Detection of Toxoplasmosis in human and cats immunologically

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Abstract:

The aims of this research to diagnose Toxoplasmosis in aborted women and cats in cities of Al-Qadisyia governorate . So 91 blood samples were collected from clinically suspect- ed women and six samples of blood of stray cats .Latex agglutination and ELISA (IgG , IgM) tests were used . The results , 75 cases were positive for Toxoplasmosis in latex agglutination test (82%) , while the 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 11 positive cases (14.6%) from that positive to latex .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 case was negative to IgG.

تشخيص داء المقوسات في الأنسان والقطط مناعياً

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الخلاصة:

أن هدف هذا البحث هو تشخيص داء المقوسات في النساء والقطط في مدن محافظة القادسية ، ولذا تم جمع 91 نموذج دم من النساء وستة نماذج من دم القطط السائبة في الشوارع . أستعمل فحص التلازن لاتكسLatex agglutination test، وكانت النتيجة 75 حالة موجبة لفحص التلازن لاتكس (82%) كما تم أجراء فحص الأليزا (ELISA IgG , IgM) ، وكانت نتيجة فحص الأليزا IgG هي 60 حالة موجبة من مجموع الحالات الموجبة لفحص اللاتكس ، أي بنسبة (80%) . أما فحص الأليزا IgM كانت النتيجه 11 حالة موجبة (14.6%) من مجموع الحالات الموجبة لفحص اللاتكس أيضاً ، بينما كانت هناك 4 حالات (5.4%) سالبه للأختبارين المذكورين .

كما تم أجراء فحص التألق المناعي (Immunofluorescent) لـ IgG لعشرين حالة من الحالات الموجبة لفحص الأليزا للـ IgG وكان التطابق أيجابياً بـ 19 حاله أي(95 %) .

أما نماذج القطط فكانت جميعها موجبة للفحصين تلازن اللاتكس والأليزا IgG ماعدا حالة واحدة كانت سالبه للفحص الأخير .

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 toxoplas- ma in England , Wales and Northern Ireland for ten years . while Fausto G.A.*et al* (1980) used monoclonal antibodies to detect antigen of toxoplasmosis in sera of the patients acutely infected with Toxoplasma gondii . Ourth ,D.D.(1971) Produce Toxoplasma monospecific antibody was conjugated with fluorescein isothiocya-nate .This conjugate globulin made to demonstrate the Toxoplasma cysts in pepsine 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* proteins in sera from patients with acute infection , while Patel ,B .*et al* (1993)found that 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 retinochorditis for all people in Britain was 0.4/100.000 / year and for black people born in west Africa 57/100.000/year. Silvia,R.R.(1999) studied the occurrence of toxoplasmosis antibodies in domestic cats in the city of Sao 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 Toxoplasma gondii in housewives, veterinarians , butchers ,urban and rural womens . Jasim ,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 toxopla-smosis in women And cats in cities of Al- Qadisyia 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 coaggulated 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 slowlythe 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 the respective wells . Leave well Al 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 off the 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 at the same rate as for the TMB solution.

The Results :

Ninty one blood samples were collected from aborted women and five samples (control) heal-thy women . Six blood samples were collected from stray cats in the street of city 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 time abortion (46.6%)while the lowest was the five and six abortion (1.2%) table (1).

These cases distributed geographically depend on the cities of the patients (seven cities of Al-Qadisyia 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%)of 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 cases 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.

