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Aetiology and Risk Factors Associated with Septicaemia of Dogs in Malaysia

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Abstract: Septicaemia is an important health problem in both veterinary and human medicines. This study reports on the aetiology and risks of septicaemia in dogs submitted to the Post-mortem Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia between 2006-2016. It was found that the most common point of entry of microorganisms leading to septicaemia in dogs was via the gastrointestinal tract (57%, $p<0.05$). Commonly isolated aetiological agents were *Escherichia coli* (65%), *Streptococcus* sp. (15%), *Staphylococcus* sp. (12%), *Klebsiella pneumoniae* (3%), *Pasteurella* sp. (2.5%) and *Salmonella* sp. (2.5%). The septicaemia was associated with risk factors which included underlying infection (60.8%), stress (36.7%), malnutrition (21.7%), tumor (7.5%) and traumatic injury (5%). Analysis of association between age group and risk factors showed that paediatrics were very susceptible and this was closely associated with underlying viral infection. Other important risk factors included injury to the integument and malnutrition. Reasons behind these observations were subsequently discussed.

Key words: Septicaemia, risk factors, aetiological agent, dog, point of entry, infection

INTRODUCTION

Septicaemia is a condition in which there is persistent presence of microorganism and its toxin in the bloodstream that spread throughout the body leading to multiple organ failure and septic shock. It is a fatal condition due to the exaggeration of inflammatory response by the host (De Backer *et al*., 2014). In veterinary medicine, septicaemia is the most important health problem among dogs and the fifth among cats (Rathiymaler *et al*., 2017). Dogs with underlying disease died acutely of septicaemia while those without underlying disease are capable of surviving longer hours (Isola *et al*., 2013). Bacterial septicaemia is known to be the most common type of septicaemia among human and animals (Ramachandran, 2014). Most septicaemia among animals is caused by Gram-negative bacteria (Osterbur *et al*., 2014) while in humans it is predominated by Gram-positive bacteria (Ramachandran, 2014). This identification of aetiological agent is vital especially in employing antibiotic treatment (Johnson *et al*., 2009).

Pathogenesis of septicaemia requires entry of the organism into the host's circulatory system (Annas *et al*., 2015). Common entry points involve the mucosal surfaces which include the lining of respiratory, digestive, reproductive and urinary tracts while the skin is compromised due to insect bite, cuts, scrapes or other wound (Kenney *et al*., 2010). The important risk factors leading to septicaemia include poor health condition, underlying diseases, malnutrition, low birth weight and prolonged labour in newborns (Yancey *et al*., 1996; Angus *et al*., 2001; Martin *et al*., 2003; Hamer *et al*., 2015). Thus, it is important to establish the common risk factors as it could assist in planning preventive measures against septicaemia (Mugalu *et al*., 2006). This study identifies the causative agents and risk factors associated with septicaemia among dogs.

MATERIALS AND METHODS

Data collection: Records of post-mortem cases on dogs were collected from the Post-mortem Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) between 2006 and 2016. The age, sex and breed of animal, the history, bacteriological findings and laboratory results were obtained. Cases diagnosed as septicaemia were selected for further analyses. Septicaemia was diagnosed based the successful isolation of the same bacterium from at least 3 samples of internal organs of a carcass showing

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moderate to severe congestion. Cases with no definitive diagnosis, missing or incomplete reports were excluded.

Data were analysed to determine cases of septicaemia in different sex, breed, age, point of entry, isolation of microorganism and risk factors associated with septicaemia. Subsequently, the association between these risk factors were analysed.

Age: Age of the dogs were classified into three groups; pediatrics (≤3 months old), adult (3 months, 9 years) and geriatrics (>10 years) (Hosgood and Scholl, 1998; Joubert, 2007). The number of septicaemia cases were compared to the total number of cases sent for post-mortem laboratory according to the age group for a fair comparison.

Determination of point of entry: The possible point of entry was based on the reports written by the pathologists. The point of entry were classified based on the body systems (respiratory, Gastrointestinal (GIT), urinary, integumentary tracts). The one identified as others may include reproductive, musculoskeletal system or which has unknown point of entry. The reproductive and musculoskeletal systems were categorised under others because they have <10 cases from the overall septicaemic cases.

Aetiological agents: Common microorganisms isolated in cases of septicaemia in these cases were then noted. In cases where multiple organisms were isolated, the main causative agent of septicaemia was presumed based on the isolation of the same organism from all organs submitted for bacteriological isolation.

Risk factors: Following assessment of records, the risk factors were divided into underlying infection, stress, malnutrition, tumour, traumatic injury and miscellaneous (e.g., post-surgical complication, poisoning, lightning strike, idiopathic gastritis).

