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Laboratory diagnostics of selected feline respiratory pathogens and their prevalence in the Czech Republic

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Abstract: Respiratory problems in cats have a multifactorial character. Therapy without the detection of pathogen is often ineffective. Our study was therefore focused on the detection of important feline respiratory bacterial pathogens such as *Mycoplasma felis*, *Chlamydia felis* and *Bordetella bronchiseptica* and viral pathogens such as *Felid alphaherpesvirus-1* and feline calicivirus. The goal of this study was to map the occurrence of these pathogens in cat populations in the Czech Republic with the aim of introducing rapid and highly sensitive methods into routine diagnostics and to provide consulting services to animal health professionals based on the acquired data. A total of 218 cats were investigated in the study: 69 were outdoor and 149 were indoor cats. Three groups of animals were compared: up to one year of age (60 cats), one to three years of age (68 cats) and more than three years of age (90 cats). Samples were taken from conjunctiva and/or the oropharynx. Samples originated from cats with various forms of respiratory disease or from healthy cats from different parts of the Czech Republic. Real-Time RT-PCR, multiplex Real-Time PCR, nested PCR and sequencing analyses were performed. Outdoor cats were infected more often (84 detected pathogens in 69 cats) than indoor cats (110 detected infections in 149 cats). More than one pathogen was detected in a total of 38 cats, and six cats were infected with more than two pathogens. The difference was statistically significant in the case of co-infections, but not for mono-infections ($P < 0.05$). Kittens and young adults up to the age of one year were the most common reservoirs of respiratory infections (only 19 cats out of 60 were negative and positive cats often harboured coinfections). The difference in age groups were not statistically significant ($P > 0.05$). Concerning the site of the sampling, feline calicivirus, *M. felis* and *B. bronchiseptica* were detected more often from oropharynx than from conjunctival swabs. *M. felis* was slightly more common in clinically diseased animals (39.6%) than in healthy ones (26.1%). The obtained results reveal the frequency of individual pathogens and their co-infections in cats kept on the territory of the Czech Republic, data which can be used to make the treatment of respiratory infections and breeding measures more effective. Therefore, the diagnostic methods are now available to veterinary surgeons with the possibility of consultation and discussion of the results.

Keywords: respiratory disease complex; polymerase chain reaction; diagnostics; *Mycoplasma felis*; *Chlamydia felis*; *Bordetella bronchiseptica*; *Felid alphaherpesvirus-1*; FHV-1; feline calicivirus; FCV

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Respiratory infections in cats are considered to be multifactorial diseases with nonspecific clinical signs that very often present as conjunctivitis, inflammation of the upper respiratory tract and less commonly also of the lower respiratory tract. They are characterised by serous to mucopurulent discharge from conjunctiva or the upper respiratory tract (Konvalinova et al. 2016). The feline respiratory disease complex is thought to be mainly caused by feline herpesvirus (*Felid alphaherpesvirus 1*, FHV-1) and feline calicivirus (FCV). The main bacterial pathogens involved in feline respiratory disease complex are *Mycoplasma felis*, *Chlamydia felis* and *Bordetella bronchiseptica* (Sykes 2014; Litster and Leutenegger 2015). Some of these pathogens have zoonotic and epidemiological potential (*B. bronchiseptica*, *M. felis*, *C. felis*), mainly in immunosuppressed individuals (Hartley et al. 2001).

Mycoplasma felis (*M. felis*), class *Mollicutes*, family *Mycoplasmataceae*, genus *Mycoplasma*, is one the most common pathogens of the upper respiratory tract in cats. Most feline-associated mycoplasma species are common microflora of conjunctiva and the upper respiratory tract, and they usually exercise pathogenicity as a secondary source of infection in co-infection with other pathogens of the upper respiratory tract. It is disputable if *Mycoplasma felis* is only present in the upper respiratory tract as a secondary pathogen; however, in the lower respiratory tract, *M. felis* causes primary infection resulting in the inflammation of the lungs and pleural space. However, *M. felis* has not been isolated from the lower respiratory tract in healthy individuals (Lee-Fowler 2014). A vaccination against *M. felis* infections has not yet been developed, and, therefore, therapy is based on prolonged antibiotic treatment and on the improvement of cat welfare. The antibiotics of choice for *M. felis* infections of the conjunctiva are tetracyclines such as eye ointments containing oxytetracycline, chloramphenicol or fluoroquinolones.

