Detection of Sphingomonas paucimobilis Infections in Domestic Animals by VITEK® Compaq 2 and Polymerase Chain Reaction

Detección de infecciones por Sphingomonas paucimobilis en animales domésticos por VITEK Compaq® 2 y PCR

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INTRODUCTION

Sphingomonas spp. is a Gram-negative, aerobic, non-fermentative and oxidase positive rod, originated from the environment. Although the bacterium is not well known, it has high pathogenicity and causes severe clinical signs (Hsueh et al 1998, Ryan and Adley 2010). Because it has properties such as well as bacterial growing temperature, being widespread in environment and hanging on air by holding on dust, the bacteria can be presented in animal and human tissues and can cause infections particularly in the respiratory system (Hsueh et al 1998, Koskinen et al 2000, Tokajian et al 2008). In contrast, recent research on the detection of the bacterium has reported its high pathogenicity, showing it causes severe disorders with various clinical symptoms in different body systems (Maragakis et al 2009).

The study aimed to detect S. paucimobilis using different methods and bring the attention on this unusual bacterium. Additionally, the first case of S. paucimobilis found in animals in Turkey is reported.

MATERIAL AND METHODS

Two cows (both of two years old), a calf and a lamb (both of the 45 days) from different herds were admitted to the Veterinary Control Institute in Erzurum, Turkey. The calf presented general septicemia findings, the first cow had clinical signs related with chronic and repetitive pneumonia which was resistant to antibiotic treatment, the second cow had foot disease with clinical symptoms (lameness, swelling, etc.) and was resistant to antibiotic therapy, and the lamb had severe diarrhea and high body temperature. The autopsy of the animals was performed after euthanasia and several organs such as lung, liver, spleen, joint fluid and joint swaps were sampled for bacteriology. Samples were incubated on blood agar for 24 to 48 h at 37 °C. Gram stain and biochemical tests were done according to Quinn et al (1999). Antibiogram tests were done according to CLSI (2006) recommendations to define antibiotic resistance. Therefore lincomycin (2 μg), gentamicin (10 μg), ceftazidime (30 μg), ampicillin-sulbactam (10-10 μg), cefoperazone

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(30 μg), enrofloxacin (5 μg), amoxicillin-clavulanic acid (20-10 μg) discs were used for the analyses. At the same time, VITEK Compaq® 2 system and PCR method were used to definitive identification (Tokajian et al 2008).

The PCR mixture was prepared in 25 μl total volume at final concentrations of 1μM primers (S. paucimobilis F; AAGTCGTAACAAGGTAACC, S. paucimobilis R; GGGTTBCCCCATTCRG), 200 μM dNTP and 3 mM MgCl₂, then 2 μl of extracted bacterial DNA was added to the mixture. Thermocycling protocol consisted of 5 min of preliminary denaturation at 94 °C followed by 30 cycles of 30 second denaturation at 94 °C, 30 second of primer binding 48 °C and 1 min of polymerization at 72 °C and finally 5 min of polymerization at 72 °C. Amplification products were subjected to electrophoresis in a fixed 120 V electrical field; the resulting bands were examined under ultraviolet transilluminator and photographed.

RESULTS AND DISCUSSION

Gram-negative rods were isolated from all animals (Quinn et al 1999), but the isolates could not be completely identified by using conventional bacteriologic methods. Then, all isolates were examined by VITEK Compaq® 2 system and PCR method were used to definitive identification (Tokajian et al 2008). The PCR mixture was prepared in 25 μl total volume at final concentrations of 1μM primers (S. paucimobilis F; AAGTCGTAACAAGGTAACC, S. paucimobilis R; GGGTTBCCCCATTCRG), 200 μM dNTP and 3 mM MgCl₂, then 2 μl of extracted bacterial DNA was added to the mixture. Thermocycling protocol consisted of 5 min of preliminary denaturation at 94 °C followed by 30 cycles of 30 second denaturation at 94 °C, 30 second of primer binding 48 °C and 1 min of polymerization at 72 °C and finally 5 min of polymerization at 72 °C. Amplification products were subjected to electrophoresis in a fixed 120 V electrical field; the resulting bands were examined under ultraviolet transilluminator and photographed.

In previous studies, S. paucimobilis was isolated from both human and animals (Hsueh et al 1998, Maragakis et al 2009). In the current study, the bacterium was isolated from different animal infections with various clinical signs such as sepsicaemia, pneumonia and foot disease. S. paucimobilis was reported to be resistant to some antibiotics, disinfectants and some chemical agents (Hoquet et al 1985, Koskinen et al 2000). As compatible with the clinical history, isolated strains were also found resistant to most antibiotics. This result showed that antibiotic usage strategy must be considered in field conditions and excessive antibiotic therapies cause resistance in environmental bacteria such as S. paucimobilis. It is clear that treatment for some likeliest infections especially those caused by environmental bacteria may be more difficult in the future.

In conclusion, S. paucimobilis should also be taken into the consideration as causative agent in pneumonia and...
foot diseases when antibiotics could not provide recovery. Additionally, convenient bacteriologic approaches must be arranged for identification. In addition to conventional methods, advanced diagnostic tests such as Api, VITEK Compaq® 2 systems and PCR can be used for the identification of *S. paucimobilis* infections in animal species. As it was shown in the study, usual environmental bacterium can cause disease and death. Therefore, veterinarians and/or the owner of the animals must be ensure that animals have protected housing in appropriate conditions and also must pay attention to suckling.

REFERENCES


