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Prevalence of *Toxoplasma gondii* and *Neospora caninum* contaminations in poultry eggs: molecular surveillance in three different geographical regions of Iran

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Abstract

Background *Toxoplasma gondii* and *Neospora caninum* are important protozoan parasites with worldwide distribution among warm-blooded animals. Moreover, *T. gondii* is a zoonotic parasite that infects humans. Poultries are important intermediated hosts of *T. gondii* and *N. caninum*. However, little is known about the contamination of poultry eggs with these parasites. We aimed to investigate the molecular frequency of *T. gondii* and *N. caninum* among the eggs of chicken, domestic duck, and quail from three different geographic regions of Iran. *T. gondii* and *N. caninum* were detected by PCR targeting the *RE* and *Nc5* genes, respectively.

Findings Overall contamination rates with *T. gondii* and *N. caninum* were 10.7 and 5.9%, respectively. The overall contamination rates of *T. gondii* among chicken, duck, and quail were 12.2, 15.5, and 4.4%, respectively; while *N. caninum* was detected in 11.1, 3.3, and 1.1% of the same samples, respectively. The contamination rates were increased with increasing humidity across three different regions.

Conclusions Taken together, this study indicates the contamination of poultry eggs with *T. gondii* and *N. caninum*. The possibility of egg-born transmission of *T. gondii* should not be neglected by consuming raw soft-boiled eggs. Furthermore, contamination of poultry eggs could be an indicator for environmental contamination by these parasites.

Keywords *Toxoplasma gondii*, *Neospora caninum*, Poultry, Eggs, Humidity, Chickens, Ducks, Quails

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Introduction

Toxoplasma gondii and *Neospora caninum* are two closely related cyst-forming protozoan parasites with worldwide distribution among warm-blooded animals (de Barros et al. 2018; Dubey and Schares 2011a; Frenkel and Smith 2003a; Schlüter et al. 2014). Poultries are among the most important intermediate hosts for *T. gondii* (Dubey 2002; Dubey et al. 2020) and *N. caninum* (de Barros et al. 2018; Dubey and Schares 2011a). However, little is known about contamination poultry eggs by *T. gondii* and *N. caninum*. The objective of this study was to investigate the molecular detection of *T. gondii* and *N. caninum* in eggs of chicken (*Gallus domesticus*), domestic ducks (*Anas platyrhynchos*), and quails (*Coturnix coturnix*) from three different geographic regions of Iran.

Results

As shown in Table 1, *T. gondii* and *N. caninum* were detected in 29 (10.7%) and 16 (5.9%) out of 270 samples. Regarding the sampling site, the total frequency of *T. gondii* and *N. caninum* DNAs were higher in Astara (16.6 and 7.7%, respectively) than Kermanshah (11.1 and 5.5%) and Jahrom (4.4 and 3.3%). Regarding the poultry species, the total frequencies of *T. gondii* among chicken, duck, and quail were 12.2, 15.5, and 4.4%, respectively; while, the total frequencies of *N. caninum* were 11.1, 3.3, and 1.1%, respectively (Table 1). We did not detect any co-contamination of *T. gondii* and *N. caninum* in the samples.

Discussion

Both *T. gondii* and *N. caninum* are important protozoan parasites that infect a wide variety of warm-blooded animals, including bird species. However, *T. gondii* is more important because of their zoonotic potential for human health. Poultries could be an important reservoir of these parasites for their final host (Dubey and Schares 2011b; Schlüter et al. 2014). Indeed, humans can become infected by *T. gondii* by consuming undercooked avian tissues, including chickens and ducks (Dubey 2010; Dubey et al. 2020). Experimental studies revealed that chicken embryonated eggs could be an experimental model for *T. gondii* (Setasimy and Namavari 2016; Zuckerman 1966) and *N. caninum* (Furuta et al. 2007; Mansourian et al. 2009). However, in earlier reports with parasitological methods, only a few eggs had viable *T. gondii* following experimental infection of hens (Biancifiori et al. 1986; Boch et al. 1966; Dubey 2010; Jacobs and Melton 1966; Sokolov 1970), and there are no available data regarding *N. caninum* in this regard. *T. gondii* has been isolated from different organs of naturally infected hens, including ovaries and oviducts (Foster et al. 1969; Jacobs and Melton 1966); hence, the route of egg contamination may originate from ovaries and oviducts. Although we only detected the parasite DNA in avian eggs and did not perform methods for isolation of viable parasites (e.g., mouse bioassay), the possibility of egg-born transmission of these parasites should not be neglected. Contamination of avian eggs could also be an indicator for their host infection by *N. caninum* and *T. gondii*. Hence, detection of these parasites in avian eggs might be an alternative method for assessment of infection in their host. Inasmuch as the parasite oocysts are prevalent in the environment (Maleki et al. 2021), poultry could get the infection be feeding in the contaminated environment, therefore, infection of poultry might represent environmental contamination with the parasite's oocyst. Likewise, the feline and canine definitive host of the parasites could get the infection by eating the infected intermediated hosts of the parasites.

