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The prevalence and avidity of *Toxoplasma gondii* IgG antibodies in pigs from Brazil and Peru

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Abstract

Raw or inadequately cooked pork is an important source of *Toxoplasma gondii* infection, and the infection rate in animals used as human food, is an important risk predictor. The prevalence of this infection was estimated in 396 sera from 5-month old pigs obtained at abattoirs in São Paulo, Brazil (300) and Lima, Peru (96). The seroprevalence was higher in pigs from Peru (32.3%) as compared to Brazil (9.6%), as detected by ELISA and Western blot. Hemagglutination gave poor resolution which was not useful for the diagnosis of *T. gondii* infection. Specific antibody avidity is correlated with infection time, as shown in experimentally infected piglets. Using an arbitrary cut-off of 50% avidity index, Brazilian pigs were found to be more recently infected than Peruvian pigs. Pork should be considered a significant source of human *T. gondii* infection both in Brazil and Peru. Avidity assays could help in the detection of the time of *T. gondii* infection in pigs, allowing preventive management.

Keywords: *Toxoplasma gondii*; Pigs; Prevalence; Avidity; Brazil; Peru

1. Introduction

Toxoplasma gondii, an Apicomplexa protozoan parasite, infects man and other warm-blooded animals (Dubey, 1993). Human toxoplasmosis is generally asymptomatic in immunocompetent individuals, with occasional eye involvement, but causes devastating disease resulting in immune system immaturity or deficiency (Beaman et al., 1995). This

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infection has acquired great importance due to the HIV epidemic, causing toxoplasmic encephalitis, a disabling and lethal disease in 20% of AIDS patients (Luft and Remington, 1992). Therapeutically immunosuppressed patients, such as patients with cancer or transplant histories, may also have toxoplasmic encephalitis, mostly due the reactivation of tissue cysts (Israelski and Remington, 1993).

Toxoplasma gondii infection in women during pregnancy can result in fetal infection, with severe problems, including abortion, encephalitis, mental retardation and blindness, due to infection through the placenta and immune immaturity (Frenkel, 1990; Remington et al., 1995). Toxoplasmosis is also an important cause of neonatal deaths and abortion in sheep, goats and pigs.

The infection can be transmitted to humans by ingestion of oocysts in food and water contaminated with cat feces or by consuming tissue cysts in undercooked meat (Dubey and Beattie, 1988). Raw or inadequately cooked pork is considered to be an important source of *Toxoplasma* infection in the USA (Dubey, 1991). Infective tissue cysts have been found repeatedly in commercial cuts of pork from both experimentally and naturally infected pigs (Dubey et al., 1986; Dubey, 1988).

Toxoplasmosis in pigs was first reported in 1951 on a farm in Ohio (Sanger and Cole, 1955). *Toxoplasma gondii* infection in pigs can be confirmed by definitive histological examination or by bioassays that require isolation of organisms from fresh tissue. Usually, livestock can also be analyzed by more practical serological surveys. By this approach, the prevalence of *T. gondii* infection was found to range from as low as 4% to as high as 69% in pigs from the USA (Dubey, 1990), with similar variable results in other countries (Van Knapen et al., 1995). The prevalence of infection was also related to the type of pig production, such as intensive or extensive management, contact with pasture and age of sows. Few studies have been conducted in South American countries, with sporadic prevalence estimates in Brazilian pigs (Jamra, 1969) showing a prevalence of over 40% in 1986 (D'Angelino and Ishizuka, 1986). The main serological tests used for these assays was indirect immunofluorescence, hemagglutination and microagglutination of fixed tachyzoites, with no attempts to use ELISA or antibody avidity, a useful technique for the detection of the time of the infection (Jenum et al., 1997). The purpose of the present work was to standardize an ELISA test for the determination of seroprevalence of toxoplasmosis in pigs from Brazil and Peru. We also developed an avidity assay for the detection of early infection in these animals to facilitate better preventive management.

