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Detection of *Mycoplasma felis* from the kenneled cats with pneumonia

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Abstract

Two cats were obtained from a cat kennel. Over the previous 7 days, the cats had shown cough, anorexia, depression and nasal discharge. In this study, the consensus PCR was able to detect successfully Mycoplasma species in nasal swab samples of the cats. To identify feline mycoplasma species from the lung tissue of the cats with pneumonia, Mycoplasma species-specific PCR reactions were conducted. As the results, we could identify *M. felis* by the positive amplified DNAs. On the other hand, we could not detect any positive reactions with the PCR reaction for *M. arginini*, *M. canis*, *M. edwardii*, *M. cynos*, *M. gateae*, *M. maculosum*, *M. molared*, *M. opalescens*, *M. spumans* and Mycoplasma HRC-689. In conclusion, we detected *M. felis* from the kenneled cats with pneumonia. We suggested that this consensus PCR would be useful and effective for monitoring Mycoplasma species in various kinds of animals including cats. The application of preceding consensus PCR before the species-specific PCRs may be the most recommended strategy for the identification of *Mycoplasma spp*.

Key words : Mycoplasma, *Mycoplasma felis*, PCR, Cat, Pneumonia

INTRODUCTION

Mycoplasma species are small bacteria that lack a peptidoglycan cell wall. They belong to the normal commensal flora of the conjunctiva and upper airways (pharynx, larynx, oral cavity, nasal cavity) in cats and are a well-recognized cause of conjunctivitis and upper respiratory infection in this species (Bannasch and Foley, 2005; Johnson et al, 2005; Hartmann et al, 2010). However, their role as a primary cause of lower respiratory disease has been debated for several years. Mycoplasma species have never been isolated from the trachea, bronchi, or lung of healthy cats (Padrid et al, 1991; Randolph et al, 1993a). In most reported canine or feline cases with isolation of Mycoplasma species in the lower airways, a co-infection with other bacteria or an underlying disease leading to aspiration of gastric content, impairment of local defense mechanisms, or systemic immunosuppression were identified (Randolph et al, 1993a; Randolph et al, 1993b; Jameson et al, 1995; Foster et al, 1998; Chandler and Lappin, 2002). Thus, Mycoplasma colonization and proliferation were considered a secondary event.

Few reports argue for a primary pathogenic role of Mycoplasma species in lower respiratory disease in cats. A case series identified Mycoplasma species as the sole bacterial isolate cultured from airway washings in 3 cats with clinical and radiographic signs of bronchitis (Chandler and Lappin, 2002). However, the role of Mycoplasma species as primary pathogens was unclear because of the suspicion of concomitant feline bronchial disease (feline asthma/chronic bronchitis) in these 3 cats. A retrospective study on lower respiratory tract infections in cats reported a pure airway growth of Mycoplasma species in 11/21 cats (Foster et al, 2004). A case of primary severe Mycoplasma pneumonia with

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reversible respiratory failure was recently reported in a cat (Trow et al, 2008).

M. felis is associated with conjunctivitis (Campbell et al, 1973; Haesebrouck et al, 1991) and pneumonia (Rosendal, 1979) in domestic cats and several diseases in a variety of other mammals, including pleuritis (Ogilvie et al, 1983; Hoffman et al, 1992) and lower respiratory tract disease (Wood et al, 1997) in horses, severe respiratory disease in servals (Johnsrude et al, 1996) and arthritis in immuno-compromised cats (Hooper et al, 1985) and humans (Bonilla et al, 1997). In addition *M. felis* is thought to be a commensal of several species also being isolated from clinically healthy cats (Tan et al, 1977), dogs (Rosendal, 1979) and horses (Wood et al, 1997). However, there are conflicting reports of the presence of *M. felis* in clinically healthy cats, with some authors describing the frequent isolation of *M. felis* from healthy cats (Tan et al, 1977), and others reporting *M. felis* isolation from the conjunctiva of diseased cats only (Haesebrouck et al, 1991). Despite reproduction of *M. felis* induced disease in both horses (Ogilvie et al, 1983) and cats (Haesebrouck et al, 1991) the methods of pathogenesis of *M. felis* in equine and feline respiratory disease remains undefined.

It has been reported that polymerase chain reaction (PCR) assays is easy and useful method and can be performed even on nasal swabs as a noninvasive means of rapidly screening large numbers of animals for Mycoplasma species (Harasawa et al, 1991; Chalker et al, 2004c). However, those kinds of PCR assays require multiple assays because of a lot of Mycoplasma species (McAulifffe et al, 2003; Chalker et al, 2004c).

