Role of Toxoplasma Oocysts for Spread of Swine Toxoplasmosis

By SHINGO ITO and KIYOSHI TSUNODA

First Research Division, National Institute of Animal Health

Swine toxoplasmosis is one of the most important diseases of pigs in Japan. Toxoplasma is a causal agent of zoonoses infecting not only swine, but also many kinds of mammals, including man. Therefore, routes of infection have been examined in toxoplasmosis of man and animals. Congenital transmission has already been confirmed in man and animals, but no acquired infection has been fully understood as yet. It is well known that animals are infected by eating fresh meat containing Toxoplasma cysts. However, the presence of any other route for toxoplasmosis has not been determined in swine as in some other animals until recently.

More recently, the coccidial stage of Toxoplasma was clarified[1]. When cysts of Toxoplasma were given orally to a cat, oocysts began to be discharged in the feces of the cat 4 or 5 days after cyst inoculation. The oocyst of Toxoplasma was identical with that of the small type of *Isospora bigemina*[2]. Toxoplasma oocysts, as well as other coccidial oocysts, showed a remarkable resistance to various kinds of disinfectants and other chemicals[4]. Moreover, they survived more than 1 year under the usual climatic conditions in Japan. In the authors' unpublished data, they retained their infectivity to mice even after storage at 0–4°C for more than 3 years. Therefore, the oocysts seemed to be among the most important sources of Toxoplasma infection. Then, the authors inoculated Toxoplasma oocysts into piglets to examine the role of oocysts in the spread of toxoplasmosis.

Inoculation experiments of piglets with Toxoplasma oocysts

Oocysts of two strains of Toxoplasma were used in the experiments. One was the O–1 strain which had been isolated in the form of oocyst from the feces of a naturally infected cat (No. 139). The other was the Beverley strain obtained from experimental infection.

1) Inoculation with O–1 strain oocysts[3]

Eight Landrace piglets were inoculated orally with sporulated oocysts of the O–1 strain in the following numbers: 1.7×10^5 for piglet Nos. 1 and 2, 1.7×10^4 for Nos. 3 and 4, 1.7×10^3 for Nos. 5 and 6, and 1.7×10^2 for Nos. 7 and 8. As a result, all the experimental piglets began to fall ill 3 to 7 days after infection. They showed typical clinical symptoms of toxoplasmosis, such as fever (41–42°C), trembling, decrease or loss of appetite, vomiting, diarrhea, discharge of muddy or sometimes bloody feces, nasal discharge, coughing, discharge of sputum and other respiratory symptoms, including dyspnea with rapid respirations of the abdominal type, and cyanosis of the mouth, nose, ears, legs, and abdominal regions. Finally, they lay down on the floor and were not able to stand up. All of them died of toxoplasmosis 9 to 14 days after inoculation. In the postmortem examination, they revealed typical pathological changes of

Table 1. Detection of Toxoplasma from organs of piglets

Organ affected	Piglet No. and Inoculum size													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14*1
	1.7×10^5		1.7×10^4		1.7×10^3		1.7×10^2		Cohabitation		1×10^6		4×10^6 *2	
	O-1 strain										Beverley strain			*3
Liver	##	##	##	##	##	##	##	##	##	##	+	+	+	−
Spleen	##	##	##	##	##	##	##	##	##	##	⊕	−	−	−
Lung	##	##	##	##	##	##	##	##	##	##	−	−	−	−
Kidney	+	+	##	##	##	+	##	##	+	⊕	−	−	+	−
Heart	+	+	⊕	+	+	+	+	+	+	⊕	+	−	−	−
Diaphragm	+	⊕	+	+	+	+	+	+	+	+	−	−	−	−
Intercostal muscle	+	+	+	+	+	+	+	+	⊕	+	−	−	−	−
Gluteal muscle	+	⊕	⊕	⊕	+	⊕	+	+	+	+	−	−	−	−
Pancreas	##	−	−	##	⊕	−	+	−	⊕	−	ND	−	+	−
Brain	+	+	+	+	##	##	##	ND	##	⊕	−	−	+	+
Adrenal gland	##	##	##	##	##	##	##	##	##	##	⊕	+	+	+
Genital organ	CM	+	⊕	##	+	CM	+	⊕	CM	CM	−	−	−	−
Ileum	##	##	##	##	##	##	##	−	##	##	⊕	−	+	⊕
Mesenteric lymph node	##	##	##	##	##	##	##	##	##	##	−	−	−	−
Peritoneal fluid	+	+	⊕	+	+	+	+	+	+	+	NE	NE	NE	NE
Pleural fluid	⊕	+	+	ND	+	+	+	+	+	⊕	NE	NE	NE	NE
Pericardial fluid	ND	+	−	+	⊕	⊕	+	+	+	⊕	NE	NE	NE	NE

##: Toxoplasma was detected by direct microscopic examination.
+: Toxoplasma was detected from dead mice by mouse inoculation method.
⊕: Cysts were detected from the brain of the surviving mouse.
−: Toxoplasma was negative both in direct microscopic and mouse inoculation methods.
ND: Not done.
NE: Not examined because the body fluid did not increase.
CM: Castrated male which could not be examined.
*1: Piglet No.
*2: Inoculum size.
*3: Strain of Toxoplasma inoculated.

toxoplasmosis. Trophozoites of Toxoplasma were detected (##) directly with ease under the microscope in fresh suspensions of some organs. They were also demonstrated from most of the other organs examined by the mouses inoculation method (+ and ⊕) (Table 1).

