Veterinary Parasitology, 15 (1984) 39—46
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

HISTOPATHOLOGY OF THE DUODENUM AND RUMEN OF GOATS DURING EXPERIMENTAL INFECTIONS WITH *PARAMPHISTOMUM CERVI*

R.P. SINGH, B.N. SAHAI and G.J. JHA*

*Department of Veterinary Parasitology, and *Department of Veterinary Pathology, Faculty of Veterinary Science, Birsa Agricultural University, Ranchi-834007 (India)*

(Accepted for publication 10 August 1983)

ABSTRACT

Singh, R.P., Sahai, B.N. and Jha, G.J., 1984. Histopathology of the duodenum and rumen of goats during experimental infections with *Paramphistomum cervi*. *Vet Parasitol.*, 15 39—46.

On microscopic examination after experimental infection with *Paramphistomum cervi*, tissue reactions in the duodenum were more pronounced during early stages of the infection (20th day post-infection (DPI)). Immature parasites were seen migrating to the muscularis layer, and focal infiltration of macrophages and lymphocytes was observed in the lamina propria and in the interstitial tissue of Brunner's gland. At places, there was cystic dilatation of Brunner's gland. At 40 DPI, the parasite was not present in the duodenal sections, and cellular infiltration was more diffuse and consistent With the passage of time, the tissue reactions and cellular infiltration in the duodenum became less pronounced, but at 80 days parasites were attached to the villi of the rumen. Infiltration of mononuclear cells in the supporting connective tissue of the rumen was also observed. Thus, it is concluded that the immature forms of *Paramphistomum cervi* caused more severe damage in the duodenal tissue, whereas the adult form inflicted mild tissue damage in the rumen of the experimental kids.

INTRODUCTION

In India, paramphistomiasis is a serious helminthic problem and fatal outbreaks of the disease in goats have been reported from Assam (Pande, 1935), Bihar (Kuppuswamy, 1946; Varma, 1957), Punjab (Chhabra et al., 1978) and Uttar Pradesh (Katiyar and Varshney, 1963). Although 44 species of amphistomes have been reported, *Paramphistomum cervi* is the species most common in goats (Dutt, 1980; Sahai, 1981), and histopathological alterations in the duodenum and rumen of sheep and goats naturally and experimentally infected with various species of amphistomes have been studied (Pande, 1935; Srivastava, 1938; Mudaliar, 1945; Nobel, 1956; Varma, 1961; Katiyar and Varshney, 1963; Sharma Deorani and Katiyar, 1967; Sharma Deorani and Jain, 1969; Nath, 1970; Boray, 1971; Prasad

0304-4017/84/$03.00

et al., 1974). There has been no systematic study of the histopathology of goats experimentally infected with *P. cervi* to assess the impact of developing flukes. The present paper deals with the periodic tissue alterations in the duodenum and rumen of goats experimentally infected with *P. cervi*.

MATERIALS AND METHODS

Experimental design

Thirteen 2–3-month-old, healthy parasite-free kids, were selected for the present study. The kids were taken from known sources where the chance of amphistome infection was extremely remote. At random, one kid from the experimental lot was killed and the gastro-intestinal tract thoroughly examined for the presence of any developing stages of the amphistome. The remaining 12 kids were divided into 2 groups of 8 and 4 kids. Each kid in Group I was infected with 2500 laboratory-maintained metacercariae of *P. cervi*. The other 4 kids, Group II, were maintained as controls. Faecal samples from each kid were examined 3 times prior to infection, to confirm the presence of parasitic infections, if any. All experimental kids were kept under parasite-free conditions and on identical diets throughout the period of the experiment.

Histopathological technique

At 20, 40, 60 and 80 DPI, 2 infected kids and one control were killed. The gastro-intestinal tract was thoroughly examined, and tissue samples from duodenum and rumen were collected and fixed in 10% buffered formalin. These were then processed through conventional methods of washing, dehydration, clearing and infiltration; 6-μm thick paraffin sections from both groups were stained simultaneously with haematoxylin and eosin.

Photomicrographs were taken using an "Olympus" research microscope with 35 mm photomicrographic equipment.

