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Toxoplasma gondii serosurvey in domestic ducks (*Anas platyrhynchos domesticus*) and long-legged buzzard (*Buteo rufinus*) Golestan Province, North of Iran

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ABSTRACT

One of the most prevalent zoonotic diseases is toxoplasmosis which is caused by an obligate intracellular parasite, *Toxoplasma gondii*, with a high global dissemination. Some species of birds are susceptible to *T. gondii* infection. To indirectly survey on *T. gondii* contamination of the rural and wild ecosystem of the Golestan Province, the presence of *T. gondii* antibody was examined in serum sample of 60 domestic rural ducks (*Anas platyrhynchos domesticus*) and 40 long-legged buzzard (*Buteo rufinus*) by MAT. Results: Out of 60 (32=male, 28=female) domestic ducks' serums, 38 (63.3%) samples (20 male and 18 female) were diagnosed positive. There was no significant difference in the prevalence of anti-*T. gondii* antibody in male (60.6%) and female (62%) ducks. Anti *T. gondii* antibody frequency was detected in 77.5% (31 cases) of the sampled long-legged buzzards. Anti *T. gondii* antibody frequency in long-legged buzzard was significantly higher than domestic ducks. Conclusion: High seroprevalence of *T. gondii* infection in long-legged buzzards indicates high risk of *T. gondii* infection in rural and wild ecosystems of Golestan Province. Besides, the high levels of *T. gondii* contamination of sampled domestic duck highlights the importance of villagers' knowledge improvement about the important role of wild and domestic birds in the life cycle of *T. gondii*.

1. Introduction

Zoonotic diseases are considered the most important health problem in many countries. One of these diseases is toxoplasmosis, which is caused by an obligate intracellular parasite, *Toxoplasma gondii*, with high global dissemination. *T. gondii* contamination has been documented in scientific reports higher than other zoonotic diseases in susceptible animals worldwide (Dubey and Beattie, 2010).

Felids that repel the resistant *T. gondii* oocysts in the feces are known as the final hosts, and warm-blooded animals, including mammals and birds, are the intermediate hosts (Dubey, 2008). The *T. gondii* transmission to its hosts is mainly through consumption of oocysts contaminated food and water, as well as eating raw or semi-raw meat of the intermediate hosts infected with *T. gondii* cysts (Dubey, 2008).

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Most *T. gondii* contaminated animals do not show any specific signs of toxoplasmosis. However, there is danger of abortion or negative impact on fetus if warm blooded animals' contamination takes place during pregnancy (Dubey, 2010).

It is said that sensitivity of different species of the birds to *T. gondii* contamination is different and *T. gondii* contamination does not cause any specific symptoms in most species of the birds (Dubey, 2010). However, some of them such as turkey, parrot and canary are susceptible to *T. gondii* infection and severe mortalities have been reported due to *T. gondii* infection in these species (Dubey, 2010). Nevertheless, the truth is that studies on the different species of birds that have different diets (carnivores and herbivores) are limited, and the symptoms and severity of toxoplasmosis in most wild species of birds are unknown (Howerth and Rodenrotho, 1989).

There are several ways to detect *T. gondii* contamination in animals. Detection of anti-*T. gondii* antibody in the serum sample indicates the level of contamination with *T. gondii* in the lifetime of the animal. Among the various serological tests for detection of anti-*T. gondii* antibody, the modified-agglutination test (MAT) is one of the simplest tests with high specificity and sensitivity (Dubey, 2002).

Studies on the seroprevalence of *T. gondii* in different species of animals in Iran indicated high *T. gondii* contamination of sampled animals, especially in the northern regions of Iran (Behineh et al., 2017; Namroodi et al., 2016; Namroodi et al., 2015). Most regions of Golestan Province, in North Iran, have moderate and humid climate condition. These kind of climatic conditions help to the survival of *T. gondii* oocysts and also led to the presence of many wild and domestic birds (in rural areas) in Golestan Province (Sharbati, 2012). As felids' food, wild and domestic birds play an important role in the life cycle of *T. gondii* (Dubey, 2002).

