Middle-East Journal of Scientific Research 11 (11): 1530-1535, 2012
ISSN 1990-9233
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DOI: 10.5829/idosi.mejsr.2012.11.11.6578

Comparison of Dot-Elisa with Ifa Test for Diagnosis of Human Toxoplasmosis and Seroepidemiological Evaluation of the Disease

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Abstract: Toxoplasmosis is a common parasitic disease of public health importance. Dot-ELISA is a solid phase diagnostic method for detection of antigen or antibody that is used widely for diagnosis of protozoan and metazoan diseases of human and animals. The diagnosis of the disease by conventional serological methods lacks accuracy because of false negative and false positive results. The current study was undertaken to compare the agreement rate of Dot- ELISA as a field screening method with Indirect Immunofluorescent Antibody (IFA) for diagnosis of toxoplasmosis. Five hundred and sixty primary school children were randomly bled and their serum were examined for detection of Toxoplasma antibody by both IFA and Dot-ELISA methods and their agreement rate was compared by kappa test. The agreement rate between IFA and Dot- ELISA was determined as 90% and by both methods the prevalence of toxoplasmosis was 23.39%. Also, the results were analyzed based on sex, age and locality. Dot-ELISA is a simple fast and cheep method with an agreement rate of 90% compared to IFA test. It can be easily replaced IFA for diagnosis of toxoplasmosis even in field screening studies.

Key words: Dot-ELISA · Indirect Immuno-Fluorescent Antibody (IFA) · Toxoplasmosis

INTRODUCTION

In spite of the advances made in diagnosis of bacterial, viral and protozoan diseases, diagnostic methods have to be renewed to be more rapid, sensitive and specific. During the past few years, there has been an increased interest in the diagnosis of parasitic diseases using techniques, which are rapid, simple and inexpensive.Conventionalserological tests such as indirect haemagglutination [1, 2], complement fixation [3], counter immuno-electrophoresis [4, 5] and immuno-fluorescence [6, 7] aretedious, difficult to standardize, conduct and interpret. Also the reagents are consumptive and require highly trained technicians as well as expensive instruments such as fluorescent microscope. *Toxoplasma gondii* is a coccidian parasite of the cat and its infection may lead to major public health problems [4, 5]. Human infection results from ingestion of soil contaminated with cat litter, ingestion of raw or insufficient cooked meat (lamb, pork and beef) and transmission from a mother to a fetus through the placenta (congenital infection) or by blood transfusion or organ transplantation [8]. Though most human infections are subclinical and even clinical infections are rarely fatal, however, in pregnant women this organism may cross the placenta and infect the fetus with serious consequence [9]. Since the diagnosis of clinical toxoplasmosis is often difficult, serodiagnostic methods have been employed. Seroprevalance and risk factors of toxoplasmosis was evaluated among pregnant women in district Kohat, Khyber Pakhtunkhwa Pakistan [10]. The most used assay; Indirect Immuno-Fluorescent Antibody (IFA) test, requires expensive fluorescent microscope [6, 11, 12] and trained technicians.The Dot-ELISA has been employed by

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some researchers for detection of *Toxoplasma* antigen or antibody. Yamamoto *et al*. [13] used this method for detection of IgG, IgM and IgA antibodies against excreted-secreted (E/S) antigens of *Toxoplasma gondii.* In their study E/S antigens from peritoneal exudates of infected mice were precipitated with 40% ammonium sulphate and then were used in immune-blot assay and Dot-ELISA., So the present research is plannedto standardize the Dot-ELISA, which is simple to perform and doesn't need expensive equipment to detect IgG and IgM specific antibodies against *Toxoplasma gondii* compared with the IFA test and to evaluate the prevalence of toxoplasmosis in the elementary students.

MATERIALS AND METHODS

Toxoplasma Antigens: *Toxoplasma* tachyzoites (RH. Strain) were collected from peritoneal cavities of Balb-c mice inoculated 3 days previously and separated from peritoneal exudate cell by centrifugation at 3000g for 15 min.The tachyzoites were adjusted to 50 organisms per high power field of microscope.Twenty microliter of this suspension were loaded on glass slides (BehringWerkA.G.), dried up and stored at -20°C, until use. Antigen was prepared for Dot-ELISA by washing parasites as above. Then the fluid was centrifuged and the parasites were suspended inextracting buffer and ruptured with the sonicator set at 60 cycles for the periods of 30 seconds. After checking the suspension under microscope and being sure that no more intact parasites remained, it was centrifuged and the supernatant was decanted.

Protein Determination: Protein content of antigen was determined using Lowry technique [9], then by adding deionized distilled water the final protein concentration was adjusted to 2mg/ml.

