocrit values and reduced weight gains in the infected calves compared with the controls. There was also a change in the distribution of albumin from the extravascular to the intravascular pool and a significant increase in the plasma and blood volumes of infected calves although the blood and albumin loss via the gastrointestinal tract recorded in this study was similar in both groups.

INTRODUCTION

Many studies on haemonchosis have focused on the pathophysiology of experimental and natural infections of *Haemonchus contortus* in sheep. Early reports on the blood-sucking activities of the larval and adult stages indicated that this parasite causes haemorrhage into the host's abomasum, resulting in anaemias of varying severity (Veglia, 1915; Fourie, 1931; Baker et al., 1959). Clark et al. (1962) showed that the severity of the anaemia is related to worm

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burdens and Dargie and Allonby (1975) studied red cell loss and the ferrokinetics in single experimental infections in sheep using ^{51}Cr-labelled red cells and ^{59}Fe-labelled transferrin; these studies showed that there was a significant loss of red cells into the abomasum and an increase in the rate of red cell production in infected animals.

There has been little work on haemonchosis in cattle and there are no reports of the pathophysiology of *Haemonchus placei* infection.

The present study was conducted to provide information on the pathophysiology of *H. placei* infection in young calves. Bovine albumin labelled with ^{125}I was used to study changes in plasma albumin pools and the catabolic rate of albumin while ^{51}Cr-labelled red cells and ^{59}Fe-labelled transferrin were used to study red cell loss into the abomasum together with synthesis of red cells and utilization of iron in both infected and uninfected animals.

MATERIALS AND METHODS

Experimental design

The experiment involved seven 4- to 6-month-old Friesian calves that had been reared parasite-free from birth. The animals were divided into one group of four calves and one of three animals, based on age and weight. The group of four calves each received 500 infective third stage larvae (L_3) of *H. placei* kg^{-1} body weight, while the other animals remained as non-infected controls. Body weights were recorded before infection immediately prior to the radioisotope study and at necropsy. The animals were fed on hay and a commercial balanced concentrate ration (750 g twice a day).

Five weeks post-infection, after patency was established, the calves were placed in metabolic crates and fitted with faecal bags which allowed separate collection of urine and faeces. The calves were injected with ^{125}I-labelled albumin, ^{51}Cr-labelled red cells and ^{59}Fe-labelled transferrin. Blood samples were taken at intervals after injection and daily collections made of the total urine and faeces excreted by each calf.

The radioactivity of plasma, blood, urine and faeces were measured and the rates of turnover of plasma albumin, ferrokinetics and red cell loss into the gastrointestinal tract were calculated.

Two weeks after injection of the radioisotopes the calves were killed and the abomasal worm burdens recorded.

Parasitological techniques

Infective *H. placei* larvae were cultured from the faeces of an experimentally infected calf which had previously been reared and maintained parasite-

free. The infective dose was 500 larvae kg^{-1} body weight: 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 and Whitlock (1939) once a week starting a week before the infection until the beginning of the patency, when the animals were put in the cages.

At slaughter the abomasum was removed intact then opened, the contents collected in a bucket and the free surface of mucosae washed with tap water into the same bucket. The abomasum was then placed in a water bath at 42 °C with saline for 12 h to digest the mucosa and release any worms. The worms present in the total abomasal contents and the mucosal digest were counted.

Radioisotopic techniques

A solution of 2% bovine serum albumin (Sigma) was labelled with ^{125}I (New England, NH, U.S.A.) as described by McFarlane (1958) and each calf received 18.5 MBq of ^{125}I-albumin.

Twenty ml of heparinized blood was collected from each calf and the plasma was removed. The red cells were then labelled with ^{51}Cr-sodium chromate (Dupont, U.S.A.), 18.5 MBq per calf, as described by Gray and Sterling (1950).

The transferrin present in the plasma was labelled with 37 kBq ^{59}Fe-ferric citrate (Amersham, U.K.) kg^{-1} body weight as described by Pollycove and Mortimer (1961).

The calves were dosed orally each day with 10 ml of 0.75% (w/v) potassium iodide to saturate the thyroid and ensure rapid excretion of ^{125}I released by catabolism of ^{125}I-albumin.

