First report on seroprevalence and risk factors of *Toxoplasma gondii* on some traditional poultry farms in north central Algeria

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Abstract

Toxoplasma gondii is an obligate intracellular protozoan parasite which affects humans and a wide range of animals. The aim of this study was to investigate the seroprevalence of *T. gondii* in domestic birds from family farms in north central Algeria. Blood samples of 121 chickens, 14 geese and 7 ducks were collected and examined for the presence of *T. gondii* antibodies by MAT. Sera were diluted 2-fold serially from 1:20 to 1:160. Titers equal or higher than 20 were considered positive. Multivariable logistic regressions were used to evaluate risk factors. The overall *T. gondii* seroprevalence in the examined domestic birds was 51% out of which chickens, ducks, and geese seroprevalences were 50%, 57% and 50%, respectively. Female birds were 1.5 times more infected than male birds (OR = 2.52; p = 0.047), and Blida area has 3.4 higher prevalence then Algiers and Médéa areas (OR = 4.42; p = 0.003). The present study revealed that *T. gondii* infection was highly prevalent in chickens, ducks and geese in north central Algeria.

Key words: chickens, geese, ducks, prevalence, modified agglutination test

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Introduction

Toxoplasma gondii (*T. gondii*) is a cosmopolitan obligate intracellular protozoan affecting approximately 30% of the populations worldwide and a wide range of animals (Dubey, 2010; Zhang et *al*., 2014; Tan et *al*, 2016; Spalenka et *al.*, 2017). Cats and other wild felids are the only definitive hosts for the parasite and shed the environmentally-resistant oocytes (Hill &Dubey, 2018). Almost all warm-blooded species and birds including humans are intermediate hosts (Dubey, 2010) who become infected by ingesting food or water contaminated with the oocytes excreted by infected felids or by consumption of undercooked meat containing parasite tissue cysts (Dubey, 2014). The prevalence of *T. gondii* in domestic birds like chickens and ducks is a good indicator of environmental contamination with *T. gondii* oocytes owing to their feeding behaviors (El-Massry et al., 2000; Bártová et al., 2009).

Several studies have been carried out on *T. gondii* infections in various bird species, including domestic species. Their seroprevalences were summarized by Dubey (2010) and more recently by Rouatbi et al. (2019). However, there is no information about *T. gondii* infection in poultry in Algeria. Therefore, the investigation of the seroprevalence of *T. gondii* in chickens, ducks and geese on the family farms in north central Algeria was undertaken, and risk factors associated with this infection were estimated.

Material and methods

Study areas

Our study was carried out on the family farms in three regions of north central Algeria. They are from north to south: Algiers (36° 46′ 34″ North, 3° 03′ 36″ East), Blida (36° 29′ 00″ North, 2° 50′ 00″ East) and Medea (36° 16′ 03″ North, 2° 45′ 00″ East). Algiers is the capital of Algeria, and Blida and Algiers are located in the plain of Mitidja. Médéa is a mountainous region located at 981 m altitude. The north central region receives on average annual amounts of rainfall ranging between 600 and 1150 mm (National Office of Meteorology, 2019).

Blood samples

Blood samples of 142 domestic birds were collected and examined for the presence of *T. gondii* antibodies. The blood was centrifuged and the sera were stored at -20 °C until assayed. Sera of 121 chickens, 14 geese and 7 ducks were tested using the modified agglutination test (MAT).

Serological assay

Antibodies to *T. gondii* were detected in the sera by MAT using a commercial kit: “Toxoscreen DA” (bio-Merieux, France). Sera were diluted 2-fold serially from 1:20 to 1:160. Titers equal or greater than 20 are considered positive.

Statistical analyses

Statistical analyses were done using R (version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria) via RStudio (version 1.1.383, RStudio Inc., Boston, MA). In order to incorporate into account, the sensitivity (Se) and specificity (Sp) of the diagnostic assay, the 'epi.prev' function in epiR package (Stevenson et al., 2013) was used to adjust the prevalence estimate. Associations between individual bird serostatus and independent variables were assessed using multivariable logistic regression. The multivariable models were built by manual stepwise backward elimination, and the final multivariable model was chosen using the Akaike's information criteria (AIC). Variables included in the final multivariable model were: bird sex (male vs. female), species (chickens vs. others) and areas (Algiers, Blida and Médéa).

