

## RESEARCH ARTICLE

## Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* infections in aborted cattle in Hamedan, Iran

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### ABSTRACT

The aim of current study was to assess the seroprevalence of *Neospora (N.) caninum* and *Toxoplasma (T.) gondii* infections in aborted cattle and subsequent estimation of role of these parasites in cattle abortion in Hamedan province, Iran. Blood samples (n=85) of aborted cattle were collected from different locations of Hamedan province for a period of two years from January, 2010 to December, 2012. All the samples were evaluated for the presence of IgG-antibodies against *N. caninum* and *T. gondii* using Enzyme Linked Immuno Sorbent Assay (ELISA). The results showed that 61.2% (n=52/85), 5.9% (n=5/85) and 3.5% (n=3/85) animals were positive for *N. caninum*, *T. gondii* and co-infection of these two, respectively. There was no significant correlation between seroprevalence rates, age groups and breeding ( $p>0.05$ ). However, a significant difference was found between *T. gondii* infection and the type of cattle ( $p=0.05$ ), unlike to *N. caninum* ( $p=0.52$ ). This is the first report of *N. caninum* and *T. gondii* co-infection in cattle in Iran. Although the rate of *T. gondii* infection was lower but this parasite might partly be responsible to be transmitted to humans. Further investigations and appropriate control strategies in management of cattle farms are highly recommended.

### Keywords:

*Neospora caninum*, *Toxoplasma gondii*, co-infection, seroprevalence, abortion, cattle, Hamedan

### INTRODUCTION

*Neospora (N.) caninum* and *Toxoplasma (T.) gondii* are two species of heteroxenous coccidia from the Sarcocystidae family having a wide host-range and global distribution (Dubey et al., 2007). These parasites are mainly transmitted either transplacentally or through food contaminated with their oocysts (Gharekhani and Tavosidana, 2013; Heidari et al., 2013). The oocysts come to environment through feces and enter the final hosts. Thus, the oocysts act a risk factor for the occurrence of abortions and stillbirths associated with *N. caninum* and *T. gondii* infections in cattle (Dubey and Schares, 2011; Gharekhani et al., 2013a). Both of these diseases are responsible for major economic losses in livestock industry worldwide (Gharekhani, 2013; Gharekhani et al., 2013a). The diagnosis of both infections is based largely upon bioassays, histopathology and serological examinations including the Enzyme Linked Immuno Sorbent Assay (ELISA) (Gharekhani and Tavosidana, 2013; Gharekhani et al., 2013a).

Several studies indicated that a wide range of animals had been exposed to *N. caninum* and *T. gondii* in Iran (Hashemi-Fesharki, 1996; Sadrebazzaz et al., 2006; Raeghi et al., 2011; Gharekhani et al., 2012; Gharekhani, 2013; Gharekhani and Tavosidana, 2013; Gharekhani et al., 2013a, 2013b, 2013c; Heidari et al., 2013). The presence of *N. caninum* DNA in brains of aborted bovine fetuses was first detected in Iran in 2007 (Razmi et al., 2007). However, there is no published data on cattle abortion caused by *N. caninum* and *T. gondii* co-infection in this region. The aim of this study was to assess the seroprevalence of *N. caninum* and *T. gondii* infections in aborted cattle and subsequent estimation

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of the role of these parasites in the abortion of cattle in Hamedan province, Iran.

## MATERIALS AND METHODS

**Study area:** Hamedan province (a mountainous and mild climatic region) is located in the western part of Iran (34.77°N and 48.58°E). It covers an area of 19,546 km<sup>2</sup> and average annual temperature is 11.3°C. This province is economically important for crops and animal husbandry.

**Sample collection and serology:** Blood samples (n=85) were collected from aborted cattle for a period of two years from January, 2010 to December, 2012. Information about age (<2, 2-4 and >4 years old), breed (native, hybrid and holstein), and type of cattle (rural or industrial dairy cattle) were taken from the owners and physical examination (Table 1). All the sera samples were collected after centrifugation at 1500×g for 15min and stored at -20°C until laboratory testing. Anti-*Neospora* and anti-*Toxoplasma* IgG-antibodies in the sera samples were detected by using commercially available ELISA kits (HerdCheck® and CHEKIT-TOXOTEST®, IDEXX, Switzerland) according to the manufacturer's instructions.

**Statistical analysis:** Statistical analysis was performed by using the software package SPSS version 16.0 for windows. The differences among variables were evaluated by Chi-square test. All the values with  $p \leq 0.05$  were considered statistically significant.

## RESULTS AND DISCUSSION

IgG-antibodies against *N. caninum* and *T. gondii* were found in 61.2% (50.9% <95% CI < 64.2%) and 5.9% (0.9% <95% CI < 10.9%) samples, respectively. A simultaneous infection of *N. caninum* and *T. gondii* was found in 3 (3.5%) samples (Table 1). The association between infection rates between *N. caninum* and *T. gondii* was statistically significant ( $\chi^2=69.946$ ,  $p < 0.0001$ ). A statistically significant difference was found between *T. gondii* infection and type of cattle ( $\chi^2=3.718$ ,  $p=0.05$ ); unlike to *N. caninum* infection ( $\chi^2=0.4$ ,  $p=0.52$ ). There was no significant correlation between age groups ( $\chi^2=0.042$ ,  $p=0.978$  and  $\chi^2=0.001$ ,  $p=0.999$ ) and breed ( $\chi^2=0.076$ ,  $p=0.962$  and  $\chi^2=0.315$ ,  $p=0.854$ ) in both *N. caninum* and *T. gondii* infections, respectively.