Injection of labelled materials

For each calf a 20-ml syringe was filled with the ^{125}I-albumin and weighed. A 1.0-ml syringe was also filled with ^{125}I-albumin and weighed for preparation of the standard.

In the case of ^{51}Cr-labelled red cells, for each calf a 20-ml syringe was filled with a suspension of its own ^{51}Cr-red cells in physiological saline. A 1.0-ml syringe was also filled with each calf's suspended labelled red cells for preparation for the ^{51}Cr standards.

Seven 20-ml syringes were filled from the pool of plasma labelled with ^{59}Fe-transferrin and weighed. A 1.0-ml syringe was also filled and weighed for the ^{59}Fe standard.

The three radioisotopes were injected in rapid succession into the jugular vein using a multi-three-way tap assembly fixed to a catheter and subsequently flushed through with saline. After the injections the syringes were reweighed and the weight of the injected materials noted. The standard sy-

ringes were expelled into volumetric flasks, diluted to 100 ml and the syringes reweighed.

Several 1.0-ml aliquots of each standard solution were put in counting vials and diluted to 3.0 ml with 0.01 N NaOH.

Blood sampling

Blood samples were taken at 10, 20, 30, 45, 60, 90, 120, 180 and 240 min after injection and twice daily for the first 4 days post-injection then daily for the remainder of the experiment. The samples were collected from the jugular vein which had not been used for injection into vacutainer tubes containing di-potassium EDTA. One-ml aliquots of blood and plasma were pipetted into counting vials and diluted to 3.0 ml with 0.01 N NaOH.

Collection of faeces and urine

Faecal bags were emptied twice daily and the total daily faecal output from each animal was weighed. Eight samples of ~ 3 g were put into pre-weighed counting vials and the exact weight of faeces in the vials ascertained by re-weighing prior to counting.

The daily total output of urine from each calf was collected in a pre-weighed container. The container was then reweighed to obtain the total amount of urine excreted by each calf day^{-1}. Three aliquots of 3 ml were transferred to weighed vials and reweighed to find the exact weight of each urine sample to be counted.

Radioisotope determinations

The vials containing the samples of blood, plasma, urine and faeces and the standards were assayed in a bi-channel gamma spectrometer (Berthold LBMAG-312). By analysis of the ratios of the ^{125}I, ^{51}Cr and ^{59}Fe standard count rates in each counting channel, the count rate of each sample was ascertained.

Plasma volume and albumin turnover calculations

The plasma volume was measured with ^{125}I-albumin using the dilution principle and this together with the appropriate serum albumin value enabled the intravascular albumin pool (CA) to be calculated. The determination of the extravascular pool (EA) was based on the 'equilibrium method' described by Campbell et al. (1956). The catabolic rate of albumin was assessed from the apparent half-life as described by Sterling (1951) and more precisely by calculation of the intravascular catabolic rate (F(CA)), i.e. the frac-

tion of the intravascular pool degraded each 24 h. F(CA) was calculated from the total radioactivity excreted every 24 h divided by the total intravascular radioactivity (Campbell et al., 1956).

Red cell indices

Red cell volumes were measured using ^{51}Cr-red cells and the dilution principle. Blood volumes were calculated as the sum of plasma and red cell volumes. The half-life of the circulating ^{51}Cr-red cells was used as an index of red cell survival.

The rate of red cell synthesis was obtained from the following measurements: the half-life of the circulating ^{59}Fe-transferrin; the plasma iron turnover rate (PITR) in mg kg^{-1} day^{-1}; the percentage incorporation of iron into red cells (Dargie and Mulligan, 1970)

Daily packed cell volume (PCV) measurements were made and on the day prior to the commencement of the radioisotope study serum albumin, serum total protein and serum iron concentrations were determined for each animal.

Statistical methods

Student's *t*-test was used to evaluate data and significance was considered where $P<0.05$. Variations around the mean were expressed as the standard error (SE).

RESULTS

Body weight and worm burdens

Body weight gains in the infected and control animals during the period of the experiment are shown in Table 1. A mean gain of 21.25 ± 3.77 kg was recorded in the infected group compared with 34.33 ± 6.27 kg in the control calves but the difference was not statistically significant. Worm burdens at

TABLE 1

Mean weight gain (kg), infective dose and worm burdens in calves of infected and control group

Experimental group	Number of calves	Weight gain (kg)	Infective dose (500 L_3 kg^{-1})	Worm burdens
Infected	4	21.25 ± 3.77	$51\,750 \pm 2066$	3075 ± 1255
Control	3	34.33 ± 6.27	–	–

necropsy are also shown in Table 1. A mean of approximately 3000 worms established in the infected calves.

