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RESEARCH ARTICLE

Seroprevalence and Risk Factor Association of Feline Immunodeficiency Virus in Cats

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ABSTRACT

Feline immunodeficiency virus (FIV) causes immunocompromising diseases in both domestic and wild cat species *(Felis catus)* around the world. Many characteristics of cats can affect the uptake of this virus including health status, age, gender, environment, lifestyle etc. The diagnosis of FIV can be confirmed by detecting antibodies in serum/plasma samples of infected cats. Despite its importance, the status of FIV infection is still poorly known in Pakistan. Therefore, the present study was devised to investigate the seroprevalence of FIV in Faisalabad, Pakistan. Blood samples of cats (n=90) were collected from outdoor facility of Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad and sera samples were analyzed for the presence of antibodies against FIV by ELISA Kit (Agrolabo S.p.A, Italy). The ELISA based results obtained by measuring the optical density of the substrate utilization showed the overall prevalence of FIV in cats as 28.9% in Faisalabad with a prevalence of 33.9% in male cats and 20.6% in female cats. The overall seropositivity for FIV was higher in adult cats (30.9%) than in young cats (22.7%). Risk factor association by Odd's ratio revealed that the disease is positively associated with adult age and male gender in cats. In conclusion, the factors of age (adult) and gender (male) were found to be the positively correlated (P=0.0164, OR=2.37, CI=0.74-7.55, RR=1.28, CI=0.94-1.73) with the presence of anti-FIV antibodies in the studied cats indicating the status of FIV infection in cats.

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INTRODUCTION

Feline immunodeficiency virus (FIV) belongs to the family *Retroviridae* genus *Lentivirus* and is among the most common infectious agents of immunosuppression in felids. It was first isolated and identified in 1986 from an outbreak of immunodeficient cats in Petaluma, California, USA. Due to its slow replication cycle, microbiologists categorize FIV as lentivirus (slow virus). Importantly, it resembles to human immunodeficiency virus (HIV) and causes syndrome by depleting $CD4^+$ T-lymphocytes in domestic as well as wild cats (Tompkins and Tompkins, 2008; Moench, 2014). Management and housing conditions may affect the progression of the immunodeficiency disease in cats (Bęczkowski *et al*., 2015). Due to their resembling morphological and pathogenetic characteristics, FIV may act as an animal model for the study of HIV (Bienzle, 2014).

Specific surface proteins and receptors of the virus on the host cells, respectively, describe the cell tropism and pathogenesis of the viral diseases (Lecollinet and Richardson, 2008; Willett and Hosie, 2008). After receptor-facilitated entrance in various immune cells, RNA of FIV undergoes reverse transcription and the resulting DNA is integrated into the host genome, now known as provirus. Like other proviruses of the family, genome of FIV contains three most important genes along with accessory genes, envelope (*env*), polymerase (*pol*), and group specific antigen (*gag*). The *env* gene codes the viral glycoprotein (gp120) and the transmembrane protein (gp41), the *pol* gene codes the capsid protein p24 and the *gag* gene codes for enzymes used in different purposes like replication (Dunham and Graham, 2008). Translation of transcribed products is increased by ORF (open reading frame), which is transactivator of FIV but ORF upholds both transcription and translation in different methods as compared to the other lentiviruses (Chatterji *et al*., 2002).

The progression of the disease makes the infected cats vulnerable to different opportunistic infections (Kang *et al*., 2014). The diseases caused by FIV can prevail both in free roaming and domestic feline species. The transmission of FIV is usually associated with bites from infected feline species through saliva. Infected mothers can also transfer virus to young ones by infected milk (Sellon and Hartmann, 2006). A very good agreement of detection of FIV with ELISA and PCR has been reported in a recent study reported by Nichols *et al*. (2017). Retroviral vaccines are being evaluated in different field studies but efficacy of FeLV and FIV vaccine (Fel-O-Vax FIV®) is still under doubtable concerns (Westman *et al*., 2016). Although, there are several studies carried out to investigate the prevalence of FIV in different countries, but a very little is known about the status of FIV prevalence in felids in Pakistan. The present study was designed to investigate seroprevalence of FIV infections in domestic cat population in Faisalabad, Pakistan and to evaluate the possible risk factors associated with this retroviral infection in cats.

MATERIALS AND METHODS

Study area: This study was designed to observe seroprevalence of FIV infection in cats in Faisalabad, Pakistan. The cat population can be distinguished into different groups depending upon their residence *i.e.* free-roaming or stray cats, wild cats, and domestic cats or the cats kept as pets in homes. Most of the cat population in Faisalabad, Pakistan like other major cities comprises of the pet cats and free-roaming or stray cats. The study was conducted in compliance with the Institutional Bioethics Committee (IBC), UAF.

