DIAGNOSIS OF TOXOPLASMOsis USING BENTONITE FLOCCULATION METHOD AND COMPARING WITH IF METHOD

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ABSTRACT
Toxoplasmosis is a parasitic disease caused by the protozoan Toxoplasma gondii. The parasite infects most genera of warm-blooded animals, including humans, but the primary host is the felid (cat) family. 10 microliter of prepared kit is poured by sampler on slide and mixed with 10 microliter of rabbit immunized serum against toxoplasmosis (positive control) and flocculation was measured. This was executed by human and rat serum positive as well as negative human, rat and rabbit serums for negative control. Also, testing result for 61 people affected by toxoplasmosis in flocculation bentonite method was positive and for 8 people affected normally by toxoplasmosis incorrect in flocculation bentonite and for 98 people affected by toxoplasmosis diseases in normal case, 17 cases were fake positive. Sensitivity of flocculation bentonite test to diagnose toxoplasmosis will be determined 100% further to above results and implemented positive tests.

KEY WORDS: Bentonite Flocculation, IF Toxoplasmosis.

INTRODUCTION
Infection by the protozoan parasite, T. gondii, is wide spread in humans and many other species of warm blooded animals (Anganga et al., 1981). Although the course of disease is generally begun, it can cause significant morbidity and mortality in the developing fetus and in immune compromised individuals, including humans with Acquired Immunodeficiency syndrome AIDS (Burney., 1996). Toxoplasmosis is a major zoonotic disease and is a major cause of abortion; the infection does not usually cause clinical symptoms. The organism is cosmopolitan in distribution. It is estimated that over one third of the world population have contracted the infection (Kean., 1972). After ingestion by any host the organism escape from its cyst and penetrate intestinal wall and emerge either as tachyzoites or sporozoites. In an immune competent individual, the organism will encyst itself in the brain, muscles and eyes as a bradyzoites (Burney., 1996). However, if the host becomes immune suppressed, the organism may reactivate and cause disease. Toxoplasmosis causes respiratory, alimentary and neurological disturbance in canines in conjunction with viral infection and stress factors. Poly myositis- poly radiculitis has been reported due to toxoplasmosis (Suter et al., 1984). No much local informations on canine toxoplasmosis are available except (Ahmed et al., 2001). conducted a serological survey of toxoplasmosis in dogs but sample size was very small. The present study was designed to record the seroprevalence of toxoplasmosis in canines having reasonable number.

MATERIALS AND METHODS

Procedure
Preparation of positive and negative serums to perform Bentonite flocculation test
We used positive and negative serums of rabbis and rats immunized with toxoplasmosis as positive and negative control. The patients’ positive toxoplasmosis serums is used for positive control. Also, in order to determine flocculation bentonite assignment in diagnosis of toxoplasmosis, there was used non-toxoplasmosis patients serum like Auto-immune patients of Anti-nuclear antibody (ANA), listeriosis, Rheumatoid arthritis factor, leptospicosis as well as negative toxoplasmosis patients serum.

Testing method for flocculation bentonite by using positive and negative serum samples in order to determine sensitivity of above method
10 microliter of prepared kit is poured by sampler on slide and mixed with 10 microliter of rabbit immunized serum against toxoplasmosis (positive control) and flocculation was measured. This was executed by human and rat serum positive as well as negative human, rat and rabbit serums for negative control.
Flocculation bentonite test procedure in order to determine flocculation bentonite assignment by different diseases’ serums including those affected by anti-nuclear antibody (ANA), listeriosis, Rheumatoid arthritis factor and sample of negative toxoplasmosis serums.

10 microliter of prepared kit is poured by sampler on slide and mixed with 10 microliter of a person affected by ANA and then, flocculation was measured. This procedure was also performed for listeriosis, Rheumatoid arthritis factor and negative toxoplasmosis were executed together with measurement time.

