Toxoplasma Gondii Infection in Domestic Animals in Hamedan, Iran: A Sero-Epidemiological Study

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Bulletin UASVM Veterinary Medicine 71 (1) / 2014, 68-72
Print ISSN 1843-5270; Electronic ISSN 1843-5378

Abstract

Toxoplasma gondii is an important zoonotic parasite with global distribution. This parasite is responsible for major economic losses in livestock; also it is a public health problem owing its transmission to humans. The main aim of current investigation was to determine the seroprevalence of *T. gondii* in domestic animals (cattle, sheep, horses, donkeys and dogs) from Hamedan province, western Iran. From 2010 up 2012, blood samples were collected randomly in animals in this region. The Enzyme Linked Immuno Sorbent Assay (ELISA) and Modified Agglutination Test (MAT) assays was used for serum evaluation in ruminants (cattle and sheep) and other animals (hroses, donkeys and dogs), respectively. Antibodies to *T. gondii* were found 2.3% (32/1406) in cattle, 9.7% (68/700) in sheep, 13.3% (16/120) in horses, 47% (47/100) in donkeys and 10.7% (29/270) in dogs. This study is the first report of *T. gondii* infection in donkeys from Iran. There is *Toxoplasma* infection in cattle, sheep, horses, donkeys and dogs in Hamedan province, Iran. Therefore, it is necessary to take integrated strategies for prevent and control of infection in animals, which could help to reduce humans infection in this region.

Key words

Toxoplasma gondii, animal, MAT, ELISA, Iran.

INTRODUCTION

Toxoplasma gondii is an important zoonotic heteroxenous Apicomplexan parasite with global distribution. felins are final hosts; humans, birds and other animals such as cattle, sheep, horses, donkeys and dogs are intermediate hosts (Hosseininejad *et al*., 2011). This parasite is responsible for major economic losses in livestock; also it is a public health problem owing its transmission to humans (Raeghi *et al*., 2011).

Toxoplasmosis may contaminate with the consumption of uncooked or raw meat containing parasite cysts; contact with food, water or sand involving the oocysts that are spread by infected cats and transplacentally (Cuclu *et al*., 2007).

Toxoplasmosis is subclinical in cattle, equine and dogs (Hosseininejad and Hosseini, 2011; Miao *et al*., 2013); but the abortion is the most economic losses of toxoplasmosis in sheep worldwide (Dubey, 2009).

The diagnosis of toxoplasmosis is based largely upon the application of histopathological, bioassay, and serological examination including the Enzyme Linked Immuno Sorbent Assay (ELISA) and Modified Agglutination Test (MAT) (Habibi *et al*., 2012).

Numerous studies have been performed of toxoplasmosis rate in animals from Iran and other countries; but the seroprevalence of this parasite within animal populations has never been the object of researchers in western Iran.

The main aim of current investigation was to determine the seroprevalence of *T. gondii* in domestic animals (cattle, sheep, horses, donkeys and dogs) from Hamedan province, western Iran.

MATERIALS AND METHODS

Study area:

Hamedan province by mountainous and mild climate is located in west part of Iran (34.77°N and 48.58°E). The mean annual rainfall and temperature is 317.7 mm and 11.3°C, respectively. This region is economically impressed by an agricultural and animal husbandry.

Sampling:

A cross-sectional study was performed during 2010 up 2012. Blood sampling were collected randomly from 1,406 cattle, 700 sheep, 120 horses, 100 donkeys and 270 dogs from different rural regions of Hamedan province (Table 1). All sera were removed after centrifugation at 1000×g for 15min and stored at -20°C until laboratory testing. The ELISA and MAT assays was used for serum evaluation in ruminants (cattle and sheep) and other animals (hroses, donkeys and dogs), respectively.

ELISA:

Anti-*Toxoplasma* IgG-antibodies of cattle and sheep samples were detected using a commercially available *T. gondii* ELISA kit (CHEKIT-TOXOTEST®; IDEXX). The presence of antibody was determined by calculating of Value% (≥100% = positive) according to the manual formula.

