Experimental Toxoplasma Infection of Pigs with Oocysts of *Isopora bigemina* of Feline Origin

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Abstract. Twelve pigs were divided into 2 groups: the 1st group of seven and the 2nd group of five. Each of them was given orally with 1.7×10^6 sporulated *Toxoplasma* oocysts, which had been isolated from the feces of an unwanted cat and identified as those of *Isopora bigemina*. All the pigs were examined for body temperature and symptoms every day. The 2nd group was fed a ration containing 500 ppm sulfamonomethoxine (6-methoxy-4-pyrimidinyl sulfanilamide monohydrate) and 25 ppm pyrimethamine (2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine) for 7 consecutive days before the inoculation of oocysts and the following 58 days. All the pigs of the untreated 1st group showed an increase in body temperature, loss of vigor, anorexia, ocular discharge, coughing, tachypnea, diarrhea and cyanosis. All of them except one which was killed for pathological examination on the 7th day after inoculation died some time between the 9th and 15th day after inoculation. Histological examination of the 1st group revealed necrotic foci in some visceral organs, including lymph nodes and intestine, and encephalitis and inflammatory edema in both lungs. Such lesions as these were seen in no pigs of the 2nd group. *Toxoplasma* was detected from the pigs of the 1st group, but not from these of the 2nd group, and identified by Giemsa's staining and the mouse inoculation test.

Recently Hutchison et al. [4] and Frenkel et al. [3] reported the oocyst form of *Toxoplasma* found in the feces of cats infected with this organism. The morphology of this oocyst coincided approximately with that of *Isopora bigemina*, a species of *Coccidium* parasitic to cats [1, 2].

The authors supposed that *Toxoplasma* oocyst discharged in the feces of cats might play a role in the occurrence of toxoplasmosis in pigs. They gave the Beverley oocysts to pigs orally. In these pigs infection took

place without causing symptoms [5]. In this experiment, however, the administration of *Toxoplasma* oocysts was not regarded as an important cause of toxoplasmosis in pigs.

After that, the authors found that oocysts isolated from unwanted cats and identified those of *I. bigemina* had been different from those of the Beverley strain in pathogenicity for several species of experimental animals. Then, oocysts of *I. bigemina* were inoculated orally to experimental pigs, which were examined for clinical symptoms and patho-

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logical changes.

Materials and Methods

Twelve pigs (2 Landraces and 10 Large Yorkshires) born from 3 mothers and weighing from 20 to 30 kg were used. Hemagglutinating (HA) antibody titer against *Toxoplasma* was under 1:64 in them, which present no abnormalities clinically.

Oocysts of the 0-1 strain which had been isolated from an unwanted cat and identified as those of *I. bigemina* were used for inoculation [6, 7, 11]. They were given to a cat weighing under 500 g in order to obtain a large number of oocysts. Then newly discharged oocysts showed sporulation when kept in a 2% potassium dichromate solution at 25°C for 3 days. Each pig was injected with 1.7×10^8 sporulated oocysts into the innermost part of the oral cavity by the aid of a 1 ml syringe.

The 12 pigs were divided into 2 groups: the 1st group of seven and the 2nd group of five. They were examined for body temperature and symptoms every day after the inoculation of oocysts. For the 2nd group, 500 ppm sulfamonomethoxine (6-methoxy-4-pyrimidinylsulfanilamide-monohydrate) and 25 ppm pyrimethamine (2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine) were added to the diet and given continually over a period from 7 days before the inoculation of oocysts to 58 days after that.

Pigs which had succumbed during the experimental period were submitted for autopsy without delay. Those which had survived after the experimental period were sacrificed 58 days after infection and examined for the presence of lesions and *Toxoplasma*. The existence of *Toxoplasma* was demonstrated by observing Giemsa-stained smears of liver, lung, lymph node, brain, gluteal muscle, diaphragm, heart muscle, and intestine (the lower part of the small intestine and the colon), or by injecting mice with the emulsion of these organs.

When an organ was found macroscopically to have pathological changes, it was stained with Giemsa's stain at first. When an organ was proved to be positive for *Toxoplasma* by microscopic observation, the mouse inoculation test of the organ was omitted.

