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Full Length Article

Serosurveillance of *Neospora caninum* and *Brucella* species in Dairy Cattle of Konya, Turkey

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Abstract

This study reports the seroprevalence of *Neospora (N.) caninum* and *Brucella* sp. in aborting and non-aborting dairy cattle in Konya province of Turkey. To this end, blood samples were collected from 560 cattle, 66 of which were not aborting and 494 were aborting, and sera were isolated from these samples through standard protocol. Antibodies against *N. caninum* were determined by using a commercial competitive ELISA (cELISA) kit. *Brucella* sp. antibodies were determined using the Rose Bengal Plate Test (RBPT). According to cELISA results, 222 of 560 cattle (39.64%) were seropositive for *N. caninum* antibodies. Of 494 aborting cattle samples, 213 (43.11%) were positive for *N. caninum* antibodies. Through RBPT, 89 of the 560 cattle tested were positive for *Brucella* sp. Of 494 aborting cattle, 79 (15.99%) were positive for *Brucella* sp. The seropositivity differences between *N. caninum* and *Brucella* sp. were statistically significant in aborting cattle ($p< 0.001$). The co-infection rate of *N. caninum* seropositivity with *B. abortus* was detected 9.5% in aborting cattle. In conclusion, seroprevalence of neosporosis and *Brucella* sp. was 39.64% and 15.89% through cELISA and RBPT, respectively in cattle of Konya.

Keywords: Abortion; *Brucella* sp.; Dairy cattle; Konya; *Neospora caninum*

Introduction

The dairy cattle industry suffers economic losses due to abortion. Protozoan, bacterial, viral and fungal agents directly affect the reproductive health of cattle. In cattle, *Neospora (N.) caninum* and *Brucella* sp. are the important abortive agents generally causing abortion during the last 3 months of gestation (Radostits *et al*., 1997).

Neospora caninum, an apicomplexan protozoan, causes abortions, reproductive failure or stillbirth in many warm-blooded animals e.g. cattle, horses, sheep, deer and goats and neurological alterations in dogs and cattle (Barber and Trees, 1996; Dubey and Lindsay, 1996; Dubey, 1999). Neosprosis was reported to increase the susceptibility of the infected hosts to other infectious agents e.g. Bovine Viral Diarrhoea Virus (BVDV) and Bovine Herpes Virus 1 (Bjorkman *et al*., 2000; Mineo *et al*., 2006). For the diagnosis of neosporosis in cattle, clinical findings, immunohistochemical methods, serological tests, tissue culture and molecular techniques are used. In the serological diagnosis of the disease, specific antibodies, which react against *N. caninum* ticisolide antigens, are determined by methods such as the commonly used ELISA and IFA test (Sanderson *et al*., 2000). Serological studies with these methods report seroprevalence of antibodies against *N. caninum* as 12.5% in England, 30% in Canada, 18% in Spain and 39.4% in the Netherlands (Davison *et al*., 1999).

Brucellosis, a zoonotic infection, is induced by the bacteria of *Brucella* genus which are small aerobic intracellular coccobacilli localizing the reproductive organs of the animals causing abortions and sterility. Brucellosis is often spread by infected material at the time of calving or abortion. Bovine brucellosis, usually caused by *Brucella (B.) abortus* (OIE, 2009), is a well-recognized cause of abortion in dairy cattle (Shabbir *et al*., 2011). The buffered *Brucella* antigen tests such as Rose Bengal Plate agglutination Test (RBPT) are appropriate for screening individual animals and herds (Gall and Nielsen, 2004). Numerous epidemiologic studies of neosporosis and brucellosis have been reported worldwide (Shabbir *et al*., 2011; Lucchese *et al*., 2016). However, in Turkey, the epidemiological studies about the seroprevalence of both *Brucella* sp.

