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Prevalence of bacteremia in dogs admitted to an intensive care unit with intravenous catheterization

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Abstract

Bacteremia is a common complication in hospitalized patients, and intravenous catheterization is a routine intervention in dogs in intensive care units (ICU). This study aimed to investigate bacteria associated with bacteremia in an ICU of a veterinary teaching hospital during 2013-2014. Fifty dogs admitted to the ICU were included in this study. Twenty-four dogs (48.00%) presented bacteremia by blood culture. Bacterial species were identified, including Klebsiella pneumoniae (8/24), Pseudomonas aeruginosa (7/24), Staphylococcus pseudintermedius (3/24), Escherichia coli (2/24), Bacillus sp. (1/24), Acinetobacter sp. (2/24) and Burkholderia cepacia (1/24). Only five dogs had positive catheter colonization by identical bacterial species with those isolated from blood. Risk factors and clinical presentation were not associated with bacteremia in the dogs and need further evaluation. The study showed that commensal flora and environmental saprophytes were sources of bacteremia in canine patients admitted to the ICU.

 $\textbf{Keywords:} \ \text{bacteremia, canine, intravenous catheter, intensive care unit}$

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Introduction

Bacteremia is a common complication in patients that increases morbidity, mortality and duration of hospitalization. This condition results in a worse outcome of diseases because of the presence of bacteria in the bloodstream which evokes an inflammatory response (Hotchkiss and Karl, 2003). In humans, nosocomial pathogens such as Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Acinetobacter baumanii, Klebsiella spp. and Enterococcus spp. are common causes of bacteremia (Abebe et al., 2014). Common predisposing causes, including destruction of organ epithelium and use of medical indwelling devices, result in damaged body barriers and facilitate bacterial translocation to bloodstream. An intravenous catheter colonized by bacteria can cause bacteremia, termed catheter-related bacteremia (CRB), in humans (Abebe et al., 2014). Blood culture is the gold standard method for diagnosis of bacteremia and concurrent and identical bacterial species isolated from both blood and a catheter can definitively identify CRB (Fletcher, 2005). Likewise, bacteremia-induced sepsis results in fatal outcomes in dogs. The occurrence can be transient in healthy animals that can eliminate bacteria from the bloodstream (Dahlinget et al., 1997; Unterer et al., 2015). Patients in an intensive care unit are prone to have bacteremia by primary diseases and medical intervention (Burrows et al., 1982; Rello et al., 1994). Intravenous catheterization is routinely used in dogs for multiple purposes during treatment. Intravenous catheter colonization is reported in dogs ranging from 15.4% to 26.5%, but there is still a lack of research on the causes of bacteremia (Lobetti et al., 2002; Marsh-Ng et al., 2007; Sequela and Pages, 2011). Furthermore, information about bacteremia and associated bacterial species is comparatively rare in veterinary medicine. This study, therefore, aimed to investigate the prevalence of bacteremia and bacterial species associated with bacteremia in dogs with intravenous catheterization admitted to an intensive care unit of a veterinary teaching hospital between 2013 and 2014.

Materials and Methods

Study population: Fifty client-owned dogs admitted to the intensive care unit (ICU) of a veterinary teaching hospital between October 2013 and August 2014 were included in the study following signed consents by the owners. The protocol was approved by the Animal Care and Use Committee (No. 13310051). All patients needed intravenous catheterization for treatment performed in the ICU, and none had previous intravenous catheter treatment before the time of admission. Signalment, catheter insertion site, duration of catheter placement, body temperature, white blood cell (WBC) count, duration of hospitalization, antimicrobial administration, and presence or absence of local inflammation identified by swelling, erythema or abscessation of the skin around the insertion site were recorded.

