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positive (15.18%) (16). In this study, didn't observe significant difference between Holstein (14.8%) and Brown Swiss (19.6%) breeds. also no difference between different age groups was seen. In this study from 139 aborted cows 19.42% was infected to *N. caninum* (16). According to Dubey findings, the rate of daily milk production in seropositive cows (with antibiotic therapy against N. caninum) was less (2.5 liter) than seronegative cows (without antibiotic therapy against N. caninum) (10). Also revealed that abortion risk in seropositive cows in endemic places is two up three times more than epidemic places. In herds that abortion storm is seen, BVD infection has to be considered also (10). So we suggest that do another test for survey of B.V.D infection in samples for distinguishing between neosporiasis and BVD.

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Table 1- Prevalence of *Neospora caninum* in 6 groups.

Center NO	All dairy cattles	All dairy cattles (without history of abortion)	All dairy cattles (with history of abortion)	Number and percent of dairy cattles (without history of abortion) seropositive	Number and percent of dairy cattles (with history of abortion) seropositive	Number and percent of all dairy cattles seropositive	Number and percent of seropositive dairy cattles under 2 years old	Number and percent of seropositive dairy cattles between 2-4 years old	Number and percent of seropositive dairy cattles between 4-6 years old
1	130	97	33	9 9.2%	7 21.2%	16 12.3%	4 3.07%	7 5.3%	5 3.8%
2	80	59	21	8 13.5%	4 19%	12 15%	3 3.7%	5 6.2%	4 5%
3	95	65	30	6 9.2%	6 20%	12 12.6%	2 2.1%	4 4.2%	6 6.3%
4	140	102	38	15 14.7%	7 18.4%	22 15.7%	7 5%	10 7.1%	5 3.5%
5	205	150	55	22 14.6%	8 14.5%	30 14.6%	9 4.3%	13 6.3%	8 3.9%
6	150	106	44	9 8.4%	6 13.6%	15 10%	5 3.3%	4 2.6%	6 4%
All	800	579	221	69 11.9%	38 17.19%	107 13.37%	30 3.7%	43 5.37%	35 4.25%

Table 2- Descriptive statistics of 6 groups

Center	Number	Subset for Alpha=0.05 Ratio	SD	Standard error %	95% confidence interval for Mean	
					Lower Bound	Upper Bound
1	130	0.1231	0.32980	0.02893	0.0658	0.1803
2	80	0.1500	0.35932	0.04017	0.0700	0.2300
3	95	0.1263	0.33397	0.03426	0.0583	0.1943
4	140	0.1571	0.36524	0.03087	0.0961	0.2182
5	205	0.1463	0.35431	0.02475	0.0976	0.1951
6	150	0.1000	0.30101	0.02458	0.0514	0.1486
Total	800	0.1338	0.34060	0.1204	0.1101	0.1574
Sig.		0.263				

11), therefore we used a commercial iscom ELISA kit (IDEXX Lab. Inc. Westbrook, Maine, USA) for diagnostics of bovine *neospora*-species antibodies in blood serum.

Due to the lack of information about the prevalence of infection in the definitive host, the dog, in Iran, it is not possible to know which method of transmission (horizontal or vertical) is the main route of infection.

On base of this study, prevalence of *Neospora caninum* in dairy cattle without abortion history and with abortion history was 11.9% and 17.19% respectively. Comparing these numbers reveal that *N. caninum* has a role in increasing the abortion rate in dairy cattle (5.1% more). Voural et al (2006) in turkey studied 3287 sera of dairy cattle and reported the rate of prevalence of *N. caninum* (13.96%). The results in this study declare that the prevalence of *N.caninum* in samples with abortion history is higher than samples without abortion history. So there are direct relationship between positive sera and abortion (18). Our study confirmed these result also. Regarding to our survey there is not a significant difference between prevalence rates of *N.caninum* in different age groups. as percent of prevalence in under 2 years old group, between 2 up 4 years old group and up to 4 years old group were 3.7% , 5.37% and 4.25% respectively. Dijkstra et al (2001) verified post natal transmission of *N. caninum* in Netherlands (7).