2. Materials and methods

2.1. Parasites

The RH strain of *T. gondii* was maintained as frozen stabilates in liquid N_2 or by successive intraperitoneal (i.p.) passages in Swiss mice, in the Laboratory of Protozoology of the Instituto de Medicina Tropical de São Paulo. All plastic ware, reagents and conjugates were purchased from Sigma (St. Louis, MO) and solutions were prepared with MilliQ high quality water.

2.2. Swine sera

We analyzed 396 serum samples from 5-month-old pigs obtained at abattoirs in São Paulo, Brazil (300), and Lima, Peru (96). Blood was collected during slaughter, immediately after killing. After clotting, serum was separated from the clot by centrifugation at $1000 \times g$ for 10 min, aliquoted and stored at -20°C.

2.3. Experimental infection

An anti-*Toxoplasma* IgG-negative piglet was bought from a Duroc pig producer, with clean facilities and good management. Several piglets were tested for specific anti-*T. gondii* IgG and a seronegative piglet was maintained by a team of veterinarians of the Veterinary Hospital of USP, receiving commercial chow, water and mineral supplements ad libitum throughout the experimental period.

A blood sample was collected before *T. gondii* injection and used as negative control. The animal was then inoculated subcutaneously (s.c.) with 10^7 viable forms of tachyzoites of the RH strain of *T. gondii*, obtained from an infected mouse. The pig was carefully observed for 140 days, a period during which it did not present any clinical signs of the infection, with normal growth. Blood samples were collected 60, 80, 100, 120 and 140 days after infection. The samples obtained at Day 0 and Day 140 after infection were used, respectively, as negative and positive standards in all reactions.

2.4. Antigen

Toxoplasma gondii antigen was prepared as described elsewhere (Camargo et al., 1978). Briefly, tachyzoites were harvested from mouse peritoneal cavity in phosphate buffered saline (PBS), recovered by centrifugation, washed, counted and submitted to sonication for four periods of four cycles per 30 s in an ice bath, with phase contrast microscopy control, and the procedure was repeated as necessary. When most parasites were lysed, 0.3 M NaCl was added and the suspension was cleared by centrifugation at $10000 \times g$ for 3 min. The protein content of the supernatant was determined (Bradford, 1976) and aliquots were maintained at -70°C until used as purified antigen.

2.5. Indirect hemagglutination

In this assay we used a commercial kit, HAP Toxoplasmose (Salck®), according to manufacturer instructions. Briefly, the serum sample was inactivated for 30 min at 56°C, diluted 1:16 and added to 96 multiwell V bottom plates (25 μl per well) after the addition of 25 μl of a 1% suspension of red blood cells sensitized with *T. gondii* antigens. The plates were homogenized and incubated for 1 h in a humid chamber without shaking. The test was positive when a layer of agglutinated erythrocytes was formed in more than 50% of the bottom of the V-shaped well, and negative when a small dot of non-agglutinated RBC was formed in the center of the well. Titration was also performed using the highest dilution that induced agglutination in this test.

2.6. Enzyme-linked immunosorbent assay — ELISA

The ELISA was performed as described elsewhere (Venkatesan and Wakelin, 1993). Briefly, ELISA plates (Multiwell Plate/polystyrene, Sigma®) were coated with 100 μl/well of a solution containing 10 μg/ml of the antigen extract diluted in 0.1 M carbonate buffer, pH 9.5, and incubated overnight at 4°C. The plates were then washed five times with PBS containing 0.02% Tween 20 (PBS-T), and blocked for 1 h at 37°C with PBS containing 2% fat-free lyophilized milk in a humid chamber. After this step, 100 μl of serum sample, diluted 1:100 in PBS-T, was added to each well and the plate was incubated for 1 h at 37°C. After additional washing with PBS-T, 100 μl of peroxidase-conjugated anti-swine immunoglobulin G (IgG) diluted 1:10,000 in PBS-T, was added to the wells, with incubation at 37°C for 1 h, followed by five washings with PBS-T. The reactions were developed with 100 μl per well of OPD solution (1 mg/ml *o*-phenylenediamine and 0.03% H_2O_2 in 0.2 M phosphate–citrate buffer, pH 5.0). The reaction was stopped with 25 μl per well of 4 N HCl and the optical density (OD) at 492 nm was measured in a microplate reader (Labsystems Multiskan MS). The cut-off value (0.2706) was determined using 10 negative sera samples form piglets, representing the 99% upper confidence level.

