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Toxoplasma gondii serosurvey in Golden Jackals from Golestan province, Iran

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ABSTRACT

Toxoplasma gondii as an apicomplexan zoonotic parasite is able to infect humans and almost all other warm-blooded animals. Wild and domestic felids are the main definitive hosts. Also, canids such as dogs and jackals have been introduced as mechanical vectors since *T. gondii* oocysts can pass their intestinal tract and spread by feces in environment. Toxoplasmosis is a common zoonotic disease in Iran especially in North humid regions of Iran with 70% prevalence. The goal of this study was to evaluate the *T. gondii* antibody in jackals from Golestan province in East-North of Iran in order to analyze wild canids' role in *T.gondii's* life cycle. Blood samples obtained from 40 killed golden jackals. After separation of serum samples, the presence of *T.gondii* antibody was evaluated by ELIZA kit. *T.gondii* antibody has been detected in 31 out of 40 (77.5%) serum samples. Most positive jackals' sera belong to older jackals. The *T. gondii* seropositivity was the same in male and female jackals.The relatively high prevalence of *T. gondii* in the samples of golden jackals gives valuable insight to *T. gondii* epidemiology and is useful for managing practical prevention and control programs.

1. Introduction

Toxoplasma gondii is an apicomplexan intracellular protozoan parasite occurring worldwide in humans and other wild animals. *T.gondii* is one of the most common zoonotic protozoa in humans, with the prevalence ranging from 20–80% worldwide (Tenter et al., 2000). Hosts acquire infection following ingestion of sporozoites from oocysts excreted by wild and domestic felids, the definitive hosts that can excrete oocysts in the feces, or by feeding on cyst-infected tissues of warm-blooded hosts. Also, congenital *T. gondii* infection in humans and canids such as dogs has been reported as an important factor, the fetus may get firstly *T.gondii* infection during pregnancy in female canines (Bresciani et al., 2009). Besides, a study revealed that dogs can vertically transmit *T.gondii* to their offspring by semen (Arantes et al., 2009). *T.gondii* can lead to wide range of symptoms in other infected hosts which they have both veterinary and medical sciences importance. As it was mentioned *T. gondii* may be transmitted vertically by tachyzoites that are passed to the fetus via placenta and lead to abnormalities in infected fetus and also abortion in infected women. There is not so many data

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about the clinical significance of toxoplasmosis in free-ranging canids. Fatal toxoplasmosis has been reported in several species of foxes including the red fox (Vulpes vulpes) and the arctic fox (Vulpes lagopus) (Murphy et al., 2007; Sorensen et al., 2005).

T. gondii oocyst has been detected in feces of jackals (Takcs et al., 2014). So it is said that Jackals, foxes and other wild carnivores might play an important role in the maintenance of sylvatic cycle of *T.gondii* (Karbowiak et al., 2010). On the one hand, wild canid populations may suffer from severe epizootics and declines related to zoonotic diseases such as toxoplasmosis there are species like jackals that are highly adaptable to different ecosystems and human-impacted environments and can introduce zoonotic agents to humans (Alonso et al., 2009).

Sporulated *T. gondii* oocysts stay viable and infectious in warm-humid environment for a number of years, because they can largely struggle threats such as heat and coldness. It is demonstrated that the prevalence of antibody against *T. gondii* is related to the interaction between temperature and rain (Afonso et al., 2006). The infection risk increases when the weather is both warm and humid, or moderated and less humid. Because mean winter temperature is rising, the parasite survival is likely to increase worldwide. This can have consequences for prevalence of *T. gondii* in wild and domestic hosts (Afonso et al., 2006; Frenkel et al 2006).

Golestan province locating in East-North of Iran has Mediterranean climate in most parts which makes it suitable for *T.gondii* oocyst growth (Weather Centre 2007). Mammalian wildlife of Golestan province comprises a large variety of species of mammals, acting and interacting in different ways as reservoir, intermediate or definitive hosts for zoonotic pathogens such as *T. gondii* that can be transmitted to farm animals and people in rural areas. Also there are numbers of wild animals such as corsac fox and Persian leopard which are facing extinction danger in Golestan province. Jackals are more likely to surround villages and interact with domestic dogs because of their free roaming in residential areas, potentially acting as bridge reservoir host between rural and wildlife ecosystem in Golestan province (Ziaie, 2006).

