Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado Integrado em Medicina Veterinária

Dissertação

Effects of plasma transfusions in the outcome of acute gastroenteritis in young dogs with leucopenia: a retrospective study

Maria Rui Pereira de Magalhães de Castro Oliveira

Orientador(es) | Sandra Maria Branco

Carolina Torres de Almeida

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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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Abstract

Effects of plasma transfusions in the outcome of acute gastroenteritis in young dogs with leucopenia: a retrospective study

Acute gastroenteritis represents one of the leading reasons for seeking veterinary assistance in young dogs. When associated with leucopenia, it is mostly due to parvoviral infection. Supportive therapy is the mainstay of treatment protocols in dogs with parvoviral enteritis, but plasma transfusions are commonly used because of theoretical and anecdotal benefits. There is no substantial evidence to support their use, and, at the same time, there isn't any to refute it.

In this retrospective study, the influence of fresh frozen plasma transfusions on outcome and duration of hospitalization of dogs with a presumptive diagnosis of parvoviral enteritis was assessed. Moreover, several variables were evaluated as predictors of outcome.

Plasma transfusions were found to have statistically significant relationships with worst outcomes and longer hospitalization times; however, it should be taken into consideration that their use was also significantly associated with dogs in worst health conditions (lower neutrophil nadir and worst pulse quality). In addition, lower neutrophil counts and worse pulse quality also resulted in longer hospitalization times and the presence of fever was significantly linked to worse outcomes.

***Keywords*:** Gastroenteritis; Plasma; Leucopenia; Transfusion; *Parvovirus*

Resumo

Efeitos das transfusões de plasma no prognóstico da gastroenterite aguda em cães jovens com leucopenia: um estudo retrospetivo

A gastroenterite aguda representa um dos principais motivos de consulta em cães jovens. Quando associada a leucopenia, deve-se principalmente a infeção por *Parvovirus*. Em cães com parvovirose, a terapia de suporte é a base dos protocolos de tratamento, mas as transfusões de plasma também são frequentemente usadas devido aos seus benefícios teóricos. Não há evidência substancial para apoiar o seu uso, mas também não a há para o refutar.

Neste estudo retrospetivo, foi avaliada a influência das transfusões de plasma fresco congelado no prognóstico e duração da hospitalização em cães com diagnóstico presuntivo de parvovirose. Adicionalmente, foram avaliadas outras variáveis para testar a sua correlação com os mesmos parâmetros.

As transfusões de plasma revelaram uma relação estatisticamente significativa com piores resultados e tempos de internamento mais longos; contudo, o uso destas também esteve diretamente relacionado com cães que estavam em piores condições de saúde (nadir de neutrófilos mais baixo e pior qualidade de pulso). Contagens mais baixas de neutrófilos e pulsos fracos ou palpáveis também resultaram em tempos de internamento mais longos, e a correlação da febre com piores prognósticos revelou-se estatisticamente significativa.

