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Prevalence of antibodies against feline panleukopenia virus in client-owned cats in Southern Germany

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ABSTRACT

Feline panleukopenia is a frequent and commonly fatal disease of cats. Recent published studies have raised suspicions that some cats fail to develop antibodies after vaccination. The purpose of this study was to assess the prevalence of antibodies against feline panleukopenia virus (FPV) in cats in Southern Germany, and to identify factors that are associated with a lack of antibodies. In total, 350 cats presented to the Clinic of Small Animal Medicine, Ludwig-Maximilians-Universitaet were randomly included in the study. Information regarding signalment, origin, environment, lifestyle, housing conditions, health status, chronic diseases, glucocorticoid therapy, and vaccination status were collected. Antibodies were detected by haemagglutination inhibition test. Asymptomatic chi-squared tests and univariable logistic regression were used to investigate associations between a lack of antibodies and the different variables. Associations determined to be statistically significant at $P < 0.1$ were verified by a multivariable logistic regression analysis.

Of the 350 cats, 103 (29.4%) had no antibodies against FPV. Chronic kidney disease, neoplasia, glucocorticoid therapy, and vaccination status were significantly associated with a lack of antibodies. The cats with no antibodies were likely to have inadequate immunity against panleukopenia and those with chronic diseases or receiving glucocorticoids were less likely to be protected.

Introduction

Feline panleukopenia is a disease of high morbidity and high mortality for all members of the Felidae family (Barker et al., 1983; Scott, 1987; Kruse et al., 2010). Consequently, vaccination is strongly recommended for all cats and belongs to the core category according to the American Association of Feline Practitioners (AAFP) and other expert groups (Richards et al., 2006; Truyen et al., 2009; Day et al., 2010; Ständige Impfkommission Veterinär, 2013).

Vaccine-induced antibodies correlate with protection against infection with feline panleukopenia virus (FPV) and measurement of antibodies can be used to evaluate the specific immune status of individual cats (Scott and Geissinger, 1999; Lappin et al., 2002). The presence of antibodies indicates previous vaccination or exposure to the virus. One study in the USA showed that 67% of 267 client-owned cats had antibodies against FPV (Lappin et al., 2002). However, the current protection of the population in Germany is unknown and feline panleukopenia is still commonly diagnosed despite widespread vaccination (Kruse et al., 2010).

Vaccination against FPV does not always seem to be effective. In 2008/2009, several outbreaks of feline panleukopenia in Norwegian Forest cats (NFC) were reported in Germany (Hoffmann et al., 2010) and a subsequent field study revealed that 36.7% of kittens did not develop antibodies despite three basic vaccinations at the age of 8, 12, and 16 weeks. Maternally derived antibodies (MDA) that interfered with primary vaccination and prevented antibody development were detected until 20 weeks of age (Jakel et al., 2012). Recently, it was shown that cats entering an animal shelter in Florida were more likely to have antibodies when they were neutered or older than 6 months (DiGangi et al., 2012). However, studies of associated factors in client-owned cats with a known history (including vaccination status) are missing.

It is also currently unknown whether vaccination is effective and safe in immunocompromised cats that receive immunosuppressive drugs or suffer from chronic diseases (Hosie et al., 2009; Lutz et al., 2009; Truyen et al., 2009). In a study with dogs, different doses of glucocorticoids over a short period of time did not affect responses to immunization (Nara et al., 1979). However, cats that are infected with feline leukemia virus (FeLV) do not respond adequately to vaccination (Franchini, 1990) and a similar situation might occur in cats infected with feline immunodeficiency virus (FIV).

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The objectives of this study were (1) to provide information about the prevalence of antibodies against FPV in the field to estimate the protection rate in cats and (2) to identify factors associated with a lack of antibodies to determine groups that are particularly at risk.

Materials and methods

Cats

The protocol of this prospective cross-sectional study was approved by the ethical committee of the Ludwig-Maximilians-Universitaet (approval number 3-5-10-2012).

In total, 350 cats that were presented from December 2011 to June 2012 to the Clinic of Small Animal Medicine and to the Clinic of Small Animal Surgery and Gynaecology of the Ludwig-Maximilians-Universitaet were randomly included into the study. In each of these cats blood had been taken for various unrelated reasons. Cats were excluded if (1) serum preparations containing antibodies against FPV had been administered within 6 months of presentation, or (2) if no historical data were available (i.e., in stray cats). Information about signalment (breed, age, sex, neutering status), origin (breeder, animal shelter, foreign country, adopted stray cats, or private household), environment (urban or rural), lifestyle (currently indoors or outdoors), housing conditions (single- or multi-cat household), health status (healthy, acute or chronic diseases), and vaccination status were recorded.

A complete vaccination against FPV according to current guidelines (Richards et al., 2006; Truyen et al., 2009; Day et al., 2010; Ständige Impfkommission Veterinär, 2013) was defined as a completed primary vaccination including a booster at 16 weeks of age and a further booster after 1 year, followed by regular vaccinations on a triennial basis. The health status was judged by history and physical examination on the day of presentation. Any disease that had been present for at least 4 weeks (according to the owner or known time point of diagnosis) was classified as chronic, others were defined as acute. In addition, FIV and FeLV status and current therapy with glucocorticoids were recorded.

