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Prevalence of immunoglobulin G (IgG) antibody to *Neospora caninum* in dairy cattle of Hamedan province, west of Iran

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Article Info	Abstract
Article history:	Bovine neosporosis caused by the apicomplexan protozoan parasite <i>N. caninum</i> , was initially recognized in 1989 and is now reported as a leading infectious cause of reproductive
Received: 08 July 2012	failure in dairy cattle in world wide. The aim of this study was to determine the seroprevalence
Accepted: 05 January 2013	of N. caninum infection in industrial dairy cattle of Hamedan province (west of Iran) by ELISA
Available online: 15 June 2014	method. Blood samples were collected from 492 cattle in 41 farms. Antibodies to N. caninum
	were found in 63(12.80%) sera. A Significant difference was observed between seropositive
Key words:	cattle and dog presence in farm, dog contact with herd, abortion history and herd population.
	No significant differences were found between seropositive cattle and age as well as breed. This
Cattle	study is the first report of N. caninum infection in dairy cattle farms in Hamedan province. As
Hamedan	per our knowledge, Neospora is an important factor in abortion of cattle in this region.
Iran	Therefore, comprehensive studies for control strategies and improving management of dairy
Neospora caninum	farms is necessary.
Prevalence	© 2014 Urmia University. All rights reserved.

شیوع آنتی بادی ایمونو گلوبین G علیه *نئوسپورا کانینو*م در گاوهای شیری استان همدان، غرب ایران

چکیدہ

نئوسپوروزیس گاوی با عامل *نئوسپورا کانینوم* تک یاخته انگلی از اپی کمپلکسا برای اولین بار در سال ۱۹۸۹ تشخیص داده شد و امروزه بعنوان یک عامل عفونی نارسایی تولید مثلی در گاوهای شیری از سراسر دنیا گزارش می شود. هدف از این مطالعه تعیین شیوع سرمی آلودگی به *نئوسپورا کانینو*م در گاوهای شیری صنعتی استان همدان (غرب ایران) به روش الایزا بود. ۴۹۲ نمونه خون گاو از ۴۱ گاوداری اخذ گردید. آنتی بادی علیه *نئوسپورا کانینو*م در ۳۶ نمونه سرم (۸/۱درصد) مشاهده شد. اختلاف معنی داری بین گاوهای سرم مثبت و حضور سگ در گاوداری، ارتباط سگ با گله، سابقه سقط و جمعیت گله مشاهده شد. اختلاف معنی داری بین گاوهای سرم مثبت و حضور سگ در *کانینو*م در گاوداری های شیری استان همدان می باشد. با توجه به یاف*سپورا کانینو*م در را ۲۸ درصد) مشاهده شد. اختلاف معنی داری بین گاوهای سرم مثبت و حضور سگ در و بهبود مدیریت در گاوداری های شیری استان همدان می باشد. با توجه به یافته های ما*، نئوسپورا* یک عامل مهم سقط جنین در گاو در این منطالعه اولین گزارش آلودگی به *نئوسپورا* و بهبود مدیریت در گاوداری های شیری استان می باشد. با توجه به یافته های ما*، نئوسپورا* یک عامل مهم سقط جنین در گاو در این منطقه است. بنابراین، مطالعات جامع برای استراتژی های کنترل

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Introduction

Neospora caninum is a protozoa that causes abortion and economic losses in cattle worldwide.¹ In cattle, transplacental transmission is the main mechanism by which the parasite persists in a herd.² After recognizing the dog as the definitive host of the parasite, epidemiological work established the association between the presence of dogs and the disease in cattle.^{3,4} Additionally, the association of canids with cattle on their premises, has been postulated as a risk factor for the disease.⁵ Similarly, it has been established that intensive herd management was associated with increased seroprevalence to N. caninum.6 The presence of wild canids have also been related with high prevalence of sero-active cattle.7 Neosporosis of cattle has been associated with abortion, neonatal mortality and decrease in the volume of milk production that cause yearly economic loss.8

The seroprevalence of *N. caninum* infection in cattle varies largely, depending on the country and region.⁸ Several assays are available for detecting antibodies to *N. caninum* in cattle.^{9,10} Some serological studies in dairy herds have done in some part of Iran. However, there is not published information of *N. caninum* infection in the cattle of this province.

This study was performed to determine the prevalence of antibodies to *N. caninum* in industrial dairy cattle in Hamedan province, using enzyme-linked immuno-sorbent assay (ELISA).

Materials and Methods

A cross-sectional study was performed in the first half of year 2010. Blood samples were taken from 492 dairy cattle in the 41 industrial farm of Hamedan province, Iran. The animals were randomly selected. The owners were questioned about age, breeding, dog presence and its contact with the herds, abortion history, and herd population.

All samples were immediately transported to the diagnostic laboratory of Hamedan Veterinary Office, Hamedan, Iran. Serum was removed after centrifugation at 1000 g for 15 min. All sera were stored at -70 °C until laboratory testing.⁸

The samples were analyzed for antibodies against *N. caninum* using ELISA kit. Anti-*Neospora* antibodies were detected using a commercially available *N. caninum* ELISA kit (Herdcheck, Maine, USA). The kit was used according to the manufacturer's instructions. The presence or absence of antibody was determined by calculating of sample to positive ratio (S/P ratio according to the formula mentioned inside the manual). A S/P ratio more than 0.5 and less than 0.5 was considered positive and negative, respectively.

