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Original Article

Survey on the Role of Environmental Factors in the Spatial Distribution of the *Toxoplasma gondii* Antibody in Hosts (Rural Dogs and Cats) Using GIS Software: A Case Study in Golestan Province

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Abstract

Background and aims: Toxoplasmosis, a zoonotic parasitic disease of warm-blooded animals, is notably influenced by environmental features. Recognizing spatial pattern of *Toxoplasma gondii* distribution in environment can provide significant contributions to public health and toxoplasmosis control. This study was designed to survey the frequency of *T. gondii* antibody in rural dogs and cats and also analyze possible relation between environmental factors and spatial distribution of *T. gondii*antibody in Golestan province, using GIS.

Methods: From 2015 to 2016, 106 rural cats and 154 rural dogs were randomly sampled. Serum samples were tested for presence of *T. gondii* antibody through modified agglutination test (MAT). The relation between *T. gondii* antibody frequency and environmental factors was surveyed in ArcGIS and Idrisi Selva software with multiple linear regression model.

Results: From sampled rural cats and rural dogs, 85 (80.1%) and 99 (64.2%) were positive for *T. gondii* antibody, respectively. The highest frequency was seen in areas with temperate climate, Gorgan (82.4%) with a humidity of 78%, rainfall of 551 mm and temperature of 12.5°C. The lowest rate was in Maravetappeh (60.8%) with a humidity of 70%, rainfall of 418 mm and temperature of 15.37°C. About 53% of the difference in the *T. gondii* antibody frequency in sampled population was explained by climatic condition of each region.

Conclusion: The output presented here can facilitate the identification of high risk areas, based on climate condition, to apply effective planning control measures.

Keywords: *Toxoplasma gondii*, Golestan, GIS.

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Introduction

Toxoplasma gondii is one of the most successful protozoan parasites with complex lifecycle comprising sexual replication only in felids, and asexual propagation in all warm-blooded animals.[1] Herbivores are contaminated by consuming *T. gondii* oocyst, while carnivores may acquire toxoplasmosis by ingesting contaminated raw meat, foods and water.[2] Infected felids enter unsporulated oocysts, without the infecting capability, into the environment with faeces in high numbers. Oocysts become sporulated and infectious under favorable humidity and temperature conditions.[3] Indeed, oocyst sporulation and life period are affected by climate condition.

Oocyst-containing faeces, which are usually taken into soil by cats, are small enough to be broadly disseminated by water flow. Also, mechanical transmission by contaminated invertebrate hosts can play an important role in oocyst dissemination. This type of *T. gondii* life cycle results in worldwide contamination of the environment by this parasite.[4,5]

Toxoplasmosis which is caused by *T. gondii*, is a health issue in warm-blooded animals globally. Toxoplasmosis is widely known to cause severe

symptoms in immunosuppressed people or fetuses infected via congenital transmission.[6-8]

In 2000, one-third of the world's population was reported to be potentially exposed to *T. gondii*.[6] The prevalence of *T. gondii* contamination varies from 12% to 90% in different parts of the world.[6,9]

Data on *T. gondii* spatial distribution in the environment can help to estimate the danger of toxoplasmosis outbreak in each region and manage control programs. However, predicting *T. gondii* antibody spatial distribution and role of environmental factors in it remain unclear in Iran.

Geographical information systems (GIS) have been widely applied to obtain environmental data and analyze the relationship between different factors. This method makes it possible to create map for the spread of diseases.[10]

Golestan province, located in the north-east of Iran, has heterogeneous geographical condition with different types of climate. There are many small villages with high number of free living cats and dogs and also poor sanitary condition. It has been documented that northern Iran has the highest prevalence of human *T. gondii* contamination in this country.[11]

As disease frequency can provide data for health care planning, and free living rural dogs and cats' populations can act as indicator of *T. gondii* exposure of animals in their ecosystem, this study was designed to survey the frequency of *T. gondii* antibody in free living rural dogs and cats and also analyzed possible relation between environmental factors and spatial distribution of *T. gondii* antibody in Golestan province, using GIS framework.

