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HAEMATOLOGICAL, SERUM BIOCHEMICAL ALTERATIONS AND EFFICACY OF DIFFERENT THERAPEUTIC REGIMENS IN HAEMORRHAGIC GASTRO-ENTERITIS WITH SPECIAL REFERENCE TO CANINE PARVO VIRUS INFECTION

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ABSTRACT

The present study was conducted on 120dogs at the Teaching Veterinary Clinical Complex (TVCC), C.V.Sc. and A.H., OUAT, Bhubaneswar showing the symptoms of haemorrhagic gastro enteritis were made against CPV infection to reveal the haematological, serum biochemical alterations and efficacy of different therapeutic regimens. Significant variations were found in both hematological and sero-biochemical parameters with respect to different age groups. In spite of aggressive treatment of dogs showing the symptoms of hemorrhagic foul smelling diarrhea along with profuse vomition was done with parenteral fluid therapy, broad spectrum antibiotics, antihaemorrhagics, anti-emetics with other supportive therapy, young pups of age groups between 1-3 months succumbed to death.

KEY WORDS: Canine Parvo Virus, efficacy, haematological, haemorrhagic gastroenteritis, serum biochemical, therapeutic regimen.

INTRODUCTION

Gastroenteritis is one of the most common ailments in the canine population irrespective of breed, age and sex of the animal. Among various infectious and non-infectious diseases Canine parvovirus (CPV) is one of the challenging diseases in our society which is a highly contagious and fatal hemorrhagic gastroenteritis (HGE) in dogs of all ages. This disease can cause havoc in the canine population in case of outbreak, if it is not treated properly. All known parvoviruses that infect and cause disease in carnivore species, is a small eukaryotic, nonenveloped and single stranded DNA virus. The CPV has been well established as an enteric pathogen of dogs throughout the world with high morbidity (100%) and frequent mortality up to 10%. (Appel and Scott, 1979; Black and Holscher, 1979).CPV is one of the viral diseases showing hemorrhagic enteritis with severe leukopenia, nausea and myocarditis in puppies over the age of 2 months. Infection in dogs is mainly seen in young puppies between 6-20 weeks of age (Mercks, 2006) and less in adult dogs. The prevalence study in India was first reported by Balu and Thangaraj in 1981 in Madras. Dogs can get infection through direct contact with contaminated faeces and indirectly by contact with contaminated fomites such as clothings, hospital benches and tables, food pans and kennel floors. Indirect transmission is due to the hardy nature of the virus to adverse environmental condition and can remain infectious in contaminated environment for months. Infection can also be through insect and rhodent vectors (Foster and Smith, 2011). Predisposing factors associated with the development of clinical parvovirus disease includes tressors such as weaning, overcrowding and parasite load, insufficient passive or active immunity, geographical region and the presence of co-pathogens. Hence, keeping in view of the importance of parvo virus

disease, the present study was undertaken to study haematological and serum biochemical alteration and efficacy of different therapeutic regimens in affected dogs.

MATERIALS & METHODS

The present study was conducted on 120 dogs at the Teaching Veterinary Clinical Complex (TVCC), C.V.Sc. and A.H., OUAT, Bhubaneswar after the permission of Animal Ethical Committee, C.V.Sc. and A.H., OUAT, Bhubaneswar showing the symptoms of haemorrhagic gastro enteritis were made against CPV infection to reveal the haematological, serum biochemical alterations and efficacy of different therapeutic regimens. The total no. of dogs were divided into 3 groups according to their ages viz. i.e.1-3 months of age (Group-A), 4-12 months of age (Group-B) and more than 12 months of age (Group-C) having 16,71,33 numbers of dogs respectively. Further, according to breed wise the total number of suspected dogs were divided into four groups viz. small breed (German Spitz, Cocker Spaniel, Chihuahua and Pug) ; Medium breed (Pitbull, Beagle and Boxer); Large and Giant Breed (Dalmatian, Doberman, German Shepherd, Labrador, Golden Retriever, Rottweiler, Great Dane and Neapolitan Mastiff) and Indigenous breeds having 19,10,63 and 28 numbers of dogs respectively. With respect to sex wise study, the total number of dogs were divided into two groups as male (Group A) and female (Group B) having 71 and 49 numbers of animal respectively.