When an organ was negative for *Toxoplasma* by the staining method, approximately 5 g of its tissue were homogenized by a Homoblender. Then 20 ml of homogenate emulsion was made in physiological saline containing 3 antibiotics (10,000 IU of penicillin G, 100 mg of streptomycin, and 100 mg of kanamycin per 1000 ml). Four mice were injected intraperitoneally with 1 ml of the emulsion and ob-

served for 4 weeks thereafter.

HA antibody titer was measured in mice from which *Toxoplasma* had been isolated. After macroscopic examination all the organs were fixed in 10 per cent formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for pathological examination.

Results

1. Clinical observation

The body temperature and clinical symptoms of the infected pigs examined with the lapse of time are charted in Figs. 1 and 2 for the 1st and 2nd groups, respectively.

All the pigs of the 1st group manifested an increase in body temperature, loss of vitality and appetite, discharge from the eyes, coughing, acceleration of the respiration, and incoordination of the hind quarters 4 days after the inoculation of oocysts and later. Their feces contained mucus or were watery. On the 7th day of infection and later, these pigs had cyanosis at the apices of the ears and noses, and in the lower part of abdomen. Some of them found it difficult to stand up. One of them was killed for pathological examination on the 7th day. The remaining six pigs died some time between 9 and 15 days after the inoculation of oocysts.

All the pigs of the 2nd group presented a temporary rise in body temperature 3 days after the inoculation. Thereafter they showed no other symptoms than occasional coughing and discharge from eyes. They grew normally after that.

2. Macroscopical findings

Various pathological changes were found only in the infected pigs of the 1st group. No remarkable changes were noticed in any pig of the 2nd group.

Cyanosis: This pathological change was seen in all the 7 pigs of the 1st group. Reddish purple spots were present at the apices of the ears and nose, and in the lower jaw, lower part of the abdomen, and 4 limbs.

Fig. 1. Body temperature and clinical symptoms of each pig of the 1st group

Intraperitoneal and intrapleural fluids: Yellowish red intraperitoneal and intrapleural fluids increased in quantity in all the pigs of the 1st group.

Liver: The liver swelled slightly. Yellowish or light gray spots of pinhead size were densely distributed in the lobes in all the pigs of the 1st group.

Spleen: In pig No. 35 killed 7 days after the inoculation of oocysts the follicles were obscure, though on other changes were observed. In 6 dead pigs the spleen was enlarged moderately and turned reddish brown or dark red in color. Its cut surface

Fig. 2. Body temperature and clinical symptoms in each pig of the 2nd group

was soft and protruded like tar. The follicles were obscure.

Lung: Severe pulmonary edema was found in 4 pigs of the 1st group. A large quantity of yellowish red exudate was deposited under the pleura and in the interlobular tissue. In the remaining 3 pigs of the 1st group, the cut surface of the lung was moist and a small quantity of exudate with bubbles leaked when the surface was pressed. Three severe cases had pneumonic foci occupying more than half of both lungs. Two of them were affected with pleuritis simultaneously.

Lymph node: The lymph nodes were swollen and enlarged. The mesenteric lymph node enlarged most conspicuously and its cut surface was as long as 7 cm in diameter. Marked enlargement was also observed in the hepatic hilus, submandibular, and inguinal lymph nodes. In these lymph nodes the cut surface was wet showed diffuse hemorrhage and yellowish white focal necrosis.

Digestive organs: Pathological changes were found in the lower part of the small

intestine and in the large intestine in all the pigs of the 1st group. The pseudomembrane formation was noticed in the lower one-fourth or one-fifth part of the small intestine. At the upper end of this part only yellow crust-like pseudomembranes of rice grain or little-finger tip size were scattered. Pseudomembranes became so dense in the lower part of the small intestine that they covered the intestinal mucous membrane completely in the ileocecal region. The mucous membrane covered with pseudomembranes was thickened. Hemorrhage was seen in some parts of it. Hemorrhage, ulceration and nodulation were observed on the mucous membrane of the cecum and colon.

Others: Some pigs of the 1st group exhibited light gray spots of millet or rice grain size in the renal cortex, gray spots of red bean or soybean size under the surrounding membrane and interstitial substance of the pancreas, necrosis of tonsil, and systemic jaundice.

3. Histological findings

The results of histological examination were nearly corresponding to those of macroscopic observation.

Liver: Multiple focal necrosis with or without cellular reaction was observed in the liver lobules. Necrotic foci contained eosinophilic substance without structure and were infiltrated with histiocytes, lymphatic cells, and polykaryocytes. Infiltration of lymphocytes, histiocytes, and plasma cells was found in the interstitial tissue.