MAT:

In brief, MAT was performed with a suspension of *Toxoplasma* tachyzoites fixed with formalin, serum samples which had been diluted in phosphate buffered saline (PBS, pH:7.2), positive and negative control sera, antigen diluting buffer containing bovine serum albumin (BSA), 2-mercaptoethanol for deplete the sera of nonspecific IgM. MAT titers of 1:20 or higher were considered as positive, and those sera with dubious results were re-tested. Positive and negative controls were incorporated in each test and tested at the same dilutions of sera samples. Sera were tested on 96-well plates with a U shaped bottoms (25 µl of prepared mix antigen plus 25 µl of serum), based on two-fold serial dilutions, from 1:10 until 1:160 beside of negative and positive controls and antibody titer of ≥1:20 was considered positive (Raeghi *et al*., 2011; Wu *et al*., 2011).

Statistical analysis:

Statistical analysis was performed by using the software package SPSS version 16.0 for windows. Odds ratios (OR), confidence interval (CI), X^2 and *p*-value were calculated separately for each variable. *p*-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Antibodies to *T. gondii* were found 2.3% in cattle, 9.7% in sheep, 13.3% in horses, 47% in donkeys and 10.7% in dogs (Table 1). There was significant difference in age groups in cattle (P<0.0001, OR=7.5), sheep (P<0.0001, OR=3.4) and dogs (P<0.0001, OR=8), unlike to horses (P=0.77) and donkeys (P=0.871). Also, there was statistical differences among gender in sheep (P<0.0001, OR=11.4) and donkeys (P=0.001, OR=5.4), unlike to cattle (P=0.316), horses (P=0.067) and dogs (P=0.618). In cattle, the most rate of seropositive was determined 5.25% (21/400) in rural cattle, following 1.7% (9/514) in beef and 0.4% (2/492) in dairy cattle (P=0.559).

The seroprevalence rate was reported 24.3% (17/70) and 6% (12/200) in stray and shepherd dogs, respectively (P<0.0001, OR=5).

T. gondii infection is common in many species of animals, and antibodies against this parasite has been reported worldwide (Dubey, 1986). Seropre-

Tab. 1 Comparison of *T. gondii* seroprevalence in different variables in animals.

No.(P%)	Age groups (year)		Gender		Total	CI 95%
	<2	**>2**	**Male**	**Femal**		
Cattle	415(5.8)	991(0.8)	514(1.7)	892(2.6)	1406(2.3)	1.52-3.08
Sheep	226(17.7)	474(5.9)	192(27.1)	508(3.1)	700(9.7)	7.5-11.9
Horse	49(12.2)	71(14.1)	43(20.9)	77(9.1)	120(13.3)	7.3-19.3
Donkey	63(47.6)	37(45.9)	22(77.3)	78(38.5)	100(47)	37.2-56.8
Dog	156(3.2)	114(21)	158(9.5)	112(12.5)	270(10.7)	7-14.4

valence of toxoplasmosis has been found to range from 0% (some regions in Australia, Canada, Egypt, Italy, Mexico, Indonesia and Vietnam) to 100% (Tennessee and Iowa regions in USA) in cattle worldwide (Dubey, 1986). This rate were reported between 1.6-15.9% in Northwest, and 0% in North of Iran (Ghazaei, 2005; Sharif *et al*., 2007; Nematollahi and Moghadam, 2008; Raeghi *et al*., 2011). Also, in Hashemi-Fesharaki (1996) study, *T. gondii* was not detected in cattle using Latex Agglutination (LAT), Indirect Hemagglutination Tests (IHAT), direct microscopy and bioassay in mice from Iran. The similar of our study, infection rate was reported in Brazil (2.3%) (Vieira-Fajardo *et al*., 2013). The seroprevalence per animal is considered low compared to those observed in other studies (Dubey, 1986).

This study showed that the highest seroprevalence in age group of <2yr (5.8%); the difference was statistically significant (Table 1, P<0.0001), similar to study in Northeast Iran (Nematollahi and Moghadam, 2008).

Nematollahi and Moghadam (2008) was reported significant difference in gender in Northeast Iran (P<0.01), unlike to our study (20). Pita *et al* (1999), reported females cattle were more seropositive in Brazil. This difference are probably due to different management methods in breeding of animals.

In sheep, seroprevalence rate were reported between 3% (Pakistan) to 95.7% (Kars) worldwide, and 13.8% to 72.6% in Iran (Hashemi-Fesharaki, 1996; Bonyadin *et al*., 2007; Sharif *et al*., 2007; Bahrieni *et al*., 2008; Dubey, 2009; Raeghi *et al*., 2011). The similar rate of infection was reported in Turkey (9.5%) (Oz *et al*., 1995).

