Seroepidemiological study for the prevalence of *Neospora caninum* in Dairy & Beef cattle in some Iraqi provinces

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Abstract

A Seroepidemiological study of *Neospora caninum* was conducted in Al-Muthana and Al-Nasseria provinces, Iraq on 800 cows serum sample by using commercial Elisa kit. the overall seroprevalence ratio of *Neospora caninum* was 17.5%, on provincial basis *Neospora caninum* infection was present in these provinces that was 16 %, 18.4% in Al-Muthana, and Al-Nasseria provinces respectively,which non significant differences between provinces (P<0.05), Comparisons of *N.caninum* serological status with age groups (5-8 y) showed seropositive rate 21.32% that higher thanother groups with significant differences (P<0.05). antibodies of *N.caninum* showed in aborted cows 32.29% higher than non aborted cows 7.53% with significant differences (P<0.05). Also the infection rate in dairy cows 19.17% higher than beef cows 12.5% with significant differences (P<0.05).

Introduction :

Neosporosis is a parasitic disease caused by *Neospora caninum*, a protozoan that until1988 was misdiagnosed as *Toxoplasma gondii* because of close structural similarities(1,2). Phylogenic studies showed that it is very closely related to *Toxoplasma gondii* and it is now placed as the sister group of *Toxoplasma gondii* (3).some studies have been conducted to assess the prevalence and to identify factors related to the disease, Prevalence's have been estimated in ranges between 16.8% and 70% . (4,10,11).*Neospora caninum* infections have been reported from most parts of the world and seroprevalences for each host were tabulated recently, quantitative studies involving a large number of fetuses in many countries indicate that 12% to 42% of aborted fetuses from dairy cattle are infected with *Neospora caninum*, also in serologic prevalence in cattle varies, depending on the country, region, type of serologic test used, and cutoff level used to determine the exposure. (8). Seroepidemiological studies have assessed the increased risk for abortion in seropositive cows (5,41,11) .The risk of being seropositive may increase with age or parity number in beef and dairy cattle due to horizontal transmission of *N. caninum* by ingestion of oocysts shed by definitive hosts (7). However, the age effect might be influenced by management practices such as replacement rate, which influences the time cattle maybe exposed to horizontal transmission, or by selective culling of seropositive animals (16).Vertical transmission of *Neospora* through generations of cattle appears to be the major method by which *Neospora* infection is maintained in herds and the role of congenital transmission of neosporosis was supported by evidence of the familial distribution of seropositive cattle through successive generations. (15, 10). The direct losses include cost of loss of the fetus, decrease in milk yield and weight gain, while the indirect costs include time for rebreeding, health costs and costs associated with culling. neosporosis is estimated to cause a loss of $35 million per year to the Californian dairy industry alone (45), $85 million to the australian dairy industry and $25 million to the Australian beef industry. (1).

Materials and Methods

The study was done in Al-Muthana and AL-Nasseria provinces .800 cows(dairy & beef) cows) ranged between 1y - 16 year during the period of March 1-3-2010 to June 1-6-2011.

Blood samples collection :

The blood samples were collected from aborted and non aborted cows to detect *Neospora caninum* after cleaning the area by using denatured 70% medical spirit, Five ml of venous blood(Jugular Vein) was taken in a 10 ml vacutinar disposable tube, the blood samples were then centrifuged at 3000 rpm for 5 minutes and serum samples then transferred to 3 ml sized micro test tube with screw cap and stored at 4 – 8°C for 24–48 hrs. then the sera kept in deep freeze at 20 °C After that the samples were transported to the laboratory in Al-Samawa hospital by cooling box. at laboratory, sera samples were examined by Elisa test according to manufacturer's instructions as follow: (w w w.diagnostics.b e).

Assay Procedure:

1. The reagents were allowed to come to room temperature (18-25°C) at least 30minutes before use.

Individual serum: Individual serum and controls have to be diluted 1/100 in sample diluent solution. The positive and negative controls must always be run in duplicate.

20 µl of prediluted 1/20 positive control was added to wells A1 and B1.

20 µl of prediluted 1/20 negative control was added to wells A2 and B2.

20 µl of prediluted 1/20 samples were added for testing to the remaining wells.

80 µl of sample diluent solution was added to each well occupied by controls and samples.then Mixed gently and the plate was covered with an adhesive plate cover (included in the kit). then Incubated for 1hour at 37±2°C.

2- The adhesive cover was remove and the plate was washed 4 times with diluted washing solution. then all the wells were filled to the top for each wash (volume per well: 300 µl). all liquid from the wells were empted and the plate was taped hard to remove the last traces of liquid. Alternatively, the plate was washed 4 times on a automatic plate washer using a well volume of 300 µl.

3. 100 µl of Conjugate Solution was added to each well.

4. The plate was mixed gently and covered with a new adhesive cover and incubated for 1 hour at 37±2°C.

5.The adhesive cover was removed and the plate was washed 4 times with diluted washing solution, all the wells were filled to the top for each wash (volume per well: 300 µl). all liquid from the wells were empted and the plate was taped hard to remove the last traces of liquid. Alternatively, the plate was washed 4 times on a automatic plate washer using a well volume of 300 µl.

