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Prognostic indicators of haemorrhagic gastroenteritis in puppies

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Abstract
Canine haemorrhagic gastroenteritis (HGE) is a common condition manifested by vomiting and diarrhoea in puppies. The aim of the study was to identify the prognostic markers in puppies below 6 months of age with HGE. A total of ten apparently healthy puppies were taken as positive control (Group I). Stool sample obtained from sixty two affected puppies were screened using polymerised chain reaction for infectious agents and classified as follows, puppies with viral enteritis (Group II, n=forty one), bacterial enteritis (Group III, n=six), combined bacterial and viral enteritis (Group IV, n=fifteen). Among the clinical parameters the total WBC, serum albumin, potassium, blood lactate and blood pressure were statistically highly significant ($P \leq 0.01$). A total WBC count ≤ 4100 cu.mm, serum albumin concentration of <2 g/dL, serum potassium of ≤ 3.4 mEq/L, blood lactate levels of >3.2 mmol/L and systolic blood pressure of ≤ 90 mmHg were identified as the prognostic values Their interquartile range and median values will be discussed critically.

Keywords: Canine haemorrhagic gastroenteritis (HGE), infectious cause, clinical parameters, prognostic levels

1. Introduction

Acute haemorrhagic diarrhoea is one of the most serious clinical manifestations of the GI failure faced by small animal practitioners (Dow, 1996) [4]. Haemorrhagic gastroenteritis (HGE) is an acute, severe form of diarrhea that occurs in dogs. Infectious agents associated with diarrhoea in young dogs are typically bacterial or viral (Magne, 2006) [10]. HGE syndrome is characterised by the acute onset of bloody diarrhoea and vomiting accompanied by marked haemoconcentration Unterer *et al.* (2011) [14]. Identification of various haematobiochemical parameters in survivors and non-survivors of haemorrhagic gastroenteritis will help in arriving at better prognostication.

2. Materials and methods

Puppies below 6 months of age presented with the history of progressive vomiting and haemorrhagic diarrhoea were subjected for faecal PCR and haematobiochemical tests. The apparently healthy puppies found negative for enteropathogens and their toxins were selected to serve as Group I- healthy control (n=ten). Faecal samples from affected puppies were screened for the common enteropathogens such as *Escherichia coli* – Shiga and enterotoxin (LT), *Clostridium perfringens* – alpha and enterotoxin (cpa and cpe) *Clostridium difficile* and its toxin B, *Salmonella* sp., *Campylobacter* sp., Canine Parvovirus strain 2b (CPV-2b), Canine Distemper Virus (CDV), Enteric Canine Coronavirus (CCoV) and Rotavirus. They were classified into various groups based on the underlying etiology as follows: Group II: puppies with viral enteritis (n=forty one), Group III: puppies with bacterial enteritis (n=six) and Group IV: puppies with combined bacterial and viral enteritis (n=fiffteen). The haematobiochemical parameters were interpreted among the survivors and non-survivors of each group.

2.1 Haematology and Serum Biochemical Profiling

About 3 ml of blood samples were collected from each puppy by venepuncture of either the cephalic or the jugular vein.

1ml of the sample was transferred to vaccutainers coated with 10% ethylenediamine tetracetic acid (EDTA) for haematologic analysis using the haematology analyser (MINDRAY-BC-2800 VET). Remaining, 2 ml of the blood was transferred to plain vaccutainers (without anticoagulant), centrifuged at 1500 rpm for 20 minutes and serum samples separated. Commercial biochemical kits (Aggappe Diagnostics, India) were used for quantitative estimation of BUN, creatinine, total protein, albumin, globulin, calcium, phosphorous, magnesium, glucose, sodium, potassium and chloride. Their values were measured in a semi-automated biochemical analyser (A-15 Biosystem Random Access Analyzer, Biosystems).

The data obtained in the study were subjected to statistical analysis using SPSS16.0. The haematobiochemical profile of the survivors and non-survivors of Group II, Group III Group IV were statistically analysed by one-way ANOVA, and compared with the healthy control (Group I). Receiver operating characteristic curve (ROC) analysis was performed using the Medcalc 16.4.3 statistical package to obtain sensitivity, specificity and predictive values of critical parameters.

