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Seroepidemiology of *Toxoplasma gondii* (Apicomplexa) in meat producing animals in Costa Rica

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Abstract: A serologic screening for *Toxoplasma gondii* antibodies by IgG indirect fluorescent antibodies test (IgG-IFAT) was carried among 496 swine and 601 cattle serum samples from throughout Costa Rica, to study the possible role of their meat in the transmission of the parasite. The overall prevalence of antibodies was 34.4% in cattle and 43.8% in swine. No significant differences were found in the antibody prevalence between males and females in both animal groups, which acquire the infection early in their lives. Swine did not present significant differences between age groups. In cattle there was a high percent of seropositivity between the first and third years of age. The provinces that showed a greater number of seropositive animals were Limón and Puntarenas for both animals, and Guanacaste for cattle.

Key words: *Toxoplasma gondii*, cattle, swine, meat producing animals.

Toxoplasma gondii is a globally recognized infectious agent of warm blooded animals. In humans this parasite is acquired mainly by ingestion of oocysts (Wallace *et al.* 1974, Barbier *et al.* 1983), or ingestion of tissue cysts (Feldman and Miller 1956, Walls *et al.* 1967), However, transmission through the placenta (Dubey 1970) is the most important route of infection for humans, and extensive screening programs have been initiated to control congenital infection (Desmonts and Couvreur 1974). Most cases of toxoplasmosis are subclinical but some times serious consequences may result, especially in individuals with suppressed or defective immune system (Skart *et al.* 1973, Desmonts and Couvreur 1974, Tadros and Laarman 1982, Wanke *et al.* 1987, Cimino *et al.* 1991, Zangerle *et al.* 1991).

Animals as swine, cattle, sheep, mice and poultry can act as intermediate hosts for *Toxoplasma* (Jacobs and Frenkel 1970). They become infected either by ingestion of tissue cysts or oocysts from cat feces; the infection persists and can be demonstrated by their serological response.

Since man can become infected through meat animals, it is important from medical and public health aspects to be aware of the routes of transmission and potential reservoirs of the infection. In Costa Rica, there is partial knowledge about prevalence of this parasite in meat producing animals (Ruíz 1966, Rodríguez *et al.* 1991, Torres *et al.* 1991)

The aim of the present study is to determine the level of seropositivity to *T. gondii* in cattle and swine indicating that meat is a potential source of infection to man.

MATERIAL AND METHODS

Population: 496 swine from 33 farms and 601 cattle from 40 farms, all through Costa Rica, were included in the study. The number of animals tested in each flock ranged from 12

to 18. This sample collection was done from May to September, 1991, with the gentle collaboration of the staff of Ministerio de Agricultura y Ganadería MAG. Information regarding the age, sex, race (in cattle) and origin of the animals was collected. Swine were 4 to 6 months fattening pigs of both sexes (castrates).

Blood samples were collected by jugular vein punction of cattle, and stick wound of swine. The samples were transported the same day to the laboratory, located in the Faculty of Microbiology, immediately centrifuged and serum withdrawn and kept at -70°C until the analysis within 3 months.

Serologic Analysis: The sera were tested by indirect fluorescent antibody technique (IFA) (Garin and Ambroise 1963).

Sera were examined in twofold serial dilutions starting from 1:10 dilution. A positive reaction at the dilution 1:20 or more was considered as significantly positive (Boch and Neurohr 1982, Sousa *et al.* 1988).

Antigen was prepared from *T. gondii* RH strain cultered intraperitoneally in outbred Swiss albino mice (NIH, Bethesda, MD). After 3 days, peritoneal exudate was collected and treated according to the method of Garin and Ambroise-Thomas (1963). Briefly, this method consists of washing the peritoneal exudate with PBS buffer, separating by centrifugation the host cells and suspending a dilution of the parasite in multispot slides (Bio Meriaux, Lyon, France), which were dried and stored at -20°C until use.

Immunofluorescence: The IF method was carried according to the method of Garin and Ambroise-Thomas (1963). Briefly, acetone fixed tachyzoites were covered with different serum dilutions, washed and covered again with an appropriate solution of fluorescent antiswine IgG immunoglobulin (Fluorescein-conjugated rabbit anti-swine immunoglobulins Dakopatts, Copenhagen, Lot #119, F/P ratio=2.3) or anti-cow IgG immunoglobulin (Fluorescein-conjugated rabbit anti-cow immunoglobulins Dakopatts, Copenhagen, Lot #117, F/P ratio =2.3) respectively, of known titer, diluted in 0.2% Evans blue PBS solution.

