

Studies on Strongyloidiasis of the Peacock

IV. Observations on Pathogenicity of *Strongyloides pavonis* Sakamoto et Yamashita, 1970 for Various Birds*

Tsukasa SAKAMOTO

(Laboratory of Veterinary Pathology)

Received for Publication September 10, 1981

Introduction

Some considerable number of reports¹⁻¹⁰⁾ have been made of avian *Strongyloides* in the past. Most of the reports, however, are concerned with the morphological description of the parasites themselves. The brief descriptions regarding the pathological findings of avian *Strongyloides* were found only in a few reports. It is the purpose of this paper to give a detailed account of the pathogenicity of *Strongyloides pavonis*, based on the histopathological investigations of experimentally infected baby chickens and peacocks.

Materials and Methods

To obtain the filariform, infective larvae of *Strongyloides pavonis* Sakamoto et Yamashita, 1970⁹⁾, the feces from peafowls and chickens artificially infected with the parasite were cultured on the clay plate in the Petri dish with water at 28°C. The infective larvae having migrated into water of the Petri dish were collected with the water 4 and 5 days after incubation, and were placed into a test tube. The test tube was allowed to stand for 15 minutes. The supernatant was removed from the test tube. The sediment was dispersed with saline solution added 100 units of penicillin G and 100 γ of streptomycin sulfate per ml, and was placed in a new tube. Those procedures were repeated 5 times to eliminate the admixtures. The suspension containing about 10,000 larvae per ml was prepared by regulating the quantity of supernatant.

The present experiment includes three series as follows.

Experiment 1: The pathogenicity of the parasite for baby chickens was observed in this series. Three groups of baby chickens 5- to 7-days old were given 1000 to 3000 infective larvae by means of oral administration, subcutaneous injection and skin penetration, respectively. Those chickens were dissected in the course of time. The chickens which died during the period of the experiment were also subjected to pathological examination. At autopsy, one-sided caecum of all cases was examined under the binocular microscope. The larvae were collected from a half part of the liver, lung, kidney, intestine, colon and brain of several cases with Baermann's apparatus, and were examined morphologically. After removing the brain, the head was minced with scissors. Cranial and nasal cavities and paranasal sinuses were washed in saline solution at 37°C using

* This work was presented at the 56th Meeting of the Japanese Society of Veterinary Science, on 19th-20th October, 1963 in Tokyo, Japan

pipette. The larvae in the saline solution were examined in the same manner. All the organs were fixed with Carnoy's and 10% neutral formalin solutions, and were sectioned. For histological observations, the sections were stained with hematoxylin-eosin (H. E.) and by other routine procedures such as Azan method and periodic acid-Schiff reaction (PAS).

Experiment 2: Each of the three young Indian peacocks (*Pavo cristatus* Linnaeus), 2 months old, was once orally, subcutaneously and percutaneously inoculated with about 1000 infective larvae. The birds were clinically observed for 3 months, and then were sacrificed for pathological examination. Otherwise, an Indian peacock, 3 months old, was orally given a dose of about 3000 infective larvae once a month for 6 months. The bird was clinically observed, and died 120 days after infection. The bird was dissected and was examined pathologically.

Experiment 3: Japanese quails (*Coturnix coturnix japonica* Temminck et Schlegel), domestic pigeons (*Columba livia domestica* Linnaeus), domestic ducks (*Anas platyrhynchos domestica* Linnaeus), Japanese tree-sparrows (*Passer montanus kaibatoi* Munsterhjelm) and silver pheasant (*Gennaes nyctemerus* Linnaeus) were orally inoculated with about 3000 infective larvae. The birds were dissected 6 to 15 days after inoculation and were examined for the parasite.

Results

Experiment 1.

Five to 7 days old chickens belonging to the three groups were infected with 1000 to 3000 larvae by oral, subcutaneous and percutaneous inoculations, respectively. The baby chickens were observed clinically, and were dissected in the course of time for the pathological examination. The distribution and the development of the larvae in their bodies were observed.