Twenty-four-gauge, 3/4-inch or 22-guage, 1-inch ethylene tetrafluoroethylene catheters (Safelet Cath®, Nipro, Thailand) were inserted into the patients' cephalic or saphenous vein. The purposes

were for fluid therapy and drug administration. Catheterization was performed by attending veterinarians and according to the hospital's standard guidelines. Before catheter placement, hair around the vein was shaved, and skin over the insertion site was wiped using sterile cotton soaked with 70% ethyl alcohol until no dirt was apparent to prevent contamination of the insertion site. The skin was airdried before a catheter was inserted into the vein. After placement, the catheter was secured to the limb with adhesive tape, and an intravenous extension set was attached to the catheter. The insertion site was assessed daily to detect the presence of local inflammation.

Sample collection: The intravenous catheters were aseptically collected for culture by one of the authors (S.R.). The distal 1.5-2.0 cm of the catheter was cut off with sterile scissors and kept in a sterile tube at 4°C. Criteria for replacement or removal of the intravenous catheter included non-functional operation, discontinuation of fluid therapy, and presence of local inflammation or development of fever without an obvious cause. The samples were submitted for aerobic bacterial culture within 2 hours.

Blood was collected for culture by venipuncture from the jugular vein immediately after completion of catheter removal (performed by S.R.). Hair around the area over the jugular vein was removed, and skin over the site of venipuncture was wiped using sterile cotton soaked with 70% ethyl alcohol. After air drying, a volume of blood (1-3 mL) was collected using a 21-guage sterile needle attached to a 5 mL disposable syringe. Double needle technique was conducted by changing to a new sterile needle, and the top of the blood culture bottle (Peds Plus™/F Culture Vials, Becton Dickenson and Company, NJ) was wiped with 70% ethyl alcohol prior to inoculation. The blood culture bottles were sent within 2 hours to a microbiological laboratory for incubation.

Bacterial culture: The blood culture bottles were incubated and monitored for bacterial growth using an automated blood culture system for 5 days (BACTEC™ 9120 Blood Culture System, Becton Dickenson Microbiological System, MD). The medium in positive culture bottles was transferred onto a Brain Heart Infusion (BHI) agar with 5% sheep blood (BHIB agar) (Difco®, France) for isolation of single colonies. Bacterial culture from the catheter tips was performed following the semi quantitative (roll plate) method (Maki et al., 1977). Briefly, the catheter tip was transferred onto on a BHIB agar and rolled repeatedly at least 3 times across the surface. The agar plates were incubated at 37°C and observed for bacterial growth every day for 1 week. Pure colonies were cultivated for species identification based on Gram's staining and biochemical tests using an automated bacterial identification system (Vitek® 2 Compact system, Biomérieux, SA).

Definition: Bacteremia was defined when the blood displayed a positive culture. Hypothermia was the body temperature <100°F (<37.8°C), and fever was >102.5°F (>39.2°C). Leukopenia and leukocytosis ranged <6,000 cells/mL and >16,000 cells/mL,

respectively (Miller et al., 2004). Catheter colonization was characterized by a yield of >15 colonies on a catheter culture by the roll plate method (Maki et al., 1977).

Statistical analysis: Univariate statistical analyses were performed using chi-square or Fisher's exact test for qualitative data and a student's *t* or Mann-Whitney U test for quantitative data. Risk factors for bacteremia and bacterial colonization such as clinical signs, duration of hospitalization, catheter insertion site, and presence of local inflammation at the site of catheter placement were also evaluated using chi-square or Fisher's exact test. A *P* value <0.05 was considered statistically significant.

Results and Discussion

A total of 50 dogs, including 24 females and 26 males, were enrolled in the study. Age of the population ranged from 3 months to 20 years (median, 9 years). There were 12 cross-bred dogs and 38 purebred dogs, including Poodle (n=16), Golden Retriever (n=4), Shih Tzu (n=4), Labrador Retriever (n=2), Pomeranian (n=2), French Bulldog (n=2), Chihuahua (n=1), Chao Chao (n=1), Pug (n=1), German Shepherd (n=1), Saint Bernard (n=1), Yorkshire Terrier (n=1), Miniature Pinscher (n=1) and Dachshund (n=1). Catheters were inserted into the cephalic vein of 40 dogs (80%) and the saphenous vein of 10 dogs (20%). The median duration of the catheter in placement was 4 days (range, 2-5 days). Nineteen dogs (38%) showed local inflammation at the site of catheterization. All dogs received antimicrobial therapy and 4/50 dogs had episodes of fever over the period of hospitalization. Six dogs died, and the remaining 44 dogs were discharged.

