Short Communication

Bacteremia and Multidrug Resistance in Naturally Parvovirus Infection Dogs

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Abstract

Antimicrobials are not indicated for treating viral infections; however, secondary bacteremia is an important complication of canine parvovirus infection. The present study aimed to identify the bacteremia and multidrug-resistant bacteria in naturally parvovirus-infected dogs. A total of 50 dogs with canine parvovirus infection were enrolled in the present study. Blood samples were serially collected from the jugular vein with a sterile technique on days 0, 3, 5 and 7 of hospitalization, until the dog died or was discharged, to perform aerobic bacterial hemoculture. The disk diffusion method was used for the antimicrobial susceptibility test. Overall, 13 of 83 blood samples (15.7%) tested positive for bacterial culture [11 of 50 parvovirus-infected dogs (22.0%)]. Bacteremia and multidrug-resistant bacteria were found on each of the days during the period of parvovirus infection and more than one kind of bacteria was present in individual dogs. The isolated bacteria were *Escherichia coli, Enterobacter species, Pseudomonas aeruginosa, Klebsiella pneumonia,* Coagulase-negative staphylococci, and non-hemolytic streptococci group D (enterococci). In conclusion, bacteremia and multidrug-resistant pathogens were present on each day during canine parvovirus infection. Performing hemoculture in each case of canine parvovirus infection must be encouraged to enhance the therapeutic outcome.

Keywords: Bacteremia, Dog, Hemoculture, Multidrug resistance (MDR), Parvovirus infection

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Introduction

Canine parvovirus (CPV) is the main cause of gastrointestinal infection in unvaccinated or incompletely vaccinated dogs (Pollock and Coyne, 1993). Supportive therapy and preventing secondary infections are the recommended treatment (Nandi and Kumar, 2010). The frequently used antimicrobials are amoxicillin-clavulanic acid, ampicillin, cephalosporin, and enrofloxacin (Lobetti *et al.,* 2002; Judge, 2015; Mylonakis *et al.,* 2016). Escherichia coli, Serratia spp., Acinobacter anitratus, Citrobacter freundii, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter spp., Staphylococcus intermedius and Streptococcus spp. can be found with CPV infection as the result of bacteria in gastrointestinal tract penetrated into the blood stream or nosocomial infection (Isogai *et al.,* 1989; Lobetti *et al.,* 2002; Prittie, 2004). In veterinary practice, there is an increased abundance of pathogenic multidrug-resistant (MDR) bacteria, the same as in humans (van den Bogaard and Stobberingh, 2000). This causes increased severity and a prolonged hospitalization period (Trott *et al.,* 2004; Iris *et al.,* 2010). The present study aimed to identify the bacteria that cause bacteremia and determine the MDR bacteria among naturally-infecting canine parvoviruses.

Materials and Methods

Study dogs: A total of 50 dogs infected with CPV were enrolled between September 1st, 2016 and March 31st, 2017. The inclusion criteria were the presence of clinical signs of CPV infection, no history of parvovirus vaccination or the use of antimicrobials in the previous month, and a positive result in the polymerase chain reaction test for CPV. The study protocol was approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Chiang Mai University (Ref. no. S34/2559).

Sample collection: BCS Serial blood collection was performed for 50 dogs with CPV on days 0, 3, 5 and 7 of hospitalization. In total, 12 and 5 dogs died during days 1–3 and 4–5 of hospitalization, respectively. In addition, 12, 12 and 11 dogs were discharged during days 1–3, 4–5 and 6–7 of hospitalization, respectively. Overall, 83 blood samples were collected from the jugular vein using a sterile technique.

Hemoculture: BCS One milliliter of blood was transferred into a bottle of brain, heart infusion broth (blood to broth ratio 1:9) (Lanna Lab. Co., Thailand). The sample bottle was immediately transferred to the laboratory and incubated at 35°C for 24 h; then, it was withdrawn and inoculated on to a blood agar medium for aerobic culture. Bacterial identification was based on colony type and morphology, gram staining characteristics and standard biochemical tests (Carter, 1990).