On the 2nd day after inoculation, many of the chickens of all the groups suffered from lack of appetite, became much weakened, and showed panting breath. Some of the severely affected chickens died. Many larvae were found in the lumen of small and large intestines. The histological appearance of lung showed the larvae having penetrated into alveoli and peribronchial and peribronchiolar tissue, and revealed pneumonia catarrhalis which was associated with remarkable hyperemia, petechiae and infiltration of histiocytes and granulocytes. The degeneration and desquamation of epithelia of the mucous membrane were recognized in the small intestine. Caeca showed congestion and degeneration of epithelia and lamina propria. In the caeca, a number of the larvae were recognized in their lumen, considerable number penetrated into their crypts and lamina propria, and a few invaded into their submucosa and subserosa. Many histiocytes and a few plasma cells were recognized infiltrating into lamina propria and submucosa of caeca. Colon revealed the desquamation of epithelia, and the infiltration of granulocytes and plasma cells in lamina propria. In the liver and spleen of the subcutaneously injected cases, the proliferation of reticulo-endothelial cells were recognized. Some considerable number of larvae were found in the saline solution in which the minced head was washed.

On the 4th day, the birds discharged thin washy feces mingled blood. The larvae obtained from mucous membrane of caeca of the chickens infected in the three ways, appeared the features of parasitic female. In the histological examination of the orally inoculated cases, some accumulations of histiocytes and plasma cells were recognized in the liver. The mild proliferation of reticulo-endothelial cells were seen in the spleen. The lung revealed remarkable hyperemia, petechial hemorrhage and nodular accumulations of plasma cells and histiocytes. The epithelia of small intestine were degenerated and partially desquamated. The lymphoid tissue of small

intestine was hyperplastic. In their caeca, a number of parasites were seen in the lumen, and some considerable number of worms were found in the layer of epithelial cells, crypts and lamina propria. Plasma cells, lymphoid cells and histiocytes were seen infiltrating in their submucosa and lamina propria. In the colon also, several parasites were found in the layer of epithelial cells and crypts, and the infiltration of lymphocytes and the hyperplasia of lymphoid tissue were seen in its submucosa and lamina propria. A small number of larvae were found in the saline solution in which the minced head was washed.

On the 6th day, the birds were weakened and became emaciated due to obstinate diarrhoea caused by severe typhlitis. The severely affected birds discharged bloody and mucous feces. At autopsy, a number of matured adult females were obtained from the caeca of all of the chickens inoculated by the three means. The embryonated eggs were seen in uteri of the females. Histologically, also the mild proliferation of reticulo-endothelial cells were seen in the liver and spleen. Some nodular aggregations consisting of histiocytes occurred at the area adjacent to the blood vessels in liver. A few petechial hemorrhages were often found in the lung and subendocardial myocardium. A few larvae are rarely recognized in the blood of atrium cordis and the lung. The lung was congested. The nodular accumulations and the filtration of histiocytes were recognized in it. In the cases in which they were subcutaneously or percutaneously inoculated, their lungs revealed catarrhal pneumonia, which was accompanied by infiltration of reticulocytes, giant cells, heterophils and eosinophils in the peribronchial and bronchiolar areas and parenchyma of lung. The mild desquamation of epithelia of small intestine was seen. The lumen of caeca were occupied with desquamated epithelial cells, infiltrated leukocytes and many female parasites having many eggs in uteri. A number of female were found in the crypts, epithelia and lamina propria of caeca. The infiltration of histiocytes and plasma cells and the proliferation of lymphoid tissue were seen in the lamina propria. The proliferated lymphoid tissue was often exposed because of the desquamation of the epithelia, villi and lamina propria. A few parasites were found sometimes penetrating into the lymphoid tissue. The wall of caeca was thickened by the proliferation of connective tissue and lymphoid tissue in submucosa. A few parasites were recognized in the colon also.

