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Histopathological changes during experimental infections of calves with *Cooperia punctata*

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Abstract

Eleven male two-month-old Holstein calves were used to determine the pathological changes induced by a *Cooperia punctata* infection. After weaning, ten calves received a single oral dose of 45,000 *C. punctata* infective larvae. One calf remained as a non-infected control. Groups of two calves were killed on days 7, 14, 21, 28 and 35 post-infection (p.i.) for determination of worm burdens and histopathological evaluation. The small intestine was sub-divided into three sections of approximately equal length, and representative samples of mucosa were fixed in 10% formalin, cut, and stained with haematoxylin-eosin. Samples of intestinal contents and mucosal digests were taken and fixed in 10% formalin for an estimation of total worm burdens. An increase in the number of adult parasites and a decrease in the number of larvae were observed with time ($P < 0.001$). A higher concentration of worms was found in the first segment of the small intestine during the five weeks of observation. Histology showed larvae in the intestinal mucosa on day 7 p.i., with a discrete increase in the cellular response. Adult worms and a marked cellular infiltrate with eosinophils and neutrophils were present on day 21 p.i., and these persisted until day 35 p.i. Microcysts resulting from worm destruction were observed from day 21 p.i.

Introduction

Among the trichostrongylids that infect livestock, the genus *Cooperia* is cosmopolitan and comprises species that preferentially parasitize calves, impairing their performance by inducing loss of appetite, diarrhoea, nutritional deficiency, and reduced weight gain (Alicata & Lynd, 1961; Armour *et al.*, 1987).

The most prevalent trichostrongylid among cattle in Brazil is *C. punctata*, followed by the species *C. pectinata*, *C. oncophora* and *C. spatulata* (Honer & Vieira-Bressan, 1992; Lima, 1998). Observations by Bianchin *et al.* (1990) on beef calves in Mato Grosso do Sul (Brazil) demonstrated that 75.8% of the parasites recovered belonged to the genus *Cooperia*, with a predominance (92%) of the species *C. punctata*. Lima (1998) studied the epidemiology of nematodes on naturally contaminated pastures in Minas Gerais state (Brazil) using tracer calves and observed a higher frequency of the genus *Cooperia*, representing 74.4% of the total parasites, with 93.3% of the *Cooperia* belonging to the species *C. punctata*.

The first descriptions of pathological lesions were reported by Ransom (1920) and Hung (1926), who observed damage to the intestinal mucosa of animals naturally infected with *C. punctata*. Bailey (1949), also using histopathology, observed marked changes in the

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intestinal epithelium of calves experimentally infected with the third stage larvae (L3) of *C. punctata*, with lymphocyte and eosinophil infiltration of the lamina propria of the mucosa and the presence of worms between the crypts and villi, with necrosis of surrounding tissues. Armour *et al.* (1987) observed the destruction of the intestinal epithelium of calves experimentally infected with *C. oncophora*, with villous atrophy and the presence of worms between the villi and crypts. The presence of *Cooperia* may aggravate existing symptoms of parasitism, as confirmed by Vieira-Bressan *et al.* (1995) in studies on calves concurrently infected with *C. punctata* and *Haemonchus placei*.

Due to the high prevalence of *C. punctata* in Brazil, the production losses induced and the scarcity of information about this nematode worldwide, the objective of the present study was to study the histopathological changes in the small intestine of calves experimentally infected with *C. punctata*.

Material and methods

Eleven Holstein male calves, ten experimentally infected and one uninfected control were used for the study. Calves were purchased immediately after birth and kept in pairs in stalls with a concrete floor, to prevent contact with any helminth infection. All calves were fed 4 l milk *in natura* twice a day, 2 l in the morning and 2 l in the evening until weaning, which occurred when the calves were about two months old. At 10 days of age, calves were offered 250 g of a maize and soya-bran concentrate per day. From day 30, the quantity of concentrate increased to 500 g, and from day 45 increased to 1 kg. Coast-cross hay (*Cynodon dactylon*) was offered *ad libitum* from day 10. Calves were experimentally infected with a single dose of 45,000 L3 of *C. punctata* obtained by total culture from a donor animal. Larvae were used within 48 h following recovery from culture. Each group of two calves was necropsied on days 7, 14, 21, 28 and 35 post-infection (p.i.) for worm recovery and histopathological studies.

