



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2021; SP-10(1): 46-48

© 2021 TPI

www.thepharmajournal.com

Received: 15-11-2020

Accepted: 22-12-2020

Sukanta Datta

M.V.Sc Scholar, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Vinod Kumar K

Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Vijaya Kumar K

Professor and Head, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Justin Davis K

Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Mini M

Professor and Head, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Corresponding Author:

Sukanta Datta

M.V.Sc scholar, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Identification of aetiological agents of upper respiratory tract infections in domestic cats

Sukanta Datta, Vinod Kumar K, Vijaya Kumar K, Justin Davis K and Mini M

Abstract

Upper Respiratory Tract infections are common disease conditions encountered in both household and shelter cats. The most frequent etiological agents reported are Feline Herpes virus-1 (FHV-1), *Chlamydia felis* (*C. felis*) and *Mycoplasma felis* (*M. felis*). Twenty five cats with clinical signs of upper respiratory tract disease were screened for the presence of FHV-1 *C. felis* and *M. felis* by polymerase chain reaction. Twenty two out of 25 cats were found to be positive for the presence of FHV-1 whereas none of the samples had DNA of *C. felis* and *M. felis*.

Keywords: Domestic cats, upper respiratory tract infections, feline herpes virus-1

1. Introduction

Upper respiratory tract infections (URTI) has become one of the most common and important diseases of cats in both households as well as catteries. URTI has been identified as the second most leading cause of euthanasia in cat shelters¹. Various pathogens have been identified as the etiological agent for URTI like the bacteria², virus³, fungi⁴ and miscellaneous causes. Among the viruses Feline Herpes virus-1(FHV-1) is considered as one of the most important pathogen¹ and among the bacteria *Chlamydia felis* (*C. felis*) and *Mycoplasma felis* (*M. felis*) are considered as the important pathogens⁵. FHV-1 is a member of the *Varicellovirus* genus of the herpesvirus subfamily *Alphaherpesvirinae* while *C. felis* are intracellular bacteria that replicate in conjunctiva cells, and *M. felis* are wall less bacteria that reside on the mucous membrane of respiratory tract. Even though there are numerous reports of identifying FHV-1, *C. felis* and *M. felis* as etiological agents for feline URTI, literature regarding their prevalence in India is scarce. This study was designed for screening the presence of FHV-1, *C. felis* and *M. felis* among cats with symptoms of URTI.

2. Materials and methods

The study took place in Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. Twenty five animals with clinical signs of URTI were selected from among the cats presented to University Veterinary Hospitals, catteries and local hospitals of the Animal Husbandry Department. Signalment and results of detailed clinical examination were recorded for each animal. Sterile swabs with screwed cap containers were used to collect ocular, nasal and oral swabs and two millilitre blood was collected for performing complete blood count using automated haematology analyser (Orphee, Mythic Vet 18). Statistical analysis of variations in haematological parameters was done by SPSS version 24 software using t-test. DNA was extracted using QIAamp® DNA Mini Kit and subjected to polymerase chain reaction (PCR) for amplification of specific parts of the genome of FHV-1, *C. felis* and *M. felis* using primers designed by Henzel *et al.* (2012)^[6], Sykes *et al.* (2001)^[7] and Chalker *et al.* (2004)^[8] respectively. Details of the primers used and PCR protocols are given in Tables 1 to 4. The PCR products were sequenced at Agrigenome, Kochi. A phylogenetic tree was constructed using the neighbour-joining algorithm with molecular distance estimation. Clustal V method with MegAlign programme (LaserGene/DNAStar) software was employed for this purpose.

Table 1: Primers used for identification of FHV-1, *C. felis* and *M. felis*

Organism	Target region	Primer	Primer sequence (5'-3')	Amplicon size (bp)	Reference
FHV-1	Thymidine kinase gene	Forward	GACGTGGTGAATTATCAGC	287	(Henzel <i>et al.</i> , 2012) [6]
		Reverse	CAACTAGATTTCCACCAGGA		
<i>C. felis</i>	<i>ompA</i> gene	Forward	ATGAAAAAACTCTTGAAATCGG	1094	(Sykes <i>et al.</i> , 2001) [7]
		Reverse	CAAGATTTTCTAGACTTCATTTTGT		
<i>M. felis</i>	16S/23S intergenic rRNA	Forward	CACCGCCGTCACACCA	187	(Chalker <i>et al.</i> , 2004) [8]
		Reverse	AGGCATCCACCAAACTCT		

Table 2: PCR protocol used for FHV-1

Sl. No.	PCR programme	Temperature (°C)	Time	Cycles
1.	Initial denaturation	94	5 min	1 cycle
2.	Denaturation	94	45 sec	40 cycles
3.	Annealing	56	30 sec	
4.	Extension	72	45 sec	
5.	Final extension	72	7 min	1 cycle
6.	Hold	4	Until use	

