Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας

Τόμ. 71, Αρ. 2 (2020)

Seroprevalence and associated risk factors of Toxoplasma gondii infection in stray cats in Algiers urban area, Algeria

A. MOHAMED-CHERIF, K. BENFODIL, S. ANSEL, K.H. AIT-OUDHIA

doi: 10.12681/jhvms.23638

Βιβλιογραφική αναφορά:

MOHAMED-CHERIF, A., BENFODIL, K., ANSEL, S., & AIT-OUDHIA, K. (2020). Seroprevalence and associated risk factors of Toxoplasma gondii infection in stray cats in Algiers urban area, Algeria. *Περιοδικό της Ελληνικής Κτηνιατρικής Εταιρείας*, *71*(2), 2135–2140. https://doi.org/10.12681/jhvms.23638

J HELLENIC VET MED SOC 2020, 71(1):2135-2140
ΠΕΚΕ 2020, 71(2): 2135-2140

Research article
Ερευνητικό άρθρο

Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in stray cats in Algiers urban area, Algeria

A. Mohamed-Cherif, K. Benfodil, S. Ansel, K.H. Ait-Oudhia

Higher National Veterinary School, Oued Smar, Algiers, Algeria

ABSTRACT: Toxoplasmosis is a zoonotic parasitic disease caused by the protozoan *Toxoplasma gondi*i. Human infections are common and generally asymptomatic, but they can become very dangerous in immunosuppressed and HIV-positive patients. The infection can also be serious if it is transmitted to the fetus during pregnancy. Infection in untreated mothers can lead to abortion, birth defects and blindness of the fetus. The aim of this study is to determine the seroprevalence of Toxoplasma IgT antibodies in cats in the urban area of Algiers. From December 2017 to August 2018, blood samples from 184 stray cats were collected and analyzed for IgG antibodies against T. gondii using an ELISA method. Overall, the prevalence of T. gondii infection in stray cats was 58.15% (107/184). There was no statistically significant difference between male and female cats. The rate of seropositivity of T. gondii increased with age ($p < 0.05$). There was no statistically significant difference between the different regions from which the samples were taken. The results of the present study showed the high seroprevalence of toxoplasmosis in Algiers rather than in other countries. Due to the high seroprevalence of Toxoplasma IgG antibodies in cats, it is recommended to include in the prenatal evaluation, together with the adoption of a screening test and the determination of the IgG antibody titer in the high-risk populations (young girls, pregnant women) public information programs on the disease and measures that can contribute to prevention

Keywords: *Toxoplasma gondii*, seroprevalence, cat, ELISA, Algiers, Algeria

Corresponding Author:
Khatima Ait-Oudhia, ENSV, PB 161 Rue Issad Abbes, Oued Smar, Algiers, Algeria.
E-mail address: khatima.aitoudhia@gmail.com

Date of initial submission: 09-06-2019
Date of revised submission: 20-01-2020
Date of acceptance: 12-03-2020

INTRODUCTION

Toxoplasmosis is a worldwide zoonotic infestation caused by *Toxoplasma gondii*, which is transmitted to humans through ingestion of oocysts in contaminated water, soil and oocysts in raw and undercooked meat (Fayer et al., 2004).

The prevalence of toxoplasmosis in humans has increased significantly due to the high incidence of immunosuppressive infections such as AIDS. It can infect all warm-blooded animals and about 30% of the human population is a carrier of the parasite (Petersen et al., 2001).

Cats are considered the key to the transmission of *T. gondii* to humans and other animals because they are the only hosts that can excrete environmentally resistant oocysts in their feces (Dubey et al., 1995; Dubey, 2010). Compared to companion cats, stray cats are particularly important for public health, since they are considered to be the best sentinels of the level of *T. gondii* in the environment (Wang et al., 2012).

Serological prevalence data are important in determining the epidemiological significance of *T. gondii* infection in cats, since oocysts are rarely found in cat feces (Dubey et al., 1995). Since toxoplasmosis is a major concern for pregnant women, sero-epidemiological studies in young girls are useful for designing prevention policies before pregnancy.

