# Detection of Toxoplasma gondii Antibodies in Different Meat Juices

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### ABSTRACT

Toxoplasmosis is one of the most important zoonotic diseases worldwide and it is caused by the protozoan *Toxoplasma gondii*, humans can get infected post-naturally either by uptake of sporulated toxoplasma oocysts or by ingestion of tissue cysts upon consumption of raw or undercooked meat.

The study included cattle, sheep and chicken of different age groups and housing conditions whenever possible and applicable. Direct Latex Agglutination test was used to detect *T. gondii* specific antibodies and to determine seroprevalences in meat juice of slaughtered animals. The total samples are 300 of domestic animals (cattle, sheep and chicken 100 samples each of them).

The results of Latex agglutination test are positive in 17% of cattle, 37% of sheep and 9% of chicken from meat juice. The examination of specific IgG and IgM antibodies was detected by Hydroxy ethylmercaptan (2-ME) test with used of meat juices and Compound of 2- Hydroxy ethylmercaptan which appear in cattle 17.9% of IgM and 82.1% IgG, in sheep 16.2% of IgM and 83.8% IgG and in chicken 11.1% of IgM and 88.9% IgG.

The aim of this study was to approximate the risk of human infection via meat consumption by estimating the seroprevalence of T. gondii in slaughtered animals in Hamdania.

Keywords: Toxoplasma gondii; Toxoplasmosis; Antibodies, meat juices .

Toxoplasma gondii

100	100		100	300		
37%	17%					
2- Hydroxy ethy	lmercaptan					9%
17.9% IgM	82.1%	IgG			IgM	IgG
88.9% IgG		16.2% IgM	]	IgG 83.8%		
				.11.1 %	b IgM	

### **INTRADUCTION**

*Toxoplasma gondii* is an obligate intracellular protozoan that infect human and a wide range of mammalian and bird (Smith and Reduck, 2000) the parasite is known to cause congenital disease and abortion both in humans and livestock (Dubey and Beattie 1988; Remington and Desmonts, 1990). Maternal toxoplasmosis during early pregnancy of human may leads to death of fetus or cause chorioretinitis, hydrocephaly, microcephaly and jaundice in neonates (Joynson and Wreghitt, 2001; James, 2003).

In its life cycle, domestic and wild felines are definitive hosts while human, other mammals and birds are its intermediate hosts, Intermediate hosts acquire the infection by many main routes like Ingestion of oocysts in soil, sand or any other place where cats defecate, being disseminated by means of transport hosts such as flies, cockroaches and worms; ingestion of cysts in raw or undercooked meat and transplacentary infection (Dubey and Beattie, 1988; Ruiz and Frenkel, 1980).

The tissue parasitism during the proliferative phase may occur without symptoms (Joan, 2005). It may lead to a transient illness characterized by lymphadenopathy, fever, fatigue, arthralgia, dermatosis, malaise, headache, and myalgia (Pinard *et al.*, 2005), any meat from warm-blooded animals and birds traditionally has been considered a major source of *T. gondii* infection in the world (Asgari *et al.*, 2006).This idea stems from small outbreaks associated with the consumption of undercooked meat and several epidemiological studies, the consumption of undercooked meat is the most likely source of infection (Choi, 1997; Cook, 2000; Tenter *et al.*, 2000).

### **MATERIAL AND METHODS**

### **1.** Meat samples

Meat samples (300 samples) were selected from a population collected for market and Hamdania slaughter the amount collected meat samples (100 grams) from each animals of cattle 100 samples, sheep 100 samples and chicken 100 samples, then deep freezing of these

meat samples at -18 °C and thawing at 20-25 °C and collect the meat juices about 2-5 ml in the tube. The study was approved in libratory of Dept. of Pharmacy, Institute of Technical in Mosul.

### 2. Serological test

#### **2.1.** Latex Agglutination Test (LAT)

Determination of *T. gondii* Antibody by latex agglutination test, depending on kit called Toxocell-latex with meat juices of cattle, sheep and chicken at room temperature by freezing individual meat samples at -18 C overnight, then thawing at 20-25 C and using a commercial test kit. This kit uses formalin-fixed whole tachyzoites as antigen, and has been validated for use with pork samples (Gamble *et al.*, 2005; Dubey *et al.*, 2005 ) for testing cattle, sheep and chicken tissue fluids of 0.05 ml of meat juices and 0.05 ml of kit in clean slights by micropipette and then mixed by circle movement for 5 minutes and reported the result by macro examination or micro examination.

