PATHOGENESIS OF *ASCARIS SUUM* IN REPEATED INFECTION OF LAMBS

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Received March 27, 2000
Accepted July 27, 2000

Abstract

Dubinský P., E. Švický, G. Kováč, Ľ. Lenhardt, I. Krupicer, Z. Vasilková, E. Dvorožňáková M. Levkut, I. Papajová, D. J. Moncol: *Pathogenesis of Ascaris suum in Repeated Infection of Lambs.* Acta Vet. Brno 2000, 69: 201–207.

After a repeated long-term infection of lambs with the dose of 100 and 1 000 eggs for 23 days only sporadical larvae were detected in the intestines, but they migrated into the liver and the lungs. Clinical signs and biochemical changes were little expressive. Numerous small disseminated greyish white nodules were forming in the liver during infection, the space around the bile ducts and vessels was infiltrated with lymphocytic cells and developing foci were made up of lymphocytes and polymorphonuclear cells. The changes in the lung parenchyma occurred later than in the liver, appearing as disseminated greyish blue nodules. The peribronchial area and interalveolar septa were infiltrated with lymphocytic cells. After infection has been ceased, the restoration of changes in the liver was very rapid, while in the lungs it was slower. An increased alkaline phosphatase and acid phosphatase activities in the intestinal wall induced by larval penetration were persisting and suggested a long-term malabsorption. Despite the mentioned lesions, the infected lambs were able to compensate for the negative influence of migrating *Ascaris suum* larvae without any conspicuous changes in the concentrations of plasma proteins, albumins, total immunoglobulins, bilirubin and marker enzyme activities.

Lambs, non-specific hosts, Ascaris suum, pathogenesis

Postinfective larvae of some nematode species of the suborder Ascaridata are able to migrate also in the organism of non-specific hosts (McDonald and Chevis 1965; Borella et al. 1966). This ability is also possessed by larvae of *Ascaris suum*, which cause pathomorphological changes in the liver and lungs of lambs and calves (Aitken and Sanford 1968). Some larvae in sheep are even able to reach their maturity. The migration ability of *Ascaris suum* larvae in ruminants was also confirmed experimentally, when lambs and calves were infected with a single large dose of infective eggs (Fitzgerald 1962; McCraw 1973, 1975). Such an infection, however, occurs only rarely in practice. Non-specific hosts usually come into contact with infective *Ascaris* eggs in joint enclosures or on pasture grounds manured with pig slurry (Borland et al. 1980; Gunn 1980; Mitchel and Linklater 1980; Gibson and Lanning 1981), or when pigs and cattle are grazed on the same pasture grounds (Thansborg et al. 1999). The eggs in turf and soil develop, survive, and remain infective for a long time (Jurášek et al. 1993). Grazing animals are therefore repeatedly infected usually with small numbers of eggs.

Changes in the organism of lambs were studied after their repeated long-term infection with two different doses of *Ascaris suum* eggs.

Materials and Methods

Experiments were conducted on four-month-old lambs of improved Valaška breed. The animals were divided into three groups. Each of six lambs in group 1 was daily infected with 100 eggs and each of 13 lambs in group 2

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with 1 000 eggs for 23 days. Control group consisting of five lambs was not infected. The lambs were housed separately in pens and fed with an optimal feeding dose with free access to water.

The eggs were isolated from distal portions of *Ascaris suum* uteri. They were sedimented and placed in 0.5 N NaOH for 15 min, repeatedly washed with distilled water and incubated in 0.1 N H_2SO_4 at 26 °C for 30 days.

Prior to the experiment and then at 7-day intervals until day 56, the lambs were weighed, their body temperature and respiration rate taken and blood and fecal samples collected. On days 7, 14, 21, 28 and 35, one animal of group 2 infected with 1 000 *A. suum* eggs was sacrificed. On days 42 and 56 experiment, i. e. on days 19 and 33 after the last infection, each time three lambs of group 1 and four lambs of group 2 were sacrificed. On day 28 of experiment one lamb and on days 42 and 56 two lambs from control uninfected group were killed each time.