The prevalence of *T. gondii* differs from country to country depending on the socioeconomic model of cat accommodation, their feeding behavior and the education level of people (Al-Kappany et al., 2010). Infection rates in stray cats are an indirect indication of *T. gondii* in the environment (Wang et al., 2012).

Although *T. gondii* infections are generally asymptomatic, toxoplasmosis can be a serious disease in humans (Montoya and Liesenfeld, 2004). Infection with *T. gondii* can cause toxoplasmic encephalitis in immunocompromised patients, blindness, abortion, fetal abnormalities or even prenatal death in congenital cases (Cook et al., 2000).

The ingestion of ecologically robust stages (sporozoites in oocysts), the consumption of raw or undercooked meat or meat products containing tachyzoites or bradyzoites are the main routes of transmission of *Toxoplasma* to humans (Tenter et al., 2000; Dubey et al., 2009).

In Algeria, cats mainly live outside like stray dogs where they hunt for food or live on waste. Even indoor cats are allowed to roam. Thus, the environment is likely to be contaminated by oocysts excreted by these cats. In recent years, the cat population has increased due to the accumulated attention paid to animal welfare in cities. On the other hand, animals are highly respected in Algerian culture, so stray cats live freely in our environment. The aim of this study was to determine the seroprevalence of *Toxoplasma* IgG antibodies in cats in the urban area of Algiers, Algeria.

MATERIALS AND METHODS

Ethic statement

Risk assessment was submitted to and approved by the ethics committee and decision board of Hygiène Urbaine d'Alger (HURBAL). HURBAL is an institution affiliated with the Algerian Ministry of the Interior, the Local Government and the Algerian Ministry of Agriculture and Rural Development. By decision of the Ministry of the Interior and in the context of the National Program for Rabies Control, HURBAL captured stray cats and dogs from Algiers area. Once captured, the stray animals were housed in cages, euthanized after the expiration of the legal waiting period (7 days, to allow owners to claim their pets). To facilitate the work in the field, the veterinary surgeons and their assistants working in this establishment collaborated with us.

Samples collection

Samples were taken from December 2017 to August 2018. A total of 184 blood samples were taken from stray cats (109 males, 75 females) living in various places and neighbors (12 in stables, 15 in zoos, 17 in hatcheries, 19 in slaughterhouses, 21 in farms and 100 in a canine furrier in the city of Algiers (Algeria), located in the north of Algeria (latitude 36 ° 42 '00' 'N, longitude 3 ° 13 '00' 'E) . Sampling was performed in a room devoted to veterinary activities. Blood samples were taken from the heart immediately after the animals were euthanized.

The approximate age of each cat was estimated based on the teeth. Cats were classified as juveniles (<2 years) and adults (> 2 years) (García-Bocanegra et al., 2010). Sex was also recorded for each animal. The serum was separated from the blood clot by centrifugation for 10 min at 1900 x g and was stored at -20 ° C until analysis.

Serological examination

The presence of anti-T.gondii antibodies was test-

ed using an ELISA test (ID Screen® Toxoplasmosis Indirect ELISA Multi-species, ID.VET. Innovative Diagnostics. Montpellier, France) according to the manufacturer's instructions. The sensitivity of this ELISA test reaches 100% while the specificity has been determined at 96% (manufacturer's data).

The results were expressed in optical density (OD); the 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 is formed using the peroxidase conjugate which is added later. Positive and negative controls were provided by the manufacturer and used to validate each test.

The samples were considered positive if they had a value ≥ 50%; doubtful for values between 40% and 50% and negative if ≤ 40%. This percentage was calculated as follows: Percentage of positivity = 100 * OD of the sample / OD of the PC. The sensitivity and specificity of this ELISA test are 100% and 96% respectively (information provided by the manufacturer).

