Serological survey of *Toxoplasma gondii*, feline immunodeficiency virus and feline leukaemia virus in urban stray cats in Belgium

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Three hundred and forty-six serum samples taken between 1998 and 2000 from urban stray cats in the city of Ghent were tested for antibodies to *Toxoplasma gondii* and feline immunodeficiency virus (FIV), and antigens of feline leukemia virus (FeLV). Of these 346 samples, 243 (70·2 per cent) were seropositive for *T gondii*. Thirty-nine cats (11·3 per cent) had antibodies against FIV and 13 (3·8 per cent) had circulating antigens of FeLV. Fewer of the female cats had FIV and heavier cats had a higher seroprevalence of FIV. Exact logistic regression showed that cats that were infected with FIV were more likely to be infected with *T gondii* (P=0·04), and the cats with FIV had a higher titre of *T gondii* antibodies than FIV-negative animals. However, FeLV was not associated with either *T gondii* or FIV.

THE protozoan parasite *Toxoplasma gondii* is a ubiquitous pathogen of warm-blooded animals, including man. In man it is responsible for fetal damage and is a common cause of death in acquired immune deficiency syndrome patients, and is therefore considered as a major zoonosis (Dubey 1994). Because cats disseminate millions of infectious stages of *T gondii* into the environment, they are considered to be pivotal in its life cycle. People are infected through the accidental ingestion of sporulated oocysts or through the consumption of meat containing cysts. A recent study has shown that the consumption of undercooked meat and contact with soil are the main factors associated with the infection of preg-

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nant women in European cities (Cook and others 2000). In Belgium, 50 per cent of pregnant women have antibodies against *T gondii*, and 0·8 per cent acquire the infection during pregnancy (Luyasu and others 1997). There is, however, a decreasing trend in the incidence of human infection in industrialised countries, resulting from better information and advice on the risk factors to pregnant women and from a decrease in the prevalence of *T gondii* in meat-producing animals in areas of intensive farming (Tenter and others 2000).

Few data are available on the prevalence of *T gondii* in domestic cats in Belgium. Van Beeck and others (1985) found that 2·7 per cent of cats were excreting oocysts and 50 per cent had antibodies against *T gondii*, suggesting that they had been exposed to the organism. The cat population in Belgium is estimated at 1·5 million, an unknown number of which live as strays in urban areas. This subpopulation is most exposed to infection with *T gondii* because they feed on rodents, which are considered to be a reservoir for the parasite (Webster 1994). Stray cats may therefore be an important source of infection for man because they defecate in urban gardens.

The city of Ghent has a population of 350,000 and, in 1998, it launched a programme to control the stray cat population. The programme included clinical examinations, serological analyses, and the euthanasia or spaying of captured cats. This paper describes a study of the seroprevalence of toxoplasmosis in the stray cat population of Ghent. The cats were also tested for immunity-comprising viral infections because they may affect the outcome of toxoplasmosis in cats (Davidson and others 1993).

MATERIALS AND METHODS

Cats

Between October 1998 and February 2000, 346 stray cats were trapped; they were considered to be strays because they had no indications of previous ownership. The sample consisted of 149 males and 197 females. On the day they were captured they were weighed and thoroughly examined, and a blood sample was taken from the jugular vein into an EDTA tube. The blood samples were centrifuged at 1811 *g* for 15 minutes, the plasma was collected and screened immediately for feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV); a sample was also stored at –20°C until analysed for antibodies to *T gondii*. Cats which were showing obvious signs of illness, or were in bad general condition and/or had a positive result for one of these viral infections were euthanased by an intravenous injection of embutramide and mebenzonium iodide (T61; Intervet). The healthy cats were spayed, treated against fleas with fipronil (Frontline; Merial) and marked. They were released the day after the surgery at the place where they had been caught.

Serological analyses

A commercially available direct microagglutination assay was used to detect *T gondii* IgG antibodies in plasma (Toxo-Screen DA; bioMérieux). This test uses formalin-fixed tachyzoites. Plasma samples were tested at two dilutions in phosphate-buffered saline (pH 7·2), 1:40 and 1:4000, according to the manufacturer's instructions. The minimum positive titre was fixed at 1:40. Serum collected from a naturally infected cat that had previously shed oocysts of *T gondii* was used as a positive control, and serum from an uninfected cat was used as a negative control. This test has been validated in cats by Dubey and Thulliez (1989) and Dubey and others (1995a, b).