3. Results and discussion
The results of the study are presented in table 1-6 and fig.1,2. The mean total WBC count, serum albumin, potassium concentrations and blood pressure were less, whereas the blood lactate concentrations were more in the affected puppies than the healthy animals (P≤0.01). The mean haemoglobin and PCV were significantly lower in the affected puppies than healthy animals (P≤0.05). The results were in accordance to Appel et al. (1978) [1], Greene (1984) [5] and Macartney et al. (1984) [9] wherein a moderate to severe fall in the total leucocyte count was detected in dogs suffering from parvoviral infection. The severe intestinal blood loss results in anaemia and panhypoproteinemia (Hackett, 2011) [6]. The low systolic blood pressure and high blood lactate levels were attributable to the dehydration, poor perfusion to tissue and build of lactate. Boag and Hughes (2005) [2] suggested that although a systolic arterial blood pressure (ABP) less than 80 mmHg and mean ABP less than 60 mm Hg implies severe hypoperfusion and requires immediate treatment, ABP was an insensitive indicator of mild to moderate hypoperfusion, because the body's homeostatic mechanism act to maintain ABP within a narrow range by changing heart rate, stroke volume, and systemic vascular resistance. Hypokalemia is caused by loss of potassium in

vomitus and repeated bouts of diarrhoea in the affected puppies. Hackett (2011) [6] identified that the concentrations of electrolytes including sodium, chloride, potassium, and magnesium dropped precipitously in anorectic animals with diarrhoea.

The ROC curve analysis indicated the sensitivity (%), specificity (%), positive predictive value (%) and negative predictive value (%) of significant parameters (table 5, fig.1) and the interquartile range and median values of these significant parameters were tabulated and represented graphically using the box whisker plot analysis (table 6, fig. 2). In the present study, a total WBC count in survivors ranges from 1.2×10^3-26.7×10^3/cu.mm with a median value of 7.6×10^3/cu.mm. This is in contrary to the findings of Haligur et al. (2009) [7], as the values $< 2 \times 10^3$ were reported to be prognostic in the puppies with parvoviral enteritis. Sharkey and Wellman (2013) [12] reported that the lactate concentration is used as an indicator of tissue hypoperfusion and hypoxia, particularly in critical care or perioperative settings to determine the severity of underlying disorder, assess response to therapy, and predict outcome. The present study identified blood pressure and blood lactate as prognostic indicators of HGE in the Grade III puppies, which was related to septic shock and hypovolemia resulting in tissue hypoxia/hypoperfusion as suggested by the above authors. The blood lactate levels in non-survivors, ranges from 3.4 to 6.7 mmol/L with a median value of 4.8 mmol/L which is higher when compared with the reference range (0.3 to 2.5 mmol/L) in puppies aged >2 months, given by McMichael et al. (2005) [11] which is indicative of hyperlactemia. The blood lactate values >3.2 mmol/L and systolic blood pressure ≤90 mmHg were identified to be the cut-off values in determining the prognosis. In the non-survivors the blood pressure ranges from 60 to 90 mmHg, with median value of 76 mmHg, which was related to severe sepsis in combination with organ dysfunction, hypoperfusion, or hypotension as suggested by Silverstein et al. (2007) [13]. The serum potassium values in non-survivors, ranges from 2.1 to 5.2 mEq/L with a median value of 3.2 mEq/L which were lower when compared with the reference range (4.5 to 6.3 mEq/L in puppies between 0-3 months and 3.9 to 6.1 mEq/L in puppies between 3-6 months of age) given by Kraft et al. (1996) [8] thus indicative of hypokalemia. The serum albumin levels in non-survivors, ranges from 1.3-3.1 g/dL with a median value of 2.2 g/dL which were lower when compared with the reference range (2.6-3.7 g/dL) given by Dehn (2014) [3], indicative of hypoalbuminemia.