Statistical analysis

Statistical analysis as performed using SPSS Statistics 22.0. The degree of significance of the correlation between seroprevalence, age and sex was achieved by the χ test. These correlations were considered significant for $p < 0.05$. Confidence intervals (95%) were calculated according to Toma et al., 1996.

RESULTS

Out of 184 serum samples, 58.15% (107/184) were positive and 41.84% (77/184) were negative for the presence of antibodies to *T. gondii*. The percentages of seropositivity of the samples taken at different sites were as follows: 75% of the 12 samples from the stables, 46.6% from the 15 samples from zoos, 64.7% from the 17 samples from hatcheries, 73.6% from the 19 samples from the slaughterhouses, 61.9% of the 21 farm samples and 53% of the 100 canine fur samples. (Table 1)

There was no statistically significant difference between male and female cats. The seropositivity rate of *Toxoplasma gondii* increases with age (**p** = 0.05), but there is no statistically significant difference between the age groups (Table 1).

Table 1. Analysis of risk factors related to *T. gondii* seroprevalence in stray cats (*n = 184*) in Algiers urban area

Variables		N	No. of positive	Percentage (%)	95% CI	*P-value*
Age group (year)	Young	32	12	37.5	20.7 – 54.3	0.0589
	Adult	152	97	63.8	56.2 – 71.4	
Gender	Male	109	66	60.5	51.3 – 69.5	0.0656
	Female	75	44	58.6	47.5 – 69.7	
Region (sample collect location)	Stables	12	09	75	50.5 – 99.5	0.3130
	Zoos	15	07	46.6	22.3 – 71.7	
	Hatcheries	17	11	64.7	42 – 87.4	
	Slaughterhouses	19	14	73.6	53.8 – 93.4	
	Farms	21	13	61.9	11.55 – 29.45	
	Canines furrier	100	53	53	43.2 – 62.8	

N: number of animals tested; No.: number; CI: Confidence Interval;

DISCUSSION

The size of the cat population has increased in recent years due to the improvement of the human lifestyle and the awareness of animal welfare. Stray cats live freely in the environment and feed on waste thrown around the houses during the night. The cat plays an important role in the spread of toxoplasmosis because it is the only animal to excrete oocysts in the environment (Silva et al., 2002).

Due to the close contact of cats with humans and the fact that children play outside on the ground, cats can be an important potential source of transmission of zoonotic parasites such as Toxoplasma as they are the only hosts that can excrete resistant *T. gondii* oocysts in the environment (Asthana et al., 2006; Dubey et al., 2006; Pas and Dubey, 2008)

Toxoplasmosis is a zoonotic parasitic disease caused by the protozoan *T. gondii*. Human infections are common and generally asymptomatic, but they can become very dangerous in immunocompromised

and HIV-positive patients. The infection can be serious if it is transmitted to the fetus during pregnancy. In unexposed mothers, it can lead to abortion, birth defects and blindness of the fetus.

Toxoplasmosis is one of the most important zoonotic diseases in Algeria and anti-Toxoplasma antibodies have been detected in humans, cattle, goats, sheep, wild and domestic birds (Keshavarz Valianand Ebrahimi, 1970) (Ghazaei, 2006; Sharif et al., 2010; Mohamed-Cherif et al., 2015; Mohamed-Cherif et al., 2019). The seroprevalence of *T. gondii* in cats varies according to their type (stray or domestic), age, test method and geographic location (Dubey et al., 2002; Silva et al., 2002; Mohan et al., 2002).