Antimicrobial susceptibility testing and the selected Antimicrobials: All isolated bacteria were tested for antimicrobial susceptibility using the disk diffusion method on Muller–Hinton agar. The antibiotic disks were not selected on the basis of the identified microbial agents but based on 10 kinds of antimicrobials that are commonly used in small-animal practice. The intrinsic antimicrobials resistance of each bacterium (Giguere *et al.,* 2013; Mackie, 2017) was indicated (Table 2).

Results and Discussion

Overall, 13 out of 83 blood samples (15.7%), which were from 11 out of 50 parvovirus-infected dogs (22.0%), tested positive for bacterial isolation. The bacteria isolated from blood samples were found on each of the days during the period of infection and more than one kind of bacteria was present in individual dogs (Table 1). The results regarding antimicrobial resistance and MDR for bacteria isolated from cases of CPV infection are presented in Table 2 (intermediate isolates were included in the resistant isolate group).

The gram-negative bacteria found in the blood samples were *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumonia*, which is similar to the findings in previous studies (Prittie, 2004; Sykes, 2012). Most *Escherichia coli, Pseudomonas spp., Klebsiella spp.,* and *Proteus spp.* have been reported to be opportunistic pathogens causing animal infection when host defenses or immune systems are impaired in most animal species (Sykes, 2012). CPV damages the intestinal crypts, which results in small-intestinal epithelial villous collapse and bloody diarrhea, which increases the risk of bacteria entering the body and causing widespread infection (Nandi and Kumar, 2010). Therefore, those gram-negative bacteria need a short period of time before entering the bloodstream (from day 3 onwards in the present study). Coagulase-negative staphylococci (CoNS) were the gram-positive bacteria most commonly isolated here. CoNS and enterococci were reported as the major nosocomial pathogens that have a high impact on the mortality rate and dramatically increase the treatment cost (Moses *et al.*, 2012; Becker, 2014). Therefore, the presence of CoNS and enterococci in the parvovirus-infected dogs was suspected to reflect the contamination of bacteria from the medical personnel/procedure, such as intravenous catheter placement (Lobetti *et al.,* 2002; Moses *et al.,* 2012; Becker, 2014). These were found in the bloodstream from day 0 in the present study. Bacteremia was not the factor that was significantly associated with death in parvovirus infected dogs but hemoculture and a drug sensitivity test had to be performed in every patient which made veterinarians able to choose the right antimicrobial (Sunghan, 2019).

MDR was identified here, the same as in previous studies, especially in Escherichia coli (Habib *et al.,* 2016) and enterobacteriaceae (Trott *et al.,* 2004; Gronvold *et al.,* 2010). The possible reasons for the presence of MDR in the parvovirus-infected dogs include the following: firstly, they received previously normal flora from their mother when in the uterus, which is the reservoir for resistance genes (Newman and Seidu, 2002; De Graef *et al.,* 2004); secondly, the transmission of MDR could have occurred from medical personnel/procedures during hospitalization (Lobetti *et al.,* 2002; Becker, 2014). Thirdly, the direct transmission of MDR between the owner and the dog could have occurred through direct contact or from the