Table 1: Mean ± S.E values of Haemogram in puppies under various groups.

Parameters	Group I Apparently healthy animals (n=10)	Group II Survivors (n=31)	Group II Non-survivors (n=10)	Group III Survivors (n=3)	Group III Non-survivors (n=3)	Group IV Survivors (n=11)	Group IV Non-survivors (n=4)	F value
Haemoglobin (g/dL)	7.13ª± 0.22	9.38ab±0.50	10.83b±0.67	8.80ab±0.63	7.01ª±0.40	8.39ab±0.63	8.78ab±1.32	2.771*
PCV (%)	21.87ª±0.52	29.14ab±1.31	32.74b±1.79	26.73ab±3.46	26.57ab±4.44	25.20ab±1.72	25.58ab±4.59	3.038*
RBC (×10⁶/cu.mm)	4.28±0.09	5.20±0.25	5.52±0.32	5.17±0.50	4.20±0.41	4.61±0.25	4.58 ±0.70	1.779NS
WBC (×10³/cu.mm)	11.90b±1.75	8.69ab±0.90	3.99ª±1.40	18.50c±4.10	21.30c±5.10	7.47ab ±1.10	6.20ab±1.33	7.656**
Platelets (×10⁵/cu.mm)	3.02±0.42	2.34±0.26	2.50±0.47	2.12±0.22	2.51±0.43	2.82±0.46	2.12 ±0.69	0.483NS

Mean values bearing the same superscript in the row do not vary significantly,
** - statistically highly significant (P≤0.01), * - statistically significant (P≤0.05), NS – statistically non-significant (P>0.05)

Table 2: Mean ± S.E values of serum biochemical parameters in puppies under various groups.

Parameters	Group I Apparently healthy animals (n=10)	Group II		Group III		Group IV		F value
		Survivors (n=31)	Non-survivors (n=10)	Survivors (n=3)	Non-survivors (n=3)	Survivors (n=11)	Non-survivors (n=4)	
BUN (mg/dL)	15.46±1.98	22.19 ± 2.12	14.72± 1.18	23.01±8.12	16.21±5.88	17.37±1.89	14.41 ± 1.28	1.547^{NS}
Creatinine (mg/dL)	0.57 ± 0.03	0.95 ± 0.12	0.66 ± 0.08	1.03 ± 0.14	0.86 ± 0.24	3.30 ± 2.62	0.59 ± 0.02	0.836^{NS}
Total protein (g/dL)	6.14 ± 0.23	5.91 ± 0.23	5.84 ± 0.19	6.47 ± 0.68	5.33 ± 0.49	5.77 ± 0.34	5.62 ± 0.28	0.432^{NS}
Albumin (g/dL)	3.04^b ± 0.14	2.13^a ± 0.11	2.26^a ± 0.12	2.06^a ± 0.17	1.77^a ± 0.29	2.19^a ± 0.13	2.42^{ab} ± 0.16	4.666**
Globulin (g/dL)	3.11 ± 0.11	3.59 ± 0.20	3.58 ± 0.16	4.40 ± 0.75	3.57 ± 0.20	3.57 ± 0.30	3.20 ± 0.14	0.941^{NS}

Mean values bearing the same superscript in the row do not vary significantly,
** - statistically highly significant (P≤0.01), ^{NS} – statistically non-significant (P>0.05)

Table 3: Mean ± S.E values of Blood lactate and Blood pressure in puppies under various groups

Parameters	Group I Apparently healthy animals (n=10)	Survivors (n=14)	Non-survivors (n=9)	F value
Blood lactate (mmol/L)	0.89^a ± 0.11	2.55^b ± 0.25	4.81^c ± 0.35	52.063**
Blood pressure (mm Hg)	104.50^b ± 4.50	105.14^b ± 3.72	74.78^a ± 3.46	17.78**