Palavras-chave: Gastroenterite; Plasma; Leucopenia; Transfusão; *Parvovirus*

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GI - Gastrointestinal

GIP – Gastric Inhibitory Peptide

CCK – Cholecystokinin

CBC – Complete Blood Count

PCR – Polymerase Chain Reaction

ELISA – Enzyme-Linked Immunosorbent Assay

AHDS – Acute Hemorrhagic Diarrhea Syndrome

CPV – Canine Parvovirus

CCoV – Canine Coronavirus

CDV – Canine Distemper Virus

CDI – *Clostridium difficile* infection

TcdA - C. *perfingens* Toxin A

TcdB - C. *perfingens* Toxin B

CPE – C. *perfingens* enterotoxin

ETEC – Enterotoxigenic *E*. *coli*

EPEC – Enteropathogenic *E*. *coli*

EHEC – Enterohemorrhagic *E. coli*

NTEC – Necrotoxigenic *E.coli*

EIEC – Enteroinvasive *E*. *coli*

AIEC – Adherent-invasive *E. coli*

EAEC – Enteroaggregative *E. coli*

NSAID – Non-Steroidal Anti-Inflammatory Drug

CRTZ – Chemoreceptor Trigger Zone

CNS – Central Nervous System

HGE – Hemorrhagic Gastroenteritis

PCV – Packed Cell Volume

WBC – White Blood Cell Count

HSC – Hematopoietic Stem Cell

SCF – Stem Cell Factor

IL-3 – Interleukine-3

IL-6 – Interleukine-6

GM-CSF – Granulocyte-Macrophage Colony Stimulating Factor

G-CSF - Granulocyte Colony Stimulating Factor

TNFα/β – Tumor Necrosis Factor α/β

C5a – Complement Component 5a

IL-1 – Interleukine-1

MNP – Marginal Neutrophil Pool

CNP – Circulating Neutrophil Pool

MVC – Minute Virus of Canines

VP-1 – Viral Protein 1

TfR – Transferrin Receptor

MDA – Maternally Derived Antibody

DIC – Dissimulated Intravascular Coagulation

SIRS – Systemic Inflammation Response Syndrome

PTT – Partial Thromboplastin Time

PT – Prothrombin Time

cPLI – Canine Pancreatic Lipase

NPO – *Nil Per Os*

BPI – Bactericidal Permeability Increasing protein

FFP – Fresh Frozen Plasma

FP – Frozen Plasma

RBC – Packed Red Cells

HSA – Human Serum Albumin

IVIG – Intravenous Immunoglobulins

CRYO – Cryoprecipitate

CPP – Cryo-poor Plasma

vWf – von Willebrand's Factor

fVIII – Factor VIII

fVII - Factor VII

fIX - Factor IX

COP – Colloid Oncotic Pressure

DOH – Duration of Hospitalization

EDTA – Ethylenediaminetetraacetic Acid

Preface

This dissertation was written following a six-month traineeship that took place from September 2019 to March 2020 in Centro Hospitalar Veterinário in Porto, Portugal, as part of the sixth and last year of the integrated master's degree in veterinary medicine at the University of Evora.

During the externship, the author contacted with several areas of small animal medicine, such as emergency and critical care, internal medicine, soft tissue and orthopedic surgery, neurology, anesthesiology, oncology, and diagnostic imaging.

Parvoviral enteritis and other cases of severe gastroenteritis were very common in the length of this traineeship. This raised the author's interest in the subject and led to the writing of this dissertation, which resulted from the existence of many questions that remained unanswered.

I- Literature review

1- Acute gastroenteritis in young dogs

Acute gastroenteritis stands for the acute onset of vomiting and diarrhea caused by inflammation of the stomach and intestinal tract's mucosa, thus representing a co-occurrence of gastritis and enteritis.[1,2]

According to studies[3,4], it represents one of the leading reasons for seeking veterinary assistance in young dogs. Usually, the cause remains unknown because there is spontaneous recovery, but it is often associated with infectious pathogens, like viruses, bacteria or parasitic agents.[5,6] Nevertheless, it can have a wide selection of other underlying causes like dietary indiscretions, toxins, or metabolic disorders, for instance.[7]

1.1- A brief review of the anatomy and physiology of the dog's gastrointestinal tract

The dog's digestive system is formed by the primary gastrointestinal (GI) tract, which includes the oropharynx, the esophagus, the stomach, the small intestine and colon, and the accessory glands, the pancreas and the liver (Figure 1).[8,9]

The GI tract's primary role is to digest food and provide nutrients, electrolytes, vitamins and water to the body while protecting it against undesirable substances, such as toxins and microbes, and excreting waste products that are not useful for the organism.[10] It absorbs 99% of the water presented to it. Hence, any damage it suffers can result in major alterations in fluid and acid-base balances.[7]

It is a tubular structure that extends from mouth to anus, and every part serves a specific purpose: some are only for the passage of food and temporary storage, and others perform digestion and absorption.[11,12]

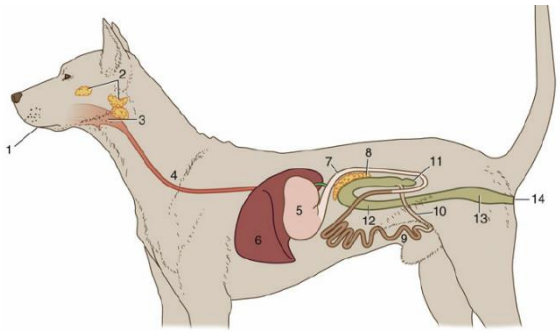

Figure 1- Dog's gastrointestinal tract. 1- Mouth; 2- Salivary glands; 3- Pharynx; 4- Esophagus; 5- Stomach; 6- Liver; 7- Duodenum; 8- Pancreas; 9- Jejunum; 10- Ileum; 11- Cecum; 12- Colon; 13- Rectum; 14- Anus.(Reproduced from Dyce, Sack and Wensing's Textbook of Veterinary Anatomy, Singh, 2018)[13]

