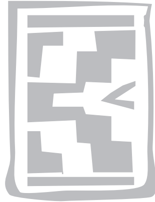

A macro- and light microscopical study of the pathology of *Calicophoron microbothrium* infection in experimentally infected cattle

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ABSTRACT

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Twelve Tuli weaner steers aged 1 year were randomly subdivided into three groups of four animals and infected with different doses of *Calicophoron microbothrium* metacercariae. Each animal in Group I received a low dose (LD) of 5 000 metacercariae, Group II a medium dose (MD) of 15 000 metacercariae, Group III a high dose (HD) of 25 000 metacercariae and one additional animal was kept as an uninfected control (C). After infection, one animal from each group was slaughtered on Day 28, 42, 56 and 84 post infection (pi) and samples from the ileum, jejunum, duodenum, abomasum and the rumen were collected for histopathological and cytological examination.

On Day 28 pi, the gross pathological lesions observed in the duodenum of the LD and the MD animals were similar and comprised duodenal thickening, corrugation, hyperaemia, petechiation and ulceration. In the HD animal the duodenal lesions were similar but more severe. The abomasal folds were severely oedematous in the MD group and nearly occluded the abomasal lumen. Moderate oedema of the abomasal folds was also present in the LD and HD animals. The gross pathological lesions regressed in all the infected groups with increasing age of infection and had disappeared completely by Day 56 pi.

On Day 28 pi the histopathological lesions in the duodenum and jejunum of the LD and MD groups were similar, comprising subtotal villous atrophy, hyperplasia of Brunner's glands and Peyer's patches and moderate infiltration of eosinophils, mast cells and a few globule leukocytes, basophils and lymphocytes in the lamina propria. The HD group had total villous atrophy, severe hyperplasia and cystic dilatation of Brunner's glands, which had expanded to cover the entire submucosa. On Day 42 pi the histopathological lesions were still present in the MD and the HD groups comprising subtotal villous atrophy and hyperplasia of Brunner's glands. Heavy infiltrations of eosinophils, moderate amounts of mast cells and a few basophils, globule leukocytes and lymphocytes were still present in the lamina propria of all three groups. On Day 56 pi, a few glands were still cystic in the MD and the HD groups. Moderate cell infiltrations were still present in the lamina propria of all the three groups and by Day 84 pi complete regeneration had occurred in all animals.

Keywords: *Calicophoron microbothrium,* cattle, pathology, intestinal tract, metacercariae, rumen, Tuli

INTRODUCTION

Ruminant amphistomosis is an infection of cattle, sheep, goats and other wild ruminants caused by severe infections of immature amphistomes in the small intestines of immunologically incompetent hosts (Horak 1967; Dutt 1980; Gupta 1993). The disease is characterised by sporadic epidemics of acute parasitic gastroenteritis and loss of production associated with high mortality and morbidity particularly in young stock (Butler & Yeoman 1962; Boray 1969; Horak 1971; Singh & Lakra 1971; Dutt 1980).

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Infection follows ingestion of herbage heavily contaminated with amphistome metacercariae (Dutt 1980; Boray 1985). The adult ruminal amphistomes are not associated with clinical disease and have been described as commensals living on ruminal contents of the host (Boray 1985; Cheruiyot & Wamae 1988).

The amphistome species responsible for the majority of outbreaks of immature amphistomosis in ruminants and their related snail intermediate hosts have been recorded in many countries (Dinnik & Dinnik 1962; Dinnik 1964; Horak 1971; Dutt 1980; Over 1982; Boray 1985; Eduardo 1982a, 1983, 1988; Sey 1991; Mukaratirwa, Siegismund, Kristensen & Chandiwana 1996; Mukaratirwa, Kristensen, Siegismund & Chandiwana 1998; Chingwena, Mukaratirwa, Kristensen & Chimbari 2002; Madzingira, Mukaratirwa, Pandey & Dorny 2002).

In Zimbabwe, amphistome species recovered from domestic and wild ruminants include *Calicophoron calicophorum, Calicophoron microbothrium, Calicophoron clavula, Calicophoron phillerouxi, Calicophoron sukumum, Calicophoron raja, Paramphistomum cervi, Cotylophoron cotylophorum* and *Cotylophoron jacksoni* (Eduardo 1982a, 1983, 1988; Jooste 1989; Sey 1991; Madzingira *et al.* 2002).

In southern Africa, including Zimbabwe, *C. microbothrium,* with the snail species *Bulinus tropicus* as the main intermediate host, has a wide distribution and has been incriminated in the majority of outbreaks of immature amphistomosis in ruminants (Dinnik & Dinnik 1962; Swart & Reinecke 1962a; Dinnik 1964, 1965; Horak 1971; Over 1982; Chingwena *et al.* 2002).

The clinical and pathological manifestations of amphistomosis have been studied in both field and experimental infections particularly in sheep and goats (Horak & Clark 1963; Deorani & Katiyar 1967; Horak 1967; Boray 1969; Panda & Misra 1980; Singh, Sahai & Jha 1984; Rolfe, Boray & Collins 1994). Studies in cattle are limited and information on the clinical and pathological manifestations of the disease has been derived mainly from field outbreaks (Butler & Yeoman 1962; Singh & Lakra 1971; Kelly & Henderson 1973) and no experimental infections have been conducted to follow the course of the disease and establish its pathogenesis.

The objective of this study was to describe the pathology of *C. microbothrium* in experimentally infected cattle.

MATERIALS AND METHODS

Study area and selection of experimental animals

The study was conducted at Grassland Research Station, a State owned farm in Marondera about 80 km south east of Harare, Zimbabwe. The farm is amphistome-free and mainly raises Tuli cattle for commercial beef production. It is subdivided into paddocks where cattle can graze and have access to clean borehole water provided in troughs. Prior to the commencement of the study, faecal samples were collected from 30 1-year-old Tuli weaner steers for coprological examination using the sedimentation technique and the modified McMaster technique (MAFF 1986) to screen for trematode and nematode infection respectively. Thirteen animals were randomly selected for the study from the parasite-free weaner steers, on the basis of age and mass.

The animals selected were allowed to graze in paddocks and had access to clean borehole water provided in concrete troughs and were dewormed every month using 10 % fenbendazole.

