

Analysis of feral cats role in dissemination of *Toxoplasma gondii* infection in rural area, Golestan province, North-East of Iran

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ABSTRACT

Background and aims: Cats carry a lot of zoonotic parasites such as *Toxoplasma gondii* (*T. gondii*) which causes toxoplasmosis in warm-blooded animals. Toxoplasmosis includes wide range of symptoms in its intermediate and definitive hosts but the worst symptoms have been seen in pregnant women and also on immunocompromised hosts. Considering the importance of the fact that cats are as the only definitive host that spread *T. gondii* oocysts in environment, it is necessary to obtain comprehensive information about the role of cats in life cycle of *T. gondii*.

Methods: This comparative study was done during the January 2012 to February 2014, a total of 210 feral cats were captured by hand made traps. Age, sex, season and locality were documented. Blood samples from carotid vein were collected. Sera were separated by centrifugation at 3000 rpm for 10 min and stored at 20°C until laboratory examination. Serum sample of 210 feral cats from 20 villages of Golestan province were obtained and analyzed for presence of Ig G antibody against *T. gondii* by ELIZA Kit.

Results: The frequency of *T. gondii* infection in serum samples was 85% (180/210). Percentage of *T. gondii* infection was similar in male and female and different seasons but significant difference was recorded between sampled villages. Also the seropositivity rate of *T. gondii* had positive relationship with age ($P < 0.005$).

Conclusion: High seropositive results of the current study highlight the risk of endemic toxoplasmosis in the sampled rural ecosystem. Performing suitable screening test in rural population, keeping feral cats far from the villages, controlling of feral cats population by sterilization and making villagers aware of toxoplasmosis danger are recommended.

Keywords: Feral cats, *Toxoplasma gondii*, ELIZA kit, Golestan province.

INTRODUCTION

Toxoplasma gondii is a zoonotic protozoan with worldwide distribution which causes a well-known disease, toxoplasmosis, in human and other warm-blooded animals.¹ Only members of Felidae are definitive hosts and can excrete million oocysts by feces.

Oocysts can remain infective for many months in cold-humid climates and can spread to soil, water, food, or anything mixed with the feces.² Cats may be infected by ingesting tissues cyst from intermediate hosts, such as small rodents which are accessible in most ecosystems in

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high numbers.³ Toxoplasmosis is mainly introduced to intermediate host by usages of oocyst contaminated foods and water. *T. gondii* generally does not produce clinical symptoms in healthy adult human. Although, women newly infected with *Toxoplasma* during pregnancy and with a damaged immune system should be aware that toxoplasmosis can seriously make ill, and it can sometimes be fatal.⁴

Congenital toxoplasmosis specially during the first or second trimester (up to week 27 of the pregnancy) can have bad effects on fetus including: hydrocephalus, epilepsy, jaundice, eye infections and reduce vision, an enlarged liver or spleen, growth problems and cerebral palsy.⁵ Fetal mortality of other intermediate host such as sheep and cattle by *T. gondii* infection has been reported. Regarding the effect of *T. gondii* on animal production and the importance of safety of human being, toxoplasmosis is in economic importance and public health concern for both veterinary and medical sciences.⁶

Golestan province is consist of three areas of mountain, foothill and plane and has a Mediterranean and desert climate. This kind of climate makes it suitable for presence of many villages and also different species of mammal (which can act as intermediate and definitive host of *T. gondii*).⁷

In the villages of Golestan province, large numbers of stray cats are found roaming residential streets and increasing the risk of public health for animals and humans.⁷

Also, human and cat habitats are overlapped in rural areas of Golestan province. So analysing *T. gondii* infection in feral cats can give suitable data to calculate the degree of environmental contamination and risk of toxoplasmosis outbreak in villagers of Golestan province.