Histologically, the gastrointestinal wall has four main layers, from the lumen to the outer surface: the mucosa, the submucosa, a muscle layer, and the serosa. These are then divided into sublayers within them: the mucosa includes the epithelium cells, the lamina propria, and the mucosal muscle, and the muscular layer splits into 2 – the deeper one is circular, and the outer is longitudinal, between them lies the myenteric plexus.[10,12,13] Interestingly, and contrarily to other species like humans or cats, the muscular layer is composed of smooth muscle throughout the dog's whole GI tract except in the esophagus, where it is striated muscle.[14,15]

The dog has a small and simple stomach with five different areas: the cardia (connected to the esophagus), fundus, body, pyloric antrum, and the pylorus (connected to the duodenum). Each area serves specific functions.[8,13] Its mucosa is filled with gastric pits (invaginations) that hold openings to gastric glands.[16]

The intestine is formed by the small and large intestine, divided into three parts each: the small intestine consists of the duodenum, jejunum and ileum and the large intestine is formed by the cecum, colon (ascending, transverse and descending) and rectum. In the small intestine, the mucosa contains a single layer of epithelial cells called enterocytes.[7] The luminal epithelium is covered with countless tiny projections, called the villi, that allow for a much larger absorption surface. Microvilli then form the "brush-border" and increase even more the absorption area. There are also small intestinal glands between the villi bases, the crypts, essential for digestion and protection of the intestine. The large intestine mucosa, on the other hand, does not have any vill.[7,13,17] Figure 2 is a simplified illustration of a cross-section of the intestinal wall.

When ingested, food is usually too complex to be digested right away.[18] It is exposed to several mechanisms, such as movements and secretions, throughout the digestive tract. Mastication

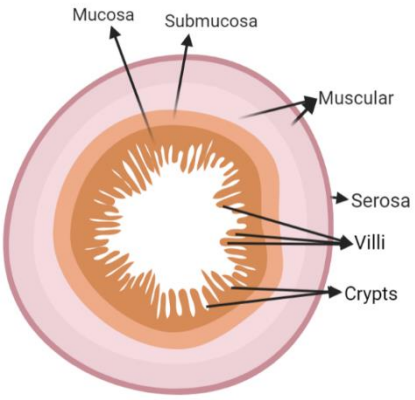

Figure 2- Cross section of the intestinal wall (original figure created with Biorender.com)

(chewing) and deglutition (swallowing) are the first steps in the digestion process and occur in the upper part of the GI tract, specifically in the mouth, pharynx and esophagus. Chewing is limited to the mouth and consists of preparing food for swallowing by breaking it down into smaller particles and mixing it with saliva, a secretion produced by salivary glands that lubricates food and allows for formations of easier to swallow boluses.[16,19,20] Swallowing involves a voluntary

phase in the mouth and an involuntary one, first in the pharynx and then in the esophagus.[20] The esophagus is also responsible for peristalsis - propulsive movements that take the ingesta from the pharynx to the stomach. Notwithstanding, these movements (peristalsis) take place in all parts of the GI tract.[14]

Further into the tract, the stomach has the role of storing food and processing it into a fluid mixture, also known as the *chyme*, by mixing it with gastric secretions.[20] The stomach's lumen epithelium is covered in mucous cells and glands that secrete hydrochloric acid (HCl), pepsinogen, intrinsic factor and mucus.[16,21] Peristaltic waves are also present and propel food, grind it and mix it until small enough and with the right consistency to be released into the duodenum.[14]

Once in the small intestine, slow-wave, segmental and peristaltic contractions serve three main functions: mixing the ingesta with digestive enzymes and other secretions, moving the intestinal contents to facilitate contact with the intestinal mucosa and propulsion of the intestinal content into the next part.[8] The intestinal epithelium is specialized for membrane brush-border digestion, fluid and electrolyte secretion, and absorption. The crypt is the germinal area of the epithelium where stem cells differentiate into crypt or villus epithelia[8,22] The secretions that act in the intestine are bile, pancreatic juice and mucus, which has almost no enzymes and is secreted by glands in the intestinal mucosa mainly to protect the wall against the highly acid juice from the stomach.[21] It's in the small intestine that the digestion of main dietary constituents happens: carbohydrates, proteins and triglycerides are hydrolyzed into smaller molecules to be absorbed through the mucosa. This digestion occurs thanks to the bile salt emulsification and hydrolysis by the pancreatic enzymes; brush border enzymes also perform terminal hydrolysis of proteins and carbohydrates.[22]

