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Full Length Research Paper

Seroprevalence of *Neospora* spp. in horses from Central Province of Saudi Arabia

Abdullah D. AL Anazi[1] and Alyousif Mohamed S.[2]*

[1]Department of Biological Sciences, Faculty of Science and Human Studies, Shaqra University, P. O. Box 1040, Ad-Dawadimi 11911, Saudi Arabia.
[2]Department of Zoology, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia.

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From October 2010 to May 2011, blood samples were collected from 229 healthy horses from six different regions of the Central Province of Saudi Arabia. Sera were tested for *Neospora* spp. antibodies by a competitive inhibition enzyme-linked immunosorbent assay and confirmed by an indirect fluorescent antibody test. 23 horses out of 229 (10%) reacted positively to *Neospora* antibodies in competitive-inhibition enzyme-linked immunosorbent assay; five of them had ≥50% inhibition. Samples were confirmed with indirect fluorescence test (IFAT) test and only two samples were positive with final titers of 50 and 100, while other samples were negative. This study is the first investigation to determine *Neospora* spp. in horses from semi arid areas in Saudi Arabia which indicates that horses in Saudi Arabia are exposed to this parasite.

Key words: Horse, *Neospora caninum*, competitive-inhibition enzyme-linked immunosorbent assay (cELISA), indirect fluorescence test (IFAT), Saudi Arabia.

INTRODUCTION

Neospora spp. are coccidian parasites and has been identified in a wide range of animal species, being associated with neonatal mortality and abortion in cattle, sheep, goats, dogs and horses (Dubey, 2003). Horses can be infected by *Neospora caninum* and *Neospora hughesi* (Lindsay, 2001). Domestic dogs (*Canis familiaris*) and coyotes (*Canis latrans*) are known to be the definitive hosts of *N. caninum* (Dubey et al., 2007). *N. hughesi* was first described in the brain and spinal cord of an adult horse in California, USA (Marsh et al., 1998) but the definitive host of *N. hughesi* is still unknown (Dubey et al., 2007). However, different serological methods were used to detect *Neospora* spp. antibodies in horse. *Neospora* spp. antibodies have been reported to be 2 to 31% in North America (Cheadle et al., 1999; Dubey et al., 1999, 2003), 2.5 to 15% in South America (Hoane et al., 2006; Villalobos et al., 2006), 1 to 28% in Europe (Pitel et al., 2001; Ciaramella et al., 2004; Jakubek et al., 2006; Bártová et al., 2010), 2% in New Zealand (Vardeleon et al., 2001) and South Korea (Gupta et al., 2002). Although, there are some reports related to survey of *Neospora* infection in cattle (unpublished data) and camels (Al Anazi, 2011) in Saudi Arabia, but there is no information on the prevalence of this infection in horse. Therefore, the objective of this study was to determine the seroprevalence of *Neospora* spp. in horses from central province of Saudi Arabia using two different serological tests [competitive-inhibition enzyme-linked immunosorbent assay (cELISA) and indirect fluorescence test (IFAT).

*Corresponding author. E-mail: myousif@ksu.edu.sa.

Abbreviations: IFAT, Indirect fluorescence test; **cELISA,** competitive-inhibition enzyme-linked immunosorbent assay.

MATERIALS AND METHODS

Blood collection and preparation

Blood samples were collected from 229 clinically healthy adult horses from six different localities within the Central Province of

