PATHOLOGICAL AND HISTOCHEMICAL ALTERATIONS IN THE SMALL INTESTINE OF SHEEP IN THE COURSE OF EXPERIMENTAL INFECTION WITH STRONGYLOIDES PAPILLOSUS

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It was demonstrated that partogenetic Strongyloides papillosus females living in the small intestine of sheep infected with L3 S. papillosus larvae caused the necrosis of the mucosa and, in some cases, destruction of the intestinal villi. Furthermore, the enzymatic activity of the epithelial cells of the small intestine was strongly correlated with the morphological picture of the intestine. As early as 11 d after the infection a decrease in alkaline phosphatase in the epithelium, stroma and base of the villi was noted. A reduced activity of the enzyme in the epithelium was continued but in the stroma the reaction was negligible.

Key words: sheep, Strongyloides papillosus, strongyloidosis, intestines, pathology, experimentation.

Strongyloides papillosus is a nematode which may occur in lambs as early as the first weeks of life. The occurrence early nematode infection in these animals results from the possibility of the infection not only through the skin but via the colostrum, milk or placenta as well (4, 5, 10-12, 16). Additionally, the per os infection is also possible when infectious larvae migrated into the circulatory system through the mucous membrane of the oral cavity, throat and oesophagus. The larvae reach the small intestine after 3 d and then locate under the epithelium and mature 6 to 8 d thereafter. In females the larvae can penetrate into the udder and infect the suckling through the colostrum and milk. Moreover, the larvae can migrate with the blood stream into the foetuses. The animals which died from strongyloidosis demonstrated the signs attributed to the inflammation of the intestinal mucosa. The leukocyte infiltration and oedema were found microscopically in the intestine (17). However, alterations in the lungs such as pulmonary inflammation and emphysema were found by Turner (14) only in one of the lambs examined. The literature concerning strongyloidosis in sheep describes fragmentary the pathogenesis and pathology of this disease. Considering the results of investigations into other invasions caused by intestinal parasites in various host species it is obvious that the invasions often result in the occurrence of
malabsorption syndrome irrespective of the location of the invasion in the alimentary tract. The irritation of the mucosal cells by the parasites, organism intoxication and alterations in gastrointestinal flora are also of importance. Consequently, the malnutrition resulted from disturbed digestion and absorption occurs in affected animals. The above changes are evidenced not only by morphological alterations in the intestinal mucosa but also by a decrease or lack of the activity of alkaline phosphatase that plays a key role in absorption (1, 8, 9, 13). Considering the current knowledge of the life cycle of *S. papillosus* and the pathogenesis of strongyloidosis in sheep we carried out the studies aimed to understand the dynamics of morphological and histochemical alterations in the small intestine resulting from *S. papillosus* infection.

**Materials and Methods**

**Animals.** Twelve 6-month old rams from a breeding farm free of *S. papillosus* infection were used. The animals were arranged into two groups: Group 1 (9 sheep) infected subcutaneously into a lateral abdominal wall with $5 \times 10^4$ of $L_3 S. papillosus$ larvae per animal and Group 2 (3 sheep) consisting uninfected controls. All animals were weighed before the arrangement and every other week thereafter.

**Coproscopic examinations.** Qualitative Fullerborn’s method and quantitative McMaster’s method modified by Raynaud (7) were used to examine the presence of parasite eggs in faeces.

**Postmortem examination.** On days 11, 47, and 83 after the infection infected animals and 1 control were slaughtered and necropsied. Small intestine segments were sampled, fixed in a 10% formalin and paraffin sections stained with hematoxylin and eosin were prepared. Some intestinal samples were fixed in Baker’s fluid and frozen sections were prepared to demonstrate the alkaline phosphatase activity. The enzyme was localised according to Gomori’s method (2).

**Parasitologic examinations.** *S. papillosus* females from the infected animals were isolated by scraping off the intestinal mucosa from the total length of the small intestine. Scrapped mucosa were mixed with digestive fluid (1.3 g pepsin + 1.8 mL HCl and 100 mL H$_2$O and kept for 4 to 5 h at 37°C in a water bath with a shaker. The fluid volume was measured and one tenth of the volume was overflowed using a 75 µm sieve followed by a careful rinse; then the parasite were poured into a beaker and counted using a binocular magnifying glass. The calculated number of parasites was multiplied by 10 to get the total number of parasites in infected animals.

**Results**

The infection of sheep with *S. papillosus* was effective. The excretion of the parasite eggs was noticed 9 d after the infection. No pathognomonic signs were observed during the experiment. However, polyuria and a decrease in a body weight gain were found.

For example, a control sheep increased their body weight gain by 5.5 kg, whereas infected sheep failed to gain any weight in a 11-week period.

The excretion rate of the parasite eggs in infected animals was high since the second day of ovulation and continued at a high level for the whole experimental period (an average of 13 200 to 17 770 eggs/1 g faeces). The number of *S. papillosus*
females found in the intestinal mucosa was 1110 to 6160 (on an average of 2710/animal) after slaughter and was similar in selected stages of infection.

