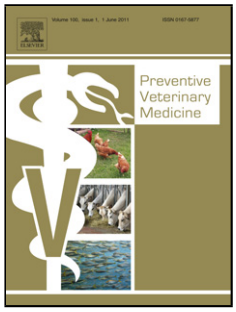

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Original article

Prevalence of serum antibody titres against feline panleukopenia, herpesvirus and calicivirus infections in stray cats of Milan, Italy

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HIGHLIGHTS

- In the majority of stray cats in Milan the seroprevalence for calicivirus is high whereas less than 50% of cats were seropositives against feline panleucopenia virus and herpesvirus
- The feline calicivirus represents the most common circulating pathogen in Milan, as observed in other studies worldwide
- An increase of antibody protective titres from kitten to senior is generally observed for the three pathogens

Abstract

The aim of the study was to determine the seroprevalence of feline panleukopenia virus (FPV), feline herpesvirus type 1 (FHV-1) and feline calicivirus (FCV) in stray colony cats from Milan, Italy. Cats were divided in groups based on age, gender, reproductive status, health status and colony of origin. Blood samples were tested with an in-clinic ELISA test. The possible presence of a link between the antibody titre or the presence of seropositive results and the independent variables (age, gender, reproductive status, health status and colony location) was assessed by means of multinomial and univariate logistic regression models, respectively. Seroprevalence of 85.4% was reported for FCV. The diffusion of the other two pathogens in the cat population was much lower compared to FCV, with 45.7% and 37.1% seroprevalence observed for FPV and FHV-1, respectively. An increase of antibody titres from kitten to senior was generally observed for the three pathogens. Age was a statistically significant variable for FHV-1, with senior cats significantly associated with higher antibody titres and higher percentages of seropositive animals compared to younger age groups. Neutered cats had significantly higher antibody titres and showed significantly higher FHV-1 seroprevalences compared to sexually intact cats. Colonies from two of the nine administrative districts of Milan showed significantly higher FPV seroprevalences compared to the other. No other significant differences were observed. Our results, based on cats belonging to 70 different colonies located in urban areas far from each other, suggest that the three viruses circulate in the feline population of stray cats in Milan. The feline calicivirus represents the most common circulating pathogen, as observed also in other studies worldwide. Finally, our results

suggest that stray cats may be not adequately protected against FPV, FHV-1 and FCV and vaccination could be a possible strategic solution, especially for FPV.

Keywords: stray cats; feline panleukopenia virus; feline herpesvirus; feline calicivirus; serum antibody titres; in-clinic ELISA test

Introduction

Feline panleukopenia virus (FPV), feline herpesvirus type 1 (FHV-1) and feline calicivirus (FCV) are three of the main infectious pathogens of the cats worldwide (Gaskell et al., 2012; Greene, 2012; ABCD, 2017a; ABCD, 2017b; ABCD, 2017c). FPV is a highly contagious and lethal virus that mainly affects kittens (3-5 months old) but also adult cats. Transmission occurs by direct (orofaecal) or indirect contact, due to its long persistence (1 year or more) in a contaminated environment. Clinical signs include diarrhoea with severe dehydration, lethargy, fever, anorexia and immunosuppression (Leisewitz, 2016; Greene, 2012; Horzinek et al., 2013; Stuetzer and Hartmann, 2014; Garigliany et al., 2016; ABCD, 2017c). FHV-1 is the etiologic agent of the feline viral rhinotracheitis. As for the other herpesviruses, recovered cats become latently infected carriers and stress and other conditions could lead to viral reactivation. Early signs of illness are depression, anorexia, drooling followed by sneezing, coughing, ocular and nasal discharge and severe conjunctivitis. In the environment the virus is labile (Afonso et al., 2016; Gaskell et al., 2012; Horzinek et al., 2013; ABCD, 2017b; Monne Rodriguez et al., 2017; Munks et al., 2017). FCV has a significant genetic variability responsible for different clinical patterns: oral ulcers, chronic stomatitis and respiratory symptoms. As FPV, FCV is highly resistant in the environment (Afonso et al., 2016; Gaskell et al., 2012; Battilani et al., 2013; Horzinek et al., 2013; ABCD, 2017a; Afonso et al., 2017). Together with FHV-1, FCV is involved in the pathogenesis of Upper Respiratory Tract

Disease (URTD) (Afonso et al., 2016; Horzinek et al., 2013; ABCD, 2017b; Monne Rodriguez et al., 2017).

