

SERODIAGNOSIS OF BOVINE NEOSPOROSIS IN MOSUL CITY, IRAQ

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ABSTRACT

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One hundred and eighty four sera of local breed cattle in Mosul city , Iraq were collected for serodiagnosis of neosporosis by indirect enzyme linked immunosorbent assay (Indirect ELISA). The total percentage of seropositive was 17.4% (32 \ 184), while the percentages of *Neospora caninum* antibodies were 12.9 , 13.1, and 26.2 in (62) healthy non pregnant animals, (61) aborted cows and (61) pregnant cows respectively. This is the first study with regards to serodiagnosis of neosporosis in cattle in Mosul city , Iraq.

Key words : *Neosporosis, Serology, Bovine.*

INTRODUCTION

Neospora caninum is a heteroxenous cyst – forming Dubey (2003), that was first recognized in dogs in 1984 (Bjerkas *et al.*, 1984). and was described as a new genus *Neospora*, type species *Neospora caninum* in 1988 (Dubey *et al.*, 1988). Cattle and other ungulates such as sheep , goats, horses, white – tailed deer, camels and water buffaloes may act as natural intermediate hosts (Dubey, 2003; Rodrigues *et al.*, 2004 ; Chavez-Velsquez *et al.*, 2004). Canids such as dogs are the definitive host (McAllister *et al.*, 1998). This parasite is new recognized as an important cause of reproductive problems and abortion in dairy and beef cattle worldwide (Dubey, 2003; Dubey *et al.*, 2006). Infection in cattle is transmitted either transplacentally or by ingestion of sporozoite – containing oocysts shed by definitive host (Gondim *et al.*, 2004).

Generally, the diagnosis of *Neospora caninum* associated abortion has relied on the histological examination of infected fetuses (Dubey *et al.*, 2006). Other methods used to study *Neospora caninum* include isolation of the parasite in cell culture (Lei *et al.*, 2005), an indirect fluorescent antibody test on various body fluid (Rahman *et al.*, 2011), immunohistochemistry (Boger and Hattel, 2003) and a variety of enzyme linked immunosorbent assay (Jenkins *et al.*, 2005; Gaturaga *et al.*, 2005). The detection of specific anti-*neospora caninum* antibodies in sera of cows has been useful for the diagnosis of disease and may also prove suitable for seroepidemiologic investigations (Koiwai *et al.*, 2005; Nourollahi *et al.*, 2008; Alan *et al.*, 2011), therefore, this is the first study to investigate the serodiagnosis of bovine neosporosis in Mosul , Iraq.

MATERIALS and METHODS

A total of 184 sera (62 sera from healthy non pregnant cows, 61 sera from aborted animals and 61 sera from pregnant cows) were collected from local breed cattle

(3 – 10 years old) from different regions in the Mosul city , Iraq during the period from July 2011 to April 2012.

A commercial ELISA kit (Bio – X *Neospora caninum*, Indirect ELISA kits) for detection of antibodies against *Neospora caninum* in bovine serum was used , the kit has been supplied from Bio – x Diagnostics – Belgium. The test uses 96- well microtitration plates sensitized by a purified *Neospora caninum* protein. The plates odd columns (1, 3 , 5, 7, 9 and 11) contain the purified protein , where as the even columns (2, 4, 6, 8 , 10 , 12) contain a control antigen. We thus have agenuine negative control. Using such a control reduce the number of false positive considerably.

All sera were tested according to the manufacturers instructions, then read the optical densities in the microwells using a microplate reader at a wave length of 450 nm. ELISA optical density (OD) reading were transformed to serum / positive percentage (S / P) according to a specific equation cited by manufacturer. The sample considered positive if it gives $S / P \% > 15 \%$, $10 \% < S / P \% < 15 \%$ considered doubtful , but $S / P \% < 10 \%$ considered negative.

S / P= Delta OD sample / Delta OD positive X 100

The data management and statistical analysis by two – ways analysis of variance were performed using SPSS version 11.5 (2002) software for windows.

RESULTS

The results of study showed that the total percentage of seropositive of *Neospora caninum* antibodies was 17.4 (mean 32 seropositive out of 184 sera). Among the 61 pregnant cows, 16 sera (26.2 %) were seropositive, where as among 61 aborted cows, 8 sera (13.1 %) were seropositive. Of the 62 healthy non pregnant cows, 8 (12.9 %) were seropositive (Table1).

The percentage of seropositive values (S / P %) was significantly higher in the aborted animals than in other animals (Table 2).