In this study, we detected Mycoplasma infected cats by a genus-specific PCR analysis method using 16S ribosomal DNA gene to detect. Thereafter, *Mycoplasma felis* was identified by a species-specific PCR.

MATERIALS AND METHODS

Study populations and sampling

Two cats, 5-month-old female Persian, were obtained from a cat kennel in Iksan City, Korea. The cats were presented with a 7 days-history of cough, anorexia, depression and nasal discharge. Nasal swabs were collected from the cats. Samplings were performed from the nares with a dry and unmoistened swab. The tip of the collection swab was inserted into the nares and rolled five times in each nostril. Collected specimens were transported and stored at room temperature. Specimens were processed for PCR analysis within 24 h of being collected. Each collection swabs were put into 2ml of 0.1M PBS buffer and vortexed and discarded, and the PBS was submitted to extract genomic DNAs for Mycoplasma consensus PCR assay.

The cats were euthanized and necropsied. Tissue specimens were collected from lungs. For Mycoplasma identification, some of the tissues were collected freshly and submitted to processing Mycoplasma species-specific PCR analysis.

DNA extraction

Lung tissues of the cats was homogenized and resuspended in PBS. Then, the lung tissue suspension and the nasal swab specimens were submitted to DNA extraction as described previously (Cho et al, 2011). Briefly, genomic DNA was isolated using an *AccuPrep* Genomic DNA extraction kit (Bioneer Corporation, Daejeon, Korea) according to the manufacturer's instructions. The DNA was eluted in Tris-EDTA buffer (pH 8.0), and an aliquot was used for PCR amplification. All DNA samples were stored at −20°C until the PCR assays were performed.

Mycoplasma consensus PCR

For screening assay of mycoplasma infection, we conducted Mycoplasma consensus PCR with the extracted DNAs from the nasal swabbing samples, as described previously (Hong et al, 2011). Briefly, amplification of the V3 region of the 16S ribosomal DNA performed with consensus primers GC-341F (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG) and 534R (5'-ATT ACC GCG GCT GCT GG). The template DNA (50 ng) and 20 pmol of each primer were added

to a PCR mixture tube (*AccuPower* PCR PreMix; Bioneer Corporation) containing 2.5 U of Taq DNA polymerase, 250 μM each deoxynucleoside triphosphate, 10 mM Tris-HCl (pH 8.3), 40 mM KCl, 1.5 mM $MgCl_2$, and the gel loading dye. The final volume was adjusted to 20 μl with distilled water. The reaction mixture was subjected to denaturation at 94°C for 5 min followed by 30 cycles of 95°C for 1 min, 55°C for 45 s, and 72°C for 1 min and a final extension step of 72°C for 10 min, and samples were kept at 4°C until analysis. Reactions were conducted using My Genie 32 Thermal Block PCR (Bioneer Corporation). Eight microliters of each sample were mixed with 2 μl of loading buffer, and electrophoretically separated on 1.2% agarose gels stained with 0.5 μg/ml ethidium bromide. DNA bands were observed under ultraviolet light.

Mycoplasma species identification

To identify canine mycoplasma species, we use species-specific PCR reactions for *M. arginini*, *M. canis*, *M. cynos*, *M. edwardii*, *M. felis*, *M. gateae*, *M. maculosum*, *M. molared*, *M. opalescens*, *M. spumans* and Mycoplasma HRC-689. Due to the high similarity of the 16S rRNA genes of canine Mycoplasma species (Chalker and Brownlie, 2004a), species-specific PCR tests were developed and used for the species listed in Table 1 to species-specific regions identified in the 16S/23S rRNA intergenic spacer region (Chalker et al, 2004b). Primers and amplification parameters are also listed in Table 1. All PCRs commenced with an initial denaturation at 95°C for 5 min and were followed by the specific annealing step listed in Table 1 and then by final extension at 72°C for 5 min. PCR reactions included 0.025 μg forward primer (Myc1; 5′-CACCGCCCGTCACACCA-3′), 0.025 μg reverse primer (Table 1), template DNA (50 ng) and 20 pmol of each primer were added to a PCR mixture tube (*AccuPower* PCR PreMix; Bioneer Corporation) containing 2.5 U of Taq DNA polymerase, 250 μM each deoxynucleoside triphosphate, 10 mM Tris-HCl (pH 8.3), 40 mM KCl, 1.5 mM $MgCl_2$, and the gel loading dye. The final volume was adjusted to 50 μl with distilled water. Reactions were conducted using My Genie 32 Thermal Block PCR (Bioneer Corporation). Eight microliters of each sample were mixed with 2 μl of loading buffer, and electrophoretically separated on 1.2% agarose gels stained with 0.5 μg/ml ethidium bromide. DNA bands were observed under ultraviolet light.