The following histological alterations were found: thickening of the alveolus walls in the lung and the presence of cellular infiltration, comprising mainly lymphocytes, around bronchioles and necrosis of the intestinal villus surface, which in several cases extended up to ½ of the villi (Fig. 1). Examinations performed after a 6-week infection showed no alterations in the lungs; however, the intestinal villus necrosis, eosinophilic cells among the villi, and parasites in the intestinal lumen and mucosa were still present (Fig. 2).

**Fig. 1.** Surface necrosis of the villi (H&E, x300).

**Fig. 2.** Fragments of *S. papillosus* in the intestinal mucosa (H&E, x300).
No pulmonary changes were showed after a 11-week invasion. Two animals from this group demonstrated villus atrophy, extended cellular inflammation, typical mucosa denudation and the presence of a retained secretion in several glands. The secretion containing exfoliated glandular epithelial cells caused a marked enlargement of the glands (Fig. 3). The observed pathomorphological lesions were less evident in one sheep as compared to those in the two remaining animals. No macroscopic changes were noticed in the liver in the whole invasive period.

![Fig. 3. Intestinal gland enlarged by secretion. Cell nuclei are visible in the secretion (H&E, x300).](image)

**Alkaline phosphatase (AP) activity.** The controls revealed a strong enzymatic reaction in the intestinal mucosa cells. A weaker response was observed in parabasal segments of the epithelium covering the villi, Lieberkuhn’s crypts and the villus stroma (Fig. 4).

![Fig. 4. Reaction to alkaline phosphatase in the small intestine of non-infected sheep. Positive reaction is visible in the epithelial cells of the villi and intestinal glands (Gomori method, x80).](image)
The animals infected and slaughtered 11 d after the infection showed a small drop in AP activity both in the epithelial cells and the villus stroma; a markedly weaker reaction was observed in the epithelial cells of the basis of the villi.

After a 6-week invasion period a distinct decrease in AP activity in the intestinal epithelial cells and stromal connective tissue was observed. A stronger reaction was observed in the crypts of Lieberkühn.

After a 11-week invasion period a weaker reaction to AP activity persisted in the epithelial cells; however, the reaction was hardly visible in the villus stroma (Fig. 5).

![Fig. 5. Disappearance of alkaline phosphatase activity in the majority of intestinal glands Gomori method, x80).](image)

**Discussion**

Histological examinations of selected organs of the sheep infected with *S. papillosus* demonstrated that the parasites caused morphological changes evidenced by thickening of the alveoli and cell infiltration, comprising mainly lymphocytes, around the bronchioles in the first phase of the parasite migration through the lungs. Then, morphological alterations in the small intestine expressed as villus necrosis in the first stage of invasion (11 d after infection) and villus atrophy thereafter (6 and 11 weeks after infection) were observed.

Few reports have described morphological alterations in the intestinal mucosa of sheep affected with strongyloidosis. Histological alterations in the small intestine on days 13 and 15 after infection were only shown by Turner (14, 15) who studied the migration of *S. papillosus* larvae L₃ in the sheep infected experimentally. The author found the inflammation and oedema of the intestinal mucosa. The intensity of the changes varied in the two experiments although the number of larvae administered was the same.
On the other hand, rabbits infected with a lethal dose of \(2 \times 10^4\) L$_3$ of *S. papillosus* larvae developed pulmonary tissue lesions, vacuolation of Brunner’s glands and changes in intestinal villus epithelium (6).

In course of the majority of parasitic diseases of the gastrointestinal tract diffuse and non-specific inflammation occurs in the small intestine irrespective of the kind of invasion. The alterations, however, are negligible macroscopically and it is often difficult to decide whether the observed changes bear pathological features. Chronic and serious forms of the parasite disease may be attributed to profound pathological alterations involving a visible atrophy of the intestinal mucosa as it was found in the present studies. The atrophy of the intestinal mucosa explains the syndrome of impaired absorption in the cases of chronic and serious parasitic diseases (1, 3, 9).

Measurements of enzymatic activity in the epithelial cells of the small intestine reflect the functioning of the intestinal mucosa. The enzymatic activity of these cells and the morphological picture of the intestine are strongly correlated.

The location and activity of alkaline phosphatase in both the epithelium and villus stroma of the controls were in accordance with those reported by others (1, 9). In the normal intestinal mucosa the enzymatic activity is lower at the basis of the villi than in the upper parts because the epithelial cells originate from Lieberkühn’s crypts and then migrate along the villus up to the top where they are shed. In infected animals a decrease in AP activity both in the epithelial villus, stroma and basis was found as early as 11 days after infection. A weaker reaction to AP activity in the epithelial cells and a negligible one in the stroma continued 11 weeks postinfection.

Considering the above results of the experimental strongyloidosis in sheep caused *S. papillosus* larvae it should be stressed that the parasite females living in the intestinal mucosa produce morphological alterations and a decrease in AP activity in the epithelial cells, stroma and basis of the villi.

**References**