** - statistically highly significant (P≤0.01)

**Table 4:** Mean ± S.E values of electrolytes and minerals in puppies under various groups

Parameters	Group I Apparently healthy animals (n=10)	Group II		Group III		Group IV		F value
		Survivors (n=31)	Non-survivors (n=10)	Survivors (n=3)	Non-survivors (n=3)	Survivors (n=11)	Non-survivors (n=4)	
Sodium (mEq/L)	142.97 ± 2.76	138.38±1.97	143.94±2.26	139.36±3.63	143.30±5.72	141.89±3.45	136.05±5.38	0.721^{NS}
Potassium (mEq/L)	5.83^c ± 0.17	4.84^{bc}± 0.17	3.50^a ± 0.33	3.80^{ab}±0.35	3.47^a± 0.59	4.62^b ± 0.13	3.30^a ± 0.69	9.120**
Chloride (mEq/L)	113.06 ± 0.92	112.52±1.30	111.83±2.42	116.50±3.26	101.87±2.71	112.53±2.31	105.98±3.00	1.944^{NS}
Calcium (mg/dL)	11.31 ± 0.20	10.95 ± 0.14	11.13 ± 0.27	10.56 ± 0.86	10.12 ± 0.61	11.26 ± 0.14	10.83 ± 0.31	1.394^{NS}
Phosphorous (mg/dL)	8.15 ± 0.41	6.48 ± 0.34	6.88 ± 0.58	9.40 ± 1.34	7.60 ± 0.32	7.30 ± 0.71	6.60 ± 0.44	1.994^{NS}
Magnesium (mg/dL)	4.05 ± 0.44	4.40 ± 0.29	3.81 ± 0.46	3.74 ± 0.21	4.06 ± 1.06	3.18 ± 0.39	3.08 ± 0.65	1.248^{NS}

Mean values bearing the same superscript in the row do not vary significantly,
** - statistically highly significant (P≤0.01), ^{NS} – statistically non-significant (P>0.05)

Table 5: Receiver operating characteristic curve (ROC) analysis of significant parameters in puppies with haemorrhagic gastroenteritis

Parameter	Criterion/cut off value*	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Younden index J
Total WBC (cu.mm)	≤4100	58.8	91.1	71.4	85.4	0.4993
Albumin (g/dL)	>2	76.5	48.9	36.1	84.6	0.2536
Serum potassium (mEq/L)	≤3.4	64.7	95.6	84.6	87.8	0.6026
Blood lactate (mmol/L)	>3.2	100.0	85.7	81.5	100.0	0.8571
Blood pressure (mmHg)	≤90	100.0	78.6	75.0	100.0	0.7857