In our study, the highest seroprevalence was reported in <2 yr, the difference was statistically significant (Table 1); opposite to study in Kurdistan, Iran (Khezri *et al*., 2012). Dubey (2009) reported that age is an important factor in sheep toxoplasmosis. Toxoplasmosis in sheep is a potential risk of its transmission to humans through consumption of meat contaminated with tissue cysts of *T. gondii* in Iran (Shahmoradi *et al*., 1993).

The seroprevalence were reported from 0% to 90% in horses worldwide (Miao *et al*., 2013). Also, this rate were 11.5% and 71.2% from Urmia and Qazvin provinces in Iran, respectively (Hajialilo *et al*., 2010; Raeghi *et al*., 2011). The similar rate of infection was reported in Portugal (13.3%) (Lopes *et al*., 2013).

In agree with our finding, no significant difference was reported in age group and gender in Turkey (Guclu *et al*., 2007), China (Miao *et al*., 2013), Portugal (Lopes *et al*., 2013) and Mexico (Alvarado-Esquivel *et al*., 2012); which is the opposite to that reported in previous studies in Tunisia and North India (Chhabra and Gautam, 1980; Boughattas *et al*., 2011).

In Miao *et al* (2013) research, donkeys infection was reported 20.3%; there was no significant difference in age contrast to our work. In Iran, the donkey carcasses are being used for carnivore food in the zoo and are fed to stray canids and felins in suburb of villages; it may contribute to transmission of infection between animals and thus indirectly to humans.

The seroprevalence rate were reported 10.1-26.8% and 19.6-91% in dogs from Iran and worldwide, respectively (Hosseininejad and Hosseini, 2011; Hosseininejad *et al*., 2011; Khanmohammadi and Ganji, 2012). In our study, this rate (10.7%) was less than what has been reported 22.47% in Tehran, Iran (Hosseininejad *et al*., 2011), 26.8% in Central of Iran (Hosseininejad and Hosseini, 2011), and similar to Northwest Iran (10.1%) and Northwest China (10.8%) (Wu *et al*., 2011; Khanmohammadi and Ganji, 2012). The infection rate in stray dogs (24.3%) was five fold higher than shepherd dogs (6%, P<0.0001), unlike to Khanmohammadi *et al* (2012) finding (Khanmohammadi and Ganji, 2012). In Hosseininejad *et al* (2011) study, infection rate in stray dogs (31%) was significantly higher than household dogs (9%, P<0.05), due to higher exposure to contaminated food, soil, and water source with sporulated oocysts.

In our study, a higher seroprevalence rate was detected in the group of >2 yr (Table 1, P<0.0001) agreement with other researchers (Hosseininejad and Hosseini, 2011; Hosseininejad *et al*., 2011). High infection has been related to a greater probability for exposure to *Toxoplasma* over time, increasing the susceptibility in older dogs (Hosseininejad and Hosseini, 2011). In Khanmohammadi *et al* (2012) and Wu *et al* (2011) studies, there was no significant difference in age groups (P>0.05). High seroprevalence in lower age may be due to congenital infection, the fetus may acquire initially *Toxoplasma* infection during pregnancy in female canines (Wu *et al*., 2011).

There was no significant difference in gender (Table 1, P=0.618). This finding agrees with study conducted by other researchers, suggesting that gender is not a crucial factor for infection (Hosseininejad and Hosseini, 2011; Hosseininejad *et al*., 2011; Wu *et al*., 2011; Khanmohammadi and Ganji, 2012). Most of the farmers tend to have male dogs in their farms. Therefore, the male dogs might have been infections more than the female dogs.

A role for dogs in the transmission of toxoplasmosis to human has been postulated, basedon observations that dogs ingest cat feces and often roll in cat feces and other foulsmelling substances (Hosseininejad *et al*., 2011).

Differente serological tests, climatic variations and frequency of felines and rodents in the farms are main cause of varied results (Dubey, 1986; 2009). Older animals have had more opportunities to come in to contact with felins; so they may be more likely to aquire toxoplasmosis.

Cats are capable of roaming areas, including food storage areas and stalls. Oocyst-contaminated pastures, fodder, and drinking water are regarded as potential sources of postnatal infection in animals (Heidari *et al*., 2013).

CONCLUSION

This study is the first report of *Toxoplasma* infection in donkeys from Iran. Our finding indicate an important circulation of infection in animals from this region. Therefore, it is necessary to take integrated strategies for prevent and control of infection in animals, which could help to reduce toxoplasmosis in humans.

Acknowledgements: This study has not been supported by any foundation. The author declare that there is no conflict of interests.

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