In the current study, the total frequencies of *T. gondii* and *N. caninum* were 10.7 and 5.9% in three geographic regions of Iran. In our previous study, we detected *T. gondii* DNA by PCR method among 11% of chicken eggs in the south of Iran (Khademi et al. 2018). Both *T. gondii* and *N. caninum* have been detected among chickens and ducks in a number of studies (de Barros et al. 2018; Dubey 2010; Dubey and Schares 2011a; Dubey et al.

Table 1 Detection of *T. gondii* and *N. caninum* by PCR in eggs of chicken, quail, and duck from three regions of Iran

Sampling site	Parasite	Chicken	Duck	Quail	Total
Astara *N* = 90 (30 samples from each bird species)	*T. gondii*	5/30 (16.6%)	7/30 (23.3%)	3/30 (10%)	15/90 (16.6)
	N. caninum	4/30 (13.3%)	2/30 (6.6%)	1/30 (3.3%)	7/90 (7.7%)
Kermanshah N = 90 (30 samples from each bird species)	*T. gondii*	4/30 (13.3%)	5/30 (16.6%)	1/30 (3.3%)	10/90 (11.1%)
	N. caninum	3/30 (10%)	1/30 (3.3%)	0/30 (0%)	4/90 (4.4%)
Jahrom N = 90 (30 samples from each bird species)	*T. gondii*	2/30 (6.6%)	2/30 (6.6%)	0/30 (0%)	4/90 (4.4%)
	N. caninum	3/30 (10%)	0/30 (0%)	0/30 (0%)	3/90 (3.3%)
Total	*T. gondii*	11/90 (12.2%)	14/90 (15.5%)	4/90 (4.4%)	29/270 (10.7%)
	N. caninum	10/90 (11.1%)	3/90 (3.3%)	1/90 (1.1%)	16/270 (5.9%)

2020). In our study, contamination with *T. gondii* was detected in 12.2, 15.5, and 4.4% of chicken, duck, and quail, respectively. Studies indicate that chickens are resistant to toxoplasmosis, but there are few reports on confirmed clinical toxoplasmosis in chickens (Reviewed by (Dubey 2010)). Among the poultry species, quail eggs had the lowest contamination rate of both *T. gondii* and *N. caninum* in our study (4.4% vs. 1.1%, respectively). In two previous studies in China, the molecular frequency and seroprevalence rates of *T. gondii* among quails that intended for human consumption were reported to be 6.41% (Cong et al. 2017b) and 9.52% (Cong et al. 2017a), respectively. However, we only detected *N. caninum* DNA in one (1.1%) out of 90 quail eggs. Data regarding the prevalence of *N. caninum* among quails are scarce; nevertheless, an experimental study revealed that quails are resistant to infection with *N. caninum* (Oliveira et al. 2013).

Climate conditions are important factors for maintaining the parasite oocysts in the environment. While a relatively mild climate with humid conditions provides a suitable circumstance for oocysts to be active longer in the soil (Maleki et al. 2021). Hence, exposure and infection of animals in the regions with humid conditions are increased. Consequently, the biological transmission rates of these parasites could be increased in this condition. The higher prevalence of *T. gondii* and *N. caninum* in Astara County probably related to the mild temperature and higher humidity of this region than the two other sampling sites. A relatively mild temperature and higher humidity favor the survival and sporulation of coccidian oocysts, resulting in an increased risk of infection in human and animals (Dubey et al. 2007). The results of the current study have shown that the overall prevalence of *T. gondii* and *N. caninum* was higher in Astara county (which has a humid subtropical climate with an average relative humidity of 82%) than Kermanshah province (which has a Mediterranean climate with an average relative humidity of 49%) and Jahrom county (which has a hot semi-arid climate with an average relative humidity of 46%). Our recent meta-analysis revealed that increasing relative humidity (≥76%) is associated with an increasing trend in the prevalence of *T. gondii* oocysts in the soil of public places (Maleki et al. 2021). In our previous study (Khademi et al. 2021), we assessed the molecular prevalence of *T. gondii* in slaughtered sheep in two high- and low-humidity regions in the south of Iran (Hormozgan Province). The results revealed that the rate of *T. gondii* was 10.7% of the samples from the highly humid region, while no positive samples were detected in the low-humidity region. Our another investigation in the same region of Iran was performed the molecular detection of *T. gondii* in chicken eggs (Khademi et al. 2018). The results demonstrated that *T. gondii* was positive among 11% of the chicken eggs in the area with hot and humid weather; whereas, no *T. gondii* was detected in the chicken eggs in the area with warm and dry weather (Khademi et al. 2018). Moreover, our recent reports among pregnant women in the same region of Iran (Hormozgan Province) revealed that pregnant women are at high risk of congenital toxoplasmosis (Khademi et al. 2019; Khademi et al. 2022).