The slides were mounted in alkaline buffered glycerol and examined in an epifluorescence microscope.

Positive and negative controls were gently provided by Arvid Uggla, from the Veterinary Faculty of the Swedish University of Agricultural Sciences.

RESULTS

The overall prevalence of antibodies towards *T. gondii* was of 34.4% in cattle and of 43.8% in swine (Table 1).

The most common antibody titers to *T. gondii* found in cattle were 1/20 (23.5%) and 1/40 (8.3%) and in swine 1/20 (26.2%) and 1/40 (10.3%).

No statistical difference was found in the prevalence of antibodies to this parasite in males and females, both in cattle or swine.

Also, there was no statistical difference between the prevalence of antibodies and the age groups in swine (Table 2).

In cattle the highest percent of seropositivity was obtained between the first and third years of age (Table 3), after which a decrease in the percent of seropositivity was observed.

Puntarenas and Limón were the provinces with the highest number of seropositive swine (Table 4), while these same provinces and Guanacaste had the highest number of seropositive cattle.

The prevalence of antibodies to the parasite according to the race of cattle is shown in Table 5. In this sample, the "criollas" or national cattle presented 40.8% of positivity, Holstein 34.4% and Cebú 23.8%.

TABLE 1

Origin of 601 cattle and 496 swine included in the study

Province	Cattle percent	Swine percent
San José	14.6	15.3
Alajuela	15.1	15.5
Heredia	14.3	15.7
Cartago	13.8	7.1
Guanacaste	13.6	13.9
Puntarenas	14.1	18.8
Limón	14.3	13.7

TABLE 2

Prevalence of IFA antibodies to T. gondii *in swine from Costa Rica, according to age*

Age (months)	Posit./total
4.0-4.5	16/38
4.6-5.0	65/122
5.1-5.5	61/134
5.6-6.0	74/201

TABLE 3

Prevalence of IFA antibodies to T. gondii *in cattle from Costa Rica, according to age*

AGE(YEARS)	Posit./total
1	54/152**ab**
1-2	7/26**a**
2-3	53/155
3-4	49/171**b**
4-5	18/59

a. There is statistical difference between 1 year and 1-2 years of age
b. There is statistical difference between 1 year and 3-4 years of age

TABLE 4

Prevalence of IFA antibodies to T. gondii *in swine and cattle from Costa Rica, according to the province of origin*

Province	Cattle Posit/total	Swine Posit/total
San José	26/88	37/76
Alajuela	23/91	33/77
Heredia	25/86	28/78
Cartago	26/83	10/35
Guanacaste	33/82a	22/69
Puntarenas	33/85a	53/93a
Limón	41/86a	39/68a

a. $p<0.05$ respect to the other provinces

TABLE 5

Prevalence of IFA antibodies to T. gondii *according to the race of cattle*

Race	Posit./total
"Criolla"	87/213
Brahman	46/141
Gyr	5/27
Holstein	54/157
Cebú	15/63

DISCUSSION

Cattle and swine raising for meat production is economically important in Costa Rica (IFAM 1983). Earlier surveys of *T. gondii* in the country report prevalences of 12.4% in cattle by carbonimmunoassay (CIA) (Rodríguez *et al.* 1991), and 12% and 26% in swine by biological isolation and CIA respectively (Ruíz 1966, Torres *et al.* 1991), lower than the obtained in this study. The average prevalence in this study was of 34.4% in cattle and 43.8% in swine. Our results are similar to those obtained in many countries of the world (Boch *et al.* 1982, Uggla and Hjort 1984, Dubey 1985). We therefore conclude that the previously reported lower prevalences in Costa Rica reflect the low sensitivity of the assays used. The high sensitivity and specificity of the assay used is well documented (Suzuki *et al.* 1965, Munday and Corboud 1979).

