

## Evaluation of Different *Toxoplasma gondii* Isolates as Antigens Used in the Modified Agglutination Test for the Detection of Toxoplasmosis in Camels and Donkeys

<sup>1</sup>R.M. Shaapan and <sup>2</sup>A.M. Fathia Khalil

<sup>1</sup>Department of Zoonotic Diseases, National Research Centre, Dokki, Giza, Egypt

<sup>2</sup>Department of Parasitology and Animal Diseases, National Research Centre, Dokki, Giza, Egypt

**Abstract:** Different isolates of *Toxoplasma gondii* (RH, equine, camel and sheep) strain were evaluated when used as antigens in the modified agglutination test (MAT) for the detection of antibodies to *T. gondii* in sera of slaughtered camels and donkeys from Egypt. The camel and sheep strains of *T. gondii* revealed a higher prevalence of toxoplasmosis in camels (30.7 and 27.3%) than that showed by RH and equine strains (18 and 20%) respectively. Conversely, in donkeys the RH and equine strain antigens revealed high prevalence of toxoplasmosis (44.5 and 52%) than that found by camel and sheep strain antigens (36 and 39%) respectively, when used in MAT at titers of 1:25. The difference in antigenic potency between the different local isolates of *T. gondii* in this study illustrates the necessity of investigating the suitable isolates of *T. gondii* as antigens for detection of toxoplasmosis in every animal species, moreover our findings suggested that the slaughtered camels and donkeys were frequently infected with *T. gondii* so their meat may be an important source of *T. gondii* infection for human and wild zoo-animals.

**Key words:** Toxoplasmosis % Local antigen % MAT % Camels % Donkeys

### 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 and excrete environmentally-resistant oocysts in their feces. Hosts become infected by ingestion of food or drink contaminated with oocysts or by ingesting undercooked meat from infected animals with *T. gondii* which causes mental retardation and loss of vision in congenitally-infected children and abortion in pregnant women and animals [1].

The prevalence data for the determination of *T. gondii* infection in camels at the different localities of the world is extremely variable. In Egypt, the infection rates among slaughtered camels were 46% using DT [2], 54.2% using IHAT [3], 16.7% and 18% using IHAT and IFAT, respectively [4], 17.4% using MAT [5] and recently by bioassay in mice and cats a total of 23 out of 90 camel meat samples were found to be infected with an incidence rate of 25.6% [6]. The infection rate was 16% (IHAT) in Saudi Arabia, [7], 31.4% (ELISA)[8] and 22.4%(MAT) [9]

in United Arab Emirates and 67% (latex agglutination test) in Sudan [10].

Although antibodies to *T. gondii* have been reported in equines from many countries based mainly on different serological tests as in North America, 6.9% using MAT [11], in Argentina 97.1% using IHAT [12], in Nigeria 37.1% using IHAT [13], in Turkey 20.6% using DT [14]. In Egypt, there is a little known investigations about the prevalence of the infection in donkeys, the *T. gondii* antibodies was found in 65.6% of 121 sera from adult donkeys using ELISA [15], while the infection rate in horses ranged from 31.7- 51.7% using ELISA and MAT [16,17]. Recently by bioassay in mice in Egypt, 79 out of 150 horse meat samples were found to be infected with an incidence rate of 52.6% [18].

Little is known about *T. gondii* types of isolated strains distribution in Egypt, especially in slaughtered animals, which will be later processed for food. The prevalence of *T. gondii* antibodies was studied in camels and donkeys using different locally isolated strains [6, 18, 19]. The vast majority of *T. gondii* isolates has been classified into three major genotypes I, II and III which are remarkably different in severity and

geographical distribution, genotype II is probably predominant in animals [20, 21]. Little is known about the biological and molecular characteristics of isolates of *T. gondii* from the Middle East and Africa, in Egypt, genotyping of 20 chicken isolates of *T. gondii* indicated that 17 isolates were type III and three were type II. The duck isolate of *T. gondii* was type III [22] and recently in UAE, genotyping of isolates from serologically positive camels was done by nested PCR at the SAG2 locus followed by restriction fragment length polymorphism (RFLP) and results indicated that most strains typed were genotype I or II; none was type III [9].