Levels of total proteins, albumins, immunoglobulins and total bilirubin as well as activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) and alkaline phosphatase (ALP) were determined spectrophotometrically using Bio-Lachema tests. Samples from the intestine, liver and lungs of dissected animals were taken for histopathological, histochemical and parasitological examination. The intestinal content and mucosal scrapings from the intestine and samples of liver and lungs were examined by the Baerman method for the presence of *A. suum* larvae. Faeces and the intestinal content were examined by a flotation method (Manual of Veterinary Parasitological Techniques 1986). A histopathological examination was performed on paraffin sections stained with haematoxylin-eosin.

Intestinal samples for histochemical examination were frozen within 10 min and cut into 7 μm thick sections on cryostat. Enzyme activities in the intestinal wall were determined by the azocopulation method, with a 30 min incubation at room temperature for alkaline phosphatase and a 15 min incubation at 37 °C for acid phosphatase (Lojda et al. 1979). The enzyme activities were evaluated on an integrating microdensitometer VICKERS-M-86 (Germany). The reaction product density was measured with standard use of objective ×20 and screen with diameter of 2 μm at optimum length wave (alkaline phosphatase at 480 nm and acid phosphatase at 520 nm). Each enzyme was measured on four intestinal section from each lamb, on 10 intestinal villi and at 10 points. The measured values were evaluated by a one-way ANOVA. Significance of differences was evaluated by Tukey's test.

Results

Changes in weight and other parameters of the clinical and immunological status of infected and control lambs were published earlier (Krupicer et al. 1999; Levkut et al. 1999).

Examination of the content and scrapings of the digestive system and of feces

Two infective larvae *A. suum* were found in the ruminal content on day 28 of experiment. In the content and scraping from the small and the large intestine and in feces sporadical larvae and eggs *A. suum* were observed from day 7 to day 28 of experiment. No higher developmental stages of *A. suum* were found in the intestines.

Table 1
Number of *Ascaris suum* larvae per gram (LPG) in liver and lungs of lambs infected for 23 days with 1 000 *Ascaris suum* eggs a day

Days of experiment	Liver (LPG)	Lungs (LPG)
7	2.4 ±0.69	1.43 ± 0.75
14	1.2 ±0.26	1.21 ± 0.38
21	0.78 ± 0.39	1.02 ± 0.07
28	0	0
35	0	0
42	0	0
56	0	0

Examination of liver and lungs

Samples of the liver and the lungs weighing 3 g, from lambs of experimental group 2 infected daily with 1 000 larvae, were examined by Baerman method in three repetitions. The results (Tab. 1) suggest that the maximum LPG (larvae per gram) value was recorded on day 7 of experiment. The number of larvae in the liver was decreasing more rapidly than

in the lungs. Larvae were detected only on day 7, 14 and 21 of experiment. Control animals showed no presence of larvae.

Table 2
Statistical significance of increased alkaline phosphatase (ALP) and acid phosphatase (ACP) activities in the small intestine of lambs infected with 100 and 1 000 *Ascaris suum* eggs for 23 days compared with control on days 42 and 56 after the last infection.

Dose of *Ascaris suum* eggs	ALP		ACP	
	Day 42	Day 56	Day 42	Day 56
100	$P > 0.05$	$P > 0.05$	$P < 0.01$	$P > 0.05$
1000	$P < 0.001$	$P > 0.05$	$P < 0.001$	$P < 0.01$

Biochemical examination of serum

Biochemical parameters in the blood plasma of control and infected lambs showed no significant changes. Infection of lambs with 100 and 1 000 *A. suum* eggs per day for 23 days neither influenced total proteins which were within the lower range of the standard, nor the levels of albumins and total immunoglobulins. Total bilirubin was standard in all animals (Fig. 1), but in infected lambs higher than in control. Among enzymes studied (ALT, AST, GGT and ALP), infection influenced only ALP activity. Compared with control, the group infected with 100 eggs exhibited statistically insignificantly lower ALP activity (Fig. 2) from day 14 to day 42, but a statistically significant decrease in the activity was observed on days 49 and 56 ($P < 0.01$ and $P < 0.05$, respectively). Administration of 1 000 eggs had no effect on ALP activity in lambs.

