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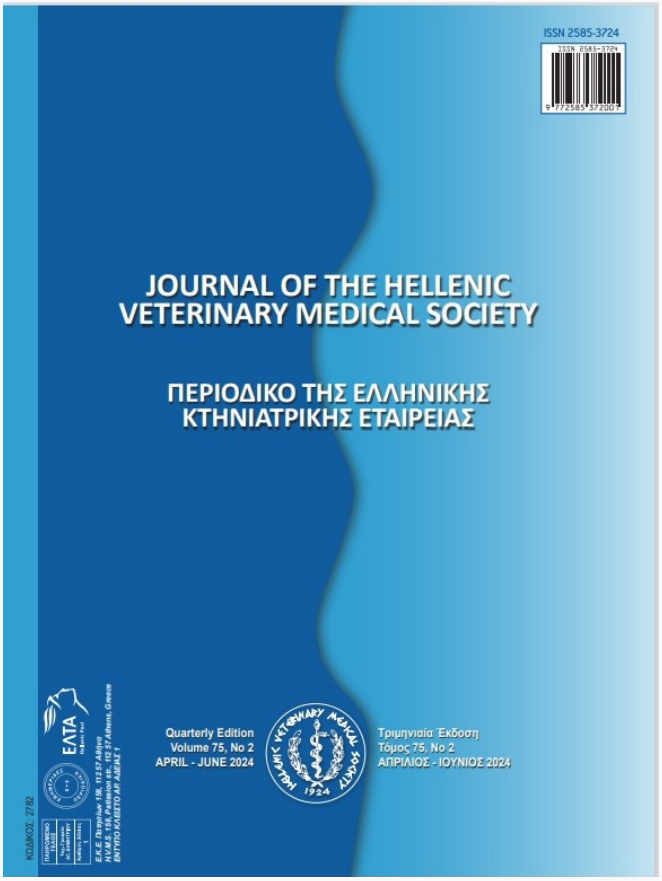

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Research article
Ερευνητικό άρθρο

Detection of anti-*Toxoplasma gondii* antibodies in wild animals from two zoological parks in Algiers, Algeria

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ABSTRACT: Toxoplasmosis is the most frequent parasitic diseases in the world. It is caused by the protozoan *Toxoplasma (T.)gondii* and can affect most warm-blooded animals. However, only felids are considered its definitive hosts. Eighty-six blood samples were collected from different animal species raised in captivity within two Zoological Parks in the Algiers urban area. Sera from eighteen birds and sixty-eight mammals were assayed for the presence of *T. gondii* antibodies by the ELISA test. The overall prevalence of *T.gondii* infection was 58.1% (47. 7 - 68. 6). By ELISA, *T. gondii* antibodies were found in 38.9% (16. 4 - 61. 4) of birds, in 75% (56 - 94) of primates, in 75% (32. 6 - 117. 4) of carnivores, and in 56.8% (42. 2 - 71. 5) of herbivores. Moreover, all seropositive animals were apparently healthy. The results have shown a statistical link between mammary age and the presence of anti-*Toxoplasma gondii* antibodies (Pvalue=0.0002), as well as a significant link between diet and the presence of anti-*Toxoplasma gondii* antibodies in birds (Pvalue=0.0002).This contribution represents the first report of *T.gondii* seroprevalence in captive wild animals from Algiers zoological Parks. The obtained results indicate a widespread exposure of Algerian zoo animals to *T. gondii*.

Keywords: *Toxoplasma gondii*; ELISA; wild birds; wild mammals; zoological parks; Algeria.

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INTRODUCTION

Toxoplasma (T.) gondii, a protozoan parasite, is of significant medical importance as it has the ability to infect a wide range of endothermic vertebrates including farm animals, pets, and wildlife (Hill et al., 2005; Dubey, 2010). *T. gondii* has been associated with morbidity and mortality in various bird and mammal species (Silva et al., 2002). Furthermor, humans can also be infected by this parasite (Tenter et al., 2000). The global prevalence of *T. gondii* infection remains unknown, although estimates suggest that approximately one third of the world's population is infected (Dubey, 2010; Montoya and Liesenfeld, 2004).

Transmission to humans occurs through the ingestion of oocysts shed by cats, the primary host, in their feces, or through the consumption of undercooked or raw meat from infected intermediate hosts such as mammals and birds, as well as congenital transfer (Hill et al., 2005; Petersen et al., 2010). Understanding the transmission dynamics of *T. gondii* between humans and animals in the environment is crucial. While the parasite cycle is well understood in domestic animals and humans, it remains poorly studied in the wild (Wendte et al., 2011; Rendón-Franco et al., 2014).

