

***Toxoplasma gondii*: Comparison of Some Serological Tests for Antibody Detection in Sera of Naturally Infected Pigs**

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Abstract: A total of 180 blood samples collected from pigs of different ages, sexes slaughtered for food in the main abattoir at Cairo, Egypt were used for the comparative serologic diagnosis of *T. gondii* infection. The modified agglutination test (MAT) revealed a higher prevalence of toxoplasmosis (56.6%), followed by the enzyme linked immunosorbent assay (ELISA) (52.2%) and the indirect hemagglutination test (IHAT) (42.7%), while the lowest prevalence was detected with the Methylene blue dye test (DT) (35.5%). When the data from the first three serological tests were compared with that of the DT test, which was used as a reference test for toxoplasmosis MAT had the highest sensitivity (95.3%), followed by ELISA (90.6%) and IHAT, which demonstrated the lowest sensitivity (80.4%). Conversely, IHAT had the highest specificity (85.3%), followed by MAT (82.8%) and ELISA (80.1%). The results of the present survey recommended the use of MAT and ELISA as more sensitive and specific serological tests in diagnosis of toxoplasmosis in pigs. Consequently this study scopes the public health significance of pig's meat as source of human infection.

Key words: *Toxoplasma gondii* · Pigs · DT · IHAT · ELISA · MAT

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite that can infect almost all mammals including man. It has a worldwide distribution. Cats, including all felines, are its definitive hosts and excrete environmentally-resistant oocysts in their feces [1]. The importance of this parasite in food safety, human health and animal husbandry has been well recognized. Although the parasites remain dormant in people with normal immune competence, they do pose threats to individuals who are immunocompromized as patients with AIDS or organ transplantation and causes mental retardation and loss of vision in congenitally-infected children and abortion in pregnant women and livestock [2].

Pigs are important to the economy of many countries because they are a source of food for humans. Infected pig meat is a source of *T. gondii* infection for humans and

animals. Most pigs acquire the infection postnatally by ingestion of oocysts from contaminated environment or ingestion of infected tissues of animals [3]. While, *T. gondii* infections in pigs are subclinical, outbreak of toxoplasmosis is characterized by high temperature, gradually loss of appetite, high morbidity reached 57% and mortality of approximately 2% [4]. Higher abortion rates, up to 44% may occur in sows infected during pregnancy and transplacentally infected pigs may be born premature, or weak, dead over a very short period of time [5].

Although the importance of *T. gondii* in worldwide pigs breeding and public health, there is a little surveys of *T. gondii* in Egyptian pigs, which was shown to be 50% using Sabin Feldman Dye test (DT) [6], 22% using indirect fluorescent antibody test (IFAT) [7] and 16 and 49% using indirect hemagglutination test (IHAT) and modified agglutination test (MAT), respectively [8].

Numerous studies have been reported in many countries for the detection of *T. gondii* infection in pigs and the epidemiological assessment is based mainly on serodiagnosis by different antibody detection methods. Using methylene blue dye test (DT), the prevalence of *T. gondii* was 5.9% in pigs in the region of South Bohemia, Czech Republic [9], 28.1 in Chile [10] and 70.2% in Uruguay [11]. Using indirect hemagglutination test (IHAT), the prevalence of infection was 36% in California swine in USA [12], 15.6% in Malaysia [13] and 80% in Indonesian swine [14]. Using enzyme linked immunosorbent assay (ELISA), the prevalence of infection was 10.4% in pigs bred in Sicily, Southern Italy [15], 25% in farmed pigs in Maryland, USA [16], 40.6% in Ghana [17] and up to 58.1% in China's southern Guangdong Province [18]. Using modified agglutination test (MAT), the prevalence of infection was 9.3% in domestic and some wild game species of pigs from Zimbabwe [19], 50% in Swedish swine [20] and 70.8% in USA [16].

In view of the economic importance of domestic pigs in Egypt and their potential role in the zoonotic transmission of toxoplasmosis, therefore, the main objectives of this study were studying the prevalence of *T. gondii* infection among slaughtered pigs using DT, IHAT, ELISA and MAT serological tests and the comparison, advantages and disadvantages of each test were also determined to assess their potential as reservoirs of infection.

MATERIALS AND METHODS

Blood Samples: Blood samples were collected from 180 apparently healthy pigs of different ages, sexes slaughtered for food in the main abattoir of Cairo, Egypt. Sera were separated, labeled in serial numbers and kept at -20°C until use.

Toxoplasma gondii Strain: The local strain of *T. gondii* used for antigen preparation was isolated by feeding kittens with meat samples obtained from freshly slaughtered pigs at the Cairo abattoir, Egypt according to the procedure described by Dubey [21].

Serological Assay: Detection of *T. gondii* antibodies in the collected sera was examined using the following tests:

Methylene Blue Dye Test (DT): Viable freshly harvested *T. gondii* tachyzoites Ag was prepared using the method

of Sabin and Feldman [22] and the test procedure was modified according to the micro-titration technique of Feldman and Lamb [23].

Indirect Hemagglutination Antibody Test (IHAT): The IHAT was adopted using soluble tachyzoite antigen coated in tanned red blood cells and the procedures were carried out according the serology manual prepared by Palmer *et al.* [24].

Enzyme Linked Immunosorbent Assay (ELISA): The optimum antigen, serum and conjugate concentrations were determined by checkerboard titration and test procedures carried out according to the method described by Lind *et al.* [25].

Modified Agglutination Test (MAT): The formalized killed whole tachyzoites antigen of *T. gondii* (RH strain) was prepared according to the method described by Desmonts and Remington [26]. The procedures were carried out according to the method described by Dubey and Desmonts [27] at a dilution of 1:25.