Table 3: PCR protocol used for *C. felis*

Sl. No.	PCR programme	Temperature (°C)	Time	Cycles
1.	Initial denaturation	94	5 min	1 cycle
2.	Denaturation	94	1 min	40 cycles
3.	Annealing	54	40 sec	
4.	Extension	72	1 min	
5.	Final extension	72	5 min	1 cycle
6.	Hold	4	Until use	

Table 4: PCR protocol used for *M. felis*

Sl. No.	PCR programme	Temperature (°C)	Time	Cycles
1.	Initial denaturation	94	5 min	1 cycle
2.	Denaturation	94	1 min	40 cycles
3.	Annealing	54	40 sec	
4.	Extension	72	1 min	
5.	Final extension	72	5 min	1 cycle
6.	Hold	4	Until use	

granulocytopenia, normocytic normochromic anaemia and thrombocytopenia.

Sequencing of the PCR product revealed close similarity of the FHV-1 found in this study with the FHV-1 organisms already reported by Nunberg *et al.* (1989) [9] and Tai *et al.* (2010) [10]

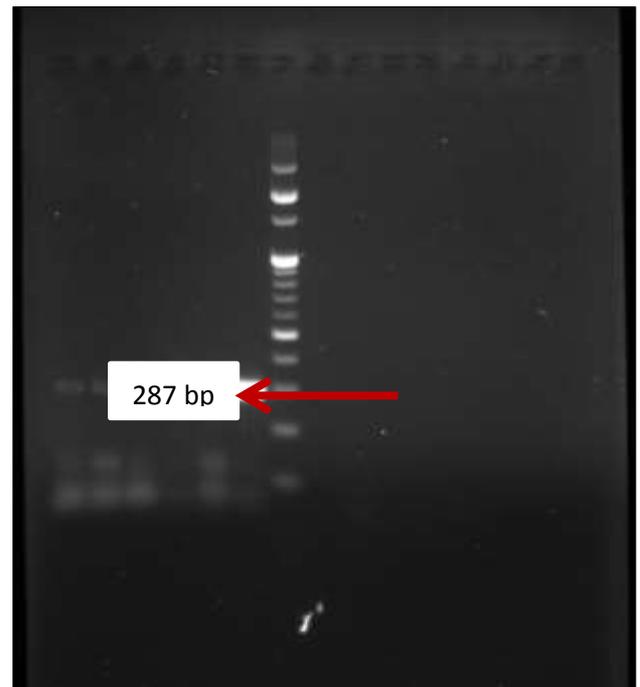


Fig 1: Agarose gel electrophoresis of the PCR amplified products of FHV-1

3. Results

The targeted product of 287 bp for FHV-1 was amplified in total of 22 samples out of 25, however *C. felis* and *M. felis* were not amplified from any of the samples. Haematological analysis revealed leucocytosis, monocytosis,

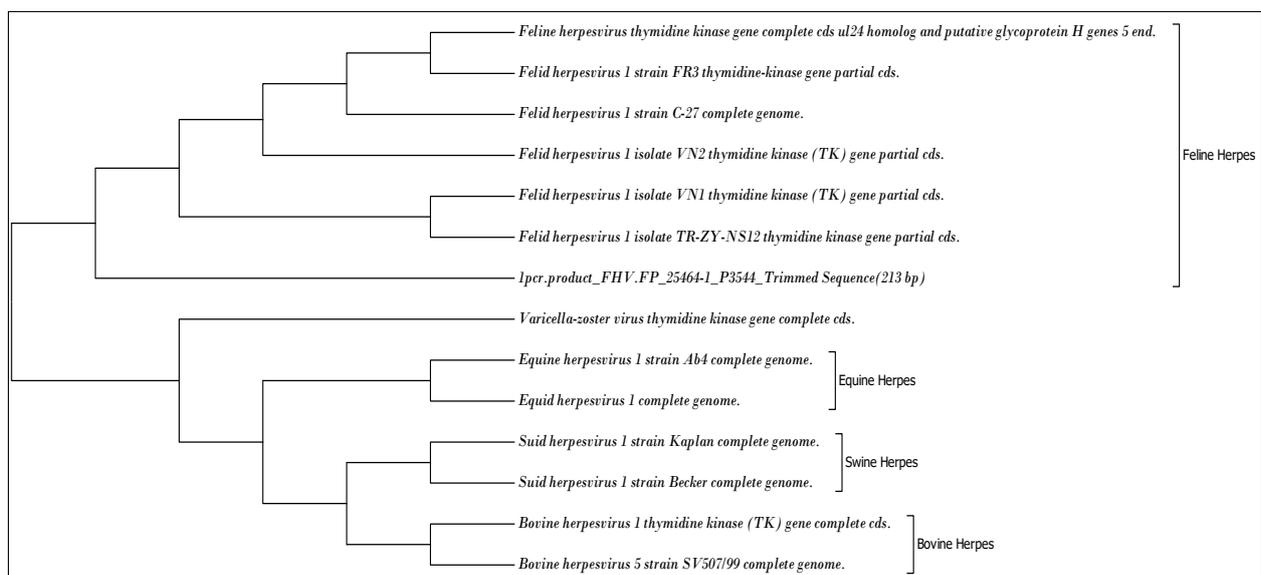


Fig 2: Phylogenetic tree constructed using thymidine kinase gene of Herpes virus. The evolutionary history was inferred using the Neighbour-Joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

