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Seroprevalence of *Toxoplasma gondii* in Cattle in the Province of Kars, Turkey as Determined by ELISA

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Abstract: This study aimed to determine the seroprevalence of *Toxoplasma gondii* infection in cattle from five localities in the province of Kars, Turkey. A total of 216 cattle serum samples were tested using an in-house ELISA, which was developed and optimised using sonicated tachyzoite antigens of RH strain *T. gondii*: 202 of the samples were found to be seropositive (93.5%). This result, which is consistent with findings from horses and sheep in previous studies undertaken in the area that *T. gondii* is highly prevalent in cattle in the region.

Key words: *Toxoplasma gondii*, cattle, seroprevalence, ELISA, serum, sample

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the obligate intracellular parasite *Toxoplasma gondii*, which has a world-wide distribution. While a wide range of warm-blooded animals including human beings may act as intermediate hosts for the parasite the only known final hosts are cats and other felids (Dubey and Beattie, 1988; Dubey, 1994). Although, infection is generally asymptomatic in healthy human beings the parasite may cause important health problems in pregnant women and in immunocompromised people (Montoya and Liesenfeld, 2004). Similarly in sheep and goats toxoplasmosis is a principal cause of abortion and neonatal death (Dubey and Jones, 2008). In contrast, in cattle which have high natural resistance to the parasite, *T. gondii* causes subclinical infection (Dubey and Thulliez, 1994). Therefore, diagnosis of the infection is based largely upon the application of several types of serological test including the Sabin-Feldman Dye Test, the Indirect Fluorescent Antibody Test, the Latex Agglutination Test, the Modified Agglutination Test and the Enzyme Linked Immuno Sorbent Assay (ELISA). In serological surveys undertaken in many countries, the seroprevalence of toxoplasmosis has been found to range from 0-92% (Tenter *et al*., 2000). Likewise in Turkey, it has been reported to range from 2.6-70.5% (Altintas, 1996; Inci *et al*., 1999; Yildiz *et al*., 2000; Nalbantoglu *et al*., 2002; Aslan and Babur, 2002; Karagenc *et al*., 2005). In the Province of Kars, previous studies have found 20.6% seroprevalence in horses and 51.5-95.5% prevalence in sheep (Akca *et al*., 2004; Aslantas and Babur, 2000; Mor and Arslan, 2007). However the prevalence of the infection in cattle has not been determined in the region. Therefore, the aim of the study was to determine the seroprevalence of *T. gondii* in cattle in the Province of Kars, using an ELISA test developed in-house.

MATERIALS AND METHODS

Serum samples: Blood samples were obtained from 216 cattle randomly selected from the villages of Cerme, Kumbetli, Yucelen, Akyaka and Buyuk Pirveli in the Province of Kars, which is the major cattle breeding area of Turkey and is situated at high altitude in the North-East of the country. The villages were visited in 2003 and the blood samples were collected by jugular venapuncture. Serum was removed from the clotted blood samples by centrifugation at 4000 rpm for 10 min and stored at -20°C until tested by ELISA for *T. gondii* antibodies.

ELISA: The ELISA test was performed using sonicated tachyzoite antigens of RH strain *T. gondii*, supplied by the Refik Saydam Hifzissihha Institute in Ankara and sera known to be positive (n = 2; Dye Test positive) or negative (n = 8; commercial Latex Agglutination Test negative). The tachyzoites were washed three times in physiological saline, frozen and thawed twice sonicated at 7000 MHz at 30 sec intervals on ice and then centrifuged for 30 min at 14000 g. The supernatant was

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removed and the protein concentration was measured at 280 nm using a standard spectrophotometric method (Harlow and Lane, 1988).

The optimum concentrations of antigen and serum for the ELISA were determined using a chequerboard assay with serial dilutions of antigens (10, 5, 2.5, 1.25, 0.63, 0.3 μg mL^{-1}) and of sera (1:100, 1:200, 1:400, 1:800). On the basis of the optical density readings with the greatest range between positive and negative, the optimum antigen and serum concentrations were selected as 2.5 μg mL^{-1} and 1:400, respectively. The ELISA test was performed essentially as described for the detection of anti-*T. gondii* antibodies in sheep with some modifications (Akca, 1995). Briefly, sonicated tachyzoite antigens were diluted in coating buffer (0.015 M carbonate, 0.035 M bicarbonate, pH 9.6) to give a final volume of 2.5 μg mL^{-1} and 100 μL of the diluted antigen was then added to each well of 96-well ELISA plates. The plates were incubated for one h at 37°C or overnight at 4°C. Following six washes (three short, three 5 min. long) with 0.01 M phosphate buffer 0.15 M saline (PBS, PH 7.2) containing 0.05% Tween 20 (BDH Laboratory Supplies, Poole, England) the wells were blocked for one h at 37°C with 200 μL blocking buffer (3% skimmed milk [Marvel] (Premier Beverages, Stafford, UK) in 0.05% PBS-Tween 20) and the washing step was then repeated.

