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ON THE ISOLATION OF *TOXOPLASMA GONDII* FROM HUMAN FOOD OF ANIMAL ORIGIN. PARTIAL RESULTS IN THE CITY OF SÃO PAULO (BRAZIL)

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SUMMARY

An effort is being made to evaluate the importance of food of animal origin as source of toxoplasma infection for the human population of the city of São Paulo. The methods used are described together with results so far obtained, in food purchased in markets or butcher shops.

A total of 292 samples of meat, viscera and eggs was examined. The parasite was isolated from 6.8% of the pork samples. The possibility is discussed that the low titer dye tests among mice receiving the digest of other samples (another 6.8% of the pork, 8.1% of the beef and 1.9% of the eggs) represented specific responses to inoculation of dead parasites. The rates of infection found among the livestock or in samples collected shortly after the animals are slaughtered may not reflect the actual risk of getting the infection from meat or viscera bought in markets or butcher shops.

INTRODUCTION

Among the several methods of transmission of *Toxoplasma gondii* that might occur in nature, the most satisfactorily confirmed in laboratory is the ingestion of parts or products of infected animals [7, 8, 11, 18]. Circumstantial evidence that this may be also an effective mechanism among humans, is furnished by repeated isolations of the parasite from muscles, viscera, milk and eggs widely used as human food [2, 12, 14, 17, 21, 24]. According to some Authors [3, 7, 26] non-congenital toxoplasmosis would be acquired chiefly by consuming raw or undercooked meat. However, feeding habits being extremely variable, toxoplasmosis of common meat animals may have little significance for some populational groups.

In Brazil, a few surveys have been made using the dye-test (DT), either alone [23], or with the skin test [5, 6, 19]. They included extremely poor, sparse populations of the equatorial Amazon region, whose diet (chiefly manioc and fish) cannot be compared with that of the cosmopolitan population of the Southern city of São Paulo — not even with that of the latter's crowded slums. Although the percentage of positives has been of about 70 for each group, the epidemiology probably differs from one place to the other.

In trying to evaluate the importance of food as source of toxoplasma infection for the human population of the city of São Paulo, we started by testing working methods. The methods used and the results so far obtained are presented and discussed in this paper.

MATERIAL AND METHODS

Our purpose being to evaluate the importance of meat and other animal products as used for food, samples for examination were

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purchased in markets and butcher shops, such as they are by the population. No effort was made to know about the source of the animals, but we assume that they were slaughtered, processed and stocked within city limits, except for the mutton, that — so we were informed — was imported frozen from another state.

The choice of types of meat or viscera was based on known people's preferences for undercooked pieces. Mutton was included only when a campaign was being made to popularize its consumption in the city. Types of meat (e.g. chicken), viscera and other foods that are usually well cooked or pasteurized (dairy products) were not included, but samples of raw pork sausages were examined. As for eggs, part of them were from chicken farms and bought in markets, the rest were of the "caipira" type, purchased from courtyard breeders in the low income urban areas.

Since the samples were obtained in different shops and on different days through more than a year, each one comes, most probably, from a different animal. In the laboratory, before being processed for inoculation, they were kept in a refrigerator for periods of not more than 30 hours.

A sample of about 100 g was cut of each piece of meat, finely ground and submitted to peptic digestion according to JACOBS & MELTON'S method [14]; treatment of the digest followed the same method but, at final resuspension in saline, total volume was kept at about 5 ml, 1 ml being inoculated into the peritoneum of each of 2 adult mice. Fresh preparations of the inoculum were examined under the microscope.

The following scheme was tried for the follow-up of the inoculated mice:

a) daily observation; examination of the peritoneal exudate of mice dead or dying after 3 days of inoculation;

b) on the 15th day one mouse was killed, blood collected for the DT, and fresh preparations of peritoneal exudate and crushed pieces of brain examined; pieces of liver and brain were fixed for sectioning and the rests were ground in a mortar, suspended in saline and inoculated into the peritoneum of 2 clean mice which were followed as above;

c) on the 30th day, the 2nd mouse of the lst passage (30 days after inoculation) and one of the 2nd passage (15th day after subinoculation) were killed and examined as above, but not subinoculated;

d) on the 60th day, the last mouse of the 2nd passage (30 days after subinoculation) was killed and examined as above.