Detection of antibodies by haemagglutination inhibition test

Serum samples as well as positive and negative serum controls for test validation were heat inactivated (56 °C, 30 min), diluted with borate buffered saline (BBS) 1:5, and pre-adsorbed to 15 µL of a 50% suspension of swine erythrocytes in phosphate buffered saline (PBS) for 1 h at 4 °C. After centrifugation at 12,000 *g* for 5 min, the supernatant was serially diluted twofold over 12 steps beginning at 1:10 in 96-well V-bottom plates (Greiner Bio-One). Eight haemagglutinating units of FPV-b (strain 292), as described previously (Scott et al., 1970a; Parrish and Carmichael, 1983) in BBS were added to each well and incubated for 1 h at room temperature. A 0.5% suspension of swine erythrocytes in phosphate buffered saline (PBS) was then added and incubated at 4 °C overnight. The following day, plates were read by two independent evaluators (KM and an experienced laboratory technician). Divergent results were re-checked by a further laboratory technician who was blinded to the results of the other two individuals. All three were blinded to the history of the cats.

The antibody titre was expressed as the reciprocal of the highest dilution of serum displaying haemagglutination inhibition (HI). As antibody titres $\geqslant$1:40 are suggestive of resistance to infection with FPV, results $\geqslant$1:40 were defined as positive (Scott et al., 1970b; Scott and Geissinger, 1999; Lappin et al., 2002; Mouzin et al., 2004).

Statistical analysis

Statistical analysis was performed in PASW version 18.0. A power analysis was conducted before beginning of the study to estimate the required sample size, assuming a prevalence of 70% and a desired precision of 5%. In order to achieve a power of $\geqslant$80%, a sample size of at least 323 animals was required.

Prevalence of antibodies was calculated as the proportion of positive results in HI of the total number of tested sera. To quantify uncertainty, 95% confidence intervals (CI) were calculated. Analysis of association of antibodies with breed was restricted to the two most common breeds, namely, European short hair and Maine Coon. A factor 'exposure risk' was introduced. Cats with current access to outdoors or a history of cattery or cat show were categorized as 'high exposure risk' in contrast to 'low exposure risk'. Diseases with frequencies of >5% in the study population were analysed and included chronic kidney disease (CKD), diabetes mellitus (DM), neoplasia, and FIV infection. A receiver operating characteristic (ROC) analysis was performed to determine the best cut-off for the duration of glucocorticoid therapy (Fawcett, 2006).

An univariable analysis was performed to investigate associations between the antibody status of the animals and the different variables. All categorical variables with two categories were analysed using asymptotic chi-squared tests, unless an expected number in one of the cells in the contingency table was <5, in which case Fisher's exact test was used to assess statistical significance ($P < 0.05$). All categorical variables with more than two categories were analysed using univariable logistic regression. Odds ratios (OR) and 95% CI were calculated to examine the strength of the associations between the categorical variables and the status of the animal. All variables with $P < 0.1$ in the univariable analysis were offered to a multivariable logistic regression analysis with backwards stepwise selection using a Wald $P < 0.05$ as a selection criterion. The variable breed was forced into the model, despite not fulfilling the selection criterion, as it was supposed to be a risk factor in earlier reports (Hoffmann et al., 2010). Variables that were dependent on other variables (duration of glucocorticoid therapy and time after vaccination) were excluded from the multivariable analysis, as they would have substantially reduced the dataset. Due to missing data in the variables, the final dataset for the multivariable analysis contained complete data for 212 cats. The significance level was set at $\alpha = 0.05$.

Results

Antibody prevalence

Antibodies against FPV were not detectable in 29.4% (103/350; 95% CI, 24.6–34.2%) and were detectable in 70.6% (247/350; 95% CI, 65.8–75.4%) of cats. Of all 350 cats, 47 (13.4%) had been vaccinated adequately and 238 inadequately according to the current guidelines. Thus, only 47/285 (16.5%) vaccinated cats had been vaccinated following the new guidelines (Richards et al., 2006; Truyen et al., 2009; Day et al., 2010; Ständige Impfkommission Veterinär, 2013). Of those, 11/47 (23.4%) had no antibodies. However, antibodies were found in 8/28 cats (28.6%) that had never been vaccinated.

Associated factors

In the univariable analysis, the factors origin, exposure risk, neoplasia, glucocorticoid therapy, and vaccination status were all significantly associated with a lack of antibodies against feline panleukopenia (Table 1). The duration of glucocorticoid therapy was categorized as short (<11 weeks) and long ($\geqslant$11 weeks) by ROC analysis. A duration of glucocorticoid therapy $\geqslant$11 weeks was significantly associated with a lack of antibodies ($P = 0.024$).

The multivariable logistic regression analysis confirmed presence of neoplasia ($P = 0.010$), glucocorticoid therapy ($P = 0.016$), and vaccination status ($P < 0.001$) as associated factors. In addition, CKD was significantly associated with a lack of antibodies ($P = 0.037$). The factors origin and exposure risk were not retained in the multivariable logistic regression analysis (Table 1).

Discussion

In this study, 29.4% of the cats in this study had no antibodies detectable, indicating that they were not protected against infection with FPV at the time of presentation and at risk of acquiring a disease that is potentially life-threatening.

Former comparable studies have shown conflicting results. A comparatively high prevalence of antibodies was found in domestic cats in a metropolitan area of Costa Rica (92.8%) (Blanco et al., 2009), although only 17.8% of the cats were reported to have been previously vaccinated. Remarkably lower antibody prevalence rates (39.8%) were observed in cats entering a Florida animal shelter (DiGangi et al., 2012) but these were mainly young, sexually intact, healthy animals, and most (67%) were strays of unknown vaccination status. A low antibody prevalence was also found in a study of 15 separated rural populations of non-vaccinated cats in France with an average antibody prevalence of 25.0% (private cats 36.6% vs. stray cats 15.9%) (Hellard et al., 2011).

A study including 267 client-owned cats in the USA (Lappin et al., 2002), however, found an antibody prevalence of 67%, which is similar to the present study. Different vaccination rates between countries and study populations might in part explain these divergent results. In addition, differences in the frequency of natural