In addition to being essential in digestion and body fluid homeostasis, the small intestine is also the largest immunological organ in the body, protecting the body from environmental threats.[22,23] Its microbiome, formed by bacteria, protozoa, viruses and fungi, is critical for the proper function of nutritional, developmental and immunological processes in the body, mainly helping keep the balance of the mucosal immune system and preventing invasion/colonization by pathogens.[22,24] It is essential in maintaining the health and well-being of the dog.[25]

The colon's main functions are water and electrolytes absorption (proximal colon), storage and coordinated evacuation of feces (distal colon). It also performs mucus production, microbial fermentation, immune surveillance and motility.[26]

To carry out its functions, the gastrointestinal system is regulated by two control systems: intrinsic and extrinsic.[12] The intrinsic regulation is made by the enteric nervous system, the GI tract's nervous system, composed of the myenteric plexus, responsible for controlling the gastrointestinal movements, and the submucosal plexus, which manages secretion and local blood flow.[11] Furthermore, intrinsic regulation is also performed by the GI hormones gastrin, gastric inhibitory peptide (GIP), cholecystokinin (CCK), secretin and motilin. Extrinsic regulation is performed by the vagus and splanchnic nerves and the hormone aldosterone.[12]

1.2- Approach to the patient with acute gastroenteritis

1.2-1. History and physical examination

When we think of vomiting and diarrhea, we immediately think of gastroenteritis. Typically, the reason for the vet visit is either one of these signs or both. These are, however, very unspecific and can be associated with a large variety of disorders, not only gastrointestinal.[7] Therefore, performing a complete investigation is essential to reach the correct diagnosis, starting with the most critical steps in any medical workup: the history and the physical examination. [27]

The first step in these patients' approach should be to rule out any extra-GI causes.[27] A thorough history is essential to identify the underlying cause and reach an accurate diagnosis. The assessment of the history should be methodical, making sure no vital question is forgotten.[28] It is important to note that specific questions should be asked to identify sings that are unrelated to primary GI disorders.[27] Table 1 features the most important subjects to cover with the owner. This assessment alone often includes the information necessary to make a presumptive diagnosis or help rank differential diagnoses.[6]

Table 1 – History assessment and questions to ask the owner.[1, 21]

Parameter	Questions
Signalment	Age, breed, sex
Places visited	Visits to dog parks, lakes, or ponds
Prior ownership	Since we are referring to a young dog, it is important to know information about the breeder or the shelter from which the patient was adopted, or any previous owner
Current household	Indoor or outdoor pet; rural or urban environment; access to toxics; unusual foods, foreign objects or drugs; exposure to other animals; health status of other animals and people in the household
Diet	Type of diet (raw food, homemade, commercial diet) and whether there have been any recent dietary changes
Vaccination and deworming status	What vaccines has the pet received and when; history of worm infections, treatments, and prophylactic measures
Prior medical problems	If there were any: what was the problem, the treatment, and the outcome
Present status	Appetite, defecation patterns and characteristics (consistency, large or small quantities, presence of blood/mucus), activity level and overall attitude, drinking and urination patterns
Current condition	A chronological description of the symptoms (from the last time the patient was normal); disease onset (acute or chronic*): clinical signs, progression, severity and duration

**chronic = duration > 14 days*

After a detailed history collection, the next step in the diagnostic approach is the physical examination.[27,29] Even though gastroenteritis cases don't usually present any pathognomonic findings in the physical examination, performing a complete one is an essential practice that must

not be overlooked.[2,27,30] Indeed, it can show signs or abnormalities that suggest that the problem may be related to other issues other than just gastrointestinal.[2]

When examining these patients, the most critical steps are assessing their hydration status by checking skin turgor, moistness of mucous membranes and capillary refill time, and abdominal palpation. A dehydrated patient will have dry mucous membranes, a prolonged capillary refill time and poor skin turgor.[2,31] It can also have enophthalmos.[6] Depending on the level of dehydration, patients will have less or more pronounced alterations (5% will show minor alterations, whereas 10 or 12% will show extreme alterations). Abdominal palpation may reveal discomfort and pain, effusion, gas distension, or organomegaly.[32] Apart from this, it is also essential to check the patient's peripheral pulses, heart rate, temperature. Weak or absent pulses, tachycardia and cool extremities are consistent with hypovolemia and shock.[2,6,7] Respiratory signs like tachypnea, dyspnea, or coughing can be indicative of a systemic condition or complications of vomiting like aspiration pneumonia, and therefore should be noted as well.[33]