These three pathogens are widespread worldwide in the cat population, with a higher prevalence in multicat households, shelters, and younger cats. Vaccination is a relatively successful measure in controlling such diseases and vaccines against FPV, FHV-1 and FCV are considered the core vaccines (Afonso et al., 2016; Leisewitz, 2016; Gaskell et al., 2012; Greene, 2012; Day et al., 2016; ABCD, 2017a; ABCD, 2017b; ABCD, 2017c).

In Italy, stray cats live free in small or big groups called "colonies", recognised and protected by National and Regional laws. During 2017, in the city of Milan (northern Italy) 6,039 stray cats from 877 cat colonies have been identified, ranging from 1 to 226 stray cats per colony, but probably these numbers are underestimated. Colony stray cats are captured by official Veterinary Service generally only for mandatory sterilisation procedures for the control of births, and consequently their health status and vaccination history are often unknown. Stray cats may socialise with owned cats that share the same territory. Therefore, it would be very important to obtain as much information as possible about their health status, in order to improve their health and wellness and, at the same time, to protect domestic cats.

Gold standard methods for the detection of antibodies against the three pathogens (haemagglutination inhibition assay for FPV and serum neutralisation assay for FHV and FCV) have to be performed in specialist diagnostic laboratories and are time-consuming. Recently, an in-clinic ELISA test has been validated, showing high diagnostic accuracy in comparison to the gold standard assay for the detection of antibodies against the three pathogens; this test can be performed in less than half an hour and can be applied in veterinary practice (Di Gangi et al., 2011, Mende et al., 2014b, Day et al., 2016).

The aim of this study was i) to evaluate the occurrence of antibodies against FPV, FHV-1 and FCV in stray colony cats living in Milan using a commercially available in-clinic ELISA test, ii) to correlate the antibody titres and the presence of seropositive results with independent variables (age,

gender, reproductive status, health status and colony location), and iii) to compare our results with worldwide available data.

Material and methods

Animals and sample collection

Colony stray cats were included in this study (approved by Ethic Committee of the University of Milan, approval number 137/2017). One hundred and fifty one cats were captured by veterinarians of the official Veterinary Service using one-door animal traps and were admitted to the official Veterinary Service of the Health Protection Agency (ATS) of Milan for mandatory sterilisation or medical reasons in the period January 2017-January 2018. Data and other important clinical information were recorded by the official veterinarians of the ATS of Milan for each cat: estimated age by dental method (Berman, 1974) (kittens [<1 year of age], adults [1-8 years], and seniors [>8 years]), gender and reproductive status (sexually intact or neutered), breed, health status (healthy or unhealthy with several clinical problems ranging from mild to severe), and colony location (grouped in the 9 administrative districts of Milan).

Blood samples (0.5 mL) were collected by jugular venipuncture from each anesthetised animal during sterilisation surgery. Samples were immediately centrifuged (1,000 x *g* for 10 min) and sera were separated and stored at -20 °C until analysis.

In-clinic ELISA test

Each serum sample was tested using an in-clinic ELISA test (Feline VacciCheck Antibody Test Kit, Biogal Galed Labs, Israel[1]), following the manufacturer's instruction. The kit is a rapid dot-ELISA-based system licensed to determine the titre of antibodies against FPV, FHV-1 and FCV. The test has been previously validated showing good values of specificity and sensitivity for each pathogen

[1] supplied in Italy by Agrolabo

and can be applied in practice, as indicated in the WSAVA guidelines (DiGangi et al., 2011; Mende et al., 2014b; Day et al., 2016).

The concentration of specific antibodies in serum samples was defined by the colour intensity of the spots measured in 'S' units, on a scale from 0 to 6. An S value of 0 was standardised by the manufacturer as being equivalent to an antibody titre of <1:20 for FPV, <1:4 for FHV-1 and <1:8 for FCV. An S value of 3 (S3) was standardised by the manufacturer to be the equivalence of 1:80 for FPV, 1:16 for FHV-1 and 1:32 for FCV. Following previous studies that validated the kit for the detection of antibodies against FPV, FHV-1 and FCV, antibody titres equal or higher than S3 values were considered indicative of a significant positive response (DiGangi et al., 2011; Mende et al., 2014b) .

