

Incidence of Feline Haemotropic Mycoplasmosis in Chennai, Tamil Nadu

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Abstract

The present study was conducted at Madras Veterinary College Teaching Hospital to report the prevalence of feline infectious anemia. The diagnosis was confirmed by the presence of pleomorphic parasites at the marginal parts of erythrocytes in stained blood smear examination and PCR. Detailed clinical examination was done for all the suspected cats. Among thirty-seven cats screened, 2 animals were found positive for haemotropic mycoplasma organisms by blood smear examination. The blood samples were further subjected to DNA extraction followed by Polymerase Chain Reaction for confirmation of Haemoplasmosis with a positivity of 5 animals. The highest rate of infection was noticed in cats of one to two years of age group with 40.00 per cent while 3 among 5 cats were non-descript cats. Male cats were high incidence with 60.00 percent. Young male non-descript cats are more susceptible for feline mycoplasmosis.

Key words : Anemia, PCR, feline mycoplasmosis

Mycoplasmas are one of the smallest bacteria and are divided into two groups, haemotropic and non-haemotropic mycoplasmas.

Haemotropic mycoplasmas are also referred to as haemoplasmas and are uncultivable bacteria without cell wall and were previously known as *Haemobartonella* and *Eperythrozoon* species. They are gram-negative, obligate, epierythrocytic organisms and are considered as pleomorphic. They are seen as rod, spherical or ring-shaped organisms and are found individually or in chains across the red blood cell surface. Haemoplasmas attach and grow on the surface of red blood cells and cause infectious anaemia in various mammalian species.

Haemoplasmas were first identified in anaemic cats in South Africa in the year 1942 by Clark and was named as *Eperythrozoon felis*. Later in the year 1955, Flint and McKelvie proposed the name *Haemobartonella felis* to these organisms. It was previously classified as *Haemobartonella spp.* under Rickettsial organisms due to its obligate parasitism, small size, affinity towards the erythrocytes and suspected arthropod transmission. Increasing knowledge on the bacterial genomic sequences and phylogenetic relationship has led to the reclassification of these organisms within the genus *Mycoplasma* by 16S rRNA analysis because of their small sized genome and lack of cell wall. Subsequently all members of the *Haemobartonella* genus and the majority of the members of the closely related *Eperythrozoon* genus were reclassified as *Mycoplasma* species (Foley and Pedersen 2001 and Neimark *et al.*, 2001). Very few reports are available in India pertaining to the disease. Hence, the present study has been designed to investigate the occurrence, associ-

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Table I : Primers employed for the PCR reactions and their specifications

PCR	Target Gene	Primers	Product size	Reference
Species specific <i>Mycoplasma hemofelis</i> (Forward)	16srRNA	ATGCCCTCT- GTGGGGGATAGCCG	274 bp	Watanabe <i>et al.</i> , 2008
Species specific <i>Mycoplasma hemofelis</i> (Reverse)	16srRNA	ATGGTATTGCTC- CATCAGACTTTTCG	274 bp	Watanabe <i>et al.</i> , 2008

Table II : PCR protocol for amplification of *Mycoplasma hemofelis*

Step No.	Step	Temperature	Time
1	Initial denaturation	94 ° C	5 Min
2	Denaturation	94 ° C	45Sec
3	Annealing	61 ° C	45Sec
4	Extension	72 ° C	45Sec
5	Steps 2 to 4 repeated 35 times		
6	Final extension	72 ° C	7 Min
	Hold	4°C	∞

ated with haemotropic mycoplasmosis.

Materials and Methods

A total of 37 cats with clinical signs like anorexia, pyrexia, pale or icteric mucous membranes, weight loss, tiredness etc., which were presented to Madras veterinary college teaching hospital formed the study group. Detailed clinical examination of suspected cases was done. Blood samples were collected from suspected cats under aseptic conditions from cephalic or femoral vein and subjected for laboratory examination like haematology and serum biochemical analysis. A thin peripheral blood smear stained with Giemsa stain was observed under oil immersion objective for primary identification of haemoplasma. Further, EDTA treated blood samples were subjected to molecular diagnostic technique such as conventional Polymerase Chain Reaction. Isolation of DNA was done from whole blood using commercially available kits (Bio-Rad’s® Blood Quick Pure kit) using protocol recommended by the manufacturer. Polymerase chain reaction amplification was carried out in 20µl reaction mixture containing 12µl Ready Mix® PCR Master Mix, 4µl of nuclease free water, 1µl of each forward and reverse

primers and 2µl of extracted DNA. The cycling was carried out in Bio Rad’s™ Thermal Cycler, Applied Biosystems, by using the primers with the details mentioned in Table 1. PCR amplification procedure in mentioned in Table II.

Results and Discussion

Among the 37 cats presented with clinical signs such as pale mucus membrane, lethargy, icterus were screened and 5 (13.5%) cats diagnosed as positive for feline mycoplasmosis by PCR and 2 (5.4%) cats among 5 were positive on blood smear examination. Total incidence of feline mycoplasmosis was 13.5% which was diagnosed based on the PCR while 5.4% based on blood smear examination.