Long-legged buzzards (*Buteo rufinus*) are a common wild raptor bird species with a carnivorous diet in forests and plains of Golestan Province. Breeding and meat consumption of domesticated duck (*Anas platyrhynchos domesticus*), an omnivorous species, is common in the rural areas of the Golestan Province (Porter, 2010).

As wild carnivorous birds are food for wild carnivorous species, the level of *T. gondii* contamination of wild carnivorous birds such as long-legged buzzard can be indicative of the *T. gondii* contamination level of many wild carnivorous species inhabiting in the wild ecosystem of Golestan Province.

Also, *T. gondii* contamination rate of omnivorous birds such as domestic-rural birds will indirectly indicate the *T. gondii* contamination of the rural ecosystem.

To indirectly survey on, *T. gondii* contamination of the rural and wild ecosystem of Golestan Province, the presence of *T. gondii* antibody was examined in serums sample of domesticated rural ducks and long-legged buzzard by MAT.

2. Materials and Methods

2.1. Sampling

In this study (2016 to 2018) 60 free living domestic ducks (*Anas platyrhynchos domesticus*), from 10 villages with similar climatic conditions (humidity and temperate), and 40 long-legged buzzard (*Buteo rufinus*), which were referred to the Wildlife Clinic of the Environment Office of the Golestan Province, were sampled. After recording bird's characteristics such as sex (domestic ducks), time and place of sampling and applying appropriate physical restraint, blood samples were collected from the wings vein.

2.2. Micro-agglutination test

After blood centrifugation and serum separation, anti-*T. gondii* antibody was detected using MAT. Suspension of killed *T. gondii* tachyzoites (prepared by the Pasteur Vaccination Institute) was used as antigen and the serum sample was mixed with 2-mercaptoethanol to remove non-specific agglutinins. Dilutions equal or above 1.25 were considered as positive titers (Dubey et al., 2010). The results were analyzed using SPSS (20) and Chi square test.

3. Results

Out of 60 (32=male, 28=female) domestic ducks' serums, 38 (63.3%) samples (20 male and 18 female) were diagnosed positive. There was no significant difference in the prevalence of

anti-*T. gondii* antibody in male (60.6%) and female (62%) ducks. Anti-*T. gondii* antibody was detected in 77.5% (31 cases) of the sampled long-legged buzzard. Anti-*T. gondii* antibody frequency in long-legged buzzard was significantly higher than domestic ducks.

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4. Discussion

As expected, similar to most studies conducted in Iran and Golestan Province, the results of this study also indicate that both long-legged buzzards and domestic ducks are faced with high risk of *T. gondii* infection (Mostafavi et al., 2012). There is no information about the rate of *T. gondii* infection mortality in long-legged buzzards.

The results of limited studies indicate that toxoplasmosis has been associated with severe clinical symptoms in domestic and wild ducks (Howerth and Rodenrotho, 1989). Considering that toxoplasmosis may cause death of some long-legged buzzards and ducks, which results in the removal of dead animals from the studied population, higher incidence of *T. gondii* antibody frequency in studied birds' population seems possible.

Sampled ducks can be contaminated with *T.gondii* in two ways (consumption of *T. gondii* cysts contaminated tissues and *T.gondii* oocysts) But sampled long-legged buzzards, as carnivorous species, can be mostly faced with *T.gondii* through consumption of cysts infected meats. So it can be imagine that *T.gondii* contamination rate should be higher in omnivorous birds than carnivorous birds. But our results were opposed to the mentioned theory.

The high rate of *T. gondii* contamination in wild rodents, which are the dominant food of the long-legged buzzards, can be one explanation for detection of higher frequency of *T.gondii* contamination in sampled buzzards than ducks (Porter, 2011). Also use of clean and prepared foods for domesticated ducks by breeders can be another reason for the detected difference in the rate of *T. gondii* contamination between sampled birds (Matsuo et al., 2014).

The results of the previous studies indicate that the growth conditions and nutrition status of the birds play very important roles in *T. gondii* contamination. For example the lowest (0%) *T. gondii* infection rate has been reported in the chicken bred in closed system (Matsuo et al., 2014) and the highest (100%) infection rate was reported in chicken have been raised in open areas (Dubey et al., 2010).