RESULTS

Consensus PCR with nasal swabbing samples

A consensus PCR analysis method using the 16S ribosomal DNA was employed to detect Mycoplasma species. The 16S r DNA gene (340 bp) was specifically amplified by PCR with the Mycoplasma genus-specific primers (GC-341F and 534R). The target nucleic acid fragments were specifically amplified by consensus PCR with 16S ribosomal DNA primers. As the result, the na-

Table 1. Canine Mycoplasma species PCR primers and reaction conditions

Species	Primer sequence	Cycle conditions (×30)	Product size (bp)
M. arginini	GTTGTATGACCTATTGTTGTC	95°C 1 min, 50°C 30s, 72°C 1 min	312
M. canis	CTGTCGGGGTTATCTCGAC	95°C 1 min, 55°C 30s, 72°C 1 min	247
M. cynos	GATACATAAACACAACATTATAATATTG	95°C 45s, 55°C 30s, 72°C 20s	227
M. edwardii	CTGTCGGGTTATCATGCGAC	95°C 45s, 55°C 30s, 72°C 20s	250
M. felis	GGACTATTATCAAAAGCACATAAC	95°C 45s, 51°C 30s, 72°C 20s	238
M. gateae	GTTGTATGACCTATTGTTGTC	95°C 1 min, 50°C 30s, 72°C 1 min	312
M. maculosum	CCTATGATTGTTACAGATG	95°C 1 min, 50°C 30s, 72°C 1 min	432
M. molare	AGCCTATTGTTTTTGATTTG	95°C 1 min, 55°C 30s, 72°C 1 min	397
M. opalescens	TAAGCTTTGTAGACCATAA	95°C 1 min, 50°C 30s, 72°C 1 min	236
M. spumans	GTTGTATGACCTATTGTTGTC	95°C 1 min, 50°C 30s, 72°C 1 min	312
Mycoplasma HRC-689	CTTGCGACCTAACAAGTCC	95°C 45s, 51°C 30s, 72°C 20s	227

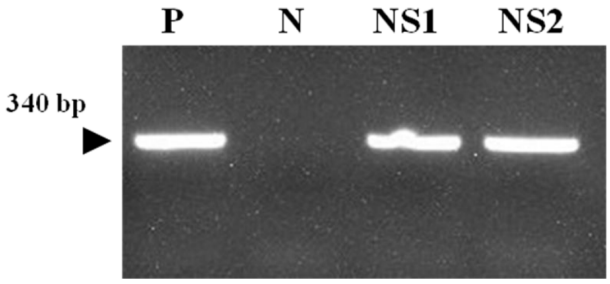

Fig. 1. Amplification of 16S rDNA gene by Mycoplasma consensus PCR was identified on a 1.2% agarose gel electrophoresis. Lane P: positive control, N: distilled water, NS1: Nasal swab sample of No. 1 cat, NS2: Nasal swab sample of No. 2 cat.

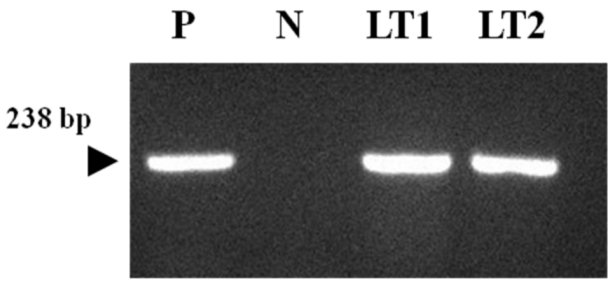

Fig. 2. Amplification with *Mycoplasma felis*-specific primers in the 16S/23S rRNA intergenic spacer region was identified on a 1.2% agarose gel electrophoresis. Lane P: positive control, N: distilled water, LT1: Lung tissue of No. 1 cat, LT2: Lung tissue of No. 2 cat.

sal secreting Mycoplasma species was detected by the positive PCR reactions in the nasal swabbing samples of two cats (Fig. 1).