The serum samples were diluted 1:400 with dilution buffer, which was the same as the washing buffer with the addition of 1% skimmed milk and added to the wells in duplicate at a volume of 100 μL each. Following incubation at 37°C for 1 h the plates were washed as before and incubated with 100 μL per well of anti-bovine IgG (whole molecule) antibodies conjugated to horse radish peroxidase (Sigma) diluted 1 in 10000 with the dilution buffer and incubated for a further hour at 37°C. After a final washing step as before, 100 μL of the freshly prepared substrate solution (TMB) was added to each well and the plates were left in darkness at room temperature for 10 min before the enzyme-substrate reaction was stopped with 50 μL of 4 N sulphuric acid. The colour changes were then recorded at a wavelength of 450 nm on an automated ELISA reader (Tekan-Spectra, Austria). Positive and negative control sera were run on each plate. In order to minimise plate to plate variation, the OD values obtained from the samples were expressed as the Percent Positivity (PP) of the positive control values. The cut-off point for the test was determined by calculating the mean PP plus two standard deviations of 8 known negative sera as detailed above, i.e., 15% PP.

Statistical analysis: Seroprevalence was expressed as the percent positive and the differences in seroprevalence between the villages were analysed by the Chi-square test, using SPSS version 11.

RESULTS AND DISCUSSION

Two hundred and two (93.5) serum samples out of 216 sera were found to be positive. When the villages were considered highest prevalence were recorded in the village Kumbetli with a prevalence of 97.29% and the lowest in Cerme with 88% (Table 1). But there was no statistical difference between the seroprevalences among the localities (villages) (x^2 = 3.919, p>0.05). Since toxoplasmosis is generally a latent infection in cattle as in many other animals, it is rather difficult to diagnose clinically. Therefore, diagnosis is still usually made using serological methods both in cases where infection is suspected clinically and in order to confirm the presence of latent infections in the field. In serological surveys in cattle, undertaken in many countries world-wide, the seroprevalence of toxoplasmosis has been reported to range from 0-92% (Tenter *et al*., 2000).

Similarly, in various regions of Turkey, seroprevalence in cattle has been found to range from 2.6-70.5% (Altintas, 1996; Inci *et al*., 1999; Yildiz *et al*., 2000; Nalbantoglu *et al*., 2002; Aslan and Babur, 2002; Karagenc *et al*., 2005). In 1967, in the first epidemiological study carried out in Turkey, Ekmen (1967) established that the level of seropositivity in cattle in the vicinities of Kars and Ankara was 22.3% by the SF test and 16.1% by the CF test. Weiland and Dalchow (1970) found a level of positivity of 40.5% in serum collected from cattle in different regions of Turkey. Likewise the level of seropositivity for *T. gondii* in cattle has been reported to be 66.0% in Kayseri (Inci *et al*., 1999), 34.7% by the SF test and 30.6% by IFAT in the Turkish Republic of Northern Cyprus (Nalbantoglu *et al*., 2002), 49.1% in Sanliurfa (Aslan and Babur, 2002) and 45.2% in Aydin (Karagenc *et al*., 2005).

The figure of 93.5% reported in this study is the highest rate of seroprevalence recorded to date in Turkey. This variation in prevalence may be contingent upon factors such as the tests used in the research the selected serum dilution levels or more importantly upon differences between the regions studied. In Turkey, the SF test has tended to be the preferred method of research. Although, this test has maintained its position as the gold standard

Table 1: Seroprevalence of *T. gondii* in cattle in the Province of Kars and Its vicinity as determined by ELISA

Villages	Serum sample tested	Positive		Negative	
		n	%	n	%
Cerme	50	44	88.0	6	12.0
Kumbetli	37	36	97.3	1	02.7
Yucelen	29	27	93.5	2	06.9
Akyaka	50	48	96.0	2	04.0
Buyuk pirveli	50	47	94.0	3	06.0
Total	216	202	93.5	14	06.5

χ^2 = 3.919; p>0.05

test for the diagnosis of toxoplasmosis in human beings in animals the specificity and sensitivity of the test are known to be rather low (Dubey and Beattie, 1988). Furthermore, the high level of prevalence found in this study is consistent with results reported previously in horses (Akca *et al*., 2004) and sheep (Mor and Arslan, 2007) in the region from research involving either the SF test or ELISA. In fact, the levels of seroprevalence reported in these studies were again the highest recorded for Turkey. Thus it appears likely that Kars and its vicinity is the region of the country in which toxoplasmosis is most endemic. Nonetheless, more sensitive tests are still required for the diagnosis of toxoplasmosis in domesticated animals and more comprehensive epidemiological studies undertaken in order to substantiate this assertion.

The high levels of prevalence found in this study and in the previous research in horses and sheep indicate that toxoplasmosis in animals poses a significant risk to human health in the region. Moreover, the scale of animal husbandry in the region and the fact that the livestock raised here is marketed throughout almost the whole of Turkey suggest that this risk is not just confined to the local populace but that it extends to all Turkish citizens. Since it has been demonstrated that in cattle the parasite may remain viable and virulent for a period of over one year, the risk that infection may be transmitted via beef products should not be underestimated (Dubey and Thulliez, 1994).

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