The avidity was determined by ELISA using an additional incubation with 6 M urea, pH 7.0, for 5 min after test serum incubation, followed by PBS-T washing and routine conjugate and development procedures. Sera of positive and negative pigs were included as controls in all assays. The avidity index was determined as the percent of resistance of OD to urea washing. This analysis was performed only in positive ELISA sera.

2.7. Western blot

The Western blot was performed as described elsewhere (Hudson and Hay, 1989). Purified antigen was submitted to SDS-PAGE in a 12.5% running gel, with subsequent electrophoretic transfer to nitrocellulose membranes. The membranes were blocked with 5% fat-free milk, lyophilized in PBS-T, cut into 3 mm strips and stored until use. Sera from animals were reacted with strips containing *T. gondii* antigens re-hydrated with PBS-T, for 18 h at 4°C. After careful washing with PBS-T, bound antibodies were detected by sequential incubation with peroxidase-conjugated anti-swine IgG for 60 min followed by washings, and developing solution containing 0.6 mg/ml 4-chloro-1-naphthol and 0.03% hydrogen peroxide in PBS.

2.8. Statistical analysis

Frequencies were compared by the χ^2-test with Yates correction, using the EpiInfo 6.01 statistical package, and were considered significantly different when the probability of equality was less than 0.05 ($P<0.05$), with estimation of the 95% confidence interval.

Table 1
Distribution of the ELISA reaction to *Toxoplasma gondii* in Brazilian and Peruvian Swine

ELISA	Brazil			Peru		
	n	%	CI[a]	*n*	%	CI[a]
Positive	29	9.6	6.6–13.6	31	32.3	23.1–42.6
Negative	271	90.4	86.4–93.3	65	67.7	57.4–76.9
Total	300	100		96	100	

[a] Confidence interval (CI)=95%, $P<0.001$ (χ^2(Yates)=27.23) (Brazil vs. Peru).

3. Results

3.1. Serological analysis

The results obtained with anti-*T. gondii* IgG detected by ELISA demonstrated 9.6% positivity in the Brazilian pig sera and 32.3% in the Peruvian pig sera (Table 1), indicating higher infection levels by *T. gondii* in Peruvian pigs.

Hemagglutination using commercial *T. gondii* antigen RBC demonstrated higher frequencies of positive sera in both populations (Table 2) as compared to those obtained by ELISA. The cut-off dilution used was compared to standard sera and most samples gave a positive reaction at lower dilutions. The kappa agreement between tests was very low, between 0.20 and 0.15, suggesting that this hemagglutination assay may not be useful for anti-*T. gondii* antibody detection in pig sera.

All ELISA-positive sera were tested against strips of SDS-PAGE separated *T. gondii* antigens, as described in Section 2. All Peruvian serum samples tested by ELISA recognized several *T. gondii* antigens by Western blotting, and the intensity of stained bands correlated well with IgG antibody titers. Some of the Brazilian pig sera showed a weak and undefined reaction in this test, probably due to low titers, being clearly positive only at higher titers. Hemagglutination-positive and ELISA-negative sera gave no reaction, showing a probably undefined cross-reaction in the unrelated erythrocyte assay.