6. 100 µl of substrate solution was added a to each well,then mixed gently for 2 seconds.

7. The chromogenic reaction was developed for 10 minutes at room temperature (18-25 ºC) in the dark. the plate didn't cover.

8. 100 µl of stop solution was added to each well, the stop solution was added in the same order as the substrate solution was added, the plate was mixed by gently for 2 seconds.

9. The under-surface of the plate free was wiped of dust with a soft tissue. Finally ,the plate was read using a microtiter plate reader at 450 nm, or at dual wavelength 450-620 nm on a microplate reader.

Calculations

For the interpretation of results, an IRPC value is required (Relative Index x100). The following formula is applied to obtain the IRPC value (using mean DO_{405} values obtained for controls).

$$IRPC = \frac{(OD_{405}\ \text{Sample Mean} - OD_{405}\ \text{NegativeControl})}{(\text{Mean}\ OD_{405}\ \text{PositiveControl} - \text{Mean}OD_{405}\ \text{NegativeControl})} \times 100$$

Interpretation of results:

INDIVIDUAL SERUM

SAMPLE	IRPC VALUE
Negative	≤ 5.0
Positive +	5 < IRPC < 25
Positive + +	25 < IRPC < 50
Positive + + +	50 < IRPC < 100
Positive + + + +	> 100

Statistical analysis:

Statistical analysis were conducted to determine the statistical differences among different groups using ready – made statistical design statistical package for social science (SPSS)., Probabilities of (P< 0.05) were considered statistically significant.

Serological Results:

1.indirect Enzyme linked immunsorbent assay:(iELISA):

The results of serological examination by iElisa of *Neosopora caninum* in cattle (Dairy & beef), that shown the total rate of infection was 17.5%(140/800) ,that the results found in AL-Muthana & Al-Nasseria provinces with percentage ratio 16% ,18.4% respectively which was significant difference(P>0.05). (Table 1) .

Table (1): Positive number and total percentage of infected cows

Provinces	Total samples	Positive sample	Negative sample	Seropositivity rate %
AL-Muthana	300	48	252	16
Al-Nasseria	500	92	408	18.4
Total	800	140	660	17.5

-Significant differences (P>0.05).

Table (2) Showed the distribution of seropositive cows in different age groups.the results of serpositivity rate in the age groups were highest rate was 21.32% in group (5-8) years old and the lowest rate was 12.94%in group (13-16) years.

Table (2): Seroprevalence of *N.caninum* infection in different age of cows.

Age (Year)	Total No.	Positive No.	Seropositivity rate %	Negative No.
1-4	245	38	15.5102	207
5-8	347	74	21.32565	273
9-12	123	17	13.82114	106
13-16	85	11	12.94118	74
Total	800	140	17.5	660

-Significant differences (P<0.05)

Table(3) Showed the results showed highest rate of Seropositivity In aborted cows 104(32.29%) than non aborted cows 36(7.53%), which was significant difference (P<0.05).

Table (3): Seroprevalence of *N.caninum* infection in aborted and non aborted cows

Aborted – Non aborted Cows	Total No.	Positive No.	Seropositivity rate %	Negative No.
Aborted	322	104	32.29814	218
Non aborted	478	36	7.531381	442
Total	800	140	17.5	660

-Significant differences (P<0.05).

As Shown table(4) the results showed the seropositive rate in dairy cows 115 (19.17%) greater than from beef cows 25(12.5 %). which was significant difference (P<0.05).

Table(4): Seroprevalence of *N.caninum* infection in dairy &beef cows.

Dairy/Beef Cows	Total No.	Positive No.	Seropositivity rate %	Negative No.
Dairy	600	115	19.17	485
Beef	200	25	12.50	175
Total	800	140	17.50	660

-Significant difference (P<0.05).

