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Prevalence of Antibodies to *Toxoplasma gondii* among Meat Animals Slaughtered at an Abattoir in Hyogo Prefecture, Japan

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Abstract

Antibodies to *Toxoplasma gondii* was surveyed in 599 swine and 600 cattle sera collected at the Kobe Abattoir, Japan, to reveal the present state of the prevalence among these slaughter animals. The overall prevalence rate was 0.33% in swine and 1.33% in cattle as determined by enzyme-linked immunosorbent assay, and the positive cases did not concentrate on any single breeder. The low prevalence is probably attributed to the improved hygenic environments at their breeding grounds that may prevent natural infection with such a zoonosis. Discussion was made on epidemiological importance of the oocyst form of this parasite for its transmission to humans in Japan.

Key words: *Toxoplasma gondii*, Seroepidemiology, Swine, Cattle, Enzyme-linked immunosorbent assay

Introduction

Toxoplasmosis, a well-known human parasitic disease, is also important in domestic animals used for meat for human consumption from both aspects of medical and veterinary sciences. The cyst form of *Toxoplasma gondii* in the edible flesh of meat animals was suggested as a source of infection in persons who consumed undercooked or raw meat (Desmonts *et al.*, 1965; Feldman, 1965; Walls *et al.*, 1967; Stray-Pedersen and Lorentzen-Styr, 1980). Frequent contacts with animals or meat may relate to the higher prevalence among veterinarians (Tizard and Caoili, 1976), veterinary students (McCulloch *et al.*, 1963), abattoir workers (Kobayashi *et al.*, 1963), and people who handle raw meat (Kozar, 1958). In addition to its exposure to humans, infection in farm animals itself has been a serious economic problem, which causes abortion in swine and sheep (Siim *et al.*, 1963; Report of a WHO meeting of investigators, 1969).

Prevalence of antibodies to *Toxoplasma* among domestic animals ranged from 0 to 86% in previous surveys throughout the world as reviewed by Remington and Desmonts (1982). In Japan, swine and cattle are the main animals slaughtered in abattoirs. Kobayashi (1977) has reported that they were seropositive at 24% and 8%, respectively, with isolation of the organism from muscle in 5 to 20% of swine. It is generally agreed that these animals mainly become infected by ingestion of sporulated oocysts shed in the cat feces under natural conditions.

A recent survey in the Netherlands (Knapen *et al.*, 1982) pointed out a decreased prevalence of *Toxoplasma* among some kinds of farm animals, which is probably due to the age of the animals examined and a change in farming methods. The sanitary environments at breeding grounds have been also improved in Japan, so that animals may be protected from possible infection with the oocyst contaminating in soil. The objective of this study was to reveal the current situation of *Toxoplasma* antibody prevalence among swine and cattle in Japan. As one of a series of our seroepidemiological surveys in Hyogo Prefecture (Takahashi *et al.*, 1985; Konishi *et al.*, 1986; Konishi and Takahashi, 1987), the present study would lead us

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Table 1 Sample source used for this survey

Species	Strain	Sex		Total
		Castrated Male	Female	
Swine	Landrace Complex	225	374	599
Cattle	Japanese Black	244	317	561
	Japanese Brown	2	7	9
	Holstein Friesian	1	29	30
	Total	247	353	600

to reconsider the significant position of meat in transmission of this parasite to humans.

Materials and Methods

Sample Population

Swine and cattle sera, each 50 in number, were collected every month from November 1984 through October 1985 at the Kobe Abattoir, a major slaughter house in Hyogo Prefecture, Japan. A total of 599 swine and 600 cattle sera corresponding to about 2% and 6%, respectively, of the overall population slaughtered anually at this Abattoir, were used for this survey; one swine sample was lost at a process of serum isolation. Their breeding grounds were distributed not only in Hyogo Prefecture but also over several other prefectures. The species and sex ratios of source animals were described in Table 1.