## **2.2.** Indirect agglutination test

Positive samples of meat juices for toxoplasma antibody which selected in the first test, examined by Indirect agglutination test and 8 dilutions for selected meat juices were prepared.

### **2.3.** 2-Hydroxy ethylmercaptan

The compound 2-Hydroxy ethylmercaptan were used with selected positive meat juices for toxoplasma antibody, the 2-Hydroxy ethylmercaptan test identify the type of immunoglobulin IgG or IgM (Desmonts and Remington, 1980), 0.14 ml of (0.2 M) 2-Hydroxy ethylmercaptan were added to 10 ml volumetric flask and volume completed using phosphate buffer saline (pH=7.2) (Gould and Clegg, 1987) 0.1 ml of 2- Hydroxy ethylmercaptan were added to 0.1 ml of meat juices in clean test tube and incubated at (37 °C) for one hour.

### RESULT

The results appeared in table (1) of serological examination by direct latex agglutination test which appeared 17% were positive in cattle and 37% in sheep and 9% in chicken.

the results and of the direct latex agglutination test in table (2) showed that (17) sample positive in cattle, the titration of antibodies in meat juices, and in table (3) were show (37) sample positive of direct latex agglutination test in sheep meat juices, and in table (4) were show of (9) sample positive of direct latex agglutination in chicken meat juices The examination of specific IgG and IgM antibodies was detected by 2- Hydroxy ethylmercaptan test by using of meat juices and compound of 2- Hydroxy ethylmercaptan which appear in table (5) were positive of (17) of direct latex agglutination test in cattle 17.9% of IgM and 82.1% IgG, in sheep 16.2% of IgM and 83.8% IgG and in chicken 11.1% of IgM and 88.9% IgG.

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Table 1: Numeration study of meat juices of cattle, sheep and chicken samples infect	ted
with Toxoplasma gondii using Direct Latex Agglutination test.	

Meat juices	No. of samples	No. of positive	% of positive	No. of negative	% of negative
cattle	100	17	17%	83	83%
sheep	100	37	37%	63	63%
chicken	100	9	9%	91	91%

Table 2: Titration of antibody in meat juices cattle for positive infected with *Toxoplasma*<br/>gondii using Indirect Agglutination test.

No. of	No. of	Titration of antibody										
samples	positive samples In(LAT)	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
100	17	-	-	1	1	2	4	3	2	3	1	-

Table 3: Titration of antibody in meat juices sheep for positive infected with *Toxoplasma*<br/>gondii using Indirect Agglutination test.

No. of	No. of	of Titration of antibody										
samples	positive samples In(LAT)	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
100	37	-	-	1	3	2	4	5	13	7	1	1

Table 4: Titration of antibody in meat juices chicken of positive infected with *Toxoplasma*<br/>gondii using Indirect Agglutination test.

No. of	No. of		Titration of antibody									
samples	positive samples In(LAT)	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
100	9	-	-	-	1	2	5	1	-	-	-	-

Meat juices	No. of positive In (LAT)	No. IgM	%	No. IgG	%
cattle	17	3	17.9%	14	82.1%
sheep	37	6	16.2%	31	83.8%
chicken	9	1	11.1%	8	88.9%

Table 5: specific IgG and IgM antibodies in cattle, sheep and chicken of positive infected with *Toxoplasma gondii* by using 2- Hydroxy ethylmercaptan test

#### DISCUTION

Toxoplasmosis is one of the most common human infections throughout the world, infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions (James, 2003). Sporulated T. gondii oocysts are very resistant to environmental conditions, and remain infective in humid soil for more than 18 months. However, they do not survive long under cold or dry conditions (Dubey et al., 2000). There are two basic forms of toxoplasma organism: the "oocyst," which is shed in the cat feces, and the toxoplasma tissue stages, which found in cattle and sheep (Khadi et al., 2009). A person who inadvertently eats either of these forms of toxoplasma is expose to become infected. When the infected person is a pregnant woman, the toxoplasma organism may cross into the placenta (Remington and Desmonts, 1990), the amount of damage done to the mother and the fetus baby depends on the stage of pregnancy at the time of infection. Infection in early pregnancy may result in miscarriage or stillbirth or in a child with varying degrees of blindness and/or various severe neurological conditions including hydrocephalus, microcephaly, and retardation (Mead et al., 1999). The ingestion of infected uncooked pork was believed to be a major meat source of T. gondii infection for humans in the world (Joan, 2005: Mead et al., 1999).