In the 8th day cases, the breath of the birds returned to be normal. The feces of the birds were bloody, and contained a number of embryonated eggs, large quantities of epithelia, inflammatory exudate and pseudomembrane eliminated from caeca. The histological findings of the heart, liver, lung and spleen were similar to those of the 6th day cases. The small intestine revealed the desquamation of the epithelial cells and villi. Caecal lumen was filled up with large quantities of inflammatory exudate containing desquamated tissue and infiltrated cells. The parasites and eggs were recognized in the contents of caeca. Most of the epithelial cells lining the crypts were necrotic and collapsed. A few intact epithelia were seen only in a deep part of the crypts. Most part of the lamina propria of caeca was exposed by the desquamation of mucous membrane. The parasites were sometimes found penetrating into remarkably thickening submucosa of caeca. The penetrates are surrounded with cellular accumulation mainly consisting of eosinophils. The lymphoid tissues remarkably proliferated were seen in the wall of caeca. Some parasites were found penetrating into the lamina propria of colon too.

On the 10th day after inoculation, there was little change in clinical appearance of the chickens. The eggs, blood and inflammatory, caseous exudate in their discharge increased continuously. At autopsy, the lumen of caeca was filled with caseous clot. The mucous membrane was covered with fibrinous pseudomembrane containing coagulated deposit of inflammatory exudate, blood, desquamated necrotic tissue and a number of the parasites and their eggs. Histologically, also

the lumen of the caeca was filled up with caseiform clot as mentioned above. Many eggs and larvae were found in the clot. A large number of the parasites penetrated into the crypts and lamina propria of caeca. Lamina propria and lamina muscularis thickened remarkably. The highly massive hyperplasia of lymphoid tissue was recognized in lamina propria of caeca. A small number of the parasites penetrated into the crypts and lamina propria of colo-rectum. Such a remarkable cellular reaction as that in caeca was not seen in colo-rectum. No development of the lesion of liver, lung and small intestine was recognized. In some cases, however, hyperemia, severely cellular infiltration and massive accumulation of histiocytic elements were seen in lung.

On the 12th day after inoculation, some of the affected chickens revealed loss of appetite, so much weakness and emaciation. In the autopsy, the caeca were swollen by thickening of the wall and by the dilation of lumen with a great amount of the caseiform deposit stuffed up in it. Histologically, the liver revealed congestion and proliferation of endothelial cells and nodular accumulations of histiocytes. Granulomatous nodules were often recognized in the lung. Some considerable number of the larvae in addition to many adult females and their eggs were recognized in the caseous clot stuffed up in the caecal lumen. In the caecal wall, a large part of lamina propria and epithelial cells were noted to be necrotic. Hyaline degeneration and focal necrosis of muscle fiber in the lamina muscularis were recognized. The remarkable hyperplasia of connective tissue was observed in the lamina propria and submucosa.

In most of the 14th day cases, little change was recognized in the clinical appearance. However, a few of the birds appeared to be slightly recovering. The lesions of the birds were noted to be similar in macroscopical findings to those in the previous days. However, the number of the parasites harboring in caeca was noted to be decreasing slightly. Histologically, no development of lesions in the liver and small intestine was recognized. The caecal lumen was stuffed up with the caseous clot as well as on the previous day. Necrotic change was spread over the most part of the mucous membrane of caeca. Numerous round cells infiltrated and exudate were seen in the necrotic lesion. The adult parasites were seen penetrating into the submucosa. The surface of mucous membrane of caeca was covered with fibrous exudate containing blood cells. The remarkable hyperplasia of lymphoid tissue and cellular infiltration in serosa were seen in caeca. Many eggs of the parasites were seen in the lumen. The parasites were seen penetrating into the epithelial cells, crypts, lamina propria, submucosa and lymphoid tissue in submucosa. In colon, some considerable number of the parasites were found penetrating in the epithelia and lamina propria. Focal hemorrhage was seen in the lamina propria and muscular layer. Lymphoid tissue of colonic wall manifested hyperplasia.