Identification of *C. microbothrium* and harvesting of metacercariae

Adult *B. tropicus* naturally infected with *C. microbothrium* were collected from a single natural habitat and maintained under laboratory conditions for periodic shedding of metacercariae. The identity of the *C. microbothrium* metacercariae was confirmed through the infection of a single sheep and recovery of mature amphistomes from the rumen for identification. The amphistomes were identified as *C. microbothrium* using the methods described by Eduardo (1982b, 1983) and Gupta (1993).

For mass production of metacercariae and to avoid the ingestion of the shed metacercariae by the snails, small nylon gauze bags with an aperture size of 2 mm, each capable of holding 20 snails, were used to confine snails during shedding. The open end of each snail bag was stapled close. Each bag was immersed in individual plastic containers holding 500 mℓ of dechlorinated pond water and exposed to direct sunlight for 3 h. The cercariae encysted at the water meniscus. Shedding was induced every 2 days whereafter the snails were placed back in the dark aquaria. The metacercariae were stored in individual plastic containers with dechlorinated pond water added to just below the level of the band of metacercariae. They were kept at a temperature of between 24–25 °C for a minimum of

6 days before they were harvested with a fine tooth-brush and dosed to the experimental animals.

Infection of experimental animals

The numbers of metacercariae dosed to cattle in this study were an extrapolation from Rolfe, Boray, Nichols & Collins (1991) and metacercaria viability studies conducted by Swart & Reinecke (1962b), which showed that less than 50 % of metacercariae administered to ruminants survive. Three dosing regimens were therefore designed: each animal in Group I received 5 000 metacercariae and acted as the low dose group (LD), in Group II each animal received 15 000 metacercariae representing the medium dose group (MD) and in Group III each animal received 25 000 metacercariae representing the high dose group (HD). The dose for individual animals was estimated by counting the metacercaria in several aliquots, as described by Rolfe *et al.* (1994) and was administered by direct inoculation into the rumen using a trocar and canula. One animal remained as an uninfected control (C).

The animals were monitored daily for the development of clinical signs. One animal from Groups I–III was randomly selected, stunned and slaughtered on Day 28, 42, 56 and 84 post infection (pi). The control animal was slaughtered on Day 28 and used as a reference for comparing pathological lesions.

Sample collection from slaughtered animals

A midline incision from the xyphoid cartilage to the inguinal region was made and the rumen and intestines were exposed by cutting through and reflecting the abdominal and paracostal muscles on either side of the midline incision. The small intestines of each animal slaughtered were separated from the rest of the organs with minimal manipulation. Starting from the distal end of the jejunum, one metre long small intestinal loops were tied at both ends, cut and placed in labelled plastic containers for fluke recovery. This was followed by the collection of samples of the intestines for histopathological examination. The samples were rinsed in physiological saline to remove excess faecal material before each segment was preserved in Bouin's fixative. Samples were also removed from the cranial ruminal pillar and the fundus of the abomasum and preserved in Bouin's fixative fluid.

Fluke recovery

To determine the distribution of flukes established along the small intestines, the intestinal segments were scraped to the level of the muscular tunic using glass slides to recover the embedded flukes.

The contents of the abomasum, omasum and the reticulum were washed into separate containers and serially sieved under pressure through 2400 µm and 850 µm sieves, respectively. The intestinal mucosal scrapings were similarly sieved and the flukes were collected after sedimentation at the bottom of a jar of 10 ℓ capacity. Excess water was decanted and the recovered flukes were preserved in 70 % alcohol for identification and enumeration.

Gross pathology

At each necropsy the intestinal portions were opened separately, examined for gross pathological lesions before their mucosal surfaces were scraped to remove embedded flukes (Boray 1969). In assessing the gross pathology of the small intestine, the duodenum, proximal jejunum, distal jejunum and the ileum were demarcated on the basis of their distances from the pylorus. The duodenum constituted the first meter from the pylorus, the proximal jejunum extended for 3 m from the distal end of the duodenum while the distal jejunum extended for 4 m from the distal end of the proximal jejunum. The ileum extended up to the ileo-cecal junction. A checklist of gross pathological lesions was drawn up based on the severity of abomasal oedema, intestinal wall thickening, mucosal corrugation, hyperaemia, petechiation and ulceration. The severity of the gross pathological lesions present were compared between the different experimental groups.

Histopathology

The samples preserved in Bouin's fluid were embedded in paraffin wax. Triplicate 4 µm thick sections were cut from each segment of the small intestines, abomasum and rumen and each section stained with haematoxylin and eosin (H&E), toluidine blue-ferric sulphate-ferrioxamine B or Giemsa-chromotrope stain. The toluidine blue stain was used to differentiate mast cells while the Giemsa-chromotrope stain was used to differentiate eosinophils and basophils. The H&E stain was used to stain globule leukocytes and the evaluation of general histopathology of the gastrointestinal regions. Similar to the assessment of the gross pathology, a checklist of histopathological lesions was drawn up based on the severity of villous atrophy, cryptal hyperplasia, goblet cell hyperplasia, dilatation of Brunner's glands and hyperplasia of Peyer's patches. The histopathological lesions observed were compared between the different experimental groups.

Data analysis

The villous crypt unit method for counting eosinophils, mast cells, basophils and globule leukocytes as described by Miller & Jarret (1971) was used. For each intestinal segment, three intact villous crypt units were identified and the different cell types estimated. The populations of tissue eosinophils, mast cells, globule leukocytes and basophils in the different intestinal sections were estimated using a four point scale (0, +, ++, +++).

RESULTS

Clinical signs

Animals in the LD and C groups remained clinically normal during the study period. An effortless and voluminous foul smelling projectile diarrhoea developed in three of the four animals in the MD group on Day 21 pi and persisted for 3 days in two of the animals. In the third animal, the diarrhoea persisted for a week and the animal became inappetant, dehydrated, emaciated, lagged behind the group and never fully recovered until it was slaughtered on Day 56 pi. No clinical signs were evident in the fourth animal in the group.

In the HD group, a non-projectile diarrhoea developed in all animals. The diarrhoea developed on Day 21 pi and persisted for 3 days before all the animals recovered. Thereafter no clinical signs or changes in body condition were observed in any of the animals until Day 84 pi when the last animal in the group was slaughtered.