Zoological parks, characterized by the higher density and intermingling of different animal species, could potentially serve as optimal environments for the transmission of infectious diseases (Morikawa et al., 2014). This knowledge is essential for species conservation and public health. However, in Algeria, there is currently no official monitoring program for the diagnosis of toxoplasmosis in animals, and limited information exists regarding the seroprevalence of *T. gondii* in wildlife.

The aim of this investigation is to determine the circulation of *Toxoplasma gondii* antibodies among captive wild animals in two zoological parks located in the urban area of Algiers. By examining the seroprevalence of *T. gondii* in these settings, this study aims to contribute to our understanding of the parasite's prevalence and transmission dynamics, thus advancing our knowledge in this field.

MATERIALS AND METHODS

Animals surveyed

Blood samples were collected from eighty-six (86) wild animals in two zoological parks, El-Hamma and Ben Aknoun (located at 36° 44′ 53″ N and 3° 04′ 34″ E; 36° 45′ 34″ N and 3° 00′ 33″ E, respectively) situated in the center of Algiers urban area; Fig 1.

Blood was collected from the jugular vein in herbivorous mammals, from the cephalic vein in carnivores, and from the brachial vein in birds, using sterile dry tubes. After coagulation, the samples were

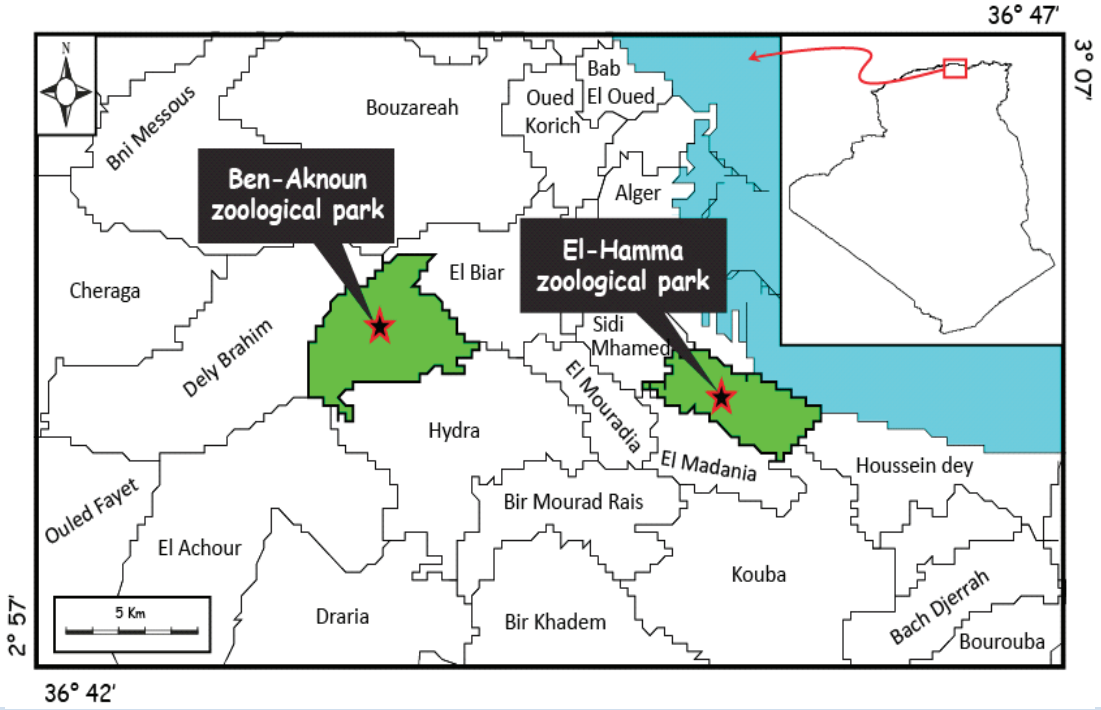

Figure 1: Map of the Algiers wilaya showing El-Hamma and Ben-Aknoun Parks geographic location.

then centrifuged at 2500 rpm in10 minutes to obtain serum, which was stored in a freezer at -20°C until examination.