Results

Immunoglobulin G (IgG) antibodies to *N. caninum* were found in 63 of 492 (12.80%) sera (CI = 0.12 ± 0.03). With regard to seropositivity, significant differences were found regarding herd population (X² = 13.15, df = 1.00 and *p* < 0.001), abortion history (X² = 48.06, df = 1.00 and *p* < 0.001), dog presence in farm (X² = 9.45, df = 1.00 and *p* = 0.002), dog contact with herd (X² = 5.73, df = 1.00 and *p* = 0.01); and stray canids presence in farm (X² = 37.17, df = 1.00 and *p* < 0.001). There were no significant differences between seroprevalence and age (X² = 3.96, df = 3.0 and *p* = 0.262), as well as breeding (X² = 0.88, df=1.00 and *p* = 0.346), (Table 1).

Discussion

This study was the first report of *N. caninum* infection in industrial dairy cattle in Hamedan proviance. There were only a few reports on *N. caninum* seroprevalence in dairy cattle of Iran.¹⁰⁻¹³ The seroprevalence rate were reported 32.00% in Babol (north of Iran), 46.00% in Mashhad (northeast of Iran) and 12.60% in Kerman (southeast of Iran) using ELISA.¹⁰⁻¹² The similar rate of infection was reported in Brazil, Greece, Peru, Australia, Canada, Ireland, Korea and Spain.^{39,14}

In the present study, the herd seroprevalence was similar to that study in Thailand and different to other countries.^{3,13} Difference of management in farms, study design and sample size are main cause of varied results.

In current study, there was no significant difference in seroprevalence between the different age groups which was similar to result of Nourollahi *et al.* in Kerman and other researchers.^{9,10,15-18} Razmi *et al.* reported statistically

Table 1. Comparison of N. caninum seroprevalence in different variables.

	Age groups			Breed		Herd capacity		Dog presence	Dog contact	Stray canids	Abortion history	Total	
	<2	2-3	3-4	>4	Hybrid	Holstein	≥100	< 100	in farm	with herd	presence in farm		
Number of	55	113	147	177	38	454	216	276	360	192	144	35	492
sample (%)	(11.20)	(23.0)	(29.90)	(36.00)	(7.70)	(92.30)	(43.90)	(56.10)	(73.20)	(53.30)	(29.30)	(7.11)	(100)
Number of positive (%)	11 (20.00)	15 (13.30)	14 (9.50)	23 (13.00)	3 (7.90)	60 (13.20)	41 (19.00)	22 (8.00)	36 (10.00)	26 (13.50)	39 (27.00)	20 (57.10)	63 (12.80)

significant in different age groups.¹² Sadrebazzaz *et al.* and Wouda *et al.* reported equal levels of seroprevalence in all age groups for most herds.^{13,19} Jensen *et al.* suggested seroprevalence increased with age and depended on sample size.²⁰ Lower seroprevalence in cattle of < 2 age is due to a decrease of antibody in congenital infections. It seems relationship between age and seroprevalence rate is speculative.

In a French study, similar to our study, there was not association between seropositivity and breed.²¹ The prevalence of *N. caninum* in dairy cattle was reported higher than beef cattle in Spain.^{22,23} This might be related to different production systems for dairy and beef cattle rather than to breed differences. Comprehensive research on the impact and role of different breed in the prevalence of infection is essential.

In this study, there was a 2.70 fold increase of seroprevalence in farms with more than 100 individuals [p = 0.0005, OR = 2.70(1.55-4.70)]. Kyaw *et al.*, reported that cattle in larger farms (\geq 21) had a higher infection than small herds (< 21) in Thailand, (p = 0.03); opposite to Davison *et al.* study.^{17,24}

In a study in Italy, the risk of seropositive case increased with the herd size due to increasing number of dogs.²⁵ Our results provide strong support for the hypothesis that increase of herd capacity is a risk factor of *Neospora* infection.

In present study, 57.10% of cattle with abortion history were seropositive (p = 0.0005). Razmi *et al.* reported, that the abortion prevalence in seropositive cattle was higher than seronegative (p < 0.05, OR = 1.78) in Mashhad.¹² This is similar to our and other results.^{2,3,9,18} Evaluation of seropositivity in previous studies showed that the risk of abortion were 4.00, 5.30 and 8.00 fold higher than seronegative cattle.²⁶⁻²⁹ Our result taken together with previous investigations supports the notion that the seropositivity rate is correlated with abortion.

In current study, *N. caninum* infection was reported 10.00% (36/360) in the farms with dog presence [p = 0.002, OR = 0.43(0.25-0.74)]. However, 13.50% (26/122) of cattle in contact with dog were seropositive. A 2.47 fold increase in the rate of infection was observed in cattle with dog contact [p = 0.02, OR = 2.47(1.16-5.30)]. Around 27.00% (39/144) of cattle in contact with stray canids (fox and jackal) were seropositive (p = 0.0005).

Studies in Spain and France have also found positive associations between the seropositivity of cattle and the presence or the number of farm dogs.²¹ Barling *et al.* observed the presence of dog in farms was a putative protective factor.³⁰ Our result is the opposite of Kyaw *et al.* finding.¹⁷

The presence of dogs in farm has been assumed to provide the greatest chance of horizontal transmission through the ingestion of oocysts shed by infected dogs. In addition, dogs kept in the neighborhood farms may pose an infection risk. The results of this research can provide baseline information for the future studies. There are both horizontal and vertical transmissions of *N. caninum* in Hamedan province. Therefore, evaluation of *Neospora* infection in other intermediate hosts and also definitive hosts are necessary for control strategies.

In conclusion, *N. caninum* is an important factor of economic loss in industrial dairy cattle in Hamedan province. Therefore, it warrants a complete overhaul of management in dairy farms.

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