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and *N. caninum* are limited (Yildiz *et al*., 2009). Therefore, the aim of this study was to detect the serological prevalence of *N. caninum* and *Brucella* sp. in dairy cattle of Konya province, Turkey using competitive ELISA and RBPT, respectively.

Materials and Methods

Sampling of Animals

The present study was performed in Konya province of Turkey. Cattle were selected from dairy cattle farms having a history of high abortion rates. Relevant information about the host-related determinants was recorded in a pre-designed questionnaire, which was tested through informal and formal ways (Thrusfield, 2007). Blood samples were obtained from 560 dairy cows with aborting (n = 494), and unknown history of abortion (n = 66).

Blood Collection

Blood samples (7−8 mL) were collected from the jugular vein of cattle in plain vacutainer tubes using standard blood collection procedure. The samples were transported to the University of Selcuk, Department of Parasitology, Konya, Turkey within 12 h of collection. After centrifugation, sera samples were separated and stored at -20^{o}C until assayed.

cELISA for *N. caninum*

Sera samples were assayed using commercial *N. caninum* antibody test kit (VMRD, Inc., Pullman, WA, USA) based on competitive ELISA (cELISA). The test was performed according to the manufacturer's recommended protocol. Results were calculated as percent inhibition (% I) using following formula:

% I= 100 - (Sample O.D. X 100)/Mean Negative Control O.D

Kit manufacturer mentioned that the sensitivity and specificity of cELISA tests are 96% and 99%, respectively (VMRD, Inc., Pullman, WA, USA). For interpretation of the results of cELISA, the tested sera samples were declared positive if they caused ≥ 30 inhibitions; and negative if they caused < 30% inhibitions.

Rose Bengal Plate Test for *Brucella* sp.

Brucella sp. antibodies were determined using RBPT as given by the OIE (2009). Briefly, equal volumes of test serum and RBPT antigen (Vetal AS) were mixed on a clean glass slide with the sterilized toothpick and incubated at room temperature for an hour. Formation of clumps was an indicative of the positive reaction.

Statistical Analysis

A chi-square (χ^2) test was performed to detect significant differences, a probability of less than 0.05 was considered statistically significant. The statistical software package MINITAB 14 was used.

Results

Most of the cattle surveyed in this report aborted in the second or third trimesters of their gestation periods. The age of the cattle sampled ranged from one to seven years. Specificity and sensitivity of cELISA test were 99% and 96%, respectively. The overall seroprevalence of *N. caninum* and *Brucella* sp. was detected as 39.64% (222/560) and 15.89% (89/560), respectively. Of 494 aborting cows, 213 sera (43.12%) had positive absorbance values in the cELISA and 79 sera (15.99%) were detected positive in RBPT. The seropositivity differences between *N. caninum* and *Brucella* sp. were statistically significant in the examined cattle ($p< 0.001$). Table 1 details the serological prevalence of *N. caninum* and *Brucella* sp. in aborting and non-aborting dairy cattle population of Konya, Turkey.

Discussion

Neospora caninum was first detected in 1984 in a dog with encephalomyelitis and myositis. During an abortion epidemic in Mexico in 1987, dairy cows were identified in cattle with *N. caninum,* which is notorious for being among the most important abortion causing pathogens of cattle in the world. Conrad *et al.* (1993) isolated the agent from waste fetus. Damages caused by *N. caninum* in cattle are abortions and associated infant deaths. It is reported that abortion risk in seropositive animals is 3−7.4 times higher than in other animals (Thurmond and Hietala, 1997; Davison *et al*., 1999). It has been observed that in the recent years, it has caused considerable abortions all over the world (Landmann *et al*., 2011, Shabbir *et al.*, 2011, Mazuz *et al*., 2014). Neosporosis, which is cosmopolitan in distribution, is quite common in North America (Anderson *et al*., 2000). Seropositivity of neosporosis was 12.5% in England and Wales, 36.8% in Spain, 15.5% in Poland, 56.9% in Argentina, 43.8% in Pakistan, 35.5% in Israel, 16.7% in Ethiopia and 59% in Mexico (Campero *et al*., 1998; Davison *et al*., 1999; Quintanilla-Gozalo *et al*., 1999; Wladyslaw *et al*., 2000; Vazquez *et al*., 2002; Shabbir *et al*., 2011; Asmare, 2014; Mazuz *et al*., 2014). Campero *et al.* (2003) reported that 7.30% of waste fetuses are positive for *N. caninum*. Anderson *et al*. (1995) found the cause of 45.5% abortions, indicating that *N. caninum* is an important waste cause in cattle. In Pakistan, *N. caninum* antibodies were determined in 43.8% dairy cattle and the prevalence of antibodies against *B. abortus* ranged from 0% to 23.8% in different farms (Nasir *et al*., 2014).