During necropsy, it was ensured that the *in situ* topography of organs in the peritoneal cavity was preserved. The alimentary tract was removed and the small intestine divided into three sections of equal length. For histology, three small pieces of mucosa (5 cm) representative of each section were taken and fixed in 10% formalin for the preparation of histopathological slides. Following fixation, pieces of mucosa were cut and embedded in wax. Sections (5 μm thick) were cut with a microtome and stained with haematoxylin-eosin (HE).

The contents of the small intestine and mucosal scrapings of each section were washed with running water and the volume of each section made up to 2 litres with water. After thorough mixing, duplicate 200 ml samples (10% of the total volume) were collected and preserved in 10% formalin for total worm counts and the identification of larval stages present. To release the worms which were adhered to the mucosa, each section of the small intestine was incubated in distilled water at 32°C for 24 h. After this period, the intestine was removed and the total contents preserved in 10% formol for the identification and counting of worms. Adult females were characterized by the presence of eggs *in utero* and adult males by the presence of dark brown spicules. All other stages were classified as larvae.

The number of worms present in each section of the small intestine over the 5-week period and the changes between the different weeks were analysed statistically by ANOVA, and the means, transformed to ln, were compared by the Tukey test using the SAS (Statistic Analysis System) software. Values of $P < 0.05$ were considered to be significant.

Results and Discussion

The genus *Cooperia* has a direct life cycle and the infective L3, when swallowed by the host, lose their sheaths and penetrate into mucosa of the small intestine, where they change to fourth stage larvae. After about 8–10 days p.i. immature adult worms return to the small intestine and develop into mature adults in about 4 days, which corresponds to the pre-patent period of 14–16 days (Leland, 1995). *Cooperia punctata* larvae are present in the small intestine of an infected calf on day 7 p.i. (fig. 1), deeply localized in the villi close to the crypts, with adult worms present on the surface of villi (figs 3, 4 and 6) in the jejunum of calves at days 21 and 35 p.i.

The present histopathological findings are comparable to those reported by Coop *et al.* (1979) and Armour *et al.* (1987) in calves experimentally infected with *C. oncophora*, and Ransom (1920), Hung (1926), Bailey (1949) and Oliveira (2001), in calves experimentally infected with *C. punctata*. However, there was no evidence of intensive destruction of the small intestinal epithelium, as reported by Coop *et al.* (1979) who observed only the compression of villi in contact with the parasites.

The number of adult worms increased significantly with time ($P < 0.01$). Similarly, there was a significant decrease in the number of larvae during the same period, reflecting their development in the intestinal mucosa. There was also a significantly greater concentration ($P < 0.01$) of both larvae and adult worms in the first section of the small intestine regardless of time, i.e. the first section remained the preferential site of worm location and development (fig. 7). Studies with calves naturally infected with *C. punctata* (Ransom, 1920), calves experimentally infected with *C. punctata* (Bailey, 1949) and calves with concurrent infection with *C. punctata* and *Haemonchus placei*, also demonstrated that the number of worms was larger in the upper part of the small intestine.

Similar results were reported by Coop *et al.* (1979) and Armour *et al.* (1987) for calves experimentally infected with *C. oncophora*. However, Armour *et al.* (1987) observed an increase in the number of worms in the middle and final sections of small intestine over a period of 3 to 12 weeks as the result of an immunological reaction, increase in peristalsis induced by the inflammatory reaction (Bailey, 1949), or crowding effect (Satrija & Nansen, 1992).

An intense cellular infiltrate, with a large number of eosinophils and neutrophils, was observed (figs 2, 4 and 5), and their presence demonstrates the involvement of cellular immunity as the host attempts to expel and/or destroy the intestinal worms (Balic *et al.*, 2000; Claerebout & Vercruysse, 2000; Garside *et al.*, 2000). The presence