RESULTS AND DISCUSSION

Duodenum

20 DPI

There were slight tissue changes at this stage. The parasite was seen embedded in the Brunner's gland just beneath the muscularis mucosae (Figs. 1 and 2). There was a cystic dilatation of the Brunner's gland in which the parasite was seen, but not much cellular reaction (Fig. 3). In some sections, the parasite had migrated as far as the muscularis layer (Fig. 4). There was a focal infiltration of macrophages and a few lympho-

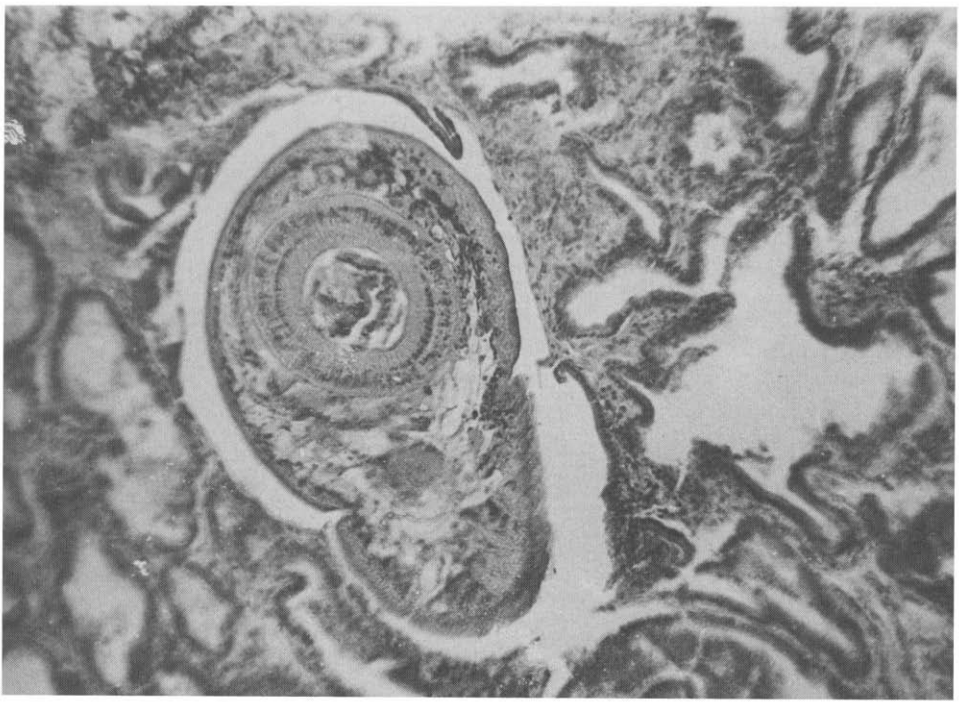

Fig. 1. Section of the duodenum of a kid 20 DPI, showing a cut section of the parasite in the Brunner's gland. H. & E. × 100.

cytes in the lamina propria and in the interstitial tissue of the Brunner's gland. In general, the blood vessels appeared congested. The muscularis proper and the serosa showed no reaction to the presence of the parasite.

During the present investigation, the immature flukes were primarily in the submucosa, whereas Varma (1961) observed some worms reaching the mucosa, submucosa and even into the muscular layer. Sharma Deorani and Katiyar (1967) and Sharma Deorani and Jain (1969) observed more worms in the submucosa than in the mucosa. Horak (1967) did not find any *P. microbothrium* embedded in the mucosa and not a single worm in Brunner's gland. In contrast to the present findings, Prasad et al. (1974), in a study of goats infected with *Cotylophoron cotylophorum*, observed more worms in the mucosa, although many were also found in submucosa.

40 DPI

Tissue reaction was more intense at 40 DPI. The cellular infiltration was more diffuse and consistent than at the earlier stage. In addition to the infiltration of mononuclear cells (macrophages) and lymphocytes in the lamina propria and interstitial tissue of Brunner's gland, there was also denudation of epithelial cells of the intestinal mucosa, with proliferation of the Brunner's gland.

