ABSTRACT
Toxoplasmosis is a disease caused by a single-celled parasite called Toxoplasma gondii (T. gondii). Toxoplasmosis is one of the most common parasitic diseases and has been found in nearly all warm-blooded animals, including pets and humans. Despite the high prevalence of T. gondii infection, the parasite rarely causes significant clinical disease in cats or any species. In the first, immunization of rabbit and mice with Toxoplasma antigen was carried out to prepare negative and positive controls of this method. Then, counter carnet method was used to examine the serum of these animals. After collecting serum samples of sheep from animal husbandry, blood serum of the sheep infected with toxoplasmosis and also the blood serum of healthy sheep were separated. One microliter of positive serum was placed on the slides and mixed with 10 microliter of BF antigen solution. We have measured the flocculation time and did it for negative serum. Studying 40 samples of positive sheep serum and 43 samples of negative sheep serum in respect of affected by toxoplasmosis in indirect immune-fluorescence comparing flocculation bentonite, both methods were completely compatible. Therefore, further to their compatibility and sensitivity, characteristic and hundred percent efficiency of flocculation Bentonite can be used to screening country poultry regarding affected by toxoplasmosis disease.

KEY WORDS: Bentonite flocculation, IF, livestock, Method, Toxoplasmosis, Veterinary.

INTRODUCTION
The coccidian parasite Toxoplasma gondiiis currently considered to be the most common cause of retinal infection throughout the world (Holland ., 2003). Recurrences of ocular toxoplasmosis are frequently observed. A long-term follow-up of patients recently showed that eventually 24% of the affected eyes become legally blind (Bosch-Driessen et al., 2002). Although anti-Toxoplasma drugs are available, it is not yet clear whether they are effective in the treatment of ocular toxoplasmosis. reviewed the literature on this subject in immune competent patients and came to the conclusion that only a few well-designed studies have been performed in this field and that to date none of the trials has shown a beneficial effect of treatment (Stanford et al., 2003). Taking the above factors into account, it is obvious that more attention should be paid to the prevention of Toxoplasma infection. Sources of Toxoplasma infection include the ingestion of undercooked or inadequately cured meat containing encysted parasites or the uptake of soil, fruit, vegetables, or water contaminated with oocysts shed from infected cats (Tenter et al., 2004). A multicenter epidemiologic study among pregnant women in Europe identified meat ingestion as the major source of Toxoplasma infection (30%–63% of cases) (Cook A.J et al., 2000). Of the meat sources, pork has always been considered to be a major source of Toxoplasma infection, whereas beef has not been shown to contain infectious Toxoplasma parasites. Because of changes in pig production systems, the incidence of infection has declined rapidly over the past decades. In the late 1960s, pigs were often kept outdoors, and up to 75% of animals were shown to be infected with Toxoplasma gondii (Tenter, 2004). Dubey (Dubey, 1986) has shown that all edible parts of an infected pig may contain toxoplasmacysts. Because of the indoor housing systems used today, the infection rate has dropped below 1%. Indoor housing of animals is not regarded as beneficial for the animal’s welfare, and due to social pressure, the bioindustry in several European countries has been urged to reintroduce outdoor housing.

The effect of the introduction of animal-friendly production systems on the incidence of Toxoplasma infection in slaughter pigs is not yet known and was therefore the subject of this study. In our results, outdoor housing was indeed associated with a small but significant increase in the rate of Toxoplasma-infected animals.
MATERIALS AND METHODS
In the first, immunization of rabbit and mice with Toxoplasma antigen was carried out to prepare negative and positive controls of this method. Then, counter carnet method was used to examine the serum of these animals. Performing counter carnet Immunoelctrophoresis (CCIE) test to examine serum samples of immunized animals: Counter carnet immunoelectrophoresis method is a powerful technique based on the use of antibody-antigen reactions used to separate the proteins. To perform this test, the agarose gel prepared first was poured evenly on glass slide with at the plane by a Pasteur pipette with 1mm in thickness.

- After cooling and hardening of the gel by a punch, create a number of parallel rows of sink on them.
- The serum of immunized and non-immunized rabbits was then down of 20 microliter was poured in the sinks non against Toxoplasma antigen.
- For positive control, the serum of a patient with hydatidosis was used; and for negative control, distilled water was used instead of serum.
- Then place the slide on the platform of electrophoresis tank such that the row of sinks containing antigen are in the negative pole and the row of sinks containing serums on the positive pole.
- Barbital buffer is poured into the tank and 25 relationships between the gel and barbital buffer was established by Whatman paper.
- place the ampere of power supply device on 25 mA and turn on electrophoresis for 90 minutes; then turn off the machine and analyze the results.

