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Short Communication

Prevalence of *Neospora caninum* and *Toxoplasma gondii* Antibodies in Bulk Milk of Dairy Cattle, Mashhad, Iran

Razmi [1, *], G., Barati [2], M.

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

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Corresponding Author: Razmi@um.ac.ir

ABSTRACT

Neospora caninum (*N. caninum*) and *Toxoplasma gondii* (*T. gondii*) are both obligate intracellular protozoan parasites, which have gained considerable attention because of their role in bovine abortion. This study aimed to detect anti-*N. caninum* and -*T. gondii* in bulk milk of dairy cattle, Mashhad, Iran. The bulk milk samples were collected from July 2014 to June 2015 and analyzed for anti-*N. caninum* and -*T.gondii* antibodies using enzyme-linked immunosorbent assay (ELISA). Out of 123 bulk milk samples, 44 (35%), 14 (11.38%), and 3 (2.4%) samples had *N. caninum*, *T. gondii*, and mixed infection with these two parasites, respectively. According to the results, the prevalence of *N. caninum* infection was more than *T.gondii* infection in dairy cattle of Mashhad, Iran.

Keywords: Bulk milk, Cattle, ELISA, Mashhad, *Neospora caninum*, *Toxoplasma gondii*

Estimation de la prévalence des anticorps dirigés contre les antigènes de *Neospora caninum* et *Toxoplasma gondii* dans le lait des réservoirs des fermes laitières dela ville de Mechhed

Résumé *Neospora caninum* et *Toxoplasma gondii* sont deux parents protozoaires intracellulaires constituant un risque sanitaire majeur en raison desavortementsqu'ils peuvent engendrés chez les vaches laitières. Cette étude avait pour objectif la détection des anticorps spécifiques aux antigènes de *Neospora caninum* et *Toxoplasma gondii* dans le laitprovenant des réservoirs des fermes laitières dans la ville de Mechhed en Iran. Pour ce faire des prélèvements de lait ont été effectués à partir desréservoirsdes fermes laitières de Mechhed entre juillet 2014 et juin 2015 et ont été soumis à un test d'ELISA afin de détecter les anticorps spécifiques aux antigènes de *Neospora* et de *Toxoplasma*. Sur un total de 123 prélèvements , 44 (35%), 14 (11,38%) et 3 (2,4%)échantillons étaient respectivement positifs au *Neospora caninum*, auToxoplasma gondii etaux deux espèces simultanément . Nos résultats ont révélé untaux d'infection plus important auxNeospora comparé auxToxoplasma dans les fermes laitières de la ville de Mechhed. Ces résultats ont démontré que le test ELISAsur des échantillons provenant des réservoirs de lait peut être utilisé en tant qu'un test quantitatif approprié afin d'estimer le taux dinfection aux*Neospora* et *Toxoplasma* dans les fermes laitières.

Mots-clés: Lait de réservoir, test ELISA, *Neospora caninum*, *Toxoplasma gondii, vache*, ville de Mechhed

