

## SEROPREVALENCE OF *NEOSPORA CANINUM* IN CATTLE IN SOME PROVINCES IN IRAQ

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### ABSTRACT

A seroepidmiology study of *Neospora caninum* was conducted in Dawania , Nasseria and Basrah provinces, Iraq on 92 cows by using commercial ELISA kit .The overall seroprevalence of *Neospora caninum* was 19.56% on provincial basis *Neospora caninum* infection was present in the three provinces Antibodies to *N.caninum* was found in 13(40.625%) of 32 aborted cows . The prevalence of *N.caninum* was significantly higher in the aborted cows than in non-aborted cows ( $p<0.05$ ). Comparison of *N.caninum* serological status with age groups, year , 2 -4  $\geq$ 5 years) showed seropositivity prevalence rate 33.33% in 2-4 years age group while greater than 5 years was lowest. Our result indicate that *Neospora* infection is widespread in Iraq.

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**Keywords:** *Neospora caninum*, Seroepidemiology; Abortion, ELISA

### INTRODUCTION

*Neospora caninum* is considered as one of most important causes of abortion and infertility in cattle world wide (**Dubey and Lindsay, 1993 ; Anderson et al. ,1995; Barr et al. , 1997 ; Dubey et al. , 2007**). Abortion in some herds up to 88%. of one infected (**Campoero et al. , 1998**). *Neospora caninum* is obligate intracellular apicomplexa protozoan parasite .

It has been detected in several mammalian species , i.e. , sheep , goat , horses , deer (**Dubey and Lindsay , 1993** ) water buffaloes (**Guarino et al. , 2000** ) , rhinoceros (**Williams et al. , 2002**) and foxes (**Almeria et al. , 2002**) . Dog were the first definitive host of *N. Caninum* (**Mc Allister et al. , 1998**) also coyotes (*Canis Latrans* were also demonstrated to be definitive hosts of the parasite (**Gondim et al. , 2004 ; Mc Allister et al. , 2004**) .Other possible hosts of interest are of course , human , sera famers and aborting women have been examined for presence of antibodies but no clearly positive samples were found (**Petersen et al. , 1999; Graham , 2006**) . However a recent study found antibodies to *N. Caninum* in 38% of HIV infected patient (**Lobato et al. , 2006**) . These findings might bring a new concern for the actual role of *N.Caninum* infection in immuno-compromssed patients .

Cattle can be infected with *N. Caninum* in two ways the first by transmission of parasite from the cow to her fetus during gestation , second by transmission of the parasite through faces from definitive host (**Pare et al. , 1997 ; Wouda et al., 1998; Davison et al., 1999**) and In experimental study new born calves were infected through colostrums mixed with tachyzoites (**Venturini et al. , 1999**) . The parasite elicits an antibody response in infected animals , detection of these antibodies is a sign of exposure to the parasite but not necessarily predictive of abortion (**Dubey et al. , 1996**) .

ELISA has become one of the most commonly used assay for the serologic diagnosis of *N. Caninum* infection in cattle (**Anderson et al. , 1995**) . ELISA enables rapid analysis of samples and extremely useful for large-scale screening of cattle herd (**Atkinson et al. ,2000**). Serological diagnosis for *N. Caninum* are needed to obtain information about epidemiology of life cycle differentiate between recent and chronic infection and determine seroprevalence in regions and countries (**Bjorkman and Ugglä , 1999**) . They have been no reports of *N.Caninum* infection in Iraq .So, the objective of the present study was determine of seroprevalence of *N.Caninum* antibodies in healthy and aborted cattle with ELISA for the first time .

## **MATERIAL AND METHOD**

### **Field study area**

The study samples were collected from healthy and aborted cows in three Iraqi provinces (Dawania, Nasseria and Basrah) .Table (1), indicate that, the first province was located in the middle of Iraq and the second and third were located in south of Iraq. The climate is moderate and cold winter and very hot summers, the temperature reach 55C at summer.

### **Serum collection**

Ninety two blood samples were taken on Jan. and June 2009 by using disposable needle from jugular vein in sterile tubes .All samples were immediately transported to the diagnostic laboratory. Serum was obtained after centrifugation at 2000 rpm for 15 minutes and stored at -20 C until serological tests were conducted.