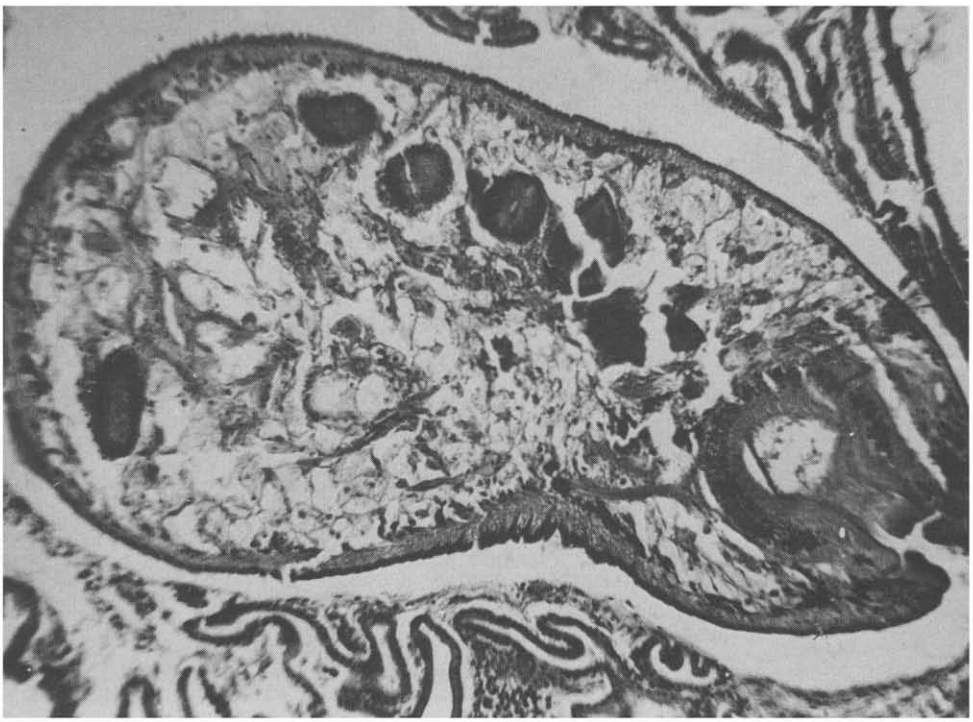

Fig 3

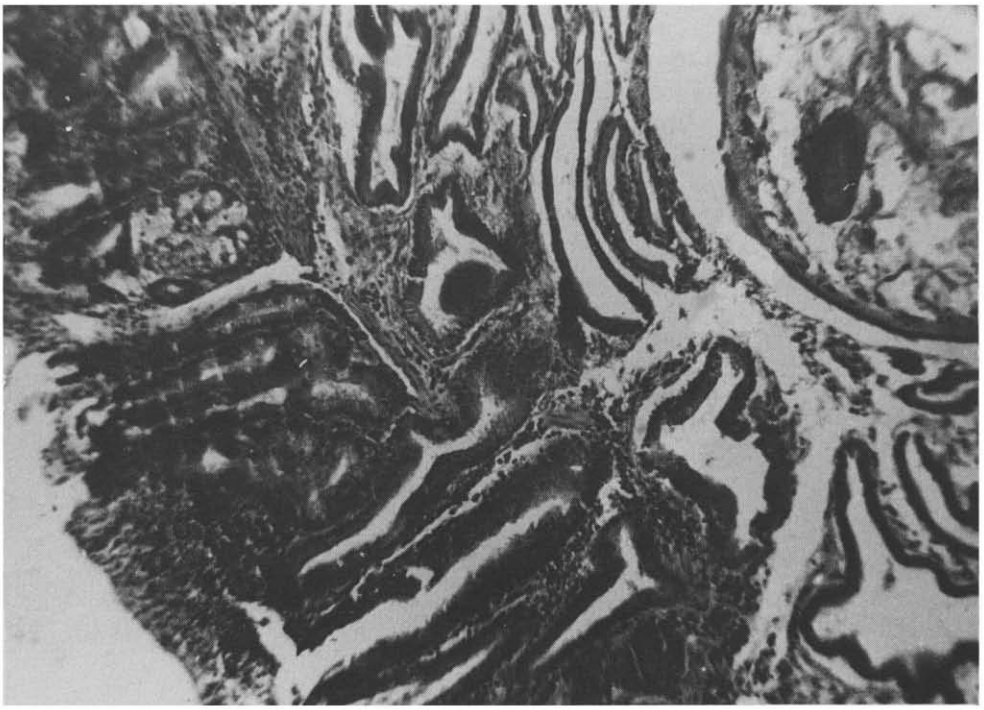

Fig. 2

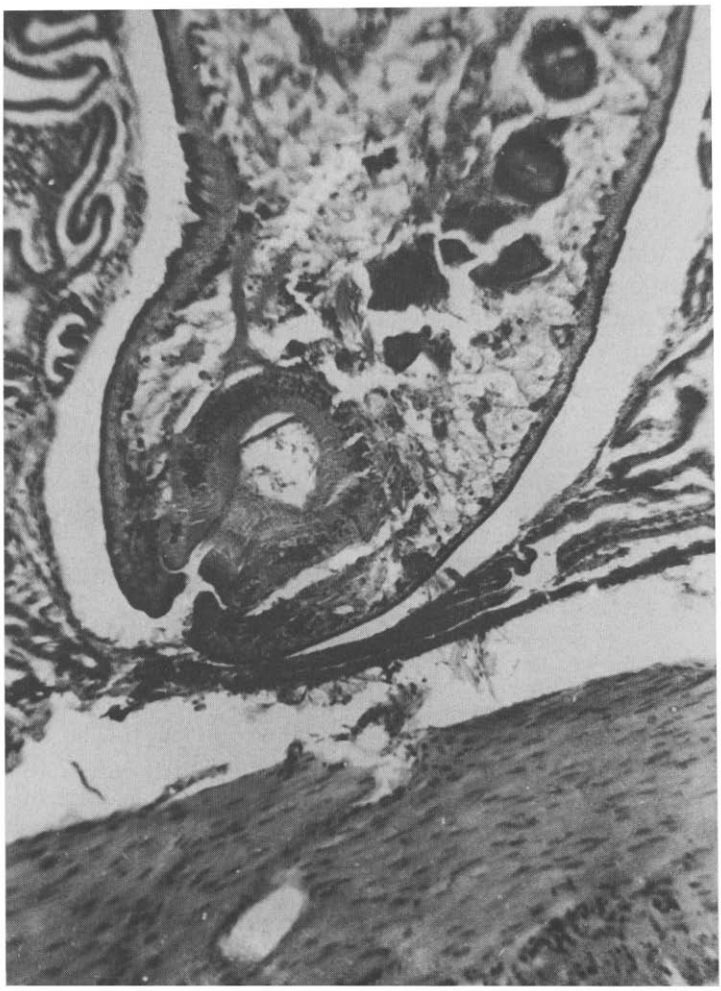

Fig. 4. Section of the duodenum of a kid 20 DPI, showing a section of the parasite reaching up to the muscularis layer. H. & E. × 100

60 DPI

The fluke was now absent from sections of intestinal tissue, but the tissue alteration persisted. Desquamation of intestinal villar epithelium was persistent, causing atrophy of the mucosa. Infiltration of macrophages and lymphocytes was also seen, particularly in the mucosal tissue. On

---

Fig. 2. Section of the duodenum of a kid 20 DPI, showing a portion of the parasite in the submucosal tissue. H. & E. × 100.

Fig. 3. Section of the duodenum of a kid 20 DPI, showing a section of the *P. cervi* in the cystic cavity of the Brunner's gland. H. & E. × 100.

the other hand, Brunner's gland showed degenerative changes of the epithelial cells, and the interstitial tissue of the Brunner's gland was infiltrated with many macrophages and a few lymphocytes. The submucosa appeared to have an increased thickening due to connective tissue growth. The muscularis layer and serosa, even at this stage of experimentation, were not involved in the reaction against the parasites.

These observations are in agreement with those reported from a cow infected with *P. cervi* (Maqsood, 1944), in goats with *C. cotylophorum* (Mudaliar, 1945), in cattle with a mixed infection of *P. cervi* and *C. cotylophorum* (Guilhon and Priouzeau, 1945), in sheep and goats with immature amphistomes (Katiyar and Varshney, 1963), in sheep and goats with immature amphistomes (Sharma Deorani and Katiyar, 1967), in sheep and goats with *C. cotylophorum* (Sharma Deorani and Jain, 1969) and in goats with *C cotylophorum* (Prasad et al., 1974). Srivastava (1938), working with experimentally infected sheep, reported that the metacercariae of *C. cotylophorum*, on excystation in the duodenum, attached firmly to its mucosa only. In contrast to these findings, Nobel (1956) observed cellular infiltration, mainly eosinophilic, in *P. cervi* infection in cattle and sheep. However, none of the earlier workers mentioned the age of the fluke.