The main purpose of this study is to prepare antigens from different locally isolated *T. gondii* strains to be used in modified agglutination test (MAT) and evaluating them when used in the investigation of the *T. gondii* antibodies in camel and donkeys intended for slaughter in Egypt in order to obtain the accurate assessment of the serological prevalence of the *T. gondii* antibodies.

## MATERIALS AND METHODS

**Blood and Serum Samples:** A total number of 150 and 200 blood samples were collected from apparently healthy camels and donkeys intended for slaughtering at the main abattoir of Cairo (El-Bassatin) and Giza-Zoo abattoir, respectively. Sera were separated from both types of blood samples and stored at -20°C until used for serological testing.

### *Toxoplasma gondii* Strains

***T. gondii* RH Strain:** Virulent RH strain of *T. gondii* was obtained from colony maintained in Department of Zoonoses, National Research Center.

**Local Isolated *T. gondii* Strains:** The local camel [6], Equine [18] and Sheep [19] isolates of *T. gondii* were successfully obtained after many trails of bioassay of the suspected infected camel, horse and sheep tissues respectively in cat and mice as the procedures described by [23,24] in order to isolate the *T. gondii* infective stages.

**Maintenance of *T. gondii* Strains:** The RH and locally isolated sheep, camel and equine *T. gondii* strains were maintained in the laboratory by serial passage in mice according to the procedures of [25], briefly as follows:

about  $2 \times 10^6$  tachyzoites of different *Toxoplasma* isolates were inoculated into 3 to 5 Swiss albino mice about 1 month-old, then, they are ether anesthetized 2–3 days after inoculation and the mouse peritoneal cavity washed by about 5 ml of sterile normal saline using sterile Pasteur pipette. The peritoneal wash is examined microscopically for tachyzoites and then collected. The washing process repeated until almost all parasites are recovered, then peritoneal wash containing tachyzoites are pooled and stored at 4°C. Further inoculation was done every 2-4 days depending up on the ambient temperature and the condition of the inoculated mice.

### Modified Agglutination Test

**Antigens Preparation:** The tachyzoites of RH, sheep, camel and equine *T. gondii* strains had been recovered with high concentration and purity by removing of contaminating host cells from the infected mice peritoneal exudates according to [26] and the formalized killed whole tachyzoites of the each *T. gondii* strain was prepared [27].

**Test Procedure:** All camel and donkeys sera were tested for the presence of anti-*T. gondii* antibodies using MAT at dilution of 1:25, the mercaptoethanol was incorporated in the antigens and the procedures were adopted according the method described by [28].

## RESULTS

**Prevalence of *T. gondii* Antibodies in Camels:** A total number of 150 serum samples of slaughtered camels tested by MAT revealed that 27 (18%), 30 (20%), 46 (30.7%) and 41 (27.3%) had antibodies against *T. gondii* in titers of 1: 25 using RH, equine, camel and sheep isolates of *T. gondii* as antigens, respectively, (Table 1).

**Prevalence of *T. gondii* Antibodies in Donkeys:** Examination of the 200 serum samples of slaughtered donkeys by MAT revealed that 89 (44.5 %), 104 (52 %), 72 (36 %) and 78 (39 %) had antibodies against *T. gondii* in titers of 1: 25 using RH, equine, camel and sheep isolates of *T. gondii* as antigens, respectively, (Table 2).

**Comparison Between the Different *T. gondii* Strain Antigens:** The camel and sheep *T. gondii* strain antigens showed high prevalence of toxoplasmosis in camels (30.7 and 27.3%), in contrast the RH and equine strain antigens which showed the lowest prevalence

Table 1: Prevalence of *T. gondii* in Camels by MAT using different *T. gondii* isolates as antigens

Antigen	No. of examined sera	Sero-prevalence by MAT			
		Positive reactors		Negative reactors	
		No	%	No	%
RH strain Ag	150	27	18.0	123	82.0
Equine strain Ag	150	30	20.0	120	80.0
Camel strain Ag	150	46	30.7	104	69.3
Sheep strain Ag	150	41	27.3	109	72.7