Chlamydia felis (*C. felis*) belongs to class *Chlamydiales* (family *Chlamydiaceae*, genus *Chlamydia*) which number among the obligatory intracellular bacteria with a unique developmental cycle, and the pathogenesis of *C. felis* infections in cats is still not fully clear. Serological studies have shown the existence of a few different strains of *C. felis* with differing virulence (Kuroda-Kitagawa et al. 1993). DNA fingerprinting has been used to reveal the existence of two important strains of this species in cats (Pudjiatmoko et al. 1997). Using other DNA analyses, a highly conserved gene for the ompA protein (major outer membrane protein) has been discovered among the different isolates of *C. felis* (Di Francesco and Baldelli 2002).

The disease is often complicated by co-infections with other pathogens such as FCV, which causes ulceration of the oral cavity together with conjunctival infections. Feline immunodeficiency virus often complicates recovery and prolongs therapy. Moreover, *Mycoplasma felis* and *Bordetella bronchiseptica* are also involved in the worsening of clinical signs. The prevalence of *C. felis* in asymptomatic cats is very low (< 5%) (Di Francesco et al. 2004). *C. felis* is sensitive to tetracycline antibiotics, erythromycin, rifampicin, fluoroquinolones and azithromycin. Doxycycline is the drug of choice but is contraindicated in pregnant queens and kittens. The immune response after *C. felis* infection is usually short and weak, and there are different types of monovalent and polyvalent vaccines. *Bordetella bronchiseptica* (*B. bronchiseptica*), class *Betaproteobacteria*, family *Alcaligenaceae*, genus *Bordetella* is a strictly aerobic Gram-negative, motile, rod-shaped bacteria. Clinical and experimental studies have shown that *B. bronchiseptica* is a primary pathogen of the respiratory tract of cats (Hoskins et al. 1998). *Bordetella bronchiseptica* is sensitive to doxycycline and other possible drugs of choice are co-trimoxazole, enrofloxacin, pradofloxacin and cefovecin. It is also possible to use macrolide antibiotics such as erythromycin or clarithromycin; however, these drugs are not licensed for use in cats (Konvalinova et al. 2016). Live intranasal vaccine (Nobivac BB) can be used as prophylaxis, mainly in animals in shelters.

Felid alphaherpesvirus 1, feline herpesvirus also known as feline rhinotracheitis virus (FHV-1) belongs to family *Herpesviridae*, (enveloped DNA viruses), subfamily *Alphaherpesvirinae*, genus *Varicellovirus*, and represents a typical alphaherpesvirus. Clinical signs are most obvious during the primary infection of kittens. The disease is also described as "cat flu" and can reoccur and affect one or both eyes (Stiles 2014). Treatment of disease caused by FHV-1 is mainly symptomatic, even though there are studies showing promising results with the use of immunomodulators (Fiorito et al. 2016) and antivirals (Thomasy et al. 2016). *Felid alphaherpesvirus 1* is commonly present in most polyvalent vaccines together with feline par-

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vovirus and FCV, but no vaccine can protect the animal from infection and they only help to reduce its consequences.

Feline calicivirus (FCV) is small non-enveloped RNA virus belonging to family *Caliciviridae*, genus *Vesivirus*. A simple genome consisting of single-stranded RNA enables FCV to exhibit great adaptability and variability under the selective pressure of the host immune system. The clinical signs of FCV can therefore vary substantially (adaptation to the new receptor systems), and the development of new vaccines is complicated (antigen variability of the strains) (Radford et al. 2007). Nevertheless, Afonso et al. (2017) proved a neutralising effect of vaccinated cat sera on nearly all field strains of FCV isolated in six European countries. The vaccination strain used was F9. The resistance of the virus in the environment allows the virus to survive even the use of common sanitary agents. The sequencing of FCV and evidence of circulating strains of FCV in particular geographical areas could greatly facilitate the selection of appropriate vaccines in particular in breeding programmes (Ossiboff et al. 2007). Vaccination strains of FCV are usually included in the core vaccination schemes for cats; however, due to the high variability of the virus, the level of immunity is not always protective but rather reduces the consequences of FCV infection. Unfortunately, sometimes the pathogenic strain completely differs from the vaccination strain, and, therefore, vaccines with two and more FCV strains are currently preferred.