Pathological examination of liver and lungs

The liver parenchyma of lambs infected with 1 000 eggs and necropsied on days 7 and 14 after infection showed small, inconspicuous greyish white nodules 0.1–0.2 mm in size. However, on days 21 and 28 even more pronounced greyish white foci, 2 × 3 mm large, were observed. On day 35 only sporadical almost invisible greyish white foci were recorded. In

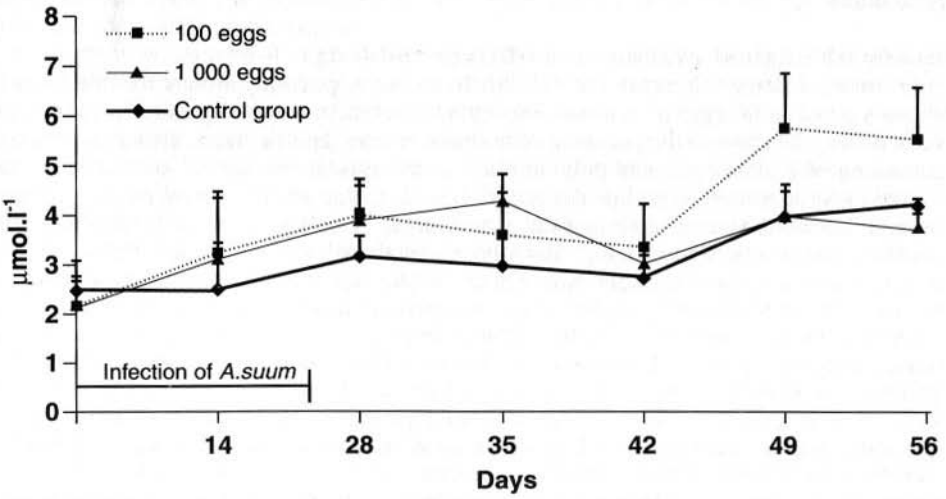

Fig. 1. Total bilirubin in the plasma of lambs infected for 23 days with *Ascaris suum* eggs

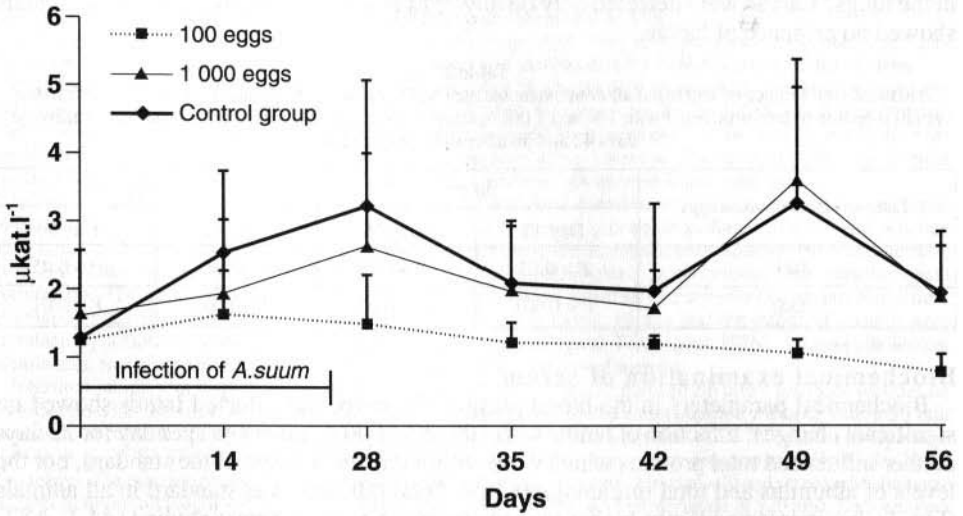

Fig. 2. Alkaline phosphatase in the plasma of lambs infected for 23 days with *Ascaris suum* eggs

most lambs of both experimental groups examined on days 42 and 56 the liver parenchyma was without visible macroscopical changes, only one animal showed the presence of conspicuous narrow bands, 5-10 mm long.

