Prevalence of antibodies to *Toxoplasma gondii* in horses in the province of Kars, Turkey

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ABSTRACT: This study investigates the seroprevalence of *Toxoplasma gondii* in horses from seven villages in the province of Kars in north-eastern Turkey. A total of 189 serum samples from clinically healthy, local crossbred horses were tested for anti-*T. gondii* antibodies using the Sabin-Feldman dye test. Antibodies to *T. gondii* were found in 39 (20.6%) horses, with specific titres of 1 : 16 (27), 1 : 64 (11) and 1 : 256 (1). The 95% confidence interval for the population proportion ranged from 13.3 to 27.9%. The number of seropositive horses in Yucelen village (40%) was considerably higher than in other villages, whereas in the villages of Caglayan (8%) and Cerme (10.5%) the seroprevalence was lower than elsewhere. However, the differences in seroprevalence between the seven villages were not statistically significant ($P > 0.05$). In contrast, the overall seroprevalence in the province of Kars was significantly higher than that reported previously in studies carried out on army and private horse stud farms elsewhere in Turkey ($P < 0.05$). The relevance of these findings to the epizootiology of toxoplasmosis in Kars and Turkey is discussed.

Keywords: *Toxoplasma gondii*; horse; seroprevalence; dye test; Turkey

Toxoplasma gondii is an intracellular protozoan parasite capable of infecting a wide variety of tissues in a number of mammals and birds. The parasite has a worldwide distribution and is of both medical and veterinary importance. Although symptoms such as fever, ataxia, retinal degeneration and severe encephalomyelitis may be observed occasionally (McDonald and Cleany, 1970; Beech, 1974; Cusick *et al*., 1974), in general *T. gondii* infections in horses progress subclinically and, therefore, diagnosis relies largely on serological techniques to detect the parasite-specific antibodies.

The seroprevalence of toxoplasmosis in horses varies with country, ranging from 1% to 74% (Zardi *et al*., 1968; Eugster and Joyce, 1976; Tizard *et al*., 1978; Beyer and Shevhunova, 1986; Uggla *et al*., 1990; Hejlicek and Literak, 1994). Toxoplasmosis in horses was reported for the first time in Turkey by Weilland and Dalchow (1970), with a seroprevalence of 14.3% in 154 horses from different regions. Subsequently, seroprevalence ranging from 1.9% to 8.3% was reported in studies carried out at army stud farms or specialist horse farms using the Sabin-Feldman dye test (SFDT) (Inci *et al*., 1996; Zeybek *et al*., 1998; Babur *et al*., 1998; Aktas *et al*., 1999). However, since the first study in 1970 by Weilland and Dalchow, no report has been published on toxoplasmosis in horses kept by peasant farmers in Turkey. The province of Kars is located in north-eastern Turkey at an altitude of approximately 2 000 metres and is relatively underdeveloped when compared to the other parts of the country. The region has the largest horse population in Turkey and horses are still widely used for short distance transportation, agriculture and in traditional sports and ceremonies. Therefore the area was felt to be an appropriate choice for our study which aims to establish the seroprevalence of toxoplasmosis in horses maintained under extensive farming conditions and to discuss its epizootiological relevance.

MATERIAL AND METHODS

Study area and the design of the study

Turkey is divided into administrative provinces which generally bear the name of their central city. The central city and its associated provincial towns are each directly responsible for the administration

of a number of villages in their respective vicinities. Kars province in the north east of Turkey is the major animal (mainly cattle) breeding area of the country and has the largest horse population. A recent programme for the control and eradication of Glanders infection recorded an overall population of approximately 32 000 horses in the province (personal communication, Governmental Animal Health Unit of Kars Province). In this study, an estimate of the seroprevalence of *T. gondii* is made for the population of approximately 7 000 horses in 62 villages spread within the 1 400 km^2 area directly administered by the city of Kars. Seven villages (10%), at points north, south, east and west of the city, were selected to carry out the study and were visited between February and April, 2001. A one-stage cluster sampling method was applied according to Thrusfield (1995). Each village was considered to be a cluster and an attempt was made to sample all the horses in each cluster, resulting in a total of 189 horses sampled. This preliminary study is assumed to be representative of the rest of the province because the animal management practices of farmers in the region are very similar.