Sample collection: Blood samples were collected from domestic cat patients coming to outdoor facility of Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad. Blood samples (1-3 mL) from diseased domestic cats were collected from either jugular or cephalic veins and were further processed to collect the serum. A total of 90 serum samples (n=90) of cats were processed for the detection of FIV antibodies. Among these samples, 68 were from male and 32 were from female cats. Furthermore, the samples were divided into two age groups; young (≥1year) and adult (<1 year). A total of 22 samples (15 males and 7 females) were from group young, and 68 samples (43 males and 25 females) were from group adult.

Detection of FIV antibodies by ELISA: The proteins p24 and p17 are core proteins of FIV and infected cats produce antibodies against these two viral expression proteins. The detection of antibodies against these proteins in cat serum samples confirms the detection of FIV infection. The antibodies against p24/p17 were detected in cat serum samples using an indirect enzyme linked immunosorbent assay (indirect ELISA) kit (FIV Ab ELISA 96, Agrolabo S.p.A, Italy). The utilization of the substrate by peroxide conjugated secondary antibodies was observed by detecting optical density (at 450nm), which showed the presence of FIV antibodies in feline sera as described previously (Little *et al*., 2009).

RESULTS

In the present study, 90 feline serum samples were processed to detect antibodies against FIV. The ELISA test was performed in triplicate including negative and positive controls as per supplier's instruction. The optical density was measured at 450 nm and cut off value for positive control was calculated as 0.97. Our results revealed that out of the 90 cats tested, 28.9% (26/90; 95% CI=20.5–38.9) were positive for FIV antibody (Fig. 1, Column 1). Risk for FIV seropositive status is significantly higher in male cats (33.9%) than female cats (20.6%) (Fig. 1, Column 2 and 3). In this study, the association of FIV positive cats with their age and sex was observed. The seropositivity of FIV was more frequent in adult cats (<1-year-old) than young cats (≥1-year-old), (Fig. 2, Column 1 and 4). Additionally, among the age groups, male cats are more likely to be test positive than female cats (Fig. 2, Column 2 *vs* 5 and Column 3 *vs* 6) in both young and adults. However, the overall seropositivity for FIV was higher in adult cats (30.9%) than young cats (22.7%) as shown in Fig. 2. The statistical analysis of FIV seroprevalence in cats and association of risk factors (age, sex) were determined (Odd's ratio and Relative Risk) and presented in Table 1. Briefly, the risk factor association revealed that the disease was positively associated with adult aged cats (p value=0.0017, OR=1.52, CI=0.50-4.67, RR=1.12, CI=0.85-1.48) and male gender (p value=0.0186, OR=1.98, CI=0.73-5.38, RR=1.20, CI=0.93-1.55) as compared to female cats (Table 1). The adult age and male gender were found to be positively correlated (p value=0.0164) factors for the persistence of anti-FIV antibodies or FIV infection in the studied cats (Table 1).

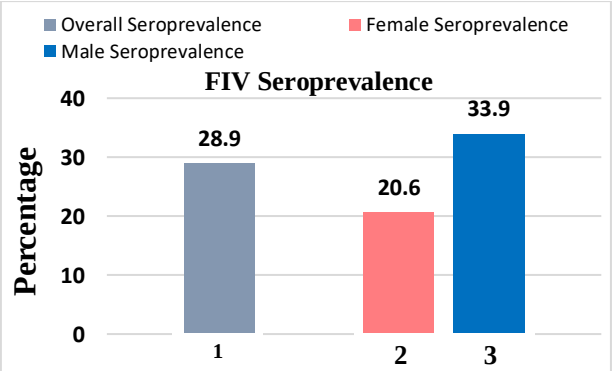

Fig. 1: Overall and sex-based seroprevalence of FIV in cats (n=90) by ELISA.

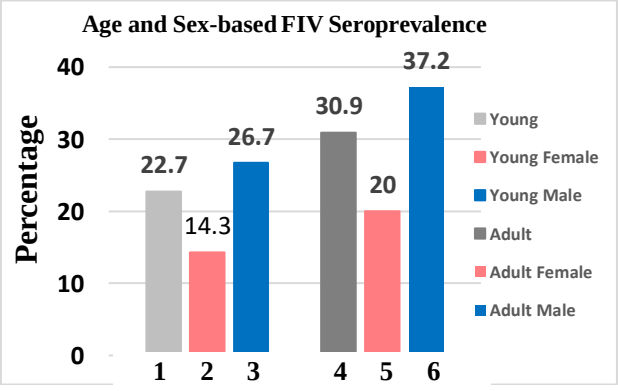

Fig. 2: Age and sex-based seroprevalence of FIV in cats (n=90) by ELISA.