Spleen: Hemorrhage in red and white pulp, focal necrosis, a decrease in number of lymphocytes, and reduction in size of follicles were common in all the pigs of the 1st group. In some instances large basophilic cells proliferated.

Lung: Exudates were observed in the alveoli and interstitial tissue. Alveolar epithelial cells dropped out. Infiltration of

cells was seen in the interstitial tissue. Small necrotic foci were found in the alveolar wall in some pigs. Inflammatory edema too mild to be observed macroscopically was found in 3 pigs of the 1st group.

Brain: Such encephalitis as represented mainly by glial nodule formation was noticed. Small necrotic foci without infiltration of glial cells were produced in a pig (No. 35) killed on the 7th day and a pig (No. 19) found dead on the 9th day after the inoculation. Perivascular cell infiltration also occurred to 2 pigs (Nos. 32 and 34) found dead on the 12th and 15th day after the inoculation, respectively.

Lymph node: Small necrotic foci were present in a lymph node which showed slight swelling. Moreover, basophilic cells increased in number in some of them. In lymph nodes enlarged most conspicuously, normal lymphatic cells, cell debris, vitreous degenerated substances, or exudates were not found among scattered reticuloendothelial cells.

Intestine: The wall of the lower part of the small intestine was covered with a pseudomembrane which contained fibrous substances and necrotic cells. The intestinal mucosal villi were affected with necrosis, ulceration, and infiltration of histiocytes, lymphocytes, and plasma cells. In the submucosal tissue, edema and hyperplasia of connective and lymphatic tissues were seen. Necrosis, ulceration and hemorrhage were also observed in the upper part of the small intestine and large intestine.

Adrenal gland: Small necrotic foci with infiltration of lymphocytes and histiocytes were scattered in the cortex.

Others: Some pigs showed accumulation of cells in the renal interstitial tissue, necrosis of fatty tissue around the pancreatic parenchyma, necrosis of the tonsil, and accumulation of small round cells in the heart

Table 1. Results of histological examination

Group	1st							2nd				
Pig No.	35	19	21	30	29	34	32	31	33	38	26	27
Necrotic foci in liver	++	+++	+++	+++	+++	+++	+++	—	—	—	—	—
Necrotic foci in spleen	+	++	++	++	+	+	++	—	—	—	—	—
Inflammatory edema in lung*	+	+	+++	+++	++	+++	++	—	—	—	—	—
Encephalitis	+	+	++	++	+	++	+	—	—	—	—	—
Necrosis in visceral lymph nodes	++	+++	+++	+++	+++	++	++	—	—	—	—	—
Necrosis in subcutaneous lymph nodes	+	+++	++	++	++	+++	++	—	—	—	—	—
Necrosis in intestinal mucous membrane	+++	+++	+	++	+++	++	+++	—	—	—	—	—
Necrosis in adrenal glands	+	++	+	++	+++	++	++	—	—	•	•	—
Fat necrosis of pancreas	—	—	+	—	—	++	+	—	—	—	—	—
Cell infiltration in renal interstitial tissue	—	+	+	—	—	++	++	—	—	—	—	—

Remarks.

* : In a region free from SEP-like pneumonia.

• : Not examined.

Table 2. Results of detection of *Toxoplasma gondii*

Group	1st							2nd				
Pig No.	35	19	21	30	29	34	32	31	33	38	36	27
Liver	+	+	+	+	+	+	+	—	—	—	—	—
Lung	+	+	+	+	+	+	+	—	—	—	—	—
Lymph nodes	+	+	+	+	+	+	+	—	—	—	—	—
Blood	⊕	⊕	•	•	•	•	⊕	•	•	•	•	•
Brain	⊕	•	⊕	⊕	⊕	⊕	⊕	—	—	—	—	—
Diaphragm	⊕	⊕	(+)	—	(+)	(+)	—	—	—	—	—	—
Heart muscle	⊕	⊕	⊕	—	⊕	(+)	⊕	—	—	—	—	—
Gluteal muscle	⊕	⊕	—	—	⊕	⊕	⊕	—	—	—	—	—
Intestinal wall	⊕	•	⊕	•	+	+	•	—	—	—	—	—
Feces	•	•	•	•	—	•	•	•	•	•	•	•

Remarks.

+ : Detected directly from smears.

⊕ : Detected by injecting tissue emulsion into mice.