The blood from 120 no of suspected dogs was collected with the help of syringe either from the saphenous vein or recurrent tarsal vein. Then blood sample was transferred to EDTA vials or blood collection tube with anticoagulant. The haematological studies were conducted on the collected blood samples for the estimation of different blood parameters such as haemoglobin as per Sahli's acid haematin method (Jain, 1986), packed cell volume by micro hematocrit method (Jain, 1986), total leucocyte count (Thomas fluid and hemocyto meter as per Jain, 1986), total erythrocyte count (Jain, 1986) and differential leucocyte count (Jain, 1986), Means Corpusular Volume (MCV), Means Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH). Enzymatic estimations for the study of biochemical parameters like ALT, AST, and BUN were estimated by autoanalyser using commercial reagent kits (CORAL PVT LTD) in the department of Biochemistry C.V.Sc. and A.H, OUAT, Bhubaneswar.

All the breeds were treated with broad spectrum antibiotic as Gentamicin, Ceftriaxone, Ceftriaxone + Tazobectum, Amikacin. Allthetype of antibiotic drugs were administered twice daily to check the secondary bacterial infection along with fluid therapy to maintain the electrolytic balance to check dehydration and shock and simultaneously oral intake of water and food materials were withdrawn. The antiemetic drugs i.e. Metacloperamide, Ondasetron were given to check the vomition and further, the H₂ blockers i.e. Ranitidine and Cemetidine were administered to check the acidity, Using the Haemacel to overcome the anemic condition and also using B-complex to restore the energy. This therapy was continued for 7-10 days to get better result.

Design of therapeutic trial

120 nos. of dogs were divided into four group's viz. group- (A), group-(B), group-(C) and group-(D) consisting of 19, 10, 63 and 28 animals respectively. Further, group-(A), group-(B), group-(C) and group-(D) include small, medium, large and indigenous breeds of dogs respectively. **Treatment schedule**:

(a) Broadspectrum Antibiotic - First treat with Gentamycin @4-mg/kgb.wt,then with Amikacin@5-10mg/ kgb.wt, then Ceftriaxone +Tazobectum @15-25mg/kg b.wt twice daily.

(b) Fluid Therapy - Lactate Ringer (R.L)@30-40ml/kg b.wt I/ V twice D.N.S @20-30ml/kgb. wt I/V twice daily

(c) Plasma Expander - Haemaccel @20ml/kg b.wt I/V twice daily.

(d) Antiemetic – Metoclopramide @ .2- .4mg/kg b.wtI/V twice daily.

(e) Antacid (H2 blocker) - Ranitidine@2-4 mg/kg b.wtI/M twice daily

(f) Antihaemorrhagic - Vitamin-K (Kaplin) @5mg/kgb.wt Haemacoagulase (Botoropase) @.5-1ml/animal I/Vtwice daily

(g)Vitamin-Bcomplex (Polybion) I/V with saline

RESULTS & DISCUSSION

The present study of hematological examination comprised 120 numbers of cases out of which 58 cases were found positive by KIT Ag test method with respect to breed, sex and age. The average hemoglobin value was found to be 10.60 \pm 1.6gm% with a minimum of 7.8gm% to maximum of 14gm%. The mean hemoglobin value indicated that there was low in Hb % value. The average TLC value was found to be 12182 \pm 491.6 per cu. mm. with a minimum of 2100 to maximum of 29600. The mean TLC value indicated that there was fall in TLC value. The average

TEC value was found to be 4.88 ± 0.118 million/cu. mm. with a minimum of 2.1 to maximum of 6.8. The mean TEC value indicated that the TEC was low. The mean and standard error value of PCV was found to be 31.53 \pm .757% with a minimum of 18% to maximum of 50%. The mean PCV value indicated that the PCV value was low.

