CASE REPORT

ISOLATION OF *MYCOPLASMA GATEAE* FROM A CAT SUSPECTED WITH FELINE *PARVOVIRUS* INFECTION

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ABSTRACT. Recently, diagnostic cases of *Mycoplasma* screening from feline kept rising. In 2018, there were two cases in felines that were positively diagnosed as *Mycoplasma* co-infection in which one of the two cases was due to mycoplasmosis. Diagnosis of mycoplasmosis was based on isolation and identification using biochemical testing. *Mycoplasma* was successfully isolated from both cases. Based on the feline death case, the result indicated that *Mycoplasma gateae* was isolated from the blood-stained trachea sample. In contrast, none was isolated from the lung. As the infection was associated with other microorganisms, this case concluded that the cause of death of the cat was due to *Parvovirus* with secondary infection of *Mycoplasma gateae*.

Keywords: diagnostic, *Mycoplasma gateae*, feline, pathogenic

CASE HISTORY

The carcass of a domestic short-haired (DSH) feline was received by Central Region Veterinary Laboratory with a body condition score of 2/5 and with a history of loss in appetite, loss in weight, flu and cough. The duration of symptoms was not clarified. At the perineal region, it was stained with pasty faeces. Necropsy finding showed that there was a slight amount of blood-stained fluid along the tract. The mucosal surface of the trachea had striations of hyperemia and the lung was soft with fluid oozing out upon palpation. Hyperaemia of the cardiac region of the stomach and pseudomelanosis were also observed during necropsy. The kidney had also been autolysed. Following that, Central Region Veterinary Laboratory processed the samples for viral and bacterial isolation and identification. *Parvovirus* was detected from the intestine by Polymerase Chain Reaction (PCR) method while the bacterial isolation and identification showed mild growth of *Pseudomonas aeruginosa*, *Streptococcus canis* and *Escherichia coli* from the lung, heart, kidney, liver and trachea. Parts of the trachea and lung samples were also sent to Veterinary Research Institute (VRI) for *Mycoplasma* isolation and identification.

Mycoplasma Isolation and Identification

Organ samples such as trachea and lungs were received and processed in Biosafety Cabinet Level 2. The isolation method was done as described in OIE Terrestrial Manual (2018) Chapter 3.3.5. Samples were cut into small pieces and cultured into PPLO broth and on PPLO agar. The culture was then incubated at 37°C in an anaerobic condition and monitored daily. After the broth culture showed some changes in pH, colour or turbidity, it was filtered using a 0.45 µm filter and inoculated onto PPLO agar which was prepared as stated by Stalheim (1976). Subsequently, the cultured agar was examined daily under

a stereomicroscope for suspected colonies up to a maximum of 14 days. Any identified *Mycoplasma*-like colony was then further tested using digitonin and a few biochemical tests.

Mycoplasma and *Acholeplasma* are highly similar in terms of colony morphology on solid agar media which is a fried egg-like appearance (Holt *et al*., 1994). Digitonin test was performed to differentiate between *Mycoplasma* and *Acholeplasma* in which *Acholeplasma* is highly resistant towards 1.5% digitonin (Holt *et al*., 1994).

Figure 1 shows the effect of digitonin of the inoculated culture on PPLO agar where *Mycoplasma* is susceptible towards digitonin. Digitonin test showed an 18 mm radius of clear zone area that confirmed it as *Mycoplasma*.

After the confirmation of *Mycoplasma*, the identification of the species was continued with biochemical tests. Biochemical tests that were performed included glucose, arginine, tetrazolium chloride, phosphatase and film and spot tests. All test media were prepared conventionally as stated by Aluotto *et al.* (1970) and Stalheim (1976) in Special Microorganisms Section, Veterinary Research Institute (VRI). Biochemical reactions towards the identification of *M. gateae* are shown in the following Table 1.

Biochemical test results showed that the presumptive isolate was unable to break down glucose where there was no change in colour of the glucose media. No colour change in tetrazolium

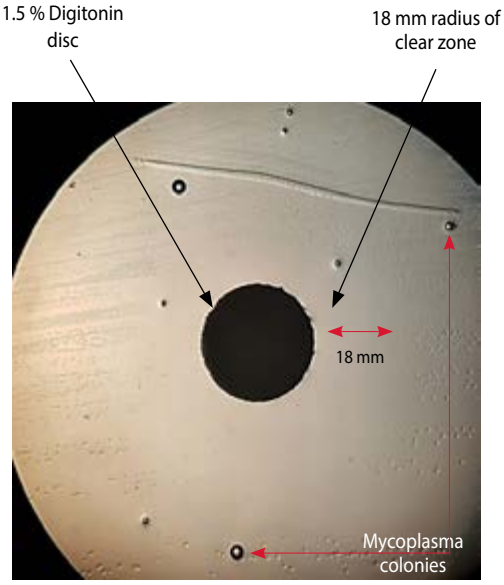

Figure 1. The effect of digitonin of the inoculated culture on PPLO agar.