80 DPI

The epithelial cells lining the villi were now either denuded or atrophied. However, mild mononuclear cell infiltration was still visible in the lamina propria and in the interstitial tissue of Brunner's gland. Once again, the muscular and serosal layers appeared almost normal. However, Sharma Deorani and Jain (1969) believe that the immature forms of amphistomes enter the duodenal wall to protect themselves from the acid medium of the abomasum. Again, Sharma Deorani and Katiyar (1967) also attributed the possible reason for the tissue changes to the migratory habits and feeding behaviour of the parasite. Prasad et al. (1974) stated that the flukes caused damage to the duodenal wall not only by their embedding habit, but also because they exert an additional pulling action and consequently increased tissue damage with the help of their powerful acetabulum.

Rumen

40, 60 and 80 DPI

There were no significant tissue alterations of the rumen even 40 or 60 DPI. However, at 80 DPI, there was an interesting tissue alteration. The parasites were lying on the mucosal surface of the rumen, either in between, or attached to, the villi (Fig. 5). The villus papilla showed epithelial desquamation, and there was also infiltration of a few mononuclear cells in the supporting connective tissue of the rumen.

Histological examination of the duodenum and rumen of the control kids showed no significant tissue alterations at any stage of study.

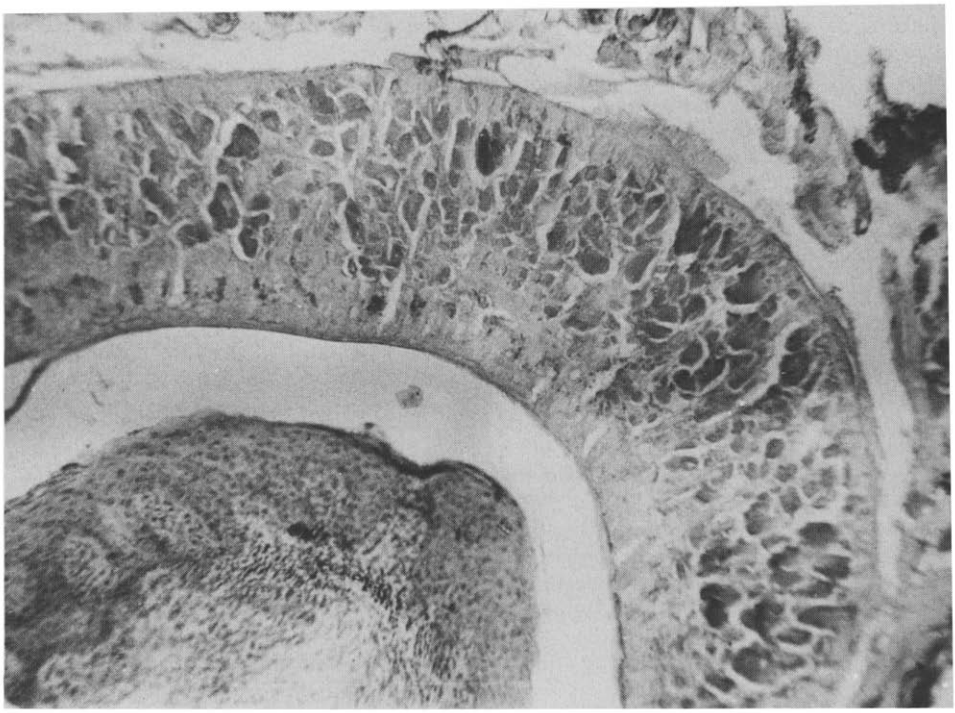

Fig. 5. Section of the rumen of a kid 80 DPI, showing encircling of the ruminal papilla by the anterior sucker of the parasite. H. & E. × 100.

Mukherjee and Sharma Deorani (1962) described proliferation of epithelium and occasionally mild hypertrophy of the corneum, rarely necrosis and sloughing of the mucosa, but no cellular reaction. Cankovic and Batistic (1963) also observed lymphocytic infiltration in the lamina propria, and sometimes in the epithelium and submucous layer of the rumen infected with *P. cervi*. Graubmann et al. (1978) reported a proliferated papillary body with cellular infiltration at the point of adhesion.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. H.R. Mishra, Dean of the Faculty, for facilities provided, and to the Indian Council of Agricultural Research, New Delhi, for financial assistance.