Despite presence of wild hosts such as corsac fox that are facing danger of extinction in Golestan province, harmful impact of toxoplasmosis on vulnerable animal species and also highest prevalence of human toxoplasmosis in humid regions of North Iran, the role of wild canids such as jackals that can act as an important reservoirs for *T. gondii* in Golestan province is not clear (Mostafavi et al., 2012; Sorensen et al., 2005).

Public health concerns associated with *T. gondii* clearly indicates the need for epidemiological investigations of this infection in wild canids, particularly those that can be sentinel for human infection, to establish effective control programs.

T.gondii infection was serologically analyzed in jackals as this species can serve as indicator for the presence of *T.gondii* in environment; however, transmission risk to livestock, humans and other susceptible hosts in Golestan province.

2. Materials and Methods

40 golden jackals (*Canis aureus*) (18 females and 22 males) were shooted (2013) around the villages with Mediterranean climate in Golestan province. Age was determined by the way that Root and Payne has been described (Root and Payne, 1984). Blood samples were collected from the heart immediately after shooting and transferred on ice to research laboratory of Gorgan University of Agricultural Sciences & Natural Resources. Clotted bloods centrifuged 3000 rpm for 10 min and after separation of sera, they were kept at -20^{0}C until used. For all sampled jackals, age, sex and capture locality were recorded.

In order to detect anti-*T. gondii* IgG antibody in the serum samples, ID Screen Indirect® (ID-VET Company, France) ELISA kit was used.

Briefly, first, 90 µl of Dilution Buffer 2 was added to each microwell, then 10 µl of negative control was added to wells A1 and B1 and 10 µl of positive control was added to wells C1 and D1 and 10 µl of each sample was added to remaining wells. Then, microwells were kept for 45 min ± 4 min at room temperature, after that, they were washed with the wash solution for 3 times. Then 100 µl dilution conjugate 1x was added to each microwell and after re-incubation of them for 30±3 minutes at the room temperature, each well was washed again for 3 times with the wash solution. In the next step,

100 µl of the substrate solution was added to all microwells and then was incubated for 15 min ± 2 min in the dark. After taking microwells out, 100 µl of the stop solution was added to each well and as a final point they were read at 450 nm by ELISA reader. The results were interpreted according to the manufacture's instruction by calculating S/P value (Table 1).

Table 1. Interpretation of *T.gondii* IgG antibody base on instruction of ELIZA kit.

Results	Grade
S/P 40%	Negative
40% < S/P < 50%	Doubtful
50% S/P < 200%	Positive

SPSS software (version 20) was used for statistical analysis. Measured data were expressed as mean ±standard deviation. P value of < 0.05 was taken to be significant.

3. Results

T.gondii antibody has been detected in 31 out of 40 (77.5%) serum samples in positive range. Most positive jackals' sera belong to older jackals (Table 2). The *T. gondii* seropositivity was 77.7% and 77.2% in female and male jackals, respectively (P > 0.05) (Table 2).

4. Discussion

51 parasites reported from human beings in Iran that are common between man and animals and one of the most prevalent of them is *T.gondii* (Eslami et al., 2005). Study on the *T. gondii* infection of humans and domestic animals has been the subject of several surveys in Iran, detecting prevalence rate ranging from 22.4% to 77.7% in different dogs' population according to the region and also highest humans toxoplasmosis prevalence (70%) rate in humid regions of North of Iran (Hoseinnejad et al., 2011; Shadfar et al., 2012, Mostafavi et al., 2012). Higher prevalence rate of toxoplasmosis in warm and moist areas of North Iran compared to cold and dry ones is attributed to the lengthening of the *T. gondii* oocysts viability in moist or humid environments (Mostafavi et al., 2012, Afonso et al., 2006)

Among 29 species of wild carnivores detected in Iran, golden jackals are one of the most abundant species that are characterized by extremely high adaptability with a variety of habitats and human proximity (Ziaie, 2006). Therefore, they are a potential source for producing parasitic infections in farm animals and human. Zoonotic parasites such as *T. gondii* with wildlife reservoirs represent a major public health problem (Eslami et al., 2005).