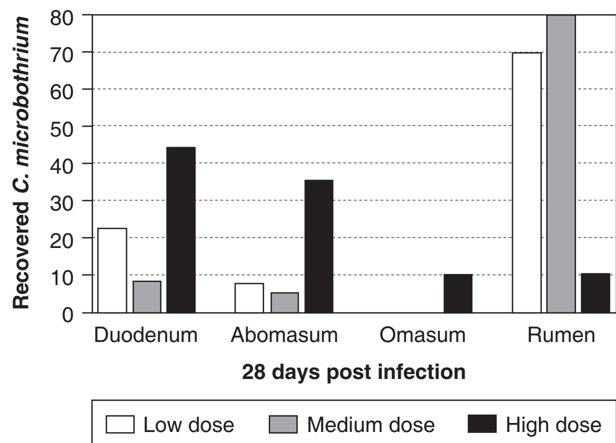

FIG. 1 Distribution patterns of recovered *Calicophoron microbothrium* (%) in cattle infected with different doses of metacercariae and slaughtered on Day 28 post infection

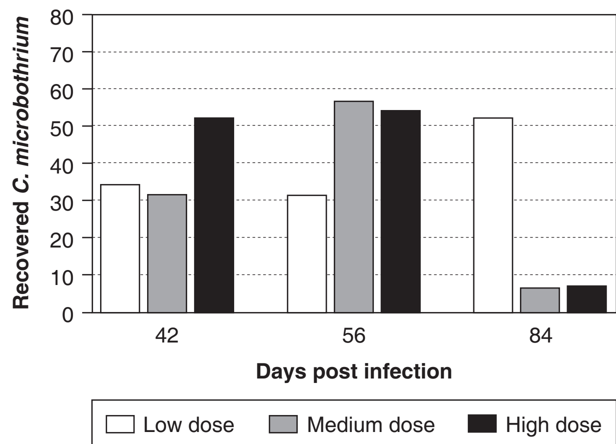

FIG. 2 Percentage establishment of *Calicophoron microbothrium* recovered from the rumen of cattle infected with different doses of metacercariae and slaughtered on Day 42, 56 and 84 post infection

Fluke recovery and distribution

The distribution patterns of flukes recovered from the various gastrointestinal sites among the different experimental groups on Day 28 pi are presented in Fig. 1. In the LD group, 781 (15.6 %) of juvenile flukes were recovered from the different regions of the gut of the animal slaughtered on Day 28 pi. Of the recovered flukes, 22.4 % were from the proximal third of the duodenum, 7.9 % from the abomasum and the remaining 69.7 % from the rumen along the oesophageal groove and the cranial pillar of the rumen. In the MD group, 4 044 (27 %) of juvenile flukes were recovered from the various intestinal sites of the animal slaughtered on Day 28 pi. Of the flukes present, 8.3 % occurred in the proximal third of the duodenum, 4.9 % in the abomasum and the remaining 86.8 % were recovered along the oesophageal groove and the cranial pillar of the rumen.

In the HD group, 965 (4 %) of juvenile flukes were recovered from the various gut sites the animal slaughtered on Day 28 pi. Of the flukes present, 44.4 % were recovered from the proximal half of the duodenum, 35.3 % from the abomasum, 10.4 % from the omasum and the remaining 10 % from the oesophageal groove and the cranial pillar of the rumen.

All the flukes were recovered from the cranial pillars of the rumen of all the animals slaughtered on Days 42, 56 and 84 pi, and the percentage that had become established in the slaughtered animals is presented in Fig. 2.

The percentage of flukes recovered at each slaughter occasion varied between animals, with recovery rates ranging from 4 % to 56 %.

Pathology

Gross pathology

The gross pathological lesions of the gut observed in all the infected groups at different stages of infection were confined to the abomasum, the duodenum and the jejunum and are summarized in Table 1.

Histopathology

The histopathological lesions of the gut observed in all the infected groups at different stages of infection were confined to the duodenum and the jejunum and were characterized by villous atrophy, goblet cell hyperplasia, cystic dilatation of Brunner's glands and hyperplasia of Peyer's patches.

There was subtotal villous atrophy in the duodenum of the LD animal slaughtered on Day 28 pi. The villous atrophy was characterized by fusion of villi, transformation of absorptive enterocytes into low cuboidal, and moderate hyperplasia of the crypts which were elongate and emptying into the intestinal lumen.

The goblet cells were hyperplastic and occurred in large numbers in the crypts and along epithelia of the villi. Necrotic debris and free-lying flukes were observed in the lumen. The lamina propria was moderately infiltrated by eosinophils, mast cells and a few globule leukocytes, basophils and lymphocytes. In the submucosa Brunner's glands were hyperplastic, and occupied nearly half of the submucosal area. Some cystic glands were surrounded by eosinophils and a few lymphocytes. The Peyer's patches were hyperplastic and expanded into the mucosal area in some sections. In the jejunum, subtotal villous atrophy characterized by shortening and blunting of the villi with elongate crypts emptying into the intestinal lumen was present. The lamina propria was mildly infiltrated by eosinophils, mast cells and low levels of globule leukocytes and basophils.

The duodenal and jejunal lesions in the MD animal were similar to those in the LD animal slaughtered on Day 28 pi. However, infiltration of eosinophils and mast cells into the lamina propria tended to be heavier.

In the HD animal, Brunner's glands were hyperplastic and cystic and had expanded to cover the entire submucosal area on Day 28 pi. The cystic glands were surrounded by an intense eosinophilic cell reaction and a few lymphocytes. Total villous atrophy characterized by severe shortening of the villi and elongate crypts opening into the duodenal lumen was evident. Goblet cell and Peyer's patch hyperplasia were present and comparable to those in the LD and MD animals. The lamina propria in both the duodenum and the proximal jejunum was heavily infiltrated by eosinophils, moderate amounts of mast cells and a few basophils, globule leukocytes and lymphocytes. The histopathological lesions in the distal jejunum were similar to those in the LD and MD animals slaughtered at the same time.

On Day 42 pi the duodenal glands of the LD animal were hyperplastic, non-cystic and contained little mucin. Moderate villous atrophy and goblet cell hyperplasia were still present. The lamina propria was moderately infiltrated by eosinophils, mast cells, lymphocytes and a few globule leukocytes while basophils were not present. Similar cell infiltrations were present in the jejunum.