Albumin pools and catabolic rates

The plasma volumes, albumin pool sizes and albumin turnover rates based on the half-life ($T_{1/2}$) of the ^{125}I-albumin together with the fractional catabolic rate measurements in the infected and control calves are shown in Table 2.

Whilst there was no significant difference in the $T_{1/2}$ or the catabolic rate of the circulating albumin there was a significant increase in the plasma volume of the infected calves and a significant change in the distribution of albumin from the extravascular to the intravascular pool in the infected group, compared with the controls.

Erythrokinetics and ferrokinetics

The results of red cell kinetics are shown in Table 3 and ferrokinetics in Table 4.

Evidence of anaemia was shown by the significantly lower packed cell vol-

TABLE 2

Albumin metabolism of calves after infection with 500 *H. placei* larvae kg^{-1} and the respective controls (mean ± SE)

Experimental group	Number of calves	^{125}I-albumin $T_{1/2}$ (days)	Plasma volume (ml kg^{-1})	IA (g kg^{-1})	EA (g kg^{-1})	EA/IA (g kg^{-1})	F(CA)
Infected	4	15.7 ± 1.0	57.7 ± 5.3	1.90 ± 0.05	2.22 ± 0.02	1.17 ± 0.04	0.069 ± 0.005
Control	3	13.9 ± 2.2	47.7 ± 1.3	1.63 ± 0.14	2.36 ± 0.21	1.44 ± 0.03	0.074 ± 0.006

IA = intravascular pool of albumin; EA = extravascular pool of albumin; F(CA) = intravascular catabolic rate.

TABLE 3

Erythrokinetic values of calves after infection with 500 *H. placei* larvae kg^{-1} and the respective controls (mean ± SE)

Experimental group	Number of calves	Packed cell volume (%)	Red cell volume (ml kg^{-1})	Blood volume (ml kg^{-1})	^{51}Cr-red cells $T_{1/2}$ (h)	Clearance of ^{51}Cr-red cells by the faeces (ml day^{-1})
Infected	4	27.0 ± 1.9	16.4 ± 1.5	74.1 ± 2.7	199 ± 21.3	34.8 ± 14.7
Control	3	34.5 ± 1.8	15.6 ± 1.1	62.6 ± 2.2	221 ± 20.0	21.2 ± 7.9

TABLE 4

Ferrokinetic values of calves after infection with 500 *H. placei* larvae kg^{-1} and the respective controls (mean ± SE)

Experimental group	Number of calves	^{59}Fe $T_{1/2}$ (min)	PITR (mg kg^{-1} day^{-1})	% utilization of ^{59}Fe by the red cells
Infected	4	94.12 ± 5.29	0.72 ± 0.02	59.40 ± 1.59
Control	3	117.86 ± 24.19	0.48 ± 0.07	42.80 ± 6.30

^{59}Fe $T_{1/2}$ = half-life of ^{59}Fe in the blood; PITR = plasma iron turnover.

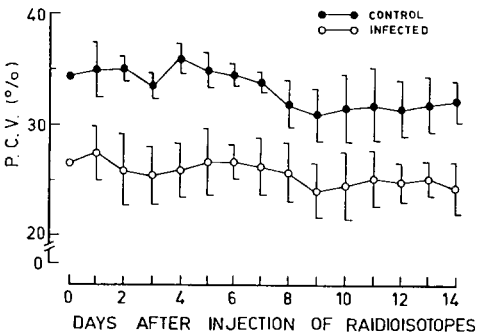

Fig. 1. Mean daily PCV (%) values of the calves infected with 500 *H. placei* larvae kg^{-1} and the respective controls (mean ± SE) after injection of the radioisotopes.

umes (PCV) in the infected calves (Fig. 1). This was reflected to some extent in slightly reduced ^{51}Cr-erythrocyte $T_{1/2}$ values of 199 ± 21.3 h in the infected animals compared with 221 ± 20.0 h in the controls. There was also evidence of an increase in faecal red cell clearance in the infected animals but the difference between these values in the two groups of calves was not statistically significant. Whilst there was no significant difference in the red cell volumes there was a significant increase in the blood volume of infected calves, i.e. 74.05 ± 2.7 ml kg^{-1} compared with 62.56 ± 2.18 ml kg^{-1} in the control group, which is reflected in a substantial increase in the plasma volume in the infected animals.