In this study, it was hypothesised that the treatment of cats with respiratory disease is not always effective due to inaccuracies in diagnostics. Further, there was no relevant data about the prevalence of causative agents in the Czech Republic. Such data could help veterinary surgeons to apply the correct treatment procedures.

Therefore, the objective of our study was to introduce highly specific and sensitive multiplex real-time PCR and real-time RT-PCR assays for the detection of the prevalence of the main feline respiratory pathogens in the Czech Republic, and to analyse the obtained data with respect to age, clinical status and history in the cat household.

MATERIAL AND METHODS

Tested samples. A total of 218 cats were investigated, including 149 indoor and 69 outdoor cats. Three groups of animals were compared: up to one year of age (60 cats), one to three years of age (68 cats) and more than three years of age (90 cats). A total of 177 oropharyngeal swabs and 70 conjunctival swabs were collected. In 189 cats, we swabbed only one site, while in 29 cats both sites were swabbed. Samples originated from 157 cats with respiratory disease. The problems included acute upper respiratory tract inflammation, keratoconjunctivitis, chronic rhinitis and oropharyngeal lesions. A total of 61 (28%) healthy cats selected during preventive care examination or randomly chosen healthy cats at cat shows and cats after treatment of respiratory diseases were also tested in this study. In 13 (5%) tested cats, history data were missing. We also discovered that only 26 cats had been vaccinated with Purevax RPC vaccine and two cats with Felocel CVR vaccine. The other cats had not been vaccinated at all, or the anamnestic data about the vaccination were missing. We collected 60 cats (28%) from single-cat households and 158 (72%) cats from multi-cat households. However, exact data specifying the number of cats in the cat household and their living style were missing. Shelter cats were not included in this study.

Control strains. As positive controls, the following type strains were used: *Mycoplasma felis* field strain confirmed by sequencing; *Chlamydia felis* vaccine strain Purevax (Merial, France); *Bordetella bronchiseptica* CCM 6047 (Collection of microorganisms, Brno, Czech Republic); FHV-1 positive control TC strain confirmed by sequencing; FCV positive field strain "Holubice" WSV Bio 84 (Bioveta, Ivanovice na Hané, Czech Republic); FCV F9 type strain (Bioveta, Ivanovice na Hané, Czech Republic).

Detection of bacterial and viral pathogens. DNA and RNA were extracted using commercial extraction kits according to the manufacturers' instructions: viral RNA using the NucleoSpin® RNA II kit (Macherey-Nagel) and bacterial and viral DNA using the NucleoSpin Blood DNA (Machery-Nagel); simultaneous extraction (DNA/RNA) was carried out using the High Pure viral nucleic acid kit (Roche).

PCR identification of *Mycoplasma felis*, *Chlamydia felis* and *Bordetella bronchiseptica* using multiplex qPCR. Detection and quantification of bacterial DNA were done using Xceed qPCR Probe 2× Master Mix in a total volume of 20 µl according to the manufacturer's instructions on a

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Light Cycler® 480 II (Roche). Xceed qPCR Probe 2× Mix No-Rox (IAB, Czech Republic) was used for the reaction together with primers (400 nM, 6×) and probes (200 nM, 3×).

Primers were designed using the IDT PrimerQuest Tool and optimized for multiplex qPCR in our department.

M. felis: sequence of 16S ribosomal RNA and the ITS-1 (*Mycoplasma felis* strain MF6 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence, sequence ID: AY274256.1, product length: 103 bp).

5'-CTACGGAGACAACTGCCTAAG-3' *M. felis* For (positions 53–73); 5'-GTACTCCGTAGAAAGGAGGTAATC-3' *M. felis* Rev (positions 133–156); 5'-ACAAGGTATCCCTACGAGAACGTGGG-3' *M. felis* probe Cy5-BHQ2 (positions 103–128); *C. felis*: sequence of the ompA protein (*Chlamydophila felis* strain 02DC26 outer membrane protein A (ompA) gene, sequence ID: KP165540.1, product length: 77 bp).

