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ORIGINAL ARTICLE

Bentonite Flocculation (BF) Method Evaluation comparing to Elisa, Indirect Immunofluorescence (IFA) and Latex Agglutination (LA) in Diagnosis of Toxoplasmosis

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ABSTRACT
Toxoplasmosis is a common disease among human and livestock with a worldwide distribution. This disease will be generated by a unicellular parasite called toxoplasmosis Gondii. The disease usually is diagnosed in laboratory through several methods such as; ELISA, Immunofluorescence and Chemo luminescence. The mentioned methods have sufficient sensitivity and trait, but since they are need to use expensive equipment's, mostly a lot of time and noticeable costs, the flocculation bentonite can be considered as a screening way to quickly and efficiently diagnosis this disease depends in case it has acceptable sensitivity, trait and efficiency. In this paper as a descriptive/ analytical research, 127 patrons who referred to two reliable medical Labs.in Tehran, so 127 suspected samples were collected and evaluated by using of ELISA, IFA, LAT and BFA methods. Also, in the present paper we measured the ability and efficiency of flocculation bentonite way to evaluate the epidemiologic parameters. The results showed that comparing to other methods such as; ELISA, immunofluorescence and latex agglutination, the flocculation bentonite has 100% sensitivity, 85.71% trait and 90.95% efficiency. Hence, as for the almost good sensitivity, trait and efficiency rate of flocculation bentonite, we can use this system to evaluate and take a significant population under study in respect of spread and indication of toxoplasmosis disease and screening its positive cases, then in order to avoid wasting high time and cost, we can prove the result by using other methods such as immunofluorescence and ELISA.
Keywords: sensitivity, trait, efficiency, flocculation bentonite, toxoplasmosis

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INTRODUCTION

Toxoplasmosis is a common disease among human and livestock. The disease has a widespread share all over the world including Iran [1, 2, 3, 13, 27]. It is estimated that a third of people around the world are have been infected by this disease [7, 8]. The infection generated by this disease has usually no sign except of people who suffer from their defected immune system. In this case, the disease appears as neck lymphadenopathy, retina-choroiditis (eye disease), joint pains, myocarditis and encephalopathy. Mainly, human is affected by this unicellular through the foods contaminated by cysts or ascite [7, 12]. Toxoplasmosis has different clinical sings, and therefore, it can be misunderstood as other diseases. So, it is essential to use reliable serv immunology tests in order to detect this kind of parasite disease [15]. The most common serv immunology tests to detect toxoplasmosis are Sabin-Feldman dye test, indirect fluorescent antibody, Complement Fixation-CF, Immuno sorbent Assay-ELISA Enzyme Linked and also, agglutination especially indirect hemagglutination [19, 15]. At the present, serv immunology and molecular methods (PCR) are considered as the sensitive ways as well [15, 16]. According to existing studies, it is shown that some serologic methods such as CF, I.H.A due to necessity of antigen solution during their performance, they may be weak positive or even negative. Hence, disease diagnosis cannot be definitive only based on a serologic way [8, 9]. Most of the researchers agree that IFA test's trait are equivalent to dye test, but still there have seen the pseudo-positive reactions in some serums which are seen positive for ANA [11, 14].

Since the serologic diagnosis of toxoplasmosis is performing through ELISA and indirect immunofluorescence methods in some laboratories, and this possibility cannot be accessed by small laboratories and even the ones in counties, so in this case, we suggest using the more simple ways such as

agglutination to diagnosis toxoplasmosis. Indeed this needs agglutination with a suitable sensitivity and trait rate. Since toxoplasmosis is a widespread parasite factor and its generated infection remains no sign in human body, in this paper, we evaluate the sensitivity, trait and efficiency of flocculation bentonite in compare with other seroimmunology methods is considered as a fast and screening way to detect and evaluate toxoplasmosis once its efficiency and performance is proved.

MATERIALS AND METHODS

In this descriptive/analytical study, we evaluated 127 serum samples of the patients suspected to toxoplasmosis together with their filled questionnaires including personal and epidemiologic information which were collected through both Danesh medical and Tehran Central pathobiology laboratories. First, in this study, the serum samples of latex agglutination and flocculation bentonite and their positive and negative points were clarified. Then, all the samples were studied by using both ELISA and indirect immunofluorescence methods. At the next step, the samples which were positive during agglutination and flocculation tests but negative in direct immune fluorescence analyzed through dye test.

