

Current status in Costa Rica of toxoplasmosis transmission through meat products

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Abstract

Background: Toxoplasmosis is usually an asymptomatic chronic disease which can eventually cause problems for humans, such as abortion and eye damage. Therefore, it is important to know about possible infection sources for humans. The objective of this study is to review the literature related to transmission through meat products and to compare the infection level for *Toxoplasma gondii* in meat for human consumption to that occurred 10 years ago.

Methods: laboratory animals were fed with ground meat or chorizo (a type of sausage) obtained from various suppliers of this product and immunological techniques were used to determine whether the animals' contamination with the parasite came from eating such products.

Results and conclusions: In 4% of the animals fed with ground meat or sausage, there were traces of antibodies against *T. gondii*. This result is significantly lower than the one reported twelve years ago using the same method. After a historical analysis of the subject, the conclusion is that there has been a reduction in meat infected with *T. gondii*.

Keywords: Toxoplasmosis, *Toxoplasma gondii*, transmission, epidemiology, meat.

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Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii*, belonging to the Apicomplexa group that although usually occurs as a latent-type infection in human beings, under certain circumstances it can cause important problems such as eye injuries or abortions.^{1,2}

The more severe problems will manifest in newborns² and in cases where there are significant immunosuppressants treatment-induced immune problems^{3,4} or due to weakening diseases such as cancer, leukemia and AIDS, etc.⁵ In the latter, infections tend to be disseminated and can mean the final cause of very serious injuries and even death.⁶

For the tendency towards chronic or latent stages of this disease in Costa Rica, it is not given much importance as in other countries that normally performed epidemiologic, immunologic and pathologic type studies.⁷ Thus; there are prevalence studies with some regularity in many countries¹ even in European regions. From these studies emerge a prevalence data that goes from 22 to 98%. The most recent data in Costa Rica were those from Zapata *et al*⁸ with a positivity of 58% in a study performed on adults. In relation to the transmission of *T. gondii* in Costa Rica, the most complete initial epidemiological studies leaned toward the establishment of infection by ingestion of oocysts expelled in the feces of cats, after its maturation in the ground.^{9,10} But later studies showed transmission patterns a little bit different in Costa Rica, where meat products started to represent important elements in the process of transmission.¹¹ Even these findings led to the formulation of a new hypothesis about the epidemiology of toxoplasmosis in this country.¹²

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In Costa Rica, studies performed by Madrigal *et al.*¹³ added sausages as a potential source of infection for this parasite in human beings, this aspect was also contemplated in Reyes *et al* work,¹³ proposing a new model of transmission.

Given this, grounded meat was chosen, a product derived from bovine and sausages made with pork (chorizo), both very common in the diet of a good number of people in the general population, to determine its potential as an infection source for toxoplasmosis. The results obtained in this study and the comparative analysis of these with similar observations in previous years, are the main reason for this work.

Methods

Fifty samples of 200 g of grounded meat and another 50 similar samples of chorizo were studied, collected in diverse selling points, including supermarkets and some independent butcher's shops from the great metropolitan area. In these places, the hygiene conditions of polls and the cooling condition where the meats were, as well as these polls surroundings, also the presence or absence of flies in the surrounding areas. Based on these observations, the places were graded in good, fair and poor.

An *in vivo* model was used for the possible detection of *T. gondii*, previously described¹⁴ using laboratory animals. To made this model, white Swiss mice were used, to which were administrated a certified commercial concentrate and ad libitum water, maintain in any case according to the regulations issued by No 7451 Law of Animal Welfare. A group of 5 animals for every sample were fed with uncooked meat, with a prior 18 to 24 hours fasting period, with the objective of promoting the intake of the products under study. The mice remained in contact with the supplied material in a period of time require to ensure the full consumption of it. All the animals in our laboratory were monitored regularly for the presence of *T. gondii*, so the negativity for this parasite is guaranteed.

After two months, the mice were sacrificed and bled from the heart to obtain the serum which is then subsequently be studied for the presence of antibodies against *T.gondii*. Also, under the microscope, between blade and slide, 6 portions took from different regions of the brain of each animal were checked, in order to determine if there were cysts of the parasite. Additionally, portions of brain, lung, liver, spleen, heart, kidney, diaphragm and pectoral muscle, and the eye were fixed in 10% formalin. Histological sections stained with hematoxylin-eosin were studied for the presence of parasite.

The serum obtained was studied through CIA (Carbon Immuno Assay) for sensibility and specificity,¹⁵ as well as

with Sabin-Feldman confirmatory technique.¹⁶ Both techniques are widely known and had been described in multiples studies. As a positive control, positive serums for *T. gondii* were used (American Type Culture Collection, ATCC, 50174). Tachyzoites from RH strain were used in CIA tests.

