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Prevalence of antibodies to *Neospora caninum* in cattle in Kerman province, South East Iran

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ABSTRACT

Neospora caninum is an intracellular parasite which causes abortion in cattle worldwide. The aim of this study was to determine the seroprevalence of *Neospora caninum* in cattle in the province of Kerman in South East Iran. Blood samples were collected from 285 cattle in the province of Kerman for determining the seroprevalence of *Neospora caninum*. A total of 285 serum samples were tested for anti-neospora antibodies. Serum samples were analyzed for antibodies against *N. caninum* antigen using a commercial *N. caninum* ISCOM ELISA kit. Antibodies to *N. caninum* were found in 36 of the 285 (12.6%) sera based on ELISA test results. This study is the first report of *Neospora* infection in this area. With regard to seropositivity, no significant difference was observed regarding origin, sex and age (P>0.05).

Key words: *Neospora caninum*, cattle, antibody, ELISA

Introduction

Neospora caninum (Apicomplexa) is a worldwide-distributed pathogen which causes abortions in cows leading to economic losses in the cattle industry (DUBEY, 1999a).The parasite was first detected in 1984 in dogs with myositis, lameness and encephalitis and named as *N. caninum* (BJERKAS et al., 1984; DUBEY, 1999b). *Neospora caninum* has worldwide distribution and has been known as one of the most commonly diagnosed causes of bovine abortion. The parasite was subsequently identified in aborted bovine

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foetuses (BARR et al., 1990; THILSTED and DUBEY, 1989) and is now recognized as a significant cause of economic loss in dairy and beef cattle herds worldwide, due primarily to abortion and reduced reproductive efficiency (BARLING et al., 2000; DUBEY, 1999b, WALDNER et al., 1998). The economic impact of *Neospora*-induced abortions depends on direct costs and the value of fetuses lost. Indirect costs include those associated with establishing the diagnosis, rebreeding cows that aborted and possible loss of milk yield. As clinical diagnosis is difficult, serological tests are necessary for an exact diagnosis. Several serological tests, including the enzyme-linked immunosorbent assay (ELISA), the indirect fluorescent antibody technique (IFAT), the direct agglutination test (DAT), and immunoblots (IB) can be used to detect anti *Neospora caninum* antibodies (BJORKMAN and UGGLA, 1999).

N. caninum is transmitted vertically from an infected cow to her foetus during pregnancy (ANDERSON et al., 1997). Dogs have been shown to excrete *N. caninum* oocysts (DeMAREZ et al., 1999; LINDSAY et al., 1999; McALLISTER et al., 1998).

Although neosporosis has been reported from many parts of the world (DUBEY and LINDSAY, 1996; DUBEY et al., 2005), there is only one published report available on its occurrence in Iran, Mashhad; that indicated that 123 (15.18%) of 810 cattle were seropositive by indirect fluorescent antibody test in 4 herds (SADREBAZZAZ et al., 2004). So this study was performed to determine the prevalence of antibodies to *N. caninum* in cattle in the province of Kerman in south-eastern Iran.

Materials and methods

Serum samples. Serum samples were collected from a total of 285 cattle, the animals being randomly selected. Blood samples were taken using disposable needles. The owners were questioned about animal management and age, and the information obtained was recorded. This study was performed between September 2005 and October 2006. All samples were immediately transported to the diagnostic laboratory. Serum was removed after centrifugation at 1000×*g* for 10 min. All sera were divided equally into two microtubes and stored at -20 °C until laboratory testing.

ELISA. Serum samples were stored at -20 ºC until tested. They were analyzed for antibodies to *N. caninum* using ELISA. Anti-Neospora antibodies were detected using a commercially available *N. caninum* iscom ELISA kit (Svanova Biotech AB, Sweden). The kit was used according to the manufacturer's instructions. Briefly, 100 microlitres of pre-diluted serum sample added as first antibody and the plate incubated at 37 °C on shaker for 1 hour. The wells were washed 3 times with PBS Tween Buffer and 100 microlitres of HRP conjugate added to each well and incubated for 1 hour at 37 °C. The plate was washed again and 100 microlitres of substrate solution added and incubated at room temperature for 10 minutes. Then 50 microlitres of stop solution were added to

stop the reaction and the plates were read in an ELISA microplate reader (Anthos 2020, Austria) at a wavelength of 450 nm. The optical density (OD) of the ELISA was read on an automatic plate reader and the Percent Positivity values (PP) of the test samples were calculated by the following formula:

PP = Mean OD value (sample or Negative Control) × 100) / Mean OD value Positive Control

The results were expressed as the percent positivity (PP) of the high positive control sera. The manufacturer's current recommendations for the interpretation of the test are that a test result of below 20 PP indicates a negative result, and a test result of above or equal to 20 PP indicates a positive result.

Statistical analysis. A chi-square test of independence was used to analyze associations between infection by *N. caninum* and other factors studied in the present study. For statistical analysis, the SPSS 12 computer program was used and $P<0.05$ was considered to be significant.