Table 1: The serological status of *Neospora caninun* and *Brucella* sp. in aborting and non-aborting dairy cattle of Konya, Turkey

Pathogens	Aborted		non- aborted		Total Seropositivity
	+	-	+	-	
N. caninum	213[a]	281	9[a]	57	39.64%[a] (222/560)
Brucella sp.	79[b]	415	10[a]	56	15.89%[b] (89/560)

[a, b]: different superscript letters (a, b) are statistically significant (P < 0.001, chi square)

In Turkey, various studies have been conducted to determine the seropositivity of neosporosis in cattle e.g. Akça and Gökçe (2003) reported 2% in Kars region, Öncel and Bıyıkoğlu (2003) reported 9.2% in Sakarya, Sevgili *et al.* (2005) reported 7.5% in Sanliurfa, İça *et al*. (2006) reported 7% in Kayseri region, Vural *et al.* (2006) found 5.1% to 32.7% in central Anatolian provinces, Aktaş *et al.* (2005) reported 4−15% and Şimşek *et al.* (2008) reported 8.19% in eastern Anatolia, and recently, Aytekin *et al.* (2013) found 8.83% seropositivity of *N. caninum* in Konya. Our results (39.64% prevalence of *N. caninum*) are closer to those reported elsewhere from different regions of the world and Turkey (Sevgili *et al*., 2005; Vural *et al*., 2006). Some investigators reported the seroprevalence between the groups (aborted and non-aborted) of cattle was statistically significant (Locatelli-Dittrich *et al*., 2001; Romero-Salas *et al*., 2010). On the contrary, Sadrebazzaz *et al.* (2004) and Aktaş *et al.* (2005) stated that the difference between the two groups was nonsignificant which are in accordance with our findings.

The herd and individual prevalence of cattle brucellosis is reported as 7.8 and 2.7%, respectively in Turkey (Anonymous, 2012). However, the data is limited to co-infection of cattle in Turkey with *N. caninum* and *B. abortus.* Yildiz *et al.* (2009) reported 13.82% prevalence of *N. caninum* as concurrent seropositivity with *B. abortus* in dairy cattle in Turkey. Castilleja *et al.* (2010) reported 21.2% of the brucellosis-positive cattle also had antibodies against *N. caninum* in Mexico. Similarly, Nasir *et al.* (2014) stated that 13.2% of buffaloes were infected with *Neospora* sp. as well as *Brucella* sp. in Pakistan. In agreement with the previous studies, we detected the co-infection rate of *N. caninum* seropositivity with *B. abortus* as 9.5% in aborting cattle of Konya, Turkey.

Conclusion

In conclusions, seroprevalence of neosporosis and *Brucella* sp. were found 39.64% and 15.89% through cELISA and RBPT, respectively in cattle of Konya. Hence, *N. caninum* should also be taken into account with other abortion causing pathogens. Further investigations should include identification of pathogens from the aborted fetuses. Cattle breeders and growers should be aware of and trained of effective preventive management strategies for the control of these economically significant diseases in Konya, Turkey.

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