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Prevalence of Antibodies to *Toxoplasma gondii* in Cats, Dogs and Horses in Sweden

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Uggla, A., S. Mattson and N. Juntti: Prevalence of antibodies to *Toxoplasma gondii* in cats, dogs and horses in Sweden. Acta vet. scand. 1990, 31, 219–222. – Samples of serum or plasma taken during 1986 and 1987 from 244 pet cats, 303 dogs and 219 horses, randomly selected among animals referred to the Animal Clinics of the Swedish University of Agricultural Sciences, were screened by enzyme-linked immunosorbent assay (ELISA) for antibodies to *Toxoplasma gondii*. 42 % of cats, 23 % of dogs and 1 % of horses examined were found seropositive.

parasitic infection; protozoa; zoonosis; enzyme immunoassay.

Introduction

Toxoplasma gondii is a ubiquitous coccidian parasite having felines as final hosts and an exceptionally wide range of warm-blooded animals, including man, as possible intermediate hosts (*Dubey* 1986). In Sweden, the infection is common in man (*Ljungström et al.* 1989), in meatproducing animals as pigs (*Hansen et al.* 1965, *Uggla & Hjort* 1984) and sheep (*Uggla & Hjort* 1984, *Lundén et al.* 1989), and in game such as hares (*Gustafsson et al.* 1988). However, the seroprevalence of *T. gondii* in the feline final host as well as in dogs and horses in Sweden has not previously been reported.

Materials and methods

Animals

The study comprises a random selection of 244 cats, 303 dogs and 219 horses referred during 1986 and 1987 to the Animal Clinics of the Veterinary Faculty, Swedish University of Agricultural Sciences, Uppsala, Sweden. The animals were brought to the clinics for a variety of reasons, including various infectious as well as non-infectious diseases, traumatic injuries, lameness, and health certification. No case of suspected or clinically verified toxoplasmosis was included in the study. Cats and dogs were pet animals of different ages and breeds and originating predominantly from the Uppsala area in Central Sweden. Horses were adult trotters or riding horses from Central Sweden. Venous blood was withdrawn from cats and dogs in tubes with or without anticoagulants as heparin or EDTA. Blood from horses was withdrawn in tubes without anticoagulant. Serum or plasma was prepared and then stored at –20°C until analysed for Toxoplasma antibodies by enzyme-linked immunosorbent assay (ELISA).

Toxoplasma ELISA

Cat sera or plasma and horse sera were diluted 1:400 and dog sera or plasma 1:100, in PBS with 0.2 % Tween 20 (PBS-T) and analysed in a conventional Toxoplasma ELISA system carried out principally as described by *Voller et al.* (1976). Microtitre plates (Immunoplate II, Nunc, Roskilde, Denmark) were coated overnight at +4°C with a soluble antigen preparation consisting of a

sonicated extract of purified, repeatedly freeze-thawed Toxoplasma tachyzoites (National Bacteriological Laboratory, Stockholm, Sweden). The antigen concentration was 0.34 µg protein per well in 100 µl of 0.1 mol/l carbonate buffer, pH 9.6. After washing in PBS-T, 100 µl of diluted serum or plasma was added in duplicate to the wells and the plates incubated at +37°C for 1 h. After repeated washing, 100 µl of a horseradish peroxidase (HPR) conjugated anti-cat immunoglobulin (Ig) G preparation (National Veterinary Institute, Uppsala, Sweden), diluted 1:500 (for cat sera), protein A preparation (Pharmacia, Uppsala, Sweden), diluted 1:1000 (for horse sera), or monoclonal anti-dog IgG preparation (National Veterinary Institute), diluted 1:1000 (for dog sera), in PBS-T with 2.5 % swine serum, was added and incubation performed for 1 h at +37°C. After a final wash, 100 µl of enzyme substrate hydrogene peroxide and tetramethyl benzidine (Sigma, St. Louis, Mo., USA), was added to each well and left to react at +37°C for 1 h, after which the reaction was stopped by adding 50 µl of 2 mol/l sulphuric acid. The optical density (OD) values were read at 450 nm using a Titertec Multiscan spectrophotometer (Flow Laboratories, Irvine, Scotland). Samples showing OD values exceeding 0.20, 0.30 or 0.25 for cats, horses and dogs respectively, were considered as positive. Positive and negative controls were included on each plate. Cat and horse controls were pre- and post-inoculation sera from experimentally infected animals, and dog controls were sera found positive and negative, respectively, at screening in the indirect fluorescent antibody test (*Uggla & Hjort* 1984). From several individual cats and dogs where both serum and the corresponding plasma sample were available, they were both included in the assay for comparison of OD values.

Results

42 % (101) of the 241 feline samples tested had OD values exceeding 0.20 (mean 0.91 ± S.D. 0.30). Mean values for negative samples were 0.06 ± S.D. 0.04.

