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PRESENCE OF Ig G ANTIBODIES AGAINST *CHLAMYDOPHILA FELIS* IN CATS POSITIVE TO FIV AND/OR FELV

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The aim of our study was to confirm the presence of Ig G antibodies against Chlamydophila felis (Cp. felis) in cats infected with feline immunodeficiency virus (FIV) and/or feline leukemia virus (FeLV) and possible higher incidence of any clinical symptoms. A group of 30 cats which had been exposed to FIV and/or FeLV were tested for chlamydial antibodies presence. FIV and FeLV exposure were established by commercial ELISA rapid tests. The results of serological testing to antibodies against Cp. felis in cats with immunofluorescence assay (IFA) are shown. In 16.7% (5/30) of tested cats Ig G antibodies against Cp. felis were found. No correlation with FIV and/or FeLV infection neither with clinical symptoms of upper respiratory tract could be confirmed.

Key words: cats, immune response, Chlamydophila felis, FeLV, FIV

INTRODUCTION

Cp. felis (previously *Chlamydia psittaci* or feline pneumonitis agent) was first isolated in the United States from cats with respiratory disease in 1942 (Baker, 1944). The disease is clinically characterized by conjunctivitis, sneezing and coughing, accompanied by mucopurulent ocular and nasal discharges. *Cp*. *felis* is primarily considered an ocular pathogen with or without rhinitis rather than a pulmonary pathogen (Sykes, 2001). Chlamydiae can also cause fever, lethargy, lameness, and reduction in weight gain in infected kittens (Terwee *et al.*, 1998).

A few reports of epidemiological investigations of feline chlamydiosis can be found (Pudjiatmoko *et al.*, 1996; Dovč *et al.*, 1998; Yan *et al.*, 2000). Seroepidemiological studies indicate some variability among *Cp. felis* strains (Pudjiatmoko *et al.*, 1996). Nevertheless, *Cp. felis* strains show a substantial degree of rRNA and *ompA* gene conservation and belong to a distinct serotype (Pudjiatmoko *et al.*, 1997).

The 16S rRNA genes of *Cp. felis* strains that have been sequenced and differed by <0.6% (Everett *et al.*, 1999). In 1997 Dovč *et al.* (1998) established 57.7% (15/26) seropositive cats to chlamydial infection in Slovenia by IFA. Yan and his colleagues (2000) found in Japan 45.5% stray cats and 17.3% pet cats to be seropositive to *Cp. felis* (Fe/Pu1). Pudjiatmoko *et al.* (1996) established a lower prevalence of seropositive cats. Positive rates of Ig G antibodies to *Cp. felis* were 34.4% in 1985 and 15.5-21.4% from 1993 to 1995.

FIV belongs to the lentiviruses and it was first described by Pedersen and co-workers (1987). A diagnosis of FIV infection can be establish by detection of FIV-specific antibodies in blood or salva, most commonly by ELISA, which is widely available and easy to use (Poli *et al.*, 1992). FeLV is a member of the oncornavirus subfamily of retroviruses, and it was first described in 1964 (Jarrett *et al.*, 1964). Feline viral core proteins, especially p27, are produced within infected cells, may also circulate free in the plasma or are excreted in the tears or salva, can be detected by ELISA techniques (Ghosh *et al.*, 1992). FIV and FeLV infections present a more or less serious problem in pet cats all over the world and can cause sever immunodeficiency in infected animals. The results from the Clinic for Surgery and Small Animal Medicine, Veterinary Faculty of Ljubljana show very high seroprevalence of FIV and FeLV in cats in Slovenia, 13.0-16.0% of FIV seropositive cats and 18.0-30.4% FeLV seropositive cats per year since 2000 using commercially available rapid test, confirmed by PCR (Tozon, 2000; Zemljič and Zakošek, 2005).

MATERIAL AND METHODS

Cats

A total of 30 serum samples were collected from naturally infected cats, exposed to FIV or FeLV in the period from January 2004 to January 2005. No cats were vaccinated against *Cp. felis*. They were of both sexes and different age. In fifteen cats conjunctivitis, rhinitis, laryngotracheitis, bronchopneumonia and lymphadenopathy were recorded noted.