5'-GCTAGGAGCGTCTAATGGTTAC-3' *C. felis* For (positions 387–408); 5'-GTTCCTGCAAGACCAATCAATC-3' *C. felis* Rev (positions 443–464); AGCAAGTTCTGATGCATTTAACCTTGTCG-3' *C. felis* probe FAM-BHQ1 (positions 414–442).

B. bronchiseptica: sequence of fhaB (*Bordetella bronchiseptica* FhaB gene, sequence ID: AF140678.1, product length: 92 bp); 5'-GGAACTTCAACTCCACGTATGA-3' *B. bronchi*. For (positions 821–842) 5'-ATTCGGTGTAGGTCGGATAGA-3' *B. bronchi* Rev (positions 893–913); 5'-TGGACCAGAATCGCTGGGAATACA-3' *B. bronchi*, probe HEX-BHQ1 (positions 860–883).

Amplification conditions in this multiplex qPCR were initial denaturation at 95 °C for 3 min, 45 cycles consisting of denaturation at 95 °C for 5 s and primer annealing at 60 °C for 30 s. For the positive control, strains of *M. felis*, *C. felis* and *B. bronchiseptica* (mentioned above) were added to each reaction and PCR water was used as a negative control.

Multiplex qPCR specificity. The specificity of multiplex qPCR was tested on extracts of nucleic acids from other microorganisms that could be present in the oral cavities of cats: FHV-1, FCV, *Pasteurella multocida* and *Staphylococcus epidermidis*. No positive reactions were recorded. Swabs were taken from a group of nine clinically healthy kittens (aged three to four months) from non-problematic households (flats) to avoid the possibility of primer binding inside the feline genome.

qPCR sensitivity. Reaction efficiency and sensitivity was tested on ten-fold dilutions of DNA standards. In three separate runs, all the DNA dilutions were processed in triplicate.

PCR identification of FHV-1. DNA of FHV-1 was detected using combi PPP master mix (Top-bio, Czech Republic) by nested PCR in a Piko ThermalCycler (Finnzymes instruments); amplified products were visualised on agarose gels. Primers targeted the gene for viral thymidine kinase and were used according to Reubel et al. (1993) for nested PCR. For the first round of amplification, forward primer 5'-GCATTTACATAGATGGTGCCT-3' and reverse primer 5'-ATATCTTGCGAGTGGGAAACAG-3' were used, giving a PCR product of 382 bp. For the second amplification round, forward primer 5'-CTTACTACTTCCCAGAACC-3' and reverse primer 5'-GTTCCTCACATACAACTTTC-3' were used according to Stiles et al. (1997). The length of the amplified DNA fragment was 222 bp, which was also confirmed *in silico* using the whole genome sequence of FHV-1, sequence ID: NC_013590.2.

Amplification conditions were identical in both steps: initial denaturation at 94 °C, followed by 30 cycles of denaturation 94 °C, 50 s, annealing at 50 °C, 50 s and extension at 72 °C, 50 s. The final extension took place for 5 min at 72 °C.

For the positive control, a FHV strain 1 was added into each reaction and PCR water was used as a negative control.

qPCR identification of FCV. Both detection and quantification of FCV RNA were performed using Xceed qPCR SG 1step 2× Mix Lo-ROX in a total volume of 20 µl according to the manufacturer's instructions on a Light Cycler® 480 II (Roche). The primers used for detection were described by Helps et al. (2002) and are targeted at a sequence in ORF1.

FCV reverse primer 5'-CATATGCGGCTCTGATGGCTTGAAACTG-3', FCV forwardprimer 5'-TAATTCGGTGTTTGATTTGGCCTGGGCT-3'.

Amplification conditions were initial denaturation at 95 °C for 15 min, 35 cycles consisting of denaturation at 95 °C for 30 s, primer annealing at 52 °C for 30 s and extension at 72 °C for 1 min. Immediately following the PCR, a melting curve was performed by raising the incubation temperature from 55 to 95 °C to confirm the specificity of obtained products. For the positive control, FCV

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strains were added to each reaction and PCR water was used as a negative control.