In the present study, 58.15% of the stray cats were positive for *T. gondii*. The estimated seroprevalence is comparable to the seroprevalences in other countries, for example 55% in an urban cat population in Germany (Tenter et al., 1994b) and 64.6% in Iran (Tehrani-sharif et al., 2015). The results of the present study showed a higher seroprevalence of *T. gondii* in the urban area of Algiers rather than in other countries, for example 18.6% in a population of urban cats in France (Afonso et al. , 2006), 23.1% in Japan (Nishikawa et al., 2003), 25.0% in domestic cats in Belgium (De Craeye et al., 2008), 30.5% in northern Italy (Spada et al., 2012), 32% in German (Tenter et al., 1994a; Tenter et al., 1994b), 36.9% in Spain (Miró et al., 2004), 40% in Sari-Iran (Sharif et al., 2009) and 50% in Algiers (Yekkour et al., 2017). Our results are lower than those observed in other studies: 70.2% in Belgium (Dorny et al., 2002), 85.4% in Addis Ababa, Ethiopia (Tiao et al., 2013), 90% in Tehran (Haddadzadeh et al., 2006) and 95.5% in Egypt (Al-Kappany et al., 2011). It is difficult to compare different serological surveys in felines because the seroprevalence of *T.gondii* varies between wild and domestic animals, between cat age groups, the serological test method, the sample size and the geographical location. The difference in seroprevalence in the areas where the samples are located could be explained by differences in the local reservoirs of the parasite, both in prey and in the environment, which serve as local sources of infection for cats. In Algiers, cats are generally kept outside in urban areas and often roam more freely, which increases their access to parasites.

In addition, the higher seroprevalence of *T. gondii* found in cats is likely due to the carnivorous behavior of cats living outdoors and the consumption of prey animals such as rodents and birds. The higher seropositivity with the age of cats increases the hypothesis that most cats acquire a *T. gondii* infection after weaning (Torrey and Yolken, 2013; Ahmad et al., 2014; Must et al., 2015), even if in our study, there is no significant statistical difference between the age groups.

CONCLUSION

In conclusion, the results of the present study revealed that *T. gondii* infection is highly prevalent (58.15 %) in stray cats, with 75% of them are from stables, 46.6% from zoos, 64.7% from hatcheries, 73.6% from Slaughterhouses, 61.9% from farms and 53% of 100 from canine furrier. In order to protect public health, more measures should be taken. The proper disposal of cat litter, keeping cats indoors to minimize their acquisition of infection from prey or the environment, and reducing the feral cat population are recommended.

Minimizing close contact with cats and protecting the play areas of children might potentially reduce the oocyst burden (Zhang et al., 2009). Moreover, children, pregnant women, and immunosuppressed people should adhere to hygiene principles after contact with soil and cats, as well as the good handling of meat, meat products and fruits and vegetables.

ACKNOWLEDGMENTS

The authors thank Professor Rachid Kaidi for revising the manuscript and the Algerian Ministry of Higher Teaching and Scientific Research for contributing to her PhD training.

REFERENCES

Afonso, E., Thulliez, P. and Gilot-Fromont, E. (2006) 'Transmission of *Toxoplasma gondii* in an urban population of domestic cats (Felis catus)', International Journal for Parasitology 36, 1373–82.

Ahmad, N., Ahmed, H., Irum, S. and Qayyum, M. (2014) 'Seroprevalence of IgG and IgM antibodies and associated risk factors for toxoplasmosis in cats and dogs from sub-tropical arid parts of Pakistan', Trop Biomed 31, 777–84.

Al-Kappany, Y.M., Lappin, M.R., Kwok, O.C.H., Abu-Elwafa, S. a, Hilali, M. and Dubey, J.P. (2011) 'Seroprevalence of *Toxoplasma gondii* and concurrent Bartonella spp., feline immunodeficiency virus, feline leukemia virus, and Dirofilaria immitis infections in Egyptian cats.', The Journal of Parasitology 97, 256–58.

Al-Kappany, Y.M., Rajendran, C., Ferreira, L.R., Kwok, O.C.H., Abu-Elwafa, S.A., Hilali, M. and Dubey, J.P. (2010) 'High prevalence of toxoplasmosis in cats from Egypt: isolation of viable *Toxoplasma gondii*, tissue distribution, and isolate designation', J. Parasitol. 96, 1115–18.

Asthana, S.P., Macpherson, C.N.L., Weiss, S.H., Stephens, R., Denny, T.N., Sharma, R.N. and Dubey, J.P. (2006) 'Seroprevalence of *Toxoplasma gondii* in pregnant women and cats in Grenada, West Indies', J. Parasitol. 92, 644–45.

