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Modified Agglutination Test for Serologic Survey of *Toxoplasma gondii* Infection in Goats and Water Buffaloes in Egypt

¹R.M. Shaapan, ¹M.A. Hassanain and ²Fathia A.M. Khalil

¹Department of Zoonosis,

²Department of Parasitology and Animal Diseases, Veterinary Research Division,
National Research Center, Giza, Egypt

Abstract: Blood was collected from goats and buffaloes slaughtered for food in various areas of the Giza province, Egypt. A Modified Agglutination Test (MAT) with cut-off value 1:25 was used to test sera for evidence of exposure to *Toxoplasma gondii*. Serum antibody prevalence was higher 44.3% (102 positive of 230 tested) for goats than 22.5% (36 positive of 160 tested) for buffaloes. The antibody prevalence in relation to sex and age for each species was determined. Prevalence was higher (63.3 and 27.9%) in female than (32.1 and 16.2%) in male goats and buffaloes, respectively. On other hand, higher prevalence (58 and 25.5%) was detected in aged goats (>1.5 years) and buffaloes (>4 years) than (32.8 and 14.5%) detected in younger goats (\leq 1.5 years) and buffaloes (\leq 4 years), respectively. The present study is the first to report serological evidence of *T. gondii* infection in Egyptian goats and water buffaloes by MAT and determined the effect of sex and age. Consequently the finding results obtained scope the public health significance of goat and buffalo's meat and milk as source of human infection.

Key words: *Toxoplasma gondii*, goats, buffaloes, Modified Agglutination Test (MAT), serologic survey

INTRODUCTION

Toxoplasma gondii is a protozoan parasite of warm-blooded animals including man. It has a worldwide distribution. Cats, including all felines are its definitive hosts which excrete environmentally-resistant oocysts in their feces, while the organism can infect a wide range of animal species and man as an intermediate hosts. Hosts become infected by ingestion of food or drink contaminated with oocysts or by ingesting undercooked meat from infected animals with *T. gondii*. It causes mental retardation and loss of vision in congenitally-infected children and abortion in pregnant women and livestock (Dubey *et al.*, 2005).

Goats and buffaloes are very economic food animals act as an important source of both meat and milk for humans in many countries (Dubey, 1990; Huong *et al.*, 1998). Infection with *T. gondii* in goats and buffaloes not only results in significant reproductive disorders as abortion or neonatal mortalities but also has implications for public health since consumption of infected buffaloes and goat meat or milk can facilitate zoonotic transmission (Waldeland and Loken, 1991; Tenter and Johnson, 1997).

Although the importance of *T. gondii* in worldwide is well known in goat and buffaloes breeding, there is a lack of data on its seroprevalence and factors that contribute to spread

Corresponding Author: R.M. Shaapan, Department of Zoonosis, National Research Center,
Giza, Egypt Tel: +20 2 25272439 Fax: +20 2 33371362

the infection. The surveys of *T. gondii* in goats from Egypt revealed that the rate of infection was 54% using Sabin Feldman Dye Test (DT) in goats from Assiut Province (Fahmy *et al.*, 1979). Using Indirect Hemagglutination (IHAT) and Indirect Fluorescent Antibody Test (IFAT) the prevalence of infection was 38 and 40%, respectively among slaughtered goats in Tanta abattoir (Ibrahim *et al.*, 1997). In other hand, a numerous studies have been reported in many countries revealed that the prevalence rate was 55 and 65% in USA using IHAT and Modified Agglutination Test (MAT), respectively, (Patton *et al.*, 1990), 26.8% in Ghanaian goats using ELISA (Puije van der *et al.*, 2000), 37% in barren-ground goats from northern Canada using MAT (Kutz *et al.*, 2001) and 12.3% in goats from Sardinia island, Italy using IFAT (Masala *et al.*, 2003).

Little surveys of *T. gondii* in buffaloes revealed that the rate of infection was 20.4 and 15.7% using IHAT in water buffaloes from Afghanistan and northern India (Kozojed *et al.*, 1976; Chhabra *et al.*, 1985), respectively. Using IFAT, 8.8% 34 out of 385 water buffaloes from Iran had anti-*T. gondii* antibodies tittered 1:16 and (Navidpour and Hoghooghi-rad, 1998). Using direct agglutination test (DAT), Furthermore Huong *et al.* (1998) reported the prevalence of infection was 3% in sera collected from buffaloes in southern Vietnam. While by latex agglutination test (LAT) the antibodies to *T. gondii* were found in 3.85% of water buffaloes from Brazil, at a dilution of 1:64 (Pita Gondim *et al.*, 1999). However, antibodies to *T. gondii* were not found in serum of any tested buffaloes from Egypt and China, using MAT and IHAT (Dubey *et al.*, 1998; Lin *et al.*, 1990).

Since there is little information on the prevalence of infection in Egyptian goats and buffaloes and due to their potential role in the zoonotic transmission of toxoplasmosis, this study was undertaken to determine the frequency of antibodies against *T. gondii* among goats and buffaloes in Giza Province, Egypt and its relation to sex and age and thus to help assess their potential as reservoirs of infection.

MATERIALS AND METHODS

Blood Samples

Blood samples from 230 goats and 160 buffaloes were collected from different localities of the Giza province, Egypt at the time of slaughtering. Sera were separated, labeled and kept at -20°C until use. This research project was conducted from 5-2007 to 5-2010.

Serological Assay

The Modified Agglutination Test (MAT) was performed for the detection of anti-*T. gondii* antibodies in goat and buffaloes at dilution of 1:25. The formalized killed whole tachyzoites antigen was prepared using RH strain of *T. gondii* as described by Desmonts and Remington (1980). The procedures were carried out according to the method of Dubey and Desmonts (1987).