Statistical analysis. The results were analysed using the statistical package Unistat 5.6. (Unistat Ltd., London, United Kingdom). Differences in counts were tested on the basis of a Chi-square test within an analysis of k × m and 2 × 2 contingency tables with Yates's correction (Zar 1999). When the frequencies were lower than five, Fisher's exact test was used instead of the Chi-square test. A *P*-value less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

From a total number of 218 tested cats, 87 (40%) were negative for all tested agents. The results presented in Table 1 show that outdoor cats suffered from upper respiratory tract infections in the clinical or subclinical form (84 detected pathogens in 69 cats), whereas in indoor cats only 110 pathogens were detected in 149 cats. More than one pathogen was detected in a total of 38 cats, and six cats were infected with more than two pathogens. The main combination of pathogens detected in co-infections was FHV-1 and *Mycoplasma felis* (11.7%; Table 2). Only in six cats showing clinical symptoms were co-infections with pathogens other than *M. felis* proven (3 × FHV-1 with *C. felis,* 1 × FHV-1 with *B. bronchiseptica,* 1 × FCV with *C. felis,* and 1 × *C. felis* with *B. bronchiseptica*). Statistical analysis revealed no significant difference in mono-infections ($P > 0.05$) between indoor and outdoor cats. *M. felis* was the most prevalent pathogen (56.5%) detected in both outdoor and indoor cats (48.3%).

It should be noted that *B. bronchiseptica* was responsible for the smallest percentage (4.6%) of positive samples. Helps et al. (2005) describe a rate of PCR detection of *B. bronchiseptica* in diseased cats of 5% from different European catteries. Six diseased animals were positive for *B. bronchiseptica*, and four positive cats were asymptomatic. In order to obtain more significant statistical data, more positive samples are needed. Similar results related to the effect of the environment, such as where the cats live, on pathogen prevalence have been found in the USA (McManus et al. 2014). We confirmed a significant difference between outdoor and indoor cats in terms of the incidence of co-infection ($P < 0.05$). Outdoor cats were more frequently affected by multiple pathogens (29%) than indoor cats (13%). Similar results have been published in other countries (Cai et al. 2002; Rampazzo et al. 2003; Maazi et al. 2016).

A comparison of the prevalence of the pathogens in different age groups is shown in Table 3 and demonstrates that kittens and young adults up to the age of one year are the most common reservoirs of respiratory infection; only 19 cats (31%) out of 60 were negative and there were often co-infections (23%), mostly *M. felis* and FHV-1. Several reports have shown a high occurrence of infections among cats under one year of age (Di Martino et al. 2007; Zicola et al. 2009). Although no statistical significance was found for the ages of FHV-1- and FCV-positive cats, there was a higher prevalence of *C. felis*-positive cats among younger individuals (Maazi et al. 2016). Moreover, even though the number of detected pathogens seems to be higher in one-year-old cats, our statistical analysis did not

Table 1. Positive results of qRT-PCR, multiplex qPCR and nested PCR analysis

	In total	Indoor +/(%)	Outdoor +/(%)
Total cats	218	149	69
Feline calicivirus	17	10 (6.7)	7 (10.0)
Felid alphaherpesvirus 1	36	12 (8.0)	24 (34.8)
Mycoplasma felis	111	72 (48.3)	39 (56.5)
Chlamydia felis	20	7 (4.7)	13 (18.8)
Bordetella bronchiseptica	10	9 (6.0)	1 (1.4)
Negative cats	87	67 (45)	20 (29)

Table 2. Detection of *Mycoplasma felis* (MF; 111 positive findings = 100%) in cats with respiratory disorders (Sick) and without respiratory signs (Healthy) cats

	Sick (%)	Healthy (%)
MF mono-infection	44 (39.6)	29 (26.1)
MF + *Felid alphaherpesvirus 1*	13 (11.7)	0
MF + feline calicivirus	6 (5.4)	2 (1.8)
MF + *Chlamydia felis*	5 (4.5)	0
MF + *Bordetella bronchiseptica*	2 (1.8)	4 (3.6)
MF + feline calicivirus + *Felid alphaherpesvirus 1*	2 (1.8)	0
MF + *Felid alphaherpesvirus 1*+ *Chlamydia felis*	4 (3.6)	0
Total cases of MF infection	76 (68.5)	35 (31.5)

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Table 3. Positive identification of cat pathogens depending on age

	Up to 1 year of age +/(%)	1–3 years of age +/(%)	> 3 years of age +/(%)
Total cats	60	68	90
Feline calicivirus	7 (11.7)	5 (7.4)	5 (5.6)
Felid alphaherpesvirus 1	11 (18.3)	12 (17.6)	13 (14.4)
Mycoplasma felis	35 (58.3)	33 (48.5)	43 (47.8)
Chlamydia felis	11 (18.3)	4 (5.9)	5 (5.6)
Bordetella bronchiseptica	5 (8.3)	3 (4.4)	2 (2.2)

confirm significant differences among different age groups of animals and the incidence of individual pathogens ($P > 0.05$).