Cook, A.J.C. et al. (2000) 'Sources of toxoplasma infection in pregnant women: European multicentre case-control studyCommentary: Congenital toxoplasmosis—further thought for food', BMJ 321, 142–47.

De Craeye, S., Francart, A., Chabauty, J., De Vriendt, V., Van Gucht, S., Leroux, I. and Jongert, E. (2008) 'Prevalence of *Toxoplasma gondii* infection in Belgian house cats', Vet. Parasitol. 157, 128–32.

Dorny, P., Speybroeck, N., Verstraete, S., Baeke, M., De Becker, A., Berkvens, D. and Vercruysse, J. (2002) 'Serological survey of *Toxoplasma gondii*, feline immunodeficiency virus and feline leukaemia virus in urban stray cats in Belgium', Vet. Rec. 151, 626–29.

Dubey, J.P. et al. (2002) 'Prevalence of antibodies to Neospora caninum and Sarcocystis neurona in sera of domestic cats from Brazil', J. Parasitol. 88, 1251–52.

Dubey, J.P. et al. (2006) 'Prevalence of Toxoplasma gondii in cats from Colombia, South America and genetic characterization of T. gondii isolates', Veterinary Parasitology 141, 42–47.

Dubey JP. (2010) 'Toxoplasmosis of Animals and Humans'. Second. CRC Press Inc., Boca Raton, New York 1–313.

Dubey, J.P., Lappin, M.R. and Thulliez, P. (1995) 'Diagnosis of induced toxoplasmosis in neonatal cats.', J Am Vet Med Assoc 207, 179–85.

Fayer, R., Dubey, J.P. and Lindsay, D.S. (2004) 'Zoonotic protozoa: from land to sea', Trends Parasitol. 20, 531–36.

García-Bocanegra, I. et al. (2010) 'Factors affecting seroprevalence of *Toxoplasma gondii* in the endangered Iberian lynx (Lynx pardinus)', Veterinary Parasitology 167, 36–42.

Ghazaei, C. (2006) 'Serological survey of antibodies to *Toxoplasma gondii*', Afr J Health Sci 13, 131–34.

Keshavarz Valian H.; A. Ebrahimi (1970) 'Prevalence of Toxoplasma gondii in birds of Kerman city by serological and parasitological methods', Iran J Public Health 23.

Haddadzadeh, H.R., Khazraiinia, P., Aslani, M., Rezaeian, M., Jamshidi, S., Taheri, M. and Bahonar, a. (2006) 'Seroprevalence of *Toxoplasma gondii* infection in stray and household cats in Tehran', Veterinary Parasitology 138, 211–16.

Miró, G., Montoya, A., Jiménez, S., Frisuelos, C., Mateo, M. and Fuentes, I. (2004) 'Prevalence of antibodies to *Toxoplasma gondii* and intestinal parasites in stray, farm and household cats in Spain', Veterinary Parasitology 126, 249–55.

Moahmed-Cherif, A., Ait-Oudhia, K. Khelef, D. (2015). Detection of anti-Toxoplasma gondii antibodies among horses (Equus caballus) and donkeys (Equus asinus) in Tiaret province, northwestern Algeria. Rev Méd. Vét., 2015, 166, 9-10, 271-274.

Mohamed-Cherif, A., Miroud, K., Benfodil, K., Ansel, S., Khelef, D., Kaidi, R., and Ait-Oudhia, K. (2019) Cross-Sectional Survey on Toxoplasma gondii Infection in Cattle, Sheep, and Goats in Algeria: Seroprevalence and Risk Factors. Vet. Sci. 2019, 6, 63; doi:10.3390/vetsci6030063

Mohan, B., Dubey, M., Malla, N. and Kumar, R. (2002) 'Seroepidemiological study of toxoplasmosis in different sections of population of Union Territory of Chandigarh', The Journal of communicable diseases 34, 15—22.