This study showed 48.00% prevalence of bacteremia due to positive blood culture in dogs admitted to the ICU with intravenous catheterization. Previous studies presented 0%, 2.1%, and 11.0% in dogs with immune-mediated anemia, in dogs with idiopathic acute hemorrhagic diarrhea syndrome and in experimental healthy dogs following gastroduodenoscopy and biopsy, respectively (Jones et al., 2013; Miller et al., 2004; Unterer et al., 2015). High

prevalence of bacteremia was reported in GDV patients (43%) and dogs undergoing an ovariohysterectomy (40%) (Winkler et al., 2003). The dog populations, underlying diseases and severity of illness considerably varied and were less likely to correlate with bacteremia in the studies. Nevertheless, bacteremia was considered to be relatively high and should be noted as a possible complication.

Frequency of positive catheter culture was 38.0% of the study's population. Most of the bacteria isolated from blood and catheters were commensal flora or saprophytes, which commonly cause nosocomial infection. The number of patients with bacteremia and catheter colonization are shown in **Table 1**. *K. pneumoniae* is an intestinal microflora that hospital contaminates environments opportunistically infects and causes severe clinical outcomes in humans (Podschun and Ullmann, 1998); it is also associated with severe enteritis, septicemia and death in dogs (Roberts et al., 2000). P. aeruginosa is a common nosocomial pathogen and persists in the environment (Obritsch et al., 2005). As the predominant species from catheter colonization, saprophytic Burkholderia cepacia is a consequence of contamination of medical instruments and solutions (Nasser et al., 2004). S. pseudintermedius is a major species on dog skin, and it can cause complications such as urinary tract infection, bacteremia and postoperative infection (van Duijekeren et al., 2011). This organism also infects a child with hemophilia exposed to dog, causing catheter-related bacteremia (Chuang et al., 2010). E. coli and Acinetobacter sp. were only isolated from the blood culture while Serratia marcescens and E. cloacae were only found in the catheter culture. Most microorganisms are a concern as species associated with multidrug resistance can be widespread in medical and veterinary settings (Boerlin et al., 2011; Rubin et al., 2008; Weese and van Duijkeren, 2010; Wieler et al., 2011). Possible origins of bacteremia can be the intestinal microflora translocated by destruction of the intestinal barrier and immunosuppression (Defez et al., 2004). The source of bacteremia could not be indicated in this study, but the identified species could infer that bacteria in the animal body or environment might be the source.

 Table 1
 Bacterial species isolated from blood and catheter cultures

	Number (%) of dog		
Bacterial species	Blood culture	Catheter culture	
	(n=50)	(n=50)	
Klebsiella pneumonia	8 (16%)	2 (4%)	
Pseudomonas aeruginosa	7 (14%)	1 (2%)	
Staphylococcus pseudintermedius	3 (6.0%)	5 (10%)	
Escherichia coli	2 (4.0%)	0	
Acinetobacter sp.	2 (4.0%)	0	
Bacillus sp.	1 (2.0%)	1 (2%)	
Burkholderia cepacia	1 (2.0%)	8 (16%)	
Serratia marcescens	0	1 (2%)	
Enterobacter cloacae	0	1 (2%)	
Total	24 (48%)	19 (38%)	

Five dogs had identical bacteria isolated from the catheter and blood cultures. The result could indicate that catheter colonization might be a source of bacteria in the blood. Introduction of contaminated bacteria in the environment by medical indwelling devices is also a risk for bacteremia (Abebe et al., 2014). Catheter-related bacteremia can be diagnosed by the concurrent positive culture with the same

microorganism from blood and catheter (Abebe et al., 2014; Fletcher et al., 2005). With identical bacterial species, these dogs presenting bacteremia met the criterion. The roll plate technique could be applied for the diagnosis in canine patients when bacteremia and catheter colonization are suspected.