Data analysis: The data were analysed using Statistical Packages for the Social Sciences (SPSS) Version 22. Frequencies were identified for each categorical group according to the sex, breed and age of the animals. The data were categorical and non-continuous, thus non-parametric test was used for data analysis. All tests were done at 95% confidence intervals level. Subsequently, Pearson's Chi-squared test was used to determine the point of entry and the most common bacteria isolated. The association between variables were determine using Pearson's Chi-squared test. There is also identification of relation between age group to point of entry and age group to risk factors by using Excel Version 2007. The significance is identified using Pearson's Chi-squared test as well.

RESULTS AND DISCUSSION

Prevalence: There were a total of 136 (30.6%) cases of septicaemia out of 444 cases of dogs sent for post-mortem between 2006 and 2016. Out of these 136 septicaemic cases, 16 (11.7%) cases were excluded due to inconclusive diagnosis of septicaemia, incomplete or missing reports. The remaining 120 cases were further analysed to reveal that 65 (54%) cases involved male and 55 (46%) cases involved the female dogs but the difference was insignificant ($p>0.05$) difference.

Age of animal: Table 1 shows prevalence of septicaemia according to breed. The difference, however was not significant ($p>0.05$). Out of the 120 septicaemic cases, 48 (40%) cases involving paediatric, 64 (53%) cases involved adult and 8 (7%) cases involved geriatric dogs. Similarly, there were no significant ($p>0.05$) differences between the ages of dog susceptible to septicaemia (Table 2).

Point of entry: The records revealed that 68 (57%) cases originated from the GIT, followed by 26 (22%) cases from the respiratory tract, 11 (9%) cases from the urinary tract and 5 (4%) cases from the integument while the remaining 10 cases (8%) were from other routes. The GIT was identified as the most common ($p<0.05$) point of entry.

Bacterial isolation: Following bacterial isolations, 25 (21%) cases yielded pure culture of single bacteria, 17 (14.1%) cases have a mixture of 2 bacteria and 78 (65%)

Table 1: Total number cases for different breed of dogs with septicaemia between the year 2006 and 2016

Breed	Total number of cases of septicaemia	Total number cases of post mortem	Percentage
Local	51	203	25.1
German Shepherd	15	56	26.8
Shih Tzu	12	40	30.0
Labrador	10	46	21.7
Golden Retriever	8	30	26.7
Poodle	7	19	36.8
Siberian Husky	5	15	33.3
Others	12	35	34.3
Total	120	444	27.0

Table 2: Total number cases for each age group of dogs with septicaemia between the year 2006 and 2016

Age groups	Total number of cases of septicaemia (%)	Total number cases of post mortem	Percentage
Paediatrics	48 (40)	189	25.4
Adult	64 (53)	204	31.4
Geriatrics	8 (7)	51	15.7
Total	120 (100)	444	27.0

Table 3: Total number of each point of entry associated with different types of bacteria in septicaemia cases among dogs between the year 2006 and 2016

Breed	*E. coli*	*K. pneumoniae*	*Staphylococcus* sp.	*Streptococcus* sp.	Other G. negative	Total
GIT	59	30	4	10	23	126
Respiratory	16	9	5	4	12	46
Urinary	11	3	3	2	4	23
Integument	4	3	0	2	2	11
Others	8	3	2	0	3	16
Total	98	48	14	18	44	222

Table 4: Total number of cases and percentage of points of entry associated wih their respective risk factors among the between the year 2006 and 2016

Factors	Underlying infection (%)	Malnutrition (%)	Trauma (%)	Tumour (%)	Stress (%)	Miscellaneous (%)	Total (%)
GIT	48 (42.1)	20 (17.5)*	2 (1.8)	6 (5.3)	30 (26.3)	8 (7)	114 (100)
Respiratory	18 (58.1)	0 (0)	1 (3.2)	1 (3.2)	6 (19.4)	5 (16.1)	31 (100)
Urinary	9 (64.3)	1 (7.1)	1 (7.1)	0 (0)	1 (7.1)	2 (14.3)	14 (100)
Integument	2 (25)	0 (0)	2 (25)*	0 (0)	3 (37.5)	1 (12.5)	8 (100)
Others	2 (13.3)	5 (33.3)	0 (0)	2 (13.3)	4 (26.7)	2 (13.3)	15 (100)

*Indicates that its significant

cases have 3 or more bacteria. *Escherichia coli* was significantly ($p<0.05$) more frequently isolated with 78 (65%) cases followed by *Streptococcus* sp. (15%), *Staphylococcus* sp. (12%), *Klebsiella pneumonia* (3%), *Pasteurella* sp. (2.5%) and *Salmonella* sp. (2.5%), $\chi^2(6, N = 120) = 874.34$, $p<0.05$. No significant relationship was detected between all bacteria groups.