3.2. Avidity assays

Analysis of sequential serum samples from the experimentally infected pig showed a clear increment in IgG anti-*T. gondii* production and in avidity index, as determined by urea

Table 2
Comparison of results obtained by indirect hemagglutination and by ELISA in seroprevalence study of *Toxoplasma gondii* in Brazilian and Peruvian swine[a]

ELISA	Brazil		Peru	
	Positive	Negative	Positive	Negative
Positive	14	15	25	6
Negative	49	222	39	26

[a] Kappa coefficient: 0.198 (Brazil) and 0.16 (Peru).

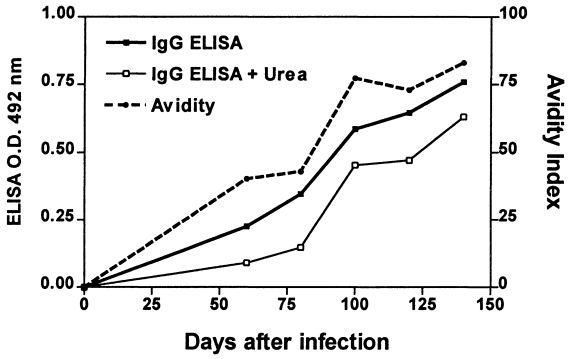

Fig. 1. Time sequence of specific anti-*Toxoplasma gondii* IgG antibody and its avidity during pig experimental infection. Solid lines show OD of IgG ELISA, solid squares total IgG, empty squares urea resistant IgG. Dotted line express the IgG avidity index as described in Section 2.

extraction (Fig. 1). Fifty percent avidity of the IgG antibodies occurred sometime between 75–100 days of infection. When the avidity index, expressed as percent of ELISA-resistant titer, was determined in Brazilian and Peruvian positive samples, a higher frequency of high avidity sera was observed in the Peruvian samples compared to the Brazilian ones, as shown in Fig. 2. When a standard cut-off of 50% was used to determine acute or recent infection, we found a higher frequency of Brazilian pigs presenting early or recent infection as compared to Peruvian pigs.

4. Discussion

Our data show that the ELISA for swine IgG specific antibodies against *T. gondii* is a useful test for the determination of the prevalence of toxoplasmosis in pigs from Brazil and Peru, as also confirmed by Western blotting detection. ELISA is a sensitive serological test,

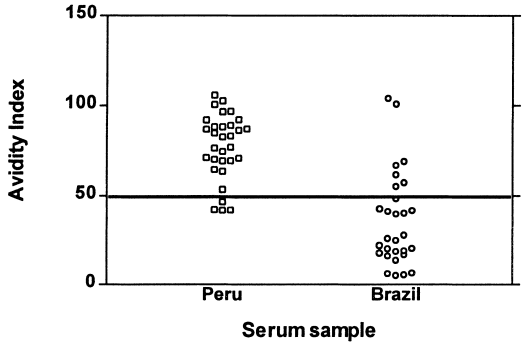

Fig. 2. Avidity index of anti-*Toxoplasma gondii* positive sera samples obtained from Brazilian or Peruvian swine. The line express the artificially defined cut-off of low and high avidity.

able to detect low antibody titers either in recent infections (Dubey et al., 1996), or in animals subjected experimentally to small numbers of infective forms for longer times of as much as 800 days (Dubey et al., 1997). Although few reports are available, ELISA is extremely efficient for the detection of IgG antibodies against *T. gondii* in swine (Hirvela-Koski, 1992; Lind et al., 1997).

The indirect hemagglutination assay employed in our experiment yielded poor detection and resolution, as also reported by others in studies in which this test gave poor resolution compared to the classical indirect immunofluorescence assay (D'Angelino and Ishizuka, 1986). This event may be ascribed to many factors, including cross-reactions with blood groups (Stormont, 1982; Shan et al., 1998), since most indirect hemagglutination reagents are made with bird red blood cells as a biological support, or to low sensitivity. The low specificity of the test emphasizes the need for proper standardization before its use in epidemiological surveys.