The studied animal species are as follows: twenty-two (22) carnivores, forty-four (44) herbivores, four (4) primates and eighteen (18) birds, from which Sera were taken. Valuable information was collected through personal interviews with the zoological parks veterinarians. Animals were classified into two groups basing on their ages (young and adult), that was determined according to their breeding history. Furthermore, domestic and stray cats are in free circulation in all zoological parks included in the study.

Serological examination

All sera were examined by the ID Screen® *Toxoplasma gondii* Indirect Multi-species Kits (ID vet) following the manufacturer's recommendations and protocols. Results were expressed as optical density (OD), absorbance was read at 450 nm (wavelength) with an EL-800 ELISA Plate reader (Biotek Instruments Inc., USA).The 96-well plate is coated with P30 *T. gondii* antigen, and the antigen-antibody complex forms with the help of the peroxidase conjugate which is added later. Positive and negative controls were provided by the manufactured and used to validate each test. Samples were considered positive if they had a value ≥ 50%,doubtful for values between 40% and 50%, and negative if value ≤ 40 %. This percentage was calculated as follow;

Percentage of positivity = (100 * OD of the sample/ OD of the PC).

The sensitivity and specificity of this ELISA test 100% and 97.8%, respectively (information provided by the manufacturer).

Statistical analysis

The association between epidemiological data and serology was analyzed using chi-squared test(x^2 test) and the Fisher's exact test, considering the significant level (α) of 5%. All statistical analyses were performed using the statistical software SPPSS version 22.0 (SPSS Inc., Chicago, IL, USA).In all analyses, P values <0.05 were considered as statistically significant.

RESULTS

The obtained results have shown that 50 out of the 86 animals (58.1%) under study tested positive for *Toxoplasma* infection. The prevalence of *T. gondii* infection in the animals tested by ELISA is shown in tables 1 and 2.

The evaluation of the prevalence of *Toxoplasma*

Table 1: Classification of wild mammals investigated for *T. gondii* antibodies according to species, common name, gender and age from Algiers's zoo.

	Family	Scientific name	Common nome	No. of samples	Sex (M/F)	Age (Y/A)	No. of positive
Carnivores	Canidae	*Vulpes vulpes*	Red fox	2	2/0	1/1	1 (AM)
		Canis aureus	golden jackal	1	1/0	0/1	1
		Vulpes zerda	Fennec fox	12	4/8	3/9	8 (6AF+2AM)
	Felidae	*Panthera pardus*	Leopard	1	1/0	0/1	1
		Panthera tigris tigris	Bengal tiger	1	0/1	0/1	1
	Hyaenidae	*Hyaena hyaena*	Striped hyena	1	0/1	0/1	1
	Viverridae	*Genetta genetta*	Commongenet	1	1/0	1/0	1
	Herpestidae	*Herpestes ichneumon*	Egyptian mongoose	1	1/0	0/1	1
Herbivores	Camelidae	*Lama glama*	Llama	1	0/1	0/1	1
	Bovidae	*Boselaphus tragocamelus*	Nilgai	2	2/0	1/1	2
		Capra aegagrus hircus	Dwarf goat	12	8/4	5/7	9 (2YF+2AF+5AM)
		Ovis aries	Black heated sheep	12	3/9	4/8	4 (1YM+3AF)
		Ammotragus lervia	Barbary sheep	1	1/0	0/1	1
		Ovis orientalis musimon	Mouflon	1	0/1	0/1	1
		Gazella leptoceros	Rhim gazelle	2	1/1	0/2	0
		Gazella dorcas	Dorcas gazelle	1	0/1	1/0	0
	Cervidae	*Dama dama*	Fallow deer	9	7/2	3/6	4 (1YF+2AM+ 1AF)
	Equidae	*Equus caballus*	Pony	3	2/1	0/3	3
Primates	Cercopithecidae	*Macaca sylvanus*	Barbary macaque	3	2/1	1/2	2 (1AM+1AF)
		Cercocebus agilis	crested mangabey	1	1/0	0/1	1
Total	**10**	**20**		68	37/31	20/48	43

Table 2: Classification of wild birds investigated for *T. Gondii* antibodies according to species, common name, gender and age from Algiers's zoo.