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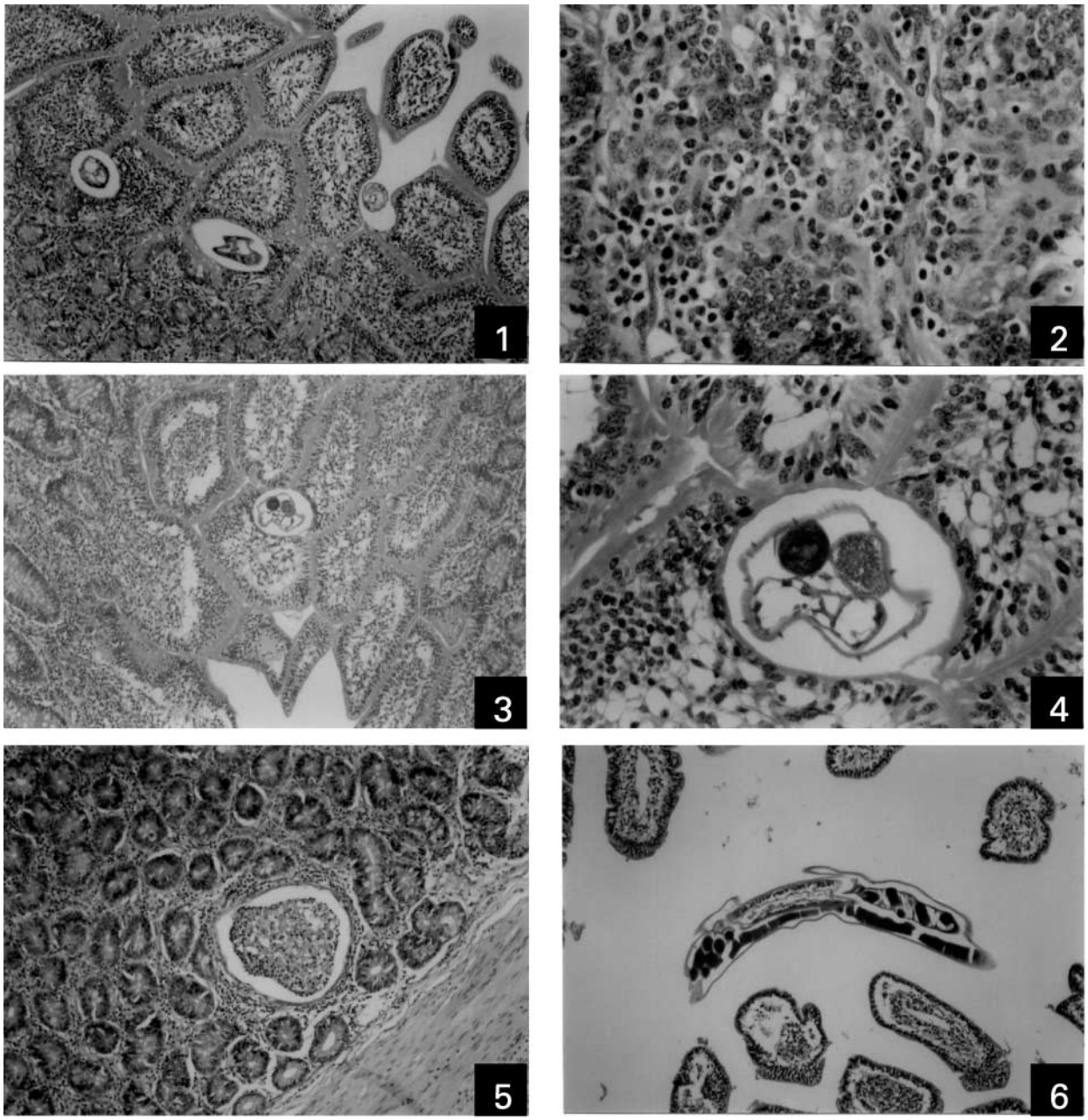

Figs 1–6. Sections of small intestine of calves infected with *Cooperia punctata* stained by HE. 1. *C. punctata* larvae deeply localized in the villi at day 7 p.i. 10 × . 2. Intestine showing an increase in the inflammatory infiltrate at day 21 p.i., especially mononuclear cells and eosinophils. 40 × . 3. Jejunum at day 21 p.i. with adult worms on the surface of the villi. 10 × . 4. Adult worms amongst the villi; connective tissue infiltrated with large amounts of inflammatory cells. 40 × . 5. Cyst formation, with capsule, inflammatory cells and cell remnants in the intestinal epithelium of an infected calf at day 28 p.i. 10 × . 6. Adult worm on the surface of the epithelium among the villi at day 35 p.i. 40 × .

of eosinophils agrees with data reported in other studies (Ransom, 1920; Hung, 1926; Bailey, 1949; Coop *et al.*, 1979; Miller, 1984; Huntley *et al.*, 1984, 1995; Armour *et al.*, 1987; Dawkins *et al.*, 1989; Rothwell *et al.*, 1993; Winter *et al.*, 1997; Oliveira, 2001), confirming their mobilization at the parasite/host interface. Activated eosinophils, as well as neutrophils, are sources of enzymes and oxygen-derived radicals that represent a powerful bactericidal and worm killer system, as observed by Wassom & Gleich (1979) and by Jones (1993).