The mean value of neutrophil was found to be 62.683 \pm .648% with a minimum of 51% to maximum of 78%. The mean value of lymphocyte count was found to be 31.34 $\pm .62\%$ with a minimum of 19% to maximum of 44%. The mean value of eosinophil count was found to be $4.33 \pm .26$ with a minimum of 1% to maximum of 14%. The mean value of monocyte was found to be 1.82 ±.66 with a minimum of 1% to maximum of 3%. Blood picture of the affected dogs showed mean values of MCV as 76.49 ±4.34 cubic micron with range of minimum 21.23 to maximum 233. 33. The mean values of MCHC percentage was found to be $36.32 \pm 1.10\%$ with a minimum percentage of 17.95 and maximum 70. Similarly, the mean values of MCH percentage were found to be 23.72 \pm .78% with a minimum percentage of 13.22 and maximum 53.63. The present study of serological examination comprised 58 cases which were found positive by KIT Ag method with respect to breed, sex and age. The average AST value was found to be 66.60 \pm 1.39U/L with a minimum of 41IU/L to maximum of 1111U/L. The mean AST value indicated that there was low in AST value. The average ALT value was found to be 102.58 \pm 2.33U/L with a minimum of 52IU/L to maximum of 154IU/L. The mean ALT value indicated that there was low in ALT value. The average BUN value was found to be 34.60 ±1.16mg/dl with a minimum of16 mg/dl to maximum of 64mg/dl. The mean BUN value indicated that there was low in BUN value.

The least squares analysis for the haematological parameters between breeds showed that the average values of differential count were found to be 62.68±.64, 31.34 \pm .62, 4.33 \pm .26 and 1.82 \pm .06 for Neutrophil, Lymphocyte, Eosinophil and Monocytes. No significant difference was observed between breeds with respect to blood differential count. The average values of TLC, TEC, PCV, Hb%, MCV, MCHC and MCH among different breeds were found to be 12182 ±491.61, 4.88 ±0.118, 31.53 ±0.75, 10.60 ± 0.14 , 76.49 ± 4.34 , 36.32 ± 1.106 and 23.72 ± 0.78 , respectively (Table 2). No significant difference was observed between breeds with respect to blood parameter. The least squares analysis for the serological parameters between breeds were found to be 66.67 ± 1.39 , 102.52 ± 2.33 and 34.60±1.16 for AST, ALT and BUN. No significant difference was observed between breeds with respect to serological parameters.

The least squares analysis for the hematological parameters between sexes showed that the average values of differential count were found to be $62.68 \pm .64$, $31.34 \pm .62$, $4.33 \pm .26$ and $1.82 \pm .06$ for Neutrophil, Lymphocytes, Eosinophil and Monocytes. No significant difference was observed between sexes with respect to blood differential count. The average values of TLC, TEC, PCV, Hb, MCV, MCHC and MCH among different sex were found to be 12182 ± 491.61 , 4.88 ± 0.11 , 31.53 ± 0.75 , 10.60 ± 0.14 , 76.49 ± 4.34 , and 36.32 ± 1.10 respectively. No significant difference was observed between sexes with respect to blood differential count. The least squares analysis for the

The least squares analysis for the hematological parameters between age group showed that the average values of differential count were found to be $62.68\pm.64$, $31.34\pm.62$, $4.33\pm.26$ and $1.82\pm.06$ for Neutrophil, Lymphocytes, Eosinophil and Monocytes. Significant difference was observed between age group with respect to blood differential count. The average values of TLC,

TEC, PCV, Hb, MCV, MCHC and MCH among different age group were found to be 12182 ± 491.61 , 4.88 ± 0.11 , 31.53 ± 0.75 , 10.60 ± 0.14 , 76.49 ± 4.34 , and 36.32 ± 1.10 respectively. Significant difference was observed between age group with respect to blood differential count. The least squares analysis for the serological parameters between age groups were found to be 66.67 ± 1.39 , 102.52 ± 2.33 and 34.60 ± 1.16 for AST, ALT and BUN (Table 3). Significant difference was observed between age group with respect to serological parameters.