	Family	Scientific name	Common nome	No. of samples	Sex (M/F)	Age (Y/A)	No. of positive
Omnivorous	Anatidae	*Anas platyrhynchos*	mallard	1	0/1	0/1	0
		Cairina moschata	Muscovy duck	1	0/1	0/1	0
		Anser anser	Greylag goose	3	1/2	3/0	0
	Phasianidae	*Pavo cristatus*	Indian Peafowl	3	1/2	0/3	0
		Phasianus colchicus	Common pheasant	2	1/1	0/2	0
Birds of prey	Accipitridae	*Gyps fulvus*	Griffon vulture	2	1/1	0/2	2
		Neophron percnopterus	Egyptian vulture	2	1/1	0/2	2
		Circaetus gallicus	short-toed snake eagle	1	1/0	0/1	1
		Aquila chrysaetos	Golden eagle	1	1/0	01	1
		Milvus migrans	Black kite	1	1/0	0/1	0
	Strigidae	*Bubo ascalaphus*	Pharaoh eagle-owl	1	1/0	0/1	1
Total	**04**	**11**	**11**	**18**	**9/9**	**3/15**	**7**

The symbols mean as follows: M: male, F: female, A: adult, Y: young.

Table 3: The seroprevalence of *T. gondii* in wild animal families from Algier's Zoo

		Sample size (N)	Seroprevalence %(CI 95%)	X^2-Value	P-Value
mammals	Carnivores	20	75 (56 - 94)	2.208	03315
	Herbivores	44	56. 8 (42. 2 - 71. 5)		
	Primates	4	75 (32. 6 - 117. 4)		
	total	**68**	**63.2 (51.8 - 74.7)**		
Birds	Omnivorous	10	0	14.318	0.0002*
	Birds of prey	8	87. 5 (64. 6 - 110. 4)		
	total	**18**	**38. 9 (16. 4 - 61. 4)**		
Global		**86**	**58. 1 (47. 7 - 68. 6)**		

Table 4: Association between epidemiological data and investigation for *T. gondii* in the studied animals (mammals)

Variable		N	ELISA +	Variable (%); CI 95%	X^2-Value	P-value
Gender	Male	37	22	59.5 (43.6 -75.3)	0.498	0.4805
	Female	31	21	67.7 (51.3-84.2)		
Age	Young	20	6	30.0 (09.9 -51.1)	13.462	0.0002
	Adult	48	37	77.1(65.2 -89.0)		
Total		68	43	63. 2 (51.8-74.7)		

infection in the zoo animals has highlighted the fact that carnivores are the most exposed to the *Toxoplasma* infection, followed by primates and herbivores.

Tables 3 and 4 summarize the result of the risk factors study using chi-squared test. *Toxoplasma* infection was associated with the family of wild animals (birds of prey were more infected than other animals) and the age of mammals ($P < 0.05$).

DISCUSSION

This investigation is considered as the first report *of Toxoplama gondii* on wild animals and birds from Algeria. The toxoplasmosis prevalence ranges between 8% and 28% on cattles (Khames et al., 2018; Abdallah et al., 2019; Djellata et al., 2019),between 25%and 35% on sheep (Abdallah et al., 2019; Benlakehal et al., 2019), between 12% and 34%on goats (Abdallah et al., 2019), 15 % on dromedaries (Abdallah et al., 2020), about 51 % on poultry farms (Tahri et al., 2020), between 58 % and 70 % on stray cats (Yekkour et al., 2017; Mohamed-Cherif et al., 2020), between 24% and 26 % on horses (Mohamed Cherif et al., 2015; Ouslimani et al., 2019), 30 % on donkeys(Mohamed Cherif et al., 2015) and about 15 %on local rabbits (Henneb et al., 2019). Humman toxoplamosis in Algeria was underestimated and little information was obtained. Furthermore, previous studies showed a prevalence of 53.2 % in the Algerian population (Schneider et al., 1977) where prevalence of 47.8 % was reported on pregnant women (Messerer et al., 2014). The wild birds and mammals could play an

important role in transmission of *Toxoplasma gondii* for domestic animals and humans. In this study, antibodies against *T. gondii* were found in 50 samples of 85 tested zoo animals. The global seroprevalence of *Toxoplasma gondi* is 58.1 %; 66.7% in El-Hamma park and 50% in the Ben Aknounon park.

All the tested wild carnivores'species were seropositive to *Toxoplasma gondii* . In addition, out of 15 carnivores were found seropositive (i.e., two felids, one golden jackal, one common genet, one Egyptian mongoose, eight fennec, one leopard and one red fox) with a prevalence of 75%. This prevalence is in accordance with the reported results from Europe and Middle East with a seroprevalence of 63 % (Lücht et al., 2019) and 66.7 % in Portugal (Tidy et al., 2017). Previous studies showed a higher prevalence of 80% in Mexico (Valenzuela-Moreno et al., 2020) and 90 % in Portugal (Lopes et al., 2011), while lower seroprevalence was found in other reports such as 50 % in Brazil (Vitaliano et al., 2014) and 33% in the Czech Republic (Bártová et al., 2018).