Enzyme-Linked Immunosorbent Assay (ELISA)

Antibodies to *Toxoplasma* in swine and cattle sera were measured by the magnetic-processing ELISA system essentially as previously described (Konishi and Takahashi, 1983), excepting the conjugate-substrate system. Solid-phase polycarbonate-coated iron beads sensitized with 15–20 μg/ml of *Toxoplasma* antigen were first reacted with 100-fold diluted test sera at 37°C for 1 h. They were then processed to the second reaction at 37°C for 1 h with the conjugate absorbed with immunoglobulin (Ig) M fraction (see below). The third reaction was performed at 37°C for 15 min with 0.4 mg of *o*-phenylene-diamine dihydrochloride per ml and 0.003% H_2O_2 in 0.1 M citrate-phosphate buffer (pH 4.8). The enzyme reaction was terminated with 3N H_2SO_4. Absorbance values at 490 nm obtained in duplicate were averaged and then adjusted with the value for the constant positive control as 1.0. Sera were diagnosed as positive, when the value was more than 0.351 for swine samples and more than 0.394 for cattle samples. The diagnostic criterion was obtained from the frequency distribution of ELISA values by calculating the confidence limit from the mean and the standard deviation by a one-tailed *t*-test at the 0.0005 probability level (Heck *et al.*, 1980).

Absorption of Commercial Conjugate with IgM Fraction

Conjugates used for this ELISA were absorbed with homologous IgM fractions as previously described (Takahashi and Konishi, 1986), to eliminate the direction of cross-reactive conjugates to natural IgM antibodies in test sera. In brief, peroxidase conjugated IgG fraction of rabbit anti-swine IgG or anti-cattle IgG (heavy and light chains specific: Cappel Laboratories Inc., USA) at 1:1,000 dilution was mixed with an equal volume of the IgM fraction at an appropriate dilution which were obtained from swine or cattle sera by sucrose density gradient centrifugation. After incubation at 37°C for 1 h, the mixture was further incubated at 4°C overnight, and then centrifuged at 8,000 × *g* for 1 h. The supernatant was checked for conjugate activity and was used as specific conjugate when its reactivity to IgM became negligible without change in

reactivity to IgG. Because γ-chain specific conjugates are not commercially available in cases for swine and cattle, absorption with IgM would be a simple way to get specific conjugates reactive only to IgG antibodies.

Results

The ELISA reaction was positive in 2 of 599 swine sera (0.33%) and in 8 of 600 cattle sera (1.33%). Detailed background information about these positive cases is summarized in Table 2. They were completely healthy at the time of slaughter as far as routine visual inspection was concerned.

Positive cattle was limited to the Japanese Black strain, which was dominant in the source population (Table 1). Prevalence rates in castrated male (0.82%; 2 of 244 heads) and in female (1.89%; 6 of 317 heads) of this strain were not significantly different by the Chi-square test ($P > 0.05$). No sex difference was also observed in prevalence among swine; 1 of 225 castrated males (0.44%) versus 1 of 374 females (0.27%).

Ages of swine (6 months) and cattle (3–4 years) in positive populations were identical with those in negative populations, corresponding to the conventional breeding periods in such meat animals. Positive results did not concentrate on any single breeder and not relate to the prefecture of their breeding grounds. They were distributed equally over the survey period and therefore monthly positivity was almost constant.

Discussion

Previous reports on the outbreak of toxoplasmosis in farm animals especially in swine (Sasaki, 1974; Ito *et al.*, 1975) have demonstrated the oocyst contamination in soil at their breeding grounds. This suggests that animals in continuous contacts with soil were simultaneously infected with *Toxoplasma*, when infected cat(s) invaded the farm, eliminated the feces and spread the oocysts. It was therefore expected that such a mini-epizootic would be effectively monitored by testing 50 serum samples for *Toxoplasma* antibodies every month, which covers several breeding grounds in different prefectures of Japan. The surveillance would be more successful for a swine population, where almost all individuals were regularly slaughtered at the age of 6 months for economical reasons. However, the seroposi-