TABLE 1. Prognosis of thera	peutic management of	parvoviral haemorrhagic	gastroenteritis in infec	ted dogs n=120

Group	Breed N=120	Treatment	After 10 Days Survival Rate N=84	Mortality Rate N=36	Percentage of Survival	Percentage of Mortality
4	Indigenous, n=28	Therapeutic management	Indigenous n=19	Indigenous n=9	22.61%	25%
1	Small, n=19	is done for 7-	Small n=11	Small n=8	13.09%	22.22%
2	Medium, n=10	10 days	Mixed n=5	Mixed n=5	5.9%	13.88%
3	Large & Giant, n=63	,	Large n=49	Large n=14	58.33%	38.88%

After the treatment, out of 120 nos. of dogs of different breeds, 86 nos. of dogs survived and rest died which is depicted in Table 1. So, the present study revealed that the percentage of survival rate is more in case of Group -(C) i.e. (Large and Giant breed). Similarly, the percentage of mortality is observed to be more in Group -(C) *i.e.* (Large and Giant breed).

The present study of hematological examination comprised 84 nos. of cases which survived after treatment according to breed, sex and age. The average hemoglobin value was found to be11.07 ±.16gm% with a minimum of 8gm% to maximum of 14.6 gm%. The mean hemoglobin value indicated that there was low in Hb % value. The average TLC value was found to be 10785 ±426.37 per cu. mm. with a minimum of 3000 to maximum of 26000. The mean TLC value indicated that there was fall in TLC value. The average TEC value was found to be 4.78 ±0.117 million/cu. mm. with a minimum of 2.4 to maximum of 7.4. The mean TEC value indicated that the TEC was low. The mean and standard error value of PCV was found to be $31.21\pm.58\%$ with a minimum of 20% to maximum of 42%. The mean PCV value indicated that the PCV value was low. The mean and standard error value of neutrophil was found to be $61.02 \pm .46\%$ with a minimum of 51% to maximum of 70%. The mean and standard error value of lymphocyte count was found to be 30.95±.43% with a minimum of 22% to maximum of 38%. The mean and standard error value of eosinophil count was found to be $5.85 \pm .27$ with a minimum of 1% to maximum of 11%. The mean and standard error value of monocyte was found to be 1.90 ±.07 with a minimum of 1% to maximum of 3%. Blood picture of the affected dogs showed mean and stand error values of MCV as 71.15±3.17 cubic micron with range of minimum 29.03 to maximum 166. 66. The mean and stand error values of MCHC percentage was found to be 36.30±.95% with a minimum percentage of 21.10 and maximum 59.09.Smilarly, The mean and stand error values of MCH percentage was found to be 24.30±.75% with a minimum percentage of 13.33 and maximum 44.66.

The least squares analysis for the hematological parameters between breeds showed that the average values of differential count were found to be. $61.02\pm.46$, $30.95\pm.$ 43, 5.85 \pm .27 and 1.825 \pm .06 for Neutrophil, Lymphocyte, Eosinophil and Monocytes. No significant difference was observed between breeds with respect to blood differential count. The average values of TLC, TEC, PCV, Hb%, MCV, MCHC and MCH among different breeds were found to 10785 ±426.37, 4.78±.117, 31.21±.580, 11.07±.168, 71.15±3.17, 36.30±.95 and 24.30±.754, 34.60 ±1.16 respectively. No significant difference was observed between breeds with respect to blood parameter. The least squares analysis for the serological parameters between breeds were found to be 58.34 \pm 1.38, 85.15 \pm 2.16 and 27.58±.88 for AST, ALT and BUN. No significant difference was observed between breeds with respect to serological parameters. The least squares analysis for the hematological parameters between sexes showed that the average values of differential count were found to be. 61.02±.46, 30.95±.43, 5.85±.27 and 1.82±.06 for Neutrophil, Lymphocyte, Eosinophil and Monocytes. No significant difference was observed between breeds with respect to blood differential count.