To the best of our knowledge this study is the first serological survey of *T. gondii* infection of feral cats in Golestan province. There are lots of tests for detection of the

positive infection with *T. gondii* such as LAT, ELISA, indirect hem agglutination assay (IHA) and PCR for various target genes. The LAT and ELISA are now widely available for serological diagnosis of toxoplasmosis but ELISA avidity method with 97% sensitivity and high specify is very useful to detect toxoplasmosis infection.^{6,7} The problem of this study was the sampling of feral cats serum and preparing appropriate ELISA kit. The aim of this study was to analyse the role of feral cats in dissemination of *Toxoplasma gondii* infection in rural area.

METHODS

Golestan province includes 14 counties with 60 townships and 1021 villages, lying within the 36°30' to 38°8' N and 53°57' to 56°22' E, is located in North-East Iran. This province with more than 1 750 000 populations covers an area of 20 893 km². Different regions of Golestan province have different climate and are notably heterogeneous. Northern parts are located in the arid and semi-arid climate, southern parts show a mountainous climate, and central and southern west parts have a moderate Mediterranean climate.⁷ Feral cats were collected from 20 villages of 5 towns locating in different climates of Golestan province (Figure 1).



Figure 1: AZ= azadshahr, AG= agh ghala, G= gorgan, BT= bandar torkaman, GO= gonbad

During the January 2012 to February 2014, a total of 210 feral cats were captured by hand made traps. Age, sex, season and locality were documented. Blood samples were obtained from carotid vein. Sera were separated by centrifugation at 3000 rpm for 10 min and stored at 20°C until laboratory examination.

Designed ELISA avidity test was used for detection of toxoplasma IgG. ES antigen (ESA) is excretory-secretory antigen that used to design ELISA avidity kit to detect toxoplasma IgG antibody.

Test procedure: 1. 100 μ l of serum sample was added to ELISA avidity wells; 2. Samples incubated for 30 minutes at 37°C; 3. Plates were washed for three times with PBS (ELISA WASHER); 4. 100 μ l enzyme conjugate added to these plates; 5. The mixture incubated for 30 minutes in 37°C; 6. Then 100 substrate (TMB) was added; 7. Stop

solution was added and then read by 450nm ELISA reader

The normal range is <0.8 but the result >1.1 is positive for toxoplasmosis.

The analyses consist of 4 main parts: age, sex, season, and locality. Chi-square tests were used to analyse the differences among the obtained results.

RESULTS

Of 210 feral cats, 90 were male and 120 were female. The age ranges of cats were from 1 to 7 \leq years old. All the feral cats were short hair and in 180 out of 210 feral cats *T. gondii* IgG was detected in positive range.

Seropositivity rate of *T. gondii* had positive relationship with age and it was higher in older groups. No difference was found in seropositivity of males and females and also seasons. Serologic results showed lower rate of *T. gondii* infection in feral cats of Agh-g hala and Bandar-Turkmen (Table 1).

Table 1: Results of the current investigation

Variable	Number tested	Number of positive cases	Percent of positive cases
Serological results	210	180	85%
Trapping Locality	Gonbad=45	Gonbad=38	Gonbad=84%
	Bandar Turkmen=40	Bandar Turkmen=31	Bandar Turkmen=77%
	Gorgan=51	Gorgan=49	Gorgan=96%
	Aghghala=49	Aghghala=38	Aghghala=77%
	Azadshahr=25	Azadshahr=24	Azadshahr=96%
Sex	Male=90	Male=75	Male=83%
	Female=120	Female=105	Female=87%
Age	1-3=35	1-3=20	1-3=57%
	3/1-5=85	3/1-5=80	3/1-5=94%
	5/1-7=90	5/1-7=80	5/1-7=88%
Season	Spring=42	Spring=36	Spring=85%
	Summer=57	Summer=50	Summer=87%
	Autumn=56	Autumn=48	Autumn=85%
	Winter=55	Winter=46	Winter=83%

In the Figure 1 it was showed that the maximum percentage of positive toxoplasmosis infection in Gorgan and Azadshahr city is shown.

