ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 5 (3): 262-264, 2006
ISSN 1682-8356

Seroprevalence of Free-Ranging Chicken Toxoplasmosis in Sub-Urban Regions of Shiraz, Iran

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Abstract: Toxoplasmosis is a widespread zoonotic disease that causes significant morbidity and mortality in the human fetus and in immunocompromised patients. Since the meat of chicken is considered one of the sources of the human infection, this study was undertaken to determine the prevalence of toxoplasmosis among free-ranging chickens (*Gallus gallus domesticus*). A total of 122 serum samples from chickens of four sub-urban properties of Shiraz city were collected and tested for toxoplasmosis by IFAT. The total prevalence of toxoplasmosis among chickens was 36.1%. The rate of seropositive chicken in titers of 1/16, 1/32, 1/64, 1/128, 1/256 and 1/512 was 20.5%, 5.74%, 5.74%, 3.28%, 0% and 0.82% respectively. The more prevalence of seropositivity was observed in Northern (56.7%) and Western (53.3%) regions. However, the prevalence in South (16.7%), and East (22.6%) of Shiraz city was low. Considering the high prevalence of toxoplasmosis in chickens, in the regions, control measures should be taken to prevent transmission of the infection to the animals and humans by Health and Veterinary organizations.

Key words: Toxoplasmosis, *Gallus gallus domesticus*, free-ranging chickens

Introduction

Toxoplasma gondii is an obligate intracellular protozoan that infects humans and a wide range of mammalian and bird (Smith and Reduck, 2000). The parasite is known to cause congenital disease and abortion both in humans and livestock (Dubey and Beattie, 1988; Remington and Desmonts, 1990). Maternal toxoplasmosis during early pregnancy of human may leads to death of fetus or cause chorioretinitis, hydrocephaly, microcephaly and jaundice in neonates (Joynson and Wreghitt, 2001). Acquired toxoplasmosis has mild flu like symptoms in immunocompetent humans, but the disease is severe in immunocompromised persons, for example 23% of HIV-positive patients will develop toxoplasmic encephalitis (Oksenhendler *et al*., 1994). Human seropositivity in northern and southern parts of Iran using indirect fluorescent antibody technique was reported to be 55% and 29% respectively and a seroprevalence of 51.8% was also reported for all parts of Iran (Ghorbani *et al*., 1978; Sedaghat *et al*., 1978; Assmar *et al*., 1997). The disease occurs mostly through ingestion of undercooked meat or by the oocyst excreted by infected cat as a definitive host (Dubey, 1998). Infected chicken is considered as an important source of *T. gondii* worldwide (Tenter *et al*., 2000). On the other hands, the rate of toxoplasmosis in chicken as an intermediate host of *T. gondii* is one of the good indicators of environmental contamination because of eating habits (Devada *et al*., 1998). The worldwide prevalence of anti-*T. gondii* antibodies in chicken were reported from nil to 40% by different methods and using different cut off points (Tenter *et al*., 2000). Since chicken breeding is common in these areas, considering that contaminated chicken is one of the sources of human infection this study was aimed to estimate the *T. gondii* prevalence among chickens (*Gallus gallus domesticus*) by IFAT.

Materials and Methods

A total 122 blood samples were cluster screening randomly collected from farms chicken of four sub-urban geographical properties of Shiraz city in 2005 year. Since female gender ranged 1-4 years old was destined for meat and egg production, they were dominated more than 96% in this work. The cut off of IFAT for *T. godii* was considered 1:16 dilution (Garcia *et al*., 2000). The sera were diluted 1:16 in PBS (0.1 M phosphate, 0.33 M NaCl, PH 7.2) for preliminary screening and the positive samples were two folds serially diluted up to 1:512 to obtain the real titer of IgG antibody. RH strain tachyzoites of *T. gondii* were used as antigen (Pasteur Institute, Tehran, Iran), fixed on wells of immunofluorescent slides. Ten micro-liters of each diluted serum was placed on the well of the slides and incubated in a humidified chamber at 37°C for 30 minutes. Slides were washed in PBS (two times 7 minutes), dried, and were

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Table 1: The toxoplasmosis titer of chickens in Shiraz

