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Seroprevalence of Toxoplasmosis in Free Range chickens in Tabriz area of Iran by using ELISA test

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Abstract

Objective: As consumption of chicken meat may be as one of the sources of human infection, this study was undertaken to determine the prevalence of *T. Gondii* among the free Range Chicken , by using ELISA in Tabriz, Northwest of Iran.

Materials and Methods: One hundred blood samples were collected from free Range chickens by a cluster random sampling method and tested for toxoplasmosis by Enzyme Linked Immunosorbent Assay (ELISA). The results were analysed by SPSS software using Chi-Square test and a P value <0.05 was considered statically significant.

Results: Results revealed that 18% of Free-Ranging chickens positive by ELISA respectively. Although the difference observed in the percentage of *T. Gondii* antibodies among different areas of Tabriz city, Iran, there were no significant differences P < 0.05 detected by the ELISA method.

Conclusion: Based on cultural and food habits in our area, the meat and viscera of chicken may be important sources of infection in human when consuming semi-cooked meats. Considering the high prevalence of toxoplasmosis in chickens, standards in chicken breeding, education of environmental health personnel and standardization for preparation and handling techniques are required by Health and Veterinary organizations.

Key words: Toxoplasmosis, Free-Ranging Chickens, ELISA, Tabriz, Iran.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan that infects humans and a wide range of mammalian and bird (1).Its high infection rates and its benign co-existence with the host, *T.gondii* is regarded as one of the most successful parasites on earth. is a global parasites with no known geographical boundaries (2).Serological surveys done in various parts of the world show that in some countries more than a third of the human population have antibodies against *T.gondii* .This high prevalence of infection in human proves the importance of toxoplasmosis as azoonotic disease(3,4).*T.gondii* infection in free-range chickens (FR) is considered important as FR chickens are one of the best indicators for soil contamination with *T.gondii* oocysts because they feed from the ground, and tissues of infected chickens are considered a good source of infection for cats. Additionally, ingestion of infected chicken meat can be a source of infection for *T.gondii* infection in humans and other animals. Rarely, toxoplasmosis can cause clinical disease in chickens (5).Soil contaminated with oocysts can be taken up by pastoral animals, such as sheep and goats, during grazing. Poultry having outdoor access will also take up considerable amounts of soil and can thus become infected with *Toxoplasma*. Therefore, free-ranging chickens are now used as sentinel animals to isolate and characterise *Toxoplasma* strains throughout the world (6). The aim of this study was to investigate the utility of enzyme linked immunosorbent assay (ELISA) for detection infections with *T. gondii* in chickens.

Material and Methods

Study area

East-Azerbaijan Province is one of the 31 provinces of Iran. It is located in Iranian Azerbaijan, bordering with Armenia, Republic of Azerbaijan, Ardabil Province, West Azerbaijan Province, and Zanjan Province. The capital of East Azerbaijan is Tabriz. East Azerbaijan Province is in Regions 3 of Iran, with its secretariat located in its capital city, Tabriz.

The province covers an area of approximately 47,830 km², it has a population of around four million people. The historical city of Tabriz is the most important city of this province, culturally, politically, and commercially (Fig. 1). Generally speaking, East Azerbaijan enjoys a cool, dry climate, being in the main a mountainous region. But the gentle breezes off the Caspian Sea have some influence on the climate of the low-lying areas.

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Temperatures run up to 8.9 °C in Tabriz, and 20 °C in Maraqeh, in the winter dropping to -10-15 °C at least (depending on how cold the overall year is). The ideal seasons to visit this province are in the spring and summer months.

Figure 1: The study area of Tabriz City, East Azerbaijan Province, North-west Iran

Samples Collection

Free range chicken samples

In 2015, a total 100 blood samples were collected from free range chickens by a cluster random sampling method in sub-urban regions of Tabriz city in East-Azerbaijan Province. The chickens were 1-4 years old females used as a source of meat and egg in the region.

Blood samples

Five millilitre (ml) of blood was drawn from each bird Wing by disposable syringe. Blood were collected in sterile plain tubes and left 30 minutes at room temperature to clot, then centrifuged at 3000 rpm for 5 minutes for serum collection which was aspirated by using micropipette and dispensed into another sterile tubes, each serum was divided into 2 tubes, and kept in deep freeze at -20˚C.

