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SEROLOGICAL INVESTIGATION ON OVINE TOXOPLASMOSIS IN EGYPT

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SUMMARY

Sheep in Egypt were investigated serologically for Toxoplasmosis. Examination of 915 apparently healthy sheep collected during slaughtering at Cairo and Giza abattoirs by IHAT revealed that 519 (56.7%) were Toxoplasma positive at titre of >1/32. Out of the 519 positive sera, 65 were positive at titre of 1/64, 92 at 1/128, 78 at 1/256, 66 at 1/512, 40 at 1/1024 and 68 at >1/2048 with percentage of 12.25%, 17.73, 15.02, 121,72, 7.7% and 13.10% respectively.

In comparative study between IHAT and IFAT, 500 serum samples were selected out of 155 IHAT negative, only 136 were IFAT negative while 19 sera were Toxoplasma positive. Also, 321out of 345 IHAT positive were IFAT Toxoplasma positive.

The comparative study between IHAT and LAT among the same 500 samples tested by IFAT showed that 140 serum samaples were LAT negative out of 155 IHAT seronegative samples, the other 15 sera revealed obvious agglutination as well as 5 samples wer latex negative out of 70 IHAT positive. However, all the IHAT positive sera at dilution of >1/64 revelaed obvious latex agglutination reaction.

The comparative study between positive and negative results obtained by IHAT, IFAT and LAT among 500 ovine sera revelaed that out of 155 IHAT negative sera only 136 and 140 sera were negative by IFAT and LAT in a percentage of agreement 87.74% and 90.32% resepctively. Also, out of 345 IHAT positive sera IFAT and LAT showed that 321 and 340 sera were positive in a percentage of agreement 93.04% and 98.55% respectively.

Lastly from this study we can concluded that for serological diagnosis of ovine Toxoplasmosis we must depend upon more than one serological test..

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by protozoan parasite named Toxoplasma gondii, It is reported as a widespread disease occurring in sheep and goats in different countries (Dubey and Kirkbride, 1984 and Munday et al., 1987). In sheep toxoplasmosis is reported as an abortifacient agent (Dubey and Kirkbride, 1984) responsible for a variety of reproductive disorders including foetal resorption (Smith, 1961), still birth (Baverly and Watson, 1961) and wealky born lambs (Perry, et al., 1979).

Diagnosis of toxoplasmosis is based mainly upon clinical picture (Groulade and Vallee, 1959), histopathology (Koestner and Cole, 1962) and serology (Patton, et al., 1990). Clinical diagnosis

is difficult since the clinical signs are not specific (Maronpot and Michael, 1968). Confirmation of clinical diagnosis by demonstration of toxoplasma in tissue lesions is also tedious, expensive and time consuming (Panigrahi, et al., 1978), therefore the detection of antibody response against toxoplasma is highly recommended for establishing perfect diagnosis.

So, different serological tests were used for the serodiagnosis of toxoplasmosis such as the dye test (Sabin and Feldman, 1948), indirect haemagglutination test (IHAT) (Jacobs and Lunde, 1957), indirect immunofluorescent test (IFAT) (Morris Goldman, 1957), Complement fixation test (CFT) (Bradstreet and Taylor, 1962) and Latex agglutination test (LAT) (Giannini and Tosti, 1968).

In Egypt previous studies on the diagnosis of ovine Toxoplasmosis were done by using different serological tests as IHAT; Maronpot and Michael (1968), Maronpot and Botros (1972) and El-Menyawy (1987), Dye test; Maronpot and Michael (1968). Rifaat, et al. (1977) and complement fixation test; Maronpot and Michael (1968) and Khawas et al., (1984).

So, the present work was carried out to apply safe, rapid and economic serological tests as IFAT and LAT in comparison with IHAT for detection of Toxoplasma antibodies in ovine sera.

MATERIAL AND METHODS

Samples:

Serum samples were collected from 915 apparently healthy sheep during slaughtering at the main abattoirs of Cairo and Giza governorates. The collected serum samples were investigated by the following tests:

1- Indirect Haemagglutination test (IHAT):

A total of 915 serum samples were conducted for detection of Toxoplasma gondii antibodies by using indirect haemagglutination test kit obtained from Behring Werke AG. Marburg, W. Germany. The test was done according to manfacturer's sheet. All the 915 ovine serum samples were serially diluted starting from the predetermined dilution 1:8 up to 1:2048.

2- Indirect Immunofluorescent test (IFAT):

500 serum samples were chosen from the previously tested sera with IFAT. Those sera were representing 155 seronegative samples and 70.60,55,40,40 and 40 sera at IHAT titres of 1/32,1/64, 1/28, 1/256, 1/512. 1/1024 and 1/2048 respectively. The test was done according to the protocl devised by U. S. department of health education and welfare Public health service, center of disease control, Atlanta, Georgia, 1976. Toxoplasma antigen coated slides were obtained from biotrol France. The antigen wells were covered with serum dilution of the tested samples in reverse order from 1/2048 - 1/32. Rabbit antisheep fluorescein labelledd conjugate IgG (obtained from Nordic, Tilburg, Netherlands) at dilution of 1:40 in Evans blue stick solution 1% was used. Negative and positive control sera were incorporated each time the test was done.

