# A Serological Survey on Toxoplasmosis in Cattle, Sheep and Goats in Iran

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***Summary***

*To investigate the prevalence of* Toxoplasma gondii *in farm animals, 2000 sera of milking, pregnant and aborted cows were tested by latex agglutination and micro-titre indirect haemagglutination tests. More than 3000 wet and stained smears, prepared from brain, liver, spleen and heart blood of 300 foetuses from cows were examined by microscope. Samples of brain, spleen, liver and lymph node from 50, randomly chosen, foetuses were homogenised and intraperitoneally inoculated into 300 white mice. These, neither resulted in isolation of Toxoplasma nor detection of antibodies to the parasite. On the other hand, 3949 randomly obtained serum samples fron sheep and goats, from different geographical areas of Iran, were tested by the same serological methods. Totally, 24.5% of the sheep sera and 19.25% of the goat sera showed positive titres. The results indicated that toxoplasmosis is not a significant disease in cattle whereas, apparantly, it is the cause of serious problems in sheep and goats. Therefore, it is suggested that further studies be carried out to find the suitable method for controlling the disease in these animals.*

## Introduction

Toxoplasmosis, a common parasitic disease in man and animals, is caused by *Toxoplasma gondii* an obligate intracellular coccidian protozoon(1, 2, 3). It has been found in various parts of the world but is more prevalent in hot and humid areas than in cold and dry regions. The protozoan parasite has been recognized to be an important cause of abortion, stillbirth, infertility

and neonatal mortalities in sheep and goats. It also causes abortion in women and some abnormalities, like mental retardation and disorders, in children(2, 4, 5,).

Different serological tests are recommended for diagnosis of toxoplasmosis, among which latex agglutination (LA) and micro-titre indirect haemaggglutination (IHA) tests have proven to be more practicable for field studies. The former is as efficient as the dye test and the latter is useful in cases of long standing infections where the common status of sheep and goats stock is required. It is indicated by Patten et al.(6) and Wilson et al.(7) that in spite of technical difficulties, such as variations in the quality of red blood cells and antigens, IHA test is to a great extent reliable. Mainly, because it is economical, simple and does not need living tachyzoites and species specific conjugates. Although, the existence of *Toxoplasma* had been previously reported in Iran(9,10,11) no comprehensive work has been undertaken to demonstrae the economical losses it causes in sheep, goats and cattle and the roles these animals play in transmitting the parasite to human beings. With this aim in mind, the present work was designed and performed.

## Materails and methods

*Serum samples*: Milking, pregnant and aborted cows, from dairy farms where a high rate of abortion had been reported as well as sheep and goats from different regions were bled for serum. The blood samples were maintained at laboratory temperature until complete coagulation took place. Sera were separated, after keeping the samples at +4°C overnight, by centrifugation at 2700 rpm for 10 minutes. Sera were distributed in 1 ml aliquotes and stored at -20°C for a later use.

*Antigens*: Antigens used in this study were LA antigen (Promezia Ltd Italy) and IHA antigen (Wellcome Laboratories, UK).

*LA test*: A series of two-fold serum dilutions (1/2, 1/4, 1/8 .... 1/64) were prepared from the test sera. A drop of each test serum, the positive control serum and the negative control serum were separately placed on a slide and a drop of antigen added to each serum sample. Antigen and serum were thoroughly mixed. Slides were kept at the laboratory temperature for 5 min before the results being recorded.

*IHA test*: A series of two-fold serum dilutions (1/64, 1/128 ...1/4096) were

prepared on plates. The antigen was added to diluted test sera, the positive serum and negative serum. The serum and the antigen were thoroughly mixed; the plates were kept at the laboratory temperature for a period of 3 h or until the cells were settled into a distinct pattern and then the results were recorded.

*Foetuses*: The aborted foetuses collected from farms were examined by the following 3 methods:

a. *Macroscopical examination*: All physical abnormalities in the foetuses organs such as brain, liver, lung, spleen as well as lymph-nodes and cotyledons of placentas were noted.

b. *Microscopical examination*: Small portions of brain and lymph-nodes were removed mixed with saline solution, arranged on microscope slides and examined under a microscope. Also, smears were prepared from different organs of the foetuses. These smears were stained by Giemsa's stain and thoroughly examined under a microscope using an oil immersion objective.

c. *Laboratory animal inoculations*: Samples of brain, spleen, liver and lymph-nodes were randomly taken from 50 aborted foetuses and homogenized in a saline solution (20%). The resulted suspensions were kept for a period of 2 h at laboratory temperature, then supernatants were inoculated into outbred white mice via peritoneal route. The inoculated mice were kept under observation for 40 days. They were killed by euthanasia on Day 10, 20, 30 and 40 post-inoculation. Their organs were then microscopically checked and their sera axamined by IHA test. Homogenised suspensions from the organs of these mice were prepared and interaperitoneally inoculated into healthy mice. Then, the same procedure was repeated to detect the presence of the micro-organism and antibodies.