Out of 44 tested herbivores, 25 (56.8 %) were seropositive to *Toxoplasma gondii* infection including 1 Lama (*Lama glama)*, 2 Nilgai (*Boselaphus tragocamelus*), 9 Dwarf goats (*Capra aegagrushircus)*, 4 Black heated sheep (*Ovisaries)*, 1 Barbary sheep (*Ammotraguslervia*), 1 Mouflon (*Ovisorientalismusimon*), 4 Fallow deer (*Damadama*) and 3pony (*Equuscaballus*).

Previous studies from Mexico on wild mouflons reported a seroprevalence ranging between 0% and 23% (Aubert et al., 2010; Ferreira et al., 2019; Valenzuela-Moreno et al., 2020).

A higher seroprevalence of *Toxoplasma gondii* was reported in a Czech Republic zoo; 29.7% in Cetartiodactyla, 27.8% in Bovidae, 32.4% in Camelidae and 45.7 % in Cervidae (Bártová et al., 2018). In this study, Two Barbary macaque (*Macacasylvanus*) and one crested mangabey (*Cercocebus agilis)* were tested positive. This result was in concordance with an investigation from Brazil zoo where one of the two primates was positive to *Toxoplasma gondii* (Vitaliano et al., 2014). Out of 18 tested birds, 38.9% (7/18 Birds) were seropositive to *Toxoplasma gondii*. From the international literature, this study from Algeria is the first serological investigation in captive wild birds. These results confirm the presence of antibodies against *T. gondii* in captive birds as it was showed in different countries. A seroprvalence reported in birds was about 22. 2 % in Brazil and 38 % in Czech Republic (Vitaliano et al., 2014; Bártová et al., 2018).

Carnivores and Primates were the most infected by *Toxoplasma gondii* with a prevalence of 75 %, followed by herbivores with 56.8%.

This difference of toxoplasmosis seroprevalence between animal varieties could be related to their diet. In most cases, zoo carnivores were nourished by mules or donkey's carcasses and occasionally by cattle or sheep. *Toxoplasma gondii* can be transmitted to carnivores by ingestion of contaminated meat, as suggested by Dubey.J.P.; et al., 2005 and Hill.; et al., 2010, Breeding management play an important role in the transmission of the parasite. Furthermore, the circulation of cats was an important factor in the transmission of the parasite to the animals. Herbivores could be contaminated by oocysts disseminated in the environment. (Dubey, 1998) The seroprevalence of this parasite suggests a high contamination of the environment (Lopes et al., 2014).

Results of univariate analysis showed a significant difference between different families of wild animals. Bird of prey were more infected than omnivorous birds (P = 0.0002). The probable ingestion of meat contaminated with *Toxoplasma gondii* oocysts may promote infection in birds of prey (Dubey J.P., et al., 2010).

In general, carnivorous birds are primarily infected by consuming prey with cysts in their tissues; however, they can also become infected with sporulated oocysts by drinking contaminated water (Dubey, 2008, Lopes et al., 2021). The age of mammals was significantly associated to toxoplasmosis seropositivity (p = 0.0002). This result could be explained by the fact that older animals were exposed to the risk of infection for longer duration compared to young animals. This result was in line with previous studies from Europe, Asia and America (Silva et al., 2002; Afonso et al., 2006; Spada et al., 2012; Lücht et al., 2019).

The gender was not demonstrated as a risk factor to be infected by *Toxoplama gondii*. In contrast, many studies reported that females were the most infected due to some physiological variation like the immune-suppression related to the gestation.

CONCLUSION

The *Toxoplasma gondii* was present with high se-

roprevalence in wild animals at Algiers zoo. This is the first study of *Toxoplasma gondii* seroprevalence in wild animals in Algeria. The highest seroprevalence was reported in Carnivores, using the ELISA test. Age of Mammals was identified to be a risk factor associated with *Toxoplama gondii* infection. More studies are recommended to isolate *Toxoplama gondii* strains in wild animals in Algeria in order to control the infection, thereby reducing the risk of human disease.

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CONFLICT OF INTEREST

None declared

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