and phosphatase activity tests indicate negative results. Besides that, other tests such as the formation of film and spot test on the egg yolk agar also showed negative reaction. However, the presumptive isolate was able to hydrolyse arginine when inoculated into PPLO media that was added with arginine that indicated colour changes of the media from pink to purple. Colour changes in the Arginine Biochemical testing further confirmed that *M. gateae* was present in the sample (Holt *et al*., 1994).

Table 1: Phenotypic identification of *M. gateae*.

Biochemical Test	Result
Glucose	Negative
Arginine	Positive
Tetrazolium chloride	Negative
Phosphatase	Negative
Film & spot	Negative

DISCUSSION

Most of the *Mycoplasma* infection is chronic and often non-fatal. In this referral case, the cat showed general symptoms such as inappetence, weight loss, flu and cough. Isolation thus proved that *M. gateae* was present in the trachea and none was successfully isolated from the lung. Diagnosis of *Mycoplasma* can be improved by practising appropriate sampling technique with proper samples to be processed. Samples such as a swab of the nasal cavity, trachea, air sacs in avian species, hock joint and lungs are highly recommended (Clothier *et al*., 2010) as *Mycoplasma* species including *M. gateae* mainly targets these areas for colonization. Having the right samples is crucial as it is one of the first steps of any diagnosis. Other than *M. felis*, *M. gateae* is also well known as *Mycoplasma arthritis* (Lemetayer and Taylor, 2014). In a study done by Moise and his team in 1983, *M. gateae* was found to be able to cause polyarthritis and tenosynovitis in a feline with clinical signs of swollen limbs and painful joints that are exhibited by sensitivity to touch, lameness and pyrexia. Experimentally, when *M. gateae* was inoculated intravenously, most of the affected areas were observed at the cat's cartilage and bones (Moise *et al*., 1983). However, in this case, *M. gateae* was only isolated from the trachea. Therefore, it was assumed that *M. gateae* could also affect the respiratory system of the cat. Further investigation is recommended on the colonization aspects of *M. gateae* in the respiratory system of feline to support the hypothesis.

From another point of view, *Mycoplasma* may act synergistically with other organisms, such as bacteria or viruses to cause disease (Robinson, 2009). This case showed that other than *M. gateae,* several other microorganisms such as *Pseudomonas aeruginosa, Streptococcus canis* and *Escherichia coli* were isolated, however, in low numbers. These bacteria are considered to be normal flora (Patricia *et al*., 1999; Seol *et al*., 2002; Frymus *et al*., 2015) and act opportunistic pathogens. There are a wide variety of microbial species that could be isolated in low numbers from clinically normal felines, suggesting that low numbers of bacteria should usually be regarded as normal flora (Patricia *et al*., 1999). Thus, the isolated *Pseudomonas aeruginosa, Streptococcus canis* and *Escherichia coli* could be considered as normal flora due to the mild growth during the isolation process and did not have a significant effect on the death of this cat.

At the same time, *Parvovirus* was detected by PCR from the intestine sample. *Parvovirus* is a common virus infecting feline of all ages and is highly contagious (Kruse *et al*., 2010). This may be one of the reasons for fatal cases of the feline though *Parvovirus* was not isolated from any of the samples in this study. The vaccination status of the cat was not included in the history as the status was unknown. However, it is suspected that *Parvovirus* might be the primary cause of the cat's death. Pasty faeces that were found around the perineal region showed that the feline might had diarrhoea. Diarrhoea may develop after 3rd or 4th days of infection, where dehydration from severe malabsorption frequently is a major contributing factor to fatal infections (MacLachlan and Dubovi, 2011). Other than that, hyperemia throughout the intestine indicated the infection of *Parvovirus* as highlighted by Greene (2012). In comparison to *M. gateae* and other isolated bacteria, those were opportunistic pathogens or widely known as secondary infection, affecting immunocompromised hosts (Little, 2011). Hence, information on vaccination status is important to provide a better diagnosis and treatment plan.

CONCLUSION

In conclusion, isolation of *M. gateae* from the trachea sample of the cat confirmed that the organism was able to contribute to inducing disease episodes in the cat infected with *Parvovirus* together with other bacteria isolated. The cat's cause of death is probably due to circulatory failure due to *Parvovirus* infection followed by respiratory failure due to mycoplasmosis and other secondary bacterial infections.

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