INTRODUCTION

Neospora caninum (*N. caninum*) and *Toxoplasma gondii* (*T. gondii*) belong to the family Sarcocystidae. Both protozoa with the same biology are capable of infecting a wide range of animals. Globally, *N. caninum* is a major cause of stillbirth and abortion during mid-gestation in dairy cattle (Dubey and Schares, 2011). If the intrauterine fetal demise occurred between the third and eighth months of gestation, the fetuses would be usually expelled showing moderate autolysis. However, those fetuses dying before the fifth month of gestation may be mummified and retained in the uterus for several months. If abortion occurs at an early stage of gestation, the fetus may be reabsorbed which causes repeat breeding. Abortions may be sporadic, endemic, or epidemic. *N. caninum*-seropositive dairy and beef cattle are more likely to abort in comparison to the seronegative ones. Nevertheless, up to 95% of calves born congenitally-infected from seropositive dams remain clinically normal (Dubey et al., 2007). Additionally, neosporosis, which is one of the infectious etiologies of abortion, is widely prevalent in dairy cattle in Iran (Sadrebazzaz et al., 2004) (Razmi et al., 2006; Razmi et al., 2007; Reza Nourollahi Fard et al., 2008; Youssefi et al., 2009; Razmi et al., 2010; Nematollahi et al., 2011; Gharekhani et al., 2014). Toxoplasmosis is a zoonotic disease with worldwide distribution that causes abortion and congenital toxoplasmosis in human and sheep. Dairy cattle are not a favored host for *T. gondii* but the consumption of beef meat and raw milk could be a risk factor for infection among mankind (Dubey, 1986; Belluco et al., 2016; Boughattas, 2017)In Iran, toxoplasmosis is observed in human, as well as wild and domestic animals. The seroprevalence of *Toxoplasama* infection in cattle from various regions of Iran was between 1.4% and 71.3% (Sarvi et al., 2015). Enzyme-linked immune sorbent assay (ELISA) is a rapid and cost-effective method, which is used to assess the frequency of *N. caninum* infection among dairy cattle. According to the results of this test, there was a significant correlation between the seroprevalence of infection in dairy cattles with the bulk milk ELISA results (Björkman et al., 1997; Bartels et al., 2005; Wapenaar et al., 2007). Generally, about 10% to 15% of the cattle in a herd are seropositive if the bulk milk ELISA results exceeded the cut-off (Bartels et al., 2005; Wapenaar et al., 2007; González-Warleta et al., 2011)This study aimed to estimate the frequency of *N.caninum* and *T. gondii* infection among dairy cattle in Mashhad, Iran, by using ELISA method.

MATERIALS AND METHODS

Area of Study. This study was performed in Mashhad, the capital city of Razavi Khorasan Province, Northeastern of Iran. The climate is semi-arid with cold winters and mild summers. The most popular breed cattle in this area were Holstein-Friesian. There are small and large dairy farms in Mashhad. The small herd was comprised of about 2 to 10 head with a low technology and milk production, while high-tech large herd consisted of 110 heads with high milk production.

Sampling . About 123 milk samples were collected from the bulk tank milk of dairy herds, which were purchased from dairy factory (Pegah Milk Co., Mashhad, Iran) and the milk collection centers of Mashhad, Iran, from July 2014 to June 2015. The samples were transferred to sterile 50 ml tubes, kept in cool boxes with ice pack, and transported directly to the laboratory. Afterward, they were centrifuged at 2000 rpm for 10 min; the floating fat at the top of liquid was removed and the rest were stored at the temperature of -20 °C.

ELISA. The skimmed milk samples were analyzed for the activity of *T. gondii* and *N. caninum* antibodies using the commercially available ELISA kits (ID screen ® Toxoplasmosis indirect Multi-species and ID screen ® *Neospora caninum* indirect Multi-species, ID Vet, Montpellier, France). Briefly, the milk samples were diluted 1:2 and the positive and negative controls determined by the test kit were used as controls at the 1:40 according to recommendations by the manufacturer. If the mean value of the optical density (OD) of positive control was greater than 0.350 and the

ratio of the mean OD values of the positive and negative controls were greater than 3, the test results would be valid. The S/P ratio was defined as the OD of the sample (S) minus the OD of the negative control (NC), all divided by the OD of the positive control (PC) minus the OD of the negative control ([S-NC]/[PC-NC]). For both kits, cut-off S/P ratios of ≤0.25%, 0.25%-29%, and ≥30 were defined as negative, doubtful, and positive, respectively.

Statistical Analysis. The differences between two sampling locations in terms of the prevalence of the infections and S/P ratios were analyzed by Chi-squared and independent t-tests, respectively. In all the measurements, P-value less than 0.05 was considered statistically significant (Petrie and Watson, 2006).