Since the majority of the cases of acute vomiting and diarrhea are due to simple dietary indiscretions that do not require extensive investigations or treatments, after the conclusion of the exam, the findings should be analyzed to decide whether the situation requires further investigation or not (Figure 3).[5]

If the physical exam shows a patient that's apparently systemically well and history assessment was unremarkable, usually symptomatic/supportive treatment is enough. However, if there are abnormalities on either history or physical exam, or if it is a reoccurring or refractory case, a list of the findings and correspondent differential diagnosis should be made to choose and prioritize the right diagnostic tests.[5,34,35]

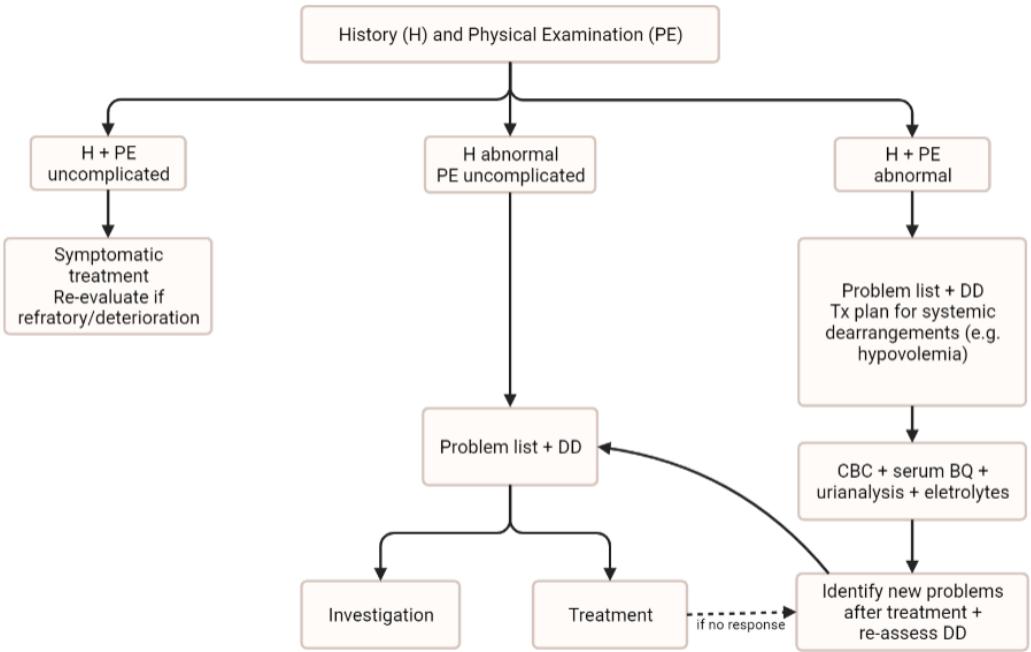

Figure 3 - Decision making in cases of acute gastroenteritis (original figure inspired by reference 5)[5]

1.2-2. Diagnostic tests

After concluding that there is a need to pursue further investigation based on history, physical exam and clinical experience, there are several diagnostic tests that should be performed, depending on the clinical suspicion, to narrow the differential diagnosis list.[6]

Testing should include a complete blood count (CBC), serum biochemistry panel (e.g. liver and renal function panels, serum glucose and albumin) and urinalysis.[5,6,35] No specific alterations in CBC and basic biochemistry will be pathognomonic to GI tract disease, and results will often be unremarkable. However, they can rule out extra GI causes like most endocrine, hepatic, and renal diseases.[27] Urinalysis will also be helpful in ruling out renal disease and assessing hydration status.[9]

A fecal examination is also of very high importance, especially in puppies and young dogs. The major causes of vomiting and diarrhea are infectious or parasitic, apart from dietary causes.[1,36] Fecal samples can be analyzed for parasites, bacteria, or viruses.[5] The available tests for parasites are microscopic parasite or protozoa identification and egg count through techniques like fecal smear, sedimentation and flotation, antigen testing, or fecal polymerase chain reaction (PCR).[37,38] These can identify parasites like helminths (e.g., *Toxocara canis*, *Uncinaria stenocephala*, *Trichuris vulpis*) and protozoa like *Giardia* or *Cryptosporidium*. Successful identification of parasites depends on the quantity and quality (freshness) of the feces, and a negative test doesn't exclude the possibility of a parasite infection.[38] Bacteria can be detected by bacterial culture of feces (*Salmonella* and *Campylobacter*), fecal antigen enzyme-linked immunosorbent assay (ELISA) (*Clostridium difficile* toxins A and B and C. *perfringens* enterotoxin), and PCR.[5,38] However, most of these enteropathogens are commensal in the GI tract and have been identified in fecal samples of healthy dogs at similar frequencies as sick ones, which lowers the diagnostic value of these tests.[39] ELISA and PCR are also used to identify *Parvovirus*, *Coronavirus*, and Distemper virus (Morbilivirus).[5]

