

Detection of Toxoplasmosis In Human and Cats Immunologically

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Abstract

The aims of this research to diagnose Toxoplasmosis in aborted women cats in cities of Al-qadisyia governorate . So 91 blood samples were collected from clinically suspected women and six samples of blood of stray cats . Latex agglutination and ELISA (IgG , IgM) tests were used . The results , 75 cats were positive for Toxoplasmosis in latex agglutination test (82%) , while results of ELISA test by using IgG reveal that 60% of the cases were positive from that of positive to the latex test . The results of ELISA to IgM were 14.6% positive cases (14.6%) from that positive to latex .The immunofluorescence was done on 20 samples that were positive to ELISA (IgG) and the result cases were positive (95%) .Six samples of stray cats , all of them were positive for both tests agglutination latex and ELISA , IgG , except one case negative to IgG .

Introduction

Toxoplasma gondii is very important protozoal parasite . It is very wide spread all over the world even in Iraq . So for the detection of antibodies of *Toxoplasma gondii* in the sera of women many researches were done by using different techniques (Gerald, D.S.and Larry ,S.R. 2006).Susan,M. Hall (1983) worked on the incidence of toxoplasmosis in England , Wales and Northern Ireland for 10 years . while Fausto G.A.*et al* (1980) used monoclonal antibodies to detect antigen of toxoplasmosis in sera of the patients who were infected with *Toxoplasma gondii* . Ourth ,D.D.(1971) Produced Toxoplasma monoclonal antibody was conjugated with fluorescein isothiocyanate-conjugate globulin made to demonstrate the *Toxoplasma* cysts in paraffin digested tissues of mice .David, K.S.and Grace, P.L.(1989) used quantitative immunofluorescence test to determine the positive antibody levels of *T.gondii* in 67 sera .Moir,I.L.*et al*(1991)studied the IgG antibody to *T. gondii* protein in sera from patients with acute infection , while Patel ,B .*et al* (1993)found detection of specific IgA of toxoplasmosis by using immunosorbent agglutination assay more sensitive than ELISA test in diagnosis of congenital toxoplasmosis .Gilbert,R.E.*et al* (1995)estimated the incidence of acute symptomatic retinochoroiditis for all people in Britain was 0.4/100.000 /year and for people born in west Africa 57/100.000/year.Silvia,R.R.(1999) studied occurrence of toxoplasmosis antibodies in domestic cats in the city of Pualo/Brazil , while Hye-Youn Kim *et al* (2008)Worked on the prevalence of *Toxoplasma gondii* in stray cats of Gyeonggi –Korea . Latex agglutination and ELISA were used for detection . The rate of infection in females was 29.2% and in males was 24%.Al-Ramahi,H.M.*et al* (2007) determined the infection rate of *Toxoplasma gondii* in housewives, veterinarians , butchers ,urban and rural women.

,G.A.*et al* (2009) studied the relation of congenital defect in children with Toxoplasmosis in women of Diwania-IRAQ. The aims of this study to diagnose toxoplasmosis in women And cats in cities of Al- Qadisiya governorate .

Materials and Methods :

Samples of blood (88) collected from aborted women , two cases of women aborted twins and one special case aborted twenty three times . Samples of sera were collected overnight from the coagulated blood.

Latex agglutination test :

- 1- Samples and reagents brought up to the room temperature.
- 2- Place one drop of undiluted serum , one drop posit-ive and one drop negative controls into different circles on the slide .
- 3- Apply adrop of Toxo latex (shaking the vial well) to the circles , mix well with sticks , and rotate slowly the slide .
- 4- After three minutes check for agglutination , at the same time compare with reaction of the control .

Toxo Latex reagent was used in this method produced by the Germany GmbH company .

IgG – ELISA :Enzyme immunoassay for the quantitative of IgG-class antibodies against *Toxoplasma gondii* in human serum or plasma .

Materials :

Reagents :

- 1- *Toxoplasma gondii* coated wells (IgG):12 breakapart 8-well snap-off strips coated *Toxoplasma gondii* antigen , in resealable aliminium foil .
- 2- IgG sample Diluent : 1 bottle containing 100 ml of buffer for sample dilution ,pH7.2+0.2 coloured yellow,ready to use , white cap.
- 3- Stop solution : 1 bottle containing 15 ml sulphric acid , 0.2 mol/1, ready to use , red cap.
- 4- Washing solution (20x conc.) : 1 bottle containing 50 ml of 20 –fold concentrated buffer for washing the wells, pH 7.2 +0.2 white cap.
- 5-*Toxoplasma gondii* anti –IgG conjucate : 1 bottle containing 20 ml of peroxidase labelled antibodies to human IgG , coloured blue , ready to use , black cap.
- 6- TMB Substrate : 1 bottle containing 15 ml 3,3 '5,5- tetramethylbenzidine (TMB),ready to use,yellow cap.