Collection of samples and serological testing

Ten ml blood samples were taken from the jugular vein of horses into evacuated tubes and transported to the Parasitology Laboratory of the Faculty of Veterinary Medicine, Kafkas University, Kars. Serum was removed from the clotted blood samples by centrifugation at 4 000 rpm for 10 minutes, and stored at –20°C. At the Refik Saydam Hygiene Centre, Ankara, the serum samples were inactivated at 56°C for 30 minutes, then tested for anti-*T. gondii* antibodies in fourfold dilutions (1 : 16; 1 : 64; 1 : 256; 1 : 1 024), using the standard Sabin-Feldman dye test as routinely performed according to the modified method of Feldman and Lamb (1966) in the toxoplasmosis laboratory of the Centre.

Statistical analysis

Seroprevalence was expressed as the percentage of animals tested positive and the 95% confidence interval (CI) for one-stage cluster sampling was calculated according to Thrusfield (1995). Statistical analysis was performed according to Petrie and Watson (1999) and Petrie and Sabin (2000). Differences in seroprevalence between the clusters were analysed using the Chi-squared test (for more than two independent categories). The overall seroprevalence found in this study was compared by Chi-squared test (for two independent groups) with each of the various figures reported previously for horses elsewhere in Turkey (Weilland and Dalchow, 1970; Inci *et al*., 1996; Zeybek *et al*., 1998; Babur *et al*., 1998; Aktas *et al*., 1999).

RESULTS

Table 1 summarises the results of the study. Serum antibodies to *T. gondii* were found in 39 out of the

Table 1. The prevalence of serum antibodies to *T. gondii* in horses of Kars province

Villages	Number of serum samples	Number of positive samples*	Antibody titres*		
			1/16	1/64	1/256
Bayraktar	40	10 (25%)	9 (22.5%)	1 (2.5%)	0
Caglayan	25	2 (8%)	2 (8%)	0	0
Cerme	19	2 (10.5%)	2 (10.5%)	0	0
Dikme	31	7 (17.5%)	5 (16%)	2 (6.4%)	0
Maksutcuk	17	4 (23.5%)	0	3 (17.6%)	1 (5.9%)
Sogutlu	32	4 (12.5%)	3 (9.4%)	1 (3.1%)	0
Yucelen	25	10 (40%)	6 (24%)	4 (16%)	0
Total	189	39 (20.6%)	27 (14.3%)	11 (5.8%)	1 (0.5%)

*per cent positive

189 horses tested. The overall seroprevalence was 20.6% with a 95% CI ranging from 13.3 to 27.9%. Antibodies to *T. gondii* were found in all 7 villages sampled. Antibody titres were found in 27 horses at a 1 : 16 dilution, in 11 horses at a 1 : 64 dilution and in 1 horse at a dilution of 1 : 256. The highest seroprevalence was observed in Yucelen village (40%), while the lowest seropositivity was found in Caglayan (8%) and Cerme villages (10.5%). Although the prevalence rate differed greatly between the clusters (i.e. villages), statistical evaluation of the data produced no significant differences ($P > 0.05$). The overall seroprevalence found in this study differed significantly from that reported previously for army and specialist horse stud farms elsewhere in Turkey ($P < 0.05$), but not from that found in 1970 in the only other report to date on horses kept by peasant farmers in the country ($P > 0.05$).

DISCUSSION

Toxoplasma gondii causes subclinical infections in horses. Therefore, the diagnosis of the infection is performed using various serological tests to detect *T. gondii* antibodies. While a number of serological tests to detect antibodies to *T. gondii* have been thoroughly studied in various hosts, in horses none of the tests has been optimised or validated to date and the test of preference has not been determined yet (Dubey and Beattie, 1988). Although the requirement for the use of live parasites means that the SFDT is not commonly used, it remains the gold standard in many hosts and we therefore selected it for our study. Using the test, we found that the overall seroprevalence of toxoplasmosis in horses in the province of Kars was 20.6%. Although a direct comparison with the results published in horses in other countries is not possible because of the variety of serological tests employed and the lack of correlation between them (Dubey *et al*., 1999), nonetheless when these figures, which range from 1% to 74%, are considered (Zardi *et al*., 1968; Uggla *et al*., 1990; Tenter *et al*., 2000), it emerges that the prevalence rate of toxoplasmosis in Kars province is higher than elsewhere with the exceptions of Nigeria (37.1%) (Aganga *et al*., 1983) and Italy (74%) (Zardi *et al*., 1968).