Table 1: Percentages of seropositive of bovine neosporosis in Mosul , Iraq

| Origin of examined sera | Number of sera tested | Number of seropositive (%) |
|--------------------------------|-----------------------|------------------------------|
| From pregnant cows | 61 | 16 / 61 (26.2) |
| From aborted cows | 61 | 8 / 61 (13.1) |
| From healthy non pregnant cows | 62 | 8 / 62 (12.9) |
| Total | 184 | 32 / 184 (17.4) |

Table 2: Distribution of the percentage of serum / positive (S/ P %) values for *Neospora caninum* seropositive cows

| Origin of examined sera | % of serum / positive |
|--------------------------------|-----------------------|
| From pregnant cows | 32 .8 ± 2.3* |
| From aborted cows | 126. 3 ± 7.2 |
| From healthy non pregnant cows | 23.5 ± 2.0 |

• Values expressed as (Mean ± S.E).

* Significantly at p< 0.05 .

DISCUSSION

This is the first serodiagnosis of bovine neosporosis in the Mosul city, Iraq. In this study an antibodies against *Neospora caninum* was detected in the 32 of 184 sera. Serological surveys indicate wide spread exposure to *Neospora caninum* in dairy and beef herds in many parts of the world (Anderson *et al.*, 2000).

The seroprevalence of neosporosis in cattle varies depending on the country and region under study (Rahman *et al.*, 2011; Koiwai *et al.*, 2005; Nourollahi *et al.*, 2008; Akca *et al.*, 2005), while there is no published information on the epidemiology of *Neospora caninum* in cattle in Mosul city, although many authors observed many serological studies of canine and bovine neosporosis in the local countries as Iran (Nourollahi *et al.*, 2008; Akca *et al.*, 2010; Malmasi *et al.*, 2007; Haddadzadeh *et al.*, 2007) and Turkey (Alan *et al.*, 2011; Akca *et al.*, 2005; Kurtdede *et al.*, 2006; Oncel and Biyikologlu, 2003; Simsek *et al.*, 2008).

In our study serodiagnosis of bovine neosporosis based on the results of the indirect ELISA test. This diagnosis of neosporosis in the live animal can be achieved by detection of anti - *Neospora caninum* antibodies using different serological tests, but ELISA is an approved serological test (Von Blumroder *et al.*, 2004). ELISA is the most suitable for high through put screening of antibodies to parasites , that has been used in epidemiological studies to estimate the prevalence of *Neospora caninum* infection and to examine the relationship between exposure to *Neospora caninum* and abortion, milk yield and

culling in cattle (Hernandez *et al.*, 2002). Some researches have recommended that the serological status of the herd be determined to obtain information about the risk of abortion and attributable to *Neospora caninum* infection.

In this study the percentages of seropositive were 12.9, 13.1 and 26.2 % in the healthy non pregnant animals, aborted cows and pregnant cows respectively. Review of all published data indicate that *Neospora caninum* is a primary abortifacient in cattle (Dubey, 2003; Dubey *et al.*, 2006) and *Neospora caninum* infection is generally latent and asymptomatic in non pregnant cattle, although bovine neosporosis in pregnant cow is associated with repeated abortion and birth of clinically healthy but persistently infected calves (Buxton *et al.*, 2002).

Several studies demonstrate that chronically infected seropositive cows have an about two – to three fold increased risk of abortion compared to seronegative dams (Pare *et al.*, 1997 ; Wouda *et al.*, 1998; Pfeiffer *et al.*, 2002). Thurmond and Hietala (1997) observed a 7.4 -fold higher risk of abortion during the first gestation of congenitally infected heifers.

The percentage of seropositive values was significantly higher in the aborted animals than in other animals which reflect the high concentration of antibodies against neosporosis, as also in the previous serological studies showed the aborted dams from herd with endemic bovine abortion have higher antibodies against specific antigens (Schaes *et al.*, 2000). Other researcher has also shown that the high antibody titer are found in post abortion sera and

during the second part of pregnancy (Schaes *et al.*, 2000; Quintanilla-Gozaló *et al.*, 2000).

In conclusion, this the first report of serodiagnosis of bovine neosporosis in Mosul, Iraq, however, further epidemiological studies are needed to provide a better understanding of neosporosis under local conditions.

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التشخيص المصلي لمرض الإجهاض البوغى (النيوسبوروسز) في مدينة الموصل – العراق

تم جمع 184 عينة مصل من الأبقار المحلية في مدينة الموصل ، العراق للتشخيص المصلي لداء الإجهاض البوغى الجديد (النيوسبوروسز) باستخدام اختبار التحليل المناعي الإنزيمي (الاليزا) غير المباشر. النسبة المئوية الكلية للعينات الموجبة بلغت %17.4 (184/32) بينما بلغت نسبة تواجد أضداد البوغ الكلابي الجديد *Neospora caninum* 12.9 ، 13.1 و 26.2 في (62) من الأبقار السليمة غير الحوامل و(61) من الأبقار المجهضة و (61) من الأبقار الحوامل على التوالي. وتعتبر هذه الدراسة الأولى من نوعها بالنسبة للتشخيص المصلي لداء الإجهاض البوغى الجديد في الأبقار في مدينة الموصل ، العراق.