The 9.6% level of infection in Brazilian pigs was lower than the 22.5% prevalence values reported by Corrêa et al. (1978) and higher values of 54% for pigs reared on human food garbage and 49.2% in pigs raised on semi-intensive crop feed management (D'Angelino and Ishizuka, 1986). In those studies, the test used was the classical immunofluorescence assay without an internal control (experimentally infected pig), as used in our study.

Serologic prevalence of toxoplasmosis presents a wide inter- and intra-region variation (Dubey et al., 1995), as described in the USA, where wide variations in the prevalence of the infection occur in different farms in the same region (Weigel et al., 1995).

We also found a higher serological prevalence of toxoplasmosis in Peruvian pigs. Despite our efforts, we could obtain no information about the type of food or environment for the Peruvian pigs. In Brazil, most swine farms are devoted to intensive management, with slaughter at 5 months of age; this might explain the lower titers found in Brazilian pigs compared to those in Peru. The rearing of swine under confined conditions without pasture contact does not exclude contamination of feed with cat feces, but a study conducted in Brazil indicated low prevalence of *T. gondii* antibodies in intensively managed pigs. We cannot exclude the possibility that in the present study the Peruvian pigs were older than Brazilian pigs. When weight and age were studied in terms of the prevalence of swine toxoplasmosis, a high proportion of infected pigs was observed among larger or older pigs (D'Angelino and Ishizuka, 1986).

Other pork-transmitted parasitic infections such as *Taenia solium* are also highly prevalent among pigs and among people in the rural areas of Peru (Diaz et al., 1992; Evans et al., 1997), suggesting a more extensive pig-rearing activity in those areas.

Our experimental infection model allowed us to study the evolution of avidity in IgG-specific anti-*T. gondii* antibodies. Since pigs have an epitheliochorial placenta, without maternal antibody transmission, specific antibody detection in serum is confirmatory of infection. Production of IgM in swine experimentally infected with *T. gondii* is short lived, with only a few weeks of positivity; it cannot be used as an index of time of contamination (Lind et al., 1997). In our model, avidity increased up to 10 weeks after infection, allowing a crude estimate of the period of contamination. This is the main importance of IgG avidity detection in pig serum, without the need for several reagents for the determination of the period of contamination and sources.

The origin of pig infection by *T. gondii* may be ingestion of cysts in refuse from infected animals, of oocysts from cat feces in pastures or of milk from infected sows. Milk is a possible vehicle of *T. gondii*, since the microorganism was also detected in pig milk (Sanger and Cole, 1955). The mammary secretion (Deyrup-Olsen and Luchtel, 1998) allows for the passage of bradyzoites in milk from chronically infected animals (Pettersen, 1984). This fact could result in early infection of piglets, which could be controlled only by elimination of *T. gondii*-infected sows. This theoretical explanation is not supported by the fact that most Brazilian pigs presented low avidity antibodies in our study, suggesting that the infection would be acquired later in life, after weaning. The access to pasture is another important risk in toxoplasmosis transmission in pigs due to the risk of ingestion of oocysts shed by cats and disseminated in the soil and crops by water/rain, as described elsewhere (Davies et al., 1998). Since Brazilian pigs are reared under confined conditions without pasture contact, the most probable cause of *T. gondii* infection is contaminated food from carcasses and other garbage products of animal origin. The virulence of specific strains of *T. gondii* is another factor that interferes with the antibody response of infected swine (Omata et al., 1994).

Our data demonstrated that consumption of raw or undercooked meat from these animals can be an important source of human *T. gondii* infection (Torres et al., 1991), and is more significant in Peru than in Brazil. The serologic assays used in our study can provide adequate information both about the prevalence of *T. gondii* infection in pigs and about the period of contamination of these animals, allowing precise intervention for the control of infection on pig farms.

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References

Beaman, M.H., McCabe, R.E., Wong, S., Remington, S.J., 1995. *Toxoplasma gondii*. In: Mandell, G.L., Bennett, J.E., Dolin, R. (Eds.), Principles and Practice of Infectious Diseases, Vol. II. Churchill Livingstone, New York, pp. 2455–2475.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Camargo, M.E., Ferreira, A.W., Mineo, J.R., Takiguti, C.K., Nakahara, O.S., 1978. Immunoglobulin G and Immunoglobulin M enzyme-linked immunosorbent assays and defined serological patterns. Infect. Immunol. 21, 55–58.