3- Latex Agglutination test (LAT):

The same 500 serum samples previously tested with IFAT were examined by latex agglutination test using toxo-Latex test kit obtained from Fumouz-France, I Ure Mechin 93450 ILE Saint Denis, France as follow:

- 25 ul of each tested serum was placed in one of the squares of the glass slide.The latex reagent suspension was shaked and added 25 ul to the serum with the special dropper provided with the

kit. The two drops were mixed together by using disposable stirrer to spread over the entire square. The slide was then rocked gently with a rotator motion and agglutination was observed within 3 minutes and before 6 minutes. Positive reaction showed agglutination visible to the nacked eye with simultanous clearing of the milky background. While no agglutination was observed in negative raction.

RESULTS

1- Serological results of toxoplasmosis in Sheep by using IHAT:

Examination of 915 apparently healthy (Balady breed) sheep by IHAT for toxoplasmosis showed that 519 (56.7%) were seropositive. The incidence was estimated at the predetermined positive titre of 1/32. Out of these 519 positive samples, 110 sera (21.19%) showed titre of 1/32 while 65,92, 78, 66, 40 and 68 sera in a percentage of 12.52%, 17.73%, 15,20%, 12,72%, 7.71 and 13.10% showed the titres of 1/64, 1/128, 1/256, 1/512, 1/1024 and 1/2048 respectively (Table, 1).

II. Serological results of toxoplasmosis in sheep by using IFAT:

Serodiagnosis of 500 tested sera with IFAT at 1/32 predetermined dilution revealed that out of 155 IHAT negative only 136 were IFAT negative. While 19 sera were Toxoplasma positive at titre 1/32 (16) and 1/64 (3). Also, 321 out of 345 IHAT positive were IFAT Toxpoasma positive. The IFAT titres of these positive sera were 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024 and 1/2048 in 100, 65, 48, 29, 45, 26 and 27 sera respectively (Tables, 2 & 3).

Hence, it could be concluded that 160 out of 500 samples were seronegative while 340 were seropositive by IFAT.

III- Serological results of toxoplasmosis in sheep by using LAT:

The same 500 serum samples previously tested by IFAT were examined by LAT using the undiluted sera, the results revealed that 140 serum samples were LAT negative out of 155 IHAT seronegative samples. The other 15 sera revealed obvious agglutination concerning dilution 1/32,5 samples were latex negative out of 70 IHAT positive sera. On the other hand, all IHAT positive sera at dilution of >1/64 revealed positive latex agglutination reaction. Hence it could be mentioned that 145 out of 500 sera were LAT negative while 355 were seropositive (Table, 4).

IV- Correlation between IHAT, IFAT and LAT among 500 tested ovine sera for toxoplasmosis:

The positive cases among 500 sera IHAT, IFAT and LAT were 345, 340 and 355 in a percentage of 69%, 68% and 71% respectively, While the negative cases were 155, 160 and 145 in a percentage of 31%, 32% and 29% respectively.

V- Comparison between nagative and positive result obtained by IHAT and the other two tests (IFAT & LAT) among 500 ovine sera:

From our study we found that out of 155 IHAT negative sera only 136 and 140 sera were negative by IFAT and LAT in a percentage of agreement 87.74% and 90.32% respectively. Also, out of 345 IHAT positive sera IFAT and LAT showed that 321 and 340 sera were positive in a percentage of agreement 93.04% and 98.55% respectively (Tables, 5 & 6).

Titres obtained by IHAT in Determination of Toxoplasma gondii antibodies in 519 ovine sera.

No. of Samples	Titre by IHAT	Percentage
110	1/32	21.19%
65	1/64	12.52%
92	1/128	17.73%
78	1/256	15.02%
66	1/512	12.72%
40	1/1024	7.71%
68	1/2048	13.10%

Table (2):

Results of Toxoplasmosis in Ovine Sera as Obtained by IFAT.

Results of IHAT		Results of IFAT			
Result	Number	Negative		Positive	
		No.	%	No.	%
Seronegative	155	136	87.75%	19	12.25%
Seropositive	345	24	6.95 %	321	93.05%
Total	500	160	32%	340	68%

Table (3):

Comparison Between IHAT and IFAT Results in 500 Ovine Serum Samples:-

Number of Samples Examined by IHAT	IHAT Titre	Results Obtained by IFAT							
		-ve	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
155	-ve	136	16	3					
70	1/32	8	49	13					
60	1/64	10	16	22	12				
55	1/128	6	10	17	22				
40	1/256		9	5	8	18			
40	1/512			5	6	5	24		
40	1/1024					5	13	19	3
40	1/2048					1	8	7	24
500		160	100	65	48	29	45	26	27

Table (4):-

Comparison Between IHAT and Latex Results in 500 Ovine Serum Samples:-

Results by IHAT		Results by Latex Agglutination Test			
		Seronegative		Seropostive	
Titres	No.	No.	%	No.	%
-ve	155	140	90.32%	15	9.68%
1/32	70	5	7.14%	65	92.86%
1/64	60			60	100%
1/128	55			55	100%
1/256	40			40	100%
1/512	40			40	100%
1/1024	40			40	100%
1/2048	40			40	100%
Total	500	145	29%	355	71%

Table (5):-

Comparison Between Negative Results Obtained by IHAT and the other two Tests (IFAT & LAT) among 500 Sera for Ovine Toxoplasmosis.