Staphylococcus and *Streptococccus* sp. were usually involved in mixed infections ($p<0.05$) rather than as a sole causative agent of septicaemia in dogs. Pearson's Chi-square analysis revealed that *Staphylococcus* and *Streptococcus* sp. involved in mixed infection sepsis at significantly ($p<0.05$) higher rate (85.7 and 83.3%, respectively) than single sepsis (14.3 and 16.7%, respectively).

Risk factors: Underlying infection was significantly ($p<0.05$) the most important risk factor leading to septicaemia in dogs, involving 73 (60.8%) of the cases compared to stress 44 cases (36.7%), malnutrition of 26 cases (21.7%), tumour of 9 cases (7.5%), traumatic injury of 6 cases (5%) and miscellaneous risk factors of 18 cases (15%) [Chi-square test, $\chi^2(5, N = 120) = 14.78$]. Furthermore, underlying infections involving bacteria were significantly ($p<0.05$) higher compared to the other aetiological agents such as virus, fungus and parasite at 59.0, 31.5, 6.8 F and 2.7%, respectively. The next most common risk factor was the stress factor which included heat, boarding, transportation and secondary stress such as poor nutrition or multi-pet household. Heat stress accounted for 22.7%, transportation stress 18.2%, boarding stress 13.6 and secondary stress 45.5%.

Association between the points of entry and the types of bacteria isolated: Septicaemia with GIT as point of entry was found to have high isolations of all type of bacteria except *Staphylococcus* sp. (Table 3). Only *E. coli* showed significance ($p<0.05$) with each point of entry. Out of the 120 septicaemia cases, *E. coli* was isolated in 98 cases.

Out of these 98 cases involving isolations of *E. coli*, 59 cases (60.2%) of *E. coli* isolated was from GIT showed significantly ($p>0.05$) higher compared to respiratory tract, 16 cases (16.3%), urinary tract 11 cases (11.2%), integumentary tract, 4 cases (4.1%) [Chi-square test, $\chi^2(4, N = 98) = 10.53$]. *Klebsiella pneumoniae*, *Staphylococcus* and *Streptococcus* sp. did not show significant correlation ($p>0.05$) with the points of entry.

Further analyses revealed significant difference between the gastrointestinal and respiratory tracts [Chi-square test $\chi^2(1 N = 94) = 7.42$, $p = 0.006$] and between urinary and respiratory tracts in successful isolation of *E. coli* [Fisher's exact test $\chi^2 (1 N = 37) = 5.79$, $p = 0.018$]. These indicate that *E. coli* predominated in GIT and urinary tract but not in the respiratory tract. There were no significant differences between the respiratory tract and integumentary [$\chi^2 (1 N = 31) = 0.63$, $p = 0.631$], between urinary and GIT [$\chi^2 (1 N = 79) = 1.64$, $p = 0.346$], between GIT and integumentary [$\chi^2(1 N = 73) = 0.18$, $p = 0.532$] and between urinary tract and integumentary [$\chi^2 (1 N = 16) = 2.35$, $p = 0.313$] in the isolation of *E. coli*.

Association between the point of entry and risk factors: The associations between the point of entry and risk factors are summarised in Table 4. There were significant ($p<0.05$) correlation between malnutrition and trauma when compared with the point of entry. Malnutirition significantly ($p<0.05$) enhanced entry of organisms through the GIT compared with the respiratory [$\chi^2 (1 N = 94) = 9.71$, $p = 0.001$], urinary [$\chi^2 (1 N = 79) = 2.0$, $p = 0.041$] and integumentary [$\chi^2 (1 N = 73) = 7.6$, $p = 0.003$]. On the other hand, traumatic injuries significantly ($p<0.05$) enhanced the entry of microorganisms through the integument compared with the GIT [$\chi^2 (1 N = 73) = 7.6$, $p = 0.003$], urinary [$\chi^2 (1 N = 16) = 2.42$, $p = 0.092$] and respiratory tracts [$\chi^2 (1 N = 31) = 0.48$, $p = 1.01$].