Conclusions

Our study indicates contamination of chickens, ducks, and quails with *T. gondii* and *N. caninum* in three different geographic regions of Iran. Our other findings revealed that increasing humidity was associated with increased prevalence of *T. gondii* and *N. caninum* contamination in the eggs of chickens, ducks, and quails. Although we did not perform methods for detection of viable *T. gondii* and *N. caninum* (e.g., mouse bioassay), the possibility of egg-born transmission of *T. gondii* should not be neglected by consuming raw soft-boiled eggs. Moreover, contamination of poultry eggs could be an indicator for environmental contamination by these parasites.

Materials and methods

Study area and sample collection

The samples were purchased from local markets in three different geographic regions of Iran (Fig. 1), including Astara county (north of Iran, border of Caspian Sea), Kermanshah province (western Iran), and Jahrom (nearly south of Iran). We selected two local markets in each region. We selected those local markets that sold traditional and local foods. The eggs came from small farms in rural areas to the markets for selling. A total of 270 eggs of chicken, quail, and duck (30 eggs from each bird from each city) was purchased during the spring and summer. Figure 1 represents the climate map of Iran and the climate conditions of each sampling site.

Sample preparation, DNA extraction, and PCR

The egg shells were washed individually before separation of the albumen and yolk. Then, albumen and yolk of each egg were completely mixed (Khademi et al. 2018) and DNA extraction was performed using the phenol–chloroform extraction method as described (Abdoli et al. 2015). Tachyzoites of the *RH* and *Nc5* strains of *T. gondii* and *N. caninum* were used as positive control for DNA extraction. Molecular detection of *T. gondii* was performed by primers targeting the repetitive element (*RE*) gene (Homan et al. 2000) as described in our previous reports (Abdoli et al. 2016; Khademi et al. 2018; Khademi et al. 2021; Rasti et al.

