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Preventive Effect of SDDS against Toxoplasmosis in Swine with Repeated Inoculations of Toxoplasma Cysts

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There is a great possibility that man may aquire toxoplasmosis by consumption or handling of cyst-bearing meat, especially the pork. In this respect, the prevention of pigs from toxoplasmosis would be beneficial to diminish swine toxoplasmosis and to prevent man from the infection through the meat as well. Continuous medication of pigs with an appropriate anti-toxoplasmic agent added to food may be of practical use for this purpose provided that the drug is assured not to accumulate in the hog meat. In the previous paper (Ohshima *et al.*, 1970; Shimizu *et al.*, 1970), it was proved that the continuous administration of SDDS (2-sulfamoyl-4, 4'-diaminodiphenylsulfone) to pigs prevented them from toxoplasmosis due to the parenteral inoculation of *Toxoplasma* trophozoites. However, there is a good reason to believe that pigs may aquire the infection by ingesting more resistant form of the parasite, e. g. the cysts (Verma and Dienst, 1965) in our present knowledge[2], and that the chance of the ingestion exists constantly. The present paper deals with the results of a successful application of SDDS mixed in the food to the pigs for the prevention of toxoplasmosis due to repeated oral inoculation of the cysts.

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[2] Another possibility of acquiring toxoplasmosis may be through ingesting the oocysts excreted by the infected cat, as supposed by the data recently accumulated (Dubey *et al.*, 1970).

Materials and Methods

Pigs and SDDS Administration

Eleven pigs of the Landrace type weighing 18 to 30 kg were employed in this experiment. They were lacking in antibodies against *Toxoplasma* in the serum as determined by the dye test twice, 8 days and 1 day before the initiation of SDDS administration. They were divided into 4 groups of 2 to 3 animals and fed 2% powder of SDDS in soy bean oil cake powder mixed in food twice daily at daily doses of 10, 5, 2.5 and 0 (control) mg/kg according to the result of previous experiment (Ohshima *et al.*, 1970), respectively. The medication was started 7 days before the 1st inoculation and continued until the day before sacrifice. The pigs were weighed every 10 days for readjustment of the dosage.

Inoculation of Toxoplasma *to the Pigs*

The pigs were each orally inoculated with the mouse brain homogenate containing cysts of the Beverley strain 4 times every 5 days, bigining on day 7 of the SDDS medication. Brains of 40 mice which had been chronically infected with the parasite were excised within an hour before inoculation and homogenized in 10 ml of saline with a belender type homogenizer. The homogenate of two brains in average was given to each pig each time in two No. 000 or three No. 00 gelatin

capsules (Eli Lilly & Co.). The number of cysts contained in the inoculum was 21,630 for the 1st ionculation, 12,800 for the 2nd, 12,610 for the 3rd and 26,880 for the 4th.

Clinical Observation

The rectal temperature was taken twice daily before feeding. The pigs were tested for parasitemia every 5 days after the 1st inoculation by injecting 1.5 ml of the blood from each pig intraperitoneally into three mice at 0.5 ml each. The positive dye test titer of the serum and/or presence of Toxoplasma cyst under microscopy in a portion of the brain of the mice 4 weeks later were the criteria for the existence of the parasite in the pig blood.

Serological Examination

The anti-toxoplasmic antibodies in pig serum were estimated by the dye- and hemagglutination (HA) tests on the day before the start of SDDS administration and every 5 days after the 1st inoculation. The method of the dye test employed was modified method by Kobayashi *et al.* (1968). The HA test was performed by the method of Hiraoka and Tsunematsu (1968) using a new diagnostic kit, TOXO-TEST 'Eiken', for toxoplasmosis, in which lyophilized, pylvic aldehyde-fixed, sensitized sheep erythrocytes are employed. The titers in this HA test agreed quantitatively and qualitatively with those obtained in the same sera in the HA test by the method of Lewis and Kessel (Hiraoka, Unpublished data).

