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Seroepidemiological Studies of *Neospora* spp. Antibodies in Arabian Horses from Riyadh Region, Saudi Arabia

[1]A.D. Alanazi, [1]A.E. Said, [2]M.S. Alhussaini and [3]H.I. Al-Mohammed

[1]Department of Biological Sciences, Faculty of Science and Humanities,
[2]Department of Laboratory Sciences, Faculty of Applied Medical Sciences, Shaqra University, P.O. Box 1678, Ad-Dawadimi, 11911, Saudi Arabia
[3]Department of Microbiology and Medical Parasitology, Faculty of Medicine, King Faisal University, P.O. Box 400, Al Ahsa, 31982, Saudi Arabia

Corresponding Author: H.I. Al-Mohammed, Department of Microbiology and Medical Parasitology, Faculty of Medicine, King Faisal University, P.O. Box 400, Al Ahsa, 31982, Saudi Arabia

ABSTRACT

Neospora spp. are an obligate intracellular protozoan parasite and are recognized as a major cause of abortion in farm animals. Epidemiological and field studies concerning *Neospora* spp are very much dependent on serology. The objective of the present study was to determine the seroprevalence of *Neospora* spp. in Arabian horses used for sporting and showing purposes in Riyadh city, Saudi Arabia. In total, 163 serum samples from physically healthy horses were analyzed for anti-*Neospora* antibodies using the *Neospora* Modified Agglutination Test (N-MAT). Antibodies to *Neospora* spp were 39 (23.9%) of the 163 horses with 1:40 serum dilution, where 1:80 serum dilution was applied as significant cut off dilution and the serum positivity was reduced to 28 (17.1%) of the 163 horses. The differences in age and horse sex were not statistically significant ($p<0.05$). However, this study is the first seroprevalence study to determine *Neospora* spp antibodies by a *Neospora* Modified Agglutination Test (N-MAT) in horses from arid areas in Saudi Arabia.

Key words: *Neospora* spp., horses, seroprevalence, *Neospora* modified agglutination test, Saudi Arabia

INTRODUCTION

Neospora spp. is protozoan parasite and have been identified in a wide range of animal species in particular farm animals such as cattle, sheep, goats, dogs and horses farm, being associated with neonatal mortality and abortion in these animals (Dubey *et al*., 2003, 2007). In horses, *Neospora caninum* or *Neospora hughesi* can be cause neurological diseases called Equine Protozoal Myeloencephalitis (EPM) (Marsh *et al*., 1998; Vardeleon *et al*., 2001). The clinical signs have been recognized for more than three decades (Beech and Dodd, 1974) and include ataxia, abnormal gait, difficulty ingesting, paralysis of muscles of the eyes, face or mouth and loss of sensation along the horse's face, neck and body (MacKay, 1997). Antibody to *N. caninum* and *N, hughesi* in horse populations were reported in many parts of the world such as in North America, with prevalence (2-31%) (Cheadle *et al*., 1999; Dubey *et al*., 1999, 2003), 2.5-15% in South America (Hoane *et al*., 2006), 1-28% in Europe (Pitel *et al*., 2001; Ciaramella *et al*., 2004; Jakubek *et al*., 2006; Bartova *et al*., 2010), 2% in New Zealand (Vardeleon *et al*., 2001) and South Korea (Gupta *et al*., 2002).

In Saudi Arabia, the No. of horses according to estimates of the Saudi Ministry of Agriculture in 2011 were more than 45,000 horse and more than 300 horse were imported per year from USA and some European countries under restricted import regulations (Ministry of Agriculture in Saudi Arabia, 2011). Although, there is lack of information about the prevalence of *Neospora* spp infection in horses in Saudi Arabia. Therefore, this study was carried out to determine the seroprevalence of such infection in Arabian horses from Riyadh city Saudi Arabia using *Neospora* Modified Agglutination Test (N-MAT).

MATERIALS AND METHODS

Blood collection and preparation: From September 2012 to March 2013, blood samples were collected from 163 physically healthy adult horses for sporting purposes from Riyadh city, Saudi Arabia. Blood samples (5-10 mL) were collected from the jugular vein of each horse in vacuum tubes without anticoagulant. The blood samples were transported to the laboratory of Parasitology, Department of Biological Sciences, Faculty of Science and Humanities, Shaqra University. After clotting, samples were centrifuged at 3000 rpm for 10-15 min, serum was decanted and stored at 20°C until assayed for the antibodies to *Neospora* spp.

Serological test for *Neospora* spp: Sera obtained was stored at 20°C and subsequently thawed at 35°C immediately before testing. For detection of antibodies to *Neospora* spp., sera were initially screened at 1:40 and 1:80 dilutions using *Neospora* Agglutination Test (NAT), as described by Packham *et al.* (1998). Sera were double diluted from 1:10-:80 with phosphate-buffered saline containing 0.2 M 2-mercaptoethanol and 50 μL of each dilution was put in a well of 96 U-bottom microtiter plates. Then 50 μL of $3.5\times107\ mL^{-1}$ suspension of tachyzoites of the NC-1 strain of *N. caninum* resuspended in alkaline buffer (7.02 g of NaCl, 3.09 g of H_3BO_3, 24 mL of 1N NaOH, 4 g of horse serum [HS] albumin [fraction V], 50 mg of eosin Y, dH2O to 1 L, 0.1% sodium azide as a preservative; pH 8.7) were added to each serum dilution of samples as well as positive and negative controls. The wells were then mixed thoroughly by pipetting them up and down several times, covered and incubated overnight at 37°C with 5% CO_2. A cut-off titer of 1:80 was considered as significant for the presence of antibodies according to Packham *et al.* (1998) and Pitel *et al.* (2001). Reactions were considered positive when the tachyzoites were spread on the entire bottom of the well of the micro titer plate and those showing button formation were considered negative.

