

## Evaluation of Lysine and Lysine-Lactoferrin Association in Cats Infected by Feline Herpesvirus-1

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**Abstract:** Feline Herpesvirus type 1 (FHV-1), the causative agent of feline infectious rhinotracheitis is a high widespread virus. At the present, treatment is aspecific and symptomatic, often based on collateral antibiotic treatment. The antiviral effect of lysine, an essential basic amino acid has been widely investigated and is related to a competitive inhibition against arginine. Lactoferrin is a glycoprotein of the transferrin family that seems able to prevent the internalization process and to inhibit viruses replication within the host cells. The aim of the present study was to verify the efficacy of lysine alone and of the association lysine-lactoferrin against FHV-1 in cats. For this purpose, four groups of five FHV-1 infected cats each were treated with different products and monitored at different time points clinically and virologically. In particular, patients received the association lysine-lactoferrin per os (group 1), the association lysine-lactoferrin via aerosol (group 2), only lysine per os (group 3) and the association amoxicillin-clavulanic acid (group 4). Four patients of group 1 (80%), 3 of group 2 (60%), 3 of group 3 (60%) and 5 of group 4 (100%), clinically improved till a complete remission of systemic condition and respiratory symptoms. Viral shedding in oculo-conjunctival samples gradually decreased in almost all cats. These data suggest that the association lysine-lactoferrin could be a first choice in FHV-1 infected cats for reducing clinical signs and viral shedding. Antibiotics should be used only when non-antibiotic products are not sufficient in limiting the secondary bacterial infections.

**Key words:** Feline herpesvirus type 1, feline infectious rhinotracheitis, cat, lysine, lactoferrin

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### INTRODUCTION

Feline Herpesvirus type 1 (FHV-1) is the causative agent of Feline Infectious Rhinotracheitis (FIR), a highly widespread infection. It has been established that 97% of cats present antibodies against FHV-1 (Maggs *et al.*, 1999, 2000). Moreover, the maternal immunity, lasting 2-10 weeks is not always able to protect the kitten from sub-clinical infections (Gaskell *et al.*, 2007). The virus has a strong tropism for nasal, upper airways and conjunctival epithelial tissues in which is also able to induce necrotic phenomena (Gaskell *et al.*, 2007). Clinical appearance of FHV-1 is very polymorphic and includes general symptoms such as anorexia and depression or signs related to respiratory apparatus as rhinitis, laryngitis and tracheitis. Also, the eye is frequently involved leading to conjunctivitis, keratitis and/or corneal ulcers; more rare is the finding of ulcerative skin lesions that are generally localized to the head, trunk and oral mucosa (Gaskell and Dawson, 2005). Infection during pregnancy could determine abortion or a severe generalized infection in newborn associated to encephalitis and necrotizing hepatitis (Gaskell *et al.*, 2007).

At the present there is no specific therapy for the disease although, the use of human antivirals has been suggested (Maggs, 2010). Thus, the treatment of FIR is generally based on symptomatic management and on reducing the bacterial complications. For this purpose, antibiotics are often used although, in some cases as showed in a study on dog's chronic bronchopneumopathies, the reduced or absent response to antibiotic therapy could be related to the presence of biofilm-producing bacteria; studies in this direction would be useful in order to verify such an occurrence also in cats (Attili *et al.*, 2012).

The antiviral properties of some non-antibiotic molecules have been often investigated. Lysine is an essential basic amino acid whose efficacy against viral infections caused by Herpes simplex has been widely studied in humans (Kagan, 1974; Milman *et al.*, 1980). The antiviral effect of lysine has been studied also in cats and other animal species. The activity is not based on a direct antiviral action but is rather principally due to the competitive inhibition against arginine that is fundamental for herpetic virus replication (Griffith *et al.*, 1981).