Statistical Analysis: The data of the various serological tests were statistically analyzed. The sensitivity and specificity of each test were determined by comparing its results with that of DT as a definitive test according to the method described by Wingstrand *et al.* [28].

RESULTS

Serological examination for the detection of *T. gondii* antibodies: examination of 180 serum samples from slaughtered pigs using DT, IHAT, ELISA and MAT revealed that 64 (35.5%), 77 (42.7%), 94 (52.2%) and 102 (56.6%), had antibodies against *T. gondii* respectively (Table 1).

Comparative Studies of the Different Serological Tests Used for Detection of *T. gondii* Antibodies: sensitivity and specificity calculations of the serological tests revealed that MAT had the highest sensitivity (95.3%), followed by ELISA (90.6%) and IHAT, which demonstrated the lowest sensitivity (79.6%). On the other hand, IHAT had the highest specificity (85.3%), followed by MAT (82.8%) and ELISA (80.1%) (Table 2).

Table 1: Prevalence of *T. gondii* antibodies among pig sera using different serological tests

Test	No. of samples examined	Positive seroprevalence	
		No	%
DT	180	64	35.5%
IHAT	180	77	42.7%
ELISA	180	94	52.2%
MAT	180	102	56.6%

Table 2: Sensitivity and specificity of serological tests compared with the DT

Test	+ve ^a	-ve ^b	Dye test		Sensitivity (%)	Specificity (%)	Total
			Positive	Negative			
IHAT	51	99	64	116	79.6	85.3	180
ELISA	58	94	64	116	90.6	80.1	180
MAT	61	96	64	116	95.3	82.8	180

a +ve means the number of positive samples recognized by both the reference test (DT) and the compared serological test (IHAT or ELISA or MAT).

b -ve means the number of negative samples recognized by both the reference test (DT) and the compared serological test (IHAT or ELISA or MAT).

DISCUSSION

Diagnosis of toxoplasmosis by demonstration of *T. gondii* in tissue is too much difficult. Therefore, the detection of antibody response by screening of slaughtered pig sera serologically appears to be the conclusive tool for proper diagnosis of toxoplasmosis [29].

The methylene Blue Dye Test used in the present study showed that 35.5% of the examined pig sera were sero-positive for *T. gondii* at a titer 1/64; similar results (28.1%) were recorded in pigs from Chile [10]. However, lower incidence rate (5.9%) was recorded in South Bohemia, Czech Republic [9] and higher incidence rates (50 and 70.2%) were recorded in Egypt [6] and in Uruguay [11], respectively.

IHAT showed that 42.7% of the examined pig sera were sero-positive for *T. gondii* using IHAT at a titer 1:64; nearly similar result (36%) was recorded in California swine, USA [12]; while a lower incidence rates (15.6 and 16%) were recorded in Malaysia [13] and in Egypt [8], respectively. However, higher incidence rate (80%) was recorded in Indonesia [14].

ELISA showed that 52.2% of the examined pig sera were sero-positive at a titer 1/100; nearly similar results (40.6%) were recorded in Ghanaian pigs [17]; whereas lower incidences (10.4 and 25%) were obtained in Southern Italy [15] and in USA [16], respectively and higher incidences (58.1%) was obtained in pigs from Southern China [18].

MAT showed that 56.6% of the examined pig sera were positive for *T. gondii* at a titer 1:25; similar incidences (49 and 50%) were recorded in Egyptian pigs

[8] and in Sweden [20], respectively; while lower incidence rate (9.3%) was recorded in Zimbabwe [19] and a higher incidence rate (70.8%) was obtained in farmed pigs in Maryland, USA [16].

Generally the higher infection rate among pigs with *T. gondii* found in this study may be attributed to the feeding habit and management of Egyptian pigs, which usually feed outdoors and in open system farms, thus are liable to contract the infection with *T. gondii* oocysts. This finding agreed with that obtained by Dubey [3], who cited that raising pigs indoors in confinement has greatly reduced *T. gondii* infection than that of outdoors and organic farmed pigs and Gamble *et al.* [29], who found that the prevalence of *T. gondii* in pigs was as high as 68% in poorly managed non-confinement systems and added that the prevalence of infection may vary strongly in some country from one locality to another due to difference in certain ecological factors and breeding system in these areas.

The variation between the results obtained from the same pig sera group using different serological tests might be due to differences in the sensitivity and specificity of the serological tests used. DT was the most specific test for *T. gondii* and this test is still considered the gold standard by which all other tests should be judged [21]. However, it has the major disadvantage of requiring the use of live organism and human serum from healthy individuals as an accessory factor. Therefore, DT had been replaced with other tests in most laboratories. IHAT is highly specific, but demonstrates low sensitivity and is a complicated test; requiring soluble antigen coated in tanned red blood cells and also detects antibodies later than DT, so acute infection likely to be missed by this test

[30]. ELISA demonstrates great sensitivity, is quantitative, low cost and may be automatically adopted, but requires further refinement with regard to procedures and standardization of the antigen used [31]. Regarding MAT, it was found that MAT has the highest sensitivity among all serological tests, is easy to perform and does not require sophisticated equipment [32, 33].

In conclusion, the results of the present work demonstrated the benefits of using the more sensitive and somewhat specific MAT and/or ELISA for the detection of *T. gondii* antibodies in pig sera. In addition, the serological tests used depended on Ag prepared from locally isolated *T. gondii*, which were much cheaper when compared with the expensive patented kits used in the previous studies in Egypt.

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