Mycoplasma species identification

To identify feline mycoplasma species from the lung tissue of the cats with pneumonia, Mycoplasma species-specific PCR reactions were conducted. As the results, we could identify *M. felis* by the positive amplified DNAs as 238 bp (Fig. 2). On the other hand, we could not detect any positive reactions with the PCR reaction for *M. arginini*, *M. canis*, *M. edwardii*, *M. cynos*, *M. gateae*, *M. maculosum*, *M. molared*, *M. opalescens*, *M. spumans* and Mycoplasma HRC-689.

DISCUSSION

Due to the specialized nature of mycoplasma culture techniques and species identification few diagnostic laboratories routinely culture *M. felis* (Chalker et al, 2004c). Therefore, the importance of *M. felis* in disease may be underestimated. The identification of *M. felis* in clinical samples is usually performed by initial cultivation of fried egg-shaped colonies on mycoplasma-specific media in 2～3 days (Chalker et al, 2004c).

Confirmation of *M. felis* identification to the species level is then achieved by growth inhibition with specific anti-sera (Tan et al, 1977), fluorescent antibody staining (Carman et al, 1997), or use of an immunobinding assay (Brown et al, 1990). Additional serological testing can confirm a recent or active infection by detecting rising antibody titres to *M. felis* with an indirect haemagglutination assay (Rosendal et al, 1986). More recently, the advent of molecular biological techniques has allowed the identification of mycoplasma species-specific regions in the 16S rRNA and 16S/23S rRNA intergenic spacer (IGS) DNA sequences (Harasawa et al, 1999; Tang et al, 2000; Kong et al, 2001). Brown et al. (1995) developed a PCR reaction for several feline mycoplasmas from domestic and wild cats that can distinguish *M. felis* when coupled with a restriction digest step. However, this PCR has not been evaluated directly on clinical samples. Chalker and Brownlie (2004) have shown that the 16S rRNA gene of *M. felis* is highly similar to that of other Mycoplasma species within the synoviae clade and the IGS is more diverse. Phylogenetic analysis with the IGS produces an identical positioning to that of 16S rRNA analysis (Chalker and Brownlie, 2004). Within this study, we sought to determine whether the IGS sequence of *M. felis* is conserved in feline isolates of *M. felis* with the aim of developing a *M. felis* species-specific PCR that can be used for the identification of *M. felis* in feline clinical samples.

Mycoplasma pneumoniae epidemic as zoonosis has been reported in the classroom where the students raised a Syrian gold hamster family (Mikola et al, 1997). Recently, Mycoplasma has been accepted an important emerging and reemerging pathogen in human and animal diseases (Rosengarten et al, 2001). There are many questions to be answered concerning Mycoplasma infection in human and animals. There is no doubt that a bacteriological culture is the best method for diagnosing

a bacterial infection. The sensitivity of the culture-isolation method is low (Harasawa et al, 1991). Therefore, a culture is not considered to be the most practical diagnostic method. PCR that is a specific and sensitive molecular method for detecting *Mycoplasma* DNA can supplement the above methods. However, PCR methods using species-specific primers require multiple assays because of a lot of *Mycoplasma* species (McAulifffe et al, 2003). In this study, the consensus PCR was able to detect successfully mycoplasma species. This consensus PCR was recommended for monitoring *Mycoplasma* species in animals.

Recently, molecular methods, such as PCR with or without a restriction enzyme digestion step, were developed for identification of canine and feline mycoplasmas. Advantages of PCR testing include the elimination of the need for complex and expensive Mycoplasma-specific culture medium and antisera, the shortened delay before diagnosis of Mycoplasma infection and identification of species, and possibly the identification of non-cultivable species (Chalker et al, 2004c; Johnson et al, 2004). Chalker et al. (2004c) tested PCR primers based on the sequence within intergenic spacer between 16S and 23S rRNA genes for specific detection of *M. felis*. The method yielded comparable to the culture approach. Previously, the consensus PCR was able to detect successfully mycoplasma species. This consensus PCR was recommended for monitoring Mycoplasma species in animals (Hong et al, 2011). In this study, the consensus PCR was able to detect successfully Mycoplasma species in cats. We suggested that this consensus PCR would be useful and effective for monitoring Mycoplasma species in various kinds of animals including cats. The application of preceding consensus PCR before the species-specific PCRs may be the most recommended strategy for the identification of *Mycoplasma spp*.