(+) : Rise of HA antibody titer in inoculated mice.

— : Negative.

• : Not examined.

and gluteal muscles. The results of histological examination mentioned above are summarized in Table 1. Pathological changes were found exclusively in the pigs of the 1st group, but not in any pig of the 2nd group.

4. Detection of *Toxoplasma* by smears

Toxoplasma was detected in the pigs of the 1st group, but not in those of the 2nd group. In 5 pigs which died some time between the 9th and 12th days after the inoculation, a large number of *Toxoplasma* organisms were observed in the stained smears of almost all the lymph nodes, liver,

Table 3. Transition of antibody titer

Group	Pig No.	Time in weeks of determination						
		1W	0	1W	2W	4W	6W	8W
1st	35	<64	<64	<64				
	19	<64	<64	256				
	21	<64	<64	1024				
	30	<64	<64	64				
	29	<64	<64	<64				
	34	<64	<64	<64				
	32	<64	<64	<64	<64			
2nd	31	<64	64	64	256	64	<64	<64
	33	<64	64	<64	256	64	<64	<64
	38	<64	<64	64	256	64	64	<62
	26	<64	<64	1024	1024	256	64	<64
	27	<64	<64	1024	1024	64	64	<64

Remarks. Antibody used were BDB-fixed and sensitized erythrocytes (Kaketsuken Co., Ltd.).

and lungs. On the contrary, in a pig killed on the 7th day and one which died on the 15th day, only a small number of *Toxoplasma* organisms were detected in stained smears.

5. Detection of *Toxoplasma* by mouse inoculation

The results of examination of smears stained with Giemsa's stain and those of the mouse inoculation test are presented in Table 2. *Toxoplasma* was isolated from all the organs and tissues examined in 7 pigs of the 1st group, whereas it was not detected at all in 5 pigs of the 2nd group.

6. Transition of HA antibody titer against *Toxoplasma*

Two pigs of the 1st group showed a titer of 1 : 256 and 1 : 1024, respectively one week after the inoculation. As all the pigs of this group died during a period from the 7th to 15th day after the inoculation, no titration could be performed continually in this group. All the 5 pigs of the 2nd group showed a tendency to rise in titer some time in the 1st or 2nd week. Then their titers fell gradually and became lower than 1 : 64

on 8 weeks after the inoculation. Table 3 indicates the transition of HA antibody titer in both groups.

Discussion

The present experiments made it clear first that oocysts isolated from the feces of unwanted cats and identified as those of *I. bigemina* could cause acute toxoplasmosis in pigs [8]. The symptoms, pathological changes, and distribution of *Toxoplasma* in the body of pigs given 1.7×10^6 sporulated oocysts of the 0-1 strain by the oral route were essentially the same as those of pigs found infected with toxoplasmosis on hog farms. In addition, the enteritis observed in the present experiment was severer than that in experiments with the vegetative form of *Toxoplasma* isolated from naturally infected pigs [8, 9]. This difference might have been derived from the difference in the type of *Toxoplasma*.

Inoculation of 8.0×10^5 oocysts of the Beverley strain caused no clinical symptom in pigs [5]. On the basis of the experience 1.7×10^6 oocysts were given to each pig in

the present experiment. Consequently, infected pigs died of typical acute toxoplasmosis. When the results of the present experiment were compared with those of experiments hitherto performed, such high fatality might be due to the fact that the 0-1 strain is highly pathogenic for pigs. It was suggested from the present experiment that even a small number of sporulated oocysts of the 0-1 strain might be able to cause toxoplasmosis.

Taking the results of the present study and the high resistance of *Toxoplasma* oocysts to the external environments into consideration [12], cats carrying oocysts were regarded as important for the spread of toxoplasmosis on a hog farm.

Furthermore, oral administration of 1.7×10^6 sporulated oocysts of the 0-1 strain caused no clinical symptoms or pathological changes in any of the 5 pigs of the 2nd group which had been fed a ration containing 500 ppm of sulfamonomethoxine and 25 ppm of pyrimethamine [9, 10]. *Toxoplasma* was not detected in any of these animals. This result suggests that these drugs may have a prophylactic effect against toxoplasmosis. The minimal effective doses of both drugs for prophylaxis and safety, however should be determined by an experiment of longer term before this method will be used for the prevention of the disease in the field. No side effects of these drugs were observed in the present work.

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