The rate of disappearance of ^{59}Fe from the plasma of the infected animals ($T_{1/2}$ = 94.12 ± 5.29 min) was faster than in the controls ($T_{1/2}$ = 117.86 ± 24.19 min) and the plasma iron turnover rates (PITR) were also greater in the infected calves (0.72 ± 0.02 mg kg^{-1} day^{-1}) than in the controls (0.48 ± 0.07 mg kg^{-1} day^{-1}). In addition the percentage utilization of ^{59}Fe by red cells showed an increase in the infected calves, i.e. 59.4 ± 1.59% compared with 42.8 ± 6.3% in the control group. These differences in the PITR and percent-

age of ^{59}Fe utilization by the red cells between the infected and control group were statistically significant.

There were no apparent differences between the concentration of plasma albumin, total plasma protein and plasma iron in the two groups and the values 3.0–3.8 g 100 ml^{-1} for albumin, 6.5–8.5 g 100 ml^{-1} for total protein and 70–250 μg 100 ml^{-1} for plasma iron are in the range of normal bovine values (Swenson, 1984).

DISCUSSION

Abbott et al. (1985a,b, 1986a,b) working with sheep, showed that variations in the phase of infection, the infective dose, the age, breed and the immune and nutritional status produced significant alterations in the pathophysiology of ovine haemonchosis.

In this study the infective dose of *H. placei* of 500 L_3 kg^{-1} body weight in calves was responsible for a moderate infection similar to that found in sheep with a dose of 125 L_3 kg^{-1} *H. contortus* and the alterations in red cell and protein kinetics were similar (Abbott et al., 1985b). With a dose of 350 L_3 of *H. contortus* kg^{-1} body weight in sheep there was an acute syndrome with significant metabolic alterations and mortality (Abbott et al., 1986b).

While there was evidence of increased faecal clearance of red cells and reduced red cell survival times, as indicated by the circulating red cell half-life, in the infected animals these differences were not statistically significant and no apparent difference in the circulating red cell volumes of the infected calves was observed. Despite this there was evidence of anaemia from the low PCV values in the infected calves.

An examination of the erythropoesis using ^{59}Fe-ferric citrate showed that the radiolabelled iron disappeared from the plasma of the infected animals at a faster rate than from the controls, but this difference was not statistically significant. However, the rate of turnover of iron kg^{-1} body weight day^{-1} and the percentage of iron utilization by red cells were significantly greater in the infected calves. This greater rate of turnover of iron in the infected animals is an indication of an increased rate of erythropoesis, which is probably the result of the blood-sucking activities of the worms in the abomasum.

There was no indication of any hypoalbuminaemia in this study in calves. These findings are in agreement with a previous study in sheep with moderate *H. contortus* infections (Kerboeuf, 1977). Also Baroni (1979) has shown that total protein levels decreased proportionally to increasing levels of infection in sheep.

The significantly higher blood volumes found in the infected calves are a consequence of the significantly higher plasma volume in these animals (see Table 2). Although there was a change in the ratio of extravascular to intravascular albumin in the infected calves, there was no clear indication of

altered albumin catabolism in this study. Allonby and Dargie (1973) found similar reductions in the ratio of extravascular to intravascular albumin in sheep chronically infected with *H. contortus*. However, Dargie (1975) showed that hypoalbuminaemia is a feature of acute ovine haemonchosis caused by greatly increased loss of plasma protein into the abomasum with a significantly increased albumin catabolism. Also 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. The situation regarding protein loss/reabsorption in cattle remains unclear and subsequent experiments are planned to examine the influence of higher levels of *Haemonchus* infection on the development and pathophysiology of bovine haemonchosis using larger numbers of experimental animals. Some of the findings in this study may have been more statistically significant had more animals been included in the two experimental groups.

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