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TOXOPLASMOSIS IN DOMESTIC ANIMALS:
The Serological Response of Swine to Toxoplasma Infection

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ABSTRACT

Swine toxoplasmosis was successfully reproduced by oral, subcutaneous and intratracheal routes of inoculation as well as through contact. The serological data of natural and experimental cases of swine toxoplasmosis were presented and discussed.

Since the report of a dye (D) test for toxoplasmosis by Sabin and Feldman (1) in 1948, the test has become a very useful tool in the experimental diagnosis of this malady. Unfortunately, the D test is not satisfactory either practically or technically (2). Information gained from D test and complement-fixation-inhibition (CFI) test (3) is significant only when a rising titer can be demonstrated. Feldman (4) showed the prevalence of antibodies in apparently normal swine, thus a single test is of limited value.

The lack of a simple and reliable diagnostic tool for toxoplasmosis and the high incidence of asymptomatic carrier makes this malady a difficult problem. A hemagglutination (HA) test was developed by Jacobs *et al.* (2, 5), and they believed that the test was reliable in the diagnosis of toxoplasmosis. However, this test has not been accepted as widely as the D test of Sabin-Feldman.

The serological response of *Toxoplasma* infected swine will be analysed in this report. The conventional D test, HAA (HA test with absorbed serum) and HAN (HA test with non-absorbed serum) tests have been used for study.

MATERIALS AND METHODS

Pigs. Eleven healthy pigs without any previous history of illness, weighing about 20 kg each, were used in this experiment. Test serums were collected from experinemtally infected pigs as well as naturally diseased pigs from the Provincial Livestock Research Institute where a serious outbreak of abortion and still-birth syndrome occurred due to a toxoplasma infection (6). Eighty-six pigs born at the time of outbreak were bled for serological testing 7 months after the peak of the outbreak.

Toxoplasma isolation. Pigs were killed for *Toxoplasma* isolation 5 months after inoculation. The left brain hemisphere was emulsified with a homogenizer to make a final 20% suspension in saline. The suspension was filtered through a single layer of fine cheese cloth and then centrifuged at 2,000 rpm for 10 minutes. The sediment was resuspended in 20 ml saline, and 1 ml aliquot of which was inoculated intraperitoneally into each of 20 mice (NIH general purpose) weighing 15-18 g. Mice were checked for parasites in ascites by paracentesis at 5 day-intervals for 3 weeks. Negative mice were killed after 2 months of observation, and their brains were similary treated and again inoculated intraperitoneally into 20 mice. This procedure was repeated and negative conclusion was based on the result of the third passage.

Dye test. The conventional D test (7) was used in all experiments, except that the accessory factor used was citrated human blood plasma. All serum samples under test were inactivated at 56 C for 30 minutes. Antibody titers at or above 1:16 were considered diagnostically significant. An isolate of *Toxoplasma* obtained in a previous study (6) was adopted as antigen for all tests.

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Hemagglutination test. The hemagglutination technique of Jacobs and Lunde (2) was used in the present study with slight modification. Goat cells were used instead of sheep. The peritoneal exudate of mice containing toxoplasma organisms was diluted with an equal volume of phosphate buffered saline (M/15), pH 7.2, and passed through a glass wool filter (about 2g of glass wool packed in a 10 ml syringe) before centrifugation in paraffin-coated tubes. By this procedure, the leucocyte content of the exudate was greatly reduced without adverse effects on the recovering of toxoplasma organisms. The final antigen solution represents the water-soluble lysate in 0.9% saline with a microbial count of 75,000 organisms per mm^3, estimated by counting in a Max-Levy counting chamber before lysis. Aliquots of antigen were kept at -23 C until use. The final concentration of the sensitized rbc in the reaction mixture was 0.25%.

The test serum and the normal rabbit serum diluent were inactivated at 56 C for 30 minutes, and were absorbed overnight with equal volume of packed goat rbc to remove heterophil antibodies (HAA). Parallel series of HA tests performed with non-absorbed test serums (HAN) was run for comparison, and the data on HAN were based on the result of duplicate experiments. Both positive and negative serums were included as controls. The last tube showing a definite agglutination pattern was taken as the end-point to avoid ambiguity in reading; doubtful reactions were considered negative.

RESULT

The antibody response of experimental pigs

Experiment 1. Four pigs were starved for 1 day, and each was fed with 1 infected mouse which had been inoculated intraperitoneally with parasites 3 days previously. Pre-infection blood samples were taken for control.

Fever started generally at 4 to 5 days after ingestion of material, and persisted for 5 to 6 days, ranging from 39.9 C to 41.7 C. Case 15 was peculiar in that the slight elevation of body temperature never exceeded 39.9 C and lasted only for 2 days. It dropped to normal level about 2 weeks later. Anorexia began 3 days after infection. Past infection of this pig prior to experiment, as evidenced by serological findings in Table I, probably accounts for the slight ill-signs observed.