Table 5: Total number of cases with percentage of age groups according to risk factors of septicaemia among dogs between the year 2006 and 2016

Factors	Pediatrics (%)	Adult (%)	Geriatrics (%)	Total
Underlying infection	29 (60.4)	39 (60.9)	5 (62.5)	73
Traumatic injury	1 (2.1)	4 (6.3)	1 (12.5)	6
Malnutrition	11 (22.9)	13 (20.3)	2 (25)	26
Tumor	0 (0)	6 (9.4)	3 (37.5)	9
Stress	21 (43.8)	9 (14.1)	4 (50)	44

Table 6: Different aetilogical agent causing septicaemia according to each age groups among dogs between the year 2006 and 2016

Age groups	Paediatrics	Adult	Geriatrics	Total (%)
Bacterial	4 (9.3%)	35 (81.4%)	4 (99.3%)	43 (100)
Viral	20 (87%)*	3 (13%)	0 (0%)	23 (100)
Parasite	5 (100%)*	0 (0%)	0 (0%)	5 (100)
Fungal	0 (0%)	1 (50%)	1 (50%)	2 (100)
Total	29	39	5	73 (100)

*Indicates that its significant compared to the other two group compared for that particular aetiological agent

Association between age group and risk factors: Correlations between the age of animal and the risk factors are summarised in Table 5. Presence of tumor was significantly ($p<0.05$) correlated with the age group, particularly the geriatrics compared with the paediatrics [χ^2 (1 N = 56) = 22.45 p = 0.025] and adults [Fisher's χ^2 (1 N = 37) = 54.21, p = 0.041]. Similarly, the adult group was significantly ($p<0.05$) correlated with tumour risk factor than the pediatrics [χ^2 (1 N = 112) = 33.87, p = 0.021]. On the other hand, the paediatric group showed significant ($p<0.05$) correlation with viral and parasite infections than the adult and geriatric groups (Table 6).

This study indicates that age, breed and sex of dogs have no significant influence on the occurrence of septicaemia. Hence, dogs of any age, breed or sex have equal chance of acquiring septicaemia. Gram-negative bacteria predominate cases of septicaemia in dogs as observed by Osterbur *et al.* (2014) with mostly the *E. coli* (McKenzie and Furr, 2001; Kenney *et al*., 2010). Similarly, *E. coli* has also been identified as an important agent leading to sepsis in neonatal foals and calves (Fecteau *et al*., 1997; McKenzie and Furr, 2001). In human medical, *S. aureus* is the most common agent isolated in community-acquired neonatal bacteraemia (Desai and Malek, 2010; Hamer *et al*., 2015). In veterinary medicine, infections of dogs by *Streptococcus* sp. may lead to streptococcal septicaemia that subsequently causing abortion and neonatal death (Lamm *et al*., 2010). Nevertheless, this study revealed that Gram-positive bacteria (*Staphylococcus* and *Streptococccus* sp.) causes mixed bacterial infections which is in agreement with a previous study where Gram-positive bacteria are often isolated in a mixed infection (East *et al*., 2000). It is possible that the endotoxin from Gram-negative organisms which is known to cause endothelial cell injury, aids in entry of Gram-positive bacteria into the circulation. When septicaemia by mixed organism occurs, the combination of endotoxin and exotoxin leads to fatal toxic shock and sepsis (Ramachandran, 2014).

The present study shows that the most common point of entry for septicaemia in dogs is the GIT. This is in agreement with findings by Kenney *et al*. (2010). Malnutrition leads to significantly high infection via the GIT due to close association of GIT with food thus lack of nutrition causes stress to the animal and eroding of gastric mucosa by gastric juice (Bresnahan and Tanumihardjo, 2014). Furthermore, malnutrition is associated with depression of the innate and adaptive immunities leading to changes in gut microbiota and infection by enteropathogens (Kau *et al*., 2011). Since, *E. coli* is the predominant bacterium in the GIT, septicaemia by *E. coli* is the most common in dogs (Cullen *et al*., 1995). However, dogs suffering from traumatic injuries tend to show the integument as the point of entry break of the integument disabled the first line of barrier to outside environment (Lebwohl and Hermann, 2005).

This study also suggests that paediatric dogs with underlying viral infections are more susceptible to septicaemia. This may occur because of their dependency on passive antibodies and under-developed immune system which may not be sufficient to protect against potent infections (Fink and Warren, 2014; Beineke *et al*., 2009). With concurrent viral infection, severe immunosuppression in the hosts results in increased susceptibility to bacterial infection including opportunistic GIT bacterial flora (Beineke *et al*., 2009).

CONCLUSION

Septicaemia is an important clinical health problem among dogs. Preventive measures should be taken in order to reduce the occurrence of septicaemia among pet animals and to avoid improper usage of antibiotics. The major causative agent in septicaemia is the Gram-negative bacteria, particularly the *E. coli* which can enter through all points of entry. However, the most common point of entry for septicaemia is the GIT. Underlying infections act as the most common risk factor for septicaemia and the pediatric group is most susceptible to septicaemia, particularly following underlying viral infections.

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