Titer	Percent
>1/16	63.9
1/16	20.5
1/32	5.74
1/64	5.74
1/128	3.28
1/256	0
1/512	0.82

Table 2: The rate of chicken toxoplasmosis in different parts of Shiraz

Part	Number	Percent
Northern	31	51.6
Southern	30	16.7
Western	30	53.3
Eastern	31	22.6
Total	122	36.1

incubated for 30 minutes, at 37°C with Rabbit anti-chicken IgG conjugate (Bethyl Co.) diluted 1:200 and Evans Blue diluted 1:10000. Slides were washed and air dried. A drop of glycerol buffer was added and each slide was covered with a cover- slip. Finally, the samples were observed under the immunofluorescent microscope (Zeiss HBO 50). The results were analyzed by SPSS software using Chi-Square test and a P value <0.05 was considered statically significant.

Results

Using the IFAT, from 122 tested samples, the average anti-IgG prevalence of toxoplasmosis among free-ranging chickens was 36.1% in four sub-urban properties of Shiraz city. The rate of seropositive chickens in 1/16, 1/32, 1/64, 1/128, 1/256 and 1/512 was 20.5%, 5.74%, 5.74%, 3.28%, 0% and 0.82% respectively (Table 1). The rate of infection from four regions is shown in Table 2. Chi-square testing showed that the higher frequency of infection was in Western (53.3%, P=0.012) and Northern (51.6%, P=0.02) regions. However, the lower seropositivity was found in Eastern (16.7%, P=.018) part.

Discussion

The sources of infection for humans, worldwide, vary greatly with culture, ethnic, geographical location and eating habits differences (Tenter *et al.*, 2000). Jacobs and Melton (1966) found *T. gondii* in ovaries, oviduct and muscle of chicken by using inoculation into mice. Boch *et al.* (1968) isolated *T.gondii* from the brain and heart of hens in Germany. Thus meat of chicken must be considered as a source of infection in human. Although the infection in ovary and oviduct is possible, chicken eggs must not be considered as a source of infection for human (Dubey *et al.*, 2005).

This study has found a high seroprevalence of 36.1% that is close to those of Ghorbani *et al.* (1990), Dubey *et al.* (2005) and Devada *et al.* (1998) found 33%, 36.3%, and 39.5% in Free-chicken from Iran, Austria, and India respectively. However the prevalence is markedly above the values detected in Brazil (Garcia *et al.*, 2000), United State (Dubey *et al.*, 2003) and Peru (Dubey *et al.*, 2004) where 10.3%, 17% and 26% rates were reported respectively.

Furthermore, all of genetic types (É, ÉÉ, ÉÉÉ) of *Toxoplasma gondii* isolates of patients that have been classified on the basis of restriction fragment length polymorphism (Howe and Sibley, 1995; Howe *et al.*, 1997) were reported in free-range chickens (Dubey *et al.*, 2003; Dubey *et al.*, 2004).

The many factors such as management and hygienic standards in breeding, density of cats and environmental conditions are effective on the acquisition of *T.gondii* oocysts by animals (Tenter *et al.*, 2000). The rate of toxoplasmosis in free-ranging chicken is an important indicator of environmental contamination because of food habits (Devada *et al.*, 1998).

Humidity and temperate temperature favor the oocyst survival. Shiraz city is situated in Southern, Iran where has dry and sub-Saharan environment with an average annual rainfall not over 350 mm. However other climatic characters such as temperature and altitude in these areas are different, for example Southern parts are warmer than others. Low seroprevalence in Southern regions is probably related to high temperature and dry climate that destroy the oocysts.

The majority of free-chicken in these areas are raised for meat and egg production by people living in villages in sub-urban of Shiraz. These products not only are consumed by residents but also sent to urban regions of Shiraz. Based on cultural and food habits in this area, meat and viscera of chicken may be important source of infection in human when consumed semi-raw.

Considering the above mentioned findings, hygienic standards in chicken breeding, education of environmental health personnel and standardization of for preparation and handing techniques are required to prevent human infection.

Acknowledgment

The authors would like to thank the Office of Vice-Chancellor for Research of Shiraz University of Medical Sciences, Shiraz University for financial support of this project.

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