Corrêa, F.M., Salata, E., Oliveira, M.R., 1978. *Toxoplasma gondii*: diagnóstico pela imunofluorescência indireta em suínos no Estado de São Paulo. Arq. Inst. Biol. 45, 209–212.

D'Angelino, J.L., Ishizuka, M.M., 1986. Avaliação da prevalência de infecção toxoplásmica em rebanhos suínos pela prova de imunofluorescência indireta e hemaglutinação. Bol. Oficina Sanit. Panam. 100, 634–644.

Davies, P.R., Morrow, W.E., Deen, J., Gamble, H.R., Patton, S., 1998. Seroprevalence of *Toxoplasma gondii* and *Trichinella spiralis* in finishing swine raised in different production systems in North Carolina, USA. Prev. Vet. Med. 36, 67–76.

Deyrup-Olsen, I., Luchtel, D.L., 1998. Secretion of mucous granules and other membrane-bound structures: a look beyond exocytosis. Int. Rev. Cytol. 183, 95–141.

Diaz, F., Garcia, H.H., Gilman, R.H., Gonzales, A.E., Castro, M., Tsang, V.C., Pilcher, J.B., Vasquez, L.E., Lescano, M., Carcamo, C., 1992. Epidemiology of taeniasis and cysticercosis in Peruvian village. The cysticercosis working group in Peru. Am. J. Epidemiol. 135, 875–882.

Dubey, J.P., 1988. Long-term persistence of *Toxoplasma gondii* in tissues of pigs inoculated with *T. gondii* oocysts and effect of freezing on viability of tissue cysts in pork. Am. J. Vet. Res. 49, 910–913.

Dubey, J.P., 1990. Status of toxoplasmosis in pigs in the United States. J. Am. Vet. Med. Assoc. 196, 270–276.

Dubey, J.P., 1991. Toxoplasmosis — an overview. Southeast Asian J. Trop. Med. Public Health 22 (Suppl.), 88–119.

Dubey, J.P., 1993. Toxoplasma, neospora, sarcocystis, and other cyst-forming coccidia of humans and animals. In: Kreier, J.P. (Ed.), Parasitic Protozoa, Vol. VI. Academic Press, New York, pp. 1–57.

Dubey, J.P., Beattie, P. (Eds.), 1988, Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, FL.

Dubey, J.P., Murrell, K.D., Fayer, R., Schao, C.A., 1986. Distribution of *Toxoplasma gondii* tissue cysts in commercial cuts of pork. J. Am. Vet. Med. Assoc. 188, 1035–1037.

Dubey, J.P., Thulliez, P., Weigel, R.M., Andrews, C.D., Lind, P., Powell, E.C., 1995. Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. Am. J. Vet. Res. 56, 1030–1036.

Dubey, J.P., Andrews, C.D., Lind, P., Kwok, O.C., Thulliez, P., Lunney, J.K., 1996. Antibody responses measured by various serologic tests in pigs orally inoculated with low numbers of *Toxoplasma gondii* oocysts. Am. J. Vet. Res. 57, 1733–1737.

Dubey, J.P., Andrews, C.D., Thulliez, P., Lind, P., Kwok, O.C., 1997. Long-term humoral antibody responses by various serologic tests in pigs orally inoculated with oocysts of four strains of *Toxoplasma gondii*. Vet. Parasitol. 68, 41–50.

Evans, C.A., Gonzalez, A.E., Gilman, R.H., Verastegui, M., Garcia, H.H., Chavera, A., Pilcher, J.B., Tsang, V.C., 1997. Immunotherapy for porcine cysticercosis: implications for prevention of human disease. Cysticercosis working group in Peru. Am. J. Trop. Med. Hyg. 56, 33–37.