REFERENCES

Boray, J.C., 1971. The pathogenesis of ovine intestinal paramphistomiasis due to *Paramphistomum ichikawai*. In: S.M. Gaafar (Editor), Pathology of Parasitic Diseases. University Studies, Lafayette, IN, pp. 209–216.

Cankovic, M. and Batistic, B., 1963. Lokalne promjene u buragu paramfistomazne Krave. Veterinaria (Sarajevo), 12: 93—95.

Chhabra, R.C., Gill, B.S. and Dutt, S.C., 1978. Paramphistomiasis of sheep and goats in the Punjab State and its treatment. Indian J. Parasitol., 2: 43—45.

Dutt, S.C., 1980. Paramphistome and Paramphistomiasis of Domestic Animals in India. Punjab Agricultural University, Ludhiana, 162 pp.

Graubmann, H.D., Grafnee, G. and Odening, K., 1978. *Paramphistomum* in red deer and roe deer. Monatsch. Veterinaermed., 33: 892—898.

Guilhon, J. and Priouzeau, M., 1945. Action of oxyclozanide on trematode parasites of cattle in a tropical environment. Rev. Elev. Med. Vet. Pays Trop., 24: 365—371.

Horak, I.G., 1967. Host—parasite relationships of *Paramphistomum microbothrium* Fischoeder, 1901, in experimentally infested ruminants, with particular reference to sheep. Onderstepoort J. Vet. Res., 34: 451—540.

Katiyar, R.D. and Varshney, T.R., 1963. Amphistomiasis in sheep and goats in Uttar Pradesh. Indian J. Vet. Sci., 33: 94—98.

Kuppuswamy, P.B., 1946. Final report of the scheme to elucidate the etiology of the 'Gillar' and 'Pitto' in sheep and goats in Bihar for the year 1945—46. (unpublished)

Maqsood, M., 1944. Acute amphistomiasis in cattle in Northern India. Indian Vet. J., 20: 266—269.

Mudaliar, S.V., 1945. Fatal enteritis in goats due to immature amphistomes, probably *Cotylophoron cotylophorum*. Indian J. Vet. Sci., 15: 54—56.

Mukherjee, R.P. and Sharma Deorani, V.P., 1962. Massive infection of a sheep with amphistomes and the histopathology of parasitised rumen. Indian Vet. J., 39: 668—670

Nath, D., 1970. Histopathological observations in immature amphistomiasis disease of sheep. Bengal Vet., 18: 1—4.

Nobel, T.A., 1956. Histopathology of paramphistomiasis. Refu. Vet., 13 : 155—157.

Pande, P.G., 1935. Acute amphistomiasis of cattle in Assam (a preliminary report). Indian J. Vet Sci., 5· 364—375.

Prasad, K.D., Sahai, B.N. and Jha, G.J., 1974. Observations on pathogenicity and histochemistry of experimental infection in *Cotylophoron cotylophorum* in goats. Proc. Natl. Acad. Sci. India, Sect. B, 44 : 202—208.

Sahai, B.N., 1981. Epidemiology, host—parasite relationship and control of paramphistomiasis in livestock. Compendium Summer Inst. Vet. Parasitol., pp. 6—39 (unpublished.)

Sharma Deorani, V.P. and Jain, S.P., 1969. Reasons for involvement of duodenum in parasitic part of the life cycle of rumen amphistomes (Paramphistomidae : Trematode). Indian J. Helminthol., 21. 177—182

Sharma Deorani, V P. and Katiyar, R.D., 1967 Studies on the pathogenicity due to immature amphistomes among sheep and goats. Indian Vet. J., 44: 199—205

Srivastava, H.D., 1938. A study on the life history and pathogenicity of *Cotylophoron cotylophorum* (Fischoeder, 1901) Stiles and Goldberger, 1910, of Indian ruminants and a biological control to check infestation. Indian J. Vet. Sci , 8: 381—385.

Varma, A.K., 1957. On a collection of paramphistomes from domestic animals in Bihar Indian J. Anim. Sci., 27: 67—76.

Varma, A.K., 1961. Observations on the biology and pathogenicity of *Cotylophoron cotylophorum* (Fischoeder, 1901). J. Helminthol , 35. 161—168