The lungs of lambs infected with 1 000 eggs were without any macroscopical changes on day 7. On day 14, sporadical greyish blue nodules, 0.2-0.5 mm large, were disseminated in the parenchyma. On days 14 and 28, the nodules were more conspicuous, more abundant and disseminated all over the lung parenchyma. From day 35 the nodules were becoming smaller, fewer and less visible, but persisted in both experimental groups for as late as day 42 when they assumed greyish white colour. On day 56, the lungs of one lamb from each experimental group showed the presence of greyish white foci, characteristic of verminous pneumonia.

Histopathological examination of liver and lungs

From day 7, largely lymphocytic cell infiltrates were appearing around the bile ducts in animals given 1 000 eggs of *A. suum*. On day 14, the infiltrates also appeared around some vessels. Day 28 showed the presence of nodules around the bile ducts, changing into foci consisting of lymphocytes and polymorphonuclear cells. From day 35, connective tissue started to form around some bile ducts. On day 42, inconspicuous eosinophilic infiltrates were still present around some bile ducts and larger vessels in 60 % of lambs from both experimental groups (Plate VI, Fig. 3). Only in a single animal nodules detected in the liver were composed mainly of lymphocytes. On day 49, the region around some liver vessels and bile ducts was permeated by the forming connective tissue (Plate VI, Fig. 4).

On day 14, the lungs were infiltrated with lymphocytic cells around the bronchi and sporadically also around the interalveolar septa. On day 21, the peribronchial lymphocytic infiltrates were more conspicuous and sporadical nodules with a cluster of lymphocytes were observed (Plate VII, Fig. 5). On day 35, the peribronchial lymphocytic infiltrate was spreading over a wider area, reaching as for as interalveolar septa. Sporadical clusters of lymphocytes occurred around the bronchi. On day 42 the lung alveoli and bronchi at sites without infiltration and nodules were airy and empty in lambs of both experimental groups. The nodules consisted mostly of lymphocytes, less of polymorphonuclear cells.

On day 56, the more expressive changes persisted only in the lungs of lambs infected with a higher dose of eggs. The peribronchial area was still infiltrated with lymphocytic cells, penetrating into a wider region as far as interalveolar septa (Plate VII, Fig. 6). The space around some bronchi was infiltrated with a newly forming connective tissue.

Histochemical examination of intestinal wall

Alkaline phosphatase (ALP) and acid phosphatase (ACP) activities (Table 2) were studied histochemically in the intestinal wall of slaughtered infected and control lambs on days 42 and 56 of experiment.

Relative to control a significantly increased ($P < 0.001$) ALP activity was observed only in lambs given 1000 *A. suum* eggs (Plate VII, Fig. 7). ACP activity was statistically significantly elevated in both infected groups on day 42 (Plate VII, Fig. 8) and in the group given 1 000 eggs for 23 days also on day 56.

The enhanced activity of both the enzymes is likely the result of a repeated migration of *A. suum* larvae through the intestinal wall and of a subsequent malabsorption.

Discussion

In studying clinical and pathomorphological changes induced by migrating *A. suum* larvae in the organism of specific and non-specific hosts, attention is usually paid to the liver and lungs and less to the intestine. However, it is the intestine where the hatched *A. suum* larvae start their migration. Observation of the damage to the intestinal epithelium and subepithelial structures is complicated as to the methods used because of the hardly identifiable site of larva penetration. A long–term administration of infective eggs to lambs, simulating the natural way of animal infection on pasture, entails a repeated damage of intestinal tissues. Such changes are likely to persist for a long time. This has been confirmed by our results, when as late as on day 42 of experiment acid phosphatase activity was increased with even a low repeated dose of eggs (100 specimens). The dose of 1 000 eggs also increased the activity of alkaline phosphatase. Acid phosphatase activity, however, remained elevated also on day 56 of experiment. These findings are surprising since changes in the liver faded away relatively early and also restoration of the lungs was observed. The signs of malabsorption after repeated infections may be a serious consequence of pasture parasitoses.