The tests were repeated in triplicate to validate the results, which were compared with those of previous studies and discussed from the epidemiological point of view of toxoplasmosis.

Results

Of the 100 CIA tests, 50 were from meat and 50 from chorizo, 4 of each groups were positive. From these eight samples, two from each product were confirmed with Sabin-Feldman test. This data indicated a positivity percentage of 4% for each meat product studied (Table 1). To the same samples studied with CIA tests, we performed the Sabin-Feldman test (dilutions of 1:2 to 1:16), where only 4 of the 8 samples were tested positive.

Looking at table 2, we can appreciate that the majority of the analyzed samples of both, meat and chorizo, came from independent establishments. Also, the positive ones with Sabin-Feldman confirmatory test came from the same type of establishments.

In relation with the histological analysis of the tested animals' organs, a direct evidence of infection for *T. gondii* was not found, however it is important to emphasize that the reviewed 6 sections from each animal brain were also negative.

In table 3, some additional data is added, according to the place's hygiene where the samples were collected. As it can be seen, from the 50 meat samples analyzed, 48% of the establishment had a good appearance and all were kept cooling chambers; on the contrary, chorizo samples from 54% of the establishments looked in fair or bad conditions. Furthermore, only 32 of 50 establishments conserved the products in cooling chambers, which leads to the presence of flies in a greater degree than with meat.

Table 1. Serology results according to CIA and SF tests on mice inoculated with meat products.

Samples	No	Positivity			
		CIA		SF	
		No.	%	No.	%
Meat	50	4	8	2	4
Chorizo	50	4	8	2	4
Total	100	8	8	4	4

CIA, Carbon Immuno Assay; SF, Sabin y Feldman also called Dye Test.

Table 2. Sample distribution by commercial establishment

Type of Sample	Total	Origin	Positivity*			
			N°	%	N°	%
Meat	50	Supermarkets	20	40	0	0
		Independent establishments	30	60	2	4
Chorizo	50	Supermercados	14	28	0	0
		Independent establishments	36	72	2	4

*Positivity for Sabin-Feldman

Table 3. Samples conditions in the establishments

Type of sample	Total of samples	Conditions									
		*Appearance						†Cooling chambers		†Flies	
		G		F		B		N°	%	N°	%
N°	%	N°	%	N°	%						
Meats	50	24	48	23	46	3	6	50	100	5	10
Chorizo	50	23	46	17	34	10	20	32	64	24	48
Total	100	47	47	40	40	13	13	82	82	29	29

†Presence of them.
 *G: good, the staff takes all the necessary hygiene measures (white coat, gloves, boots, hair cap); F: fair, although necessary measures are taken into account, the appearance of the place is not adequate; B: bad, the staff doesn't take the necessary hygiene measures (meat products manipulation without prior hand wash).

Discussion

The original concept for *T. gondii* transmission involved the ingestion of meat products as the main system, especially since Jacobs¹⁷ demonstrated the presence of the parasite in hamburgers. Subsequently and following the elucidation of *Toxoplasma* life cycle, in which it was determined that one of the developmental stages is the oocyst that is eliminated in the feces of cats;^{18, 19} it was considered that the ingestion of the oocysts from the ground, were another route of ingestion in the human being. Such hypothesis were postulated in Costa Rica in very complete epidemiological studies where meat products were not taken into consideration as a source of infection.^{9,10} Rather, it was implied that the ingestion of this parasite oocysts, were the most important system of infection for the human being. However, in latter studies, the presence of *T. gondii* in meat and pork was confirmed,^{20,21} opening the possibility for these foods as another important source of infection.

These findings, plus others found positive for the presence of this parasite in certain type of sausages, induced studies to be performed, in relation of the prevalence of toxoplasmosis

in human beings with the ingestion of meat products.²² Knowing the presence of *T. gondii* oocysts on the ground, in relation to the greater or lesser presence of cats, that disseminate those stages on the ground, also Arias *et al*²² demonstrated that such prevalence was related always with the presence of meat and sausages ingestion, independently of the number of these felines.

All of above, lead to the confirmation of the new hypothesis proposed by the authors, of a new concept for the transmission of this parasitic infection in Costa Rica¹² were the ingestion of meat and some sausages acquired increasing importance in the transmission of toxoplasmosis. However, given the increase in the export of meat, it was establish a new and more strict control measures in the management of these and consequently of sausages.^{23, 24}

The findings informed in these recent studies, although scarce, related to the conditions of the places of collection, with them seems to be demonstrated that these measures have influenced, at least in part, in reducing the presence of *T. gondii* in those foods. In fact it took 100 samples, 50 of grounded meat and 50 of chorizo to find only 4% of positivity in each of the products studied. This contrasts markedly with similar studies in the past,^{13, 21} where positivity observed was greater (8-40%), even when obtained with a lower number of samples of 38 and 40, lower than the one used in this study.

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