Table 2 Background information about slaughter animals positive for *Toxoplasma* antibodies

Species	No.	Strain	Sex	Age	Prefecture*	Month†
Swine	1	LC‡	Male§	6 mo	Shimane	Jul 1985
	2	LC	Female	6 mo	Hyogo	Sep 1985
Cattle	1	JB‖	Female	4 y	Saga	Nov 1984
	2	JB	Female	4 y	Kagoshima	Dec 1984
	3	JB	Male	4 y	Hyogo	Feb 1985
	4	JB	Male	3 y	Yamagata	Mar 1985
	5	JB	Female	4 y	Kagawa	Apr 1985
	6	JB	Female	4 y	Kagawa	Jun 1985
	7	JB	Female	4 y	Miyazaki	Aug 1985
	8	JB	Female	4 y	Saga	Oct 1985

* The place where the animal was bred.
† The time when the animal was slaughtered.
‡ Landrace Complex.
§ Castrated male.
‖ Japanese Black.

tivities obtained in this study (0.33% in swine and 1.33% in cattle) were extremely low, and the positive cases did not concentrate on any specific breeder nor any peculiar month (Table 2).

The low antibody prevalence among swine as compared with that of the previous report (24%: Kobayashi, 1977) is consistent with a remarkable decline in positive results obtained by visual inspection and by the subsequent isolation of *Toxoplasma*, which have been performed routinely at any abattoirs in Japan. The Ministry of Health and Welfare has reported the number of infected swine of 4,426 in 1975 (out of about 14 million heads) which decreased to 470 in 1985 (out of about 21 million heads: Ministry of Health and Welfare, 1986). It is most likely that this decreased prevalence among slaughter animals is attributed to the improved hygenic situation at their breeding grounds. Animals are commonly provided with artificial feeds and mainly protected from contact with soil. In particular, pigs at most farms are always isolated indoors to prevent invasion of cats. As antibody prevalence among cats in Hyogo Prefecture (21.2%) was still high in the survey of 1983 (M. Goto, personal communication), soil would be evenly contaminated with oocysts and consequently constant prevalence rate would be kept in farm animals, if there were no preventive strategy.

Our recent survey of meat inspectors for the presence of antibodies to *Toxoplasma* (Konishi *et al.*, 1986) indicated that the prevalence rate of 23.5% was similar to those obtained with other age-matched control populations, such as patients (22.3%: Takahashi *et al.*, 1985) and farmers (16.4%: Konishi and Takahashi, 1987), who do not have a chance to contact with animals or meat so frequently as those inspectors. The difference from their prevalence in the previous report (59 to 78%: Kobayashi, 1977) may relate to the dramatic decrease in prevalence among slaughter animals.

Contradiction may occur between the present result and our previous survey of a farming population (Konishi and Takahashi, 1987) in which the lifetime history of raw meat ingestion was suggested as an important factor involved in epidemiology of human *Toxoplasma* infection. A possible explanation is that the factor merely affected seropositivity in people of advanced age who had consumed raw meat in the past when prevalence among meat animals had still been high. In fact, the significant difference in antibody prevalence between people with and without raw meat ingestion was observed in males over 60 years and females over 50 years, but not in younger population. Furthermore, greater raw meat ingestion by males than by females was related to their different seropositivity at ages of 49–59, while men and women of 30–39 years were almost equal in antibody status irrespective of their significant sex difference in raw meat ingestion. More detailed studies will be necessary to further clarify the direct relation between meat and positivity in this sample population.

Natural routs for human *Toxoplasma* infection have been focused on accidental ingestion of the oocyst and cyst forms of this parasite except for congenital transmission (Kobayashi, 1977; Remington and Desmonts, 1982). Now that the importance of the cyst became negligible in accordance with the low prevalence in meat animals, the oocyst form may be the major source for human infection in this survey area.

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