20. Montoya, J.G. and Liesenfeld, O. (2004) 'Toxoplasmosis', Lancet 363, 1965–76.

Must, K., Lassen, B. and Jokelainen, P. (2015) 'Seroprevalence of and Risk Factors for *Toxoplasma gondii* Infection in Cats in Estonia', Vector Borne Zoonotic Dis. 15, 597–601.

Nishikawa, Y., Xuenan, X., Makala, L., Vielemeyer, O., Joiner, K.A. and Nagasawa, H. (2003) 'Characterisation of *Toxoplasma gondii* engineered to express mouse interferon-gamma', Int. J. Parasitol. 33, 1525–35.

Pas, A. and Dubey, J.P. (2008) 'Fatal toxoplasmosis in sand cats (Felis margarita)', J. Zoo Wildl. Med. 39, 362–69.

Petersen, E., Dubey, J.P., Joynson, D.H.M. and Wreghitt, T. (2001) 'Toxoplasmosis: a comprehensive clinical guide',

Sharif, M., Daryani, A., Barzegar, G. and Nasrolahei, M. (2010) 'A seroepidemiological survey for toxoplasmosis among schoolchildren of Sari, Northern Iran', Trop Biomed 27, 220–25.

Silva, J.C.R. et al. (2002) 'Prevalence of *Toxoplasma gondii* Antibodies in Sera of Domestic Cats from Guarulhos and São Paulo, Brazil', para 88, 419–20.

Spada, E. et al. (2012) 'Seroprevalence of feline immunodeficiency virus, feline leukaemia virus and *Toxoplasma gondii* in stray cat colonies in northern Italy and correlation with clinical and laboratory data', Journal of Feline Medicine and Surgery 14, 369–77.

Tehrani-sharif, M., Jahan, S., Alavi, S.M. and Khodami, M. (2015) 'Seroprevalence of *Toxoplasma gondii* antibodies of stray cats in Garmsar, Iran', J Parasit Dis 39, 306–8.

Tenter, A.M., Luton, K. and Johnson, A.M. (1994) 'Species-specific identification of Sarcocystis and Toxoplasma by PCR amplification of small subunit ribosomal RNA gene fragments.', Appl Parasitol 35, 173–88.

Tenter, A.M., Vietmeyer, C., Johnson, A.M., Janitschke, K., Rommel, M. and Lehmacher, W. (1994) 'ELISAs based on recombinant antigens for seroepidemiological studies on *Toxoplasma gondii* infections in cats', Parasitology 109 (Pt 1), 29–36.

Tiao, N. et al. (2013) 'An investigation into the seroprevalence of *Toxoplasma gondii*, Bartonella spp., feline immunodeficiency virus (FIV), and feline leukaemia virus (FeLV) in cats in Addis Ababa, Ethiopia.', Epidemiology and infection 141, 1029–33.

Toma, B., Dufour, B., Sanaa, M., Bénet, J.J., Ellis, P., Moutou, F. and Louzä, a. (1996) 'Epidémiologie appliquée à la lutte collective contre les maladies animales transmissibles majeures', Médecine et Maladies Infectieuses 26, 686.

Torrey, E.F. and Yolken, R.H. (2013) 'Toxoplasma oocysts as a public health problem', Trends in Parasitology 29, 380–84.

Wang, Q., Jiang, W., Chen, Y.-J., Liu, C.-Y., Shi, J. and Li, X. (2012) 'Prevalence of *Toxoplasma gondii* antibodies, circulating antigens and DNA in stray cats in Shanghai, China', Parasites & Vectors 5, 190.

Yekkour, F. et al. (2017) 'First genetic characterization of *Toxoplasma gondii* in stray cats from Algeria', Veterinary Parasitology 239, 31–36.

Zhang, H. et al. (2009) 'Seroprevalence of *Toxoplasma gondii* infection in stray and household cats in Guangzhou, China', Zoonoses Public Health 56, 502–5.