Data analysis

The possible presence of a link between the antibody titre and each factor (age, gender, reproductive status, health status, and colony location) was assessed by means of a multinomial logistic regression model. Goodness of fit was assessed by Chi square test and P >0.05 suggested an adequate model fit. Univariate logistic regression models were assessed to investigate associations between seropositive results and each separate factor (age, gender, reproductive status, health status, and colony location). Model fit was evaluated by Hosmer-Lemeshow goodness-of-fit test and P >0.05 indicated an adequate model fit. Odds ratios (OR) and 95% Confidence Interval (CI) were calculated. Statistical analysis were performed using statistical software SPSS (version 24). A P value <0.05 was considered as statistically significant.

Results and discussion

Animals

Of the 151 stray cats analysed in this study, 91 cats were females (73 sexually intact and 18 neutered) and 60 were males (45 sexually intact and 15 neutered). Fifteen cats were kittens, 123

adults, and 13 seniors. The median age was 3,3 years (5 months-16 years). All were cross-breed domestic cats. One-hundred and seven cats were healthy, while the others (44) had one or more clinical problems (above all cachexia, lymphadenopathy, chronic stomatitis, dehydration). Cats were captured from 70 colonies. Colonies were far from each other, distributed across the urban area and were from all 9 administrative districts of Milan (Figure 1). Sample size of colonies ranged from 1 to 66 cats, with female cats representing 25% to 100% of cats of the colony. The number of stray cats captured per colony and analysed in this study ranged from 1 to 11 cats. The vaccination history of cats was unknown and because vaccines was not administered to captured cats by the official Veterinarians during mandatory sterilisation procedures it was assumed that cats probably had never been vaccinated.

Positive samples and antibody titres

Serum antibody titres ranged from <1:20 to >1:640 for FPV, from <1:4 to >1:128 for FHV-1 and from <1:8 to >1:256 for FCV (Figure 2). Seropositive results for FPV, FHV-1 and FCV were observed in 69 (45.7%), 56 (37.1%) and 129 (85.4%) cats, respectively. Results confirm previous findings indicating a higher distribution and a worldwide seroprevalence of FCV in cats compared to FPV and FHV-1 (see below, "Comparison with other studies concerning stray cats"). This is probably due to FCV ability to mutate and escape the host immune response, the easy transmission with oronasal and conjunctival secretions, the carrier status of infected cats, the social behavior in the studied populations, along with its high resistance in the environment (Afonso et al., 2016; Gaskell et al., 2012; Battilani et al., 2013; Horzinek et al., 2013; ABCD, 2017a; Afonso et al., 2017). The resistance of FPV in the environment is probably responsible for the exposure and seropositivity observed in cats. However, cats can also be infected by canine parvovirus (CPV), that has been recently included with FPV in the unique species Carnivore Protoparvovirus-1 (Cotmore et al., 2014). Due to cross-reaction, CPV infected cats produce antibodies against CPV indistinguishable from those against FPV (Mende et al., 2014a, Decaro et al., 2010). Consequently,

our FPV positivity could be due to the cross-reactivity versus CPV, a very common virus widespread in the canine population in Italy. Despite the good sensitivity and specificity reported for the in-clinic ELISA test, along with its convenience and ease of use, it has to be taken into account that the accuracy of the test is lower compared to the gold standard assays, especially for FPV as reported by Mende et al. (2014b), with sensitivity ranging from 79% to 87%). Therefore, FPV seroprevalence may have been underestimated and confirmatory testing for FPV seropositive results using gold standard assays may be indicated to confirm the results of this study.

With regard to disease risk, clinical signs of FPV and high mortality rates have not been observed in colony stray cats of Milan. An excellent correlation between the presence of serum antibodies and resistance to infection has been well documented for FPV, with antibodies protecting the cat against clinical signs and strongly reducing viral excretion (Lappin et al. 2002; Day et al., 2016). Indeed, the consistency of the in-clinic test with the gold standard HI test for the detection of FPV antibodies suggests that the presence of antibodies against FPV may be a correlate of protection in the 45.7% seropositive cats in Milan and vaccination of seropositive cats would be unnecessary to control the infection (Mende et al., 2014b). However, more than half of the stray cat population of Milan was seronegative and may be at risk of disease. Considering that FPV seroprevalence may have been underestimated in the stray cat population of Milan, not all seronegative cats are at risk of disease. Indeed, FPV false negative cats would be protected against FPV by the presence of FPV antibodies (not detected by the test) and would help limit disease transmission. Despite FPV prevalence may have been underestimated, it is generally acknowledged that populations with seroprevalence <70% are at risk of epizootic disease and vaccination of seronegative cats is suggested (Stuetzer and Hartmann., 2014, Day et al., 2016). Therefore, vaccination may be recommended for FPV in seronegative colony stray cats of Milan during the hospitalisation after the mandatory sterilisation procedures, and the in-clinic ELISA test would permit to rapidly identify these kind of cats. For FHV-1 and FCV, the correlation between serum antibody and protection is less relevant than the presence of an adequate cell-mediated and mucosal immunity or neutralising