RESULTS
Flocculation bentonite test results by using positive and negative serums considering affected by toxoplasmosis

The result driven by Flocculation bentonite test which was performed by Counter Current Immuno-Electrophoresis with immunized rabbit serum (positive control) and normal rabbit serum (negative control) was positive control bow, positive and negative control, negative. The aimed resulted by Flocculation bentonite which is executed by 10 immunized rats serum and 10 normal rat serum, all immunized serum samples were positive and all normal rat serum samples were negative in this test. Also, testing result for 61 people affected by toxoplasmosis in flocculation bentonite method was positive and for 8 people affected normally by toxoplasmosis incorrect in flocculation bentonite and for 98 people affected by toxoplasmosis diseases in normal case, 17 cases were fake positive. Sensitivity of flocculation bentonite test to diagnose toxoplasmosis will be determined 100% further to above results and implemented positive tests.

Method
Bentonite Flocculation test results with the serum from non-toxoplasmosis patients and negative toxoplasmosis (determination of specificity or property of Bentonite Flocculation method) Bentonite Flocculation test results non-toxoplasmosis patients consisting of 10 serum samples from patients with rheumatoid factor, 6 serum samples from patients with positive anti-nuclear antibody (ANA) autoimmune-type and 5 serum samples of patients with listeriosis were all negative. Regarding the serum samples from patients with negative Toxoplasmosis, 17 cases out of 98 were observed to be false-positive that confirms 85.71%.specificity or property of this method.

Determination of the performance of Bentonite Flocculation
According to the tests conducted on positive and negative samples, the efficiency of Toxoplasma Bentonite Flocculation is 90.55%.
Comparison of positive and negative flocculation bentonite test and IFA
In general, for positive cases which were evaluated in IFA method, they were completely adaptive after evaluation by BFT method but regarding negative cases (non-toxoplasmosis and negative toxoplasmosis patients) 17 serums of 119 serums were fake positive based on which it can be said that they are compatible to each other in sensitivity but for assignment, BFT method assignments equals to 85.71%.

Comparing sensitivity, assignment and efficiency of flocculation bentonite test by indirect immune-fluorescence
All 61 serum sample of toxoplasmosis patients which were positive in IFA method appeared also positive in BFT method. Also, 17 cases of 98 serum samples of people who were negative toxoplasmosis in IFA method, turned into fake positive under BFT method. All 21 cases of patients affected by other diseases (rheumatoid factor, anti-nuclear antibody and listeriosis), they turned all negative in respect of affected by toxoplasmosis in IFA method as well as BFT test. Considering results of both methods it can be agreed that BFT method has 100% sensitivity, 85.71% assignment and 90.5% efficiency which is compatible with IFA method.

Measured times during evaluation flocculation bentonite
We need 90 seconds for positivity and observing the flocculated particles in immunized rabbit tests as well as rat and human serums, after passing such time and not observing flocculation it can be said that test is negative.

Table1: Comparison of sensitivity, assignment and efficiency of indirect immune-fluorescence method by using flocculation bentonite method

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Assignment</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA</td>
<td>%100</td>
<td>%100</td>
<td>%100</td>
</tr>
<tr>
<td>BFT</td>
<td>%100</td>
<td>%85.71</td>
<td>%90.55</td>
</tr>
</tbody>
</table>

Table2: Comparison of positive and negative cases in BFT and IF methods

<table>
<thead>
<tr>
<th>Cases percentage</th>
<th>Negative cases</th>
<th>Total cases</th>
<th>Cases percentage</th>
<th>Positive cases</th>
<th>Total cases</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>119</td>
<td>119</td>
<td>100</td>
<td>61</td>
<td>61</td>
<td>IFA</td>
</tr>
<tr>
<td>85.71</td>
<td>102</td>
<td>119</td>
<td>100</td>
<td>61</td>
<td>61</td>
<td>BFT</td>
</tr>
</tbody>
</table>

(Note: non-toxoplasmosis people including 21 serum related to patients affected by listeriosis, rheumatoid factor, ANA and also 98 serums related to normal people, totally 119 serums were negative.)