Results

The overall seropositivity of *T. gondii* among the examined domestic birds was 51% (72/142) out of which the ducks seroprevalence (57%) was slightly higher than that of chickens (50%) and geese (50%) (Table 1), but no significant effect of the type of species on odds of being toxoplasma seropositive was observed ($p = 0.62$; Table 2). From 72 seropositive birds, 55 (76%) had titers of ≥ 160 (Table 1). Out of 121 chickens, 61 (50%) were seropositive to *T. gondii*, and obtained titers were 20 in five samples, 40 in four, 80 in one and ≥ 160 in 51 (Table1). *T. gondii* antibodies were found in seven out of 14 (50%) tested geese with titers of 20 in three animals, 40 in two and ≥ 160 in 2 (Table 1). In ducks, antibodies to *T. gondii* were detected in 4 of 7 (57%) samples with titers of 20 in 2 and ≥ 160 in 2 (Table 1).

Table 1. Seroprevalence of *Toxoplasma gondii* infection among chickens, geese and ducks in north central Algeria by the modified agglutination test (MAT)

Animal species	No. tested	No. of positive with anti-*T. gondii*				Total positive
		1:20	1:40	1:80	1:160	
Chicken	121	5	4	1	51	61(50%)
Goose	14	3	2	0	2	7 (50%)
Duck	7	2	0	0	2	4 (57%)
Total	142	10	6	1	55	72 (51%)

Table 2. Multivariate logistic regression analysis of risk factors related to *T. gondii* infection

Variables	Classes	Prevalence (95% CI)	OR	Confidence interval (95%)	*p*
Gender	Male	39.47 (22.58 - 58.32)		Reference	
	Female	47.14 (37.02 - 57.73)	2.52	(1.02 - 6.46)	0.047
Species	Ducks and Geese	53.86 (30.36 - 74.97)		Reference	
	Chicken	51.79 (42.14 - 61.44)	1.28	(0.46 - 3.53)	0.62
Region	Algiers	50.77 (39.66 - 61.87)		Reference	
	Blida	80.95 (63.15 - 93.50)	4.42	(1.72 - 12.59)	**0.003**
	Médéa	9.81 (0.73 - 31.96)	0.10	(0.01 - 0.42)	**0.005**

CI: confidence interval; OR: odds ratio;

Results from the multivariable linear model showed that female birds were 1.5 times more likely to carry toxoplasmosis than male birds (OR= 2.52; *p*= 0.047). Also, birds in Blida area were 3.42 times more likely to suffer toxoplasmosis than birds in Algiers area (OR= 4.42; *p*= 0.003). In contrast, birds living in Médea area were 0.9 time less likely to suffer toxoplasmosis (OR= O.1; *p*= 0.005).

Discussion and conclusions

This is the first study of *T. gondii* focused on domestic birds in Algeria using the MAT technique. The results showed a high *T. gondii* seroprevalence in domestic birds (51%), out of which chickens, ducks and geese seroprevalences were 50%, 57%, 50% respectively. Results from the multivariable linear model showed that there was no significant effect of the type of species on odds of being toxoplasma seropositive.

In Algeria, approximately the same result (50%) was detected in cats in Algiers area (Yekkour et *al*., 2017). In other species studied in Algeria, the seroprevalences of *T. gondii* were considerably variable, ranging from 3 to 28.7% in cattle, from 11.6 to 25.6% in sheep, and from 11.9 to 13.2. in goats (Dechicha et al., 2015, Mohamed-Cherif et al., 2019). In equines, only one study by Mohamed-Cherif et al. (2015) showed seroprevalence of 26% in horses and 30% in donkeys. Such a high seroprevalence in birds may indicate that among all intermediate host species studied, domestic birds are more likely to be infected with *T. gondii* oocytes since they feed on the ground. A study by Liu et *al*. (2017) showed that there was a relationship between the soil contamination and high seroprevalence in free-range chickens.

In the present study, 61 (50%) of 121 chickens were seropositive to *T. gondii* (Table 1). Compared with those found in the countries with the same climate , this result was higher than those reported in Egypt (Dubey et *al*., 2003; Harfoush and Tahoon, 2010; Barakat et *al*., 2012; Ibrahim et *al*., 2016) but lower than that found in Tunisia (Boughattas and Bouratbine, 2014). These variations may be due to different serologic tests used, samples from different regions, age and the type of chickens.