Discussion

Neosporosis has been related with epizootic and sporadic abortion in dairy herds worldwide. Since the discovery of neosporosis, some studies have been conducted to assess the prevalence and to identify factors related to the disease, Prevalence's have been estimated in ranges between 16.8% and 70% .(4,10,11).In the present study the overall seroprevalence rate was 17.5% , this result was nearly the same level as reported in China (17.2%), Brazil (17.8%), Spain(17.9%) (13,36,37) Also this result is lower than that reported for cattle,Spain (36.8%), Urguay (61.3%) Iran (46%), Paraguay (35.7%), Australia (24%), Iraq (19.56%) (40,12,20,27,25,17), but higher than reported in Poland (15.6%), Turkey (13.9%), Canada (6.5%), Korea (4.1%), Italy (6%).(26,32,33,42,34). The variation in the percentage of seroprevalence in our area and other countries may be caused by different climatic and geographical conditions or may be due to characteristics (Sensitivity and Specify) of test used , that the prevalence based on serological tests could not be compared among countries because different tests and cut-off values were used .(6,4,31,41).On the other hand this prevalence might be related to presence of many dogs which consider as definitive host in farm which the sample has been collected because of it play an important role in introduction and maintenance of the infection in herds. (44). Each results in Table (1) the infection rate in Al-Muthana and Al-Nasseria don't showed significant differences (P>0.05) . this result is agreement with (17).The result of study showed an association between serological status and cow age and this study showed the positive seroprevalence of *N.caninum* increasing in age (5-8) years and this is agreement with (8,14,18) that they determined the risk of being seropositive may increase with age(4-8)years due to horizontal transmission of *N.caninum* by ingestion of oocysts shed by final host, but the result of our study is disagreement with (12,23,42,11,9) that they showed no significant difference between age group, while (35)determined the seroprevalence of *N.caninum* increasing in age 1-3 years old. In Iran, regarded(12) the higher seroprevalence *Neospora caninum* in 3-4 year old cows that suggesting post natal transmission of *Neospora caninum.*While (14) showed that seropositivity increased with age .In Iraq, Showed (17) seropositivity prevalence rate *N.caninum* was 33.33% 1n 2-4 years which greater than 5 years was lowest..The association of

infection with abortion ,in the present study showed that the prevalence of *N.caninum* was higher in the aborted group 104(32.29%) than non aborted group 36 (7.53%) which was significant difference ($P<0.05$) (Table3), this result was nearly the same level as reported in New Zealand (33.6 %) (38).but disagreement with (17) in Iraq, that reported the overall seroprevalence of *Neospora caninum* in three provinces (Dawania ,Nasseria, Basrah) was 19.56%.Also this result is different in parts of the world that reported for aborted cattle Japan 145(20%), Poland 45(15.6%), Argentina 189(64.5%), Hungary 97(10%), Sweden 70(63%)United Kingdom 95(60%). (24,26,21,22,28,29). This variation in the percentage of seroprevalence in the countries may be due to different in numbers of examined animals(aborted cattle) or to different tests were used or to the point source exposure to *N.caninum* oocysts which excreted by final host (dogs) (19).While (30) found the abortion storm in cattle due to that *N.caninum* was introduced to the region by imported cattle and therefore risk of vertical transmission to fetuses and abortion was important. A possible explanation for the fact that many non aborting cows were seropositive relates to the pathogenesis of disease and the host immune system , that these cows may have been infected with the parasite but the number of *N.caninum* tachyzoite in the host tissue may not have enough to cause clinical symptoms. abortion.(7).Observed (5) a markedly increased abortion risk in congenitally infected heifers during their first gestation but not in later gestation compared to abortion risk in seronegative controls.Seroepidemiological studies have assessed the increased risk for abortion in seropositive cows (11,41).In Table (4) the present study showed that the prevalence of *N. caninum* was higher in the dairy cows 115(19.17%) than beef cows 25 (12.50%) this result was nearly the same level as reported in Brazil (18.60 %), in dairy cows and (12.9 %) in beef cows. (19).Beef and dairy herds are managed in different production system ,beef cattle are usually raised in extensive grazing system whereas dairies are intensively exploited (39).Differences in the management between dairy and beef herds could explain the high prevalence of neosporosis found in dairy compared with that in beef cattle, also postnatal transmission could be more frequent in dairy cattle because they are more intensively exploited than beef cattle.(31).While (43) found that the differences in infection between dairy and beef cattle generally that beef cattle raised under less stressful conditions such as winter stocking density , more regular stock movement than dairy cattle , while the dairy cattle that more supplemental feeding practices ,frequent regular stock movement , high stocking density of cattle may increase the risk of horizontal transmission through a definitive host.

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دراسة وبائية مصلية لانتشار طفيلي *Neospora caninum* في أبقار الحليب واللحم في بعض محافظات العراق

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الخلاصة

تم إجراء دراسة مصلية وبائية في انتشار البوغية الكلبية الجديدة *N.caninum* في محافظتي المثنى والناصرية, على 800عينة مصل للأبقار باستخدام اختبار الاليزا التجارية وكانت نسبة الإصابة الكلية17.5%وكانت نسبة الإصابة في محافظة المثنى والناصرية % 16 , 18.4% على التوالي وبدون فرق معنوي ذو دلالة إحصائية ($P>0.05$). وأظهرت نتائج الدراسة مقارنة للأعمار إن اعلي الفئات العمرية إصابة هي الفئة (y 8-5) وبنسبة 21.32% وكانت أعلى من المجاميع الأخرى وبفرق معنوي ($P<0.05$).كذلك أظهرت نتائج الدراسة إن معدل الإصابة في الأبقار المجهضة 32.29% أعلى من الأبقار الغير مجهضة 7.53% وكذلك نسبة إصابة أبقار الحليب 19.17% أعلى من أبقار اللحم 12.5% وبفرق معنوي ذو دلالة إحصائية ($P<0.05$), نتائج الدراسة تشير إلى انتشار الطفيلي في محافظات العراق.