Discussion
Toxoplasma Gondii is a compulsory protozoan intercellular leads to toxoplasmosis which can affect an extended range of animals and human. Although, feliformia is main and ultimate host of Toxoplasmosis gondii, still a wide range of animals acts as middle hosts. Janina Carla et al. (2010), studied on Toxoplasmosis gondii affection serologic over two group of cats (stray and household cats); in this study 60 cats were tested in term of serologic for toxoplasmosis gondii antibodies by using Latex Agglutination test. 60 collected cats divided into 30 stray and 30 household cats. The results showed that 28 cats of 60 (46.67%) were positive serum. More household cats (28.33%) comparing stray cats (18.33%) were positive serum. Today toxoplasmosis gondii's coccidial parasite is the most common retinal affection all over the world. Occurring ocular toxoplasmosis is observing repeatedly. Recently a long term study of patients showed that 24% of them will be blind at the end. Though anti-toxoplasmosis medications are available, still it is not proved that they are
effective to treat ocular toxoplasmosis. Stanford et al. generated researches on these kinds of affected patients and resulted that there are only few good research in this field declared that since now no experiment has showed efficient results. Considering above factors, it is obvious that there has to be more attention to avoidance of toxoplasmosis affection. Toxoplasmosis diagnosis as treaty affection for patients’ life who have weaken immune system and more awareness of congenital toxoplasmosis has emphasized highly on necessity of assured diagnosis methods for this parasitic affection. Sabin color test and Feldman is still selected serologic test but this biology measurement which is hard technically is rarely executed out of Reference Center. Non-reference laboratory needs a reliable screening test for toxoplasmosis which is highly sensitive; its performance is simple and not taking too much time, while it is possible that reference laboratory performs a secondary experiment together with color test in order to minimize error results when many samples are processed. Latex Agglutinasition, indirect hemagglutination and indirect immune - flocculation are used in this state. Previous studies have confirmed efficiency of these experiments as screening test but the results are considerably fake positive which are presented as document. PCR technique is evaluated by different groups to diagnose toxoplasmosis. Performing none of these experiments is not easy and needs professional and expensive equipments which are only executable by expert personnel. Therefore, they can only be applied in equipped laboratories. In order to facilitate diagnosis, we are searching a method which is able to diagnose toxoplasmosis in all laboratories. Latex Agglutinsation as a fast method is one of the screening methods with efficient sensitivity to diagnose disease using globally. Latex is a bio-synthetic material importing from USA and other countries to Iran. Considering sanctions, we decided to find a new and affordable method. Flocculation bentonite test is a modified serologic approach to diagnose antibodies of different diseases such as toxoplasmosis.

Sensitivity and assignment of flocculation is similar to Latex Agglutinsation method as an affordable method. The components are used for this test can be find ready at the market. They are stocked or prepared and stable for months. The necessary equipments for this test are accessible in medium level laboratories. There is need to a little technical experience to perform this test and reading results. Therefore, since this technique is not produced in Iran, we could achieve the valuable design of BFT kit which leads to diagnose and treat the disease.

The limit distance of such research is produce and standardizing BFT method and comparing its efficiency with IFA which is accessible commercially. BFT is an efficient method and can be used to screening a wide range of samples in local areas without any advanced and expensive equipments and its results can be read less than 2 hours and is more suitable than IFA. 61 serums of people affected by toxoplasmosis executed by IFA (100%) and were positive, they showed same result by BFT too means 100% positive. Also by 21 serums of people affected by diseases other than toxoplasmosis including Rheumatoid arthritis factor, ANA, Listeriosis were negative serums and there has not been observed any positive result by IFA; we had same conclusion by BFT too and all serums turned negative, but studying 98 serums of negative toxoplasmosis people, we observed 17 false positive. Therefore, it has to be said that assignment of flocculation bentonite is 85.71%, then considering numbers of positive and negative serums and comparing positive and negative results driven by IFA method and flocculation bentonite test, BFT efficiency is estimated 90.55%.

REFERENCES