The infection rates of *N. caninum* and *T. gondii* were reported between 0.7-97.2% and 0-100% in cattle worldwide, respectively (Dubey et al., 2007; Dubey and Schares, 2011). In our study, 61.2% of samples were

seropositive against *N. caninum* infection, which is in line to several previous studies (Pare et al., 1998; Anderson et al., 2000; López-Gatius et al., 2005; Dubey and Schares, 2011; Gharekhani et al., 2012). Razmi et al. (2006) reported higher abortion rate in seropositive cattle as compared to seronegative cattle ( $p < 0.05$ , OR=1.78). The risk of abortion in seropositive cattle was reported as 4 (Václavěk et al., 2003), 5.3 (Schares et al., 2004) and 8 (López-Gatius et al., 2005) folds higher than seronegative cattle.

The higher rate of *N. caninum* infection was determined in rural cattle (64%) following the industrial dairy cattle (57.1%) (Table 1,  $p=0.52$ ). Previously, the rate was reported as 10.5% (Northwest) to 46% (Northeast) in different regions of Iran (Razmi et al., 2006; Nematollahi et al., 2011). In another study, Youssefi et al. (2010) reported that 7%, 45.2% and 57.3% of aborted cattle were seropositive for *N. caninum* infection in Ardebil (Northwest of Iran, cold climate), Garmsar (Central of Iran, warm and dry climate) and Babol (North of Iran, mild climate), respectively.

In our study, there was no association between *N. caninum* infection and age groups, similar to the findings of previous studies (Paré et al., 1998; Atkinson et al., 2000; Chanlun et al., 2002; Kyaw et al., 2004; Dubey et al., 2007); however, Razmi et al. (2006) and Gharekhani et al. (2012) reported a significant relationship within different age groups ( $p < 0.05$ ). Sadrebazzaz et al. (2004) and Wouda et al. (1998) reported equal levels of seroprevalence in all age groups for most herds. Youssefi et al. (2010) found significant increase in seropositivity against *N. caninum* in cattle of 4-5 years age group.

In our work, no association was found among cattle breeds, *N. caninum* and *T. gondii* infection rates ( $p > 0.05$ ; Table 1), similar to the studies of Ould-Amrouche et al. (1999) and Gharekhani (2013). This might be related to difference in production systems for dairy and beef cattle rather than breed differences.

In our study, *T. gondii* infection rate was 5.9% (only in rural cattle, 10%) (Table 1,  $p=0.05$ ). In other parts of Iran, for example, North and North-west Iran, the rate was reported between zero to 15.9% (Sharif et al., 2007; Nematollahi and Moghadam, 2008; Raeghi et al., 2011). However, Hashemi-Fesharaki (1996) could not detect *T. gondii* in Iranian cattle using Latex Agglutination, Indirect Hemagglutination Tests, direct microscopy and bioassay. The lower infection rate of *T. gondii* in our study might be due to the climatic variation. Gharekhani (2013) described that hot and humid environmental conditions attributed to longer viability

**Table 1: Comparison of the seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in aborted cattle in Hamedan, Iran**

| Parameter   | Age groups (years) |           |         | Breeding  |         |         | Ru      | Id        | Total     |
|---|--------------------|-----------|---------|-----------|---------|---------|---------|-----------|-----------|
|   | <2                 | 2-4       | >4      | Na        | Hy      | Ho      |         |           |           |
| No. of sample   | 10                 | 45        | 30      | 18        | 42      | 25      | 50      | 35        | 85        |
| <i>N. caninum</i> infection (%)                         | 3 (30)             | 28 (62.2) | 21 (70) | 13 (72.2) | 21 (50) | 18 (72) | 32 (64) | 20 (57.1) | 52 (61.2) |
| <i>T. gondii</i> infection (%)                          | 3 (30)             | 2 (4.4)   | 0 (0)   | 1 (5.5)   | 4 (9.5) | 0 (0)   | 5 (10)  | 0 (0)     | 5 (5.9)   |
| <i>N. caninum</i> and <i>T. gondii</i> co-infection (%) | 0 (0)              | 0 (0)     | 3 (10)  | 1 (5.5)   | 2 (4.8) | 0 (0)   | 3 (6)   | 0 (0)     | 3 (3.5)   |

Na=Native, Hy=Hybrid, Ho=Holstein, Ru=Rural cattle, Id=Industrial dairy cattle

of *T. gondii* oocysts as compared to that of cold and dry conditions.

In our findings, seroprevalence rate of *T. gondii* infection was reported 30% (3/10) and 4.4% (2/45) in the age groups of <2 and 2-4 years old, respectively; whereas, the difference was statistically non-significant ( $p=0.999$ ; Table 1) unlike to the study of Nematollahi and Moghadam (2008). It might be speculated that the cattle depleted *Toxoplasma* antibodies with the advancement of age. This might explain the reason of becoming less resistant to toxoplasmosis by the older cattle. Besides, increase of seroprevalence with age might be due to acquiring infection post-natally (Heidari et al., 2013). Moreover, difference in diagnostic methods such as serological assays, experimental strategies, climatic variations and frequency of final hosts in the farms could be the causes of varied results.

## CONCLUSIONS

This is the first report of *N. caninum* and *T. gondii* co-infection in cattle in Iran. In conclusion, *N. caninum* and *T. gondii* are important agents causing abortion in cattle and subsequent economic losses in Hamedan province of Iran. Further researches, for example, molecular and bioassay examinations and designing appropriate control strategies in improving management of cattle farms are necessary and strongly recommended.

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