Isolation of Toxoplasma *from Organs*

The pigs were bled to death between 41 and 44 days after the 1st inoculation. Isolation of the living parasite was attempted from the brain, diaphragm, lung, liver, hilar lymph nodes, and pooled sample of hepatic, gastric and mesenteric lymph nodes. The technique for the isolation was as follows: a 10 g piece of each organ was cut with scissors and homogenized under chilling in 10 ml of saline with a blender-type homogenizer and filtered through double-layered gauze. The filtrate was centrifuged at 2,000 rpm for 5 minutes and the supernatant was discarded. The sediment was resuspended in 10 ml of saline and 10,000 u. of penicillin and 20 mg of dihydrostreptomycin sulfate were added. The suspension was then injected intraperitoneally into 5 mice at 1 ml each. The inoculated mice were examined for *Toxoplasma* by the same method as that for the detection of the parasitemia. In these experiments, the mice used were all of the dd strain, female, and 6 weeks of age.

Results

Clinical Symptoms

Changes in body temperature of the pigs are shown in Figure 1. The clinical symptoms which could be attributed to the *Toxoplasma* inoculation appeared only in the control group. Pig No. 10 elevated the body temperature to 41.7°C on the 4th day and No. 11 to 41.5°C on the 5th day, the fever continuing for 4 and 5 days, respectively. Concomittantly, the loss of appetite, lassitude, coughing, constipation and occasional vomiting were observed in these non-treated pigs. The pig No. 10 excreted brownish discharge from the eyes as frequently seen in naturally *Toxoplasma*-infected pigs. Even after subsidence of the fever and some other symptoms, these non-treated pigs have had coughing for another 10–14 days. In other 9 pigs which were given SDDS those symptoms were scarcely noted. Although, a slight rise in body temperature and coughing were sometimes observed in the medicated pigs (Nos. 1, 2, 4, and 7), they had good appetite. The gross findings in the post-mortem examination revealed that these symptoms observed in the medicated groups should be attributed to swine enzootic pneumonia. Continuous coughing without fever observed in pig No. 3 was considered to be due to the infection with lung worms from the results of post-mortem examination.

Changes in body weight of the pigs are shown in Table 1. Growth of the medicated pigs were almost normal, but in the non-treated control group growth retardation was observed within 10 days after the 1st inoculation, during which time the increase in body

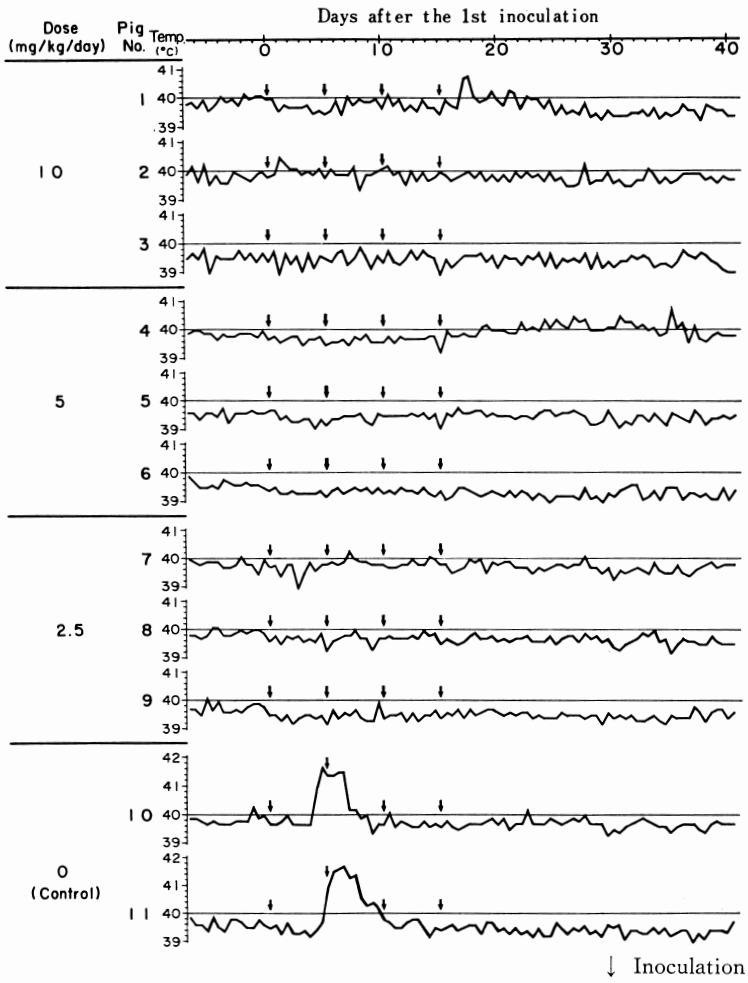

Figure 1 Graph showing body-temperature fluctuations in *Toxoplasma*-inoculated pigs with or without SDDS medication.

weight of the control group was 17.2 % in No. 10 and 7.5 % in No. 11, while that of the medicated pigs was 24.4 % on an average, ranging from 21.1 % to 29.8 %. The retardation of the growth in the control pigs was not restored until the day of sacrifice.