antibodies, respectively. For this reason, a negative antibody result against FHV-1 or FCV occurring in single cat should not be necessarily considered as indicative of lack of protection as well as a seropositive result should not be systematically indicative of protection (Lappin et al. 2002; Gaskell et al., 2012; ABCD, 2017a; ABCD, 2017b; Day et al., 2016). Indeed, even if a good consistency between neutralising antibodies titres and the in-clinic ELISA test may be expected for FCV antibodies, FCV is antigenically highly variable and the detection of antibodies against FCV antigen present in the kit does not necessarily indicate that the antibodies are able to cross-react with the circulating FCV field variants, although they generally show some cross-reactivity (ABCD, 2017a, Afonso et al., 2017). In this respect, the high FCV seroprevalence reported in this study may reflect a strong circulation of the virus in the stray cat population due to viral excretion by infected cats. Considering protection of cats in terms of reduction of viral shedding, in addition to reduction of clinical signs, our result suggest that the FCV immunity in the stray cat population is not optimal for infection control.

Overall, our results suggest that the cat population may not be well protected against FPV, FHV-1 and FCV, in terms of reduction of viral shedding and not only reduction of clinical signs.

On the whole, the lower level of seropositive cats for FPV in cats is worrying considering the high mortality of FPV compared to FHV-1 and FCV, generally causing a mild syndrome, if not complicated by immunosuppressive viruses (e.g., Feline Immunodeficiency Virus [FIV], Feline Leukemia Virus [FeLV]) or secondary bacterial infections (Gaskell et al., 2012; Greene, 2012; ABCD, 2017a; ABCD, 2017b; ABCD, 2017c).

Number and percentage of cats with specific antibody titres for each pathogen and categories are reported in Table 1. Number and percentage of seropositive cats for each pathogen and for all categories are reported in Table 2.

Age

For all the pathogens, and especially for FHV-1, an increase of antibody titres from kittens to seniors was observed.

Antibody titres were statistically different among age groups for FHV-1 (P=0.002). Higher antibody titres against FHV-1 (1:32) were more likely observed in senior cats compared to younger ones where lower antibody titres were more likely to be observed. The model fit in the multivariable analysis was sufficient (Chi square test P>0.05).

Presence of seropositive animals was significantly different among age groups for FPV and FHV-1 (P=0.018 for FPV, P=0.002 for FHV-1). The model fit in the univariate analysis was adequate for these two pathogens (Hosmer–Lemeshow goodness-of-fit P>0.05). Univariate analysis showed that kittens and adults had a lower probability of being FPV seropositive compared to senior cats (kittens vs senior cats odds ratio 0.075, 95% CI 0.012-0.457; adult vs senior cats odds ratio 0.315, 95% CI 0.08-1.26). Similarly, kittens and adults had a significantly lower probability of being FHV-1 seropositive compared to senior cats (kittens vs senior cats odds ratio 0.046, 95% CI, 0.006-0.331; adult vs senior cats odds ratio 0.251, 95% CI, 0.066-0.956). Also for FCV, senior cats showed higher FCV seroprevalence compared to other age categories but the result was not statistically significant.

The antibody titres and the percentages of seropositive cats significantly higher in the elderly suggest that age could influence the probability of encountering these viruses and therefore older animals are more likely to become seropositive with higher antibody titres (Day, 2007; Day and Schultz, 2014; Tizard, 2017).

Gender and reproductive status

Both females and males showed higher FCV seroprevalences compared to FPV and FHV-1. The percentage of seropositive males was lower than females for the three pathogens, but this result was not statistically significant. Females could have a more effective immune response due to gonadotropic hormones whereas testosterone could have an immune suppressive effect (Klein,

2000; Bilbo and Nelson, 2001; Mansfield et al., 2004; Ansar Ahmed et al., 1985). Moreover, male cats, due to their fighting and biting behavior, are more likely to be FIV-positive and therefore less immune reactive. Previous studies (Spada et al., 2012) indicated that the overall prevalence of FIV infection in colony stray cats of Milan was 6.6%, and adult age and male gender were significant predictors of FIV seropositivity.