The finding of either moderate or high antibody prevalences to *T. gondii* in swine sera correlates with the presence of tissue cysts in muscle (Friche *et al.* 1984). The association between human toxoplasmosis and the consumption of infected meat from this animal is well established (Weinman and Chandler 1956). Tissue cysts are seldom found in naturally infected cattle, despite an often recorded presence of specific antibodies in serum (Dubey 1985). This could be explained either by a lower resistance of swine to the parasite, or a greater opportunity of infection since cattle usually is farther from houses than swine. Also, pigs are omnivores and often live in unhygienic conditions, while cattle are herbivores and the hygienical conditions that surround them are better (Catar *et al.* 1969, Okolo 1985). In cattle, the sensitivity of the test used for the isolation of the parasite may have not been high enough for detecting dispersed parasites, with a more sensitive technique as PCR, probably the parasites will be found.

All animals examined from a specific particular farm (swine or cattle), with few exceptions, were either all positive or all negative to *T. gondii.* This can be explained based in the different hygienical conditions in the farms and also to other preventive measures that can be taken by the owners as the absence of cats, the use of concentrates as food source and not of garbage, etc.

No significant statistical differences were found in the antibody prevalence between females and males either in cattle and swine, which makes us conclude that the infection probabilities are the same for both species. Same has been reported in earlier works in Costa Rica (Rodriguez *et al.* 1991, Torres *et al.* 1991) and Sweden (Uggla and Hjort 1984).

Animals acquire early in their lives the infection with *T. gondii* (Tables 2 and 3), although the swine studied were young (4-7 months of age), materno-fetal transfer of antibodies can be excluded, since the pig placenta does not allow the passage of the IgG (Pond and Houpt 1978). In swine, the same prevalence was observed in young and older animals, but cattle did show statistical difference between ages. Similar results have been reported by Rommel *et al.*, Beverly *et al.*, Friche *et al.*, Dubey (Rommel *et al.* 1966, Beverly *et al.* 1977, Friche *et al.* 1984, Dubey 1985). This may be due to a decrease in the antibody level some time after infection, to a point inferior to the detection range of the assay as described by Catar *et al.* (1969).

Evaluating the prevalence of antibodies to *T. gondii* in both animal species according to the provinces, differences were found in the percentage of seropositive animals. Limón and Puntarenas in both species presented the highest percentages (Guanacaste also in cattle), (Table 4). This suggests a relationship between the positivity of the animals used as meat source and the geographical region, which can be explained based on the coastal location, the tropical climate and the low altitudes of this provinces. All these factors have been described as favorable for the parasite development (Feldman and Miller 1956, Wallace 1969).

The high prevalences reported, especially in cattle, could be due to cross reactions especially with *Sarcocystis* species. Even if we can not rule out the possibility that the low positive values are due to *Sarcocystis*, this seems unlikely since the prevalence of this parasite is low due to its life cycle that does not allow a direct infection of the definitive host (Frenkel 1974)

Criollas and Holstein races presented higher positivity, but due to the predominance of these races, differences are not significative (Table 5).

We conclude that the demonstration of a high prevalence of antibodies to *T. gondii* in animals used as meat source corroborates the important role they play in the infection mechanism of toxoplasmosis in Costa Rica. Earlier work by Frenkel and Ruiz (Frenkel and Ruiz 1980) have suggested the oocyst transmission as the most important path of *T. gondii* in Costa Rica. Since a high prevalence of antibodies to the parasite was observed, and also, the parasite has been isolated from swine (Friche *et al.* 1984) and cattle raw meat (Dubey 1992), we conclude that the infection path in Costa Rica is not only due to oocysts shed by cats but also to the consumption of tissue cysts in raw or undercooked meat.

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RESUMEN

Se analizaron 496 muestras de sueros porcinos y 601 de sueros bovinos provenientes de las siete provincias de Costa Rica, mediante la técnica de inmunofluorescencia indirecta (determinando anticuerpos contra *Toxoplasma gondii)*, para estudiar el posible papel que ejerce la carne de estos animales en la transmisión del parásito. La prevalencia total de anticuerpos en ganado fue de 34.4% y de 43.8% en cerdos, no encontrándose diferencias significativas en la prevalencia de anticuerpos entre machos y hembras de las dos especies. Aunque ambos grupos parecen adquirir a temprana edad la infección con este parásito, sólo en bovinos se encontraron diferencias significativas respecto a la edad de los animales; en éstos se presenta un alto porcentaje de seropositividad durante el primer y tercer año de vida. Las provincias con mayor número de animales seropositivos fueron Limón y Puntarenas para ambas especies, mientras que en Guanacaste el ganado bovino presentó mayor positividad.

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