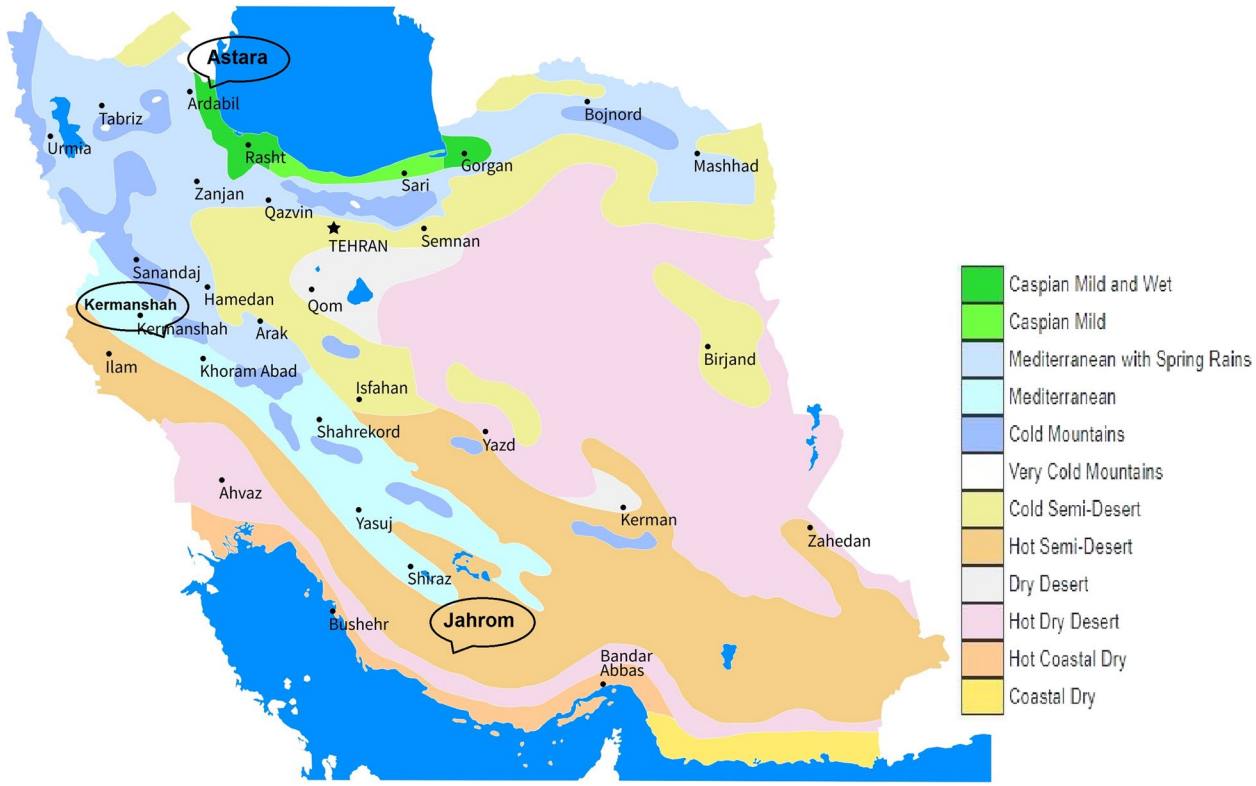

Fig. 1 The climate map of Iran. https://commons.wikimedia.org/w/index.php?title=File:Iran-climate.png&oldid=224111575. The samples were taken from Astara (north of Iran, border of Caspian Sea), Kermanshah province in western Iran, and Jahrom in nearly south of Iran. Astara: Coordinates: 38°25′45″N 48°52′19″E. Astara county has a humid subtropical climate with relatively cold, wet winters and hot, humid summers. This county has an average rainfall of 1292.5 mm per year, average rainy days (≥ 1.0 mm) of 112 days, and average relative humidity (%) of 82% (https://en.wikipedia.org/wiki/Astara,_Iran. Kermanshah province: Coordinates: 34°18′51″N 47°03′54″E. Kermanshah classified as Mediterranean climate, experiences rather cold winters and a hot-summer. Kermanshah has an average rainfall of 478.7 mm per year, average rainy days (≥ 1.0 mm) of 77.3 days, and average relative humidity (%) of 49% (https://en.wikipedia.org/wiki/Kermanshah). Jahrom: Coordinates: 28°30′00″N 53°33′38″E. Jahrom county has a hot semi-arid climate. The average rainfall is about 285 mm (11.2 in) per year. The average rainy days (≥ 1.0 mm) of 27 days, and average relative humidity (%) of 46% (https://en.wikipedia.org/wiki/Jahrom)

2018). Molecular detection of *N. caninum* was performed by nested-PCR method using specific primers that amplified the *Nc5* gene (Hughes et al. 2006) as described in our previous reports (Abdoli et al. 2015; Arbabi et al. 2016). The primer sequences and PCR cycling conditions are presented in Supplementary Tables 1 and 2. The DNAs were amplified in a 20 µl reaction mixture containing 3 µL of template DNA, 10 µl of commercial master mix containing 2x Taq DNA polymerase with 1.5 mM MgCl2 (Cat. no. A170301, Ampliqon, Denmark), 5 µL of distilled water, and 10 pmol of each primer. For nested PCR of the *NC5* gene, the same conditions were performed, except for the use of 25 pmol of primers for nested-PCR reaction (Hughes et al. 2006) and using one µL of one-tenth diluted of the first round PCR product as the template for nested-PCR. In each reaction, a positive control and a negative control (double distilled water) were included. PCR products were analyzed on 2% agarose gels stained with Safe stain (Sinaclon, Iran) that electrophoresed in Tris-acetate-EDTA (TAE) buffer and evaluated under ultraviolet transillumination.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40550-023-00103-1.

Additional file 1: Supplementary Table 1. *T. gondii* and *N. caninum* specific primers targeting the *RE* and *Nc5* genes. **Supplementary Table 2.** PCR conditions of the *Nc5* and *RE* genes.

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Authors' contributions
AA conceived the study. SM, MAB, AB, HGB, and FB were obtained the samples. HM, and AA performed laboratory tests. KS, SZK, MS and AA analyzed the results, designed the tables and fig. AA wrote the draft of the manuscript. AA and FG supervised the study. All authors approved the final manuscript for submission.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
This study was approved by the research and ethics committee of the Jahrom University of Medical Sciences, Jahrom, Iran (the ethics code: IR.JUMS.REC.1398.061).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest.

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