Parasitemia occurred only in the control pigs. Pig No. 10 showed the parasitemia on the 5th and 10th days after the 1st inoculation and No. 11 on the 5th day, both concurrently with fever.

Antibody Titers in the Serum

Marked rises of dye- and HA- test titers suggesting developing acute toxoplasmosis were found only in the control group. As shown in Table 2, the dye test titers of control pigs rose to 1 : 256 on the 10th day and to 1 : 1,024 on the 15th day. In No. 11 the titer further rose to 1 : 4,096 thereafter. On the other hand, the pigs medicated with SDDS remained to produce very low dye test titers below 1 ; 64, mostly 1 : 16 or 1 ; 4. The final titers were 1 ; 64 in one pig, 1 : 16 in two and 1 ; 4 in six pigs respectively.

The change in pattern of the HA titers was almost similar to that of the dye test titers as presented in Table 3. In the control group, the HA titers in No. 11 rose side by side with the dye test titer, but in No. 10 the rise remained at 1 : 512 level. In the

Table 1 Effect of SDDS on the growth of *Toxoplasma*-inoculated pigs

Dose of SDDS (mg/kg/day)	Pig No.	Sex*	Body weight (% of body weight at day 0)				
			Days after the 1st inoculation				
			−10	0	10	20	40
10	1	F	22.5kg (78.9)	28.5	34.5 (121.1)	40.5 (142.1)	50.5 (177.2)
	2	M	18.0 (78.3)	23.0	28.0 (121.7)	35.0 (152.2)	46.0 (200.0)
	3	F	24.0 (84.2)	28.5	37.0 (129.8)	43.0 (150.9)	53.0 (186.0)
5	4	F	20.5 (85.4)	24.0	29.5 (122.9)	36.0 (150.0)	49.0 (204.2)
	5	M	20.5 (82.0)	25.0	31.5 (126.0)	38.0 (152.0)	49.5 (198.0)
	6	M	25.0 (84.7)	29.5	36.5 (123.7)	43.5 (147.5)	55.0 (186.4)
2.5	7	F	22.5 (86.5)	26.0	31.5 (121.2)	36.0 (138.5)	48.0 (184.6)
	8	M	23.5 (82.5)	28.5	37.0 (129.8)	45.0 (157.9)	56.0 (196.5)
	9	M	30.0 (84.5)	35.5	44.5 (125.4)	49.5 (139.4)	63.5 (178.9)
0 (Control)	10	F	23.0 (79.3)	29.0	34.0 (117.2)	38.5 (132.8)	50.0 (172.4)
	11	M	27.5 (79.1)	33.5	36.0 (107.5)	42.0 (125.4)	56.0 (167.2)

* F : Female, M : Male.

Table 2 Effect of SDDS on the dye test titers of *Toxoplasma*-inoculated pigs

Dose of SDDS (mg/kg/day)	Pig No.	Days after the 1st inoculation								
		−8	5	10	15	20	25	30	35	40
10	1	N.*	1 : 4	1 : 16	1 : 64	1 : 16	1 : 16	1 : 16	1 : 16	1 : 16
	2	N.	1 : 4	1 : 16	1 : 16	1 : 16	1 : 4	1 : 4	1 : 4	1 : 4
	3	N.	N.	N.	1 : 16	1 : 4	1 : 4	1 : 4	1 : 4	1 : 4
5	4	N.	N.	N.	1 : 4	1 : 4	1 : 4	1 : 16	1 : 16	1 : 4
	5	N.	N.	1 : 16	1 : 64	1 : 16	1 : 4	1 : 4	1 : 16	1 : 4
	6	N.	N.	N.	1 : 4	1 : 4	1 : 4	1 : 16	1 : 16	1 : 4
2.5	7	N.	N.	1 : 16	1 : 16	1 : 16	1 : 16	1 : 16	1 : 16	1 : 16
	8	N.	N.	1 : 64	1 : 64	1 : 64	1 : 64	1 : 64	1 : 64	1 : 64
	9	N.	N.	1 : 4	1 : 4	1 : 4	1 : 4	1 : 4	1 : 4	1 : 4
0 (Control)	10	N.	1 : 4	1 : 256	1 : 1,024	1 : 1,024	1 : 1,024	1 : 1,024	1 : 1,024	1 : 1,024
	11	N.	N.	1 : 156	1 : 1,024	1 : 4,096	1 : 4,096	1 : 1,024	1 : 4,096	1 : 256