Materials and Equipments used :

- 1- ELISA microwell plate reader equipped for the measurement of absorbance at 450/620 nm .
- 2- Incubator 37C .
- 3- Manual or automatic equipment for rinsing wells
- 4- Pipettes to deliver volumes between 10 and 1000ul
- 5- Vortex tube mixer .
- 6- Freshly distilled water .
- 7- Timer .

Assay procedure :

One well for the substrate , four wells for standard A,B,Cand D.

- 1- Dispense 100ul of each standard (A,B,Cand D)and diluted samples in to respective wells . Leave well A1 for substrate blank .
- 2- Cover wells with the foil supplied in the kit.
- 3- Incubate for one hour +-5 min at 37 +-1C.
- 4- When incubation is completed remove the foil , aspirate the content of wells and each well is washed three times with washing solution .
- 5- Dispense 100ul Toxoplasma anti – IgG conjugate into all wells except for the blank well .
- 6- Incubate for thirty min. at room temperature (20-25C) .
- 7 – Repeat step 4.
- 8- Dispense 100ul TMB Substrate Solution into all wells.
- 9- Incubate for exactly 15 min at room temperature (20- 25C) in the dark .
- 10 – Dispense 100ul Stop solution into all wells in the same order and a same rate as for the TMB solution .

Results :

Ninty one blood samples were collectedfrom aborted women and five san (control) heal- thy women . Six blood samples were collected fromstray cats in the street c center .

Number of abortion was recorded and classified to 1 , 2 , 3 , 4 , 5 , 6 , .

Table (1): Number and percents of abortion .

Frequency of Abortion	No.of aborted women	Percents of aborted women
1	41	46.6
2	30	34
3	11	12.5
4	4	4.5
5	1	1.2
6	1	1.2
Total	88	100

Two cases aborted twins and one case aborted 23 times.

Ninety one cases was the total . So the high percent recorded for the one abortion (46.6%)while the lowest was the five and six abortion (1.2%) table

These cases distributed geographically depend on the cities of the pa (seven cities of Al-Qadisiya gove- rnorate ,therefore high percent was in Diwania (56%) while the lowest percent was in Sidear (1%)table,(2).

Table (2) distribution of aborted women in the cities

Name of the City	No. of cases	percents
Diwania	51	56
Al-hamza	11	12
Al-dagara	5	5.5
Afak	8	8.8
Sania	9	9.9
Somer	6	6.6
Sidear	1	1
Total	91	100

Seventy five cases were positive to latex agglutination test (82%) while the negative were 16 cases (18%)

Fig.(1)The ELISA test by using IgG reveal that 60 cases were positive(80%) the cases were positive from that positive to the latex test while the results of ELISA by using IgM reveal (11)cases were positive (14.6%)from that case which were positive to latex . Four cases were negative for both tests (5.4%) .

The immunofluorescent test was done on 20 samples that were positive to ELISA (IgG) and the results 19 cases were positive (95%).Six samples of stray cats , all of them were positive for both tests agglutination latex and ELISA (IgG) , except one cat was negative to ELISA (IgG) only . So there are 75 cases were positive to *T. gondii* , while the 16 cases of abortion were negative to *T. gondii* and may be other different causes.