Effect of Sex and Age

For both species, the serologic test results were grouped in classes and tabulated on the variables of sex (male and female) animals and ages (\leq and >1.5 years old) goats and (\leq and >4 years old) buffaloes.

RESULTS

A Modified Agglutination Test (MAT) at dilution of 1:25 was used to test sera for evidence of exposure to *Toxoplasma gondii* and the antibody prevalence in relation to sex and age for each species was determined.

Table 1: Serum antibody prevalence of *T. gondii* in goats and buffaloes using MAT

Species	No. of examined animals	Prevalence			
		Positive reactors		Negative reactors	
		No.	%	No.	%
Goats	230	102	44.3	128	55.7
Buffaloes	160	36	22.5	124	77.5

Table 2: Prevalence of *T. gondii* in goats and buffaloes in relation to sex and age

Species	Sex						Age					
	Males			Females			≤1.5 year (goats) ≤4 year (buffaloes)			>1.5 year (goats) >4 year (buffaloes)		
	Ex	+ve No.	%	Ex	+ve No.	%	Ex	+ve No.	%	Ex	+ve No.	%
Goats	140	45	32.1	90	57	63.3	125	41	32.8	105	61	58.0
Buffaloes	74	12	16.2	86	24	27.9	62	9	14.5	98	25	25.5

Prevalence of Anti-*T. gondii* Antibodies in Goats and Buffaloes

Examination of serum samples collected from total of 230 slaughtered goats and 160 buffaloes by MAT in titers of 1: 25 revealed that 102 and 36 had antibodies against *T. gondii*, resulting in a seroprevalence of 44.3 and 22.5%, respectively using RH *T. gondii* as antigen (Table 1).

Prevalence of Anti-*T. gondii* Antibodies in Goats and Buffaloes in Relation to Age and Sex

The prevalence was higher (63.3 and 27.9%) in female than (32.1 and 16.2%) in male goats and buffaloes, respectively. On other hand, higher prevalence (58 and 25.5%) was detected in aged goats (>1.5 years) and buffaloes (>4 years) than (32.8 and 14.5%) detected in younger goats (≤1.5 years) and buffaloes (≤4 years), respectively (Table 2).

DISCUSSION

The high prevalence of *T. gondii* antibodies using MAT 44.3% found in the goats of the our investigated study was nearly similar to 40 and 37% in goats from Egypt and Canada obtained, respectively, by Ibrahim *et al.* (1997) using IFAT and Kutz *et al.* (2001) using MAT. Meanwhile lower incidence rates (12.3 and 26.8%) were recorded, respectively, by Masala *et al.* (2003) in Italy using IFAT and Puije *et al.* (2000) in Ghana using ELISA. However higher incidence rates (54 and 65%) were reported, respectively, by Fahmy *et al.* (1979) in Egypt using DT and Patton *et al.* (1990) in USA using MAT.

Also, the high infection rate (22.5%) of water buffaloes obtained in present results was nearly similar to 20.4 and 15.7% revealed by Kozojed *et al.* (1976) and Chhabra *et al.* (1985) in Afghanistan and India, respectively, using IHAT. However, lower rates 3, 3.8 and 8.8% detected by Huong *et al.* (1998), Pita Gondim *et al.* (1999) and Navidpour and Hoghooghi-rad (1998) in Vietnam, Brazil and Iran using DAT, LAT and IFAT, respectively. On other hand, Dubey *et al.* (1998) and Lin *et al.* (1990) cannot determined anti-*T. gondii* antibodies in water buffaloes from Egypt and China, using MAT and IHAT, respectively.

The effect of age and sex on the prevalence of *T. gondii* in goats and water buffaloes obtained in our study indicated that infection rates were higher in females than males and also high in aged animal than young of both species. In this regard, Dubey and Beattie (1988), Navidpour and Hoghooghi-rad (1998) and Randall *et al.* (2000), also proved that antibody prevalence was directly related to age and sex and they ascribed this fact to the

opportunity of prolonged exposure of older animals to infection and the stresses (pregnancy and lactation) exposed by females.

The differences in seroprevalence rates of *T. gondii* in goats and buffaloes obtained during the present study and those previously reported investigations are probably due to the higher or lower degrees of feline presence in different locations shedding oocysts and/or different cut-off points in the serologic test used; initial serum dilution; the virulence and type of *T. gondii* strains which used in the Ag preparation; the immune status, age, climatic conditions and may be due to differences in management methods or species susceptibility of investigated animals in different localities (Dubey and Beattie, 1988; Bisson *et al.*, 2000).

The Modified Agglutination Test (MAT) is the major recommended test for diagnose the *T. gondii* infection in several animals and man (Garcia *et al.*, 1999). Also Dubey (1997), found that MAT has the highest sensitivity among all serological tests and this confirmed by the results obtained by Shaapan *et al.* (2008), who demonstrated the benefits of using more sensitive and specific MAT for the detection of *T. gondii* antibodies in sheep sera which is cheaper, easier than other tests and does not need special sophisticated equipment.

CONCLUSION

This study is the first report using MAT that revealed the high prevalence of anti-*T. gondii* specific antibodies in Egyptian goats and buffaloes. So it will be necessary to conduct further studies to determine the impact of toxoplasmosis on the animal industry and the potential risk of its transmission to humans through consumption of goats and buffalo's meat or milk. In order to reduce the potential for transmission of *T. gondii* to humans, meat and milk should be thoroughly cooked prior to consumption.

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