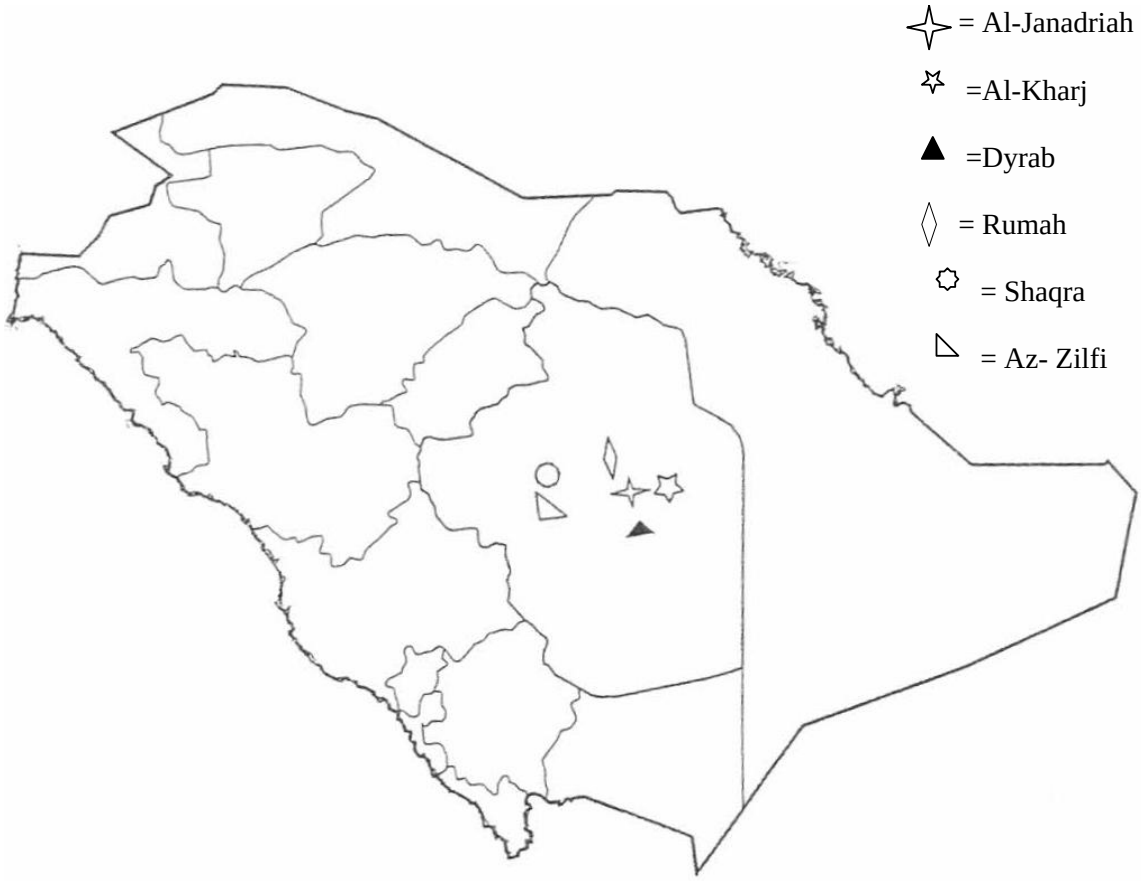

Figure 1. Map of Saudi Arabia showing the study regions (Central Province).

Saudi Arabia: Al-Janadriah (n = 89), Dyrab (n = 61), Al-Kharj (n = 31), Rumah (n = 19), Shaqra (n = 16) and Az-Zilfi (n = 13) (Figure 1). Blood samples (5 to 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 Science, Faculty of Science and Humanities, Shaqra University. Backup set of blood samples were transported to the Parasitological Laboratory at the Zoology Department, College of Science, King Saud University. After clotting, samples were centrifuged at 3000 rpm for 10 to 15 min, serum was decanted and stored at -20°C until assayed for the antibodies to *N. caninum*.

Serological test for *N. caninum*

Two serological tests were used for the detection of antibodies to *N. caninum*. Antibodies against *N. caninum* were detected by a commercial cELISA (VMRD, Pullman, USA). The sera were marked positive if more than 30% inhibition was found. Percent inhibition was calculated using the following formula: Inhibition (%) = 100-[(optical density of sample) - (optical density of negative control)]. Percent inhibition equal to or greater than 30% was considered positive and less than 30% was considered negative. Samples positive in cELISA with ≥50% inhibition were confirmed by an indirect fluorescence test (IFAT) using a commercially available NC-1 tachyzoite slides and antihorse conjugate (VMRD, Pullman, USA) was used as a “gold standard,” according to the manufacturer's recommendations. Known positive and negative sera were included on each slide. The dilutions: 1:50, 1:100 and 1:200 were used; titre ≥50 was considered positive.

RESULTS AND DISCUSSION

Twenty three (23) out of 229 (10%) horses reacted positively *for N. caninum* antibodies in cELISA. Eleven (11) horses had positive results for *N. caninum* antibodies in cELISA with 30.1 to 40% inhibition, while seven horses had 40.1 to 50% inhibition, 50.1 to 60% in 3 horses and 70.1 to 80% in 2 horses. Five samples with ≥50% of inhibition in cELISA were confirmed by IFAT; only two of them were positive (Table 1).