RESULTS AND DISCUSSION

Out of 123 bulk milk samples, the seroprevalence of *N. caninum, T. gondii*, and mixed infection was detected to be 35%, 11.38%, and 2.4%, respectively (tables 1 and 2). The seroprevalence of *N. caninum* infection in the samples collected from the milk collection centers was higher than that of collected from the milk factory (P<0.001). Nonetheless, there was no significant difference between the research contexts considering the seroprevalence of *N. caninum* infection (tables 1 and 2).

Table 1. The prevalence of *N.caninum* infection in two different sampling locations estimated by bulk milk ELISA

Sampling location	Positive No. (%)	Negative No.	Total	Mean S/P (%) (range)
Dairy factory	9 (13.8)	56	65	41.22% (ranging from 30% to 60%)
Milk collection centers	35 (60)	23	58	46.83 % (ranging from 30% to 98%)
Total	44 (35%)	79	123	

No significant difference was found between the *N. caninum*- and *T. gondii*-seropositive samples in the mentioned research contexts (P>0.05). The prevalence of *N. caninum* infection in dairy farms can be estimated by using bulk milk ELISA method to decrease the within-herd prevalence of infection by control measures. In the present study, anti-*N. caninum* antibodies were found in 35% of the samples. According to the literature, the prevalence of *N. caninum* infection based on the ELISA method, was 8.3% in Sweden (Frössling et al., 2008)0.7% in Norway (Klevar et al., 2010)1.01% in Czech (Hurkova et al., 2005)55% in Italy (Varcasia et al., 2006)19% in Irland (O'Doherty et al., 2014) 6.4%-10.4% in Canada (Wapenaar et al., 2007)2.5% in Australia (Nasir et al., 2012)and 46% in Thailand (Chanlun et al., 2002).

Table 2. The prevalence of *T.gondii* infection in the two different sampling locations estimated by bulk milk ELISA

Sampling location	Positive No. (%)	Negative No	Total	Mean S/P (%) (range)
Dairy factory	8 (14)	57	65	50.75% (ranging from 32% to 94%)
Milk collection centers	6 (12.5)	52	58	42.83% (ranging from 31% to 67%)
Total	14 (11.38%)	109	123	

Regarding the results of seroepidemiological studies, the prevalence of *Neospora* infection in dairy cattle was relatively high (ranging from 10.5% to 46%) (Sadrebazzaz et al., 2004; Razmi et al., 2006; Reza Nourollahi Fard et al., 2008) (Nematollahi et al., 2011)Gharekhani et al., 2013). In this study, anti-*T. gondii* antibodies were detected in 11.3% of the bulk milk samples. Furthermore, the prevalence of *Toxoplasma* infection was significantly high among dairy cattle in Mashhad, Iran, which might be due to the large population of cats in this area. The seroprevalence of bovine toxoplasmosis was reported to be 9%-71% in various parts of Iran (Sarvi et al., 2015). The anti-*N. caninum* and -*T. gondii* antibodies were found in 2.4% of bulk milk samples. The co-existence rate of *N. caninum* seropositivity with *T. gondii* was reported to be between 1% and 4% in Brazil

(Gondim et al., 1999) Switzerland (Gottstein et al., 1998) Czech (Bártová et al., 2015)Vietnam (Huong et al., 1998) and China (Xu et al., 2012) This results might be due to co-infection of these two pathogens or cross reactivity among them. The high prevalence of *N.caninum* infection was detected in the samples of the milk collection centers, which were collected from small herds. Therefore, the results might be attributed to the lack of sanitation and hygiene in such farms. According to the evidence, the seroprevalence of *N.caninum* infection of small farms was higher than large ones (Bartels et al., 2006) (González-Warleta et al., 2008). Regarding the high prevalence of *N.caninum* infection dairy cattle in Mashhad, ELISA is a valuable and cost-effective tool for identifying the infected farms and is a subsequent protocol with adequate control measures regarding *N. caninum* infection.

Given the results of the present study, bulk milk ELISA is a useful method for infection surveillance in dairy farms. In addition, the prevalence of *N. canium* was higher than *T. gondii* infection in dairy cattle in Mashhad, Iran. Further studies are recommended to identify specific components of infection prevention and control programs.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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