Use of positive and negative controls of bentonite method:
10 microliter of bentonite Kit was poured onto the slide using with sampler and mixed it with 10 microliter of immunized rabbit serum against Toxoplasma (positive control) and then the flocculation time was measured. It was carried out by the serum of normal and negative rabbits for negative control.

Bentonite flocculation procedure to study in animal models
After collecting serum samples of sheep from animal husbandry, blood serum of the sheep infected with toxoplasmosis and also the blood serum of healthy sheep were separated. One microliter of positive serum was placed on the slides and mixed with 10 microliter of BF antigen solution. We have measured the flocculation time and did it for negative serum.

Results
Counter carnet immunoelectrophoresis test for immunized and normal rabbits:
Performing counter carnet immunoelectrophoresis test and observation of precipitation arc between serum sample of immunized rabbit, whose immunization period against Toxoplasma has started 30 days ago (as positive control), and Toxoplasma antigen. Not observing the precipitation arc between normal rabbit serum (as a negative control) and Toxoplasma antigens confirmed the preparation of positive and negative controls for bentonite flocculation method. Note: In case of observing extremely weak precipitation arc, the positive test result is reported to be weak.

<table>
<thead>
<tr>
<th>Sheeps toxoplasmosis</th>
<th>affected percentage</th>
<th>Total number</th>
<th>Healthy sheeps percentage</th>
<th>Total number</th>
<th>Poultry serum type test</th>
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Results of evaluating positive and negative sheep serums in respect of affected by toxoplasmosis in indirect immune-fluorescence comparing flocculation bentonite method

Studying 40 samples of positive sheep serum and 43 samples of negative sheep serum in respect of affected by toxoplasmosis in indirect immune-fluorescence comparing flocculation bentonite, both methods were completely compatible. Therefore, further to their compatibility and sensitivity, characteristic and hundred percent efficiency of flocculation bentonite can be used to screening country poultry regarding affected by toxoplasmosis disease.

The time measured during the investigation of bentonite flocculation test in the serum of animals

The time is limited for positive sheep samples. In fact, we can observe floccule particles in 50 to 60 seconds in positive Toxoplasma samples. These particles are more observable in large sheep samples compared with human samples.

DISCUSSION

Transmission of toxoplasmosis gondii to human mainly occurs by accidentally swallowing spouring oocytes or eating raw meat or less cooked. Among animal meat like sheep, goat, cow and pig, there are mostly holes for toxoplasmosis gondii cysts into the eatable textures and then, these animals less cooked or raw meat results in major risk for human. In areas that people use goat milk, goat unpasteurized milk of goats which has tense disease is also an important source of affection especially for children. Toxoplasmosis gondii can also be transferred through mother placenta to children. Existence of toxoplasmosis gondii affection in domestic animals to be used by human has a great effect on economy; production and public care because this pathogen is main factor causes abortion and is able to be transferred to human. Efficient diagnosis to detect affected animal in order to reduce economical damages and decrease affection risk is necessary for human. According experiments on serum samples of 90 positive sheeps and 43 negative sheeps, it was showed that both results were similar. Therefore, this method is also functional for veterinarians. Since it is the first time in the world that bentonite flocculation test is used to diagnose Toxoplasma. But other pathogens microorganisms are used for the diagnosis of other diseases such as: tuberculosis, Schistosomiasis, bovine tuberculosis, syphilis and etc. The results of researches conducted in the past by researchers in relation with above diseases using bentonite flocculation are as follows:

Wallace and colleagues in 1966 explained a method for the detection of circulating antibodies with Mycobacterium tuberculosis using bentonite particles sensitized with human old tuberculin (OT). There was no precise distinction between the titer of the patients with active and passive tuberculosis; but gradually the titers higher than antibody increased the severity of infection. Careero et al. have performed a comprehensive study in 1973 on the value of bentonite flocculation test for the diagnosis of tuberculosis in cow within a group of 358 cows. The bentonite sensitized with human old tuberculin and a purified carbohydrate part (BCG-F1) were used. In this study, regardless of the antigen used to sensitize bentonite, it seemed there is a significant and positive correlation between the increase in tuberculin titer and the intensity of infection in tuberculin cattle.

REFERENCES