In a study by Sadrebazzaz et al (2004) in Mashhad, 810 dairy cattle were studied by IFA method for searching *N.caninum* antibody. 123 of 810 sera were

Introduction

Neospora caninum is an apicomplexan protozoan, which causes neuromuscular disease in dogs and abortion in cattle. Like all closely related protozoa (phylum Apixomplexa: family Sarcocystidae) *N. caninum* has a two-host life cycle (12). It has been demonstrated that the dog can act as a definitive host, in which a sexual development may occur, which leads to fecal shedding of oocysts (12). All other hosts, including cattle, are regarded as intermediate hosts, which only harbor asexual stages of the parasite (tachyzoites and encysted bradyzoites). In cattle, vertical or congenital transmission of *N. caninum* tachyzoites is generally considered to be the most important mode of transmission (5, 6 and 19). There is no direct transmission between cattle. However, the parasite is maintained by congenital transmission and is a major factor in providing persistence of *Neospora* to their offspring. Abortion due to neosporosis may occur over several generations. There is no known effective and economic treatment for bovine neosporosis (8, 9). However, vaccination of cattle with inactivated *N. caninum* tachyzoites was reported to prevent cattle from abortions. Humans could become exposed to *N. caninum* by accidental ingestion of oocysts shed in the feces of canid definitive hosts or following the consumption of raw or inadequately cooked meat that contains tissue cysts. Although neosporosis has been reported from many parts of the world (8, 10), there is a few published report available on its occurrence in Iran, Mashhad and Kerman (13, 14, 15, 16 and 17); so this study was performed to evaluate the significance of neosporosis in dairy cattle herds in this region of Iran.

Materials and methods

Blood samples were collected from 800 dairy cattle with and without previous history of abortion in 6 herds in Golestan province of Iran (located in the north-east of Iran, south of the Caspian Sea. Geographically, it is divided into two sections: The plains, and the mountains of the Alborz range. It has an area of 20,380 km². The major townships of the province are: Gorgan, Gonbad Kavoos, Bandar Torkaman, Bandar Gaz, Aliabad-e katul, Kord Kooy and Minoo Dasht. Golestan enjoys mild weather and a temperate climate most of the year). Jugular vein blood was collected in vacutainer tubes. After centrifugation at 3000 rpm × 15 min, sera were separated and stored at -20 °C until analysis. The samples were screened for specific *Neospora caninum* antibodies, with commercially available diagnostic kit (IDEXX Lab. Inc. Westbrook, Maine, USA) using X check software program. The diluents, wash solution, dilution buffer, and anti-bovine IgG horseradish peroxidase conjugate and substrate were provided by IDEXX. The optical density (OD) values of the wells were read with ELISA reader (Titertek Multiskan plus MK II), at a wavelength of 650 nm. The presence and absence of antibody to *Neospora caninum* were determined by sample to positive (S/P) ratio for each sample. Samples were as positive with an S/P ratio greater than 0.5. Comparison between age groups was done by ANOVA.

Results

Neospora caninum Antibody was detected in 107 (13/37±2.36%, $\alpha = 0.05$) of 800 dairy cows (Table 1). Seropositive samples (X=107) were found in 6 experimented herd (Gorgan mechanized Institute as group 1, Azadshahr semi mechanized complex as group 2, Homayoon mechanized farm as group 3 , Ali abad Agriculture institute as group 4 , Behin Talise of Kordkooy complex as group 5, Ghods research complex of Gonbad as group 6). There were not significant differences between infection to neosporosis and different age groups, so as, rate of the infection under 2 years old group was 3.7%, in 2 up 4 years old group was 5.37% and in up to 4 years old group was 4.25% (Table 1). The highest rate of prevalence to Neosporsa caninum was in group 4 (15.7%) and the lowest rate was in group 6(10%) (Table 1). Rate of positive samples in without abortion history group was 11.9% and in with abortion history group was 17.9% (Table 1).