Age estimation of horses: In this study, horses were divided in 4 age groups (= 2, 3-5, 6-8 and = 9 years) based on their dentition. In younger horses (0<2years), it was possible to make a good estimate from the number of baby and adult teeth that have erupted or emerged from the gum. Further, the baby teeth are smaller and whiter with a rounded gum line. In horses from 3-5 years old, there are two adult incisors in wear in the centre of the jaw flanked by baby teeth (Richardson *et al.*, 1995). The dental star appears in horses sequentially in more lateral teeth at 6, 7 and 8 years old (Pasca *et al.*, 2006). The length of the groove of Galwayne was the indicator which was used to estimate age greater than 9 years, (Muylle *et al.*, 1998; Pasca *et al.*, 2006). In the present study, it was difficult to estimate the age of older horses (<12) because the cups had disappeared from all the lower incisors (Richardson *et al.*, 1994; Muylle *et al.*,1998). The age groupings used in this study reduced any errors that variable dentition imposed on the judgment of age.

Table 1: Prevalence of serum antibodies to *Neospora* spp in arabian horses from Riyadh city, Saudi Arabia

		Distribution of specific antibody titres to *Neospora* spp positive reaction	
Age group	No. of horses	1:40 (%)	1:80 (%)
<2	36	11 (28.2%)	9 (25%)
3-5	41	14 (34.1%)	9 (21.9%)
6-8	29	4 (13.8%)	3 (10.3%)
>9	57	10 (17.5%)	7 (12.3%)
Total	163	39 (23.9%)	28 (17.1%)

Table 2: Distribution of NAT titres according to the horse gender

			Distribution of specific antibody titres to *Neospora* spp. positive reaction	
Horse gender	No of horses	No. of positive samples	1:40 (%)	1:80 (%)
Male	67	18 (26.8%)	18 (26.8%)	13 (19.4%)
Mare	96	21(21.8%)	21(21.8%)	15 (15.6%)
Total	163	39 (23.9%)	39 (23.9%)	28 (17.1%)

Statistical analysis: Data analyses were performed using the SPSS software (Statistical Package for Social Sciences, version 16.0, [2008] for Windows, Sydney, Australia). The Chi square test was applied to compare the rates of seropositivity between the age and the sex of the horses. Statistical significance in this study was defined as $p<0.05$.

RESULTS

Antibodies to *Neospora* spp were found in 39 (23.9%) of the 163 horses of all ages (Table 1). Also, 39 out of 163 (23.9%) horses, reacted positively for *Neospora* spp antibodies with 1:40 serum dilution, where 1:80 serum dilution was applied as significant cut off dilution and the serum positivity was reduced to 28 (17.1%) of the 163 horses. Prevalences in mares were similar to those in males, the mares showed a seropositivity of 21(21.8%) while the seropositivity for males was 18 (26.8%). These values indicated that there is not any association between the presence of antibodies to *Neospora* spp. and the sex of the horses (Table 2). The differences in age and horse sex were not statistically significant ($p<0.05$).

DISCUSSION

This is the first *Neospora* spp seroprevalence study on horses in Saudi Arabia using; *Neospora* modified agglutination test (N-MAT); however, these results can only be compared with those obtained in other countries in the same geographic area. In Turkey and Iran, it was found that 24 and 28% of horses had *Neospora* antibodies in IFAT and cELISA tests (Bartova *et al*., 2010; Yagoob, 2012), respectively. In other countries such as the Czech Republic and Italy, it was found that 9.3 28% of horses had *Neospora* spp. antibodies by Indirect Fluorescent Antibody Test (IFAT) Kilbas *et al*. (2008); Ciaramella *et al*. (2004), respectively. In South Korea, 2% of horses reacted positively for Neospora spp Gupta *et al*. (2002). In the United States, antibodies to *N. caninum* were found to be (8-12%) horses in IFAT and 21-31% in agglutination (Cheadle *et al*., 1999; McDole and Gay, 2002 and Dubey *et al*., 2003). The variation of the results may be due to the sample sizes of the different studies and also to the different methodologies used.

In the previous studies, there is a lack of sensitivity and specificity of the serological methods used to detect *Neospora* spp For example, Packham *et al*. (1998) compared the sensitivity and specificity of ELISA with modified direct agglutination test (N-MAT) and (IFAT) to detect antibodies

against *N. caninum* in cattle sera. The N-MAT had superior sensitivity (100) and specificity (97%) compared with ELISA (74 and 94%, respectively) and had a higher sensitivity but lower specificity than the IFAT (98 and 99%, respectively). Hoane *et al.* (2005) were used recombinant antigen (rNhSAG1) to detect *N. caninum* and *N. hughesi* antibodies in horse serum. In their work, the ELISA results showed the highest sensitivity and specificity at 94.4 and 95.0%, respectively. These results suggested that rNhSAG1 recombinant antigens can be used as gold promise for serodiagnosis of *N. caninum* and *N. hughesi* infection in horse serum.

While the *Neospora* spp. can cause reproductive and neurological diseases in horses (Daft *et al.*, 1997; Hamir *et al.*, 1998; Pitel *et al.*, 2003; Hoane *et al.*, 2006) but the horses in the current study were physically normal and there was no history of abortion or neurological diseases. Also, the sample size was a bit small to draw any clinical conclusion compared with previous studies.

CONCLUSION

The results of the present study confirm the presence of *Neospora* spp. antibodies in Arabian horses from Saudi Arabia. The impact of neosporosis on horse farms and production, due to clinical neosporosis and potential risk factors of its transmission to other animals in Saudi Arabia, needs further investigations.

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