Methods

Study area: 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, located in northeastern Iran. This province has a population of over 1750000 and covers an area of 20893 km^2. According to climate classification system, the province contains 5 different climates: Mediterranean in the center, arid-desert in the north, semi-arid in the coast, center and northeast, humid in the sub-south and semi-humid in the south. The lowest rainfall occurs in the north and north-east of the study area and the highest in the south. In addition, annual rainfall increases from north to south. Total annual precipitation is 250 to 700 mm (Figure 1).[12]

Sampling and modified agglutination test

During 2015 to 2016, blood samples were taken randomly from 106 rural cats (carotid vein) and 154 dogs (cephalic vein), respectively. Serum samples were separated and kept at -20°C. Presence of *T. gondii* antibody was examined through modified agglutination test (MAT). Whole formalin-fixed tachyzoites was used as antigen and serum sample was tested at dilutions of 1:25, 1:50, 1:100, and 1:500. Sera with a titer of 1:25 or higher were considered positive and those with doubtful results were re-tested.[13,14]

Geographical information systems

Sampling points were georeferenced and registered using a GPS unit. Collected coordinates in the field were transcribed into an Excel spreadsheet and then converted to dBase files (dbf) for direct use in ArcGIS 10.3 software. After formatting the data in spreadsheets, the ArcMap application was used to transform the available information in a variety of thematic maps showing the distribution of georeferenced cases in the province. The interpolation module (empirical Bayesian kriging) spatial analyst was used to design disease density maps that represented the number of cases with areas of different colors and also to explore the variability and spatial relationships between obtained data. The relation between *T. gondii* antibody frequency (as a dependent variable) with temperature, precipitation, and humidity (as independent variables), as effective parameters, was surveyed in ArcGIS and Idrisi Selva softwares with multiple linear regression model.[15] The sensitometry was done to determine the significance of the independent variables.[16]

Results

The frequency of *T. gondii* infection in serum samples was 87.6% (184/210). From the 106 sampled rural cats and 154 sampled rural dogs, 85 (80.1%) and 99 (64.2%) samples were positive for *T. gondii* antibody, respectively. Frequency of *T. gondii* antibody was different in regions with different climatic conditions (Table 1).

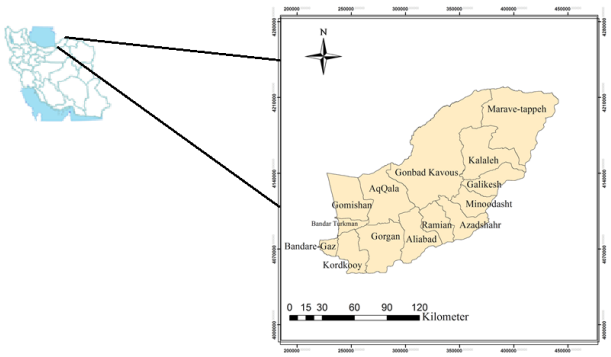

Figure 1. The Location of the Study Area in Iran.

Table 1. Frequency of *Toxoplasma gondii* in Surveyed Hosts According to the Region

Town: Village	Sample	Positive Cases	Total Positive Frequency
Gorgan Ghale Hasan Nodijeh Amir Abad	Rural dogs: 23 Rural cats: 22	17 (73%) 20 (90.9%)	82.4%
Bandar Turkman Safa Ishan Ghapaqli Qareh Su Si Joval	Rural dogs: 23 Rural cats: 17	15 (65.2%) 14 (82.3%)	73.7%
Aqghala Ghorban Abad Ghare Tape Akbar Abad Anbar Tape	Rural dogs: 23 Rural cats: 21	13 (56.5%) 16 (76.1%)	66.3%
Gonbad Kavous Gharah Ghach Vatan Ghoorchay	Rural dogs: 21 Rural cats: 9	14 (66.6%) 7 (77.7%)	72.2%
Azadshahr Khoshyeylaq Rahim Abad	Rural dogs: 23 Rural cats: 11	14 (60.8%) 8 (72.7%)	66.66%
Maravetappe Googdareh Gharahgol Sharghi Gildagh	Rural dogs: 20 Rural cats: 12	11 (55%) 8 (66.6%)	60.8%
Kordkoy Sali Kandeh Mohammad Abad Alang	Rural dogs: 21 Rural cats: 14	15 (71.4%) 12 (85.7%)	78.5%

Figure 2 Illustrates maps of average annual rainfall (*a*), average annual temperature (*b*), average annual humidity (*c*) and the interpolation results for frequency of the *T. gondii* antibody using empirical bayesian statistics in Golestan province. As illustrated, in the interpolated map *T. gondii* antibody frequency showed variation with change in temperature, rainfall and humidity. The highest frequency was seen in areas with temperate climate.