A commercially available one-step immunochromatographic sandwich assay was used to detect plasma antibodies against FIV and circulating antigen of FeLV (Speed DUO FeLV-FIV Test; Bio Veto Test). The test uses monoclonal and polyclonal antibodies prepared against the p27 epitope of FeLV core protein and a synthetic peptide that binds with the gp40 transmembrane antigen of FIV; it is highly specific and sensitive for both FIV and FeLV (Hartmann and others 2001). The test was performed on plasma.

TABLE 1: Number (per cent) of the 149 male and 197 female cats which were seropositive for *Toxoplasma gondii*, feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV)

Cats	*T gondii*	FIV	FeLV
Male	98 (65·8)	25 (16·8)	7 (4·7)
Female	145 (73·6)	14 (7·1)	6 (3·1)
Total	243 (70·2)	39 (11·3)	13 (3·8)

Statistical analysis

Each cat was either seropositive or seronegative for either *T gondii*, FIV or FeLV. To investigate the effect of, for example, the weight or sex of an animal on the probability of it being infected, a logistic regression model was used. Denoting the probability of the infection as a function of covariates by π(covariates), the model (1) can be written symbolically as:

$$\text{logit}(\pi[\text{covariates}]) = \log(\pi[\text{covariates}]/[1-\pi\{\text{covariates}\}]) = \alpha+\beta(\text{covariates})$$

The parameters α (constant) and β (which is a vector), expressing the effect of explanatory variables, have to be defined.

Conditional likelihood methods are useful for small samples. The exact inference for a parameter can be calculated by using the conditional likelihood function that eliminates all the other parameters. Since the conditional likelihood does not involve unknown parameters, one can calculate probabilities such as P values exactly rather than use crude approximations (Agresti 1996).

Exact multiple logistic regression (Mehta and Patel 1995) or Monte Carlo simulations were used to evaluate the possible risk factors for *T gondii*, FIV and FeLV status, and the possibility of interactions between *T gondii*, FIV and FeLV status. The exact logistic regression is a well-accepted method to deal with sparseness of data with a binomial response (Agresti 1996). All the analyses except one normal regression analysis were carried out in LogXact 4 (LogXact 2000).

Regression analysis was used to compare the weights of the male and female cats. A regression analysis demands that the residuals are normally distributed. The Shapiro-Wilks statistic was used to test the normality assumption of the residuals in the latter model. The latter two analyses were carried out in Stata (StataCorp 2001).

RESULTS

The Shapiro-Wilks statistic indicated that the assumption of normality was acceptable. However, the relationship between sex and bodyweight indicated that a problem due to multicolinearity could have been present when including both weight and sex in the same logistic regression model. However, the magnitude and signs of the coefficient of weight (or sex) were not changed by leaving out sex (or weight) from the model, indicating that, in fact, there were no problems with multicolinearity (Neter and others 1996).This was supported by using an adapted logistic model (2):

$$\text{logit}(\pi[\text{covariates}]) = \log(\pi[\text{covariates}]/[1-\pi\{\text{covariates}\}]) = \alpha+\beta(\text{weight} - \text{average weight})+\gamma(\text{sex} - \text{proportion of females})$$

The signs and magnitudes of β and γ were not strongly influenced by keeping or leaving out the averages in the model, which again was an indication of no multicolinearity.

In all the models that were fitted to the data, there were no discrepancies between an asymptotically simulated and an exact or Monte Carlo-simulated fit, indicating that the results obtained with an asymptotically simulated fit were valid.

The male cats weighed more than the females, their mean (sd) weight being 3·79 (0·78) kg compared with 3·37 (0·69) kg (P<0·001).

Two hundred and forty-three of the cats (70·2 per cent) were seropositive to *T gondii* (Table 1) and, of these, 174 (71·6 per cent) had a titre of 1:4000 or more. There was a trend for the females to have a higher seroprevalence for *T gondii* than the males (P=0·068), but the weight of the cats had no significant effect.

Thirty-nine of the cats (11·3 per cent) had antibodies against FIV and 13 (3·8 per cent) had circulating antigens of FeLV. Significantly fewer of the female cats had antibodies against FIV (P=0·0145) and the heavier cats had a significantly higher seroprevalence to FIV (P=0·0427). Neither sex nor weight affected the seroprevalence of FeLV.