The average values of TLC, TEC, PCV, Hb%, MCV, MCHC and MCH among different sex were found to 10785 ±426.37, 4.78±.117, 31.214 ±.580, 11.072 ±.16, 71.15 ±3.17, 36.30 ±.95, and 24.30±.75, 34.60±1.16 respectively. No significant difference was observed between breeds with respect to blood parameter. The least squares analysis for the serological parameters between sexes was found to be 58.34±1.38, 85.15±2.16 and 27.58± .889 for AST, ALT and BUN. No significant difference was observed between breeds with respect to serological parameters. The least squares analysis for the hematological parameters between different age group showed that the average values of differential count were found to be. 61.02± .46, 30.95 ±.43, 5.85±.27 and 1.825± .06 for Neutrophil, Lymphocyte, Eosinophil and Monocytes. No significant difference was observed between breeds with respect to blood differential count. The average values of TLC, TEC, PCV, Hb%, MCV, MCHC and MCH among different age groups were found to 10785 ± 426.37 , $4.78 \pm .117$, $31.214 \pm .58$, $11.07 \pm .16$, 71.15 ± 3.17 , $36.30 \pm .95$ and $24.30 \pm .75$ respectively. No significant difference was observed between breeds with respect to blood parameter. The least squares analysis for the serological parameters between different age groups were found to be 58.34 ± 1.386 , 85.15 ± 2.16 and $27.58 \pm .889$ for AST, ALT and BUN. No significant difference was observed between breeds with respect to serological parameters.

The average haemoglobin value was found to be 10.608±1.605 gm% in affected dogs fluctuated from 7 gm% to 14 gm%. The decrease value might be due to damage of vascular epithelium of intestine in parvoviral infection (Zafar et al., 1999 and Sagar et al., 2008. The mean and standard error value of PCV before the treatment was found to be $31.53 \pm .75\%$ with a minimum of 18 % to maximum of 65%. The decrease value is due to the haemorrhage in intestinal region and blood loss through the faeces and vomitus in the disease process (Biswas et al., 2005) and due to damage of vascular epithelium of the intestine (Zafar et al., 1999) In the present study it might be due to blood loss through the faeces and vomitus in the infected dogs with severe dehydration. But after the treatment the average value of PCV was found to be 31.21±.58 % with a minimum of 20% to maximum of 42%. After 7-10 days administration of fluid therapy markedly changes in PCV observed in survival dogs.

The average TLC value before the treatment was found to be 12182 ± 491.6 per cu. mm. with a minimum of 2100 to maximum of 29600. The mean TLC value indicated that there was leucopenia in the infected dogs also we got in few cases an increased TLC value up to 29600. This exceptionally increased TLC value might be due to the fact that the blood sample in the above cases were not collected at the stage of viremia rather than the samples were collected at the later stages when leucocytosis had already had developed due to secondary bacterial infection (Sagar *et al.*, 2008, Biswas *et al.*, 2005 and Haligur *et al.*, 2009)

whereas contraindicated the findings of Apple *et al.* (1979). But after treatment the average TLC value was 10785 \pm 426.37 with a minimum of 3000 to maximum 26000 in survival dog. In the present study it was observed that not markedly increased in survival dog from CPV infection.

The mean and standard error value of neutrophil was found to be $62.68 \pm .64\%$ with a minimum of 51% to maximum of 78%. The upper value of neutrophil might be due to secondary bacterial infection (Sagar et al., 2008) and low neutrophil value probably due to early stage of viremia. The mean and standard error value of lymphocyte count was found to be 31.34±.62% with a minimum of 19% to maximum of 44%. The lower limit of lymphocyte might be due to the virus replication in the lymphoid organs resulting in lymphocytolysis as reported by Biswas et al. (2005) and Me Candlish (1998). The mean value of eosinophil count was found to be 4.33 ±.264 with a minimum of 1% to maximum of 14%. The higher limit may due to endoparasitic infection. There was no significant change in neutrophil, lymphocyte, eosinophil and monocyte percentage. This suggested that there might be no effect on these parameters due to parvoviral infection.