4. Discussion

Results of the present study demonstrates that FHV-1 was the predominant one among the three pathogens for URTI in cats of the locality. Henzel *et al.* (2012)^[6] and Fernandez *et al.* (2017)^[11] have also reported high prevalence of FHV-1 (38.2 % and 28.3 % respectively) among cats with URTI under tropical climate. Absence of *C. felis* and *M. felis* among the study population was interesting, and in contradiction to Nguyen *et al.* (2019)^[12] and Cai *et al.* (2002)^[13] who have reported high prevalence of these pathogens among shelter cats in Japan and Australia respectively. The tropical conditions persisting in the study area could be a deterrent for the survival of these organisms. Again, the close confinement and high population density associated with catteries in those areas where high prevalence of these two bacteria are not encountered in the study locality. We could not find any previous reports of FHV-1 as etiological agent for URTI in cats in India.

5. Conclusion

The higher prevalence of FHV-1 infection suggested that it can be a main pathogen. Therefore, it seems necessary to implement the routine vaccination programs. Future studies should be conducted to identify all pathogens involved in this disease.

6. References

1. Litster AL, Lohr BR, Bukowy RA. Clinical and antiviral effect of a single oral dose of famciclovir administered to cats at intake to a shelter. *The Veterinary Journal* 2015;203:199-204.
2. Johnson LR, Foley JE, De Cock HE. Assessment of infectious organisms associated with chronic rhinosinusitis in cats. *Journal of the American Veterinary Medical Association* 2005;227:579-585.
3. Bannasch MJ, Foley JE. Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. *Journal of Feline Medicine and Surgery* 2005;7:109-430.
4. Malik R, McGill S, Saul N, Beetson S, Secombe C, Robertson I, *et al.* Crptococpsis in domestic animals in Western Australia: A retrospective study from 1995-2006. *Medical Mycology* 2006;47:625-639.
5. Hartmann AD, Helps CR, Lappin MR, Werckenthin C, Hartmann K, *et al.* Efficacy of pradofloxacin in cats with feline upper respiratory tract disease due to *Chlamydophila felis* or *Mycoplasma* infections. *Journal of Veterinary Internal Medicine* 2008;22(1):44-52.
6. Henzel A, Sperotto BMC, Lautert C, Mathias M, Lovato LT, Weiblen R, *et al.* Isolation and identification of feline calicivirus and feline herpesvirus in southern Brazil. *Brazilian Journal of Microbiology* 2012;43:560-568.
7. Sykes JE, Allen JL, Studdert VP, Browning GF. Detection of feline calicivirus, feline herpesvirus 1 and *Chlamydia psittaci* mucosal swabs by multiplex RT-PCR/PCR. *Veterinary Microbiology* 2001;81:95-108.
8. Chalker VJ, Owen WM, Paterson C, Caren JJ, Brownlie J. Development of a polymerase chain reaction for the detection of *Mycoplasma felis* in domestic cats. *Microbiology* 2004;150:3491-3497.
9. Nunberg JH, Wright DK, Cole GE, Petrovskis EA, Post LE *et al.* Identification of the thymidine kinase gene of feline herpesvirus: use of degenerate oligonucleotides in the polymerase chain reaction to isolate herpesvirus gene homologs. *Journal of Virology* 1989;63(8):3240-3249.
10. Tai SH, Niikura M, Cheng HH, Kruger JM, Wise AG, Maes RK, *et al.* Complete genomic sequence and an infectious BAC clone of feline herpesvirus-1 (FHV-1). *Journal of Virology* 2010;401(2):215-227.
11. Fernandez M, Manzanilla EG, Lloret A, León M, Thibault JC. Prevalence of feline herpesvirus-1, feline calicivirus, *Chlamydophila felis* and *Mycoplasma felis* DNA and associated risk factors in cats in Spain with upper respiratory tract disease, conjunctivitis and/or gingivostomatitis. *Journal of Feline Medicine and Surgery* 2017;4:461-469.
12. Nguyen D, Barrs VR, Kelman M, Ward MP. Feline upper respiratory tract infection and disease in Australia. *Journal of Feline Medicine and Surgery* 2019; 10:973-9.
13. Cai Y, Fukushi H, Koyasu S, Kuroda E, Yamaguchi T, Hirai K, *et al.* An etiological investigation of domestic cats with conjunctivitis and upper respiratory tract disease in Japan. *Journal of Veterinary Medical Science* 2002;64:215-219.