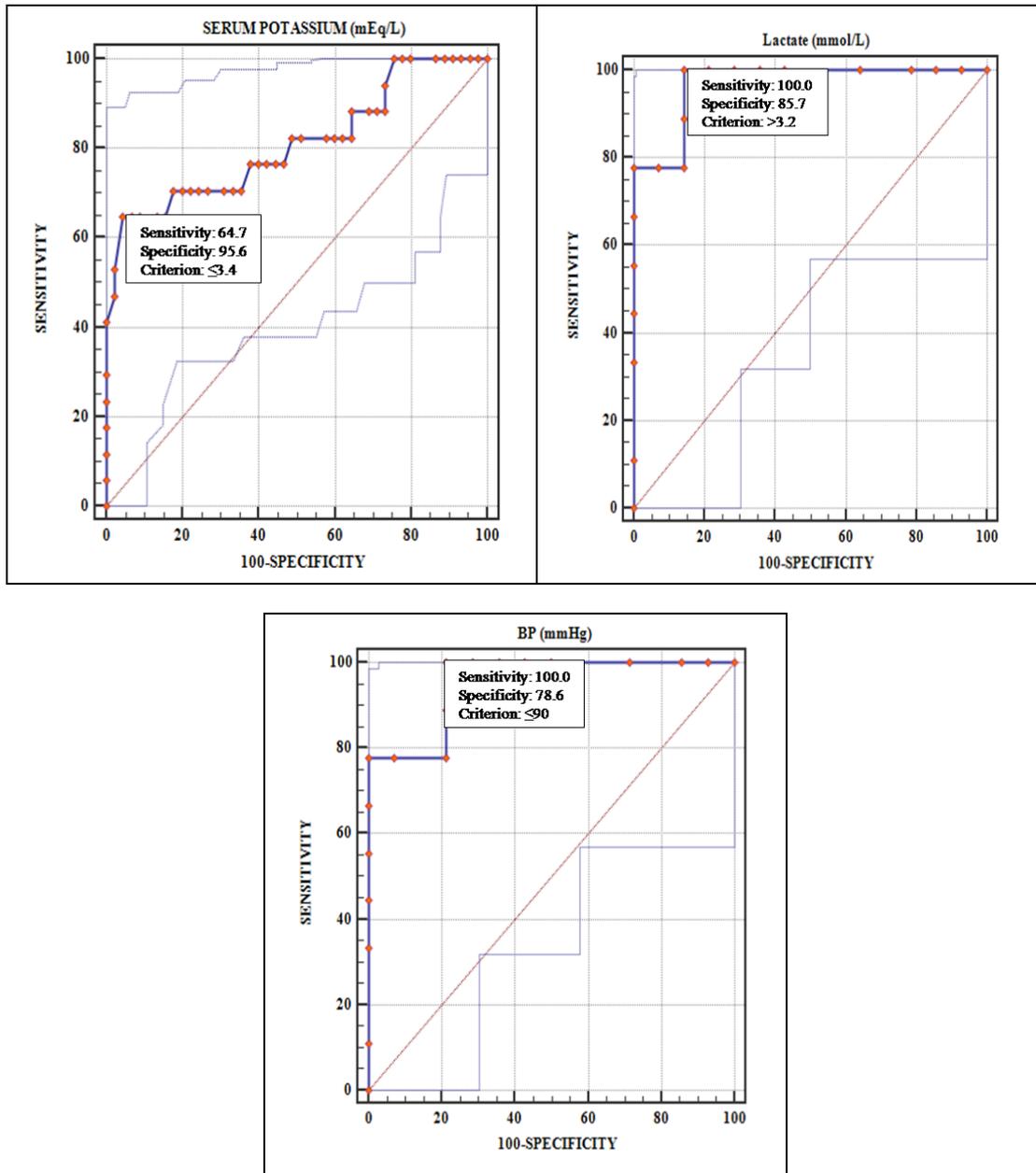*cut off value corresponding to maximal Younden index J
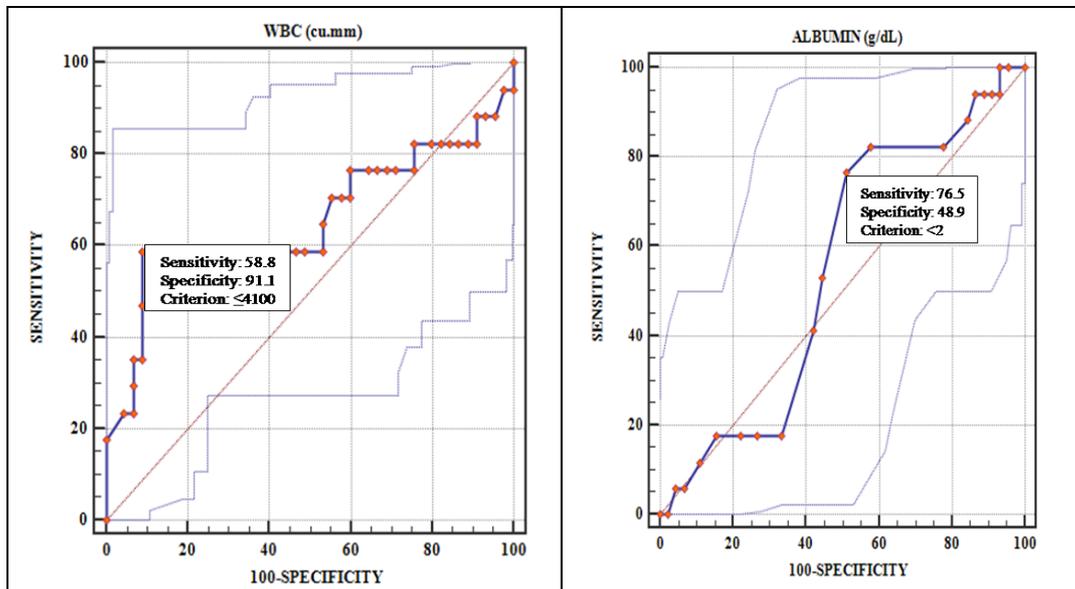
Fig 1: ROC curve - WBC Count, serum albumin, potassium, lactate and blood pressure

