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Dr. Jyothi J
Assistant Professor, Department of Veterinary Medicine, C.V.Sc, Hyderabad, Telangana, India

Padmaja K
Department of Veterinary Medicine, PVNR TVU, Rajendranagar, Hyderabad, Telangana, India

G Ambica
Department of Veterinary Medicine, PVNR TVU, Rajendranagar, Hyderabad, Telangana, India

Dr. Lakavath Ramsingh
Associate Professor, Department of Veterinary Gynecology and Obstetrics, PVNR TVU, Rajendranagar, Hyderabad, Telangana, India

Dr. M Lakshman
Professor and Head, Department of Veterinary Pathology, PVNR TVU, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
Dr. Jyothi J
Assistant Professor, Department of Veterinary Medicine, C.V.Sc, Hyderabad, Telangana, India

Diagnosis of hemorrhagic gastro enteritis in pediatric puppies

Dr. Jyothi J, Padmaja K, G Ambica, Dr. Lakavath Ramsingh and Dr. M Lakshman

Abstract
Haemorrhagic gastro enteritis is one of the most common conditions in canine animal practice**.** A total of 4064 pediatric pups upto 12 weeks of age were presented to Veterinary clinical complex, College of Veterinary Science, Rajendranagar. The first 3 months of life (Neonatal stage- birth to 2 weeks, Infant stage - 2-6 weeks and pediatric stage – 6-12 weeks) may be considered as the pediatric period in dogs and cats. Out of 4064 puppies up to 12 weeks of age were screened for hemorrhagic gastroenteritis (HGE), out of which 512 pups were presented with HGE forming the overall incidence of 12.59%. Clinical manifestations like decreased appetite, vomiting, hemorrhagic/non-hemorrhagic diarrhea and dehydration were observed. A total of 512 clinically positive dogs were screened for the presence of infectious agents i.e. Worm burden, Bacterial infection, Viral infection i.e 185 hemorrhagic gastroenteritis cases are due to worm burden (*Ancylostomiasis*, *Toxocara Canis*, *Dipylidium caninum*, *Isospora* spp.) forming 36.13%, 145 HGE cases are due to bacterial infection (*Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., and *Streptococcus* spp.) forming 28.32% and 182 cases are due to viral infection (Parvo virus, Canine distemper, Canine corona virus) forming 35.55%.

Keywords: Worm burden, PCR, rapid test, fecal culture, parasitic ova

Introduction
The first 3 months of life (Neonatal stage- birth to 2 weeks, Infant stage - 2-6 weeks and pediatric stage – 6-12 weeks) may be considered as the pediatric period in dogs and cats (Kampschmidt, 2008) [12]. Hemorrhagic gastroenteritis in puppies can have several etiologies such as parasitic, bacterial and viral. Dipylidium caninum is the most commonly encountered tapeworm, and infection is usually acquired through ingestion of fleas and lice (Battersby and Harvey, 2006) [1]. The potential enteropathogenic bacteria associated with bacterial gastroenteritis in dogs include *Clostridium difficile*, *Clostridium perfringens*, *Escherichia coli*, *Campylobacter jejuni*, and *Salmonella* Spp (Marks *et al.*, 2011) [8]. Among viral diseases canine coronavirus (CCoV) and canine parvovirus (CPV) are the most common causes and usually affect dogs up to one year old, with high morbidity and mortality in puppies from three to six months of age and which are not vaccinated (Ghiggi *et al*., 2013; Duijvestijn *et al*., 2016) [7, 6].
The intestinal micro flora of puppies is a complex and poorly understood population. The poor understanding of what truly constitutes normal versus abnormal, along with the ability to only superficially characterize the gut microbial population, limits understanding of the pathophysiology of enteritis (Weese *et al*., 2001) [11]. There is a growing body of evidence indicating bacterial translocation due to impaired gastrointestinal function, leading to the septicemia in young puppies (Biffl and Moore, 2000) [13]. Thus, the study was conducted to identify the various possible underlying etiology of hemorrhagic gastroenteritis (HGE) in puppies, which will help in better understanding of the pathogenesis and to identify the better choice of therapeutic management in most of the affected puppies, thereby to improve the survivability.

Material and Methods
A total of 512 fecal samples were collected from Paediatric puppies presented to the Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar, Hyderabad, showing the clinical signs of vomiting and bloody diarrhoea were selected for the study (Fig.1). the samples were screened for parasitic, bacterial and viral aetiologies. Microscopic examination of fecal sample was done for screening of presence of parasitic ova, Faecal

culture was carried to identify bacterial organisms and samples are screened for Rapid tests to identify Viral agents such as, Canine parvo virus, Canine corona virus and Canine distemper virus.