The antibody titres in cats were higher in the neutered group for FHV-1. The neutered status was a statistically significant variable for FHV-1 antibody titres (P=0.0008). Neutered cats were more likely to have antibody titres higher (1:32) than intact cats (<1:4). The model fit in the multivariable analysis was sufficient (Chi square test P>0.05). Neutered status was a significant variable also for FHV-1 seropositive results (P=0.0001) and the model fit in the univariate analysis was adequate (Hosmer–Lemeshow goodness-of-fit P>0.05), with neutered cats that were more likely to be seropositive (odds ratio 5.45, 95% CI 2.261-13.141) compared to intact cats. This is probably a bias due to the older age of the neutered cats (mostly adults and seniors) and to the lower immunogenicity of FHV-1, which requires a longer time for the development of a strong immune response (Munks et al., 2017).

Health status

Once again, seroprevalences in both healthy and unhealthy cats were higher for FCV compared to FPV and FHV-1. No significant differences were observed between the two groups, indicating that the three pathogens were probably not associated to the diseases observed in the unhealthy group. However, with regard only to unhealthy cats with chronic stomatitis (n=14), all cats (100%) were seropositive for FCV (14/14), whereas 64.3% (9/14) and 57.1% (8/14) were seropositive for FHV-1 and FPV, respectively. Indeed, chronic stomatitis is associated with FCV infection (ABCD, 2017a, Afonso et al., 2017).

Colonies

The examined stray cats belonged to 70 different colonies located across urban area far from each other with no contact among cats living in different colonies. In colonies where ≥3 cats were sampled (13), cats were generally seropositive against all three pathogens. In few of these colonies no cats resulted seropositive for FPV and FHV-1 (2 colonies from administrative district n. 5) or for FHV-1 alone (1 colony from administrative district n. 5 and 2 colonies from administrative district n. 7): it was then assumed that that pathogen was not circulating there.

FPV seropositive results were significantly different among cats from the 9 administrative districts of Milan (P=0.048). FPV seropositive results were more likely observed in stray cats from colonies of administrative district n. 4 and n.7 compared to cats from other administrative districts. An equal distribution of seropositive results in cats within the whole urban area was expected, as no differences in colonies of different administrative districts have been reported. This result is probably a bias due to the high presence of senior cats, that are more likely to be seropositive, from administrative district n. 7 or to the absence of kittens in colonies of administrative district n. 4, that are more likely to be seronegative, compared to cats from other administrative districts. However, despite results of statistical analysis, in our opinion it is more likely that each pathogen is equally distributed within the whole urban area. Further investigations are needed to better understand this point.

Comparison with other studies concerning stray cats

To the best of our knowledge, only few other studies on seroprevalence of these three diseases in stray cats have been published (Table 3) and confirm the results of our study (Coman et al., 1981; Yamaguchi et al., 1996; Nakamura et al., 1999; Ostrowski et al., 2003; Fischer et al., 2007; Levy et al., 2008; Hellard et al., 2011; DiGangi et al., 2012). Most cats of these studies had low or no antibodies for FHV-1 and in a lesser extent for FPV, whereas presence of antibodies against FCV was reported worldwide. The different seroprevalence reported in the previous studies probably reflect a different distribution of these pathogens among the feline population. Although it is not

possible to exclude that some cats may have received a vaccination in the past, the seropositivity reported worldwide suggested a natural exposure to these three viruses.

Conclusion

Our study reports that the most widespread virus in the colony stray cat population of Milan is FCV, followed by FPV and FHV-1, as previously observed in other studies worldwide. Since it is unlikely that the presence of antibody titres depends on vaccination of colony stray cats, that presumably have never been vaccinated, our results demonstrate the wide distribution of these pathogens within the cat colony population of Milan. Although sensitivity and specificity of the dot-ELISA test is not 100%, and the use of gold standard detection methods are important to confirm our observations, the results of our study suggest that colony cat populations living in Milan are not adequately protected against feline panleukopenia, feline viral rhinotracheitis and feline calicivirosis. In stray cat populations of Milan, where immunity following natural infection is not sufficient for adequate protection, vaccination protocols should be considered, especially for FPV. It would be very interesting to periodically repeat similar surveys on the colony stray cat population of Milan in order to monitor these very dangerous viruses.