Table 1: Results of chi-square and univariate logistic regression analyses for risk of FIV in cats

Factor	Category	Prevalence (%)	P value	OR	95% CI	RR	95% CI
Sex	Female	7/34 (20.6)	*Ref*	*NA*	*NA*	*NA*	*NA*
	Male	19/56 (33.9)	0.0186*	1.98	0.73-5.38	1.20	0.93-1.55
Age	Young	5/22 (22.7)	*Ref*	*NA*	*NA*	*NA*	*NA*
	Adult	21/68 (30.9)	0.0017*	1.52	0.50-4.67	1.12	0.85-1.48
Young & Sex	Female	1/7 (14.3)	*Ref*	*NA*	*NA*	*NA*	*NA*
	Male	4/15 (26.7)	0.1797	2.18	0.20-24.21	1.17	0.76-1.79
Adult & Sex	Female	5/25 (20.0)	*Ref*	*NA*	*NA*	*NA*	*NA*
	Male	16/43 (37.2)	0.0164*	2.37	0.74-7.55	1.28	0.94-1.73

OR=Odd's ratio; RR=Relative Risk; CI=confidence interval; *NA*=not applicable; *=statistically significant difference; *Ref*=Reference category.

DISCUSSION

American Association of Feline Practitioners (AAFP) recommends that all cats should be tested for FIV when they are acquired as pets or when they are exposed to cats known to be infected with FIV and their level of FIV infectivity is unknown or when they become ill regardless of previous test results (Levy *et al*., 2001).

Seroprevalence of FIV has been reported in various studies in different countries. In a previous study in Malaysia, cats were tested for the prevalence of FIV antibody and FeLV antigen in correlation with different risk factors. It was reported that 31.3% of cats were seropositive for FIV antibody (Bande *et al*, 2012). We also found a high seroprevalence (28.9%) of FIV in the studied cats in Faisalabad, Pakistan. Furthermore, it was also reported that seroprevalence was significantly higher in male and adult cats (Bande *et al.*, 2012). In concurrence, our results also showed that chances of seropositivity of FIV antibody are significantly higher in male and adult cats.

Cong *et al.* (2016) conducted a study to investigate the prevalence and relative risk factors for various diseases including FIV infection in stray and pet cats in China. It was concluded that seroprevalence of FIV antibody was 9.12%. Conversely to current study, there was a non-significant difference in seroprevalence in male and female cats (9.0 and 9.27%). As the samples were taken from cats visiting the veterinary clinical hospital, this may lead to higher seroprevalence of FIV antibody in the current study, as reported by Chhetri *et al.* (2015). However, it has also been observed that chances of detection of FIV antibody increases with increase in age. Previous study evidenced that the prevalence of opportunistic infections like *Toxoplasma gondii* was higher in the group with higher FIV seroprevalence (Cong *et al.*, 2016).

The seroprevalence of FIV infection amongst 90 cat samples from different cats tested in this study in Faisalabad, Pakistan was 28.9%, which is comparable to the related Asian areas such as a prevalence of 20.1% in Thailand (Sukhumavasi *et al*., 2012), and 23% in Japan (Nakamura *et al.*, 2010). However, compared to other studies out of Asia, the FIV seroprevalence was higher, as in Germany it has been reported 3% (Gleich *et al*., 2009), 6.6% in Italy (Spada *et al*., 2012), 6% in Canada (Ravi *et al*., 2010), 2% in Canada and Mexico (Munro *et al.*, 2014, Ortega-Pacheco *et al*., 2014), 8% in Australia (Norris *et al*., 2007), 6-14% in Australia (Westman, *et al.*, 2016), 10% in New Zealand (Jenkins *et al.*, 2013) and 2.5% in a sample population of 18,038 cats in North America (Levy *et al.*, 2006). A higher seroprevalence was reported in high-risk cat group as compared to low risk cat group (19 % Vs 1.2%) in Canada (Little *et al*., 2011).

History of injuries due to aggressive behavior, male gender, growing age and tiredness were linked to FIV seropositivity in cats (Chhetri *et al*., 2015). In the current study, the FIV seropositivity was significantly associated with male gender and adult age of cats.

Conclusions: A higher seroprevalence of FIV was observed in cats, Faisalabad, Pakistan. Adult male cats were found to be more prone to FIV infections as compared to the female cats or kittens, due to more exposure to outdoor environment. A continuous surveillance and effective vaccine measures should be adopted to control this immunodeficiency causing virus in cats.

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Authors contribution: SA, MIA and MS designed the study and data interpretation, FA conducted the research and collected the data. RA, GA, MA and SUR helped in data analysis and article writing, proof reading.

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