In the MD animal, the submucosal area of the duodenum was expanded to three times the size of the control animal and half the space was occupied by the hyperplastic Brunner's glands. Some of the glands were cystic and contained mucin. An intense eosinophilic cell reaction was present in the submucosa and around the cystic glands. Subtotal villous atrophy and mild goblet cell hyperplasia was still present along the crypts and epithelia of the villi. The lamina propria was heavily infiltrated by eosinophils, mast cells and low numbers of globule leukocytes and basophils. In the jejunum, subtotal villous atrophy was still present. The lamina propria was heavily infiltrated by eosinophils, moderate amounts of mast cells and lymphocytes and low numbers of globule leukocytes. In the HD animal, the duodenal and jejunal lesions were similar to the MD animal. However, infiltration of eosinophils and mast cells into the lamina propria was moderate and comparable to the LD animal.

On Day 56 pi, the villi were elongate and leaf-like with normal epithelia in both the duodenum and jejunum of the LD, MD and the HD animals. Some Brunner's glands were still cystic in the MD and HD animals. Goblet cell numbers had reduced to minimal levels in the majority of sections, while regenerative fibrous tissue deposition became evident in the lamina propria and in the submucosal area of all animals. The lamina propria of the MD animal was, however, still infiltrated by large numbers of eosinophils, lymphocytes and moderate numbers of mast cells in comparison to the LD and the HD animals.

On Day 84 pi, the villi in both the duodenum and the jejunum of all infected animals were elongate and

TABLE 1 Summary of gross pathological lesions of the abomasum, duodenum and the jejunum of groups of cattle experimentally infected with different doses of *Calicophoron microbothrium* and slaughtered on different days post infection

Days pi	Group	Gut site	Main gross lesions
28	LD	Abomasum	Mild oedema of abomasal folds present but only appreciated on cut surfaces
	MD		Severe oedema of abomasal folds present, abomasal folds swollen and nearly occluded the lumen
	HD		Moderate oedema of abomasal folds present, abomasal folds moderately swollen and could be easily flipped over
	C		No gross lesions observed
	LD, MD	Duodenum and jejunum	Duodenal wall thickened and corrugated. Height from muscularis mucosa to tip of mucosal rugae varied from 8 mm in the proximal duodenum to 4 mm in the distal end. The duodenal and jejunal contents were watery and mucoid
	HD		Duodenal wall severely thickened and corrugated. Height from muscularis mucosa to tip of mucosal rugae varied from 10 mm in the proximal duodenum to 4 mm in the distal end. The duodenal and jejunal contents similar to the LD and MD groups
	C		No lesions detected. Duodenal wall thickening and corrugation absent. Height from muscularis mucosa to tip of mucosal rugae varied from 3 mm in the proximal duodenum to 2 mm in the distal distal end. Duodenal and jejunal contents normal
42	LD, C	Abomasum	No lesions detected
	MD, HD		Mild oedema of abomasal folds present but only appreciated on cut surfaces
	LD, MD, HD	Duodenum and jejunum	Mild thickening and corrugation of duodenal wall. Height from muscularis mucosa to tip of mucosal rugae varied from 5 mm in the proximal duodenum to 3 mm in the distal end. Duodenal and jejunal contents viscous with mucus floccules present
	C		No lesions detected
56	LD, HD, C	Abomasum	No lesions detected
	MD		Mild oedema of abomasal folds present but only appreciated on cut surfaces
	LD, MD, HD	Duodenum and jejunum	Mild thickening and corrugation of duodenal wall. Height from muscularis mucosa to tip of mucosal rugae varied from 5 mm in the proximal duodenum to 3 mm in the distal end. Duodenal and jejunal contents viscous with mucus floccules present
	C		No lesions detected
84	LD, MD, HD, C	Abomasum, duodenum and jejunum	No lesions detected
	MD	Rumen anterior pillar	Fragmentation of papillary tips, Flukes firmly attached to the base of the ruminal papillae. Foci of papillae inhabited by the flukes were pale and some of the papillae had fallen off the ruminal wall leaving non-ulcerated surface patches
	LD, HD		Some papillae had fragmented tips but maintained their normal colour
	C		No lesions detected

LD = Low dose MD = Medium dose HD = High dose C = Control

covered with normal epithelium. The lamina propria was infiltrated with mainly lymphocytes, fibrocytes and a few eosinophils. Brunner's glands were normal in the LD and MD animals while in the HD animal a few were still cystic and surrounded by lymphocytes.

Histopathological lesions of the rumen were evident on Days 56 and 84 pi in the MD animals and were characterized by thickening and vacuolation of the stratum corneum. In areas where the papillae had fallen off due to parasite feeding and anchorage, microabcesses were present confined to the stratum corneum. A mild cell reaction comprising scattered eosinophils and a few lymphocytes was present around the affected areas at the dermal-epidermal junction.

No significant histopathological lesions were seen in the rumen of the LD, HD and the control animals at any stage of infection.

DISCUSSION

The pathology of amphistome infection has been systematically studied in goats and sheep (Horak & Clark 1963; Singh *et al.* 1984; Rolfe *et al.* 1994) in which experimental infections were conducted and the disease followed until after patency. Besides a few reports which describe the pathology of amphi stomes in cattle following acute field outbreaks (Butler & Yeoman 1962; Singh & Lakra 1971; Kelly & Henderson 1973) this study is the first in cattle, where periodic tissue alterations following infection with different doses of *C. microbothrium* metacercariae are described.

The clinical signs exhibited by the infected cattle in the present study are similar to experimental and field observations made in other reports (Butler & Yeoman 1962; Horak & Clark 1963; Horak 1967; Deorani & Katiyar 1967; Boray 1969; Singh & Lakra 1971; Panda & Misra 1980; Rolfe *et al.* 1994; Pillai & Alikutty 1995). However, polydypsia as described in sheep by Horak & Clark (1963) and by Boray (1969) was not observed and could have been missed since the study animals were not constantly under observation.

The occurrence of clinical amphistomosis and subsequent pathology in ruminants is dependent on the dose, the virulence and the establishment levels of the infecting metacercaria in the small intestine of the host (Horak 1966, 1967; Rolfe *et al.* 1994). The doses of metacercariae used in this study managed to reproduce the clinical disease. The parasite establishment rates obtained for the LD and MD groups are comparable to those obtained by Horak (1967) in sheep and induced the expected clinical trends of the disease. However, parasite establishment in the HD group was low and could be due to either complete failure of parasites to establish or overcrowding of the immature amphistomes in the small intestine and subsequent expulsion of unattached parasites by the host during the diarrhoeic phase of the infection (Boray 1969). The highest concentration of immature amphistomes was recovered from the small intestines of the HD group making the later theory more likely. It is also evident that fluke migration to the rumen was slow in the HD group and only a small proportion of the flukes had migrated to the rumen by Day 28 pi. Similar trends have been observed in sheep and cattle heavily infected with immature amphistomes (Horak 1967; Boray 1971; Rolfe *et al.* 1994).