Blood picture of the affected dogs showed mean and stand error values of MCV as 76.49 ± 4.34 cubic micron with range of minimum 21.23 to maximum 233. 33, which indicates an increased MCV value. The increased MCV value might be due to macrocytic normochromic anaemia. Similarly MCHC percentage was found to be $36.32\pm1.10\%$ with a minimum percentage of 17.95% and maximum 70% which indicated that value was within normal range. The mean and stand error values of MCH percentage was found to be $23.72\pm.78\%$ with a minimum percentage of 13.22% and maximum 53.63\%, which indicate the MCH is slightly high due to haemorrhagic condition.

The average TEC value was found to be 4.88 ± 0.118 millions/cu. mm. with a minimum of 2.1 to maximum of 6.8. The mean TEC value indicated that the TEC was low. The reduced mean TEC level in the present study might be due to damage of the capillaries of the villi of intestine of infected dogs (Haskins, 1998 and Sagar *et al.*, 2008).

TABLE 2. Breed wise least squares mean and standard error of Haen	meatological parameters during the treatment n=120
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Breed	PCV (%)	TEC(10 ⁶ /cu mm)	Hb%(gm %)	MCV(fl)	MCHC %	MCH %
Overall	31.533±.757	$4.88 \pm .118$	$10.608 \pm .146$	76.49±4.34	36.32±1.106	23.72±.781
Indigenous	32.392 ± 1.592	$4.807 \pm .243$	$10.585 \pm .297$	78.08 ± 8.71	34.98 ± 2.006	24.00 ± 1.64
Small	32.052 ± 1.726	$4.74 \pm .307$	$10.410 \pm .445$	80.66±11.25	34.99 ± 3.077	23.67±1.76
Large and giant	$32.920{\pm}1.053$	4.95±.162	$10.625 \pm .185$	74.01±6.03	37.14 ± 1.529	$23.54{\pm}1.12$
Medium	32.000±3.126	4.91±.454	$10.940 \pm .628$	79.77±17.03S	37.44±4.637	24.19 ± 2.68

TABLE 3. Age wise least so	juares mean and standard error of	f Serobiochemical Test dur	ing the treatment n=120

AGE	AST IU/L	ALT IU/L	BUN mg/dl
Overall	66.67±1.39	102.58 ± 2.33	34.60±1.16
1-3 Month	68.20 ± 4.60	102.93±6.65	35.80±3.13
4-12Month	69.57±1.85	108.53 ± 2.96	35.62 ± 1.58
Above 12 month	60.22±1.96	90.51±3.97	32.05±2.01

CONCLUSION

Haemorrhagic gastroenteritis is a very dangerous, emerging and challenging disease in canines of all ages as well as dog practitioners now-a-days. The dog practitioners as well as owners should consider the importance of this disease which may be a leading cause of death in puppies and more in young dogs. However, diagnosis the disease can also be managed by therapeutic measures in time. But some dogs die due to severity of viral infection and lack of therapeutic management. Analysis of blood samples collected from infected dogs recovered from CPV with the above therapeutic management revealed significant improvement in haematological parameters such as Hb %, PCV, TLC, Neutrophil, Eosinophil, Lymphocyte, Monocyte counts, MCV, MCHC and MCH. Similarly, the sero-biochemical parameters such as AST, ALT and BUN values increased after the treatment. It could be inferred from the study that the present therapeutic regimen with supportive treatment against parvoviral haemorrhagic gastroenteritis is effective in ameliorating clinical signs and gradual development of normal health status of affected dogs.

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