Diagnosis of toxoplasmosis in cattle, sheep and chicken 100 samples for each, in this study was depended upon serological test like direct latex agglutination test, it was useful in positive exploring 17% cattle, 37% in sheep and 9% in chicken to T. gondii infection through contaminated meat with parasite. The positive modified latex agglutination test used the compound 2-Hydroxy ethylmercaptan, means that IgG antibodies superimpose on IgM antibodies and that the infection was a chronic one. This suggestion could be traced by the purchased rabbit, show no symptoms of infection, so may samples acquired their infection from their infected dose, which pass their IgG antibodies through placenta and suppressing IgM antibody which response in their offsprings (Araujo and Reminogton, 1975; Aghwan et al., 2010).

The moderate titers of antibodies (up to 512) of the tested cattle, sheep and chicken may also explain the chronic of infection. This study has found a high seroprevalence in chicken are 9% that is different than those of (Ghorbani *et al.*, 1990; Dubey *et al.*, 2005; Devada *et al.*, 2005), found 33%, 36.3%, and 39.5% in Free-chicken from Iran, Austria, and India respectively. the high prevalence of toxoplasmosis in chickens was in Iran (Asgari *et al.*, 2006). However the prevalence is markedly close to the values detected in Brazil (Asgari *et al.*, 2006).

*al.*, 2006) United States (Dubey *et al.*, 2003) and Peru (Dubey *et al.*, 2004), where their reported are 10.3%, 17% and 26% rates were reported respectively. The clinic signs in birds are normally severe, ocular and cerebral lesions, which affect on canaries and turkeys to penguins. In trials involving birds, little is known on clinical signs. Several authors studied the experimental infection in wild and domestic birds. However, this kind of report involves only the immunological response of the animals to *T. gondii*, in animals are euthanized soon after the immune response is recorded, leaving a lot to be studied (Williams *et al.*, 2001; Lindsay *et al.*, 1995).

The moderate titers of antibodies (1/64 - 1/512) of the tested cattle, sheep may explain the chronic of infection. This study has found a high seroprevalence in cattle 17% and that are closet to those of Dahan *et al.*, 1983 in France (27.42%) and Knapen *et al.*, 1982 in Newsland (22%), and different to those of Horio *et al.*, 2001 in Kitakyushu city (33.9%) and Garrido *et al.*, 1972 in Spin (36%). And this study has found a high seroprevalence in sheep 37% that are closet to those of Rifaat *et al.*, 1978 (34%) in Egypt and with Amin and Morsy, 1997 (39%) in Jeddah. And deferent to those of Silva and Hangoni, 2001(7.7%) in Brazil and Chhabra *et al.*, 1982 (19.6%) in India. The causes of increase positive percentage in meat juice of cattle and sheep in hamdania slaughter are deferent environments of animal slaughter and animals are not examined by toxoplasma test before slaughter and we can't diagnosis of subclinical case and the animals not appear any clinical singes.

Many animals used for meat production show evidence of a *T. gondii* infection are measured via serum antibodies, and viable parasites have been isolated from the meat of these animals with the exception of cattle (Tenter *et al.*, 2000; Efas 2007). Meat as a source of infection in human relates to the observation that the decline in human *T. gondii* seroprevalence (Jones *et al.*, 2007), parallels the decrease in seroprevalence in animals transmitted by food (Kijlstra *et al.*, 2004), It has been suggested that the introduction of indoor farming has led to a marked drop in the *T. gondii* seroprevalence in pork, but public demand for animal-friendly outdoor production systems has led to a re-emergence of toxoplasma infection in these animals (Schulzig and Fehlhaber, 2006; Giessen *et al.*, 2007).

Many factors such as management and hygienic standards in breeding, density of cats and environmental conditions are effective on the acquisition of T .gondii oocysts by animals (Jones *et al.*, 2007). The rate of toxoplasmosis in free-ranging chicken is an important indicator of environmental contamination because of food habits (Araujo and Reminogton, 1975; Devada *et al.*, 1998).

Little is known on the clinical symptoms of toxoplasmosis in ratites in general, and specifically in ostriches, and their implications to the breeding of these animals. Then heating is the most efficient way to kill *T. gondii* tissue cysts. Meat should cooked at internal temperatures of 56- 58 °C at least for ten minutes (Dubey *et al.*, 1990). Freezing the meat at least for two days at temperatures below -18 C can also kill tissue cysts (Kotula *et al.*, 1991). Curing and treatment of meat with enhancing solutions, such as potassium or sodium lactate, can also kill *T. gondii* tissue cysts. Inactivation of tissue cysts depends on the interaction between salt concentration, maturation time and temperature.

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