Discussion

Neosporosis has been reported in many countries (2, 3, 4 and 7) with different prevalence rates since the disease was recognized in 1988. As there was not published report available on *N.caninum* infection occurrence in Golestan province we decided to obtain information on seroprevalence of *N. caninum* antibodies in dairy cattle in North East Iran (Golestan). Several serologic tests including ELISA, IFAT, and DAT can be used to detect N. caninum. At present, the two main types of serological tests most commonly used for the diagnosis of *Neospora* infection are IFAT and ELISA. Iscom ELISA for the detection of *Neospora caninum* antibodies in blood serum and milk was developed to decrease the cross-reactivity (1) and a commercial iscom ELISA kit (Svanova, Sweden) was designed for diagnostics of bovine *Neospora*-specific antibodies in blood serum. Establishing the appropriate cut-off value is a key point in all ELISA methods (11).

Characterization studies have shown that *N. caninum* NC-1 iscoms contain membrane antigens from both the cell surface and from intracellular compartments. Iscom ELISA for the detection of *Neospora caninum* antibodies in blood serum and milk was developed to decrease cross-reactivity (1,

The Seroprevalence of *Neospora caninum* antibodies in dairy cattle herds in Golestan province, Iran

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Abstract

A total of 800 sera collected from six herds of health and aborted cows in Golestan province in Iran (579 without abortion history, 221 with abortion history) were examined for presence of *Neospora caninum* antibodies by using commercial ELISA kit. The overall seroprevalence of *Neospora caninum* antibodies was 107 from 800 (13.37±2.36%, α=0.05). Comparison of *Neospora caninum* serological status in age groups (<2 years, 2-4 years, >4 years) showed no significant difference (p>0.05). The prevalence of *Neospora caninum* was higher in the aborted cows than in non aborted cows. This is the first study on *Neospora caninum* antibodies in dairy cattle herds in Golestan province in the north east of Iran. ***J. Vet. Microbiol. 7,1:60-64, 2011.***

Keywords: Seroprevalence, ELISA, *Neospora caninum*, Golestan.

بررسی سرولوژیکی آلودگی به نئوسپورا کانینوم در گاوهای شیری در استان گلستان

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چکیده

این مطالعه جهت بررسی پراکندگی عفونت نئوسپور یازیس در گاوهای شیری استان گلستان انجام شد. تعداد ۸۰۰ نمونه خون از گاوهای ۶ گاوداری صنعتی و نیمه صنعتی در استان گلستان (۵۷۹ گاو سالم و ۲۲۱ گاو با سابقه سقط جنین) جمع آوری و پس از جداسازی سرم، با روش استاندارد Elisa و توسط کیت های تشخیصی آنتی بادی نئوسپورا کانینوم، کلیه سرم های موجود، آزمایش شد. از ۸۰۰ گاو مورد آزمایش ۱۰۷ گاو (۱۳/۳۷±۲/۳۶%، α=۰/۰۵) آنتی بادی ضد نئوسپورا را در سرم خون نشان دادند. براساس این تحقیق، میزان سقط در گاوهای شیری مبتلا به تک یاخته نئوسپورا کانینوم بالاتر از گاوهای فاقد عفونت نئوسپور یازیس می باشد. همچنین تفاوت معنی داری در میزان ابتلا به نئوسپورا کانینوم در رده های سنی مختلف (زیر ۲ سال، بین ۲ تا ۴ سال و بالای ۴ سال) مشاهده نشد (p>۰/۰۵). با توجه به اینکه در مورد این تک یاخته کوکسیدیائی مطالعات اندکی در سایر استان ها صورت گرفته؛ پیشنهاد میگردد تا تحقیقات گسترده تری در این زمینه انجام شود.

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واژه های کلیدی: بررسی سرولوژیکی، نئوسپورا کانینوم، استان گلستان، الیزا.