

Estimating the rate of transplacental transmission of *Neospora caninum* to aborted fetuses in seropositive dams in Mashhad area, Iran

Razmi, Gh.R.^{1*}, Zarae, H.², Nourbakhsh, M.F.², Naseri, Z.¹

¹Center of Excellence in Ruminant Abortion and Neonatal Mortality, Ferdowsi University of Mashhad, Mashhad, Iran

²Graduated from the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

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Correspondence

Razmi, Gh.R.

Center of Excellence in Ruminant Abortion and Neonatal Mortality, Ferdowsi University of Mashhad, Mashhad, Iran

Tel: +98(511) 8788944

Fax: +98(511) 8763853

Email: razmi@um.ac.ir

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Abstract:

BACKGROUND: *Neospora caninum* is an apicomplexan protozoa, which causes abortion in cattle worldwide. **OBJECTIVES:** The present study was designed to estimate the rate of transplacental transmission of *N.caninum* to aborted fetuses in seropositive dams in dairy farms in Mashhad area, Iran. **METHODS:** Two hundred blood samples of dam with aborted bovine fetuses were collected from dairy farms from 2008 to 2010. First, the maternal sera in each aborted case were tested by using ELISA method, and then the brain samples of aborted fetuses were examined for detecting DNA of *N. caninum*, using the PCR method. **RESULTS:** In this study, 38 (19%) of dams' sera showed seropositive reaction against *N.caninum* infection, and also 23 (10.5%) aborted brain fetuses showed positive reaction by PCR. A significant association was observed between *N.caninum*-seropositive dams and infected aborted fetuses ($p<0.0001$). In the present study, 20 (86%) infected fetuses were aborted from seropositive, and 3 (13%) of the fetuses were aborted from seronegative dams. **CONCLUSIONS:** Based on the results of molecular and serology examination, the rate of transplacental transmission of *N. caninum* infection in dairy cattle were calculated as 52%.

Introduction

Neospora caninum is one of the most efficiently transplacentally-transmitted organisms in cattle and has been recognized as the most important cause of abortion in cattle throughout the world

(Dubey, 2005; Dubey et al., 2007). Most abortions occur during mid-gestation. Fetuses may die in uterus between 3 and 8 months of gestation, and they are usually expelled showing moderate autolysis; however, fetuses dying before the 5th month of gestation may be mummified and retained in the uterus for several months. Fetuses dying at an early stage of gestation may be reabsorbed and may cause repeated breeding. Abortion may be sporadic,

endemic, or epidemic (Dubey, 2005; Wouda, 2007). Dairy cattle with *N. caninum* antibodies (seropositive) are more likely to abort than seronegative cows, and this sort of abortion applies to both dairy and beef cattle. However, up to 95% of calves born congenitally-infected from seropositive dams remain clinically normal (Dubey, 2005; Dubey et al., 2007; Wouda, 2007).

In Iran, seroepidemiological studies have shown that the prevalence of *Neospora* infection is relatively high in dairy cattle (Sadrebazzaz et al., 2004; Razmi et al., 2006; Nourollahi Fard et al., 2008) and dogs (Malmasi et al., 2006; Haddadzadeh et al., 2007). *N.caninum* infection was also detected as an important causative agent of bovine abortion in dairy

farms of Mashhad area, Iran (Razmi et al., 2007, 2010). The aim of this study is to estimate the rate of transplacental transmission of *N.caninum* infection in seropositive aborted dairy cattle in large dairy farms of Mashhad area.

Materials and Methods

The study was carried out in Mashhad area, capital of Khorasan Razvi province, situated in the northeast of Iran. Khorasan Razvi province is located in northern temperature zone. The climate is semi-arid with cold winters and moderate summer. This area estimate has 25000 cattle on 110 dairy farms. The herd size varies from farm to farm with a range of 30 to 2000 cattle. The most common breed cattle are Holstein- Friesian. There are many dairy farms with over 500 dairy cattle. The present study was performed over a 2 year period in three Holstein-Friesian dairy herds with a large size. In selected dairies, all animal bred by artificial insemination and were free from tuberculosis and brucellosis, as shown by yearly tests.