According to obtained results (Figure 2), the highest number of seropositive rural hosts (rural dogs and rural cats) was detected in villages of Gorgan (82.4%) with a humidity of 78%, rainfall of 551 mm and temperature of 12.5°C and the lowest rate was in Maravetappeh (60.8%) with a humidity of 70%, rainfall of 418 mm and temperature of 15.37°*C* (Table 1).

The acquired regression equation was as follows:

Y1 *T. gondii* antibody frequency = 16.45 + 0.647*X1 temperature + 0.821*X2 humidity + 0.07*X3 rainfall

Regression statistics:

R = 0.727 R square = 0.529 F(3, 536) = 183

Table 2 contains analysis of variance (ANOVA) results and Table 3 shows regression coefficients.

The *F* value indicates overall significance of the regression. In this study, significant *F* value, (3.536) with 99% CI, was 3.78. The *F* value of the regression (183) was greater than the *F* value given in the table, so the overall regression was significant (Table 2).

The significance of the coefficient was expressed in the form of a t-statistic. The *t* test verified the significance of the variables' departure from zero (i.e., no effect) (Table 3).

The temperature coefficient had a *t*-statistic of 3.3, the humidity coefficient had a t-statistic of 4.1 and the rainfall coefficient had a *t*-statistic of 1.7 indicating that the temperature and humidity variables are highly significant (99%) while the rainfall is relatively less significant (90%). About 53% of the difference in the *T. gondii* antibody frequency was explained by climatic condition (humidity, rainfall and temperature) of each region.

Sensitivity analysis of the multiple linear regression model showed that, temperature and humidity have the highest impact on the spatial distribution of the *T. gondii* antibody frequency in sampled hosts (Figure 3).

Discussion

Frequency of *Toxoplasma gondii* in Free Living Dogs and Cats

Frequency of *T. gondii* antibody was higher in rural cats

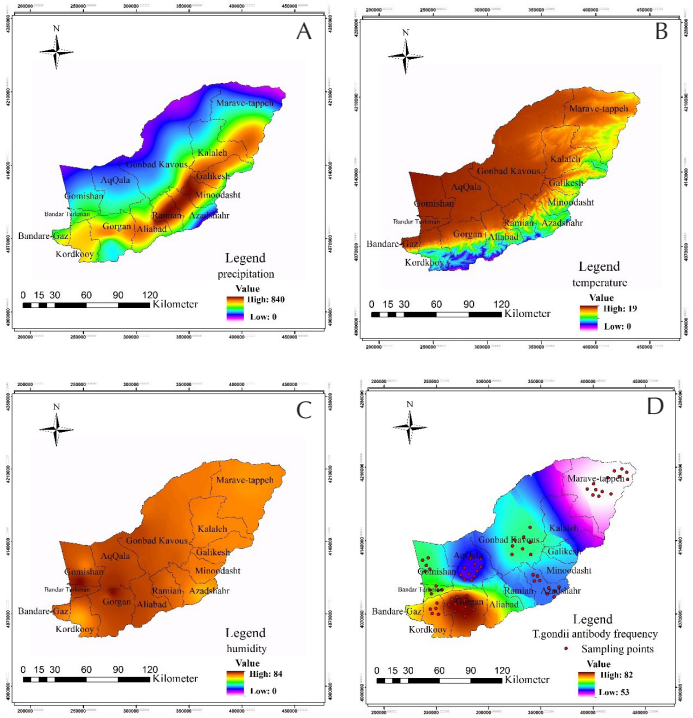

Figure 2. (A) Average Annual Rainfall; (B) Average Annual Temperature; (C) Average Annual Humidity; (D) Interpolation Module (empirical Bayesian Kriging) *Toxoplasma gondii* Aantibody Frequency.