Results

A total of 512 clinically positive dogs were screened for the presence of infectious agents i.e., Worm burden, Bacterial infection, Viral infection. Among 512, affected pups, 185 hemorrhagic gastroenteritis cases were due to worm burden (*Ancylostomiasis*, *Toxocara Canis*, *Dipylidium caninum*, *Eimeria*) forming 36.13%, 145 HGE cases are due to bacterial infection (*Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., and *Streptococcus* spp.) forming 28.32% and 182 cases are due to viral infection (Parvo virus, Canine distemper, Canine corona virus) forming 35.55% (Table-1). In worm burden highest number parasites noticed in faecal examination was *Ancylostomiasis* (74), *Toxocara Canis* (56), *Dipylidium caninum* (32) and the least was *Isospora* Spp. (23) forming 40%, 30.27%, 17.30% and 12.43% respectively (Table-2 & Fig.2). In bacterial infection most of the organisms seen was *Escherichia coli* (83), *Klebsiella* (33), *Staphylococcus* Spp. (16) and *Streptococcus* Spp. (13) forming 57.24%, 22.76%, 11.03% and 8.97%, respectively (Table. 3 & Fig. 3, 4 & 5). All the 512 samples were subjected to Rapid Parvo test, Rapid Canine distemper test, Rapid canine corona virus test to identify the viral hemorrhagic gastroenteritis Parvo virus (110), Canine corona virus (44) and Canine distemper (28), forming 60.44%, 24.16% and 15.38% respectively (Table. 4 & Fig.6).

Fig 1: HGE

Table 1: Causes of HGE in pups

SI. No		Causative agents	No. of. Pups
1	Faecal sample (Worm burden) 185 (36.13%)	*Ancylostoma* spp.	74
		Toxocara spp.	56
		D. caninum	32
		Eimeria spp.	23
2	Bacterial 145 (28.32%)	*E. coli*	83
		Klebsiella spp.	33
		Staphylococcus spp.	16
		Streptococcus spp.	13
3	Viral 182 (35.55%)	Parvo virus	110
		Canine distemper virus	44
		Canine corona virus	28
	Total		512

Table 2: Different ova from Parasitic hemorrhagic gastroenteritis in dogs

S.no	Causative agents	No. of. Pups	Percentage
1	*Ancylostoma* spp.	74	40.00
2	*Toxocara* spp.	56	30.27
3	*D. caninum*	32	17.30
4	*Isospora* spp.	23	12.43
		185	100

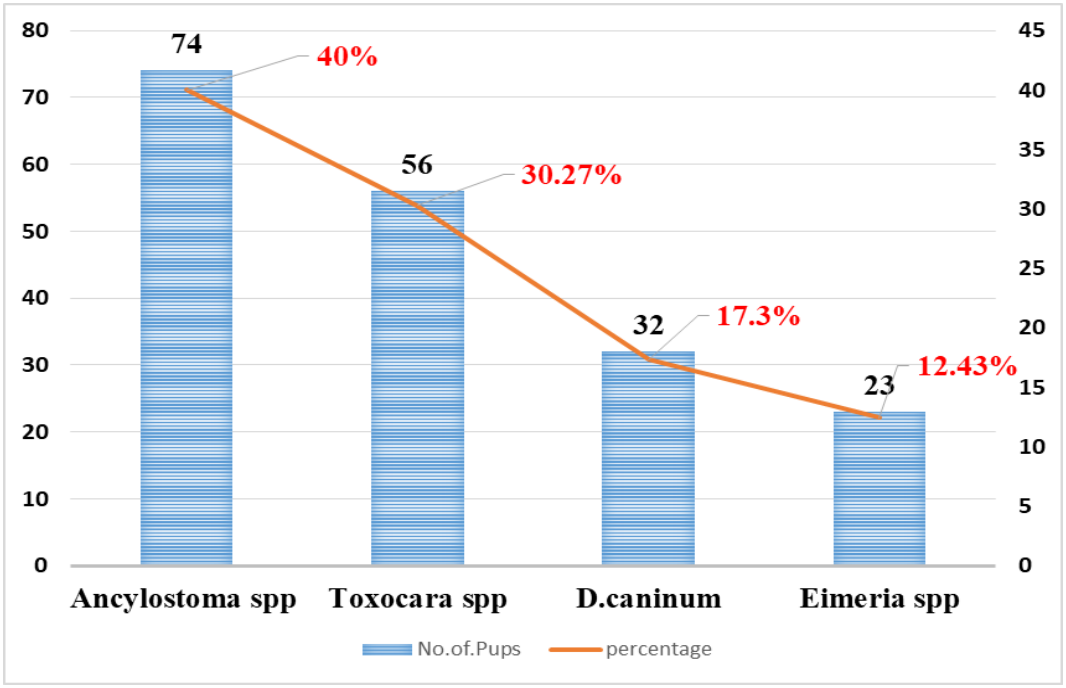

Fig 2: Different ova from parasitic haemorrhagic gastroenteritis in dogs

Table 3: Microbial isolates from bacterial haemorrhagic gastroenteritis in dogs

SI. No	Organism isolated	Number of isolates	Percentage (%)
1	*Escherichia coli*	83	57.24
2	*Klebsiella* spp.	33	22.76
3	*Staphylococcus* spp.	16	11.03
4	*Streptococcus* spp.	13	8.97
	Total	145	100