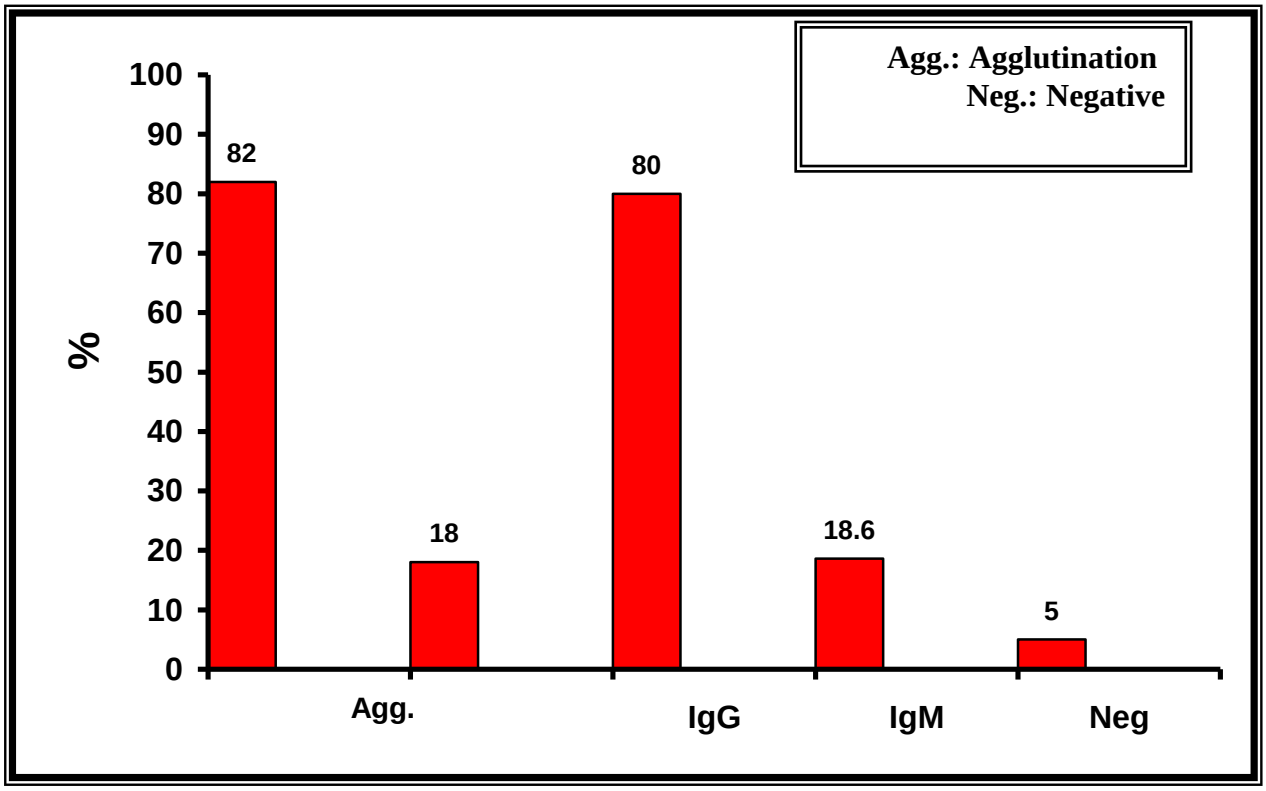

Fig (1) Comparison among the results of tests of agglutination Latex and IgG, IgM ELISA

Discussion:

Toxoplasma gondii transmitted by ingestion or drinking oocysts through contamination food or with fecal materials of cats water , or by congenital from mother to the embryo through placenta or by ingestion of infected meat with tachyzoit or bradyzoit cyst or through the milk of infected animals , therefor the chance of infection is increased (Dawood ,K.2008). Louise J. Skinner et al (1989) used of an IgM immunosor-bent agglutination assay to diagnose congenital toxoplasmo-sis . It was more sesitive in mother of infected babies . These results nearly similar to ours about aborted women .Patel,B. et al(1993) investigated that *Toxoplasma gondii* infection is a congenital disease

reactivated by (AIDS) and they detecte the T. gondii by using the IgA serologically ,while in our research IgG used for detection of *T.gondii.* Silvia , R . R . etal (1999) found Toxoplasma antibodies were higher in older cats that fed on raw meat and free in out door cats , and this agree with our results all examined stray cats were positive for agglutination (Latex). Cook , A. et al (2000)diagnosed acute toxopl-asmosis in pregnant women that were eating undercooked lamb , beef or game , contact with soil , and travel outside Europe and the United States and Canada (30%- 60% due to consump-tion of undercooked meat and 6%- 17% due to contact of the soil , there is no risk factor in contact with cats . Hye-Youn Kim ,et al (2008) recorded the prevalence of *Toxoplasma gondii* in stray cats of Gyeonggi-do,Korea . The rate of infection in females 29.2%higher than that of males cats 24.0% but examined cats in our research give 95 % positive in ELISA IgG test Jamshid ,I .and Nabila ,K.(2007) worked on acute Toxoplasmosis in early pregnancy in Kuwait women and found 61.3% women had high – avidity IgM antibodies , while Lisandr A.Suzuki et al(2001) examined 64 samples of sera For Toxoplasmosis in Brazil . 31 acute case (48.4%)from patients with *T.gondii* infection and 33 from patients (51.6%)with latent infection ,while our results quite different, 80% for latent infection , 14.6% for acute . Ramahi , H.M.et al (2007) examined sera of different people for antibodies of T. gondii , high percents recorded In butchers 68% , while the lowest was in the University students 28.27% . In our present work high percents of positive case were recorded in in Diwania city which is the main city in Al –Qadisyia governorate . In this city high number of stray cats and high cosumption of meat.

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