Futher passages were only made of positive material, to maintain the strain.

After some experience in examining meat and other materials (in a parallel work), the scheme was simplified: follow-up was as above, but the 2 mice of the lst passage were killed on the 30th day and their blood and brain collected separately; each brain was ground in a mortar with some saline, several fresh and dry slide preparations being made; the pooled material of both brains was then inoculated into the peritoneum of 2 clean mice; on the 60th day (30th of the subinoculation), the 2 mice were killed and examined as above, except that after small pieces of brain had been taken and crushed for fresh and dry preparations, the rest was fixed in formalin for eventual sectioning. No further passage was made, unless the maintainance of a strain was desired.

If the DT made with the serum of any of the 4 mice was positive, and the fresh preparations had been negative, the fixed material was throughly examined.

For comparison, part of the material was submitted to two other inoculation schemes: a) the whole washed digest was inoculated, in 4 or 5 mice which were, thereafter, followed as the others; b) two 100 g samples of the same piece of meat were processed separately and inoculated and followed as if they were from different animals.

Sausages and viscera were processed as the meat, except that brain was left in the incubator for 1 instead of 2 hours, since under the microscope the material seemed well digested within that period.

The following methods were used for the eggs: 1) the whole yolk and the membranes were poured into a flask with glass beads and

vigorously shaken before the artificial gastric juice was added; the suspension was maintained for 1 hour in an electrical shaken, at 37°C, centrifuged, washed and inoculated as the other material; or, 2) from the majority of the eggs most of the yolk was discarded and the membranes were ground with saline in a mortar and inoculated as the other samples.

DTs were made with undiluted serum first and, if positive, with increasing dilutions.

RESULTS

So far 309 samples of food have been processed and inoculated, but 17 of them were lost, chiefly because of premature death of the mice due to causes other than toxoplasmic infection. The 292 samples that could be followed were distributed as shown in Table I.

In Tables II and III the data on samples that gave positive results are detailed.

TABLE I

Results of examination of food samples to detect the presence of *Toxoplasma gondii*

Samples	no. examined	With any evidence of positivity (x)		Parasite isolated from		Probable no. of animals involved
		no.	%	no.	%	
Pork	73	9	12,3	5	6.8	83 pigs (+)
Pork sausage ..	10	—	—	—	—	
Beef	37	3	8.1	—	—	
Cow's liver	13	—	—	—	—	98 cows
Cow's brain ...	48	—	—	—	—	
Mutton	10	—	—	—	—	10 sheeps
Eggs	101	2	1.9	—	—	101 chickens

(x) Positive dye test and/or parasite found among the inoculated mice
(+) Minimus number, since meat for pork sausages probably comes from several pigs

TABLE II

Positivity of food samples as evidenced by the dye test among inoculated mice

Samples	no.	Positive in lst passage only		Positive in both passages	
		Dilutions $\leqslant$ 1/16 only	Parasite found	Dilutions $>$ 1/16	Parasite found
Pork	73	4	—	5	5
Beef	37	3	—	—	—
Eggs	101	2	—	—	—

TABLE III

Detailed results on the samples of pork from which *Toxoplasma gondii* was isolated

Sample no.	1st passage (2 mice / each sample)				2nd passage (2 mice / each sample)			
	Killed on day 15		Killed on day 30		Killed on day 15		Killed on day 30	
	DT titer	Toxo. cysts	DT titer	Toxo. cysts	DT titer	Toxo. cysts	DT titer	Toxo. cysts
9	1/4	—	1/4000	+	1/256	—	1/4000	+
12	1/1024	—	(x)		1/256	—	1/8000	+
16	1/4000	+	1/32000	+	1/1024	+	(x)	—
25	—	—	1/1024	+	(x)		—	—
			1/16000	+	(x)		—	—
218	1/1024	—	1/128000	+	—	—	1/1024	+
					—	—	1/16000	+
218 a	1/1024	+	(x)		(x)		1/32000	+