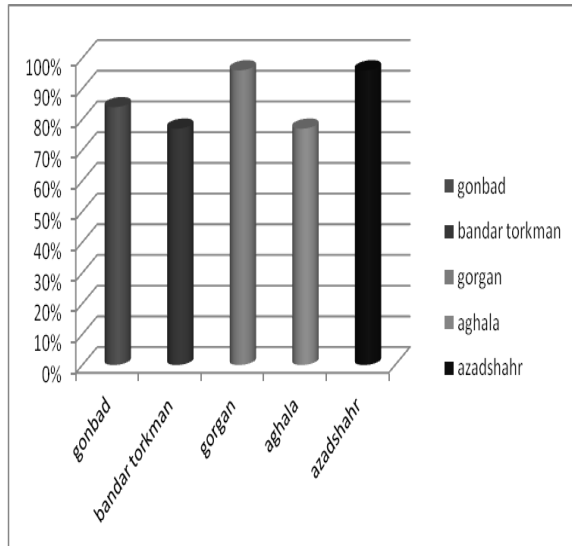


Figure 1: Percentage of positive cases with attention to locality

In Figure 2, the number of positive toxoplasmosis infection with attention to season is shown. For example, the total samples were 55 feral cats at winter season and the number of positive cases in this season was 46.

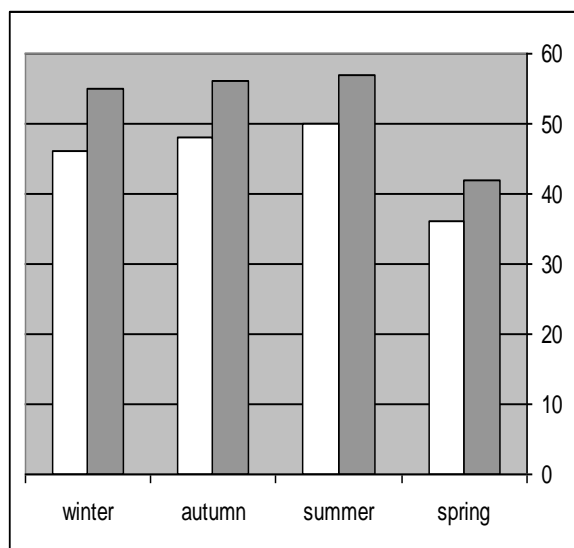


Figure 2: Number of positive cases with attention to season

In Figure 3, it shows the number of positive cases with attention to locality. For example, the total samples in Gorgan city were 51 feral cats and the number of positive toxoplasmosis infection in this city was 49.

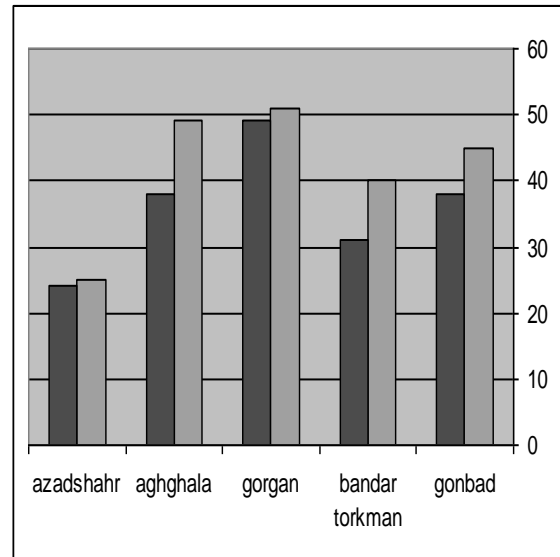


Figure 3: Numbers of positive cases with attention to locality

DISCUSSION

Despite *Toxoplasma gondii* infection of one-third of the population in developed countries, toxoplasmosis seems to be one of the neglected parasitic infections.⁸

Regarding the freely living of feral and stray cats in the cities and also the presence of them in large number in rural areas, obtaining data about *T. gondii* dynamic from cats' population in rural area is critical for the establishment of monitoring programs.