In Khuzestan, south west of Iran, *Heterophyes hetrophyes* was collected from both jackals and human revealing *Heterophyes hetrophyes* movement between jackals and humans (Massoudi et al., 1981).

Despite important role that jackals can play in *T.gondii* distribution, unfortunately, researches have been done for investigating jackals' *T.gondii* seropositivity in the world and Iran is limited and to our knowledge there are just two surveys about *T.gondii* infection of Jackals worldwide. The first time *T. gondii* antibody was detected in 1 out of 3 serum samples of jackals through latex agglutination test by Ghorbani and his colleagues in Iran (Ghorbani et al., 1983).

Second study was done by Takcs and his colleagues in Hungary. In this study *T. gondii*-type oocysts have been detected in 5% feces of 12 sampled jackals, highlighting important role of jackals in life cycle of *T. gondii* (Takcs et al., 2014)

In view of availability of limited data about *T. gondii* infection of jackals in the world, in this study wild canids considered as the same population, to compare data about *T. gondii* seropositivity of wild canids with current results simultaneously.

Table 2. *T.gondii* antibody in golden jackals regarding age, and sex

Analyzed factor	No. Tested	No. Positive	Percent of positive
Age group	A: 4	A: 1	A: 25%
	B: 25	B: 20	B: 80%
	C: 11	C: 10	C: 90.9%
Sex	Male:22	Male:17	Male:77.2%
	Female:18	Female:14	Female:77.7%
Total	40	31	77.5%

A: ≥1 year, **B:** 1-4 year**, C:** 4-10 ≤ year

Considering positive serum occurrence in 22.4% to 77.7% of different population of dogs in Iran, the results of this study indicate a rather high occurrence of *T. gondii* seropositivity (77.5%) in jackal's population in Golestan province (Hoseinnejad et al., 2011; Shadfar et al., 2012).

There are numerous reports on *T.gondii* seroprevalence in red foxes, with reports of seroprevalence of 56% of 206 red foxes in Ireland, 68% of 337 red foxes in Hungary, 20% of Austrian foxes and 35% of British foxes (Jakubek et al., 2007; Murphy et al., 2007; Hamilton et al., 2005; Wanha et al., 2005). Highest exposure rate of wild canids to *T.gondii* belonged to red foxes in Belgium and USA with seroprevalences of 85% and 98% respectively (Buxton et al., 1997; Dubey et al., 1999). 46.9% of 32 of wolves and 9% of 125 of wolves (Canis lupus) were *T. gondii* seropositive in Alaska and USA respectively (Zarnke et al., 2000). Using an immunofluorescence antibody test (IFAT), Sedla´k and Ba´rtova´ found *T.gondii* antibody in two of 10 captive wolves in Czech Republic (Sedla´k and Ba´rtova, 2006). Using an ELISA, Philippa et al. did not find *T.gondii* antibody in nine wolves from Canada (Philippa et al. 2004).

Seroprevalence for *T.gondii* was strongly related with age, with rate significantly higher in older adult jackals (90.9%) when compared to younger animals in this study.

The significant difference in antibody prevalence between age classes is a novel finding in jackals. Age of sampled wild canids such as foxes and jackals was not recorded in most other studies even though age differences were observed in dogs' population (Song-MinG, 2011). Similar results have been recorded in red foxes of Spain and Brazilian wolves too (Sobrino et al., 2007; Vitalino et al., 2004). These results suggest that the longer an animal lives the more likely it is to contact with the parasite.

T. gondii seropositivity was similar between male and female sampled jackals. Same result has been documented in dogs and it is said that sexuality is not crucial factor on *T.gondii* seropositivity but the authors couldn't find any related documented data about the role of sexuality on *T.gondii* seropositivity in wild canids.

The presence of specific *T. gondii* antibody in jackals is a sign that *T.gondii* is present in the environment and also emphasizes the danger of *T. gondii* infection in farm animals and humans.

In order to implement appropriate control and preventative strategies, it is important that efforts continue to be made to identify domestic and wild animal reservoirs.

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Refereces

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