Diagnostic tests

All cats were screened for FIV and FeLV by a commercial ELISA (IDEXX, Westbrook, Maine, USA-Feline Leukemia Virus Antigen/ Feline Immunodeficiency Virus Antibody) test kit according to the recommendations of the manufactures.

Feline Chlamydia Ig G IFA antibody kit (Fuller laboratories, California USA) is intended for the detection and semi-quantification of feline antibodies to *Cp. felis.* Substrate slides consist of teflon-masked wells containing fixed cells; approximately 7-15% of which is infected with a feline pneumonitis strain (FP-1) and contains the characteristic cytoplasmic elementary bodies. IFA was done according to the recommendations of the manufactures. The resulting reactions could be visualized using standard fluorescence microscopy, where a positive reaction (antibody titers 1:40 or higher) was seen as apple-green fluorescent elementary bodies within the cytoplasm. A negative reaction was seen either as red-counterstained cells or fluorescence, unlike the one seen in the positive control well.

RESULTS

In Table 1 are presented the results of establishing *Cp. felis*, FIV and FeLV infection with all described methods.

Table 1. Presence of Ig G antibodies against *Cp. felis* in cats positive to FIV and/or FeLV

Immunofluorescence method - *Cp. felis*	ELISA - FIV	ELISA - FeLV	Cats with clinical sings
Neg	Pos	Neg	No
Neg	Pos	Neg	No
Neg	Pos	Neg	No
Neg	Pos	Neg	No
Neg	Pos	Neg	No
Neg	Pos	Neg	No
Pos	Pos	Neg	No
Pos	Pos	Neg	Yes
Pos	Pos	Neg	Yes
Neg	Pos	Neg	Yes
Neg	Pos	Neg	Yes
Neg	Pos	Neg	Yes
Neg	Pos	Neg	Yes
Neg	Pos	Neg	Yes
Neg	Neg	Pos	No
Neg	Neg	Pos	No
Neg	Neg	Pos	No
Neg	Neg	Pos	No
Neg	Neg	Pos	No
Neg	Neg	Pos	No
Pos	Neg	Pos	No
Pos	Neg	Pos	Yes
Neg	Neg	Pos	Yes
Neg	Neg	Pos	Yes
Neg	Neg	Pos	Yes
Neg	Neg	Pos	Yes
Neg	Neg	Pos	Yes
Neg	Neg	Pos	Yes
Neg	Neg	Pos	Yes
Neg	Pos	Pos	No
(5*/16.7%)	(15*/50.0%)	(16*/53.3%)	(15**/50.0%)

* Number of positive cats
**Number of cats with clinical sings

When conjunctivitis occurred, the clinical signs were profuse serous ocular discharge with chemosis, hyperaemia of the palpebral conjunctiva, and blepharospasm on one or both eyes. Signs of respiratory tract disease were less common and included: intermittent recurrent nasal discharge, sneezing and coughing. The number and percentage of cats with clinical sings for all three agents are shown in Table 1.

The overall seropositive rate of *Cp. felis* infections was 16.7% (5/30) in the examined cats. Titers of specific Ig G antibodies against *Cp. felis* ranged between 1:40 to 1:160. Among cats (15/30) with clinical symptoms of upper respiratory tract disorders, five (16.7%) were serologically positive only to FIV, seven (23.3%) were positive to only FeLV, two (6.7%) were positive to *Cp. felis* and FIV, and one (3.3%) was positive to *Cp. felis* and FeLV.

Within cats (15/30) without clinical symptoms, six (20.0%) were serologically positive only to FIV, six (20.0%) were positive only to FeLV, one (3.3%) was positive to FIV and FeLV, one (3.3%) was positive to *Cp. felis* and FIV, and one (3.3%) was positive to *Cp. felis* and FeLV.

DISCUSSION

FIV and FeLV are common infectious diseases of cats and are a serious problem in Slovenia. At our Clinic for Surgery and Small Animals at the Veterinary Faculty of Ljubljana, the seroprevalence of FIV and FeLV has been followed since 2000. A total of 13.0-16.0% of FIV seropositive cats and 18.0-30.4% FeLV seropositive cats per year were confirmed. The results show no tendency to decrease in the last years (Zemljič and Zakošek, 2005).