Sera: Five hundred and sixty primary school children were randomly bled and their serum samples were examined for detection of *Toxoplasma* antibody. Each seum sample was divided into douple aliquots and stored at -20°C, until use.

Indirect Fluorescent Antibody Test: The sera were tested in the dilution of 1:64 using standard technique for IFA test. For the presentstudy, FITC conjugated Rabbit Anti-Human Immunoglobulin (DAKO, Denmark) was used in a dilution of 1:1500. All samples were examined under Zeiss fluorescent microscope (DAKO, Denmark). The sample was considered negative if the tachyzoites fluoresced only at their anterior end. The titers of 1:64 and above were considered as positive and the sera with titers of up to1:1024 and those of less than 1:16 were considered as golden positive and absolutenegative sera, respectively for standardization of Dot-ELISA assay.

Dot-ELISA: Nitrocellulose strips, 0.45μm Millipore were put in distilled water for 2 min, then after drying at 37°C the strips were spotted with l:l diluted antigen in which buffer?, using Hamilton syringe after determining that 1:l of antigen gives good result. After drying at 37°C for 15 min, antigen uncoated sites on the nitrocellulose strip were blocked with 3% bovine serum albumin (BSA) in phosphate buffer saline (PBS), being shaken for one hour at room temperature. Then the strips were again washed three times with PBS containing 0.05% Tween-20 for 10 min with constant shaking. Nitrocellulose strips with control positive, control negative and tested sera were incubated at 37°C for 30 min, with constant shaking. The dilution of 1:400 was determined. Then the strips were washed three times as above and a second antibody, peroxidase conjugated Rabbit Anti-Human immunoglobulin (DAKO, Denmark) was used at dilution 1:8000 and incubated at 37°C for 30 min, with constant shaking. After washing as above, the strips were exposed to a liquid 3,3´-diaminobenzidine (DAB) containing H_2O_2 at pH 7.5 (DAKO Denmark), as substrate-chromogenic system for 4 min, with gentle shaking and the color development was stopped with tap water. Positive reaction was determined by appearance of clearly defined brown dot around the sites of antigen. The reaction was considered negative, when the nitrocellulose strips had no colored dots.

Evaluation of Toxoplasmosis Prevalence: After the detection of suitable dilutions for sera and conjugated antibody, Dot- ELISA method was used to determine the toxoplasmosis prevalence in the suspicious sera. In this stage, the standard dilutions (1: 100 for serum, 1: 2000 for conjugated antibody) and, positive and negative controls were used.

Statistical Analysis: The agreement rate between Dot-ELISA and IFA, the relative abundances and the prevalence of toxoplasmosis were calculated using the software SPSS version 15.0, EPI6 and excel work sheet.

RESULTS AND DISCUSSION

In the currentstudy the Dot-ELISA was standardized for detection of antibodies against *Toxoplasma gondii* in human sera compared with IFA test. Out of 234 serum samples tested by IFA, 106 samples were positive for IgG, at the titer of 1:64 and 53 samples were positive and 181 were negative by Dot-ELISA (Table 1). Also out of 53 Dot-ELISA positive serum samples tested by IFA, 45 samples were positive (Table 1). 15Dot- ELISA negative samples were positive with IFA test. The results show that the agreement rate between two tests is 90% (P<0.000001 and Kappa coefficient = 0.73).

Table 2 shows the positivesamples based on sex and age. The positive samples based on sex and location (urban or rural); in females were 39.73% in rural and 49.24% in urban and in male samples 60.26% were in rural and 50.75% were in urban (Table 3).

The prevalence of toxoplasmosis in the students based on age is shown in the table 4 and, based on sex and location is shown in the Table 5.

Liu *et al*. [14] used the combination of Dot-ELISA and Dot-Immuno gold silver staining (Dot-IGSS) simultaneously to detect the specific IgG against *Toxoplasma*. In their study, no statistically significant differences were observed between ELISA and Dot-ELISA or between Dot-ELISA and Dot-IGSS (P>0.01). Elsaid *et al.* [15] used Dot-ELISA for diagnosis of human toxoplasmosis. Out of 538 serum samples tested by IFA-IgG as reference test, 183(34%) were positive at dilution of 1:16 and 192 (36%) were positive for Dot-ELISA at dilution of 1:256. For Dot-ELISA co-positivity was 0.94, co-negativity 0.94 and concordance 0.88 in relations to IFA-IgG. The results suggest the success of Dot-ELISA for the sero diagnosis of human toxoplasmosis.

Table 1: Compartive results of Dot-ELISA and IFA tests (P<0.000001, Kappa co. =0.73)

	IFA		
	Positive	Negative	
Dot-ELISA	Positive	45	8
	Negative	15	166

Silva *et al*. [16] evaluated the serological tests such as IFA and ELISA for the diagnosis of *Neospora caninum* infection in dogs: optimized cut off titers and inhibition studies of cross-reactivity with *Toxoplasma gondii*.