Of the 24 dogs with bacteremia, only 2 dogs (8.3%) had fever, and 11 dogs (45.8%) had leukocytosis. Fever (P=0.887) and leukocytosis (P=0.600) were not associated with bacteremia. Median duration of hospitalization was 5 days (range, 2 to 13 days), while it was also 5 days (range, 3 to 10 days) in the bacteremic and non-bacteremic dogs, respectively. Difference in the duration between the groups was not significant (P=0.062). Bacteremia was presented in 3 of the 6 dogs which died during hospitalization. Local inflammation was found in 10/24 dogs with bacteremia and 7/19 dogs with catheter colonization. Of 5 dogs with identical bacteria from the blood and catheter culture, only 2 dogs had local inflammation. The catheter insertion site inflammation was not associated with the catheter culture and bacteremia. These catheter infections are not usually associated with catheterrelated bacteremia and exist independently from catheter-related bacteremia (Dafdar and Maki, 2002). The cephalic vein was the major site for catheter placement in 15/19 (78.9%) dogs presenting catheter colonization and 25/31 (80.6%) dogs without catheter colonization. The catheter insertion site was not associated with bacterial colonization (OR, 0.900; 95% CI, 0.218, 3.715). The catheters were placed for 3.53±0.90 days for the dogs with catheter colonization and for 3.42±0.92 days for the dogs with negative catheter culture, presenting no significant difference (P=0.690). Six of 19 (40.0%) dogs with catheter colonization presented local inflammation at the site of catheter placement that was not associated with the bacterial colonization of the catheters (OR, 0.800; 95% CI, 0.227, 2.817). Catheter-related bloodstream infection (CRBSI) is a considerable complication in humans and is characterized by the presence of bacteremia with systemic signs of infection (Fletcher et al., 2005). Symptoms of CRBSI could not identify infection in the dogs because of the absence of systemic signs, and other obvious illnesses could not be ruled out. Fever is not a good predictor of bacteremia in humans (Fontanarosa et al., 1992). This study could not describe the association between the absence of symptoms and the presence of bacteremia in the dogs. Antibiotic administration might decrease the severity of the infection (Guembe et al., 2013). This study strongly suggests that blood culture is a reliable method for detecting the presence of bacteria in blood of suspected patients. This study did present the prevalence of bacteremia in dogs admitted to the ICU with intravenous catheterization. Factors and clinical signs for prediction of bacteremia in dogs could not be identified. Bacteria from the environment and normal flora are commonly associated with both bacteremia and catheter colonization that are part of nosocomial infection.

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References

- Abebe A Tener M Waller S and el Atrouni W 2014. Catheter-related bloodstream infections review. Hosp Med Clin. 3:e32-e49.
- Boerlin P Eugster S Gaschen F Straub R and Schawalder P 2001. Transmission of opportunistic pathogens in a veterinary teaching hospital. Vet Microbiol. 82(4):347-359.
- Burrows CF 1982. Inadequate skin preparation as a cause of intravenous catheter-related infection in the dog. J Am Vet Med Assoc. 180(7): 747-749.
- Chuang C-Y Yang Y-L Hsueh P-R and Lee P-I 2010. Catheter-related bacteremia caused by *Staphylococcus pseudintermedius* refractory to antibiotic-lock therapy in a hemophilic child with dog exposure. J Clin Microbiol. 48(4): 1497-1498.
- Dafdar N and Maki DG 2002. Inflammation at the insertion site is not predictive of catheter-related bloodstream infection with short-term, noncuffed central venous catheters. Crit Care Med. 30(12): 2632-2635.
- Dahlinger J Marks SL and Hirsh DC 1997. Prevalence and identity of translocating bacteria in healthy dogs. J Vet Int Med. 11(6): 319-322.
- Defez C Fabbro-Peray P Bouziges N Gouby A Mahamat A Daurés JP and Sotto 2004. Risk factors for multidrug-resistant *Pseudomonas aeruginosa* nosocomial infection. J Hosp Infect. 57(3): 209-216.
- Fletcher S 2005. Catheter-related bloodstream infection. Continuing Education in Anaesthesia, Critical Care & Pain. 5(2): 49-51.
- Fournier PE Richet H and Weinstein RA 2006. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clin Infect Dis. 42(5): 692-699.
- Fontanarosa PB Kaeberlein FJ Gerson LW and Thomson RB 1992. Difficulty in predicting bacteremia in elderly emergency patients. Ann Emerg Med. 21(7): 842-848.
- Guembe M Rodríguez-Créixems M Martín-Rabadán P Alcalá L Muñoz P and Bouza E 2013. The risk of catheter-related bloodstream infection after withdrawal of colonized catheters is low. Eur J Clin Microbiol Infect Dis. 33(5): 729-734.
- Hauptman JG Walshaw R and Olivier NB 1997. Evaluation of the sensitivity and specificity of diagnostic criteria for sepsis in dogs. Vet Surg. 26(5): 393-397.
- Jones KR Maddox CW Ridgway MD Clark-Price SC and Dossin O 2013. Incidence of bacteremia following upper gastrointestinal endoscopy and biopsy in healthy dogs before, during, and after