In our study, *M. felis* was identified from the lungs of cats with respiratory disease. For Mycoplasma identification, some of the tissues were collected freshly and submitted to processing Mycoplasma species-specific PCR analysis. As the results, we identified *M. felis* from two cats with respiratory disease.

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REFERENCES

Bannasch MJ, Foley JE. 2005. Epidemiological evaluation of multiple respiratory pathogens in cats in animal shelters. J Feline Med Surg. 7: 109-119.

Bonilla HF, Chenoweth CE, Tully JG, Blythe LK, Robertson JA, Ognenovski VM, Kauffman CA. 1997. *Mycoplasma felis* septic arthritis in a patient with hypogammaglobulinemia. Clin Infect Dis 24: 222-225.

Brown MB, Gionet P, Senior DF. 1990. Identification of Mycoplasma felis and Mycoplasma gateae by an immunobinding assay. J Clin Microbiol 28: 1870-1873.

Brown DR, McLaughlin GS, Brown MB.1995. Taxonomy of the feline mycoplasmas *Mycoplasma felifaucium*, *Mycoplasma feliminutum*, Mycoplasma felis, *Mycoplasma gateae*, *Mycoplasma leocaptivus*, *Mycoplasma leopharyngis*, and *Mycoplasma simbae* by 16S rRNA gene sequence comparisons. Int J Syst Bacteriol 45: 560-564.

Campbell LH, Snyder SB, Reed C, Fox JG. 1973. *Mycoplasma felis*-associated conjunctivitis in cats. J Am Vet Med Assoc 163: 991-995.

Carman S, Rosendal S, Huber L, Gyles C, McKee S, Willoughby RA, Dubovi E, Thorsen J, Lein D. 1997. Infectious agents in acute respiratory disease in horses in Ontario. J Vet Diag Invest 9: 17-23.

Chalker VJ, Brownlie J. 2004a. Taxonomy of the canine Mollicutes by 16S rRNA gene and 16S/23S rRNA intergenic spacer region sequence comparison. Int J Syst Evol Microbiol 54: 537-542.

Chalker VJ, Owen WM, Paterson C, Barker E, Brooks H, Rycroft AN, Brownlie J. 2004b. Mycoplasmas associated with canine infectious respiratory disease. Microbiology 150: 3491-3497.

Chalker VJ, Owen WM, Paterson CJ, Brownlie J. 2004c. Development of a polymerase chain reaction for the detection of *Mycoplasma felis* in domestic cats. Vet Microbiol 100: 77-82.

Chandler JC, Lappin MR. 2002. Mycoplasmal respiratory infections in small animals: 17 cases (1988–1999) J Am Anim Hosp Assoc 38: 111-119.

Cho SJ, Lee HA, Hong S, Kim O. 2011. Uterine adenocarcinoma with feline leukemia virus infection. Lab Anim Res 27: 347-351.

Foster SF, Barrs VR, Martin P, Malik R. 1998. Pneumonia associated with Mycoplasma spp. in three cats. Aust Vet J 76: 460-464.

Foster SF, Martin P, Allan GS, Barrs VR, Malik R. 2004. Lower respiratory tract infections in cats: 21 cases (1995–2000) J Feline Med Surg. 6: 167-180.

Harasawa R, Koshimizu K, Takeda O, Uemori T, Asada K, Kato I. 1991. Detection of *Mycoplasma hyopneumoniae* DNA by the polymerase chain reaction. Mol Cell Probes 5: 103-109.

Haesebrouck F, Devriese LA, van Rijssen B, Cox E. 1991. Incidence and significance of isolation of *Mycoplasma felis* from conjunctival swabs of cats. Vet Microbiol 26: 95-101.

Hartmann AD, Hawley J, Werckenthin C, Lappin MR, Hartmann K. 2010. Detection of bacterial and viral organisms from the conjunctiva of cats with conjunctivitis and upper respiratory tract disease. J Feline Med Surg 12: 775-782.

Hoffman AM, Baird JD, Kloeze HJ, Rosendal S, Bell M. 1992. *Mycoplasma felis* pleuritis in two show-jumper horses. Cornell Vet 82: 155-162.

Hooper PT, Ireland LA, Carter A. 1985. *Mycoplasma polyarthritis* in a cat with probable severe immune deficiency. Aust Vet J 62: 352.

Hong S, Lee HA, Park SH, Kim O. 2011. Sensitive and Specific Detection of Mycoplasma species by Consensus Polymerase Chain Reaction and Dot Blot Hybridization. Lab Anim Res 27: 141-145.