In this study, the gross pathological lesions were confined to the jejunum, duodenum and the abomasum of all infected groups and this is related to the migratory habits of the immature flukes which upon establishment in the duodenum migrate to the rumen through the abomasum and omasum causing tissue destruction (Boray 1969; Singh & Lakra 1971; Rolfe *et al.* 1994). Similar lesion distribution has also been observed in cattle (Butler & Yeoman 1962; Singh & Lakra 1971; Kelly & Henderson 1973) and in sheep (Horak & Clark 1963; Deorani & Katiyar 1967; Horak 1967; Boray 1969). The intensity of lesions appeared correlated with the establishment rate and subsequent migration of immature flukes to the rumen. Large numbers of immature flukes in the duodenum have been reported to cause overcrowding and competition among the parasites which results in delayed migration and exacerbates the disease (Horak 1967; Boray 1971; Rolfe *et al.* 1994). At the same time, poorly attached flukes, particularly those which excyst beyond the duodenal area, are ejected (Horak 1971). This scenario probably explains the massive duodenal pathology observed in the HD group compared to the other infected groups. Although overall fluke establishment was low in the HD group, the highest number of immature flukes was recorded in the duodenum of this group.

The duodenal and abomasal changes observed in the infected groups in this study are similar to observations made by Butler & Yeoman (1962), Singh & Lakra (1971) and Kelly & Henderson (1973) in cattle. The immature flukes cause tissue destruction by

anchoring and burrowing into the intestinal mucosa during migration to the submucosa for sojournment in Brunner's glands (Deorani & Katiyar 1967; Boray 1969; Singh & Lakra 1971). The lesions were present until Day 42 pi after which they regressed and by Day 84 pi no appreciable differences in gross pathology could be detected between the infected and the control groups. The disappearance of the lesions coincided with the completion of migration of the immature amphistomes from the duodenum to the rumen, confirming earlier reports that the pathogenic mechanisms of amphistomes are related to duodenal damage caused the by immature flukes (Horak 1971).

Mature amphistomes are considered non-pathogenic in the rumen (Cheruiyot & Wamae 1988), but the finding of ruminal lesions in this study seem to suggest that when present in large numbers they may cause disease directly or indirectly through systemic absorption of ruminal microflora via necrotic papillary ends. However, the dose of metacercariae used in this study appeared insufficient to cause clinical ruminal dysfunction. The ruminal lesions observed in this study have also been described in sheep and goats (Mukherjee & Deorani 1962; Singh *et al.* 1984; Rolfe *et al.* 1994). The ruminal lesions were pronounced on Day 84 pi particularly in the MD group from which a large number of flukes were recovered. This is probably attributable to the attainment of maturity and the crowding effect at a single predilection site by the flukes (Horak 1971).

Histopathological examination of the duodenal mucosa did not reveal any flukes embedded in Brunner's glands in any of the infected groups as has been observed in sheep and goats (Varma 1961; Rolfe *et al.* 1994). Brunner's glands provide an ideal environment for fluke growth until they become mature enough to withstand the acid environment in the abomasum during migration to the rumen (Deorani & Katiyar 1967; Boray 1969; Deorani & Jain 1969). Brunner's glands were, however, cystic with an atrophied basal epithelium suggesting prior occupation. Some flukes, however, could be seen in the mucosal tissue probably migrating to the duodenal surface. The absence of flukes embedded in Brunner's glands is probably related to the design of the study in which the animals were slaughtered late in the course of the infection after parasitic migration to the rumen had commenced.

There was glandular enlargement with severe villous atrophy characterized by destruction of absorptive enterocytes, fusion of villi and subsequent cryptal hyperplasia as reported in sheep (Rolfe *et al.* 1994). Villous atrophy characterized by villous and cryptal epithelial differentiation, hyperplasia and loss of function is a known feature of helminth infections and its mechanisms are obscure but could be related to immunological mechanisms against gastrointestinal parasites (Miller 1984). Similarly, with amphistome infections, the mechanisms of villous atrophy are unknown but have been ascribed to either traumatic destruction of tissues or enzymatic dissolution of the mucosa by the migrating flukes (Singh & Lakra 1971). Villous atrophy together with cystic dilatation of Brunner's glands, hyperplasia of lymphoid follicles and marked mixed cellular infiltration into the lamina propria were probably responsible for the duodenal thickening and corrugation of the mucosa observed grossly in this study. The severity of the lesion appeared correlated with the magnitude of the dose of metacercariae as lesion intensity tended to increase with increasing dose and this could be related to the different levels of sensitization that occur following metacercaria excystment and subsequent migration to the rumen.

In this study, the migration of immature flukes into the duodenal submucosa induced an acute inflammatory reaction characterized by an infiltration of eosinophils, mast cells, basophils, globule leukocytes, lymphocytes and macrophages into the mucosal parenchyma. The goblet cell population also increased and the cells appeared active. Similar responses have been observed as a feature of gastrointestinal nematode infection in sheep (Huntley, Patterson, Mackellar, Jackson, Stevenson & Coop 1995; Pfeffer, Douch, Shaw, Gatehouse, Rabel, Green, Shirer, Jonas & Bisset 1996; Winter, Wright & Lee 1997). The increase in the number of the various cell types infiltrating the intestinal mucosa during helminth infections has been associated with the development of resistance to parasitic infections, but the mechanisms of the resistance remain unclear (Huntley *et al.* 1995).