Although viable *T. go*ndii cysts were reported in some experimentally infected horses at dye test titres of 1 : 2 and 1 : 8 (Al-Khalidi and Dubey, 1979; Al-Khalidi *et al*., 1980), we preferred to test our sera at starting dilution 1 : 16 to avoid the risk of overestimating the rate of prevalence of the infection and to enable us to make a direct comparison with other studies performed in horses in Turkey where the same test and the same cut off titre were applied. In Turkey, using the SFDT, seroprevalence was found to be 1.9% in 103 horses at Gemlik Army Stud (Inci *et al*., 1996), 8.3% in 60 horses at the serum production unit of the Refik Saydam Hygiene Centre, Ankara (Babur *et al*., 1997), 2% in 50 horses slaughtered to feed carnivorous animals in Ankara Zoo (Babur *et al*., 1998), 8.2% in 194 horses sampled from stud and horse farms in different parts of the country (Zeybek *et al*., 1998), and 6.4% in 124 horses at the Government Horse Breeding Enterprise in Malatya (Aktas *et al*., 1999). When the prevalence rate found for Kars (20.6% with a 95% CI ranging from 13.3 to 27.9%) is compared statistically with each of the results cited above for elsewhere in Turkey, it emerges as significantly higher (*P*-values ranging from 0.05 to 0.001). The reason for this difference may be that in the previous studies, the serum samples were obtained from the horses of army and state studs or from specialist horse farms where good animal management practice is applied and it may therefore be assumed that measures are taken to control access to the premises by cats, the definitive host of the parasite, thus limiting the likelihood of infection. In contrast, the serum samples in our study were obtained directly from horses kept by peasant farmers under very different conditions. In particular, village farmers in Kars and the surrounding provinces keep semi-wild cats for the purpose of controlling the mouse population. These cats have free access both to stables and pastures, thus increasing the likelihood of infection. Furthermore, in the only other study to date on the prevalence of toxoplasmosis in horses kept under similar conditions in Turkey, a rate of 14.3% was reported (Weilland and Dalchow, 1970), which does not differ significantly from our result ($P > 0.05$), again indicating that the animal management conditions may be associated with the rate of prevalence.

The horse is not only an important working animal in Kars province but also it is held in people's high regard. Nonetheless, the high altitude of the area, the relative poverty of the inhabitants and the fact that the consumption of horsemeat is prohibited by law and by religious belief result in any old and unwanted horses either being culled before the onset of the long, hard winter to avoid the expense of feeding them, and being fed to village cats and

dogs, or being left to the mercy of wild animals, with the discarded carcasses remaining unburied. In consequence, although the horse is not a direct source of infection for humans in the region, it may indirectly spread the infection to humans and other herbivores via the cat, which consumes the contaminated horsemeat and sheds oocysts into the environment. The practices of the peasants may, therefore, contribute to the completion of the life cycle of the parasite. The longevity of *T. gondii* cysts in horse tissues has not been researched in detail. However, in one study, oocysts were shed by cats fed tissues of equids experimentally infected with *T. gondii* 476 days previously (Dubey, 1985).

In conclusion, the overall seroprevalence of *T. gondii* in horses in Kars province was found to be 20.6% and this rate is higher than those reported in Turkey and many other countries. It should be noted that the prerequisite for making an accurate evaluation of the prevalence of the infection remains the development of sensitive and specific serological tests for horse toxoplasmosis. Nonetheless, the high prevalence of toxoplasmosis in horses in Kars may be an indication of an abundance of infection in other intermediate hosts and humans since studies have found that seroprevalence in horses is lower than in other domestic animals tested in the same area at the same time (Uggla *et al*., 1990; Tenter *et al*., 2000). Therefore the authors are planning further research in domestic animals and humans in order to determine the extent of toxoplasmosis in Kars province.

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