Although such monitoring could give us suitable information related to the disease exposure for high risk human population as an important zoonotic disease. Different studies have been done on *T. gondii* infection in humans, cats, goats, sheep and also birds in Iran.

All of them have been introduced toxoplasmosis as one of the major zoonotic infection diseases in Iran.⁹⁻¹²

Result of the recent studies revealed that 85% of *T. gondii* infections of sampled feral cats were from 20 villages of Golestan province. Regarding to the climate characteristic of Golestan province and theoretical defined suitable conditions for growth of *T. gondii* oocysts, detection of high rate of seropositive samples was expectable.^{12,13}

Prevalence of 40% of *T. gondii* antibodies in stray cats in Sari, Northern area in Iran, was reported by Sharif and his colleagues, 2009. They detected anti *T. gondii* antibodies with latex agglutination test (LAT) on 100 serum samples collected from stray cats in 5 urban areas of Sari. Sari is located in the neighbourhood of Golestan province in North Iran and has humid climate which has been introduced suitable for *T. gondii* growth, but *T. gondii* infection of stray cats in Sari is lower than *T. gondii* infection of feral cats in Golestan province. The significant difference between these two similar studies may be due to difference in sampled population, sensitivity of applied test for surveying anti *T. gondii* antibody, degree of contamination of food of stray cats by *T. gondii* and also sampled area. Result of related studies in Garmsar 82.2%, Urmia (86%) and Tehran (89%) had the most similarity with result of current study (85%).¹⁴⁻¹⁶ Another similar study in Tehran on stray and household cats showed that 90% and 36% were seropositive, respectively.¹⁷

A study in Tabriz by Jamali clarified 36.2% *T. gondii* infection of cats by using dye test differed from our methods.¹⁸ In this study most positive samples were belonged to Azadshahr villages and also to Gorgan villages. Comparing to Bandar-Turkmen, Gonbad and Aghghala that have semi-arid and arid climates, these two regions have humid climate. So, detection of higher positive sample in Azadshahr villages and also in Gorgan villages can be due to more suitable condition for growing of *T. gondii* oocyst in

these areas. Also Mostafavi et.al. reported that the highest prevalence of human toxoplasmosis is 70%, which is in humid regions of North in Iran.¹⁹

The sex-seroprevalence pattern found in this study is similar to those found by Raeghiet et.al., and Haddadzadeh et.al.¹⁷⁻¹⁹

In Sari by contrast, differences between *T. gondii* infections were detected between male stray cats and female stray cats. Most of the studies didn't report any significant difference in *T. gondii* infection between the two sexes and the role of sexuality in *T. gondii* exposure is not clear.²⁰

Also, seropositivity of sampled cat were the same as in different seasons, but sharif et.al have reported more seroprevalence of *T. gondii* of stray cat in spring.²⁰

In this study, the results of the ELIZA test indicated seropositivity of samples grows up by age. In addition, Raeghi et al. Hamidnejat et al. and Sharif et al. have reported the significantly positive relationship between anti *T.gondii* antibody and age.^{20,21}

Given that older cats have more time to be in contact with *T. gondii* (compared to juvenile cat), obtained result is acceptable. Serological surveys are good indicators of the occurrence of *T.gondii* infection in cats because serologically positive cats probably shed oocysts but molecular test such as PCR is more sensitive and specific. So, molecular studies is recommended.²¹⁻²³

Comparing the average range of cat toxoplasmosis prevalence in Iran, which is 5.4% to 90%, the result of the current study was in the maximum degree and it highlights the danger of toxoplasmosis outbreak in villager of Golestan province.²¹⁻²³ Hence, human populations in villages need to be made fully aware of potential environmental contamination with *T. gondii* oocysts by cats and the important role of the feral cats plays in the spreading of toxoplasmosis. Controlling programs of urban stray cat populations such

by sterilization is needed in order to reduce the risk of *T.gondii* transmission to animals and humans. More study on toxoplasma infection of intermediate host such as rodents can give us useful data for managing the controlling program such as eradication of rodents in rural areas.²¹⁻²³

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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