The presence of neutrophils has not been previously reported by investigators who studied *Cooperia* infections (Ransom, 1920; Hung, 1926; Bailey, 1949; Coop *et al.*, 1979; Armour *et al.*, 1987; Oliveira, 2001) or other parasite genera (Miller, 1984; Huntley *et al.*, 1984, 1995; Dawkins *et al.*, 1989; Winter *et al.*, 1997). Mackenzie *et al.* (1981),

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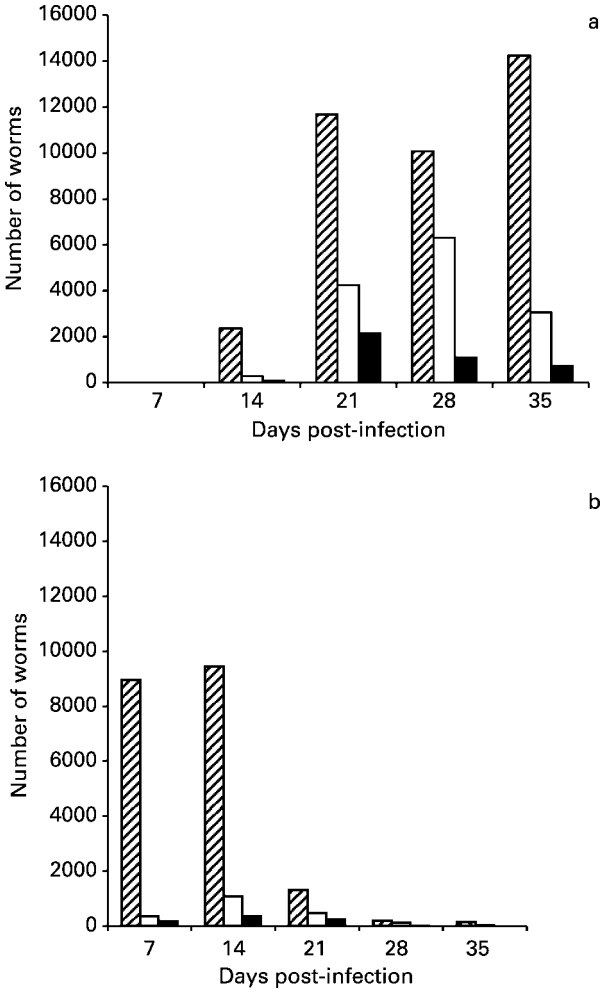

Fig. 7. The number of adults (a) and larvae (b) of *Cooperia punctata* in different sections of small intestine of calves (▨, section 1; □, section 2; ■, section 3).

however, stated that this cell type participates in the acute phase of a helminth infection and observed adherence and degranulation of neutrophils on the surface of *Nippostrongylus brasiliensis* in vitro.

In the present study, no mast cells were observed in the inflammatory infiltrate, a fact that contradicts the evidence that these are the cell types most extensively recruited in the elimination of intestinal helminths (Huntley *et al.*, 1984; Cheema & Scofield, 1985; Rothwell, 1989; Rothwell *et al.*, 1993). The present data, however, agree with those reported by Oliveira (2001), who observed the lack of mast cells in cell infiltrates in calves experimentally infected with *C. punctata*. Oliveira (2001) attributed this absence of mast cells to the fact that he did not apply specific staining techniques for visualization (toluidine blue) during the advanced stage of infection (more than 50 days). The differentiation of this cell type into globular leucocytes are also difficult to visualize with haematoxylin–eosin, and in the current study may not have been detected.

Microcysts were observed by day 28 p.i. (fig. 5), demonstrating the destruction of worms by the mobilized cell contingent. The presence of microcysts, although not demonstrated in other studies involving the genus *Cooperia* (Ransom, 1920; Hung, 1926; Bailey, 1949; Coop *et al.*, 1979; Armour *et al.*, 1987; Oliveira, 2001), was reported by Schuberth *et al.* (2000) in experimental infections of swine with *Oesophagostomum dentatum*, and the formation of these structures was related to the mobilization of neutrophils and eosinophils. However, in contrast to the present study, no destruction of worms by the cell types in question was observed, and the larvae survived and developed inside the microcysts.

In conclusion, the nematode *Cooperia punctata* preferentially occupies the first third of the small intestine of infected calves. The inflammatory reaction induced by the presence of *C. punctata* in the intestinal epithelium becomes more intense during the third week post-infection, coinciding with the population explosion of adult worms occurring on day 14 p.i. The local immunological response comprises mostly eosinophils and neutrophils.

Acknowledgements

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