Thirty-three of the cats had antibodies to *T gondii* and FIV, 11 had antibodies to *T gondii* and antigen of FeLV, and three had antibodies to *T gondii* and FIV and antigens of FeLV. The cats infected with FIV were more likely to be infected with *T gondii* (P=0·04), and they had a higher titre of *T gondii* antibodies than the FIV-negative animals (P<0·03). The presence of FeLV antigen had no significant relationship with either *T gondii* or FIV.

DISCUSSION

The results of this study show that there was a high seroprevalence of toxoplasmosis (70·2 per cent) in stray cats in the city of Ghent. Previous surveys in Belgium (Van Beeck and others 1985) and in other European countries have generally recorded a lower prevalence of toxoplasmosis in cats (Gethings and others 1987, D'Amore and others 1997, Svobodová and others 1998). The high seroprevalence in Ghent may have been due to the type of cats sampled; stray cats in an urban environment would be expected to be at a higher risk of infection as a result of their hunting habits than domestic cats fed on commercial food. The serological test used may also have affected the results. The modified agglutination test was chosen for its high sensitivity and because antibodies appear within 14 days after infection and high titres (>1:10,000) may persist for more than six years after infection (Dubey and others 1995b). More than 70 per cent of the positive samples had titres of 1:4000 or more.

The high prevalence of toxoplasmosis in a large stray cat population may seriously contaminate the urban environment. The accidental ingestion of oocysts by contact with soil was found to be among the main factors associated with the infection of pregnant women in European cities (Cook and others 2000). Reducing the stray cat population of Ghent by euthanasia and spaying may therefore help to reduce the risk of the human population becoming infected with *T gondii*.

Cats of all ages, sexes and breeds are susceptible to *T gondii* infection (Dubey and others 1977), and cats less than one year of age produce the largest numbers of *T gondii* oocysts. Cats that are born and raised outdoors usually become infected with *T gondii* shortly after they are weaned and begin to hunt (Dubey 1986). In this study, the age of the cats could not be recorded, but they were weighed and their weight did not affect the prevalence rate. Only 6·6 per cent of cats weighed less than 2·5 kg, suggesting that most of them were adult animals.

Intestinal immunity to *T gondii* is strong in cats that have excreted oocysts (Dubey 1995); most cats that have excreted oocysts once do not re-excrete oocysts if challenged within six months to one year, and intestinal immunity will last up to six years in about 55 per cent of cats (Dubey and others 1995b). Immunosuppression by high doses of a corticosteroid may cause some chronically infected cats to excrete *T gondii* oocysts (Dubey and Frenkel 1974), and immunosuppressive viral infections may also affect their immunity status to *T gondii*. An FIV infection can enhance *T gondii* infection when cats are challenged after the establishment of the FIV infection (Lappin and others 1992, Davidson and others 1993). A primary FIV infection in cats that are chronically infected with *T gondii* induces an increase in antibody titres, suggesting that bradyzoites in tissue cysts may have been reactivated (Witt and others 1989). In the present study, the FIV-infected cats had a higher titre for *T gondii* than the FIV-negative cats, and the seroprevalence of *T gondii* was also significantly higher in the FIV-infected cats. Lappin and others (1992) suggested that FIV-infected cats may have *T gondii* antibodies as part of a generalised non-specific polyclonal B cell activation, or that the direct infection of B lymphocytes by FIV may lead these cells to produce specific antibodies against certain antigens. However, experimental studies have indicated that there is no reactivation of the excretion of *T gondii* oocysts or development of clinical toxoplasmosis in chronically infected cats that experience a primary FIV infection (Lappin and others 1992). The epidemiological consequences of a coinfection with FIV and *T gondii* have therefore not yet been demonstrated.

A seroprevalence of FIV of 11·3 per cent is within the range found in healthy cat populations in Europe (Pensaert and Cox 1992). The presence of FeLV antigen and seropositivity to *T gondii* were not related. An experimental FeLV infection before a *T gondii* challenge did not appear to predispose cats to acute toxoplasmosis and had no effect on the excretion of oocysts (Patton and others 1991).

The high seroprevalence of toxoplasmosis in stray cats in Ghent suggests that they may play an important role in the contamination of the environment with oocysts. Measures to reduce the risk of human infection include informing the public about the risk of infection through contact with soil, and reducing the populations of stray cats and rodents. A vaccine for toxoplasmosis is not yet available in Europe. In the USA, a vaccine consisting of live bradyzoites of the mutant T-263 strain, which is capable of preventing the shedding of oocysts by cats, is available. The use of this vaccine may reduce the risk of infection of the intermediate hosts and man (Mateus-Pinilla and others 1999).

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