Table 2: Prevalence of *T. gondii* in donkeys by MAT using different *T. gondii* isolates as antigens

Antigen	No. of examined sera	Sero-prevalence by MAT			
		Positive reactors		Negative reactors	
		No	%	No	%
RH strain Ag	200	89	44.5	111	55.5
Equine strain Ag	200	104	52.0	96	48.0
Camel strain Ag	200	72	36.0	128	64.0
Sheep strain Ag	200	78	39.0	122	61.0

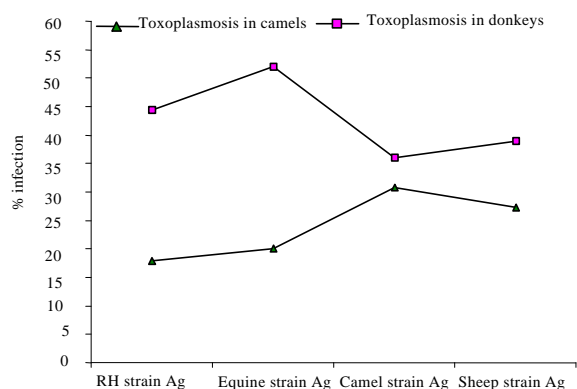


Fig. 1: Comparison between the different *T. gondii* strain isolates as antigens for the detection of *T. gondii* in camels and donkeys by MAT

(18 and 20%). On the other hand, the RH and equine strain antigens showed the highest prevalence (44.5 and 52%), while camel and sheep strain antigens revealed the lowest prevalence (36 % and 39%) in the detection of Toxoplasmosis in donkeys (Fig. 1).

### DISCUSSION

The present work is a distinct study in which local isolated strains of *T. gondii* were used as antigens in MAT for detection of toxoplasmosis and it is the first

report in Egypt for the detection of *T. gondii* in donkeys using MAT.

The prevalence of *T. gondii* antibodies of camels in the present study (18 to 27) % was nearly similar to 17.4% [5], 22.4% [9] and 25.6% [6]. Meanwhile our finding results were lower than 31.4% [8], 46% [2], 54.2% [3] and 67% [10] but higher than 16% and 16.7 % that conducted by [7, 4].

The prevalence of *T. gondii* antibodies donkeys in the present study (36 to 52) % was nearly similar to 37.1% [13], 52.6% [18], 31.7% to 51.7% [16]and [17]. Meanwhile our finding results were lower than 97.1% [12] and 65.6% [15] but higher than 6.9% and 20.6% [11, 14].

The sero-prevalence rates variation of *T. gondii* in camels and donkeys between the results obtained during the present study and those previously reported investigations is depending on 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 and management investigated animals in different localities [24].

The modified agglutination test (MAT) is the major recommended test for diagnose the *T. gondii* infection in several animals and man. MAT is cheaper, easier than other tests and does not need special sophisticated equipment [29], also it has the highest sensitivity among all serological tests [30] and the results obtained by [19], demonstrated the benefits of using more sensitive (96%) and somewhat specific (88.9%) MAT for the detection of *T. gondii* antibodies in sheep sera. In addition, the serological tests used in this work depended on Ag prepared from locally isolated strains of *T. gondii*, which were much cheaper when compared with the expensive patented kits in the previous studies in Egypt.

It is unknown whether the severity of *T. gondii* infections is due to the parasite strain, quantum of infection, host immunity, or other factors. It has been suggested that type I isolates or recombinants of types I and III are more likely to result in clinical toxoplasmosis [31]. *T. gondii* isolates differ markedly in their virulence to outbred mice. Type I isolates are lethal to mice, irrespective of the dose and virulence is genetically controlled [32]. Genetically, 85% of the chicken isolates from Egypt were type III, while type I isolates were not recovered [22]. While the prevailing genotypes in camels in UAE seemed to be genotypes I and II [9], our results in camels and donkeys, investigations are needed in progress to refine *T. gondii* infection and genotype distribution in Egyptian meat-producing animals.

It was concluded that camel and sheep strain antigens are effective than RH and equine strain antigens for the detection of toxoplasmosis in camels. On the other hand, the using of RH and equine strain antigens in the detection of toxoplasmosis in donkeys is more effective.

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