This is the first *Neospora* spp. seroprevalence study in horses in Saudi Arabia; thus, our results can be only compared with those obtained in other countries. In Turkey and Czech Republic, it was found that 9.3 and 24% of horses had *Neospora* antibodies in IFAT and cELISA tests (Kilbas et al., 2008; Bártová et al., 2010), respectively. In Italy, 28% horses had *Neospora* spp. antibodies in IFAT (Ciaramella et al., 2004). In South Korea, 2% of horses reacted positively to *Neospora* spp. (Gupta et al., 2002). In the United States, antibodies to *N.*

Table 1. Sera of horses positive for *Neospora* antibodies in cELISA with ≥50% inhibition confirmed by IFAT.

Sample number	Region	Number of horses	*N. caninum* antibodies			
			cELISA (%)	IFAT		
				1:50	1:100	1:200
1	Al-Janadriah	1	80	-	+	-
2	Dyrab	1	71.1	-	-	-
3	Al-Kharj	2	60	+	-	-
4	Rumah	1	52.6	-	-	-
5	Shaqra	7	49.3	-	-	-
6	Az-Zelfi	3	39.7	-	-	-
7	Al-Janadriah	8	33.1	-	-	-

caninum were found in 8 to 12% horses in IFAT and 21 to 31% in agglutination (Cheadle et al., 1999; McDole and Gay 2002; Dubey et al. (2003). Different results could be attributed to the different methods used with the different cut-off and the different number of horses examined.

While the *Neospora* spp. can cause reproductive and neurological diseases in horses (Daft et al., 1996; Hamir et al., 1998; Pitel et al., 2003; Hoane et al., 2006), horses in the current study were clinically normal and there was no history of abortion or neurological diseases. Also, the sample size was quite small to draw any clinical conclusion when compared with previous studies.

In veterinary medicine, ELISA techniques have been used to detect antibodies to coccidian parasites such as *Sarcocystis neurona, N. caninum* and *Toxoplasma gondii* in livestock and other animals (Baszler et al., 1996; Bjorkman et al., 1997; Williams et al., 1997; Howe et al., 2008). Moreover, a range of antigens have been used to detect antibodies of coccidian parasites in animal sera using ELISA test. These include whole-tachyzoite lysate antigens (Pare´ et al., 1995; Wouda et al., 1998), extracted tachyzoite membrane proteins incorporated into immunostimulating complexes (Björkman et al., 1997), tachyzoite surface antigens made available by chemical fixation of whole tachyzoites onto the plates (Williams et al., 1997; Wouda et al., 1998) and recombinant antigens (Louie et al., 1997).

Sensitivity and specificity of ELISA have been compared with other serological methods. 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%) when compared with ELISA (74 and 94%, respectively) and had a higher sensitivity but lower specificity than the IFAT (98 and 99%, respectively). For horses, Hoane et al. (2005) used recombinant antigen (rNhSAG1) to detect *N. caninum* and *N. hughesi* antibodies in horse serum. ELISA showed the highest sensitivity at 94.4% sensitivity and 95.0% specificity when antigen rNhSAG1 were used for serodiagnosis of equine *N. hughesi* infection. These results suggested that rNhSAG1 recombinant antigens hold promise for serodiagnosis of *N. caninum* and *N. hughesi* infection in horse serum.

In this study, we found that *Neospora* spp. antibodies have relatively low prevalence in horses in Central Province of Saudi Arabia, but it was difficult to differentiate between *N. caninum* and *N. hughesi* because the serologic methods (cELISA and IFAT) used in this study cannot discriminate between *N. caninum* and *N. hughesi* antibodies (Gondim et al., 2009).

In conclusion, in the present study, using ELISA followed by IFAT to test for false positive samples was not conclusive. The data suggest that for the Saudi horses, ELISA had low specificity and sensitivity and IFAT low sensitivity. A further relevant issue is that many tests were described prior to the differentiation between the two *Neospora* species. Without a gold standard, it is difficult to test sensitivity and specificity and publications may have overestimated these parameters. However, this study suggests that horses in Saudi Arabia are exposed to these parasites.

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