

## Seroepidemiology of Toxoplasmosis in humans: possible transmission routes in Costa Rica

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**Abstract:** A serological survey with immunofluorescence techniques to detect toxoplasma antibodies was done on a sample of 1234 Costa Ricans. The overall prevalence was 76%; it increased from 60% in donors aged 1-4 years to 90% in those over 25 years. No significant difference was found in seropositivity between males (42.8%) and females (57.2%) and between donors from urban and rural (LPI) areas. Consumption of raw meat, especially prepared meat, was significantly correlated with antibody prevalence, but cat contact was not, indicating a possible change in the usual pattern of transmission described for the country. Nevertheless, our data suggest that infectious cat feces play an important role in the transmission of toxoplasmosis.

**Key words:** *Toxoplasma gondii*, immunofluorescence, seroepidemiology, prepared meat, cats.

*Toxoplasma gondii* is an intracellular sporozoan, causing toxoplasmosis in man and other animals throughout the world. However, there are important geographical variations in its prevalence in humans. The prevalence of antibodies against this agent varies from 50 to 90% in Central America and Panamá (Gibson and Coleman 1958, Walton 1967, Remington *et al.* 1970).

Seropositivity of populations for *T. gondii* has been analyzed in reference to climate, altitude, occupation, rural or urban environment, ethnicity and individual habits (Feldman and Miller 1956, Wallace 1969). Highest prevalences are found in moist tropical areas.

The two major routes of infection are by ingestion of parasites in undercooked meat and of oocysts shed by infected cats. The latter was considered by Frenkel and Ruiz (1980) as being the principal route of infection in Costa Rica, due to the high number of stray cats found in the studied locations (Frenkel and Ruiz 1980). In contrast, ingestion of contaminated meat is a major route of infection in in-

dustrial countries (Desmonts *et al.* 1965, Feldman and Lamb 1968, Stray-Pedersen and Lorentzen-Styr 1980).

The possible role of contaminated meat in the transmission of toxoplasmosis in Costa Rica is unknown, where consumption of sausages and other prepared meats is a common practice. Processing of these types of meat that usually contain beef and pork, includes curing with salt, sugar and nitrite and/or nitrate, smoking and cooking. Federal regulations in the USA specify a final internal temperature of 58.3°C and in practice, most packers use 60°C (Anonymous 1981). However, there are no specific norms in Costa Rica for the preparation of sausages and similar products. The only general norm specifies that the total bacterial plate count should be less than 100 000 bacteria/g, and that no *Escherichia coli*, *Salmonella* sp., *Clostridium* sp. or *Staphylococcus* sp. be found in 0.1g of product (Morales 1985). Sausages are stored short periods after manufacture and purchase and may not be heated at all before consumption.

We decided to study the presence of antibodies against *Toxoplasma* in a sample of the Costa Rican population and the correlation between antibody titers and meat consumption, because recent studies have demonstrated the presence of *T. gondii* in freshly cut beef (Dubey 1992, Arias *et al.* 1994). We wanted to test the hypothesis that beef and prepared meat consumption represent another means of transmission of toxoplasmosis in Costa Rica.

## MATERIAL AND METHODS

A randomly selected sample consisting of 1234 sera from Costa Rican inhabitants was collected throughout the country from July to August, 1991. The selection was done according to the number of healthy persons under 30 years of age that assist to the clinics of the Caja Costarricense de Seguro Social. Location of residence for serum donors is given by province and county of origin and they were classified as coming from urban and rural areas according to anonymous (1983). For small children, information was obtained from their parents.

Swiss albino mice (National Institute of Health, Bethesda, Maryland) were infected with *T. gondii* RH strain and their intraperitoneal exudates were used as source of tachyzoites for IFA according to the method of Garin and Ambroise-Thomas (Garin and Ambroise-Thomas 1963). Briefly, the tachyzoites were washed with phosphate buffer saline (PBS pH 7.6); host cells were separated by centrifugation and tachyzoites were suspended in multispot microscope slides (bioMeriaux, Lyon, France), dried and stored at -20°C until use. For assays, slides were immersed in acetone for 10 min at room temperature, rinsed and covered with donor serum dilutions (1:16 to 1:4096) for 30 min at 37°C. Slides were then washed with PBS and covered with a 1:1000 dilution of fluorescent antihuman IgG immunoglobulin, (SBL National Bacteriological Laboratory, molar F/P ratio = 4.3, Lot #SH 8039905-1) and diluted in 0.2% Evans blue in PBS. The slides were covered with alkaline buffered glycerol (pH 8.6) and examined using an epifluorescence microscope.

WHO anti-toxoplasma reference IgG from the State Serum Institute, Copenhagen, Denmark was used as positive control and was in-

cluded in each slide. We regarded a serum as positive if a 1:16 dilution of serum uniformly stained the periphery of the tachyzoite. Localized fluorescence on the anterior pole only was interpreted as negative, since it represents a false positive result (Van Retgerghen and Van Nimmen 1971).

Statistical analysis was done using the chi square test, with Yates' correction for small numbers at 0.05 significance.

## RESULTS

The overall prevalence of antibodies against *T. gondii* in population sample sera was 76%. Seropositive rate increased in a statistically significant manner from 60% in children 1-4 years old to 90% in persons aged 30 or over. No statistical difference was found between males (42.8%) and females (57.2%).