- treatment with omeprazole. Am J Vet Res. 74(2): 239-242.
- Lobetti RG Joubert KE Picard J Carstens J and Pretorius E 2002. Bacterial colonization of intravenous catheters in young dogs suspected to have parvoviral enteritis. J Am Vet Med Assoc. 220(9): 1321-1324.
- Maki DG Weise CE and Sarafin HW 1977. A semiquantitative culture method for identifying intravenous-catheter-related infection. New Engl J Med. 296(23): 1305-1309.
- Marsh-Ng ML Burney DP and Garcia J 2007. Surveillance of infections associated with intravenous catheters in dogs and cats in an intensive care unit. J Am Anim Hosp Assoc. 43(1): 13-20.
- Miller SA Hohenhaus AE and Hale AS 2004. Casecontrol study of blood type, breed, sex, and bacteremia in dogs with immune-mediated hemolytic anemia. J Am Vet Med Assoc. 224(2): 232-235
- Nasser RM Rahi AC Haddad MF Daoud Z Irani-Hakime N and Almawi WY 2004. Outbreak of *Burkholderia cepacia* bacteremia traced to contaminated hospital water used for dilution of an alcohol skin antiseptic. Infect Cont Hosp Ep. 25(3): 231-239.
- Obritsch MD Fish DN MacLaren R and Jung R 2005. Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: Epidemiology and treatment options. Pharmacotherapy. 25(10): 1353-1364.
- Podschun R and Ullmann U 1998. *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 11(4): 589-603.
- Rello J Ricart M Mirelis B B Quintana E Gurgui M Net A and Trats G 1994. Nosocomial bacteremia in a medical-surgical intensive care unit: Epidemiologic characteristics and factors influencing mortality in 111 episodes. Intens Care Med. 20(2): 94-98.
- Roberts DE McClain HM Hansen DS Currin P and Howerth EW 2000. An outbreak of *Klebsiella pneumoniae* infection in dogs with severe enteritis and septicemia. J Vet Diagn Invest. 12(2): 168-173.
- Rubin J Walker RD Blickenstaff K Bodeis-Jones S and Zhao S 2008. Antimicrobial resistance and genetic characterization of fluoroquinolone resistance of *Pseudomonas aeruginosa* isolated from canine infections. Vet Microbiol. 131(1-2): 164-172.
- Seguela J and Pages JP 2011. Bacterial and fungal colonisation of peripheral intravenous catheters in dogs and cats. J Small Anim Pract. 52(10): 531-535.
- Unterer S Lechner E Mueller RS Wolf G Straubinger RK Schulz BS and Hartmann 2015. Prospective study of bacteraemia in acute haemorrhagic diarrhoea syndrome in dogs. Vet Rec. 176(12): 309-315.
- Van Duijkeren E Catry B Greko C Moreno MA Pomba MC Pyörälä S Ruzauskas M Sanders P Threlfall EJ Torren-Edo J Törneke K and Scientific Advisory Group on Antimicrobials (SAGAM) 2011. Review on methicillin-resistant *Staphylococcus*