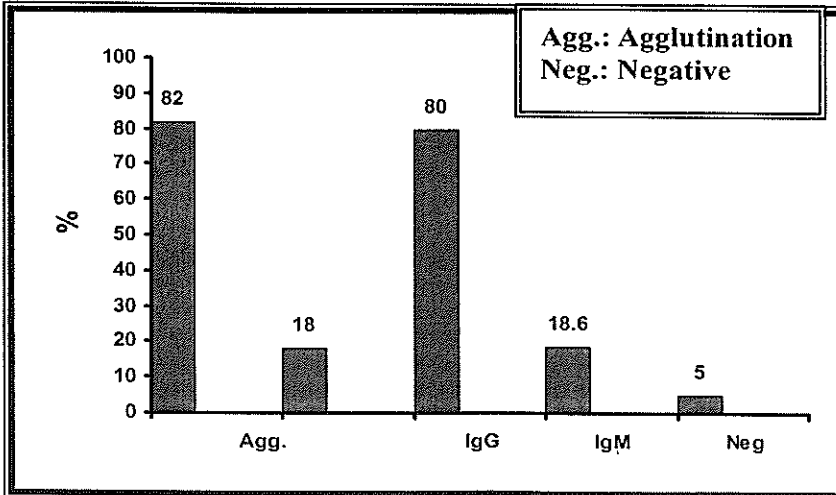


Fig (1) Comparison among the results of tests of agglutination Latex and IgM ELISA

Discussion

Toxoplasma gondii transmitted by ingestion or drinking oocysts contamination food or with fecal materials of cats water , or by congenital mother to the embryo through placenta or by ingestion of infected merozoite or tachyzoite or bradyzoite cyst or through the milk of infected animals , there chance of infection is increased (Dawood ,K.2008).

Louise J. Skinner et al (1989) used of an IgM immunosorbent agglutination assay to diagnose congenital toxoplasmosis .

It was more sensitive in mother of infected babies . These results nearly same as ours about aborted women . Patel, B. et al (1993) investigated that *Toxoplasma gondii* infection is a congenital disease reactivated by (AIDS) and they used the T. gondii by using the IgA serologically , while in our research IgG detection of *T.gondii* Silvia , R.R. et al (1999) found *Toxoplasma* antibody higher in older cats that fed on raw meat and free in outdoor cats , we agree with our results . all examined stray cats were positive for agglutination (Latex). Cook , A. et al (2000) diagnosed acute toxoplasmosis in pregnant women that were eating undercooked lamb , beef or game , with soil , and travel outside Europe and the United States and Canada 60% due to consumption of undercooked meat and 6%- 17% due to contact with the soil , there is no risk factor in contact with cats . Hye-Youn Kim , et al recorded the prevalence of *Toxoplasma gondii* in stray cats of Gyeongju, Korea .

The rate of infection in females 29.2% higher than that of males cats 24% . 24 examined cats in our research give 95% positive in ELISA IgG test . Jaafar .and Nabila ,K.(2007) worked on acute toxoplasmosis in early pregnant Kuwait women and found 61.3% women had high avidity IgM antibodies . Lisandra A. Suzuki et al (2001) examined 64 samples of sera for *Toxoplasma* in Brazil . 31 acute case (48.4%) from patients with *T.gondii* infection from patients (51.6%) with latent infection , while our results quite different for latent infection , 14.6% for acute . Ramahi , H.M. et al (2007) examined different people for antibodies of *T. gondii* , high percents recorded in 68% , while the lowest was in the University students 28.27% . In our work high percents of positive case were recorded in Diwania city which is the main city in Al-Qadisiya governorate . In this city high number of stray cats and high consumption of meat .

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تشخيص داء المقوسات في الإنسان والقطط مناعياً

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ملخصه

هدف هذا البحث هو تشخيص داء المقوسات في النساء والقطط في مدن محافظة القادسية ، ولذا تم جمع 91 وذج دم من النساء وستة نماذج من دم القطط السائبة في الشوارع . استعمل فحص التلازن لاتكس Latex agglutination test ، وكانت النتيجة 75 حالة موجبة لفحص التلازن لاتكس (82%) . كما تم إجراء فحص اليزا (ELISA IgG , IgM) ، وكانت نتيجة فحص الاليزا IgG هي 60 حالة موجبة من مجموع الحالات موجبة لفحص اللاتكس ، أي بنسبة (80%) . أما فحص الاليزا IgM كانت النتيجة 11 حالة موجبة (14%) من مجموع الحالات الموجبة لفحص اللاتكس أيضاً ، بينما كانت هناك 4 حالات (5.4%) سالبة لاختبارين المذكورين . كما تم إجراء فحص التآلق المناعي (Immunofluorescent) لـ IgG لعشرين حالة من الحالات الموجبة لفحص الاليزا لـ IgG وكان النتائج إيجابياً بـ 19 حالة أي (95%) . أما نماذج طوط فكانت جميعها موجبة للفحصين تلازن لاتكس والاليزا IgG ما عدا حالة واحدة كانت سالبة للفحص خيراً .