TABLE 1

*Age and sex profile of a study population of 1234 Costa Rican individuals, 1991*

Age group	Sex			
	Male	% Seropositive	Female	% Seropositive
1-4	126	62	97	60
5-9	91	76	66	74
10-14	76	76	76	68
15-19	26	72	70	74
20-24	74	90	135	82
25-29	77	88	134	80
30 or older	58	92	128	86
Total	528		706	

When asked about present or past cat contact or ownership, 319 persons (25.9% of total sample) admitted it, 237 (19.2% of the total population) were seropositive. The other 915 denied cat contact, but 520 (56.8% of the total population) were seropositive ( $p \leq 0.0001$ ) (Table 2). In rural localities, seropositive rates were significantly higher for individuals who denied past or present cat contact (81.1% vs. 64.2%). There was no significant difference in antibody titers in urban areas between individuals with previous or actual cat contact and those without such contact (75.1% vs. 77.0%).

When we analyzed antibody titers in the total population regarding consumption of prepa-

TABLE 2

Percent of seropositivity for *T. gondii* in 1234 serum samples according to cat contact or ownership and prepared meat consumption

		Seropositive(%)*	Seronegative(%)
Cat contact or ownership	Yes	19.2	6.7
	No	56.8**	17.3
Raw or undercooked meat ingestion	Yes	59.6***	10.0
	No	16.4	14

\* Percentage of the total population

\*\*  $P \leq 0.0001$  between seropositive and seronegative persons without cat contact

\*\*\*  $P \leq 0.0001$  between seropositive and seronegative consumers of raw or undercooked sausages.

red meats, we found that 859 subjects (70% of the total sample) admitted eating undercooked or raw sausages, and 85.7% of them were seropositive ( $p \leq 0.0001$ ) (Table 2).

Young individuals (1-19 years old), who consumed raw or undercooked prepared meats showed significantly higher seropositivity (384/491, 78.2%) than those who did not eat such meat (51/137, 37.2%), ( $p \leq 0.05$ ).

## DISCUSSION

The overall prevalence of antibodies to *T. gondii* in this sample of the Costa Rican population was 76%, slightly higher than the 61.4% reported 15 years earlier (Frenkel and Ruiz 1980). The difference in prevalence reported in the two surveys may be due to different sampling techniques and the use of different serological assays.

In this survey, no significant difference in seropositivity was found between males and females. This is consistent with previous reports (Van Retgerghen and VanNimmen 1971, Omland *et al.* 1977, Ko *et al.* 1980, Griffil and Williams 1983), although others report sex differences (Walton *et al.* 1966, Huldt *et al.* 1979, Frenkel and Ruiz 1980, Ghorbani *et al.* 1981, Konishi *et al.* 1987). Frenkel and Ruiz believe sex differences found in their study are due to greater exposure of males to oocysts during their daily activities (Frenkel and Ruiz 1980).

The younger population (1-9 years old) acquired antibodies to *T. gondii* early in life. In contrast, Swedish children have a prevalence

lower than 10% (Huldt *et al.* 1979). The population under 20 years of age in USA have a prevalence of 10% compared to 70% in the Costa Rican sample of the same age (Feldman and Lamb 1968). In Europe and the USA, toxoplasmosis is acquired mainly by the ingestion of undercooked meat, as has been noted in surveys from France, North America and Norway (Desmonts *et al.* 1965, Feldman and Lamb 1968, Stray-Pedersen and Lorentzen-Styr 1980). In some countries a major route of transmission seems to be ingestion of oocysts from cat feces, as in Guadeloupe island and Costa Rica (Frenkel and Ruiz 1980, Ruiz and Frenkel 1980, Barbier *et al.* 1983). Our results show cat contact in 25.9% of individuals, 73% of whom present antibodies to *T. gondii*, in agreement with this idea. This low percentage of people referring cat contact may be due to the fact that Costa Rica currently has a lower number of cats than the ones reported before (Ruiz and Frenkel, 1980), or some individuals may have lied or forgotten it.

Those individuals who eat raw prepared meats have a significantly higher seropositivity than those who do not; 85.7% of those who eat them are seropositive, against 53.9% of those who do not. This suggests that the actual transmission pattern of *T. gondii* can also be related to meat consumption, as it is in industrialized countries.

A higher seroprevalence was present in those who eat raw or undercooked beef or pork. In both cases, there was a significantly higher seropositivity (88.9%), compared to 73.7% in those who did not consume such meats.

We suggest that prepared meat can represent a major source of infection with *Toxoplasma* in Costa Rica. In separate studies, we found the prevalence of antibodies to this parasite in meat producing animals is high [(54.4% in swine (n=496) and 41.6% in cattle (n=601)] (Rodríguez *et al.* 1991, Torres *et al.* 1991).

Research is currently in progress on the isolation of *T. gondii* from prepared meat.

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## RESUMEN

Con el fin de determinar la prevalencia de *Toxoplasma gondii* en la población y considerar posibles rutas de transmisión, se realizó un estudio serológico utilizando la técnica de inmunofluorescencia indirecta en 1234 costarricenses. Se determinó una prevalencia de anticuerpos a este parásito del 76%, la cual aumenta de un 60% en el grupo etario de 1-4 años a 90% en los individuos de más de 25 años de edad. No se encontró diferencia significativa en seropositividad entre hombres (42.8%) y mujeres (57.2%). Tampoco se hallaron diferencias significativas entre los individuos provenientes de localidades urbanas y aquellos residentes en áreas rurales. El consumo de carne cruda, especialmente embutidos, correlacionó significativamente con la prevalencia de anticuerpos, pero no así el contacto con gatos, lo cual sugiere un posible cambio en el modo tradicional de transmisión de este parásito en Costa Rica. No obstante, nuestros datos sugieren que las heces de este animal también forman parte importante de la transmisión del parásito.

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