The response of eosinophils, mast cells and goblet cells to *C. microbothrium* infection occurred at the same time since their kinetic pattern appeared similar. The cells were seen in significant numbers in the mucosa from Day 28 pi, peaked on Day 42 pi and were maintained at more or less this level till Day 56 when they subsided in all the infected groups. This cellular response coincided with the migration of parasites from the duodenum to the rumen. A severe infiltration of tissue eosinophils was observed in the mucosa and the submucosa around the dilated Brunner's glands. A similar eosinophil infiltration pattern has been observed in sheep and

goats (Deorani & Katiyar 1967). Tissue eosinophilia has been reported to be a feature of helminth infections (Rainbird, Macmillan & Meeusen 1998). The eosinophils, following activation by Th_2 cell-derived interleukin-3 and interleukin-5 and through the mediation of parasite-induced IgE, IgG and complement C3b (Butterworth 1984; Rainbird *et al.* 1998) can bind to the parasite surface and degranulate to release potent biological molecules including major basic protein, eosinophilic cationic protein, eosinophil-derived neurotoxin, eosinophil peroxidase and toxic oxygen-derived metabolites such as superoxide anions, hydroxyl radicals, hydrogen peroxide and singlet oxygen (McLaren, McKean, Olsson, Venge & Kay 1981; Butterworth 1984; Jones 1993; Behm & Ovington 2000; Falcone, Pritchard & Gibbs 2001). The biological products released form a potent helminthicidal system believed to be responsible for parasite damage (Spry 1988; Behm & Ovington 2000). Considering the eosinophil antiparasite mechanisms as hypothesized by several workers (McLaren *et al.* 1981; Butterworth 1984; Jones 1993; Behm & Ovington 2000; Falcone *et al.* 2001) to be valid, the presence of high numbers of eosinophils in early infection in the duodenum in this study, would be regarded as part of the host defence mechanism against *C. microbothrium* infection.

Despite the presence of severe lesions in the duodenum of the HD group the mucosal eosinophilic cell infiltration was not as heavy in this group on Day 42 pi as compared to the MD group. It might be possible that the majority of the eosinophils could have been lost through desquamation and exfoliation during the antiparasite reaction that followed. Once in tissues the eosinophils can survive for several weeks (Spry 1988; Behm & Ovington 2000) and this might probably explain their continued presence in the duodenum despite the completed migration of the parasite to the rumen.

Intestinal mastocytosis has been associated with helminth infections in sheep (Miller 1984; Huntley, Newlands & Miller 1984; Huntley 1992) and similar to the eosinophils, the role of mastocytes remains unclear. In this study, the mucosal mast cell infiltration peaked at the same time as the eosinophils, showing a similar kinetic pattern. Such a kinetic pattern would be expected as the two cells are interrelated in their functional roles during helminth expulsion. The mast cells, following activation by Th_2 cell-derived interleukin-3 and interleukin-5 and cross-linkage of parasite antigens to mast cell surface bound IgE receptors (Lee, Swieter, & Befus 1986; Falcone *et al.* 2001; Kawakami & Galli 2002) degranulate and release vasoactive amines including histamin, serotonin, proteases and leukotrines C, D and E, collectively known as slow reactive substances of anaphylaxis (SRS-A) (Lee *et al.* 1986). Degranulation of mast cells provokes a Type-I hypersensitivity reaction characterized by smooth muscle contraction and increased vascular permeability and subsequent influx of fluids into the gut making the environment hostile to the parasite (Miller 1992; Falcone *et al.* 2001).

In this study the goblet cell population also increased at the same time as the mast cells and eosinophils increased. Goblet cell hyperplasia has been reported in the intestines of parasitized animals, particularly in sheep (Miller 1984) and the mucin component of their secretory products intermingles with other epithelial secretory products like IgA and IgE antibodies resulting in an increase in the viscosity of intestinal contents. The increased viscosity fixes the parasite for antibody binding and subsequent lethal attack by the effector cells (Miller 1984). In this study, increased fluidity and viscocity of the gut contents were observed till Day 42 pi and could be related to the activities of the mast and goblet cell populations. Such mechanisms would be expected to be important in the rejection of immature amphistomes excysting in the distal jejunum and ileum where the environment is unsuitable for growth and subsequent migration to the rumen.

The origin of the globule leukocyte is controversial (Gregory 1979), but it has been reported to occur in high numbers in sheep infected with gastrointestinal helminths (Miller 1984; Huntley 1992). In this study the globule leukocyte levels had a kinetic pattern similar to those of the mast cell and the eosinophils, but in lower intensities. The levels of the globule leukocyte cell infiltration seem to suggest that while the globule leukocyte might be important in sheep where it is associated with the development of resistance to parasitic infections (Douch, Harrison, Elliott, Buchanan, & Greer 1986; Douch & Morum 1993; Stankiewicz, Jonas, Douch, Rabel, Bisset, & Cabaj 1993; Rolfe *et al.* 1994; Pfeffer *et al.* 1996) the same cannot be said for cattle infected with amphistomes. The globule leukocyte kinetic pattern resembled that of mast cells. This could be expected considering the theory of its derivation as described by Huntley *et al.* (1984) where the globule leukocyte is derived from the mast cell. While the composition of the granular contents of the globule leukocyte is more or less similar to that of the mast cell granules, the mechanisms of how the globule leukocyte is involved in resistance are unknown.

Some authors, however, have linked the globule leukocyte mechanism of action to a Type-I hypersensitivity reaction (Stankiewicz *et al.* 1993).

Like the globule leukocyte, basophil numbers were found in low numbers and they appeared to be of minor importance in cattle infected with *C. microbothrium*. Presently there is very little evidence that basophils are involved in ruminant defence mechanisms against gastrointestinal parasites. However, basophils have been seen in large numbers in guinea pigs infected with *Trichostrongylus colubriformis* (Rothwell & Dineen 1972; Rothwell & Love 1975).

Although the number of experimental animals used in the present study was limited, the study has demonstrated the chronology of pathological lesions which occur in cattle experimentally infected with different doses of *C. microbothrium.* The chronology of pathological lesions observed in the present study are similar to those described in goats experimentally infected with *Paramphistomum cervi* (Singh *et al.* 1984) and in sheep experimentally infected with *P. ichikawai* (Rolfe *et al.* 1994). However, this study might not have mimicked field conditions where infection gradually builds up resulting in variations of the lesions induced by the parasite.

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REFERENCES

BEHM, C.A. & OVINGTON, K.S. 2000. The role of eosinophils in parasitic helminth infections: insights from genetically modified mice. *Parasitology Today,* 16:202– 209.

BORAY, J.C. 1969. Studies on intestinal paramphistomosis in sheep due to *Paramphistomum ichikawai* Fukui, 1922. *Veterinary Medical Review,* 4:290–308.

BORAY, J.C. 1971. The pathogenesis of ovine intestinal paramphistomosis due to *Paramphistomum ichikawai,* in *Pathology of Parasitic Diseases*, edited by S.M. Gaafar. University Studies, Lafayette.

BORAY, J.C. 1985. Flukes of domestic animals: Paramphistomata, in *World Animal Science, Disciplinary Approach Volume B2: Parasites, Pests and Predators*, edited by S.M. Gafaar, W.E. Howard & R.E. Marsh. Amsterdam: Elsevier Science Publishers.