Frenkel, J.K., 1990. Toxoplasmosis in human beings. J. Am. Vet. Med. Assoc. 196, 240–248.

Hirvela-Koski, V., 1992. The prevalence of toxoplasma antibodies in swine sera in Finland. Acta Vet. Scand. 33, 21–25.

Hudson, L., Hay, F.C. (Eds.), 1989. Practical Immunology. Blackwell Scientific Publications, Oxford.

Israelski, D.M., Remington, J.S., 1993. Toxoplasmosis in patients with cancer. Clin. Infect. Dis. 17 (Suppl. 2), S423–S435.

Jamra, L.F., 1969. On the isolation *Toxoplasma gondii* from human food of animal origin. Partial results in the city of São Paulo (Brazil). Rev. Inst. Med. Trop. São Paulo 11, 169–176.

Jenum, P.A., Stray-Pedersen, B., Gundersen, A.G., 1997. Improved diagnosis of primary *Toxoplasma gondii* infection in early pregnancy by determination of antitoxoplasma immunoglobulin G avidity. J. Clin. Microbiol. 35, 1972–1977.

Lind, P., Haugegaard, J., Wingstrand, A., Henriksen, A.S., 1997. The time course of the specific antibody response by various ELISAs in pigs experimentally infected with *Toxoplasma gondii*. Vet. Parasitol. 71, 1–15.

Luft, B.J., Remington, J.S., 1992. Toxoplasmic encephalitis in AIDS. Clin. Infect. Dis. 15, 211–222.

Omata, Y., Dilorenzo, C., Venturini, C., Venturini, L., Igarashi, I., Saito, A., Suzuki, N., 1994. Correlation between antibody levels in *Toxoplasma gondii* infected pigs and pathogenicity of the isolated parasite. Vet. Parasitol. 51, 205–210.

Pettersen, E.K., 1984. Transmission of toxoplasmosis via milk from lactating mice. Acta Pathol. Microbiol. Scand. B. 92, 175–176.

Remington, J.S., Mcleod, R., Desmonts, G., 1995, Toxoplasmosis. In: Remington, J.S., Klein, J.O. (Eds.), Infectious Diseases of the Fetus & Newborn Infant, 4th Edition. Saunders, Philadelphia, pp. 140–268.

Sanger, V.L., Cole, C.R., 1955. Toxoplasmosis. VI. Isolation of *Toxoplasma* from milk, placentas, and newborn pigs of asymptomatic carrier sows. Am. J. Vet. Res. 16, 536–539.

Shan, B., Sugiura, T., Yamashita, U., 1998. Five monoclonal antibodies against glycophorin A of human erythrocyte recognize glycoprotein of bovine erythrocyte. Hybridoma 17, 55–62.

Stormont, C.J., 1982. Blood groups in animals. J. Am. Vet. Med. Assoc. 181, 1120–1124.

Torres, A.L., Chinchilla, M., Reyes, L., 1991. Antibodies against *Toxoplasma gondii* in swine in Costa Rica: epidemiologic importance. Rev. Latinoam. Microbiol. 33, 129–133.

Van Knapen, F., Kremers, A.F.T., Franchimont, J.H., Narucka, U., 1995. Prevalence of antibodies to *Toxoplasma gondii* in cattle and swine in the Netherlands: towards an integrated control of livestock production. Vet. Q. 17, 87–91.

Venkatesan, P., Wakelin, D., 1993. ELISAs for parasitologists: or lies, damned lies and ELISAs. Parasitol. Today 9, 228–232.

Weigel, R.M., Dubey, J.P., Siegel, A.M., Hoefling, D., Reynolds, D., Herr, L., Kitron, U.D., Shen, S.K., Thulliez, P., Fayer, R., 1995. Prevalence of antibodies to *Toxoplasma gondii* in swine in Illinois in 1992. J. Am. Vet. Med. Assoc. 206, 1747–1751.