Declaration of interest

None

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Figure 1. Stray cat colony location and size of sampling in the 9 administrative districts of the city of Milan. Italy

Figure 2. Serum antibody titres for feline panleukopenia (FPV), feline viral rhinotracheitis (FHV-1) and calicivirosis (FCV) detected using in-clinic ELISA test in 151 stray cats from Milan, Italy.

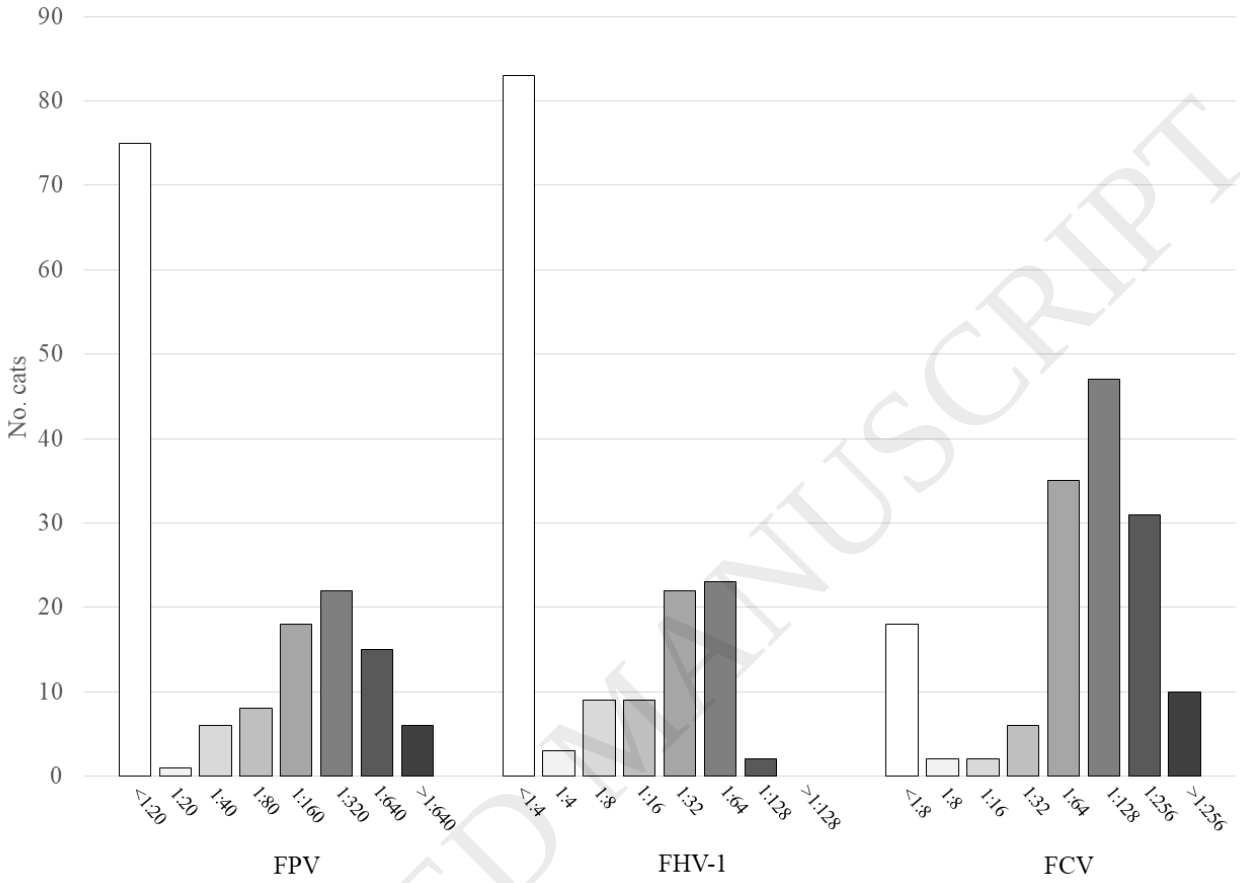

Table 1. Percentages of specific antibody titres for feline panleukopenia (FPV), feline viral rhinotracheitis (FHV-1) and calicivirosis (FCV) detected using in-clinic ELISA test and according to age, gender, reproductive status and health status in 151 stray cats from Milan, Italy.