In lambs infected with 40 000 *A. suum* eggs, Brown et al. (1984) reported the damage of the liver on day 6. This damage was manifested by small necrotic foci infiltrated with eosinophils and lymphocytes. On day 21 only peripheral lymphocytic and eosinophilic clusters persisted. Like in our experiments, also Fitzgerald (1962) reported such infiltrates around bile ducts and vessels with even much more severe *A. suum* infection (80 000 - 40 million eggs), but he observed neither significant changes nor the presence of larvae in the lamb liver. Similarly Clark et al. (1989) detected no larvae in the liver of lambs naturally infected with *A. suum*, despite conspicuous eosinophilic hepatitis. These findings testify to a different reaction of the liver tissue of pigs and sheep to migrating *A. suum* larvae. Even changes detected in slaughter pigs characterized as "white spots" were indistinct in lambs and remained unnoticed (Brown et al. 1984). Roepstorff et al. (1997) reported numerous "white spots" in the liver of pigs on day 7 post infection with low doses (100, 1 000 and 10 000) of *A. suum* eggs. Only sporadical lesions persisted from day 21 to day 56 post infection, like in our experiments.

A long–term repeated infection of lambs with 100 and 1 000 eggs neither influenced the total bilirubin level nor the activity of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyltransferase. The lamb organism was able to compensate for *A. suum* infection. A similar conclusion has also been reached by Brown

et al. (1984), considering that a single infection did not enhance the activity of gamma-glutamyltransferase, albumines and total proteins.

The most significant changes caused by migration of *A. suum* larvae were observed in the sheep lungs. They were accompanied by increased temperature from day 6 and increased respiratory rate and cough on days 6 and 7 after the first infection (Krupicer et al. 1999).

After a single infection of lambs with large doses of *A. suum* eggs, Fitzgerald (1962) reported increased temperature and dyspnoe as early as day 2, with both the signs persisting until day 8. Similar symptoms were observed by Brown et al. (1984) on day 5 after single infection but on day 15, the lambs were without clinical signs.

Unlike in pigs, where it is mostly liver that is severely damaged, migrating *A. suum* larvae in ruminants induce the most conspicuous changes in the respiratory system. The intensity of signs (dyspnoe, cough etc.) in cattle was determined by the infective dose (Morrow 1968; Greenway and McCraw 1970; McCraw and Lautenslager 1971).

Fitzgerald (1962) reported diffused petechial haemorrhages in the lungs of lambs. On day 21 after a single infection of lambs with 400 000 *A. suum* eggs, Brown et al. (1984) observed a thickening of the interalveolar septa and large clusters of lymphocytes in different parts of the lungs. Similar lesions were also seen in our repeatedly infected animals on days 42 and 56 of experiment. Histopathological changes in the lungs observed on day 42 of experiment are characterized by nodules consisting of lymphocytes. The changes affected not only the area close to the bronchi but extended as far as the interalveolar septa and the pulmonary alveoli.

In grazing cattle naturally infected with *A. suum* eggs, Morrow (1968) diagnosed diffused interstitial pneumonia. Also McCraw and Lautenslager (1971) reported a thickened alveolar wall and diffused haemorrhages in the alveoli. Both the authors are concordant in stating that interstitial parasitic pneumonia in cattle is very hard to distinguish from other respiratory diseases.

After very high single and repeated doses, *A. suum* larvae were present in the liver, but more frequently and in higher numbers in the lungs of infected ruminants (Fitzgerald 1962; Morrow 1968; McCraw 1975). Similarily in our experiment on day 7, more larvae were detected in the liver than in the lungs. This number decreased rapidly in the liver, while in the lungs the number of larvae on day 21 was three times higher than in the liver.

Ruminants, sheep in particular, are frequent hosts of adult *A. suum* localized either in the lumen of small intestine (Roneus and Christensson 1979) or in bile ducts (Pedersen et al. 1992). Such non–typical locations of *Ascaris suum*, however, were also detected in pigs (Suksaithaichana et al. 1989).