Jameson PH, King LA, Lappin MR, Jones RL. 1995. Comparison of clinical signs, diagnostic findings, organisms isolated, and clinical outcome in dogs with bacterial pneumonia: 93 cases (1986–1991). J Am Vet Med Assoc 206: 206-209.

Johnson LR, Foley JE, DeCock HEV, Clarke HE, Maggs DJ. 2005. Assessment of infectious organisms associated with chronic rhinosinusitis in cats. J Am Vet Med Assoc 227: 579-585.

Johnson LR, Drazenovich NL, Foley JE. 2004. A comparison of routine culture with polymerase chain reaction technology for the detection of Mycoplasma species in feline nasal samples. J Vet Diagn Invest 16: 347-351.

JohnsrudeJD, Christopher MM, Lung NP, Brown MB. 1996. Isolation of *Mycoplasma felis* from a serval (*Felis serval*) with severe respiratory disease. J Wildl Dis 32: 691-694.

Kong F, James G, Gordon S, Zelynski A, Gilbert GL. 2001. Species-specific PCR for identification of common contaminant mollicutes in cell culture. Appl Environ Microbiol 67: 3195-3200.

McAulifffe L, Ellis RJ, Ayling RD, Nicholas RAJ. 2003. Differentiation of Mycoplasma species by 16S ribosomal DNA PCR and denaturing gradient gel electrophoresis fingerprinting. J Clin Microb 41: 4844-4847.

Mikola I, Balogh G, Nagy A, Matyas M, Glavits R, Stipkovits L. 1997. *Mycoplasma pneumoniae* epidemic as zoonosis. Orv Hetil 138: 2933-2935.

Ogilvie TH, Rosendal S, Blackwell TE, Rostkowski CM, Julian RJ, Ruhnke L. 1983. *Mycoplasma felis* as a cause of pleuritis in horses. J Am Vet Med Assoc 182: 1374-1376.

Padrid PA, Feldman BF, Funk K, Samitz EM, Reil D, Cross CE. 1991. Cytologic, microbiologic, and biochemical analysis of bronchoalveolar lavage fluid obtained from 24 healthy cats. Am J Vet Res. 52: 1300-1307.

Randolph JF, Moise NS, Scarlett JM, Shin SJ, Blue JT, Corbett JR. 1993a. Prevalence of mycoplasmal and ureaplasmal recovery from tracheobronchial lavages and of mycoplasmal recovery from pharyngeal swab specimens in cats with or without pulmonary disease. Am J Vet Res 54: 897-900.

Randolph JF, Moise NS, Scarlett JM, Shin SJ, Blue JT, Bookbinder PR. 1993b. Prevalence of mycoplasmal and ureaplasmal recovery from tracheobronchial lavages and prevalence of mycoplasmal recovery from pharyngeal swab specimens in dogs with or without pulmonary disease. Am J Vet Res 54: 387-391.

Rosendal S. 1979. Canine and feline mycoplasmas. In: Tully, J.G., Whitcomb, R.F. (Eds.), The Mycoplasmas, vol. 2. Academic Press, New York, pp. 217-234.

Rosendal S, Blackwell TE, Lumsden JH, Physick-Sheard PW, Viel L, Watson S, Woods P. 1986. Detection of antibodies to *Mycoplasma felis* in horses. J Am Vet Med Assoc 188: 292-294.

Rosengarten R, Citti C, Much P, Spergser J, Droesse M, Hewicker-Trautwein M. 2001. The changing image of mycoplasmas: from innocent bystanders to emerging and reemerging pathogens in human and animal diseases. Contrib Microbiol 8: 166-185.

Tan RJS, Lim EW, Ishak B. 1977. Ecology of mycoplasmas in clinically healthy cats. Aust Vet J 53: 515-519.

Tang J, Hu M, Lee S, Roblin R. 2000. A polymerase chain reaction based method for detecting Mycoplasma/Acholeplasma contaminants in cell culture. J Microbiol Meth 39: 121-126.

Trow AV, Rozanski EA, Tidwell AS. 2008. Primary mycoplasma pneumonia associated with reversible respiratory failure in a cat. J Feline Med Surg 10: 398-402.

Wood JL, Chanter N, Newton JR, Burrell MH, Dugdale D, Windsor HM, Windsor GD, Rosendal S, Townsend HG. 1997. An outbreak of respiratory disease in horses associated with *Mycoplasma felis* infection. Vet Rec 140: 388-391.