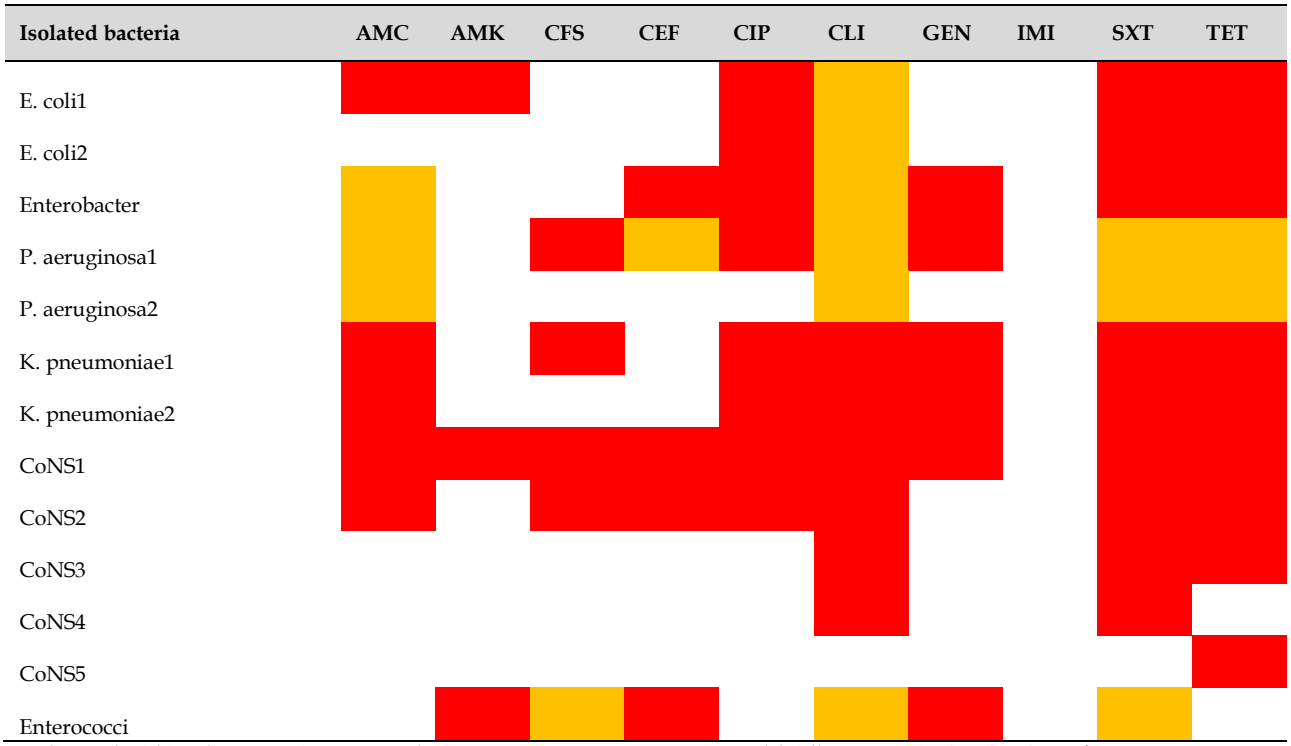
environment (Guardabassi *et al.*, 2004). Fourthly, the transmission of MDR from farm animals to humans and pets could have occurred through the food network (Ramos *et al.*, 2013). Therefore, to control MDR needs multiple compliance e.g., awareness of rational drug use, prudent infection control practices, antimicrobial stewardship and the development of new medication.

In conclusion, MDR pathogens were present in the bloodstream on each day during CPV infection. The gram-positive organisms isolated from the bloodstream were CoNS and enterococci and the gram-negative organisms were *Escherichia coli, Enterobactor spp., Pseudomonas aeruginosa,* and *Klebsiella pneumonia*. Awareness of bacteremia and MDR through performing blood culture in each case of CPV infection must be promoted to enhance therapeutic outcomes.

Table 1 Thirteen cases of bacterial identification from 83 blood samples in 50 canine parvovirus-infected dogs

Bacterial species	Day of Hospitalization			
	Day 0	Day 3	Day 5	Day 7
Escherichia coli	0	1 (dog no.2)	0	1 (dog no.7)
Enterobacter species	0	1 (dog no.3)	0	0
Klebsiella pneumonia	0	0	2 (dog no.3,32)	0
Pseudomonas aeruginosa	0	1 (dog no.28)	0	1 (dog no.4)
Coagulase-negative staphylococci	3 (dog no.33,41,46)	1 (dog no.31)	1 (dog no.31)	0
Non-hemolytic streptococci group D (enterococci)	1 (dog no.50)	0	0	0

Table 2 Antimicrobial resistance patterns isolated from blood in all groups (n = 13). Red means resistant to that kind of antimicrobial. Orange means intrinsic resistant. (AMC-Amoxicillin-clavulanate, AMK-Amikacin, CFS-Cefoperazone-Sulbactam, CEF-Ceftriaxone, CIP-Ciprofloxacin, CLI-Clindamycin, GEN-Gentamicin, IMI-Imipenem, SXT-Trimethoprim-sulfamethoxazole, TET-Tetracycline)



E. coli = Escherichia coli, P. aeruginosa = Pseudomonas aeruginosa, K. pneumoniae = Klebsiella pneumonia, CoNS = Coagulase-negative staphylococci

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