Results

Results obtained from the sera using ELISA are given in Tables 1 and 2. The results were expressed as the percent positivity (PP) of the high positive control sera. Antibodies to *N. caninum* were found in 36 of the 285 (12.6%) sera based on ELISA results. Among the 104 sera in the cattle <18 month age group, 11 (10.5%) were seropositive, whereas among the 181 sera above 18 months old, 25 (13.8%) were seropositive (Table 1). Among the 98 bulls, 10 (10.2%) were seropositive whereas of the 187 cows, 26 (13.9%) were seropositive (Table 2). There was no statistically significant relationship between seroprevalence of sex and age groups ($P>0.05$)

Table 1. Seroprevalence of *Neospora caninum* in relation to age

Age	The number of animals tested	No. of positives	Seroprevalence (%)
<18 months	104	11	10.5
≥18 months	181	25	13.8

Table 2. Comparison of *Neospora caninum* antibodies in relation to sex

Sex	The number of animals tested	No. of positives	Seroprevalence (%)
Bull	98	10	10.2
Cow	187	26	13.9

Discussion

N. caninum is considered to be one of the major causes of abortion in cattle worldwide (BARLING et al., 2000; DUBEY, 1999a). In contrast to vertical transmission, horizontal transmission involves a two-host life cycle whereby the cow is infected from the ingestion of coccidial oocyst stages shed by the definitive host. Dogs are known to be a definitive host and produce oocysts in their faeces after ingesting infected meat (McALLISTER et al., 1998; GONDIM et al., 2004).

As there was only one published report available on its occurrence in Iran on *N. caninum* infection in North East of Iran (Mashhad) (SADREBAZZAZ et al., 2004) we decided to obtain information on seroprevalence of *N. caninum* antibodies in cattle in South East Iran (Kerman). Several serologic tests including ELISA, IFAT, and DAT can be used to detect *N. caninum*. The capability of a test to distinguish infected from non infected individuals is often described by its diagnostic sensitivity and specificity. All the serological tests mentioned above are valuable for identifying sera with moderate to high levels of anti- neospora antibodies. At present, the 2 main types of serological tests most commonly used for the diagnosis of Neospora infection are IFAT and ELISA.

Characterization studies have shown that *N. caninum* NC-1 iscoms contain membrane antigens from both the cell surface and from intracellular compartments. Iscom ELISA for the detection of *Neospora caninum* antibodies in blood serum and milk was developed to decrease cross-reactivity (BJORKMAN et al., 1997; BJORKMAN and LUNDEN, 1998; FROSSLING et al., 2003), therefore we used a commercial iscom ELISA kit (Svanova, Sweden) for diagnostics of bovine neospora-species antibodies in blood serum.

The sensitivity and specificity of this technique were high (BJORKMAN and UGGLA, 1999). This study showed that the seroprevalence of *N. caninum* infection is 12.6% in Kerman's cattle was lower than 15.18% which has been reported by SADREBAZZAZ et al. (2004) in Mashhad, Iran. AKCA et al. (2005) reported that 8.2% of Simmental cows tested were positive in Kars province, Turkey. SEVGILI et al. (2005) found antibodies to *N. caninum* in 23 of the 305 (7.5%) cow sera based on ELISA test results in the province of Sanliurfa, Turkey. With regard to seropositivity, no significant difference was observed in origin, animal breed, and age ($P > 0.05$). The presence of antibodies against *N. caninum* in cows only indicate exposure to the parasite. In this study there was no significant difference in seroprevalence between the different age groups. WOUDA et al. (1998) and SADREBAZZAZ et al. (2004) reported for most herds that the seroprevalence levels were equal across all age groups. The relationship between age and seroprevalence in bovine neosporosis is speculative. JENSEN et al. (1999) suggested that seroprevalence increases with age. In contrast, SANDERSON et al. (2000) reported that cows below 3 years of age had higher CI-ELISA inhibition percentage values than cows above 6 years of age. They also suggested that infected cows can infect fetuses, and if these calves have not

been reinfected, antibody titers decline over time, resulting in an apparent decrease in seroprevalence with cow age. Due to the lack of information about the prevalence of infection in the definitive host, the dog, in Iran, it is not possible to know which method of transmission (horizontal or vertical) is the main route of infection. However, further studies on the epidemiological evidence for a relationship between *N. caninum* infection in dogs and cattle and the relationship between abortion in cows and infection with *N. caninum* in Iran are required.

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NOUROLLAHI FARD, S. R., M. KHALILI, A. AMINZADEH: Prevalencija protutijela za protozoon *Neospora caninum* u goveda u provinciji Kerman u Jugoistočnom Iranu. Vet. arhiv 78, 253-259, 2008.

SAŽETAK

Protozoon *Neospora caninum* je intracelularni parazit koji uzrokuje pobačaje u goveda diljem svijeta. Cilj istraživanja bio je odrediti seroprevalenciju neosporoze u goveda u području Kerman u Jugoistočnom Iranu. Ukupno je sakupljeno i pretraženo 285 uzoraka seruma. Uzorci su bili pretraženi komercijalnim testom *N. caninum* Iscom ELISA. Protutijela su pronađena u 36 od 285 uzoraka seruma (12,6%). U ovom je istraživanju prvi put dokazana prisutnost invazije vrstom *Neospora caninum* u pretraživanom području Irana. Seroprevalencija se nije značajno razlikovala s obzirom na podrijetlo pretraženih životinja, njihovu dob i spol ($P<0,05$).

Ključne riječi: *Neospora caninum*, govedo, protutijela, ELISA