	Age				**Gender**		**Reproductive status**		**Health status**	
	Kitten		**Adult**	**Senior**	**Female**	**Male**	**Intact**	**Neutered**	**Healthy**	**Unhealthy**
FPV	% of cats (No. of cats)									
<1:20	80.0 (12)		48.7 (60)	23.1 (3)	50.5 (46)	48.3 (29)	52.5 (62)	39.4 (13)	48.6 (52)	52.3 (23)
1:20	0.0 (0)		0.8 (1)	0.0 (0)	1.1 (1)	0.0 (0)	0.8 (1)	0.0 (0)	0.9 (1)	0.0 (0)
1:40	0.0 (0)		4.8 (6)	0.0 (0)	4.4 (4)	3.3 (2)	3.4 (4)	6.1 (2)	3.7 (4)	4.5 (2)
*1:80**	0.0 (0)		5.6 (7)	7.7 (1)	3.3 (3)	8.3 (5)	4.2 (5)	9.1 (3)	5.6 (6)	4.5 (2)
1:160	0.0 (0)		10.5 (13)	38.5 (5)	9.9 (9)	15.0 (9)	8.5 (10)	24.2 (8)	10.3 (11)	15.9 (7)
1:320	6.7 (1)		15.4 (19)	15.4 (2)	17.6 (16)	10.0 (6)	14.4 (17)	15.2 (5)	15.0 (16)	13.6 (6)
1:640	6.7 (1)		9.7 (12)	15.4 (2)	12.1 (11)	6.7 (4)	11.9 (14)	3.0 (1)	10.3 (11)	9.1 (4)
>1:640	6.7 (1)		4.0 (5)	0.0 (0)	1.1 (1)	8.3 (5)	4.2 (5)	3.0 (1)	5.6 (6)	0.0 (0)
Total	*100 (15)*		*100 (123)*	*100 (13)*	*100 (91)*	*100 (60)*	*100 (118)*	*100 (33)*	*100 (107)*	*100 (44)*
FHV-1										
<1:4	86.7 (13)		54.4 (67)	23.1 (3)	51.6 (47)	60.0(36)	63.6 (75)	24.2 (8)	61.7 (66)	38.6 (17)
1:4	6.7 (1)		3.6 (2)	0.0 (0)	1.1 (1)	3.3 (2)	2.5 (3)	0.0 (0)	1.9 (2)	2.3 (1)
1:8	0.0 (0)		5.6 (7)	15.4 (2)	6.6 (6)	5.0 (3)	5.1 (6)	9.1 (3)	3.7 (4)	11.4 (5)
*1:16**	0.0 (0)		7.3 (9)	0.0 (0)	4.4 (4)	8.3 (5)	4.2 (5)	12.1 (4)	4.7 (5)	9.1 (4)
1:32	6.7 (1)		13.8 (17)	30.8 (4)	16.5 (15)	11.7 (7)	11.0 (13)	27.3 (9)	12.1 (13)	20.5 (9)
1:64	0.0 (0)		17.0 (21)	15.4 (2)	18.7 (17)	10.0 (6)	13.6 (16)	21.2 (7)	15.0 (16)	15.9 (7)
1:128	0.0 (0)		0.0 (0)	15.4 (2)	1.1 (1)	1.7 (1)	0.0 (0)	6.1 (2)	0.9 (1)	2.3 (1)
>1:128	0.0 (0)		0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Total	*100 (15)*		*100 (123)*	*100 (13)*	*100 (91)*	*100 (60)*	*100 (118)*	*100 (33)*	*100 (107)*	*100 (44)*
FCV										
<1:8	33.3 (5)		10.5 (13)	0.0 (0)	8.8 (8)	16.7 (10)	11.9 (14)	12.1 (4)	13.1 (14)	9.1 (4)
1:8	6.7 (1)		0.8 (1)	0.0 (0)	2.2 (2)	0.0 (0)	1.7 (2)	0.0 (0)	1.9 (2)	0.0 (0)
1:16	0.0 (0)		1.6 (2)	0.0 (0)	1.1 (1)	1.7 (1)	1.7 (2)	0.0 (0)	1.9 (2)	0.0 (0)
*1:32**	0.0 (0)		4.0 (5)	7.7 (1)	3.3 (3)	5.0 (3)	4.2 (5)	3.0 (1)	3.7 (4)	4.5 (2)
1:64	26.7 (4)		22.7 (28)	23.1 (3)	20.9 (19)	26.7 (16)	22.9 (27)	24.2 (8)	22.4 (24)	25.0 (11)
1:128	6.7 (1)		30.8 (38)	61.5 (8)	34.1 (31)	26.7 (16)	28.8 (34)	39.4 (13)	29.9 (32)	34.1 (15)
1:256	20.0 (3)		21.9 (27)	7.7 (1)	23.1 (21)	16.7 (10)	22.9 (27)	12.1 (4)	19.6 (21)	22.7 (10)
>1:256	6.7 (1)		7.3 (9)	0.0 (0)	6.6 (6)	6.7 (4)	5.9 (7)	9.1 (3)	7.5 (8)	4.5 (2)