In other countries, higher seroprevalences were reported by Da Silva et *al*. (2003) in Brazil and Liu et *al*. (2017) in China. Lower infection rates were found in many countries like in the USA (Ying et *al*., 2017) and Iran with an overall estimated prevalence of 20% (Shokri et *al*., 2017).

In ducks**,** the seroprevalence (57%) of *T. goondii* was slightly higher than the prevalence reported in Egypt (47.2% and 37.5%) by El-Massry et *al*. (2000) and Harfoush and Tahoon (2010), respectively, and was much higher than that reported by Bártová et *al*. (2009) in the Czech Republic (20%) and Yang et *al*. (2012) in Northeastern China (20%). However, the very small sample size could have influenced these results. The 50% (7/14) infection rate in geese was much higher than those reported in Czech Republic (26%), China (4.7%) and Canada (7.1%) (Bartova et *al*., 2009; Yang et al. 2012; Verma et *al*. 2016). However, such an observation could have also been attributed to the small sample size.

T. gondii infection rate in chickens, ducks and geese varies around the world. It seems that the warm climate and low-lying or humid areas present the risk factors for the spread of infection, and could promote the sporulation of oocytes and their survival in the environment (Dubey, 2010). North central Algeria is located in the temperate zone and characterized by a mild, Mediterranean climate which may be favorable for the survival of *T. gondii* oocysts, and so could explain the high *T. gondii* seroprevalence from our study.

Causes for variations of *T. gondii* seroprevalence in different geographical areas of a country are not yet known but environmental conditions previously mentioned may determine the degree of natural spread of *T. gondii* infection (Dubey, 2010). In this work, birds in Blida area were 3.42 times more infected than birds in Algiers area. In contrast, birds living in Médea area were 0.9 time less prevalent. This may be due to ecological and geographical factors, age of birds and the sample size. Médéa is located at high altitude. This may explain the lower infection rate.

The present survey showed that female birds were 1.5 times more infected than male birds indicating that female birds had more opportunities for contact with *T. gondii* oocytes that may be related to the age of birds. In fact, the examined female birds originate from the family farms, which raise mainly laying birds.

Within the current work, 55 of 72 (76%) seropositive birds have titer of ≥ 160. This is an indication of acute, recent infection, and may reflect a high distribution of the parasite on the farms in the study area.

In conclusion, the results from the present study revealed that *T. gondii* infection was highly prevalent in chickens, ducks and geese in north central Algeria, and indicated a high distribution of the parasite in the study area. Our study showed that *T. gondii* was more prevalent in female than male birds, and in the Blida than Algiers and Médéa areas. The present investigation suggests to apply integrated control strategies to prevent and control *T. gondii* infection in this area.

Therefore, prevention and the public awareness measures should be taken on the modes of transmission of *T. gondii* infection. Another study is needed to assess the impact of the disease on food animal production.

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Prvi izvještaj o seroprevalenci i riziko-faktorima *Toxoplasme gondii* na tradicionalnim farmama peradi u sjevernom i centralnom Alžiru

SAŽETAK

Toxoplasma gondii spada u skupinu obligatornih intracelularnih protozoarnih parazita koji inficiraju ljude i različite životinje. Cilj našeg istraživanja jeste odrediti seroprevalencu *T. gondii* kod domaćih ptica sa porodičnih farmi u sjevernom i centralnom Alžiru. Prikupljeni su uzorci krvi od 121 pileta, 14 gusaka i 7 pataka, a potom testirani na prisustvo antitijela na *T. gondii* MAT tehnikom. Serumi su serijski dvaput razblaženi od 1:20 do 1:160. Titrovi jednaki ili viši od 20 su smatrani pozitivnim. Za procjenu riziko-fakora su korišteni testovi multivarijantne logističke regresije. Ukupna seroprevalenca *T. gondii* kod ispitanih domaćih ptica je iznosila 51%, od *čega* su pojedinačne seroprevalence kod piladi, pataka i gusaka iznosile 50%, 57% i 50%. Ženke ptica su bile 1.5 put češće inficirane od mužjaka (OR = 2.52; p = 0.047), a u području Blida seroprevalenca je bila 3.4 viša nego u područjima Algiers i Médéa (OR = 4.42; p = 0.003). Naše istraživanje je pokazalo visoku prevalencu infekcije sa *T. gondii* kod piladi, pataka i gusaka u sjevernom i centralnom Alžiru.

Ključne riječi: pilad, guske, patke, prevalenca, modificirani aglutinacijski test