The cats screened in the present study were included in the age group ranging from six months to five years. Among the 5 cats diagnosed as positive for FM by PCR, the highest rate of infection was noticed in cats of one to two years of age group with 40.00 per cent, followed by less than 6 months, less than year of age and two year group with 20.00 per cent of occurrence in each group. No animals were found to be positive for FHM in age group of five years. The age-wise occurrence of feline mycoplasmosis is presented

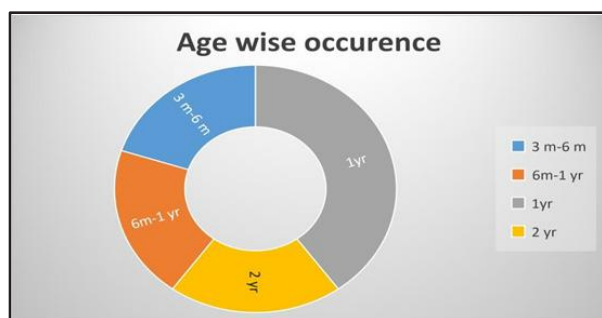


Fig 1. Age-wise occurrence of feline mycoplasmosis (FM)

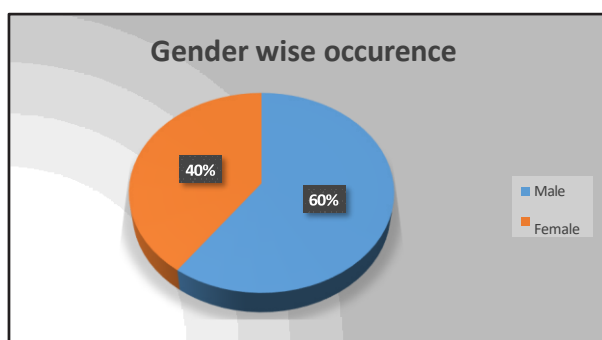


Fig 3. Gender-wise occurrence of feline mycoplasmosis

in the Fig 1. Ravagnan *et al.* (2017), Petry *et al.* (2020) and Salim *et al.* (2020) recorded a greater number of positive cases in cats older than one-year age and ≤ 3 years of age, respectively. The occurrence of haemoplasma infection was more in younger cats which might be associated with immunological immaturity of young cats resulting in higher susceptibility to infections (Sykes *et al.*, 2008; Duarte *et al.*, 2015). Some authors also suggested that trans plancetal transmission may cause a higher rate of infection in younger animals (Giroto-soares *et al.*, 2016).

In the present study, only Persian cats and non-descript breeds of cats were found positive for mycoplasmosis by PCR. Out of five cats found positive for mycoplasmosis, 3 were non-descript breed with 60.00 per cent of occurrence and occurrence of mycoplasmosis in Persian cat was 40.00 per cent. The breed-wise occurrence of feline mycoplasmosis is presented in Fig 2. Niblett *et al.* (2009) and Rosenqvist *et al.* (2016) observed higher prevalence of haemoplasma infection among domestic cats. The findings of present study are in agreement with the reports of above authors. The occurrence of haemoplasmosis in non-descript breeds of cats in the present study was more and this could be due

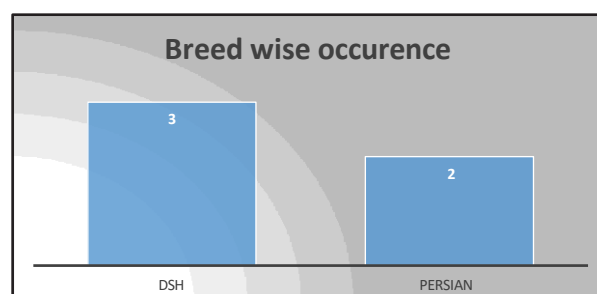


Fig 2. Breed-wise occurrence of feline mycoplasmosis (FM)

to free roaming nature of these non-descript breeds which make them vulnerable to exposure to ectoparasites and fighting nature of these cats with other stray cats.

Among the total of 5 cats positive for mycoplasmosis, 3 (60 %) cats were males and two cats (40 %) were females. Gender-wise occurrence of feline mycoplasmosis is presented in the Fig 3. Many of the researchers have documented that male cats with outdoor access have higher prevalence of infection (Petry *et al.*, 2020; Demkin and Kazakov, 2021). The bold attitude and aggressive behavior of the male cats make them more vulnerable to the disease. Increased prevalence of homeotropic mycoplasmosis in male cats together with the fighting, injury led to the horizontal transmission *via* fighting between cats (Woods *et al.*, 2005).

Conclusion

Mycoplasmosis is considered as one of the important infectious diseases of cats and a major cause of feline infectious anemia and is a major threat in the well-being of feline family. Younger, male, non-descript cats should be suspected for feline mycoplasmosis when presented with symptoms such as pallor of mucous membranes, anorexia, pyrexia, lethargy and weight loss with evidence of ectoparasites. Domestic cats with outdoor access should be routinely monitored and treated for ectoparasites to minimize risks of disease acquisition.

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