Arch Med Vet **47, 117-119 (2015)**

COMMUNICATION

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

S Cengiz[a*], S Seyitoglu[b], S Kılıç Altun[b], U Dinler[b]

[a] Department of Microbiology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey.

[b] Veterinary Control Institute, Erzurum, Turkey.

RESUMEN

Se presenta los primeros casos por *Sphingomonas paucimobilis* en Turquía, ocurridos en dos vacas, un ternero y un cordero. La bacteria fue aislada e identificada por métodos convencionales, el sistema de VITEK Compaq® 2 y la reacción de polimerasa en cadena (PCR). Se llegó a resultados compatibles entre la historia clínica, los hallazgos de necropsia y los resultados bacteriológicos. En conclusión, *S. paucimobilis* debe tomarse en consideración como agente causal, en particular en enfermedades respiratorias, septicemias e infecciones del pie, considerando su resistencia al tratamiento con antibióticos. También los métodos de detección deben ser los adecuados, no solo para los patógenos comunes, sino también *S. paucimobilis.*

Palabras clave: infecciones de animales, *S. paucimobilis*, sistema de VITEK Compaq® 2, PCR.

SUMMARY

This case report presents the first cases of *Sphingomonas paucimobilis* in Turkey, which was isolated and identified from two cows, a calf and a lamb by conventional methods, VITEK Compaq® 2 system and Polymerase Chain Reaction (PCR). Compatible findings were reached among clinical history, necropsy findings and bacteriologic results. In conclusion, *S. paucimobilis* should be considered as a causative agent particularly for respiratory diseases, septicemia and foot infections with resistance to antibiotic treatment. Also, detection methods must be arranged not only for common pathogens but also for *S. paucimobilis.*

Keywords: animal infections, *S. paucimobilis*, VITEK Compaq® 2 system, PCR.

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 pathogenity 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 pathogenity, 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), cefquinome (30 µg), ampicillin-sulbactam (10-10 µg), cefoperazone

Accepted: 13.07.2014.

* 25240 Erzurum, Turkey; seydacengiz@atauni.edu.tr

Table 1. VITEK Compaq® 2system report for the lamb isolated strain.
Informe del sistema VITEK Compaq® 2sistema para la cepa aislada de cordero.

Selected Organism	%96 Probability Bionumber: 1001711150220000				Sphingomonas paucimobilis Confidence: Excellent identification						
Contraindicating typical biopattern(s) Sphingomonas paucimobilis dMAN											
Biochemical Details											
APPA	+	ADO	–	PyrA	–	IARL	–	dCEL	–	BGAL	–
H2S	–	BNAG	–	AGLTp	–	dGLU	+	GGT	–	OFF	–
BGLU	+	dMAL	+	dMAN	+	dMNE	+	BXYL	–	BAIap	–
ProA	+	LIP	–	PLE	–	TyrA	+	URE	–	dSOR	–
SAC	+	dTAG	–	dTRE	+	CIT	–	MNT	–	5KG	–
ILATk	–	AGLU	+	SUCT	–	NAGA	–	AGAL	+	PHOS	–
GlyA	–	ODC	–	LDC	–	IHISa	–	CMT	–	BGUR	–
O129R	–	GGAA	–	IMLTa	–	ELLM	–	ILATa	–		

(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 $MgCl_2$, 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 ºC 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 these rods were identified as *S. paucimobilis* (table 1). Isolated strains were taken to PCR evaluation and disc diffusion antibiogram test (CLSI 2006, Tokajian *et al* 2008). According to PCR results, all strains produced to specific bands (350-500 bp) (figure 1) corresponding to *S. paucimobilis,* as shown in previous report (Tokajian *et al* 2008). All isolates were found to be resistant to lincomycin, gentamicin, cefquinome, ampicillin-sulbactam, cefoperozone while they were sensitive to enrofloxacin and amoxicillin-clavulanic acid.

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 septicaemia, 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

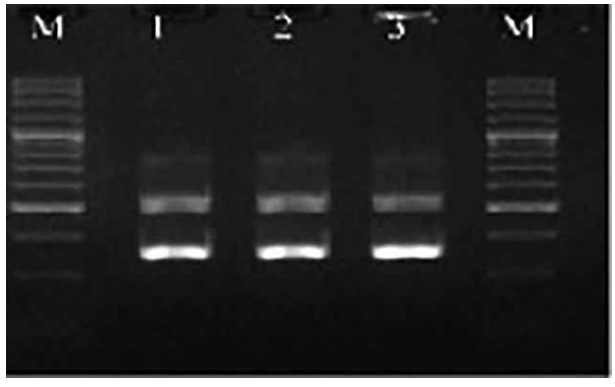

Figure 1. PCR results of *S. paucimobilis* isolated from different animals. M: Marker GeneRuler 100 bp DNA Ladder Plus (Fermantas) 1: isolate from cow's joint, 2: isolate from lamb's spleen, 3: isolate from cow's lung.

Resultados de la PCR de *S. paucimobilis* aisladas de diferentes animales. M: Marcador GeneRuler 100 pb DNA Ladder Plus (Fermantas) 1: aislamiento de articulación de vaca, 2: aislamiento de bazo de cordero, 3: aislamiento de pulmón de vaca.

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

CLSI standard. 2006. Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100-S16. 16th Informational Supplement, Wayne, PA, USA, Pp 38-39.

Hoquet F, R Higgins, P Lessard, A Vrins, M Marcoux. 1985. Comparison of the Bacterial and Fungal Flora in the Pharynx of Normal Horses and Horses Affected with Pharyngitis. *Can Vet J* 26, 342-346.

Hsueh P, L Teng, P Yang, Y Chen, H Pan, S Ho, K Luh. 1998. Nosocomial Infections Caused by *Sphingomonas paucimobilis*: Clinical Features and Microbiological Characteristics. *Clin Infec Dis* 26, 676-681.

Koskinen R, T Ali-Vehmas, P KaÈmpfer, M Laurikkala, I Tsitko, E Kostyal, F Atroshi, M Salkinoja-Salonen. 2000. Characterization of *Sphingomonas* isolates from Finnish and Swedish drinking water distribution systems. *J Appl Microbiol* 89, 687-696.

Maragakis LL, R Chaiwarith, A Srinivasan, FJ Torriani, E Avdic, A Lee, TR Ross, KC Carroll, TM Per. 2009. *Sphingomonas paucimobilis* Bloodstream Infections Associated with Contaminated Intravenous Fentanyl 1. *Emerg Infect Dis* 15, 12-18.

Quinn PJ, ME Carter, B Markey, GR Carter. 1999. Bacterial pathogens: Microscopy, culture and identification. In: *Clinical Veterinary Microbiology*. Mosby, London, UK, Pp 21-67.

Ryan MP, CC Adley. 2010. *Sphingomonas paucimobilis*: a persistent Gram-negative nosocomial infectious organism. *J Hos Infect* 75,153-157.

Tokajian S, S Al-Medawar, F Hashwa. 2008. Use of the 16-23S ribosomal genes spacer region for the molecular typing of sphingomonads. *Can J Microbiol* 54, 668-676.