Sex determination is not easily diagnosable in many species of wild birds such as long legged buzzards. Therefore, the effect of sex on *T.gondii* infection rate has been studied in a limited number of similar studies. In this study, similar frequency of *T.gondii* antibody was detected between male and female ducks. The result of the Ayinmode and his colleagues' study on broiler chicks was similar to that of the present study (Ayinmode et al., 2014).

The results of similar studies, with different diagnostic methods, on different species of birds have beenrevealed diverse contamination frequencies, fluctuating from 0.01% to 71.3% (Dubey, 2002).

For example, in studies conducted on the Anseriformes birds in Kazakhstan and Czech Republic, 1976 to 1992, *T.gondii* tissue cysts were detected in 1.1% to 28% of sampled birds (Literák et al., 1992; Pak et al., 1976).

In a study conducted in Pakistan on ducks, anti-*T gondii* antibody detected in 12% of sampled ducks (Sadia et al., 2012). *T gondii* infection of 5.7% of the surveyed ducks has been reported in Germany by use of ELISA kit (Maksimov et al., 2012). In a study conducted by El-massy et al. on ducks in Egypt, high serologic *T. gondii* contamination of ducks (50%) was reported (El-Massry et al., 2000). Alkhaled and colleagues also reported high *T gondii* contamination frequency (56%) in ducks in Iraq (Alkhaled et al., 2012). Two studies on ducks in Iran showed that 50% and 15.4% of sampled ducks were *T gondii* contaminated (Ghorbani et al.,1990; Zia et al., 2012). Comparing to other studies conducted in Iran and other parts of the world, it seems that *T gondii* contamination frequency of domestic ducks in current study is at the highest level. Such a result points to high contamination of the ecosystem of sampled villages in Golestan Province.

Unfortunately, there is not any study on *T. gondii* infection rates of raptors and carnivorous birds in Iran. Also, similar studies in other parts of the world are limited. Results of these limited studies, with the prevalence range from 8.1% to 100%, have shown very different outcomes (Literák et al., 1992; Pak et al., 1976).

In study of Litrake et al. (1992), the *T. gondii* contamination of two species of hawks in the United States were reported 41.1% and 66.7%. In a survey on serum samples of 240 eagles in Nigeria using MAT detected anti-*T. gondii* antibody detected in 64.8% of serum samples (Ayinmode et al., 2014). *T.gondii* contamination has been detected in 50% of wild carnivorous birds by Lopes, et.al. in Portugal (Lopes etal., 2012).

Studies on buzzards are very limited, and in fact the present study is the first study in the world on long-legged buzzard. One of the lowest reported rates for tissue *T. gondii* contamination of raptor birds reported by Pak, et.al. in Kazakhstan. In this study, that is the first study on buzzards, *T. gondii* tissues contamination rate was reported 8.3%, which is very different with the result obtained in this study. In the second study on common buzzards, in Czech Republic, low *T. gondii* tissues contamination rate (8.1%) has been reported as well (Literák et al., 1992). There are many factors that can affect results of similar studies on birds' population.

It seems that the density of cats' population in the studied area, the suitability of the weather conditions in the sampled areas for the survival of the oocyst, *T. gondii* identification test and the bird diet, all of which are factors that can affect the degree of similarity and difference between the results of various studies on *T.gondii* contamination of birds.

Wild rats are the dominant food for long-legged buzzards, and also these birds are the food for wild carnivorous species in the ecosystem of Golestan Province. So, high *T. gondii* contamination of sampled long-legged buzzards indicates high risk of *T. gondii* infection in wild rats and also wild carnivorous species of Golestan Province

Considering the consumption of domestic duck' meat and the high numbers of stray cats in Golestan Province, the high levels of *T. gondii* contamination of sampled domestic ducks, highlight the significance of growth in the villagers' knowledge about the important role of birds and cats in the life cycle of *T. gondii*.

The results of the recent study provide only basic information about *T.gondii* infection of birds and there is still no information on *T. gondii* mortality in long-legged buzzard and many wild species of birds in the Golestan Province. So, to understand the epidemiological condition of toxoplasmosis in the population of carnivorous and omnivore's birds in Golestan Province, more detailed comprehensive studies are needed.

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Refereces

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