3.1 Box whisker plot of significant parameters

Table 6: Box Whisker representation of median and interquartile range of significant parameters

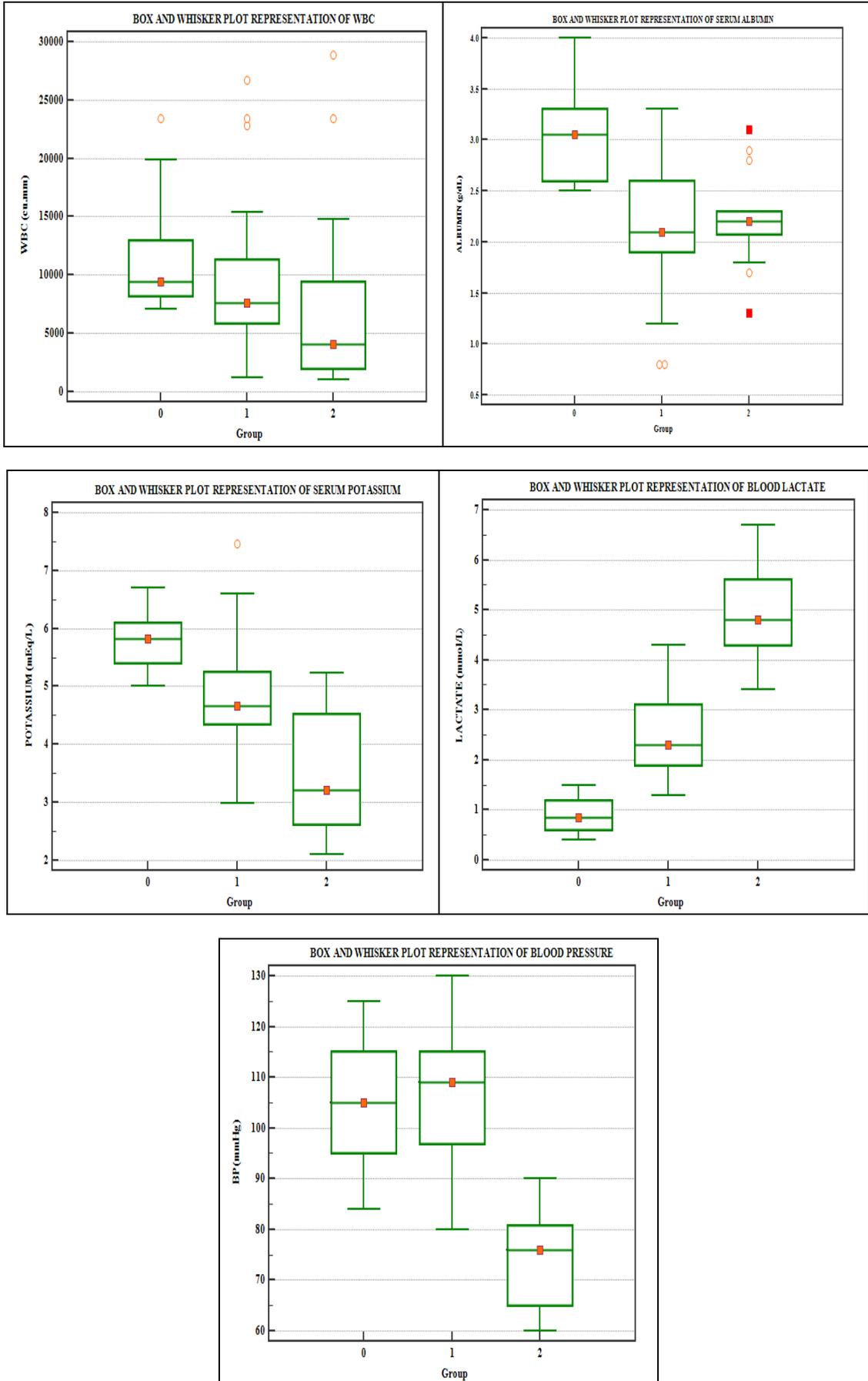| Parameter	Interquartile range			Median		
	Healthy	survivors	Non-survivors	Healthy	survivors	Non-survivors
WBC (cu.mm)	7100-23400	1200-26700	1000-28900	9385	7600	4100
Serum albumin (g/dL)	2.5-4	0.8-3.3	1.3-3.1	3.0	2.1	2.2
Serum potassium (mEq/L)	5.0-6.7	2.9-7.4	2.1-5.2	5.8	4.6	3.2
Blood lactate (mmol/L)	0.4-1.5	1.3-4.3	3.4-6.7	0.8	2.3	4.8
Blood pressure (mmHg)	84-125	80-130	60-90	105	109	76