* N. : Negative (<1 : 4)

medicated pigs the HA titers were all 1 : 32 or lower except on some days in Nos. 1 and 8.

Isolation of Toxoplasma *from the Organs*

Results of the attempt to isolate *Toxoplasma* from organs of the pigs are shown in Table 4. The parasite could not be isolated from the organs of all the medicated pigs. *Toxoplasma* was isolated from the diaphragm and lung of the non-treated pigs, and additionally from the liver of No. 10 and the brain and hilar lymph nodes of No. 11.

Discussion

It was obvious that the continuous medication with SDDS mixed in daily food at a small dosage of 2.5 mg/kg/day could prevent

Table 3 Effect of SDDS on the HA titers of *Toxoplasma*-inoculated pigs

Dose of SDDS (mg/kg/day)	Pig No.	Days after the 1st inoculation								
		−8	5	10	15	20	25	30	35	40
10	1	1 : 32	N.*	1 : 32	1 : 64	1 : 64	1 : 32	1 : 32	1 : 256	1 : 64
	2	N.	1 : 32	1 : 32	N.	N.	N.	1 : 128	1 : 32	1 : 32
	3	N.	N.	N.	N.	1 : 32	N.	1 : 32	N.	N.
5	4	N.	N.	N.	N.	N.	N.	N.	N.	N.
	5	N.	N.	N.	N.	N.	N.	N.	N.	N.
	6	N.	N.	N.	N.	N.	N.	N.	N.	N.
2.5	7	N.	N.	N.	N.	N.	N.	N.	N.	N.
	8	N.	N.	1 : 128	1 : 128	1 : 32	1 : 32	N.	1 : 32	1 : 64
	9	N.	N.	N.	N.	N.	N.	N.	1 : 32	1 : 32
0 (Control)	10	N.	N.	1 : 512	1 : 256	1 : 128	1 : 128	1 : 512	1 : 512	1 : 128
	11	N.	N.	1 : 4,096	1 : 4,096	1 : 8,192	1 : 2,048	1 : 2,048	1 : 1,024	1 : 128

* N. : Negative (<1 : 32)

Table 4 Isolation of *Toxoplasma* from the organs of the control pigs*

Pig No.	Organs examined for *Toxoplasma*					
	Brain	Diaphragm	Lung	Liver	Hilar lymph nodes	Mixture of hepatic, gastric and mesenteric lymph nodes
10	− (0 ; 0/5)**	+ (0 ; 1/5)	+ (0 ; 1/5)	+ (1 ; 5/5)	− (0 ; 0/5)	− (0 ; 0/5)
11	+ (5 ; 5/5)	+ (5 ; 5/5)	+ (1 ; 4/5)	− (0 ; 0/5)	+ (0 ; 2/5)	− (0 ; 0/5)

* Data for the pigs which had been given SDDS were omitted because no *Toxoplasma* was isolated from the organs examined.

** The first number in parenthesis, preceeding the semicolon, indicates the number of cyst-positive mice. The figure following the semicolon is the number of dye-test positive mice/number of inoculated.

pigs from toxoplasmosis which otherwise would have developed by repeated oral inoculation of the cysts. Furthermore, from the fact that the medicated pigs exhibited no parasitemia and only low titers of the dye- and HA- tests throughout the experiment coupled with the fact that they harboured no *Toxoplasma* in the organs after sacrifice, it may be assumed that the SDDS medication suppressed *Toxoplasma* infection in the pigs or, at least, killed completely the parasite which invaded into the tissues before their multiplication. In this connection, the level of the antibody titers observed in the medicated pigs was about what might be expected after inoculation of the killed parasite (Kobayashi and Jacobs, 1963; Huldt, 1966; Foster and McCulloch, 1968), and does not indicate the established infection by division or multiplication of the parasite.