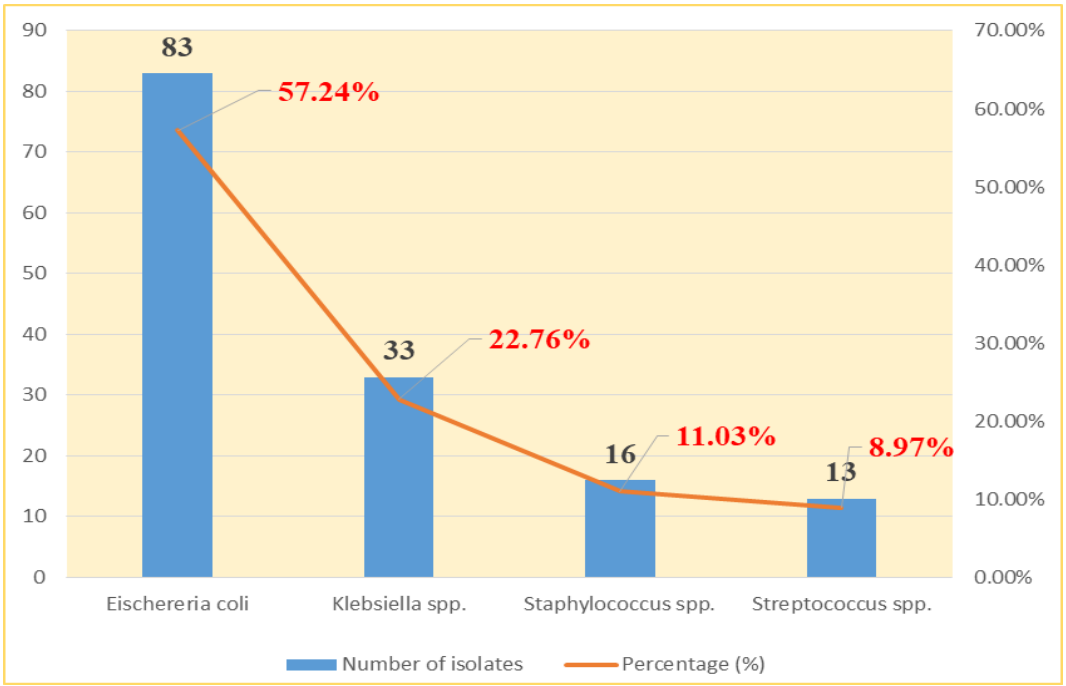

Fig 3: Microbial isolates from bacterial haemorrhagic gastroenteritis in dogs.

Table 4: Viral HGE diagnosis by Rapid Test

SI. No	Diseases	Snap test	Percentage (%)
1	Canine Parvo Virus	125	56.31
2	Canine Corona Virus	65	29.28
3	Canine Distemper Virus	32	14.41
	Total	222	100