Fig 2: Box and Whisker plot- WBC, Serum Albumin, Potassium, Blood lactate and Blood pressure

1. Group 0- Healthy group
2. Group 1- Survivors
3. Group 3- Non-survivors

4. References

1. Appel MJG, Cooper BJ, Greisen H, Carmichael LE. Status report: canine viral enteritis. J Am. Vet. Med. Assoc. 1978; 173:1516-1518.
2. Boag AK, Hughes D. Assessment and treatment of perfusion abnormalities in the emergency patient. Vet. Clin. Small Anim. 2005; 35:319-342.
3. Dehn BV. Pediatric clinical pathology. Vet. Clin. Small Anim. 2014; 44:205-219.
4. Dow SW. Handbook of small animal gastroenterology. W.B. Saunders, Philadelphia, 1996, 246-266.
5. Greene CE. Clinical microbiology and infectious diseases of the dog and cat. W.B. Saunders, Philadelphia, 1984, 437-460.
6. Hackett TB. Gastrointestinal complications of critical illness in small animals. Vet. Clin. Small Anim. 2011; 41:759-766.
7. Haligur M, Ozmen O, Sezer K, Sahinduran S. Clinical, pathological and immunohistochemical findings in diarrheic dogs and evaluation canine parvoviral and coronaviral enteritis. J Anim. Vet. Adv. 2009; 8:720-725.
8. Kraft W, Hartmann K, Dereser R. Age dependency of laboratory values in dogs and cats. Part II: Serum electrolytes. Tierarztl parx. 1996; 24:169-173.
9. Macartney L, McCandish IAP, Thompson H, Cornwell HJC. Canine parvovirus enteritis: clinical, haematological and pathological features of experimental infection. Vet. Rec. 1984; 115:201-210.
10. Magne ML. Selected topics in pediatric gastroenterology. Vet. Clin. Small Anim. 2006; 36:533-548.
11. McMichael MA, Leese GE, Hennessey J. Serial plasma lactate concentrations in 68 puppies aged 4 to 80 days. J Vet. Emerg. Crit. Care. 2005; 15:17-21.
12. Sharkey LC, Wellman ML. Use of lactate in small animal clinical practice. Vet. Clin. Small Anim. 2013; 43:1287-1297.
13. Silverstein DC, Waddell LS, Drobatz KJ, King LG. Vasopressin therapy in dogs with dopamine resistant hypotension and vasodilatory shock. J Vet. Emerg. Crit. Care. 2007; 17:399-408.
14. Unterer S, Strohmeyer K, Kruse BD, Sauter-louis C, Hartmann K. Treatment of aseptic dogs with haemorrhagic gastroenteritis with amoxicillin/clavulanic acid: a prospective blinded study. J Vet. Intern. Med. 2011; 25:973-979.