**Serology**

Serum samples were analyzed for antibody activity to *N. caninum* by using the commercially available ELISA kit (IDEXX laboratories) all reagents were in room temperature and vortexed , used diluted samples 1: 100 in phosphat buffered saline solution . Negative and positive controls were dispensed (100 µl) into recorded wells serum samples were in duplicates and incubated for 30 minutes at Room temperature., each well was washed four times, then 100 µl of anti-bovine: HRPO conjugate was dispended into each well and incubated for 30 minutes at room temperature., 100 µl of TMB substrate solution was dispensed into each well and incubated for 15 minutes at room temperature., 100µl of stop solution was dispensed into each well of test plate to stop the reaction. Measures were recorded at absorbance 630 nm. Results were calculated under the following: formula of cut- off

$$s/P = \frac{\text{sample A}(360)NCX^-}{PCX^- - NCX^-}$$

PCX<sup>-</sup> = positive control mean      NCX<sup>-</sup> = Negative control mean

Sera with absorbance values above the cut-off level of 0.20 were considered positive according to manufacture instruction. Two repetitions from each sample were perform.

**RESULTS**

The results of serological examination by IgG Elisa of *N.caninum* were defected in (18/92) 19.56% . Positive while the negative results were (74/92) 80.43% of cows. Each results were found in three provinces which were Dawania , Nasseria and Baserah , non significant differences (p>0.05) . Table.1

**Table (1):** Seroprevalance of *N.caninum* in cows in three provinces, Iraq

Provinces	Negative	Positive	Total	X2 value p<0.05
Dawania	25	5	30	Cal.x <sup>2</sup> =0.921 Tab. X <sup>2</sup> =7.814 df =3 Non significant
Nasseria	24	8	32	
Basrah	25	5	30	
Total	74	18	92	

From the 92 cows sampled ,32 had a previous record of abortion of these 18 were seropositive and 74 were seronegative , the seroprevalance of *N.caninum* was significantly differ (p<0.05) of aborted group than in non aborted group .

**Table (2)** Seroprevalance of *N.caninum* in non- aborted and aborted cows

Provinces	Non aborted cows %	aborted cows %	Seropositive rate in each Provinces	<sup>2</sup> value
Dawania	1/20 ( 5 %)	4/10 ( 40 %)	5/30 (16.66 %)	Cal.x <sup>2</sup> =5.802 Tab. X <sup>2</sup> =5.991 df = 2 Non significant p>0.05
Nasseria	3/20 (15 %)	5/12 ( 41.66 %)	8/32 (25 %)	
Basrah	1/20 (5 %)	4/10 (40 %)	5/30 (5 %)	
Total	5/60 (8.3 %)	13/32 (40.62 %)	18/92 (19.56 %)	Cal.x <sup>2</sup> =21.295 Tab. X <sup>2</sup> =3.841 df = 1 significant p<0.05

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Table(3) Showed the distribution of seropositive cows in the different age groups. The result of seropositivity in the age group were 33.33% and 8% of the age group 2-4 years group and 5-8 years respectively which was significantly differ at ( $p<0.05$ )

**Table (3) Seropositivity related to age of cows.**

Age group	2-4 years Age group %			Above 5 years (5-8) years				X <sup>2</sup> value p<0.05
Age	≤2	3	4	5	6	7	8	Cal.x <sup>2</sup> =74.08 Tab. X <sup>2</sup> =11.0705 df = 5 significant
Sera test Positive	4/12 33.3 %	4/14 28.5 %	6/16 37.5 0 %	3/23 13.1 %	1/14 7.14	0/8 0 %	0/5 0	
Total Sera test Positive of each groups	14/42 33.33 %			4/50 8.0 %				

## DISCUSSION

Neosporosis has been related with epizootic and sporadic abortion in cattle worldwid. Since the discovery of neosporosis some studies have been conducted to assess the prevalence and to identify factors related to the disease . Prevalences have been estimated in ranges between 4.3% and 70% (**Pare et al. , 1995 ; Pare et al. , 1997 ; Thurmond et al. , 1997 ; Waldner et al. , 1998**) . It has been reported in many countries with different prevalence rates since the disease was recognized in 1988 (**Buxtone et al.,1997 ; Campero et al., 1998 ; Cabaj et al.,2000 ; Dijkstra et al., 2001 ; Waldner et al.,2001; Kim et al.,2003**). In the present study, the prevalence was 19.06% . This result is higher than that reported for cattle in Poland (15.6%), Turkey (13.96%) ,Canada(9%),

korea (4.1%),but is lower than reported France (83%) , Spain (58%) , Iran (46%) and Paraguay (29.8%) (**Quintanilla – Genazalo et al. , 1999 ; Ould-Amrouche et al. , 1999 ; Waldner et al. , 2001 ; Osawa et al. , 2002 ; Kim et al. , 2003; Razmi et al. , 2006 ; Vural et al. , 2006**).

