STUDIES ON THE SEROPREVALENCE, AGE, AND GENDER ON THE DISTRIBUTION OF FELINE CORONAVIRUS IN VAN CATS KEPT IN A MULTIPLE-CAT ENVIRONMENT

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Abstract

In the present study, we aimed to investigate for the first time the seroprevalence of feline coronaviruses (FCoV) in Van cats kept in a multiple-cat environment. A total of 24 male and 46 female Van cats aged between two months and nine years were used in the study. FCoV antibodies were determined using the commercial feline coronavirus antibody ELISA kit. The cats were examined clinically before blood sampling. No clinical signs of feline infectious peritonitis diseases were observed in the cats. But, out of the 70 cats, 38 (54.3%) showed seropositivity to FCoV. When the results were examined with regard to gender, 28 (60.9%) of female cats, and 10 (41.7%) of male cats were seropositive. When the results were examined with regard to age, out of the 18 cats aged between two months and one year, three (16%) were seropositive, out of the 10 cats aged between one to two years - eight (80%), out of the 27 cats aged between two to five years - 15 (55.5%), out of the 13 cats aged between five to eight years - 11 (84%), and all cats (two) aged over eight years showed seropositive reaction to FCoV.

Key words: Van cat, feline infectious peritonitis, feline coronavirus, antibodies.

Feline coronaviruses are members of the Coronaviridae family in the newly established Order of Nidovirales (16). The viruses have so far been identified and divided into five categories: feline coronavirus (FCoV), transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus (PRCV), canine coronavirus (CCV), and human coronaviruses (5). Of these categories mentioned above, FCoVs have been reported to have two biological sub-types classified as feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV). Various researchers have also shown that the virus, which is responsible for the development of FIP emerges as a mutant during the replication of the less pathogenic FECV (5, 6, 9, 22).

It has been well-established that the disease can be contracted through oral (10, 11), respiratory, and transplacental ways (12, 13). Saliva, blood, and droppings can also function as a store from which the potential agent can be transmitted (4) and the infected cats excrete the agent for months or even years (5, 11).

FIP infection has a clinical pattern characterised by a wide spectrum of non-specific clinical symptoms like fever, exacerbated pulse, increased respiratory rate, paleness of mucous membranes, lethargy, anorexia, weight loss, ocular lesions, neurological disorders, diarrhoea, and vomiting (1, 12-14).

In the diagnosis of the disease, clinical findings should be taken into consideration (8, 12). A laboratory diagnosis can be made by ELISA, indirect florescent antibody (IFA), and reverse transcriptase-polymerase chain reaction (RT-PCR) techniques (2-5, 10, 11, 14-16, 17). In addition to such techniques, necropsy, histological, and immunohistochemical (11) findings are also useful in the diagnosis of the disease (18).

The multiple-cat environment, in-bred conditions, and age have been reported to play an important role in the development and prevalence of the disease (1, 3, 12). Gender has also been reported to have a role in the distribution of the disease (3). Van cats are one of the endangered species of cat originating from the Eastern city of Van. Therefore, they are kept in the Van Cat Research Centre, which is a multiple-cat environment. They are also in-bred. For all these reasons; they might be susceptible to this disease. Thus, the objective of the present study was to determine the seroprevalence of FCoV in these cats.

Material and Methods

Cats. The study materials constituted of 24 male and 46 female healthy Van cats, aged between two months and nine years, accommodated in the Van Cat Research Centre, (initially, 40 individual cats were taken to this centre from different private houses), which was built in 1998 and which is responsible for protecting this endangered cat species. Now, about 150 cats are kept in this location. Still, cats whose owners cannot look after
them are accepted in the Centre from time to time. Male, female, and kittens are kept in different rooms. The feeding of these cats is by commercial feedingstuff. All the cats were vaccinated against feline calicivirus, feline herpesvirus, and feline panleukopenia. No vaccination had been made against feline coronaviruses.

**Clinical evaluation.** In the cats no clinical findings related to FCoV infection have ever been observed except for conjunctivitis and diarrhoea in a few cats both during and after the period in which the blood samples were taken. After routine clinical examinations of the subjects, 2 ml of blood samples from *V. jugularis* or *V. cephalica* were taken into tubes with no anticoagulant. The samples were kept at -20°C until the analysis.

**ELISA.** The ELISA was used in order to determine the FCoV antibodies. For this purpose, FCoV commercial kits (European Veterinary Laboratory, Diagnostic Division, feline coronavirus antibody ELISA, F1005-AB02) were used. Positive and negative control sera, together with the other sera from the suspected animals, were put into microstrips covered with FCoV antigens after being diluted 1:90, 1:270, and 1:810 as reported in the kit procedure. After the other stages were followed as mentioned in ELISA procedure, the results were recorded in 450 nm by the ELISA reader. OD values above 0.5 were considered positive.

The values obtained were assessed by a χ² test in terms of gender and age.

**Results**

**ELISA results.** Out of the 70 cats examined, 38 (54.3%) were found to be seropositive for FCoV antibodies. When analysed in terms of gender, 28 (60.9%) female cats and 10 (41.7%) male cats were found to be seropositive. The mean OD values were 1,036.26 ±78.81, 789.50 ±49.99, and 648.87 ±45.51 in 1:90, 1:270, and 1:810 dilutions, respectively. Based on the gender distribution, three of the 18 (16%) cats aged between two months and one year, eight of the 10 (80%) cats aged between one and two years, 15 (55.5%) of the 27 cats aged between two and five years, 11 (84%) of the 13 cats aged between five and eight years, and both of the two cats above eight years of age were found to be seropositive for FCoV antibodies. The number of animals showing seropositivity and the percentage of age and gender distributions are given in Table 1.