Youssef *et al*. [17] evaluated the efficacy of Indirect Haem-Agglutination(IHA), IFA and Dot-ELISA in serodiagnosis of toxoplasmosis in complicated pregnancies. Investigation on 72 pregnant women having congenital anomalies, still birth and repeated abortion at first and second trimesters showed *Toxoplasma* antibodies with high-titer by IFAT and Dot-ELISA. Analysis of the results showed a positive correlation between IFA and Dot-ELISA but not with IHAT. Pappas *et al*. [18] compared Dot-ELISA and ELISA with IFA test

Table 2: Toxoplasmosis infection percentage based on sex and age of the students

	Female		Male		Total	
Age (Years)	Positive samples	%	positive samples	%	Positive samples	%
7	53	48.18	57	51.81	110	19.64
8	55	49.10	57	50.89	112	20.00
9	50	46.29	58	53.70	108	19.28
10	49	42.98	65	57.01	114	20.35
>11-13	46	39.65	70	60.34	116	20.71
Total	253	45.18	307	54.82	560	100

Table 3: Toxoplasmosis infection percentage based on sex and location of the students

	Female		Male		Total	
Location	Positive samples	%	Positive samples	%	Positive samples	%
Rural	91	39.73	138	60.26	229	40.89
Urban	162	49.21	169	50.75	331	59.1
Total	253	45.35	307	54.64	560	100

Table 4: The out-break of toxoplasmosis in the students based on age

Age (Years)	Positive	%	Negative	%	Total	%
7	25	27.22	85	77.27	110	19.64
8	26	23.21	86	76.78	112	20.00
9	28	25.92	80	74.07	108	19.28
10	27	23.68	87	68.42	114	20.35
>11-13	25	21.55	91	78.44	116	20.71
Total	131	23.39	429	76.6	560	100

Table 5: The out-break of toxoplasmosis in the students based on sex and location

	Sex		Positive		Negative		
Location	Samples number	%	Samples number	%	Total	%	Total
Urban	Female	31	19.63	131	80.36	162	29.05
	Male	48	27.29	121	72.02	169	30.34
Rural	Female	25	27.47	66	72.52	91	16.32
	Male	27	19.56	111	80.43	138	24.35
Total		131	23.39	429	76.6	560	100

for detection of IgG and IgM specific antibodies to human toxoplasmosis. The results showed that Dot-ELISA correlated with IFA test (correlation coefficient=0.895) and ELISA correlated slightly higher with IFA test (correlation coefficient=0.910) for detection of IgG antibodies to *Toxoplasma gondii*. The present study, using statistic program Epiinfo 6.0 showed these tests have a good agreement in diagnosis of toxoplasmosis with Kappa=0.8607 for IgG (Agreement rate=93.02%) and Kappa=0.8865 for IgM antibodies (Agreement rate=98.1%) ($P<0.05$). Correlation coefficient was 0.869 for IgG and 0.847 for IgM antibodies ($P<0.05$).The Dot-ELISA has been used extensively in the detection of human and veterinary protozoan and metazoan parasitic diseases [5, 6, 19-21]. It does not require special equipment and can be used as a qualitative test to screen large number of samples.

Dot-ELISA may be configured to detect antibodies or parasite antigen in either micro titer plates for large-batch testing or with dip sticks for small numbers of determinations. A slight modification of the Dot-ELISA procedure allows the determination of infection rates of vectors such as ticks and sandflies with parasites [22].

Seroprevalence of Toxoplasmosis was evaluated by Hatam *et al*. [8] in high school girls in Fasa district, Iran by ELISA method. Studies of toxoplasmosis seroprevalence have shown a statistical correlation with close contact with cats [23, 24]. In the current study, a significant association was found between *T. gondii* seroprevalence and close contact with pets including cats in urban group study, but not much for rural group. In fact those groups which showed seropositivity of about 59.39% had close contact with cats. Health managers should emphasize the importance of avoiding raw or under cooked meat, handling raw meat safely, washing hands after gardening and preventing close contact with cats [25].

ACKNOWLEDGMENTS

This work was supported by the Center of Stem Cell and Transgenic Animals Technology, Shiraz University of Medical Sciences, Shiraz, Iran. Research Improvement Center of Shiraz University of Medical Sciences and Ms. Afsaneh Keivanshekouh are also appreciated for improving the use of English in the manuscript.

CONCLUSION

According to current results, Dot-ELISA is a simple fast and cheep method with an agreement rate of 90% with IFA test. It can be easily replaced IFA for diagnosis of toxoplasmosis even in field screening studies.

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