The Diagnostic Methods

Enzyme Linked Immunosorbent Assay (ELISA)

This is kit product of the cusabio company , Chicken toxoplasma circulating antigen (TCA) ELISA, and was performed according to the manufacturer's instructions, this kit used the whole parasite protein of cell membrane as antigen and determined the antibodies in the sera of animals, and stored at ± 4ºC until used. The ELISA test has the advantage that it can be automated and is convenient for large-scale surveys. In chickens fed oocysts, Biancifiori et al. (1986) studied the kinetics of IgG-ELISA using the soluble fraction of tachyzoites. They reported IgG titres of 1 : 800 or higher starting at day 12 p.i. and titres peaked to 1 : 12 800 at day 41 when the experiment was terminated.

Statistical Analysis

The results were analysed by SPSS software using Chi-Square test and a P value <0.05 was considered statically significant.

Results

This present study showed the considerable percentage of antibody titer (18%) against *T. gondii* positive by ELISA among FR chicken (Table-1). This result indicates to high distribution of toxoplasmosis among chicken in the studied area. Our finding demonstrated that anti *T. gondii* antibodies were high in FR chickens in the studied area.

Table 1: The rate of chicken Toxoplasmosis in different parts of Tabriz city, Iran

Part	Number	Percent
Northern	27	17.6
Southern	23	18.4
Western	24	15.7
Eastern	26	20.3
Total	100	18

Discussion

Our finding demonstrated that anti *T. gondii* antibodies were high in FR chickens in the studied area. It seems that become infected mostly during feeding on the ground contaminated with oocysts. (10).The prevalence rate of anti-Toxoplasma antibody in FR chicken (18%) in present study was lower than that of EL massry et al survey (47.2%) from Giza province in Egypt (11).The role of these FR chicken as intermediate host and disseminator of oocysts (12). Since industrial chicken that reared in saloons rose for meat production in short duration and less exposed to cat feces, these chicken had the lowest prevalence compared to FR chicken. *T.* gondii antibodies were reported to be present in 53.3% of chickens in Egypt by sabin-feldman dye test ,indirect hemagglutination assay (IHA) ,or the complement fixation test (15,16). However, IHA and the dye test were found to be insensitive for detecting *T. gondii* antibodies in experimentally infected chicken The prevalence of *T. gondii* in chickens ,as determined by the modified agglutination test (MAT)(17) varies within countries, ranging from 10% to 47% (11,14,18,19,20). The many factors such as management and hygienic standards in breeding, density of cat and environmental condition are effect on the acquisition of *T. gondii* oocyst by animal.

ELISA is of a great sensitivity, objective, quantitative and may be automatically adopted, although it needs a refinement in the procedures.(24).A significant difference P≤0.05 was detected in the percentage of T. gondii antibodies among FR Chicken by ELISA, Bird

and rodents are two of the most important intermediate hosts. They become infected easily through ingestion of oocysts (25, 32).Dubey et al. (1993) studied the serologic response of four-week-old chickens to T. gondii following oral oocyst inoculation. Other workers have used an ELISA test to demonstrate that chickens and pigeons inoculated with T. gondii oocysts seroconvert within 2 and 3 weeks, respectively (26, 33).Soil is the most important source of infection for intermediate hosts and, owing to the feeding behavior of terrestrial species, e. g. chickens and partridges, the prevalence of T. gondii in these hosts is a good indicator of environmental contamination with parasite oocysts (14). which indicates that FR Chicken are more likely to get infection than those which are Industrial chicken.The prevalence rate of toxoplasmosis in FR chicken by ELISA was nearly similar to those of Chinese infected FR Chicken (34.7% (27-33).

Conclusion

Based on cultural and food habits in our area, the meat and viscera of chicken may be important sources of infection in human when consuming semi-cooked meats. Considering the high prevalence of toxoplasmosis in chickens, standards in chicken breeding, education of environmental health personnel and standardization for preparation and handling techniques are required by Health and Veterinary organizations.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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