Patogenéza *Ascaris suum* pri opakovanom infikovaní jahniat

Pri dlhodobom infikovaní jahniat dávkou 100 a 1 000 vajíčok denne počas 23 dní sa našli len ojedinelé larvy v črevách, ale larvy migrovali do pečene a pľúc. Klinické prejavy aj biochemické zmeny boli málo výrazné. Počas invadovania sa vytvárali v pečeni početné drobné diseminované sivobiele uzlíky, okolie žlčovodov a ciev bolo infiltrované lymfocytárnymi bunkami a vytvárali sa ložiská tvorené lymfocytmi a polymorfojadernými bunkami. V pľúcnom parenchýme sa zmeny objavili neskôr ako v pečeni a prejavili sa diseminovanými sivomodrými uzlíkmi. Okolie bronchov a interalveolárne septá boli infiltrované lymfocytárnymi bunkami. Po ukončení invadovania prebiehala reparácia zmien v pečeni veľmi rýchlo, kým v pľúcach pomalšie. Zvýšená aktivita alkalickej a kyslej fosfatázy v stene čreva vyvolaná penetráciou lariev pretrvávala a poukazovala na dlhotrvajúcu malabsorpciu. Napriek uvedeným zmenám boli nakazené jahňatá schopné kompenzovať negatívny vplyv migrujúcich lariev *Ascaris suum* bez výraznejších zmien hladiny plazmových bielkovín, albumínov, celkových imunoglogulínov, bilirubínu, ako aj aktivít markerových enzýmov.

Aknowledgements

This study was supported by the U. S. - Slovak Scientific and Technological Program, Grant No. 002-95 and by the Scientific Grant Agency VEGA, Grant No. 2/5012/98.

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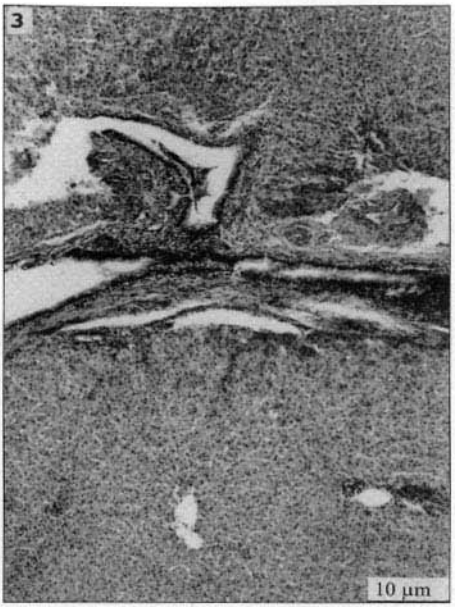

Fig. 3. Lymphocytic infiltrates surrouding the liver bile ducts and large vessels of infected lambs, haematoxylin-eosin

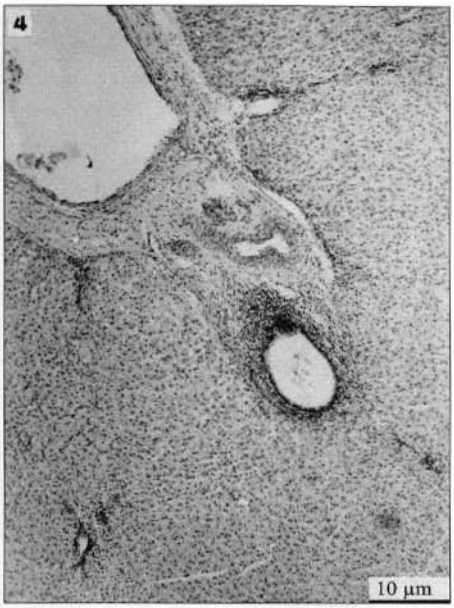

Fig. 4. The space around some liver vessels and bile ducts infiltrated with the forming connective tissue; haematoxylin-eosin