(80.1%) than rural dogs (64.2%). Higher frequency of *T. gondii* antibody in sampled cats compare to dogs can be due to difference in their diets condition.[2] The frequency of *T. gondii* antibody was different in sampled regions in this study. The lowest *T. gondii* seropositivity rate was observed in villages around Maravehtappeh (60%) where an arid climate governs. This result can be due to the climatic differences of the sampled regions as it is argued that dry and cold environment can be a challenge for the survival of *T. gondii* oocysts, and unfavorable for epidemics of toxoplasmosis.[17] Furthermore, as felids are the only hosts that can excrete *T. gondii* oocysts into the environment, number of cats roaming in each region might influence the results in different sampled regions of current study.[18] Due to the important role of dogs and cats in life cycle of *T. gondii*, there are some similar epidemiological studies in Iran.

Highest frequency of sampled rural cats belonged to Gorgan (90.9%) which is comparable with results of Namroodi Shariat Bahadori,[19] Seyed Tabaei[0] and Haddadzadeh et al.[21] Namroodi et al,[22] reported that 85% of feral cats were positive to *T. gondii* in villages of Golestan province. Seyed Tabaei and Haddadzadeh et alreported that 89%[20] and 90%[21] of sampled stray cats in Tehran were positive to *T. gondii* antibody.

Result of similar studies in Garmsar (82.2%) and Urmia (86%) had the most similarity with of *T. gondii* antibody frequency in rural cats in Bandar Turkman (82.3%) and Kordkoy (85.7%), respectively.[23,24]

Forty percent frequency of *T. gondii* antibody in stray cats in Sari, Northern area in Iran, was reported by Sharif and colleagues.[25] They detected anti *T. gondii* antibody with latex agglutination test on 100 serum samples collected from stray cats in 5 urban areas of Sari. A study in Tabriz by Jamali clarified 36.2% *T. gondii* infection of cats by using dye test.[26]

There are limited data collected on *T. gondii* antibody prevalence in dogs population in Iran. It is said that *T. gondii* seropositivity ranging from 22.4% to 77.7%, in dogs according to the region in Iran.[27,28]

The obtained results in present study, showed most similarity with result of Shadfar et al in Azarbaijan

Table 2. ANOVA Regression Results

Source	*df*	Sum of Squares	Mean Square	*F*
Regression	3	331829.98	110609.99	183
Residual	536	323492.09	603.53	
Total	539	655322.07		

Table 3. Regression Coefficients

	Coefficient	*t* test
Intercept	16.45	13.21
X1 temperature	0.647	3.3
X2 humidity	0.821	4.1
X3 rainfall	0.07	1.7

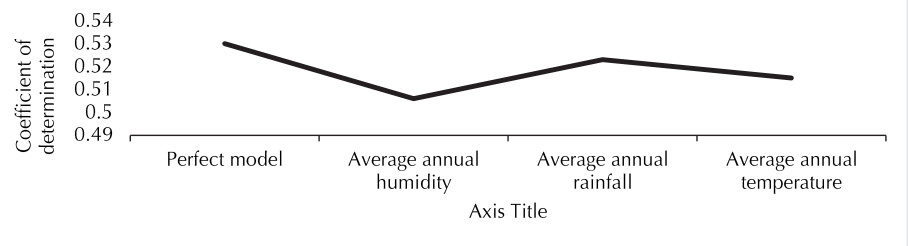

Figure 3. Sensitometry Multiple Linear Regression Model.

(77.7%) where has moist, moderate and also cold climate in some parts.[28] Also the lowest frequency of *T. gondii* antibody, 65.6%, in rural dogs that has been found in this study, is higher than results of Hosseininejad et al, (22.4%) on dogs in Tehran.[27]

Taken together, it can be concluded that the lowest results of this study is higher than the lowest results reported by Jamali (32.6%) and Sharif et al (40%) in cat population of Iran. Therefore, these results show that *T. gondii* Golestan province is a suitable province for growth and dissemination of *T. gondii*.[25,26]

Moreover, the differences between results of similar mentioned studies can be due to differences in applied serological tests, sample sizes, period of surveys, climate of sampled regions and cut-off values.