The Variation in the percentage of seroprevalance in our area and other parts may be caused by different climatic and geographical conditions , and may be reflected differences in prevalence between countries and also due to the characteristics (sensitivity , specify) of test used (**Tree et al. , 1994 ; Pare et al. , 1997 ; Pare et al. , 1998 ; Perez et al. , 1998 ; Wouda et al. , 1998 ; Barling et al. , 2000 and Bergeron et al. ; 2000** ) . On the other hand this might be related to the presence of many dogs definitive host in farms from which the samples has been collected because of it play an important role in introduction and maintenance of the infection in herds (**Dubey , 1999**) .

The association of infection with abortion ,in the present study showed that the prevalence of *N.caninum* was higher in the aborted group 13/32 ( 40.62%)than non aborted group 5/60 (8.33) which was significant differ ( $p<0.05$ ).Table ( 2 ) . A more definitive diagnosis can achieved when the abortion problem is examined on a herd. Serological approaches have been proposed to determine association between infection and abortion (**Thurmond et al. , 1997 ; Jenkin et al. , 2006**) . The result of study was in agreement with studies of **Osawa et al. (2002) ; Razmi et al. (2006)** which showed that abortion rate in total herd 56.7% , 46% respectively and seroprevalence of Abs was 262/879 (29.8%) , 85/170 (50%) , however the probability of abortion in seropositive cattle is twice that in seronegative cattle (**Moen et al. , 1998**). Several studies demonstrate that chronically infected seropositive cows can have more than twofold-increased risk of abortion compared to seronegative dams (**Wouda et al. 1998; Sager et al. 2001; Lo´pez-Gatius et al., 2004**).

Moreover, there are indications that the risk of endogenous abortion is influenced by the parity of the dams (**Lo´pez-Gatius et al., 2005**) .

**Thurmond and Hietala (1996)** observed a markedly increased abortion risk in congenitally infected heifers during their first gestation but not in later gestations, compared to the abortion risk in seronegative controls

.Seroepidemiological studies have assessed the increased risk for abortion in seropositive cows (Thurmond and Hietala, 1996; Perez et al., 1998; Waldner et al., 1998; Wouda et al., 1998) which also have higher risks for stillbirth (Waldner et al., 1998), a seropositive offspring, culling for reproductive reason (Thurmond and Hietala, 1996; Paré et al., 1997; Waldner et al., 1998) compared to seronegative ones. It is expected that infected cows have higher odds of subsequent abortions. Neosporosis-induced abortions occur year-round. Cows with *N. caninum* antibodies (seropositive) are more likely to abort than seronegative cows and this applies to both dairy and beef cattle (Dubey, 1999).

The result of our study is in agreement with studies carried out by (Osawa et al., 2002; Razmi et al., 2006), that showed the abortion rate in total herd 56.7%, 46% respectively and seroprevalence of Abs was 262/879 (29.8%), 85/170 (50%), however the probability of abortion in seropositive cattle is twice that in seronegative cattle (Moen et al., 1998). The study showed an association between serological status and cow age significantly ( $p < 0.05$ ) (Table 3). Sanderson et al., (2000) determined that seropositivity in cows under three years old which is in agreement with our study, also a higher seroprevalence for cows in age group between 1-3 years old was observed by Dijkstra et al. (2001). The differences in seroprevalence by age group might be due to point source of infection as suggested by McAllister et al. (1998). The other studies have not showed an association between serological status and cow age (Vural G., et al., 2006; Waldner et al., 1998; Davison et al., 1999).

## CONCLUSION

In conclusion this is the first seroprevalence study of bovine neosporosis in Iraq. Results showed the presence of disease; further epidemiologic studies are needed to provide a better understanding of neosporosis, and determine the incidence of abortion due to *Neospora caninum* in Iraq.

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دراسة مصلية وبائية في انتشار البوغية الكلبية الجديدة في ابقار مدن عراقية  
(الديوانية,الناصرية والبصرة)

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الخلاصة

تم اجراء دراسة مصلية وبائية في انتشار البوغية الكلبية الجديدة في مدن عراقية هي الديوانية ,الناصرية والبصرة على 92 بقرة باستخدام اختبار الاليزا التجارية وكانت الاصابة في جميع المحافظات وبنسبة انتشار 19.56 %واظهرت الدراسة ان نسبة الاجسام المضادة في الابقار ال مجهزة هي 40.625 %من 32 بقرة مجهزة وبفرق معنوي ذو دلالة احصائية عن غير المجهزة وبالمقارنة بين العمر ونسبة الاصابة اظهرت الدراسة ان نسبة انتشار المرض 33.33%بين المجموعة العمرية 2-4 سنة اكثر من المجاميع الاخرى.نتائج دراستنا تشير لانتشار المرض في العراق .