(x) Mouse found dead before this period; in those of the 2nd passage that still could be examined, the peritoneal exudate was positive

Note: Numbers 218 and 218a correspond to 2 different samples of 100 g taken from the same piece of pork; in the previous Tables they are counted as *one* positive sample of pork

The digest of practically all samples of pork, pork sausages, beef and mutton were extremely rich in *Sarcocystis spp.* sporozoites and a total of 309 mice received large quantities of this parasite in the peritoneum. On the other hand, 293 mice were inoculated with material from eggs, liver or brain, and 572 were subinoculated with organs from lst passage mice, what means that 865 mice received inocula free from *Sarcocystis*. Results of the DT among the two groups are presented in Table IV.

From each of 15 pieces of pork, two 100 g samples were processed separately; only 2 samples were positive and they both came from the same piece, that is, from a single animal. In both instances the DT was positive and the parasite was found in mice of the lst and the 2nd passages.

From 15 other samples of pork or mutton, the whole digest was inoculated, a little over 1 ml in each of 4 mice, or, more often, 1 ml in each of 5 mice. This material is also being submitted to more than 2 passages, but up to now it has been all negative.

DISCUSSION

Since we had in mind to test methods that would economize the time of a well trained technical worker (who is also helping in other programs), we felt the need to begin with the smallest number of animals that could give reliable results. On the other hand we also wanted to multiply the chances of actually finding the parasite. That is why we used 2 adult mice, both receiving about half of the total digest of each sample of meat or viscera, and 2 other mice for a 2nd passage, made before the result of the DT of the first mice was known.

In no instance did a positive result appear only in 2nd passage mice, but the latter were a useful group to confirm positive findings, to give an idea of the virulence of the strains

TABLE IV

Dye tests among 1174 mice included in the investigation, plus 100 controls. Distribution according to the presence of *Sarcocystis spp* in the inoculum and the isolation of *Toxoplasma gondii*

Sarcocystis spp. in the inoculum	Passage	Dye — tested mice			
		no.	Negatives %	Positives	
				no.	*T. gondii* isolated %
+	1st	309	92.8	22	40.9
—	1st	293	98.9	3	0
	2nd	572	98.9	8	100
Control (clean) mice		100	100		

and to point to a probable explanation for the low titres DTs among 1st passage mice.

We do not think that the inoculation of a larger number of mice would have significantly modified our results, since:

a) when there was any evidence of positivity for one mouse of the 1st passage, this was true for the other mouse, except in 3 occasions in which the only evidence was a low titer (undiluted serum, 1/4 and 1/16) dye test in one of them; b) inoculation of the entire digest into 4 or 5 mice did not improve the results; c) in a parallel work which includes isolation of toxoplasma from other animals and materials, results when positive, are so for all the inoculated mice.

Our present experience with different materials is in accordance with that of Authors who find that the DT is a very sensitive method to detect infection in mice and that several successive passages are usually unnecessary for the isolation of the parasite [20].

There are already several reports on the isolation of toxoplasma from meat animals, through mouse inoculation of ground bits of brain, or of the digest of skeletal muscles or viscera taken in slaughter houses. Some results taken from the literature are listed in Table V.

Toxoplasma has also been found in eggs: PANDE et al. [24] isolated the parasite from 4 out of 42 eggs laid by infected chickens during a toxoplasmic epizooty, and JACOBS & MELTON [13] had 1 positive out of 108 eggs taken from 108 chickens examined at a poultry processing plant; 4 of these chickens were found to have chronic infection. On the other hand, these Authors found only 1 positive out of 327 eggs laid by 16 chickens with experimental chronic infection, and none of 2214 eggs laid by 32 chickens with recent or chronic experimental infection was positive in a study by BOCH [2].