23 % (69) of the 302 canine samples tested had OD values exceeding 0.25 (mean 0.85 ± S.D. 0.39). The 238 negative samples had a mean of 0.06 ± S.D. 0.05.

Two out of the 219 equine sera tested, comprising 1 % of the material, showed OD values exceeding 0.30 (0.40 and 1.02, respectively). The 217 negative sera had a mean OD value of 0.11 ± S.D. 0.04.

There were no noticeable difference in OD values between serum and corresponding plasma samples from either dogs or cats.

Discussion

The present study is the first to describe the prevalence of Toxoplasma infection in populations of cats, dogs and horses in Sweden. The prevalence rate of Toxoplasma infection in pet cats is of particular interest since felines are the final hosts of *T. gondii* capable of spreading the infection by oocysts to other animals and man (*Dubey* 1986).

Infected prey or raw meat or organs given as feed are the main sources for cats to contract a *T. gondii* infection. After a prepatent period of 3–25 days depending on the source of infection, cats will, generally without showing any clinical signs of the infection, shed oocysts for 7–20 days and then normally be immune against reinfection and reshedding for the rest of their lives (*Dubey* 1986). The population tested in this survey comprised pet cats, many of which are allowed to hunt wild prey outdoors. Our results show that a substantial proportion of pet cats from the actual part of Sweden have not experienced a Toxoplasma infection and may therefore be potential excreters of oocysts after ingesting viable *T. gondii* in prey or feed. The

seropositivity rate of 42 % as observed in cats in this study can be compared to 63 % found in Denmark (*Work* 1969) and 24 % in Norway (*Kapperud* 1978).

Clinical toxoplasmosis in dogs is rare and has generally been associated with concomitant infection with distemper virus, which is known to produce immunosuppression (*Dubey et al.* 1989). Not more than 30 cases of toxoplasmosis-like illness were observed among 11,000 dogs necropsied at the Angell Memorial Animal Hospital in Boston, USA, during 1948–1987. In a retrospective study using immunohistochemical techniques (*Uggla et al.* 1987) only 13 of these cases were proven to be caused by *T. gondii* (*Dubey et al.* 1989). Recently, a new Toxoplasma-like parasite in dogs, *Neospora caninum,* was described (*Bjerkås & Presthus* 1988, *Dubey et al.* 1988). It is obvious that this organism has previously been frequently mistaken for *T. gondii* when found at post mortem examinations (*Dubey et al.* 1988). *N. caninum* is however antigenically distinct from *T. gondii* as judged by immunohistochemical reactions, and a possible presence of Neospora antibodies in samples tested in serological surveys for *T. gondii* should therefore not have influenced the results obtained.

In the present study, 23 % of dogs tested were found to have antibodies to *T. gondii.* Earlier studies in dogs in other Scandinavian countries revealed seropositivity rates of 45 % in Norway (*Borgen & Berg* 1957) and 43 % in Denmark (*Work* 1969). For comparison it can be mentioned that a seroprevalence of 18 % was recently recorded in women in Central Sweden (*Ljungström et al.* 1989).

Equines are regarded as being resistant to *T. gondii* infection, and, apart from a transient fever in some cases, clinical symptoms were not observed at experimental infections (*Dubey* 1985). Furthermore, exceptionally few cases of clinical toxoplasmosis in equines have been reported from the field, and it is believed that some earlier observations of equine toxoplasmosis might have been due to infection with a morphologically related organism, possibly a Sarcocystis species, causing the so called equine protozoal encephalomyelitis (*Simpson & Mayhew* 1980).

As compared to other domestic animals, relatively few serological screenings for Toxoplasma antibodies have been performed in equines. In the present study only 1 % of animals tested were found seropositive. The figures can be compared to 20 % found in the USA (*Riemann et al.* 1975) and 25 % in Brazil (*Costa et al.* 1986). *Al-Khalidi & Dubey* (1979) isolated *T. gondii* from edible tissue of 7 out of 500 horses slaughtered in Ohio, USA, and *von den Drisch* (1987) from 2 out of 108 horses in Germany. It has been demonstrated that *T. gondii* can be isolated from tissues of infected horses as long as 16 months after inoculation (*Dubey* 1985). Thus, despite a low prevalence rate of *T. gondii* as observed in this study, uncooked or undercooked horsemeat from Sweden must be regarded as a potential source of infection in humans as well as other carnivores.

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Sammanfattning

Förekomst av antikroppar mot Toxoplasma gondii hot katter, hundar och hästar i Sverige.

Serum eller plasma från 244 tamkatter, 303 hundar och 219 sporthästar som provtagits vid djurklinikerna vid Sveriges lantbruksuniversitet i Uppsala under 1986 och 1987 testades med ELISA för antikroppar mot *Toxoplasma gondii.* Prevalensen seropositiva djur befanns vara 42 %, 23 % och 1 %, respektive.

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