Role of Environmental Factors in the Spatial Distribution of the *Toxoplasma gondii* in the Hosts

When comparing the estimated serofrequency in different sampled regions, it has to be taken into account that frequency of *T. gondii* antibody in sampled hosts varied in regions with different climate condition in this study. Sensitivity analysis of the multiple linear regression model revealed that the temperature and humidity of each region are highly correlated with frequency of *T. gondii* antibody in sampled hosts, while the rainfall is relatively less effective.

Rain fall, humidity and temperature were estimated to have 53% relative percentage of influence on spatial distribution of *T. gondii* antibody in Golestan province. It means that environmental factors affect the spatial distribution of *T. gondii* antibody at 53% value and are potential risk factors contributing exposure to *T. gondii*.

In Svalbard, Norway where the felid population is absent, Jensen et al showed that warmer water temperatures increased the survival period of *T. gondii* oocysts.[29]

Similarly, Ljungström et al and Ahlfors et al analyzed the possible relationship between the *T. gondii* exposure prevalence in pregnant women and the average annual temperature using Pearson's correlation analysis. They reported a positive correlation between average annual temperature in different areas in Sweden and the incidence of toxoplasmosis in pregnant women. Ljungström et al documented that the prevalence of toxoplasmosis in pregnant women in Sweden decreased from warmer regions to colder regions.[30] One more survey in Mexico found that an increase of 0.6°C in the temperature between 2000 and 2006 was positively correlated with an increased prevalence of toxoplasmosis in humans in 21 states of Mexico ($r = 0.489$, $P = 0.029$).[9]

Similar to results of this study, Laaksonen et al documented that even minor variations in temperature may have a notable influence on the surveillance of the *T. gondii* oocysts.[32] Another explanation for positive relation between temperature and *T. gondii* contamination can be the increase of *T. gondii* hosts abundance in warmer temperature, particularly for rodents, which are considered important in persistence, spreading and transmission of *T. gondii* in ecosystems.[33,34]

As it was noted, there was also considerable relation between humidity & rainfall and frequency of *T. gondii* antibody in sampled hosts (rural dogs and cats). Similarly, Djokic et al reported concordance between higher prevalence of *T. gondii* antibody and rainfall in goats in Serbia.[35] One study used logistic regression analysis to find a significant association between *T. gondii* seropositivity in sea otters and sampling areas with a maximal freshwater outflow along the coast. They suggested that freshwater runoff has significant impact on transition of *T. gondii* oocysts from the land to the aquatic ecosystem.[36] Rainfall is one of the critical factors which influence the transport of water-borne parasites in the terrestrial environment.[37]

Additionally, rainfall can increase humidity of environment that results in oocysts survival, increase in the food accessibility and host density.[38,39]

Afonso et al[40] showed that risk of *T. gondii* contamination of cats was higher in rainy sites or during rainy years, especially when the mean precipitation per 10-day period was >25 mm. Conversely, as low rain or drought can result in poor hygiene and reduced food supply, floods contamination, and the abundance of animals, *T. gondii* contamination risk can decrease.[41]

Conclusion

High frequency of *T. gondii* contamination in sampled dogs and cats in regions with different climate condition reported in this study, can provide further evidence of extensive environmental contamination with this parasite in Golestan province. Hence, it is necessary to make human populations in villages fully aware of potential danger of toxoplasmosis and also ways to prevent *T. gondii* contamination. As noted, the frequency of *T. gondii* contamination in sampled dogs and cats depends on climate condition. Therefore, these results can be used for identification of at-risk areas and also at-risk population in Iran. They can also be used as useful data for implementation of toxoplasmosis control programs. However, further researches are necessary to obtain more knowledge about the relationship between the transmission of *T. gondii*. Accordingly, more efforts should be made to develop collaborations among parasitologists, ecologists, epidemiologists, statisticians, modelers and GIS specialists to control *T. gondii* contamination.

Ethical Approval

This basic research obtained ethical approval from the Gorgan University of Agricultural sciences and Natural resources.

Conflict of Interest Disclosures

None.

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