On the 20th day, the clinical condition of the chickens became somewhat better, their appetite recovered, and the feces appeared normal. The fecal examination for the egg of parasite showed to be positive, but the number of the eggs was conspicuously fewer than that in the previous days. At autopsy, no remarkable change was seen except several small tubercles in the mucous membrane. Histologically, the mucous membrane of caeca revealed recovering from the desquamation and necrotic change in the previous stages. Some considerable number of the parasites were found still remaining in the crypts, lamina propria and lamina muscularis. Some tubercles surrounded with connective tissue were recognized in the submucosa and lamina muscularis. The central portion of the tubercles was necrotic, and a few parasites were found sometimes penetrating into it.

On the 30th day, the clinical appearance of the chickens looked nearly normal. A small number of the eggs were still found in the fecal examination. The pathological findings are similar to those of the cases on the 20th day.

In the cases 2 months after infection, almost all the mucous membranes of caeca were re-established. A few of the parasites were found remaining in the crypts of caeca. In a few of the cases, several petechiae, desquamation of lamina propria were seen in the colon harbored by a few parasites. The histopathological change of the colon was milder than that of caeca.

Experiment 2.

Each of the three male, Indian peacocks, 2 months old, were infected by oral, subcutaneous and percutaneous inoculations with about 300 infective larvae, respectively. The birds suffered from mild occasional diarrhoea from 6 days after inoculation. The diarrhoea continued for about two weeks. The feces contained a small quantity of blood and mucous. The eggs of the parasite were found from the 7th or 8th day. The clinical disorders such as lack of appetite, weakness and emaciation were not noticed for 3 months, and then the birds were sacrificed. In the pathological examination, a small number of parasites were found in the caeca of all the birds. Histologically, a few of the parasites were found penetrating into the crypts of caeca. The infiltration of lymphocytes, histiocytes and eosinophils and slight hyperplasia of lymphoid tissue were seen in the lamina propria of caeca.

A male Indian peacock, 3 months old, was orally administered about 3000 infective larvae per once at regular intervals of a month for 6 months until his death. The bird suffered from severe obstinate diarrhoea, and discharged the feces containing varied amounts of blood and caseiform clots. The bird showed lack of appetite, extreme weakness and emaciation. His feathers got ruffled and lost their gloss. The amount of the feces decreased remarkably for the last two weeks. The feces eliminated in the last week were covered with caseiform pseudomembrane with blood. The fecal examination manifested some considerable number of rhabditiform larvae and a few of filariform larvae besides many eggs. Finally, the bird lost absolutely his appetite, became impossible to stand by himself, and was found dead 120 days after the first inoculation. At autopsy, caeca were filled up with caseiform clots. The surface of mucous membrane of caeca was covered with pseudomembrane. The caecal contents were revealed to be containing many rhabditiform larvae, a small number of filariform larvae and a number of the eggs besides desquamated necrotic tissue. In the histological findings of the liver and spleen, activated reticulo-endothelial cells were seen conspicuously. The lungs manifested remarkable congestion and cellular accumulations consisting of histiocytes in the alveoli and peribronchial area. The caseous deposit in the lumen of caeca was essentially constructed of debris of necrotic tissue and fibrinous inflammatory exudate. In the deposit, many eggs, larvae and adults of *Strongyloides pavonis* were recognized. The desquamation of epithelial cells and necrotic mucous membrane and remarkable cellular infiltration in lamina propria were seen. The infiltrating cells were mainly composed of eosinophils, lymphocytes, plasma cells and histiocytes. Many adult females were recognized penetrating into the crypts of caeca. Mild cellular infiltration and epithelial desquamation were recognized in colon also.

Experiment 3.

Japanese quails, domestic pigeons, domestic ducks, Japanese tree-sparrows and silver pheasant were orally inoculated with about 3000 infective larvae. After inoculation, the fecal examination for the eggs of parasite was performed. The eggs were recognized only from the silver pheasant of the inoculated birds. In the autopsy of the inoculated birds, considerable number of adult parasites were found in caeca of the silver pheasants. Histologically, the parasites were seen penetrating into the crypts of caeca. The infiltration of histiocytes, lymphocytes and eosinophils in

lamina propria and slight hyperplasia of lymphoid tissue were recognized in the caeca.