The clinical symptoms and parasitemia observed in the non-treated control pigs appeared after the 1st inoculation but not after the subsequent inoculations. It is thought that the protective antibody against the parasite may have been produced to some extent following the 1st inoculation as supposed from the rising titers in the dye-

and HA-tests and acted to prevent the pigs from the developement of toxoplasmosis by the subsequent challenges.

As shwon in the present experiment, if it is possible to prevent pigs from *Toxoplasma* infection and toxoplasmosis by the continuous administration of an anti-toxoplasmic agent mixed in food, such method would be promising for prophylactic use against the disease in pigs which are usually killed for meat in as short life as several months of age. For this purpose, the drug should not accumulate in pig tissues. The concentration of SDDS in the serum of pigs which were medicated with SDDS at a dose of 5 mg/kg/day for more than 20 days varied between 0.5 to 5 μg/ml during medication and remained in a trace amount 4 hours after the cessation of medication (Inami *et al.*, unpublished data).

It is unlikely that SDDS had only a static effect in pigs against *Toxoplasma* and the parasites which were alive in the pig tissues were only prevented from growing in the mice by small amount of SDDS carried over on inoculation of the tissue homogenates into the mice, though such possibility cannot be completely excluded.

There is a good reason to believe that amounts of the drug delivered to mice together with the tissue homogenate under study would have been too small to prevent the parasite from their growth, because the concentration of the drug in the homogenate should have been lowered to far less level than the minimum effective dose in the mouse after the washing procedure (Ohshima *et al.*, 1967).

In this experiment, the pattern of change in the HA test titer of the pig sera was almost similar to the pattern of change in the dye test titer. The new diagnostic kit for the HA test is thus applicable as an easier method for the diagnosis of *Toxoplasma* infection than the dye test which requires the accessory factor and live *Toxoplasma* parasites, both of which are often difficult to obtain.

Summary

Assuming that one of the main cause of *Toxoplasma* infection in pig is repeated ingestion of cysts of the parasite, 11 pigs lacking antibodies in the serum against *Toxoplasma* as determined by the dye test, were orally inoculated 4 times repeatedly every 5 days with the mouse brain homogenate containing many cysts of the Beverley strain of the parasite. Nine of them were continuously medicated with SDDS mixed in daily food each at doses of 2.5, 5 and 10 mg/kg/day from the 7 days before the 1st inoculation and its preventive effect was examined against *Toxoplasma* infection or toxoplasmosis.

The results in the medicated pigs show that clinical symptoms, parasitemia and the parasite in the organs after sacrifice were all absent and mostly the dye test titer rose only up to 1 : 16 or lower while in the control pigs the dye test titer rose to 1 : 1,024～1 : 4,096, fever and other clinical symptoms appeared, and parasitemia and the isolation of the parasite from the organs after sacrifice were both positive. It is concluded that the continuous medication of SDDS in food at a dose of 2.5 mg/kg/day could almost completely prevent pigs from toxoplasmosis by repeated exposure of the cysts.

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トキソプラズマ・シスト反復経口接種豚に対する SDDS の感染予防効果

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豚におけるトキソプラズマの自然感染経路の一つは，そのシストの反復経口摂取によるとの想定の下に，実験的に11頭の色素試験陰性子豚に Beverley 株シストを含むマウスの脳乳剤を5日毎に4回経口接種し，そのうち9頭については，第1回接種7日前から，飼料中に SDDS を，それぞれ2.5，5，10 mg/kg/day ずつ添加して連日投与し，薬剤投与によるトキソプラズマ感染・発症防止効果を検討した．その結果は，薬剤非投与対照群2頭については，色素試験抗体価は1：1,024～1：4,096に上昇し，発熱，虫血症など発症を認め，かつ臓器内生残原虫の存在を認めたのに反し，薬剤投与豚においては，全く症状の発現なく，虫血症および臓器内生残原虫の探索もすべて陰性であり，かつまた色素試験抗体価も殆ど1：16以下をしめした．これらのことから，SDDS の連続投与（2.5 mg/kg/day）は，トキソプラズマ・シストの反復経口接種による感染あるいは発症を，ほぼ完全に防止し得るものと結論された．

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