The prevalence of *Cp. felis* infection in thirty cats, positive to FIV and/or FeLV was examined. Half of them had chronic conjunctivitis, rhinitis, laryngotracheitis, bronchopneumonia and lymphadenopathy. They were studied to determine the possible causes of the disease.

Immunofluorescence for *Cp. felis* resulted in the identification of the Ig G antibody in 16.7% (5/30) of naturally infected pet cats. In the previous years we found 57.7% (15/26) seropositive cats to chlamydial infection in Slovenia (Dovč *et al.*, 1998). Tozon *et al.*, (2006) concluded, on the basis of a clinical study, that *Chlamydia sp.* can be considered to be a primary or secondary pathogen, which can potentially cause severe sings of respiratory tract infections in cats, especially in younger animals. Nasisse *et al.*, (1993) reported *Cp. felis* antibodies in 18% of tested cats using IFA. The results of Pudjiatmoko *et al.* (1996) and Yan *et al.* (2000) showed the wide prevalence of chlamydial infection in cats in Japan (up to 45,5%, up to 34.4%, respectively), and antigenic diversity in the feline strains of *Cp. felis*.

The availability of IFA is limited, antibodies induced by vaccination may interfere with the assay, and acute and convalescent phase sera may be required to obtain an accurate diagnosis in acute cases (Sykes, 2001). For this reason we tested only unvaccinated pet cats. However, several ELISA antigen kits are available for detection of human *Chlamydia trachomatis* infection. Their sensitivity and specificity for detection of *Cp. felis* are extremely variable, raging from 25% to

79% for sensitivity and 84% to 90% for specificity (Wills *et al.*, 1988; Pointon *et al.*, 1991).

In cats experimentally infected with FIV alone no clinical signs were developed (Pedersen *et al.*, 1990). Clinical sings may occur after secondary infection with any other pathogen and especially in cats co-infected with FeLV (Pedersen and Barlough, 1991). O'Dair *et al.*, (1994) studied the clinical outcome and antibody responses to *Cp. felis* in cats, experimentally infected with FIV. They confirmed that the FIV infection prolonged the duration of the clinical signs resulting from the infection with *Cp. felis* and led to the development of chronic conjunctivitis. On the other hand, no difference in antibody response between FIV infected and control uninfected cats was found. In addition, it appeared that the infection with a secondary pathogen may have accelerated the clinical progression of the FIV infection.

Similar findings in our study were established. Three (10.0%) examined cats had antibodies against FIV and *Cp. felis*, contemporarily. Two of them showed clinical simptoms. Among two cats (6.7%), which were FeLV positive and had antibodies against *Cp. felis*, only found one had clinical sings. The presence of Ig G antibodies against *Cp. felis* was not in correlation with FIV and/or FeLV infection nor with clinical symptoms of the upper respiratory tract.

Our results did not confirm a higher incidence of clinical sings in cats co-infected with FeLV and/or FIV compared with seronegative cats. The influence of *Cp. felis* infection remains still unclear.

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ZASTUPLJENOST Ig G ANTITELA USMERENIH PROTIV *CLAMIDOPHILA FELIS* KOD FIV I/ILI FELV POZITIVNIH MAČAKA

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SADRŽAJ

U ovoj studiji je ispitivano prisustvo antitela klase Ig G usmerenih protiv *Clamidophila felis* kod FIV i/ili FELV pozitivnih mačaka. Ukupno je ispitano 30 životinja kod kojih je infekcija navedenim virusima potvrđena brzim komercijalnim ELISA testovima. Serološka ispitivanja na prisustvo antitela klase Ig G usmerenih protiv *Clamidophila felis* vršena su imunofluorescentnom metodom. Od ukupnog broja ispitivanih životinja, antitela su dokazana u 16,7% slučajeva (5/30) i nije utvrđena korelacija između simptoma oboljenja respiratornog trakta i virusnih infekcija (FIV i/ili FELV).