Lactoferrin is a glycoprotein of the transferrin family, characterized by an antiviral activity against a wide range of DNA and RNA virus both in humans and in animals (biblio). Human respiratory syncytial virus is inhibited by a concentration of lactoferrin ten to twenty times lower than that found in human milk (Gonzalez-Chavez *et al.*, 2009). Also, non-enveloped viruses such as Adenovirus and Enterovirus are sensible to lactoferrin which seems also able to prevent cellular infection and intracellular replication by Human Immunodeficiency Virus (HIV) *in vitro* (Seganti *et al.*, 2004; Viani *et al.*, 1999). Lactoferrin mechanism of action against these agents has not yet been clarified but it is assumed that it could be different depending on the virus. Lactoferrin seems able to prevent the process of internalization of poliovirus, herpes simplex virus type I and II, Cytomegalovirus, Hepatitis B and C Virus (HBV and HCV) in human cells by binding specific viral receptors on target cells' surface (Marchetti *et al.*, 1999; Hasegawa *et al.*, 1994; Beljaars *et al.*, 2004; Hara *et al.*, 2002; Nozaki *et al.*, 2003). For other viruses such as rotavirus, lactoferrin seems able to inhibit replication within the host cell (Superti *et al.*, 1997).

The aim of the present study was to verify the efficacy of lysine as a single agent and in association with lactoferrin against FHV-1 in reducing both clinical healing and the viral shedding in FIR affected cats.

### MATERIALS AND METHODS

**Patients:** Twenty owned cats recovered at the Veterinary Teaching Hospital of the University of Camerino (Italy) because of respiratory and/or ocular symptoms and resulted positive for FHV-1 by PCR were included in the study.

Patients were randomly divided in four groups of 5 animals each and treated as follows; group 1: association lysine-lactoferrin, 1 mL containing 200 mg lysine base and 60 mg lactoferrin every 2 kg of BW, per os, twice a day for 1 week then once a day for 3 weeks; group 2: association lysine-lactoferrin, 1 mL containing 200 mg lysine base and 60 mg lactoferrin plus 2 mL saline solution every cat, via aerosol, twice a day for 1 week then once a day for 3 weeks; group 3: lysine alone, 1 mL containing 200 mg lysine base every 2 kg of BW, per os, twice a day for 1 week then once a day for 3 weeks; group 4: association amoxicillin-clavulanic acid, 12.5 mg kg<sup>-1</sup>, per os, twice a day for 1 week or till clinical remission. In patients presenting oculo-conjunctivitis also topical anti-inflammatory, antibiotic and/or mucolytic-anticollagenasic drugs in different association depending on clinical condition were administered till

**Table 1: Patients monitoring and samplings**

Codes	Time (days)	C	P-CS	P-OS	P-L	CBC	BIO	IFI
T0	0	x	x	x	x	x	x	x
T1	7	-	x	x	x	x	x	-
T2	14	-	x	x	x	x	x	-
T3	21	-	x	x	x	x	x	-
T4	28	x	x	x	x	x	x	x

C: Clinical evaluation; P-CS: PCR on Conjunctival Swabs; P-OS: PCR on Oropharyngeal Swabs; P-L: PCR on Leukocyte fraction isolated from blood samples; CBC: Cell Blood Count; BIO: Hemato-Biochemical evaluation of main organs' functionality; IFI: Indirect Immunofluorescence against FHV-1

clinical remission. During therapeutic trial patients were monitored and samples were collected as reported in Table 1 before the Treatment (T0) and every week for 4 weeks (named T1-T4, respectively).

The study was performed under signed informed consent of cat's owners and was approved by the ethical committee (Comitato Etico di Ateneo per la Protezione degli Animali utilizzati a fini sperimentali o altri fini scientifici-CEAPA, Prot. No. 3/2013, 25/02/2013) of the University of Camerino.