- pseudintermedius. J Antimicrob Chemother. 66(12): 2705-2714.
- Weese J and van Duijkeren E 2010. Methicillin-resistant Staphylococcus aureus and Staphylococcus pseudintermedius in veterinary medicine. Vet Microbiol. 140(3-4): 418-429.
- Wieler LE Ewers C Guenther S Walther B and Lübke-Becker A 2011. Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: Nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. Int J Med Microbiol. 301(8): 635-641.
- Winkler KP Greenfield CL and Schaeffer DJ 2003.

 Bacteremia and bacterial translocation in the naturally occurring canine gastric dilatation-volvulus patient. J Am Anim Hosp Assoc. 39(4): 361-368

บทคัดย่อ

ความชุกของแบคทีเรียในเลือดในสุนัขพักฟื้นที่มีการใช้ท่อสวนหลอดเลือดดำ ในหน่วยสัตว์ป่วยวิกฤติ

ภัทรรัฐ จันทร์ฉายทอง 1 สุกัลยา ฤทธิกุลประเสริฐ $^{2^*}$

ภาวะการพบแบคทีเรียในเลือดเป็นภาวะแทรกซ้อนที่สามารถพบได้ในสัตว์ป่วยของหน่วยพยาบาลสัตว์ป่วยวิกฤต ซึ่งมีการใส่เข็ม สวนหลอดเลือดดำให้กับสัตว์ป่วยเป็นประจำ การศึกษานี้มีวัตถุประสงค์เพื่อสำรวจหาชนิดของแบคทีเรียที่สัมพันธ์กับภาวะการพบแบคทีเรีย ในเลือดภายในหน่วยพยาบาลสัตว์ป่วยวิกฤตของโรงพยาบาลสัตว์แห่งหนึ่งระหว่างปี ค.ศ. 2013-2014 ซึ่งมีสุนัขที่เข้ามารักษาพยาบาลจำนวน 50 ตัว การศึกษาพบแบคทีเรียในเลือดจากการทดสอบด้วยวิธีเพาะเชื้อจากเลือดของสุนัขจำนวน 24 ตัว (ร้อยละ 48) ซึ่งจำแนกชนิดของ แบคทีเรียได้ดังนี้ Klebsiella pneumoniae (8 ตัว), Pseudomonas aeruginosa (7 ตัว), Staphylococcus pseudintermedius (3 ตัว), Escherichia coli (2 ตัว), Bacillus sp. (1 ตัว), Acinetobacter sp. (2 ตัว) และ Burkholderia cepacia (1 ตัว) ในจำนวนนี้มีสุนัขจำนวน 5 ตัวที่ตรวจพบแบคทีเรียจากท่อสวนหลอดเลือดดำเป็นชนิดเดียวกับแบคทีเรียที่เพาะเชื้อจากเลือด การวิเคราะห์ปัจจัยเสี่ยงและอาการทาง คลินิกไม่พบความสัมพันธ์กับภาวะการพบแบคทีเรียในเลือด จึงต้องการการศึกษาเพิ่มเติม การศึกษาครั้งนี้แสดงให้เห็นว่า แบคทีเรียประจำถิ่น และแบคทีเรียที่พบได้ในสิ่งแวดล้อมเป็นแหล่งที่ทำให้พบการปรากฏของแบคทีเรียในกระแสเลือดที่พบบ่อยในสุนัขป่วยที่รักษาพยาบาลอยู่ใน หน่วยพยาบาลสัตว์ป่วยวิกฤต

คำสำคัญ: ภาวะการพบแบคทีเรียในเลือด สุนัข ท่อสวนหลอดเลือดดำ หน่วยพยาบาลสัตว์ป่วยวิกฤต

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