## Results

Cattle sera, 1410 samples, which had been collected from Karadj, Melard , Mardabad and Hashtgerd and tested by LA test gave negative results.
Cattle sera, 590 samples, which had been collected from the suburbs of Tehran and tested with IHA test, also showed negative results.

Table 1. Latex agglutination test for detection of *Toxoplasma* antibodies in sheep and goats

| Number of sera | | Area | Number of positive sera | | serum dilution Number of | | | | Rate of sera negative sera | | positivity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sheep | goats | | sheep | goats | $\frac{1}{8}$ | $\frac{1}{16}$ | $\frac{1}{32}$ | $\frac{1}{64}$ | sheep | goats | sheep | goats |
| 1000 | 430 | Qasvin | 240 | 86 | + | + | + | + | 760 | 344 | 24% | 20% |
| 209 | - | Razi Inst. | 48 | - | + | + | + | + | 161 | - | 22.96% | - |
| 600 | - | Kordan | 150 | - | + | + | + | + | 450 | - | 25% | - |
| 400 | 100 | West Azarbaijan Province | 100 | 20 | + | + | + | + | 300 | 80 | 25% | 20% |
| Total | | | | | | | | | | | | |
| 2209 | 530 | | 538 | 106 | + | + | + | + | 1671 | 424 | 24.35% | 20% |

Table 2. Micro agglutinaton test for detection of *Toxoplasma* antibodies in sheep and goats

| Number of sera | | Area | Number of positive sera | | serum dilution Number of | | | | rate of sera negative sera | | positivity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sheep | goats | | sheep | goats | $\frac{1}{64}$ | $\frac{1}{128}$ | $\frac{1}{256}$ | $\frac{1}{512}$ | sheep | goats | sheep | goats |
| 100 | - | Kordan | 25 | - | + | + | + | | 75 | - | 25% | - |
| 602 | - | Karadj | 150 | - | + | + | + | | 452 | - | 24.91% | - |
| 400 | 108 | Fars Province | 96 | 20 | + | + | + | | 304 | 88 | 24% | 18.51% |
| Total | | | | | | | | | | | | |
| 1102 | 108 | | 271 | 20 | + | + | + | | 831 | 88 | 24.63% | 18.51% |

Samples from brains, livers, spleens and lymph-nodes from 50 randomly chosen aborted foetuses inoculated peritoneally into 300 mice did not result in isolation of the parasite, even though second blind passages were carried out.

Serum samples from inoculated mice tested by IHA and LA tests were all netagive. The results of LA and IHA tests on sheep and goats sera are shown in Tables 1 and 2.

## Discussion

Our study indicated that for sero-epidemiological surveys of *Toxoplasma gondii* infection in cattle, sheep and goats LA and IHA tests are easier and more practicable than the dye and IFA tests. Particularly, the IHA test has a place in a not so well equipped veterinary diagnostic laboratory. Moreover, though the dye test is the most sensitive and specific test for *Toxoplasma* infection in human(3) it doesn't work well with bovine and ovine sera due to a naturally occurring antibody-like substance in the sera of these animals, not infected with *Toxoplasma gondii*, and interferes with the dye test. Therefore, this test is not appropriate for screening of *Toxoplasma* antibodies in farm animals. Some scientists claim the isolatation of *Toxoplasma gondii* from cattle. Nevertheless, others have proved that these animals are resistant to the infection and can easily eliminate the micro organism in their bodies. Our study on 2000 bovine sera and examination of 300 aborted foetuses, taken from 35 dairy farms, did not demonstrate either antibodies or the micro organism. Therefore, it may be concluded that the role of this animal species in transmission of *Toxoplasma gondii* to human beings and other animals is negligible. Besides, the parasite is not a threat to the cattle industry.

The serological survey on 3949 sheep and goats sera, taken from different areas in Iran, showed respectively 24.50% and 19.25% positive reactions against *Toxoplasma gondii*. Such a high positive rate is an evidence that a considerable number of animals have come into contact with the micro organism and that they may play a role in transmitting the disease to other animals as well as to human beings. Dubey has also come to the conclusion in his study(10). We consider the distribution of *Toxoplasma gondii* to be ubiquitous among sheep and goats in Iran and that these animals, apart from cats, may play an important role in the

epidemiology of the disease. It is of vital importance to carry out studies on these animals prior to pregnancy, during pregnancy and after parturition in order to investigate the rate of abortion due to *Toxoplasma* infections. Also, development of necessary measures to reduce the incidence of the disease and to diminish the sources of infection for human beings is needed.

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