**DNA extraction and PCR:** The DNA was purified from the swabs by a commercial kit (Genomic DNA Isolation kit, Norgen Biotek Corp., Thorold, ON, Canada) following the manufacturers' instructions. PCR has been carried out following a highly sensitive and specific protocol previously validated (Weigler *et al.*, 1997). Briefly, the primers forward 5'-TGTC CG CAT TTA CAT AGA TGG-3' and reverse 5'-GGG GTG TTC CTC ACA TAC AA-3' have been used for amplifying a 322 bp sequence of the thymidine kinase of FHV-1. The mix of reaction consisted of 25 µL of Taq PCR mastermix (Qiagen GmbH, Hilden, Germany), 10 pmol of each primer and 100 ng DNA up to 50 µL total volume. The amplification protocol was 94°C for 5 min, 40 cycles at 94°C for 30 sec, 54°C for 30 sec and 72°C for 40 sec with a final extension of 72°C for 7 min. The amplification products were visualized in 1.5% agarose gel containing 0.5 µg mL<sup>-1</sup> ethidium bromide in Tris-borate-EDTA buffer (TBE; 89 mM Tris, 89 mM boric acid, 2 mM EDTA pH 8.3). The pictures were taken by a computerized camera (Gel Logic 100, Kodak) and the intensity of the bands was compared with a reference ladder (100 bp ladder, Norgen Biotek Corp., Thorold, ON, Canada). On the basis of their intensity, the bands were scored from 0-3 points (Fig. 1). Positive and negative controls were included in each run.

**Immunofluorescence test:** All sera samples, collected at T0 and T4 were submitted to an Indirect Immunofluorescence test (IFI) to verify the presence of antibodies against FHV-1. Sera, frozen at -20°C until use were diluted on PBS from 1:20 to 1:640. A commercial kit

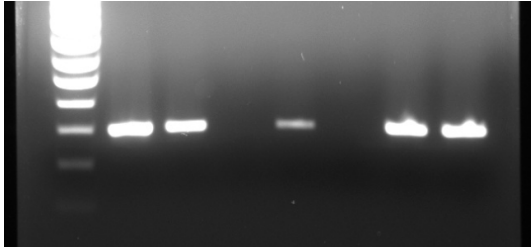


Fig. 1: PCR products for FHV-1 detection. Lane 1: ladder 100 bp; Lane 2: cat No. 5 at T0; Line 3: cat No. 5 at T1; Line 4: cat No. 5 at T2; Line 5: cat No. 10 at T0; Line 6: cat No. 10 at T1; Line 7 cat No. 16 at T0; Line 8: cat No. 16 at T1

(Fuller Lab., Fullerton-California, USA) was used. For each slide a reference positive and negative serum was used.

**Hematological evaluations:** Hemato-biochemical evaluation and CBC were performed, respectively with Automatic analyzer BT 3000 plus® (Biotecnica Instruments, Italy) and with hematology analyzer Cell-Dyn® 3500 (Abbott, USA).

**RESULTS**

All patients included in the study at the first veterinary visit were presenting clinical signs related to FHV-1 such as sero-mucous nasal discharge, sneezes, depression, disorexia, hyperthermia in variable association; all cats presented mono or bilateral oculo-conjunctivitis (Table 1).

After 1-2 weeks treatment, four patients (80%) of group 1, 3 (60%) of group 2, 3 (60%) of group 3 and 5 (100%) of group 4 improved clinically till a complete remission of systemic condition and of respiratory symptoms. Because the animal welfare, after 2 weeks of non-antibiotic treatment, one cat of group 1 and 2 cats of both group 2 and 3 were administered the association amoxicillin-clavulanic acid 12.5 mg kg<sup>-1</sup>, per os, twice a day for 7-10 days to obtain a complete remission of the clinical signs.

Conjunctivitis required in all patients 2-3 weeks of treatment before of remission with the only exception of one patient of group 2 which needed surgery because the development of an unilateral keratitis with descemetocele. Hematology at different time points did not show significant differences; almost all patients were presenting a slight increase of white blood cells at T0, gradually normalized at T4.