Sample collection: Two hundred blood of dam and aborted bovine fetuses at different stages of gestation were collected from each aborted cases in three large dairy farms from 2007 to 2009. First, the age of the submitted fetuses was estimated by crown-rump length. After that, the skulls of the fetuses were opened under aseptic condition, and then one half of the brain was sampled for PCR. The collected blood samples of dams were centrifuged at 2000 rpm for 10 min. Brain samples with sera were stored at 20 °C until they were used.

Serology: The sera of all dams were analyzed for antibody activity to *N.caninum* by using the commercially available ELISA kit (IDEXX Laboratories). Briefly, sera samples were diluted 1:100 in phosphate buffered saline solution, pH 7.4, with 0.05% Tween-20 and analyzed for the presence of IgG antibodies specific for *N.caninum* by ELISA test. Sera with absorbance values above the cut-off level of 0.20 were considered positive, according to manufacturer's instructions. Two repetitions from each sample were performed.

DNA-isolation and PCR: The brain samples were homogenized with a stirrer, and DNA was extracted from 1 g homogenate sample using the

DNATM Kit (cinnagen, Inc, Iran) according to the manufacturer's recommendations. PCR was done by the method as described by the authors (Muller, et al., 1996). The Oligonucleotide primers were for NC-5 PCR: NP21 plus (5'CCCAGTGCGTCCAATCCTGTAAC3') and Np6 plus (5'CTCGCCAGTCAACCTACGTCTTCT3'). The control positive of PCR was prepared from the institute of Parasitology, Bern, Switzerland.

Statistics analysis: The resulting data from the present study was analyzed using the chi-square test. The correlation between serostatus of dam with *N.caninum* infection in aborted fetuses was statistically significant when a P-value of less than 0.05 was observed. The rate of transplacental transmission was calculated as the proportion of seropositive dams that produced infected fetuses. The agreement between the different tests was expressed as k-value. The agreement was considered fair if k-Value was between 0.2 and 0.4; moderate if k-Value was between 0.4 and 0.6; substantial if 0.6 and 0.8; and good if it exceeded 0.8. (Petrie and Watson, 2006).

Results

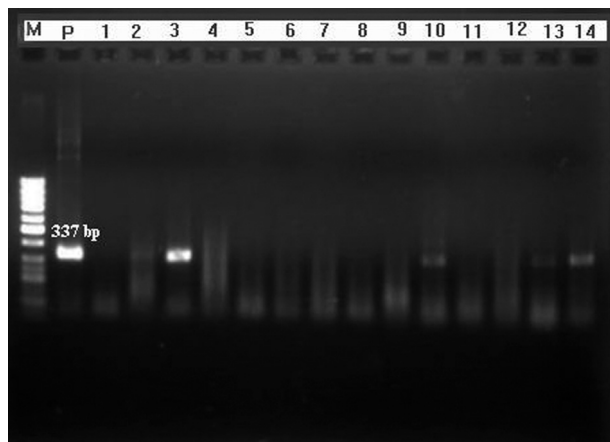
In the present study, *N.caninum* antibodies were found in 38 (19%) sera of dams. The range of OD ELISA values were from 0.6 to 2.7. In this study, 23 (11.5%) out of 200 brain of aborted fetuses were positive by PCR. (Figure 1). The results of also indicated that 20 of infected fetuses were aborted from seropositive dams and 4 from the seronegative dams. A moderate agreement (Kappa=0.59) was seen between ELISA and PCR. The risk of transplacental transmission of *N.caninum* infection in seropositive dairy cattle was 27.25 folds more than that of seronegative dairy cattle ($p < 0.0001$). Based on the serologic and molecular results in dams and aborted fetuses, the rate of trans-placental transmission of *N.caninum* infection was calculated 52% (20.38). (Table 1).

Discussion

In the present study, *N.caninum* antibodies were found in 19% of the aborted dairy cattle. The seroprevalence of *N. caninum* infection was also reported %19 (Saddrebazzas et al., 2004) and %53

Table 1. The relationship of *N. caninum* antibodies in sera of aborted dairy cattle with PCR results in their aborted fetuses.

	PCR positive	PCR negative	Total	Relative risk	95% CI	P value
Seropositive dam	20	18	38			
Seronegative dam	3	159	162			
Total	23	177	200	28.42	8.954- 116.671	0.0001

Figure 1. Detection of *N. caninum* in brain of aborted fetuses by PCR: (M) Marker 100 bp ladder; (P) positive control; (N) negative control, (3, 10,14) Positive samples.