Discussion

Cram¹⁾ conjectured that the avian *Strongyloides* might be infected by means of oral and cutaneous infection. She²⁾ described a case infected by subcutaneous injection of infective larvae in a report concerning *Strongyloides avium*. In the present experiments, the author was successful in making all the cases in which the birds were infected by subcutaneous injection and percutaneous penetration of infective larvae of *Strongyloides pavonis*. There is no report with respect to the visceral migration of avian *Strongyloides* larvae, as far as the author is aware. The author recognized that the infective larvae were distributed in all visceral organs, cranial and nasal cavities and paranasal sinuses, at the first stage, gathered in lung one day after inoculation, and settled in caeca and colon through digestive trunk.

Concerning the symptom of avian strongyloidiasis, Cram^{1,3)} stated that no clinical symptom was recognized in a case affected with a small number of the parasites, but severely infected chickens discharged thin watery feces mingled with blood in the first stage, the volume of their feces decreased remarkably in the final stage, and some of the birds lacked the elimination of feces. Noto-Soerio⁶⁾ and Freitas and Almeida⁴⁾ stated that *Strongyloides* were found from the birds having had an attack of diarrhoea. Travassos and Dudont¹⁰⁾ who described coryza and anemia as symptom caused by *S. oswaldoi*. Sakamoto et al.^{7,8)} observed that a natural case in which *S. pavonis* was harbored suffered from obstinate diarrhoea and lack of appetite, and was weakened and emaciated. In the present experiment, baby chickens showed weakness and panting breath on the 2nd day after inoculation. A part of severely affected chickens died. After then, the survived chickens recovered their usual breath, but they suffered alternately from diarrhoea. About 8 days after infection, the chickens discharged feces mingled with mucous, blood and pseudomembrane, and some of the severely affected birds died at this stage. The survived bird recovered their usual health.

As regards pathological findings of avian strongyloidiasis, Cram¹⁾ described of thickening of caecal wall and filling of caecal lumen with desquamated debris of degenerated tissue, mucous exudation and blood. She³⁾ stated that the diffuse infiltration of pseudophilic leucocytes in the mucous membrane, and the pseudotubercles containing the parasites, which are surrounded with giant cells, were recognized in the submucosa, muscle layer and subserosa of caeca of a chicken 7 days after infection. Freitas and Almeida⁴⁾ reported the same findings. In the present experiments, many pathological changes as mentioned above were observed especially in caeca and lung. However, no significant difference of pathological findings was recognized among the baby chickens infected by oral, subcutaneous and percutaneous inoculations. In the present observation, considerable number of rhabditiform and filariform larvae were found in the contents and mucous membrane of caeca of a peacock which was inoculated repeatedly at regular intervals of a month. This finding is thought to be due to the following two reasons as follows: autoinfection by larvae originated from feces which adhered in his perianal region, and accumulation of abortive larvae repeatedly inoculated. At the present, however, the author is in possession of no explanatory data for the solution of this problem.

Summary

To observe the pathogenicity of *Strongyloides pavonis* Sakamoto et Yamashita, 1970, 5 to 7