When the findings were statistically analysed with a χ² test, there were no significant differences between female and male cats in terms of seropositivity ($\chi^2$: 1.63 and P>0.05).

On the other hand, when the percentage of seropositivity of the age groups was compared, animals in the 2-month-1-year-old group had the lowest percentage ($\chi^2$: 19.50 and P<0.001).

<table>
<thead>
<tr>
<th>n</th>
<th>Seropositive (%)</th>
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<tbody>
<tr>
<td>Female</td>
<td>46</td>
</tr>
<tr>
<td>28</td>
<td>60.9</td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>41.7</td>
</tr>
<tr>
<td>2 month-1 year</td>
<td>18</td>
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<tr>
<td>3</td>
<td>16</td>
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<tr>
<td>1-2 years</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>2-5 years</td>
<td>27</td>
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<tr>
<td>15</td>
<td>55.5</td>
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<tr>
<td>5-8 years</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>84</td>
</tr>
<tr>
<td>&gt;8 years</td>
<td>2</td>
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<td>2</td>
<td>100</td>
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**Discussion**

In a study by Herrewegh et al. (12) on the prevalence of FIP infection in cats, it was demonstrated that a seropositivity rate was up to 86% by RT-PCR and to 71% by IFA tests in cats. Furthermore, Baneth et al. (1) reported seropositivity in about 60% of cats showing FIP symptoms. In addition, they also found seropositivity in up to 39%-85% in cats showing no FIP symptoms. Benetka et al. (3) observed in 37%-95% of cats viremia and in 73%-81% shedding of the virus in their droppings. In the present study, the percentage of seropositivity to FCoV was found to be 54.3% by ELISA, which was similar to the above findings. Although, the level of FCoV antibodies was found to be quite high in the present study, no clinical symptoms related to FIP infection were determined, which is in agreement with the reports made by Benetka et al. (3) and Posch et al. (21). The access to the Van Cat Research Centre by the cats whose owners could not look after them was allowed from time to time. Therefore, this situation might have some sort of effect on the high seroprevalence in the present study.

It has been reported that the closed multiple cat environment (1, 7, 8, 18), in-breeding (3, 8, 16), age (8, 16), and gender (3, 4, 20) play an important role in the aetiology and development of the disease. Foley et al. (8) studied the droppings of the multiple cat households and found 41% positivity to FCoV.

It has been reported by various researchers that FCoV has a higher prevalence among in-bred cat races. Benetka et al. (3) determined the prevalence of the disease in in-bred cats as 33.6%. In a study conducted by Kiss et al. (16) there was found 100% seropositivity in Persian cats kept in a cattery. In our study, the Van cats were also kept together for in-breeding and 54.3% were observed as being seropositive. But our study was not based on any comparative analysis of out-breeding of other races; therefore, such a comparison could not be made. However, our study supports any assumptions made. Our study supports any assumptions based on the fact that in-breeding and multiple cat environments give rise to the prevalence of the disease when compared with the results of the previous studies.

In a study conducted by Benetka et al. (3) it was reported that 52.1% of the cats under one year of age were seropositive. Similarly, Baneth et al. (1) found 75% of cats under one year of age, and 22% of cats over five years of age had FCoV antibodies. On the other
hand, Kiss et al. (16) determined 34.6% of seropositivity in cats less than one year of age; 31.6% of positivity in cats aged between one and five years, and 35.3% of positivity in cats over five years of age. In the present study, out of the 18 cats aged between two months and one year, three (16%) cats revealed seropositivity; whereas, eight (80%) out of the 10 cats aged between one and two years were found to be seropositive. Furthermore, 15 (55.5%) out of the 27 cats aged between two and five years were seropositive, 11 (84%) out of the 13 cats aged between five and eight years were seropositive, and two out of the two cats aged over eight years were seropositive. In general, the percentage obtained in the present study was lower compared to the literature results, except for the cats in the eight year and older group. Furthermore, in the cats aged two months or one year, the seropositivity was much lower than in the literature data. In the Van Cat Research Centre, newborn cats live in separate newborn small rooms for up to two months. Then, the kittens are transferred to multiple-cat environments. In the present study, the group of the youngest cats consisted of six kittens aged two months and eight kittens aged 2.5 months, which were just put into a multiple-cat environment. The other two were three and five months of age. If their mothers were not infected, the kittens’ contamination with the virus was much less. Furthermore, in the cats transferred to the multiple-cat environment a short while ago, the humoral response may not have developed yet. Therefore, the low seroprevalence in this group was most probably due to their age, since their contamination time is short and the humoral responses take time.

Kiss et al. (16) reported that gender was ineffective in the occurrence and development of FCoV infection, whereas Benetka et al. (3) pointed out that it may be effective in the aetiology of the disease. Binder and Hartmann (4), and Pollkay et al. (20) reported that FIP had a much higher prevalence in male cats than female ones. Benetka et al. (3) found that 62.4% of the male and 37.6% of the female cats was seropositive. However, there was no report about the reasons causing the male cats to be more predisposed to the disease in the above-mentioned study. In our study, 10 (41.7%) of the 24 male cats and 28 (60.9%) of the 46 female cats were found to be seropositive. This was contradictory to the findings given by Benetka et al. (3). Since wide spectrums of different male and female cats were used in this study, it may be much more tempting to have a statistical comparison; nonetheless, the difference was found to be insignificant statistically. In support of this premise, there are many researchers reporting that gender role is insignificant.

Finally, this is a novel study on the seroprevalence of FIP in the Van cats kept in a multiple-cat environment. The results obtained show that the probability of the development of FCoV infection in Van cats is quite high; therefore, protective precautions need to be taken.

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References