BUTLER, R.W. & YEOMAN, G.H. 1962. Acute intestinal paramphistomiasis in Zebu cattle in Tanganyika. *Veterinary Record,* 74:227–231.

BUTTERWORTH, A.E. 1984. Cell-mediated damage to helminths. *Advances in Parasitology,* 23:143–235.

CHERUIYOT, H.K. & WAMAE, L.W. 1988. Incidence of bovine paramphistomiasis in Kenya. *Bulletin of Animal Health and Production in Africa,* 36:55–57.

CHINGWENA, G., MUKARATIRWA, S., KRISTENSEN, T.K. & CHIMBARI, M. 2002. Larval trematode infections in freshwater snails from the highveld and lowveld areas of Zimbabwe. *Journal of Helminthology,* 76:283–293.

DEORANI, S. V.P. & KATIYAR, R.D. 1967. Studies on the pathogenicity due to immature amphistomes among sheep and goats. *Indian Veterinary Journal,* 44:199–205.

DEORANI, S. V.P. & JAIN, S.P. 1969. Reasons for the involvement of duodenum in parasitic part of the life cycle of 'rumen amphistomes' (Paramphistomidae: Trematoda). *Indian Journal of Helminthology,* 21:177–182.

DINNIK, J.A. & DINNIK, N.N. 1962. The growth of *Paramphistomum microbothrium* Fischoeder to maturity and its longevity in cattle. *Bulletin of Epizootic Diseases in Africa,* 10:27–31.

DINNIK, J.A. 1964. *Paramphistomum sukumum* sp. nov. and other stomach-flukes from cattle in the Sukumaland area of the Lake Region, Tanganyika. *Parasitology,* 54:201–209.

DINNIK, J.A. 1965. The snail hosts of certain Paramphistomatidae and Gastrothylacidae (Trematoda) discovered by the late Dr P.L. le Roux in Africa. *Journal of Helminthology,* 39: 141–150.

DOUCH, P.G.C., HARRISON, G.B.L, ELLIOTT, D.C., BUCHANAN, L.L. & GREER, K.S. 1986. Relationship of gastrointestinal histology and mucus anti-parasite activity with the development of resistance to *Trichostrongyle* infections in sheep. *Veterinary Parasitology*, 20:315–331.

DOUCH, P.G.C. & MORUM, P.E. 1993. The effect of age on the response of Romney sheep to gastrointestinal nematodes during grazing. *International Journal for Parasitology,* 23: 651–655.

DUTT, S.C. 1980. *Paramphistomes and paramphistomiasis in domestic ruminants in India*, edited by A. Singh. Ludhiana: PAU Press, Punjab Agricultural University.

EDUARDO, S.L. 1982a. The taxonomy of the family Paramphistomidae Fischoeder, 1901 with special reference to the morphology of the species occurring in ruminants. II. Revision of the genus *Paramphistomum* Fischoeder, 1901. *Systematic Parasitology,* 4:189–238.

EDUARDO, S.L. 1982b. Techniques for examining paramphistomes. *Journal of Helminthology,* 56:117–119.

EDUARDO, S.L. 1983. The taxonomy of the family Paramphistomidae Fischoeder, 1901 with special reference to the morphology of the species occurring in ruminants. III. Revision of the genus *Calicophoron* Nasmark, 1937. *Systematic Parasitology,* 5:25–79.

EDUARDO, S.L. 1988. Hosts and geographical distribution of Paramphistomid species occurring in ruminants. *Philippines Journal of Veterinary Animal Science,* 14:25–70.

FALCONE, F.H., PRITCHARD, D.I. & GIBBS, B.F. 2001. Do basophils play a role in immunity against parasites? *Trends in Parasitology,* 17:126–126.

GREGORY, M.W. 1979. The globule leukocyte and parasitic infection—a brief history. *Veterinary Bulletin,* 49:821–825.

GUPTA, N.K. 1993. *Amphistomes, systematics and biology.* New Dehli, India: Oxford and IBH Publishing Co.

HORAK, I.G. & CLARK, R. 1963. Studies on paramphistomiasis. V. The pathological physiology of the acute disease in sheep. *Onderstepoort Journal of Veterinary Research,* 30: 145–160.

HORAK, I.G. 1966. Studies on paramphistomiasis, VIII. The pathogenesis and symptoms of the disease in sheep. *Journal of the South African Veterinary Association,* 37:428–430.

HORAK, I.G. 1967. Host-parasite relationships of *Paramphistomum microbothrium* Fischoeder, 1901, in experimentally infested ruminants, with particular reference to sheep. *Onderstepoort Journal of Veterinary Research,* 34:451–540.

HORAK, I.G. 1971. Paramphistomiasis of domestic ruminants. *Advances in Parasitology,* 9:33–72.

HUNTLEY, J.F., NEWLANDS, G. & MILLER, H.R.P. 1984. The isolation and characterization of globule leukocytes: their derivation from the mucosal mast cells in parasitised sheep. *Parasite Immunology,* 6:371–390.

HUNTLEY, J.F. 1992. Mast cells and basophils: a review of their heterogeneity and function. *Journal of Comparative Pathology,* 107:349–372.

HUNTLEY, J.F., PATTERSON, M., MACKELLAR, A., JACKSON, F., STEVENSON, L.M. & COOP, R.L. 1995. A comparison of mast cell and eosinophil responses of sheep and goats to gastrointestinal nematode infections. *Research in Veterinary Science,* 58:5–10.

JONES, D.G. 1993. The eosinophil. *Journal of Comparative Pathology,* 108:317–335.

JOOSTE, R. 1989. A checklist of the helminth parasites of the larger domestic and wild mammals of Zimbabwe. *Transactions of the Zimbabwe Scientific Association,* 64:15–32.

KAWAKAMI, T. & GALLI, S.J. 2002. Regulation of mast cell and basophil function and survival by IgE. *Nature Reviews Immunology,* 2:773–786.

KELLY, D.J. & HENDERSON, W.K. 1973. *Calicophoron calicophorum* (Trematoda: Paramphistomidae) and paramphistomiasis in domestic cattle in the East Kimberley District of Western Australia. *Tropical Animal Health and Production,* 5:192–195.

LEE, T.G.D., SWIETER, M. & BEFUS, A.D. 1986. Mast cell response to helminth infection. *Parasitology Today,* 2:186–191.