The results of D and HA tests are summarized in Table I. From the table it is apparent that the D test titer of all pigs became positive

TABLE I

The antibody titer of swine fed with toxoplasma infected mice

Days	Case no.											
	15			16			17			18		
	D	HAN	HAA	D	HAN	HAA	D	HAN	HAA	D	HAN	HAA
0	64	16	4	< 4	< 4	< 4	16	< 4	nd	4	< 4	nd
2	256	< 4	< 4	< 4	< 4	< 4	4	< 4	nd	4	< 4	nd
6	64	< 4	< 4	< 4	< 4	< 4	4	< 4	nd	4	< 4	nd
8	256	< 4	< 4	16	< 4	< 4	16	4	nd	16	4	< 4
12	256	< 4	< 4	64	4	< 4	1,024	4	< 4	256	16	16
16	256	< 4	< 4	1,024	4	< 4	256	4	4	64	16	16
20	64	< 4	< 4	1,024	4	< 4	1,024	4	4	256	64	4
24	256	< 4	< 4	1,024	4	< 4	1,024	4	4	64	16	4
30	64	< 4	< 4	16	4	4	64	16	4	16	16	16
38	16	4	nd	64	4	4	256	16	4	< 4	64	64
48	64	256	256	64	16	16	256	4	4	< 4	64	64
55	64	256	256	64	16	16	64	4	4	4	64	64
63	16	256	256	64	16	16	4	4	4	4	64	64
132	16	16	64	16	< 4	< 4	< 4	4	4	< 4	64	16

D: Dye test. HAN: Hemagglutination test with non-absorbed test serum. HAA: Hemagglutination test with absorbed test serum. nd: Not done.

on the 9th day after ingestion, except case 15 which was a positive reactor already prior to the experiment.

The HA test seems to be less sensitive than D test in that positive titer invariably appeared later. Pigs 17 and 18 gave positive HA titer on day 9, pig 16 on day 12, and pig 15 as late as 38. In this connection, the sensitivity of the test appears to be hampered when absorbed serums were used for testing.

No toxoplasma organisms were isolated from brains of these pigs killed between 132 and 161 days after infection.

Experiment 2. Three pigs were inoculated subcutaneouly with 1 ml of peritoneal exudate from infected mice which were infected 3 days previously. The inoculum contained approximately 2×10^8 toxoplama organisms.

In case 19, a raised body temperature over the normal value (40.5–40.8 C) existed only on the 2nd day after infection. Moderate anorexia was observed for several days after the elevation of body temperature. It was killed 190 days after inoculation.

In case 20, a high fever of 40–41.2 C started on the 3rd day after inoculation and lasted for 5 days. Marked depression and anorexia were observed during this febric period. It was killed 198 days after inocuation. Pig 21 reacted with a high fever on day 2, which varied between 40.8–41.5 C and persisted for 7 days. This animal died 8 days after inocualtion. Marked depression, anorexia and chillness were noted.

Serial blood samples were taken at frequent intervals for serological study. The results are shown in Table II.

In D test, positive titers were obtained 7 days after inoculation in pigs 19 and 20. The serological findings of pig 21 is interesting in that a D test titer of 1:256 was obtained on day 4, but became negative on day 7, the day before the animal died. The single positive titer in this pig was probably due to *in vitro* neutralization as a result of overwhelming parasitemia, although the possibility of experimental error can not be completely excluded.

In HA test, cases 19 and 20 showed rising titer of 1:4 or higher at 4 and 7 days after inoculation respectively. Residual titers were still demonstrable with HAN test 132 days after inoculation in both cases. The inferior sensitivity of HAA was again evidenced in parallel testing of these 2 cases, the HAN titer of which usually exceeded the HAA titer.

Toxoplasma organisms were isolated from the brain of cases 19 and 20. They were also demonstrable in the smear preparation of the lymph nodes of case 21.

Experiment 3. Four pigs were used in this

TABLE II

The antibody response of swine infected with toxoplasma subcutaneously

Days	Case no.								
	19			20			21		
	D	HAN	HAA	D	HAN	HAA	D	HAN	HAA
0	< 4	< 4	nd	4	< 4	nd	< 4	< 4	nd
2	4	< 4	nd	< 4	< 4	nd	< 4	< 4	nd
4	4	64	64	4	< 4	nd	256	< 4	nd
7	16	64	64	64	16	4	< 4	< 4	nd
10	256	256	64	256	16	16	*Died on the 8th day.*		
14	256	64	64	256	64	4			
18	256	256	64	1,024	16	4			
22	256	64	64	256	16	4			
28	64	64	64	4	16	4			
36	64	64	64	16	16	16			
46	16	64	16	16	64	64			
53	16	64	16	4	64	64			
61	4	64	4	16	64	64			
132	4	4	< 4	< 4	16	4			

experiment. Two pigs (case 38 and 39) were inoculated intratracheally with 5 ml of diluted mouse ascites containing 6.5 million toxoplasma organisms. Two additional normal pigs (40 and 41) were placed in the same pen with the inoculated animals to see if contact infection would occur.