Data analysis by using statistical software

SPSS (Version 12) and McNemar were performed. In the results, significance is determined in $P<0.05$

RESULTS AND DISCUSSION

In this study, it was demonstrated that most the infected people by toxoplasmosis are 30-34 years old. Comparing flocculation bentonite with latex agglutination, both methods were matched in 69 positive cases (54.3%) and 58 negative cases (45.7%) in consideration of sensitivity and trait rate. While, they were not matched in 9 cases (7.1%); in flocculation bentonite this number is negative while positive in latex agglutination. Also, 20 cases (15.1%) were negative in flocculation bentonite and negative in latex agglutination. Hence, sensitivity and trait rate of flocculation bentonite and latex agglutination were 100% and 85.71%, respectively. As for defining the sensitivity and trait rate, this study shows that latex agglutination is match to indirect immune fluorescence among 74 positive cases (58.3%) and 53 cases of negatives (41.7%), while they were not matched in 3 cases (2.3%) as these cases were negative in latex agglutination and positive in indirect immunofluorescence. Therefore, sensitivity and trait rate of latex agglutination and indirect immune fluorescence are 100% and 94%, respectively [Table 2]. There was not seen any significant relation between disease frequency and sexuality, profession, place of residency, education and consumption of vegetables or salad [Table 1].Toxoplasmosis Gondi is considered as a mandatory cell parasite which is responsible for a worldwide infection existing in a wide range of hosts including human. The re-activity of hidden infection may threat life in patients who are facing the defected immune system [22]. This infection can be diagnosed directly or indirectly, by using serologic solutions [18]. Today, this kind of infection is detectable by using PCR, hybridization and histology directly. Despite of the fact that indirect serologic ways are widely using in the patient with healthy immune system, definite diagnosis needs mainly to detect the parasite in patient with defected immune system. Using serologic ways such as Sabin-Feldman dye test antibody measurement through indirect fluorescence (IFA), ELISA, direct agglutination, latex immunofluorescence (LAT) and ISAGA are using as diagnosis ways, among them, immunoglobulin antibodies suitable for an acute toxoplasmosis detection, are evaluated [18,19]. This study shows that sensitivity, trait and efficiency rate of flocculation bentonite were 100%, 85.71% and 90.59%, respectively comparing to indirect immune fluorescence. Considering the disease frequency at 30-34 age range, this depends on their nutrition style that almost use ready foods. Being in touch with the cat has a significant relation with the disease. The presence of cat wherever people live spreads this disease. Most of the people stated that cats were frequently seen where they live. Statistical studies do not approve any significance relation between profession and disease. Regarding patrons' profession factor, the professions which were suspected to carry the disease probability had been detected as safe within a specified interval. In seroepidemic study made on 1376, there were not also seen any significant relation between profession and this disease [4]. According to the study performed by Pahlavani comparing to indirect immunofluorescence, agglutination test had a sensitivity about 94% and a 100% specificity [5]. During the recent years, there is attention to epidemiologic relation of this parasite unicellular and causes chronic mental diseases such as schizophrenia, epilepsy (20) and migraine [17]. In spite of many studies to evaluate the human infection by toxoplasmosis [23, 24, 28], most of the studies in this respect have been based on IFA in Iran [29, 30]. In consideration of sensitivity and trait rate of understudied methods, this study suggest also other methods and solution according to costs, speed while note to priority of parasite infection diagnosis. ELISA test is considered as base test for the present study due to its high sensitivity and trait in compare with other methods. In order to detect the Neospora infection in dog and distinguish infection in crossover reaction with toxoplasmosis Gondi, Silva & coworkers used IFA and ELISA to evaluate their serologic tests [25]. In the study by Dastan& coworkers,

the sensitivity and specificity rate of agglutination compare to indirect immune fluorescence were determined 86% and 87%, respectively [6].

Table 1- Frequency and Relative frequency distribution of subjects based on age

Number	Patient's age	
Percentage	Quantity	
19%	14/96	≤ 20
20%	15/75	20-24
21%	16/54	25-29
27%	21/26	30-34
18%	14/17	35-39
22 %	17/32	≤ 20
100%	127	To
21/16 2/9	Average Standard Deviation	

Table 2- Frequency and Relative frequency distribution of the serum samples' results understudied for toxoplasmosis

Total		Negative		Positive		Test Result
Percentage	Quantity	Percentage	Quantity	Percentage	Quantity	Test
%100	127	45/7	58	54/3	69	BFT
%100	127	38/6	49	61/4	78	LAT
%100	127	32/3	41	67/7	86	IFA
%100	127	26/7	34	73/2	93	Elisa

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