IHAT Negative Results	IFAT Results				LAT Results			
	-ve	%	+ve	%	-ve	%	+ve	%
155	136	87.74	19	12.26	140	90.32	15	9.68

Table (6):-

Comparison Between Positive Results of IHAT and the Other Two Tests (IFAT & LAT) among 500 Sera for Ovine Toxoplasmosis.

IHAT Positive Results	IFAT Results				LAT Results			
	+ve	%	-ve	%	+ve	%	-ve	%
345	321	93.04	24	6.96	340	98.55	5	1.45

DISCUSSION

Diagnosis of Toxoplasmosis by demonstration of toxoplasma in tissue lesion is too much difficult just becaue the disease mainfest itself by mild clinical signs or it may be developed as an inapparent infection. (Masoud, et al., 1990). Therefore, the detection of antibody response against Toxoplasma gondii appears to be the conclusive tool for proper diagnosis of toxoplasmosis.

The present study revealed that 519 out of 915 sheep sera (56.7%) were Toxoplasma positive using IHAT. This high percentage of infection may be attributed to the uncontrolled number of stray cats around farms and slaughter houses. Also, the animals may be chronically infected with Toxoplasma. This percentage reflects the importance of such disease in sheep as while it passes asymptomatic but it may lead to a significant harm represented by abortion, still birth and neonatal deaths (Dubey and Kirkbride, 1984) and Perry, et al., 1979). Low incidence of Toxoplasmosis (9.7%, 25% and 14.8%) was recorded by IHAT in Egyptian sheep in different localities by Maronpot and Botros (1972), Michael (1977) and EI Menyawy (1984) respectively. This variation in different farms and localities from which the samples were collected (Feldman and Miller, 1956). Various authors recorded different data on ovine Toxoplasmosis in Egypt by Sabin Feldman Dye test; 24.4% - 67.9% (Michael, 1977, Rifaat, et al., 1977 and Fahmy, et al., 1977 andEl-Menyawy, 1984), Complement Fixation Test; 69.7% (Michael, 1977) and Slide Agglutination Test; 51% (Michael, 1977). The variation between our results and the afore mentioned results may be due to the difference in the sensitivity of the used test.

The present study showed a good correlation between IHAT and IFAT results (87.75% in seronegative sera and 93.05% in seropositive sera). This percentage of agreement seems to be similar with that reported by Sharma (1980) who recorded 83.78% in positive results , while higher results were reported by Panigrahi et al., 1978 who recorded 77.5% in positive cases. The later authors reported that the disagreement between IHAT and IFAT results in early infection is expected as these tests measure two different types of antibodies. The IFAT detects antibodies against surface Toxoplasma antigen while the IHAT measures antibodies to soluble cytoplasmic antigen of the parasite. This suggestion coincides with the conclusion of Karim and Laudlam, (1975b) and Sharma (1980), who stated that the disagreement among diagnostic tests could be attributed to the differences in specificity of antibodies detected by the different techniques. The quantitative comparison reflects the differences in sensitivity between the two tests specially at low titres. Whereas the IHAT negative results gave low titres by IFAT (1/32 & 1/64). Meanwhile all the IHAT results > 1/256 were positive by IFAT. These results confirm the prrevious results obtained by Pangigrahi et al., (1978) who reported that while IHAT being more sensitive than IFAT, the later is more specefic. On the contrary, this result disagreed with Maronpot and Botros (1972), who concluded that IFAT was greater in sensitivity than IHAT.

The present study revealed that 15 out of 155 IHAT negative sera was Toxoplasma positive by LAT, and the disagreement between the two tests was 9.68%. This disagreement may be attributed to the use of the undiluted serum in LAT which may give non specific reaction. Also, out of 345 IHAT positive sera only 5 sera gave LAT negative. So, the correlation between IHAT and LAT in 355 positive IHAT was 98.55%, which is in agreement with Dubey, et al. (1987) who found 100% correlation between the two tests. Moreover, the testes is easy to perform, inexpensive and the antigen is stable for long time

at 4C.

According to our results and the theory which states that the IFAT detects Toxoplasma antibodies against surface cuticular antigen (which detects early infection), while IHAT and LAT detects Toxoplasma antibodies against soluble cytoplasmic antigen (which appears later on) (Panigrahi, et al., 1978), it could be stated that the use of more than one serological test for diagnosis of Toxoplasmosis provide proper and accurate diagnosis.

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