Both inoculated pigs showed elevated body temperature 3 days after inoculation. The fever in case 38 continued for 10 days, ranging from 40–41.8 C, and dropped to normal afterward. Dyspnea and nonproductive coughing were observed during the 21-day observation period. The high fever in case 39 continued for 5 days, ranging from 40.4 to 42.2 C, and the pig died 9 days after inoculation. *Toxoplasma* was observed in stamp smears prepared from obviously pneumonic lungs of the dead animal.

Fever started in the 2 non-inoculated pigs on days 6 and 7 and dropped to normal level at days 11 and 12 after contact respectively. Fever did not exceed 41 C, and the animals recovered quickly.

Blood samples were repeatedly drawn from all animals after inoculation for testing. Serological data in Table III shows clearly that pigs responded readily to artificial inoculation as well as contact infection. Pig 39 did not show elevation of D test titer, but the HAN test became positive on the 8 th day after inoculation, the day before death. In case 38, the D test titer turned positive on day 14, while the HAN titer rose to 1:4 on day 8. The former dropped to negative level on day 29, while the latter remained at a high level of 1:64 as late as 125 days after inoculation.

The 2 animals (case 40 and 41) for contact infection experiment gave positive D test titer on days 21 and 14 respectively. It remained positive in both cases 102 days after inoculation. On the other hand, HAN tests were always negative in case 40, while a low titer of 1:4 appeared on day 14 in case 41 which disappeared quickly.

Pigs 38, 40 and 41 were killed 149, 154 and 155 days after inoculation respectively. Efforts to isolate toxoplasma organisms from the brains resulted in failure.

Serology on swine serum samples collected from field

Blood samples were collected from 86 pigs 6–7 months of age. Some pigs were born from normally farrowed mothers, and some from *Toxoplasma* affected mothers. The results of D and HAN test on these samples as shown in Table IV indicate that 25.6% of pigs gave positive D titer while 36% was positive in HAN. It is interesting that 16 pigs with negative D titer was nevertheless HAN positive. On the other hand, 7 pigs which gave positive D titer was negative in HAN.

DISCUSSION

Serological evidences presented above indicate that swine are equally susceptible to toxoplasma

TABLE III

The antibody response of swine infected by intratracheal route and by contact

Days	Case no.							
	Intra-tracheal				Contact			
	38		39		40		41	
	D	HAN	D	HAN	D	HAN	D	HAN
0	< 4	< 4	< 4	< 4	4	nd	< 4	< 4
4	4	< 4	< 4	< 4	nd	nd	nd	nd
8	4	4	4	4	< 4	< 4	< 4	< 4
14	16	4	*Died on the 9th*		4	< 4	16	4
21	16	4	*day*		16	< 4	16	4
29	4	4			64	< 4	256	< 4
102	4	< 4			16	< 4	≧64	nd
125	nd	64			nd	nd	nd	nd

infection by oral, subcutaneous and intratracheal routes and by contact. Positive D test titers appeared about 7-9 days after infection, never rose above 1:1024 in any experimental group, and the residual titers lasted no longer than 4 months.

TABLE IV

Comparison of HAN and D titers in natural cases of swine toxoplasmosis

		HAN			
		<1:4	1:4	1:16	≧1:64
DT	<1:4	48	7	2	5
	1:4		1		1
	1:16	1			1
	1:64	2	1	1	6
	1:256	3		1	3
	≧1:1024	1			2

In human cases of toxoplasmosis caused by laboratory infection where the exact time of exposure is known, D test antibodies are demonstrable on about 11 days after infection. The titer usually rises rapidly to a peak of 1:4,000 to 1:26,000, and maintains at moderate levels of 1:1,000 to 1:16,000 for many months. It takes years for the residual titer to fall off, and in some cases it persists for life. The picture is in distinct contrast to the manifestations of our swine described herein. This is rendered all the more significant in consideration of the infection dose, which is conceivably greater in our case than that of the laboratory infection in human. It remains obscure if it is also due to the difference in the susceptibility of hosts or in the virulence of strains.

HAN was more sensitive than HAA in that positive titer generally appeared earlier, lasted longer, and the response was stronger particularly when the titer was low (Table I-III). Here a question arises if a serum under test should be absorbed to remove heterophil antibodies prior to testing. We are of the opinion that the theoretical presence of heterophile antibodies does not necessarily demand the absorption of serum specimens before testing. Our findings on HAN indicate that significant amount of heterphile antibodies certainly was not present in the serum of the local swine population. Again, the adoption of a conservative end-point for reading plus the recording of a positive reaction based on a serum diluted at least 4-fold would safeguard mistaking a false-positive reaction caused by nonspecific antibodies. Early detection of a clinical case of swine toxoplasmosis depends on the use of a sensitive serologic test. For practical purposes the balance of evidences seems to be much favorable for HAN instead of HAA.

The procedure of Jacobs *et al.* (2) was followed for preparation of HA antigens in the early stage of this study. Inconsistent result was obtained with different lots of antigens prepared by this method, until a better antigen was used which had been filtered through glass-wool and standardized by counting. Apparently the decreased wbc content accounts for the better result obtained with the improved antigen, as suggested by Jacobs *et al.* (2).

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