Table 2: Results of PCR performed on conjunctival swabs. Positivity grading is on a scale from 0-3

Groups	Patient No.	T0	T1	T2	T3	T4
1	1	2.0	2.0	2	2	2
	2	2.0	1.0	0	0	0
	3	2.5	2.0	0	0	0
	4	3.0	1.0	0	0	0
	5	3.0	2.0	0	0	0
2	6	2.0	1.0	1	0	0
	7	2.0	2.0	0	0	0
	8	2.0	1.0	0	0	0
	9	3.0	2.0	0	0	0
3	10	1.0	0.0	0	0	0
	11	2.5	2.0	1	0	0
	12	1.0	1.0	1	0	0
	13	2.5	2.5	2	0	0
	14	2.0	2.0	1	0	0
4	15	2.5	2.0	1	1	0
	16	2.5	2.5	2	0	0
	17	2.0	1.0	1	1	0
	18	3.0	2.0	0	0	0
	19	2.5	2.0	2	1	0
	20	1.0	1.0	0	0	0

IFI showed in all patients a low antibody titer at T0 that slightly increased at T4. In all subjects, PCR revealed a higher presence of viral genome in ocular swab samples which were assumed as reference for monitoring the viral spread during treatment. In almost all cats the PCR score in ocular swabs gradually decreased during the study and become negative at T4 in all patients except patient 1 of group 1 (Table 2).

**DISCUSSION**

FHV-1 infection is one of the most common cat's diseases whose successful evolution considerably depends on management of disease's clinical phase. It has to be paid attention especially on hygiene and support therapy that result fundamental to reduce chances of a negative evolution of the disease. In the present study, 19 out of 20 patients clinically healed (the 20th was the one of group 2 who necessitated of ophthalmic surgery), suggesting the importance of supportive cares.

In patients of groups 1 and 2 to which it was administered the association lysine-lactoferrin respectively per os and via aerosol, it was obtained a complete remission of respiratory symptoms in 7 out of 10 patients; the other three solved the respiratory condition by adding the association amoxicillin-clavulanic acid. In particular, 4 out of 5 cats receiving the association lysine-lactoferrin per os showed a complete remission of the respiratory signs in 1-2 weeks of treatment, demonstrating a higher efficacy of the treatment per os than via aerosol and than the lysine only treatment.

Particularly interesting seems the earlier negativization of the conjunctival swabs by PCR in 9 out

of 10 patients of groups 1 and 2, treated with the association lysine-lactoferrin, comparing to the cats of the other groups suggesting a more rapid and higher efficacy of the association lysine-lactoferrin in reducing FHV-1 shedding.

It is important to underline that negativity of samples at T4 does not mean that virus was completely removed from treated animals, it is known that FHV-1 usually does not abandon the host and could remain latent in the nervous system also for patient's whole life (Gaskell *et al.*, 2007). About 80% of infected cats remain virus carrier, regardless the appearance of clinical illness and viral reactivation and subsequent shedding is possible as a result of stress or following corticosteroids administration (Gaskell and Povey, 1977; Maggs, 2005, 2007).

Although, cats remain infected for all their life because the viral latency in the ganglia, an earlier negativization of conjunctival swabs means a lower environmental viral shedding, leading to a reduction of infection risk for other animals. Specific vaccines for FIR can reduce the severity of symptoms but are not able to prevent FHV-1 infection (Maggs, 2007).

Further, studies could evaluate the role of a long-term supplement of lysin-lactoferrin in the diet to prevent the viral reactivation in stress conditions and thus the viral shedding and transmission to other cats.

### CONCLUSION

Although, the traditional pharmacological therapy has to be always considered for patients affected by FHV-1, the administration of the association lysine-lactoferrin especially per os could represent a valuable aid for clinical healing and mostly in reducing viral shedding. Considering the costs and the problems due to the use of antibiotics, first of all the selection of antibiotic resistant bacteria, the use of alternative treatments for managing viral diseases is strongly encouraged. Although, further investigations are desirable on this topic, this study suggests the use of the association lysine-lactoferrin as first choice in managing FIR affected animals, leaving as second choice the use of antibiotics in those animals not solving in 1-2 weeks their clinical conditions.

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