days baby chickens of three groups were inoculated with about 3000 infective larvae by the oral administration, subcutaneous injection and percutaneous penetration, respectively. No essential difference in pathological findings was recognized among the chickens inoculated by the three means, although there was significant difference in the migrating course and development of the parasite among the chickens of the three groups. At the initial stage of infection, many larvae were found in blood vessel and alveoli of lung. The lung showed the findings of catarrhal pneumonia. Some of the larvae in lung were surrounded with histiocytes and various leukocytes. Those cellular accumulations developed sometimes into granulation tissue. On and after the next day of inoculation, the larvae began to appear in the caeca. The larvae penetrated into the layer of epithelial cells, lamina propria, crypts and sometimes, submucosa and muscular layer. After that, degeneration, collapse and desquamation of epithelial cells, villi and mucous membrane, remarkable hyperplasia of lymphoid tissue and fibrous thickening of submucosa and muscular layer were recognized in caeca. The caeca revealed severe diphtherial typhlitis. In the 12th day cases, the caeca were remarkably swollen by thickening of the wall and dilation of the lumen stuffed with caseous clot containing fibrinous exudation, necrotic tissue, the parasites and eggs. In the severely affected cases on the 14th day, the submucosa was exposed by desquamation of necrotic mucous membrane. On and after the 20th day, the mucous membrane began to be reformed, although a few parasites were harbored in the crypts, lamina propria and muscular layer. Some cellular nodules in which a few parasites were contained sometimes, were formed by the granulation tissue repairing necrotic injured tissue in the lamina propria and submucosa.

Each of the three Indian peacocks, 2 months old, were infected by oral, subcutaneous and percutaneous inoculation with about 300 infective larvae, respectively. Those birds suffered from mild occasional diarrhoea from 6 days after inoculation. In the pathological examination, a small number of the parasites were found penetrating into the crypts of caeca of all the birds. An Indian peacock, 3 months old, was orally inoculated with about 3000 infective larvae per once a month for 6 months. The bird suffered from severe and obstinate diarrhoea caused by diphtherial typhlitis, and died 6 months after the first inoculation.

Japanese quails, domestic pigeon, domestic ducks, Japanese tree-sparrows and silver pheasant was orally inoculated with about 3000 infective larvae. Of the inoculated birds, only silver pheasant was infected with considerable number of the parasites. The pathological findings of the silver phaesants were similar to those of the inoculated chickens.

Acknowledgements

The author's sincere thanks are due to Prof. I. Kono of this laboratory for his kind advice and active interest in this problem.

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Explanation of plates

The figures except figs. 7~10 are microphotographs of specimens stained with hematoxylin-eosin. Figures 1~6 are specimens of mildly infected cases, and others are those of heavily infected cases.

Plate I

- Fig. 1 Caeca of chicken 2 days after oral infection. $\times 170$
 Fig. 2 Caeca of chicken 2 days after oral infection. $\times 350$
 Fig. 3 Caeca of chicken 4 days after oral infection. $\times 350$
 Fig. 4 Caeca of chicken 8 days after oral infection. $\times 67$
 Fig. 5 & 6 Caeca of chicken 22 days after oral infection. $\times 170$

Plate II

- Fig. 7 Hyperplasia of lymphoid tissue in the thickened caecal wall of chicken 4 days after oral infection.
 Fig. 8 Feces surrounded with the caseous pseudomembrane eliminated from peacock.
 Fig. 9 Caeca of infected peacock. The lumen of caeca are stuffed with inflammatory exudate.
 Fig. 10 The cross-sections of the caeca.
 Fig. 11 Cellular accumulation of liver of chicken 10 days after subcutaneous injection. $\times 170$
 Fig. 12 Hemorrhage in peribronchiolar tissue in lung of chicken 2 days after subcutaneous injection. $\times 170$

Plate III

- Fig. 13 Migrating larva surrounded with infiltrated cells in lung of chicken 2 days after subcutaneous injection. $\times 170$
 Fig. 14 Proliferating granulation tissue in lung 12 days after subcutaneous injection. $\times 170$
 Fig. 15 The parasites invading into the thickening lamina propria of caeca of chicken 10 days after subcutaneous injection. $\times 68$
 Fig. 16 Necrosis of mucous membrane in caeca of chicken 12 days after subcutaneous injection. $\times 68$
 Fig. 17 Desquamation of mucous membrane from caecal wall of chicken 14 days after oral infection. $\times 68$
 Fig. 18 Tubercle in caecal wall of chicken 21 days after subcutaneous injection. $\times 68$