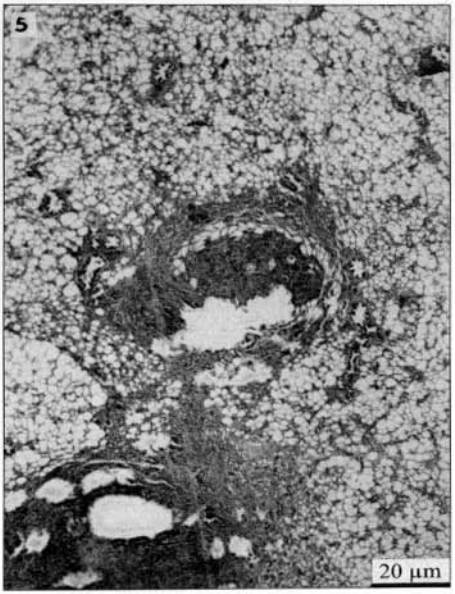

Fig. 5. Nodules consisting of lymphocytes with empty center, in the lung parenchyma; haematoxylin-eosin

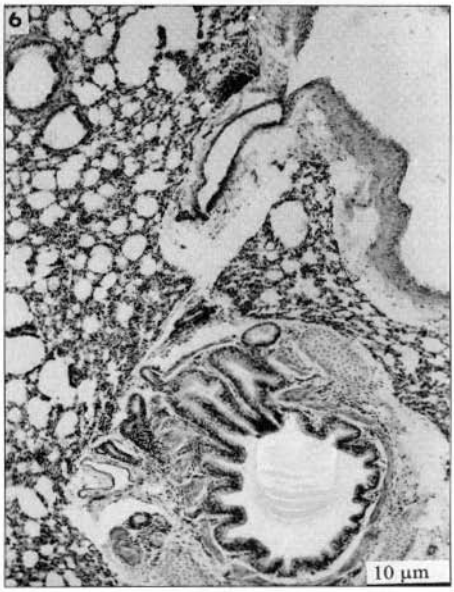

Fig. 6. Space surrounding some bronchi infiltrated with the forming connective tissue, haematoxylin-eosin

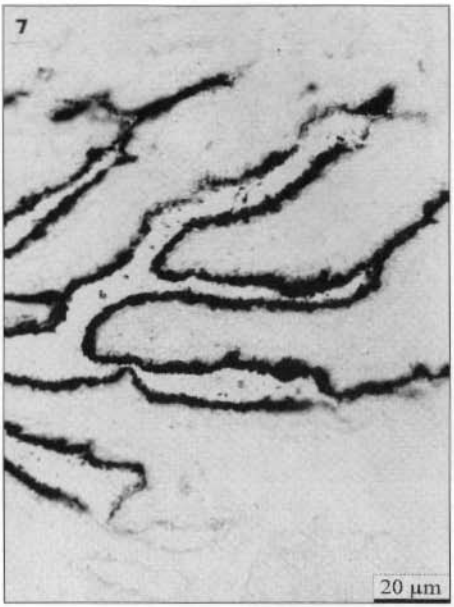

Fig. 7. The high ALP activity in the small intestine of infected lambs on day 42 of experiment; azocopulation method

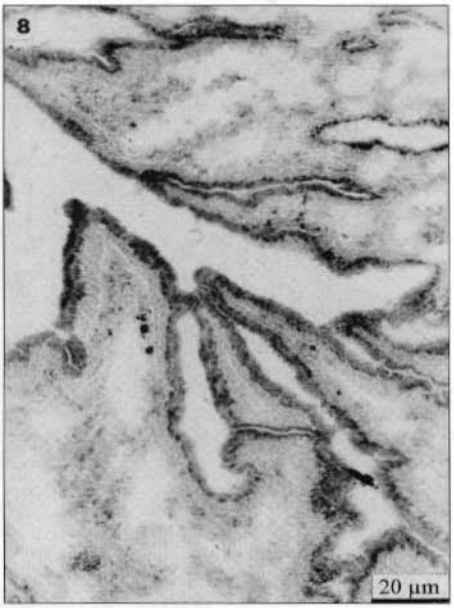

Fig. 8. Enhanced ACP activity in the small intestine of infected lambs on day 42 of experiment; azocopulation method