There was a slight decrease in positive samples with advancing age in cases of *B. bronchiseptica* and *C. felis* followed by FCV, which is in accordance with Gruffydd-Jones et al. (2009). In the case of FHV-1 and *M. felis*, similar numbers of these pathogens are detection throughout the lifetimes of cats, usually in co-infection with other pathogens (Polak et al. 2014).

Concerning the site of sampling of different pathogens (Table 4), we collected swabs from the oropharynx and from the conjunctiva in cases with clinical signs of conjunctivitis (Konvalinova et al. 2016). In the case of *B. bronchiseptica*, *M. felis* and FCV it was discovered that detection was more frequent in the oropharyngeal swabs ($P < 0.01$). All the other pathogens were detected on both oropharyngeal and conjunctival swabs ($P > 0.05$). These findings are similar to those described by Schulz et al. (2015) who reported that there is no significant difference between these two sampling sites for FHV-1 and *C. felis*. In FCV, *M. felis* and *B. bronchiseptica* there was a significant difference in the location of pathogens in favour of the oropharynx ($P < 0.01$), as shown in statistical analysis (Table 4). No significant difference was found in cases of FHV-1 and *C. felis* ($P > 0.05$).

Table 4. Positive identification of cat pathogens depending on place of collection

	In total	Oropharynx +/(%)	Conjunctiva +/(%)
Swabs	247	177	70
Feline calicivirus-positive	17	15 (8.5)	2 (2.8)
Felid alphaherpesvirus 1-positive	36	21 (11.9)	15 (21.4)
Mycoplasma felis-positive	111	87 (49.1)	24 (34.3)
Chlamydia felis-positive	20	10 (5.6)	10 (14.3)
Bordetella bronchiseptica-positive	10	10 (5.6)	0 (0)

From a total number of 29 tested animals with swabs taken from both sites, 13 (45%) cats were negative, of which six cases were both without clinical signs and without evidence of pathogens and seven cats exhibited respiratory signs without testing positive for any of the studied pathogens.

In total, 16/29 cats suffered from respiratory symptoms and tested positive for pathogens. In 5/29 cases, only mono-infection was confirmed, predominantly with *M. felis*, and in 11/29 samples co-infections were identified, predominantly *M. felis* and FHV-1. In positive cats, the presence of pathogens on both sampling sites in absolute numbers was (conjunctiva/oropharynx) *M. felis* 10/8, coincidence five cases; FHV-1 6/6, coincidence six cases; *C. felis* 4/5, coincidence two cases; FCV 2/1, no coincidence; *B. bronchiseptica* 0/1, no coincidence. Our results are in accordance with Schulz et al. (2015), where no significant difference between sampling sites for FHV-1 was found.

As shown in Table 2, *Mycoplasma felis* is a highly prevalent bacterium of the upper respiratory tract. The positive samples (39.6%) for *M. felis* mono-infection were collected from cats with clinical signs and *M. felis* was also detected in 26.1% of healthy cats without any clinical signs. In accordance with Holst et al. (2010), we can confirm that the detection of *M. felis* is possible in both healthy cats and cats with the clinical signs of an upper respiratory disease; indeed, there was no statistically significant difference between healthy and sick animals ($P > 0.05$). The other pathogens were not detected in healthy animals as mono-infections. Due to this reason, the data shown in Table 2 relate to *M. felis* and its co-infections only.

Based on differences in melting temperatures of the final products in real-time RT-PCR, we detected variability in FCV field strains.

The impact of feline vaccination on the incidence of pathogens is the subject of a future study.

In conclusion, multiplex real-time PCR, real-time RT-PCR and nested PCR have been introduced for the routine diagnostics of pathogens and by means of these methods it is possible to differentiate viral

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and bacterial pathogens in cats. A large diversity of individual FCV isolates was discovered in the feline population and needs further analysis.

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