Total	*100 (15)*		*100 (123)*	*100 (13)*	*100 (91)*	*100 (60)*	*100 (118)*	*100 (33)*	*100 (107)*	*100 (44)*

* Antibody titres equal or higher than asterisk values were considered a significant positive response

Table 2. Percentages of cats with seropositive results for feline panleukopenia (FPV), feline viral rhinotracheitis (FHV-1) and calicivirosis (FCV) detected using in-clinic ELISA test and according to age, gender, reproductive status, health status and colony location in 151 stray cats from Milan, Italy

		% of seropositive cats (No. of cats)		
	No. cats	FPV	FHV-1	FCV
All cats	151	45.7% (69)	37.1% (56)	85.4% (129)
Age				
Kittens (<1 year old)	15	20% (3)	6.7% (1)	60% (9)
Adults (1-8 years old)	123	45.5% (56)	38.2% (47)	86.9% (107)
Seniors (>8 years old)	13	76.9% (10)	61.5% (8)	100% (13)
Gender				
Female	91	44.0% (40)	40.7% (37)	87.9% (80)
Male	60	48.3% (29)	31.7% (19)	81.7% (49)
Reproductive status				
Intact	118	43.2% (51)	28.8% (34)	84.7% (100)
Neutered	33	54.5% (18)	66.7% (22)	87.9% (29)
Health status				
Healthy	107	46.7% (50)	32.7% (35)	83.2% (89)
Unhealthy	44	43.2% (19)	47.7% (21)	90.9% (40)
Colony location (No. of colonies)				
Administrative district 1 (1)	2	50% (1)	50% (1)	100% (2)
Administrative district 2 (3)	10	50% (5)	30% (3)	70% (7)
Administrative district 3 (4)	6	50% (3)	33.3% (2)	83.3% (5)
Administrative district 4 (12)	21	61.9% (13)	52.4% (11)	76.2% (16)
Administrative district 5 (16)	44	29.5% (13)	36.4% (16)	95.5% (42)
Administrative district 6 (8)	13	23.1% (3)	7.7% (1)	61.5% (8)
Administrative district 7 (12)	27	70.4% (19)	33.3% (9)	85.2% (23)
Administrative district 8 (11)	11	36.4% (4)	36.4% (4)	100% (11)
Administrative district 9 (17)	17	47.1% (8)	52.9% (9)	88.2% (15)

Table 3. Percentages of stray cats with seropositive results against panleukopenia (FPV), feline viral rhinotracheitis (FHV-1) and calicivirosis (FCV) detected in other studies worldwide.

			% of seropositive cats (No. of cats)		
	Country	No. cats	FPV	FHV-1	FCV
Coman et al. (1981)	Australia	300	79% (237)	11% (33)	77% (231)
Yamaguchi et al. (1996)	UK	45	96% (43)	100% (45)	100% (45)
Nakamura et al. (1999)	Vietnam	50	44% (22)	44% (22)	74% (37)
Ostrowski et al. (2003)	Saudi Arabia	13	8% (1)	15% (2)	39% (5)
Fischer et al. (2007)	Florida	61	33% (20)	21% (13)	64% (39)
Levy et al. (2008)	Galapagos	52	67% (35)	10% (5)	44% (23)
Hellard et al. (2011)	France	469	24.95% (117)	61.83% (290)	82.66% (386)
DiGangi et al. (2012)	Florida	347	39.8% (138)	11% (38)	36.6% (127)
Our study (2018)	Italy	151	45.6% (69)	37% (56)	85.4% (129)