MADZINGIRA, O., MUKARATIRWA, S., PANDEY, V.S. & DORNY, P. 2002. Helminth infections of cattle and antelope in a mixed farming system in Zimbabwe. *Proceedings of the 10th International Congress of Parasitology—ICOPAX: Vancouver, Canada, 4–9 August, 2002:* 627–637.

MAFF [Ministry of Agriculture, Fisheries and Food] 1986. *Manual of Veterinary Parasitological Laboratory Techniques*. London: Her Majesty's Stationery Office.

McLAREN, D.J., MCKEAN, J.R., OLSSON, I., VENGE, P. & KAY, A.B. 1981. Morphological studies on the killing of schistosomula of *Schistosoma mansoni* by human eosinophil and cationic proteins in vitro. *Parasite Immunology,* 3:359– 373.

MILLER. H.R.P. & JARRETT, W.F.H. 1971. Immune reactions in mucous membranes I. Intestinal mast cell response during helminth expulsion in the rat. *Immunology,* 20:277–287.

MILLER, H.R.P. 1984. The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. *Veterinary Immunology and Immunopathology,* 6:167–259.

MILLER, H.R.P. 1992. Mast cells: their function and heterogeneity, in *Allergy and immunity to helminths: common mechanisms or divergent pathways,* edited by R. Moqbel. London, UK: Taylor & Francis Ltd.

MUKARATIRWA, S., SIEGISMUND, H.R., KRISTENSEN, T.K. & CHANDIWANA, S.K. 1996. Population genetics and genetic variability of *Bulinus globosus* (Gastropoda: Planorbidae) from the two main river systems in Zimbabwe. *Journal of American Genetic Association,* 87:288–294.

MUKARATIRWA, S., KRISTENSEN, T.K., SIEGISMUND, H.R. & CHANDIWANA, S.K. 1998. Genetic and morphological variation of populations belonging to the *Bulinus tropicus/truncatus* complex (Gastropoda: Plarnobidae) in Southwestern Zimbabwe. *Journal of Molluscan Studies,* 64:435–436.

MUKHERJEE, R.P. & DEORANI, S.V.P. 1962. Massive infection of a sheep with amphistomes and the histopathology of the parasitised rumen. *Indian Veterinary Journal,* 39:668–670.

OVER, H.J. 1982. Ecological basis for parasite control: trematodes with special reference to fascioliasis. *Veterinary Parasitology,* 11:85–97.

PANDA, B.K. & MISRA, S.C. 1980. Observations on the aetiology, clinical pathology and chemotherapy of immature amphistomiasis in buffalo calves. *Indian Journal of Animal Health,* 19:131–135.

PFEFFER, A., DOUCH, P.G.C., SHAW, R.J., GATEHOUSE, T.K., RABEL, B., GREEN, R.S., SHIRER, C.L., JONAS, W.E. & BISSET, S. 1996. Sequential cellular and humoral responses in the abomasal mucosa and blood of Romney sheep dosed with *Trichostrongylus axei. International Journal for Parasitology,* 26:765–773.

PILLAI, U.N. & ALIKUTTY, K.M. 1995. Clinico-hematological observation in bovine amphistomiasis. *Indian Journal of Veterinary Medicine,* 1:38–39.

RAINBIRD, M.A., MACMILLAN, D. & MEEUSEN, E.N.T. 1998. Eosinophil mediated killing of *Haemonchus contortus* larvae: effect of eosinophil activation and role of antibody, complement and Interleukin-5. *Parasite Immunology,* 20:93–103.

ROLFE, F., BORAY, J.C., NICHOLS, P. & COLLINS, G.H. 1991. Epidemiology of paramphistomosis in cattle. *International Journal for Parasitology,* 21:813–819.

ROLFE, F., BORAY, J.C. & COLLINS, G.H. 1994. Pathology of infection with *Paramphistomum ichikawai* in sheep. *International Journal for Parasitology,* 24:995–1004.

ROTHWELL, T.L.W. & DINEEN, J.K. 1972. Cellular reactions in guinea pigs following primary and challenge infection with *Trichostrongylus colubriformis* with special reference to the roles played by eosinophils and basophils in rejection of the parasite. *Immunology,* 22:733–745.

ROTHWELL, T.L.W. & LOVE, R.J. 1975. Studies of the response of basophil and eosinophil leukocytes and mast cells to the nematode *Trichostrongylus colubriformis*. II. Changes in cell numbers following infection of thymectomised and adoptively or passively immunized guinea pigs. *Journal of Pathology,* 116:183–194.

SEY, O. 1991. Handbook of the zoology of amphistomes. Boca Raton: CRC Press, Inc.

SINGH, C.D.N. & LAKRA, P. 1971. Pathologic changes in naturally occurring *Cotylophoron cotylophorum* infection in cattle. *American Journal of Veterinary Research,* 32:659–663.

SINGH, R.P., SAHAI, B.N. & JHA, G.J. 1984. Histopathology of the duodenum and rumen of goats during experimental infections with *Paramphistomum cervi. Veterinary Parasitology,* 15:39–46.

SPRY, C.J.F. 1988. *Eosinophils: a comprehensive review and guide to the scientific and medical literature.* Oxford: Oxford Medical Publications, Oxford University Press.

STANKIEWICZ, M., JONAS, W.E., DOUCH, P.C.G., RABEL, B., BISSET, S. & CABAJ, W. 1993. Globule leukocytes in the lumen of the small intestine and the resistance status of sheep infected with parasitic nematodes. *Journal of Parasitology,* 79:940–945.

SWART, P.J. & REINECKE, R.K. 1962a. Studies on paramphistomiasis. I. The propagation of *Bulinus tropicus* Krauss 1848. *Onderstepoort Journal of Veterinary Research,* 29: 83–187.

SWART, P.J. & REINECKE, R.K. 1962b. Studies on paramphistomiasis. II. The mass production of metacercariae of *Paramphistomum microbothrium* Fischoeder 1901. *Onderstepoort Journal of Veterinary Research,* 29:189–195.

VARMA, A.K. 1961. Observations on the biology and pathogenicity of *Cotylophoron cotylophorum* (Fischoeder, 1901). *Journal of Helminthology,* 35:161–168.

WINTER, M.D., WRIGHT, C. & LEE, D.L. 1997. The mast cell and eosinophil response of young lambs to a primary infection with *Nematodirus battus*. *Parasitology,* 114:189–193.