(Razmi et al., 2006) in aborted dairy cattle in Mashhad area. Epidemiological studies showed that many risk factors related to climate, management, method of serology, and host could influence the range of seroprevalence of Neospora infection in every dairy farms (Wouda, 2007). The relative risk of transmission *N. caninum* infection from seropositive dams to aborted fetuses was high, and the rate of transplacental transmission was calculated 52%. The rate of endogenous transplacental route (vertical) of *N. caninum* was reported from 23% to 95% in dairy cattle (Pare´ et al., 1996, 1997; Schares et al., 1998; Wouda et al., 1998; Davison et al., 1999; Mainar-Jaime et al., 1999; Bergeron et al., 2000; Dyer et al., 2000; Romero and Frankena, 2003) and a low level rate for exogenous transplacental route (horizontal) (Bartels et al., 2007; Davison et al., 1999; Frössling et al., 2005; Hietala and Thurmond, 1999; Schares et al., 1998). With regard to high probability of Neospora transmission by endogenous trans-placental route (vertical) and any observation of epidemic abortion associated with horizontal transmission of Neospora infection during this study, it seems that the vertical route plays an important role in spreading infection in large dairy farms of Mashhad area, Iran. The estimation rate of each of the two different routes needs more seroepidemiological investigation.

In the present study, three infected fetuses were aborted from seronegative dams. This result may be due to undetectable levels of antibody against *N. caninum* in the time of blood sampling. (Dubey et al., 2007). So far, several studies have reported the abortion of infected fetuses or birth of calves from seronegative dams (Pare´ et al., 1996; Otter et al., 1997; Sagar et al., 2001; More´ et al., 2009).

In conclusion, the present study demonstrated that the *N. caninum* infection has a high rate of transplacental transmission and an important role in causing abortion in large dairy farms in the studied area.

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تخمین میزان انتقال آلودگی نئوسپورا از طریق جفت به جنین های سقطی گاوهای شیری سرم مثبت شهرستان مشهد، ایران

غلامرضارزمی^{*۱} هادی زارع^۲ محمد فواد نوربخش^۲ زهرا ناصری^۱

(۱) قطب سقط جنین و مرگ و میر نوزادان نشخوارکننده، دانشکده دامپزشکی دانشگاه فردوسی مشهد، مشهد، ایران

(۲) دانش آموخته، دانشکده دامپزشکی دانشگاه فردوسی مشهد، مشهد، ایران

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چکیده

زمینه مطالعه: نئوسپورا کاینیم تک یاخته از شاخه آپی کمپلکسا بوده که باعث سقط جنین در گاوهای سراسر دنیا می شود. **هدف:** مطالعه حاضر به منظور تخمین میزان انتقال آلودگی نئوسپورا به جنین های سقطی در گاوهای شیری سرم مثبت گاو داری های شیری مشهد انجام گرفت. **روش کار:** تعداد ۲۰۰ سرم گاوهای شیری سقط نموده به همراه جنین های سقطی طی سال های ۱۳۸۸ تا ۱۳۹۰ جمع آوری گردید. سرم گاوهای شیری سقط نموده با روش الیزا و مغز جنین های سقط شده با روش PCR آزمایش گردید. **نتایج:** در این مطالعه ۳۸ گاو شیری سقط نموده (۱۹٪) واجد عیار سرمی بر علیه نئوسپورا بوده و همچنین در آزمایش PCR در ۲۳ مغز جنین سقط شده (۱۰/۵٪) نتیجه مثبت مشاهده شد. ارتباط معنی داری بین مادر سقط نموده سرم مثبت با جنین های آلوده مشاهده گردید ($p < 0/0001$). در این مطالعه، ۲۰ جنین آلوده (۸۶٪) از مادران سرم مثبت و سه جنین (۱۳٪) از مادران سرم منفی سقط شدند. متولد شدند. **نتیجه گیری نهایی:** بر پایه نتایج سرولوژی و ملکولی میزان انتقال عفونت مادران آلوده از طریق جفت به جنین های سقط شده ۵۲٪ تخمین زده شد.

واژه های کلیدی: سقط جنین، گاو، نئوسپورا، PCR

* نویسنده مسؤول: تلفن: ۸۷۸۸۹۴۴ (۵۱۱) ۰۹۸+، نمابر: ۸۷۶۳۸۵۳ (۵۱۱) ۰۹۸+، Email: razmi@um.ac.ir