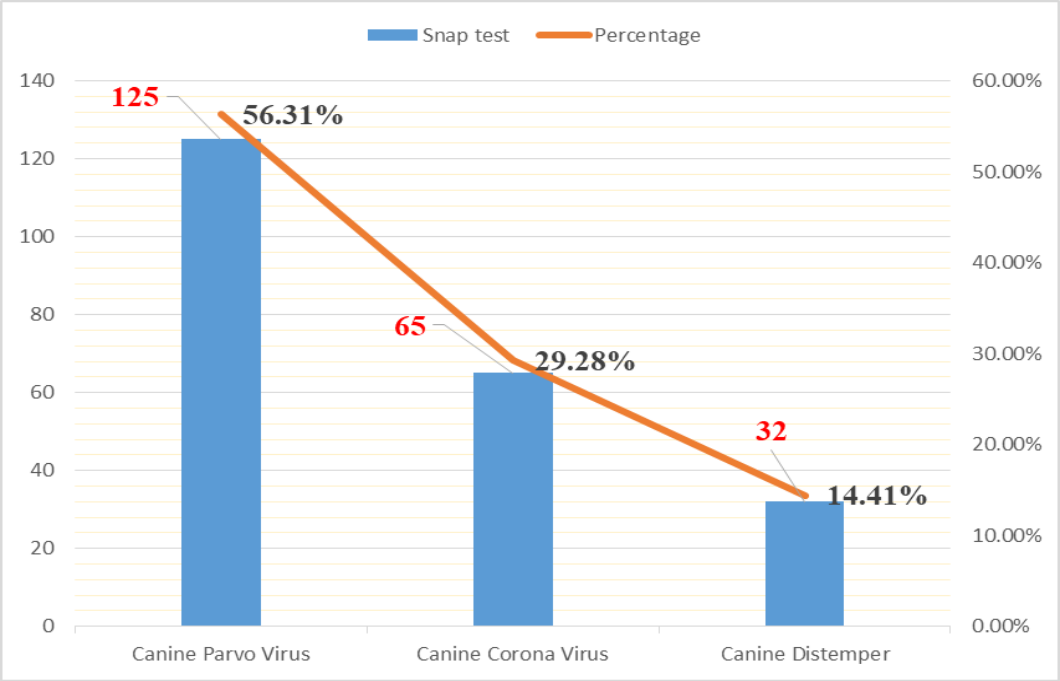

Fig 4: Viral HGE diagnosis by Rapid Test

Discussion

In worm burden highest number of parasites noticed in fecal examination was *Ancylostomiasis* (74) followed by *Toxocara Canis* (56), *Dipylidium caninum* (32) and the least was *Eimeria* Spp. (23) forming 40%, 30.27%, 17.29%, 12.43% respectively. Trans-mammary transmission of *Ancylostoma caninum* is considered to be the most important route by which puppies become infected (Raza *et al*, 2008) [14]. In healthy adult animals infected with Toxocara species, acquired immunity results in larval arrest, so clinical signs of infection are rare. Neonates may, however, be infected via maternal milk following reactivation of arrested larvae during pregnancy, by Transplacental transmission (*Toxocara canis*) or by ingestion of ova or other hosts. Dipylidium caninum is the most commonly encountered tapeworm, and infection is usually acquired through ingestion of fleas and lice (Batters by and Harvey., 2006) [1]. Priya *et al.* (2017) recorded *Escherichia coli* and *Streptococcus*. Munnicha and Lubke-Becker (2004) [15], Ali and Metwally. (2015) [9] recorded that a higher percentage of diarrheic puppies were positive for hemolytic *Escherichia coli*, with increased prevalence in young age as the intestinal epithelium appears to be more permeable than the intestinal epithelium in older dogs. Thus, the increased incidence of *Escherichia coli* in the fecal samples in puppies is explained by their normal inhabitance and abundance in gastrointestinal tract of the puppies, more commonly among the diarrheic animals.

The enteric viruses that are commonly detected in dogs with diarrhea included canine parvovirus, enteric Canine coronavirus and canine distemper virus as recorded by Schulz *et al*. (2008) [10]. Increased intestinal epithelial turnover caused by changes in the bacterial microbiota and diet (weaning) is a predisposing factor to CPV infection (McAdaragh *et al*., 1982; Hagiwara *et al*., 1996) [2, 3]. Battersby and Harvey, (2006) [1] suggested that animals presenting with clinical signs of distemper develops diarrhea during infection, but gastrointestinal signs are normally preceded by a cough, rhinitis and pyrexia. Parvovirus replicates in rapidly dividing cells as cells of GIT. Therefore, intestinal cells are affected by the virus, which results in diarrhoea and vomiting (Judge, 2015) [4]. Parvo virus infects the germinal epithelium of intestinal crypts, causing destruction and collapse of the epithelium. As a result, normal cell turnover is impaired, and the villi becomes shortened and atrophied which affects absorption, secretion, and mucosal barrier integrity. Further secondary bacterial infections cause additional intestinal damage bacteraemia and endotoxemia. All these alterations in the intestines initiated by parvo viral infection leads to haemorrhagic diarrhoea in puppies. The corona virus invades and replicates in the villi of the small intestine. Intestinal disease may be related to virus-induced apoptosis (programmed cell death) of cells of the epithelial mucosa of the small intestine (Ruggieri *et al*., 2007) [5].

Conclusion

Puppies presented with hemorrhagic gastroenteritis showed clinical signs of Bloody diarrhea, vomiting, dehydration, anorexia in the present study among parasitic etiology *Ancylostomiasis* (74) showing highest incidence, and in bacterial etiology *Escherichia coli* (83) forming highest occurrence, whereas Parvo virus (110) forming 60.43% of incidence. Various Parasites, bacteria and the toxins also play an important role as a sole enteropathogen and in combination with viruses in the etiology of HGE in puppies.

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