The above examples illustrate the great variation in infection rates, even if the same animal species be considered and the same diagnostic method employed. This variation should be expected since infection in the livestock has been found to differ from herd to herd and to be influenced by seasons [12].

Of the 73 pork samples examined by us through a period of almost 2 years, 5 were positive (4 with isolation of the parasite) among the first 25 (20%), between the months of March and August; the other 5 (one with isolation of the parasite) were scattered among the 48 other samples examined throughout many months, during which

TABLE V

T. gondii infection of meat animals examined in slaughter houses. Examples taken from the literature

Percent infected among				Country	Reference no.
Swine	Sheep	Cows	Chickens		
20.8				Brazil	1
12				Costa Rica	25
5	22.5	—		Denmark	27
24				Formosa	28
9	12	—	0.3	Germany	2
4.9				Japan	10
	50*				
	83.3**	—		New Zealand	12
24	9.3	1.7	3.7	USA	17

* among those with a positive DT at 1/16 or 1/64
** among those with a positive DT at 1/256 or over

the parasite was being isolated from other materials.

The DTs found positive only in low titers ($\leqslant$ 1/16) among inoculated mice are taken as due to the presence of specific antibody, most probably produced as a result of the inoculation of dead parasites. This assumption is justified by the data on Tables II, III and IV, which show that:

1) those DTs were found in the lst passage only; with the 3 exceptions mentioned above, they were found in both lst passage mice;

2) the DT was entirely negative in 100 control mice examined in many small lots throughout a long period (that is, from many different litters);

3) although *Sarcocystis* sporozoites were abundantly present in practically all meat samples inoculated, there was no relationship between its presence in the inoculum and positivity of the DT.

Inoculation of killed toxoplasma is known to result in positive DT [4, 27] and, since each cyst may contain thousands of organisms [22], several cysts should provide enough antigenic material to evoke a reaction at least in some of the animals [27]. On the other hand, several Authors [2, 7, 9, 13, 16, 28] have demonstrated the low resistance of *T. gondii* to the usual conditions in which meat and other animal products are preserved or prepared for human consumption. Even when the meat is stored in low temperatures, this may be not sufficient to keep the parasite viable for long [2, 23]. Furthermore there is a common practice in butcher shops and markets, of exposing the meat — especially the beef — for hours, returning the unsold pieces again to the refrigerator and repeating the exposure next day. The product is then submitted to great variations of temperature which may result in death of the parasite, thus increasing the odds against the infection of humans through the digestive tract.

The rates of infection found among the livestock or in samples collected shortly after the animals are slaughtered, may not represent the risk in which a given population actually incurs — even if it has the habit of frequently consuming raw or undercooked meat.

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RESUMO

Sôbre o isolamento do Toxoplasma gondii *de alimentos de origem animal. Resultados parciais na cidade de São Paulo*

Tenta-se avaliar a importância dos alimentos de origem animal como fontes de infecção por toxoplasma para a população da cidade de São Paulo. Neste trabalho descrevem-se os métodos usados e os resultados até agora obtidos, no exame de alimentos adquiridos em mercados e açougues.

Foram examinadas 292 amostras de carnes, visceras e ovos. O parasita foi isolado de apenas 6,8% das amostras de carne de porco. Outros 6,8% das amostras de carne de porco, além de 8,1% das de carne de vaca e 1,9% dos ovos, provocaram, nos camundongos inoculados, reações de Sabin e Feldman positivas em títulos baixos ($\leqslant 1/16$). As Autoras discutem a hipótese de que estas reações positivas (sem isolamento do parasita) resultem da inoculação de cistos inviáveis. O hábito comum nos mercados e açougues, de se exporem as carnes durante horas, retornando à geladeira as peças não vendidas, para repetir a exposição no dia seguinte, pode provocar a morte do parasita que, mesmo na forma encistada, é pouco resistente.

É muito provável que as taxas de infecção em amostras colhidas nos matadouros logo após o abate dos animais, não reflitam o risco real em que incorre uma população que normalmente se abastece de carnes em mercados e açougues, mesmo que o produto seja consumido cru ou mal cosido.

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