In addition to eight piglets, two piglets (Nos. 9 and 10), not inoculated with oocysts, were kept together with four infected ones (Nos. 1–4) for only 2 days after the inoculation of the latter, and then transferred to a clean pen. They were raised there under the oocyst-free condition. Both of them showed the same typical clinical symptoms of toxoplasmosis as the eight inoculated piglets, and finally died 13 and 14 days after the initiation of cohabitation, respectively. They revealed the same pathological changes as the eight inoculated piglets. Results of detection of Toxoplasma in the two uninoculated piglet are shown in Table 1.

It is considered that some oocysts inoculated into the four piglets may have been discharged with feces in intact conditions after passing through the alimentary tract and become infective to the two uninoculated piglets.

2) Inoculation with Beverley strain oocysts

Four Landrace piglets were inoculated orally with sporulated oocysts of the Beverley strain in the following numbers: 1×10^6 for piglet Nos. 11 and 12, and 4×10^6 for Nos. 13 and 14. They did not show so severe clinical symptoms as seen in the piglets inoculated with oocysts of the O–1 strain. Fever (40–42°C) was recognized between 3 and 7 days, and a decrease of appetite between 3 and 5 days after infection. When killed on the 29th day (Nos. 11 and 13) or the 36th day (Nos. 12 and 14) of infection, no piglets exhibited typical pathological changes of toxoplasmosis. Saline suspensions of visceral organs of each piglet were inoculated into three mice each. As a result, several mice inoculated with suspensions of some organs manifested typical symptoms and died of toxoplasmosis, indicating clearly that these organs harbored Toxoplasma organisms (Table 1). In the present experiment, however, Toxoplasma-positive results were not so frequently obtained from animals inoculated with the Beverley strain as from those inoculated with the O–1 strain.

There are clinical or burning cases and subclinical or latent ones in the natural infection of swine toxoplasmosis. The former are demonstrated by experimental inoculation with oocysts of the O–1 strain, and the latter with those of the Beverley strain.

Relationship between Toxoplasma oocysts and the natural outbreak of swine toxoplasmosis[6)]

As mentioned above, it was demonstrated experimentally that Toxoplasma oocysts played a very important role in the spread of toxoplasmosis. Therefore, the authors had an intention to ascertain that a natural outbreak of swine toxoplasmosis was caused by Toxoplasma oocyst infection.

Recently, the authors had a chance to examine an outbreak of swine toxoplasmosis which had occurred in a hoggery in Shizuoka Prefecture. In the outbreak, 103 pigs and 7 wild boars fell ill simultaneously, showing such clinical symptoms as fever (41–42°C), loss of appetite, rapid respirations of the abdominal type, and incoordination of the hind quarters. They were diagnosed as toxoplasmosis by the sanitary inspectors of Shizuoka Prefecture. Then, all of them were medicated immediately with 10 mg/kg/day of 2-sulfamoyl-4,4'-diaminodiphenyl-sulfone (SDDS) for 4 or 5 days. As a result, most of them recovered, but ten pigs and three wild boars died, and five pigs were killed because of bad prognosis. In the hoggery, leaf-moulds used to be given regularly to pigs. At that time, however, because of shortage of leaf-moulds, soil-contaminated leaf-moulds in the peripheral part of the heap were gathered and supplied with pig-feed for 3 consecutive days beginning at 1 week before the outbreak of the disease. Moreover, it became evident that many cats inhabited the hoggery and that the peripheral part of the heap was contaminated with cat feces. Therefore, an attempt was made to detect Toxoplasma oocysts directly from that soil under the microscope. Than by any other method. Toxoplasma oocysts were more frequently detected microscopically by the sugar floatation method with materials treated by the ultrasonic cleaner (Bransonic 220; frequency, 50 KHz). Moreover, Toxoplasma was isolated from mice inoculated with a soil suspension containing the oocysts. The isolate has been maintained by mouse passages until now.

An experiment was performed with two piglets to know whether such clinical symptoms as noticed in the natural outbreak were manifested by oral administration with the contaminated soil or not. When these piglets were fed a total of 2.5 kg of the contaminated soil in addition to pig-feed for 4 consecutive days, they began to reveal typical clinical symptoms of toxoplasmosis 6 days after inoculation. One was sacrificed 9 days

after inoculation for postmortem examination and detection of Toxoplasma. It exhibited typical pathological changes. In it, Toxoplasma was demonstrated by the fluorescent antibody technique and the mouse inoculation method. The other piglet recovered 16 days after inoculation and was sacrificed 27 days after inoculation. In it, Toxoplasma was demonstrated in several organs by the mouse inoculation method, although no typical pathological changes were recognized. As a result, it was reconfirmed that Toxoplasma infection was induced by soil contaminated with oocysts.

The relationship between Toxoplasma oocysts and swine toxoplasmosis was demonstrated by a series of studies on both spontaneous and experimental infection. When a cat ate Toxoplasma cysts, it generally discharged feces containing more than 10^6 oocysts per gram (OPG). Even the cysts of the isolate which developed in experimental mice were given to kittens, OPG continued to be 10^6 to 10^7 for 10 days, or 10^7 for 4 to 8 days. Therefore, it can be justified that numerous oocysts will be discharged continually from naturally infected cats for a certain period of time. The rate of Toxoplasma infection in cats was reported to be 40 to 65% or more. Toxoplasma oocysts are highly resistant to various disinfectants and changes in environmental conditions, except heating[5] and drying. Therefore, they are considered to be the most important infective source of swine toxoplasmosis.

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