Demonstration of *Toxoplasma gondii* Antigen in Stillborn Piglets using Immunoperoxidase Technique

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Congenital toxoplasmosis has been recognized in humans, puppies, lambs, mice, cattle and swine, and the exact diagnosis was based on isolation of the organism and its serological examination. Recently several investigators have successfully applied the peroxidase-antiperoxidase method to demonstrate *Toxoplasma gondii* in human [1, 10] and small animal [2] tissue sections, fixed in formalin and embedded in paraffin. The purpose of this communication is to demonstrate *Toxoplasma gondii* antigen in stillborn and live-born piglets using an immunoperoxidase technique.

Formalin-fixed and paraffin-embedded tissue blocks of 4 stillborn and one live-born piglets were obtained from two field outbreaks in Japan (Table 1). The case in Okinawa prefecture was kindly provided by Dr T. Shimabukuro. Sections were stained with hematoxylin and eosin (H-E), and avidin-biotin-complex immunoperoxidase technique (ABCIT) [3, 4]. In ABCIT, lung, brain and liver tissue sections from pigs experimentally infected with *Toxoplasma gondii*

were also used as for positive control. After deparaffinization, endogeneous peroxidase activity of each tissue was blocked with 0.3% H₂O₂ in absolute methanol. The sections were then incubated sequentially with normal goat serum, primary antiserum, biotinylated anti-rabbit IgG goat serum and ABC reagent. The peroxidase was localized with 0.05% 3,3'-diaminobenzidine tetrahydrochloride and 0.1% H₂O₂ in Tris buffer pH 7.6. Then, sections were counterstained with methyl green. The reagent for ABCIT (except for the primary antiserum) was commercially obtained from Vector Laboratories (USA). As a primary antiserum, rabbit hyperimmune serum against *Toxoplasma gondii* 0-1 strain was used at a dilution of 1:2048. The antiserum had indirect hemagglutination assay (IHA) titer of 1:512. As controls, unimmunized rabbit serum and anti-*Hammondia hammondi* serum was used for the first antiserum.

Microscopically, glial nodules and necrotic foci were scattered in the marginal part of the brain in 5 piglets. Slight pneumonia was seen in 4 piglets. In the acini of the liver, small necrotic foci were sometimes observed in 2 piglets. *Toxoplasma* cyst-like structures were seen in the cerebral

Table 1. Materials, and location of histological changes and *Toxoplasms* in each piglet

Case No.	Region (prefecture)	Condition at birth	Examined piglet (piglet number)	Microscopical lesion/ <i>Toxoplasma</i> ^{a)}		
				Brain	Lungs	Liver
1	Chiba	16 stillborn and 1 liveborn	Stillborn (2857)	+/+	+/+	+/-
			Stillborn (2858)	+/+	+/+	+/-
			Stillborn (2859)	+/+	+/+	-/-
			Stillborn (2860)	+/+	+/+	-/-
2	Okinawa	6 stillborn and 4 liveborn	Liveborn ^{b)} (86-73-A)	+/+		-/-

a) Examined by the immunoperoxidase technique.

b) Piglet was necropsied on 2 days after birth.

cortex in 5 piglets. In the lungs, terminal colony-like structures were observed in the epithelial cells of the bronchioles and the alveolar epithelium in 4 out of 5 piglets. On immunoperoxidase examination, *Toxoplasma gondii* antigen was detected as a dark-brown developed image only in antiserum immunized against *Toxoplasma gondii* (Table 1). In the brain, cysts of *Toxoplasma gondii* were clearly stained (Fig. 1) and a small number of tachyzoites were detected in glial nodules. In the lungs, many terminal colonies and tachyzoites were detected in the epithelial cells of the bronchioles, the alveolar epithelium (Fig. 2) and infiltrated macrophages. No antigen was found in the liver.

It is commonly known that the microscopic appearance of *Sarcocystis*, leishmanial forms of *Trypanosoma cruzi*, *Besnoitia besnoitiz*, *Hammondia hammondi*, and sporozoites of *Coccidia* were similar to *Toxoplasma* in animals and must be differentiated from *Toxoplasma* [5]. Although porcine congenital toxoplasmosis has been described previously [6, 7, 8, 9], these results have

Fig. 1. Cerebrum of piglet No. 2860. Encysted form of organism showing clear positive reaction against anti-*Toxoplasma* serum. Stained by Avidin-biotin-complex immunoperoxidase technique (ABCIT). $\times 1000$.

Fig. 2. Lung of piglet No. 2858. Terminal colonies in the alveolar epithelium showing positive reaction against anti-*Toxoplasma* serum. Stained by ABCIT. $\times 1000$.

come only from morphological examinations. In the present study, we could identify *Toxoplasma*-like organisms as *Toxoplasma gondii* by the ABCIT, with reference to some investigators, who detected *Toxoplasma* in human [1, 10] and small animals [2] using immunoperoxidase technique. Therefore, the congenital toxoplasmosis of pig was clearly demonstrated and the efficiency of the immunoperoxidase technique was confirmed on protozoal infection in pigs.

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要 約

死産子豚における *Toxoplasma gondii* 抗原の酵素抗体法による検出(短報): 播谷 亮・志村亀夫・岩渕 功¹⁾・小林 勝・成田 實(農林水産省家畜衛生試験場, ¹⁾千葉県家畜衛生研究所)——先天性トキソプラズマ感染を疑う死産子豚のパラフィン包埋組織切片を用い, 酵素抗体法により *Toxoplasma gondii* 抗原の検出を試みた. その結果, 脳と肺で *Toxoplasma gondii* の抗血清に対して特異反応を示す虫体が確認された.