Imaging, specifically abdominal radiographs and ultrasonography, can also be useful for the detection of several issues such as foreign bodies, mechanical obstructions, gastric dilation, and volvulus.[5,32,35] Ultrasonography is the imaging exam of choice when evaluating the GI tract, as it shows real-time images and can be used to evaluate motility. However, it requires advanced knowledge and experience to obtain a good quality image and interpretation.[40]

Endoscopy, exploratory laparotomy and laparoscopy can also be used but should only be considered when other less invasive options have been exhausted, or findings indicate them (e.g., foreign bodies).[5] They allow for mucosal observation and biopsy when needed.[7]

1.2-3. Treatment

After ruling out any extra-GI disease, cases of primary acute gastroenteritis typically respond well to supportive treatment. The aggressiveness of the treatment depends on the underlying cause and the severity of clinical signs, but these are the main options (Table 2 has a simple summary of the drugs mentioned below):[2,7,41]

Antiemetic drugs can be used to improve patient comfort and decrease the amount of fluid and electrolyte losses, they may allow for earlier enteral nutrition as well; they should not be used in cases of suspected or confirmed foreign body; this group includes drugs like maropitant, ondansetron/dolasetron, and metoclopramide.[2,7]

GI-protectants like sucralfate in cases of mucosal ulceration or erosion, famotidine/ranitidine, and omeprazole.[2]

Probiotics have been proven to be beneficial in reducing the duration of diarrhea.[42] Anti-diarrheal drugs such as Loperamide are not advised in cases of acute gastroenteritis since the diarrhea is mostly self-limiting, and there is a considerable risk of intoxication.[2]

Antimicrobial therapy should only be used in dogs with systemic signs (*e.g.,* depression, pyrexia) or who are suspected of having bacterial translocation and being in danger of developing sepsis. Although empirical use is common, it is not advised, nor does it bring any benefits.[43] Examples of antibiotics commonly used in gastroenteritis cases are metronidazole and penicillins (amoxicillin often associated with clavulanic acid).[44,45]

Nutritional management is of high importance in the management of acute gastroenteritis. Fasting may be needed in the initial period when the animal is still vomiting frequently; however, once vomiting is controlled, a return to oral intake of food is advised, except in cases of foreign bodies. A commercially available and highly digestible diet is recommended.[2]

Fluid therapy in cases of dehydration, hypovolemia, and electrolyte imbalances. This can be subcutaneous, in cases of financial concerns; oral, which may be used in cases of mild dehydration and has been proven to be beneficial and safe[46]; and intravenous, which is recommended to achieve normovolemia in cases of moderate to severe dehydration and hypovolemia.[1,2]

Table 2 - Summary of some examples of drugs used in the treatment of acute gastroenteritis.[41]

Group	Drug	Dosage	Comments
Antiemetics	Maropitant	1 mg/kg q24h SC/IV **or** 2 mg/kg q24h PO	Injection irritant
	Ondansetron	0.5-1 mg/kg q12-24h IV/PO	
	Dolasetron	0.6 to 1 mg/kg q12h PO, IV	Not extensively used in veterinary medicine
	Metoclopramide	0.25-0.5 mg/kg q12h PO/SC/IM/IV **or** CRI 1-2 mg/kg IV over 24h	Do not use in GI obstruction, perforation, or hemorrhage. Half the dose in dogs with impaired liver or kidney function
Gastroprotectants	Sucralfate	500mg-2g/dog q6-8h PO	
	Ranitidine	2 mg/kg q8–12h slow IV/SC/PO	Hypotension if administered rapidly IV
	Famotidine	0.5–1.0 mg/kg q12–24h PO	Good safety profile
	Omeprazole	0.5–1.5 mg/kg q12–24h IV/PO	Possible adverse effects include nausea, diarrhea, constipation, skin rashes and tooth fractures
Antimicrobial drugs	Metronidazole	10–15 mg/kg q12h PO/SC/slow IV infusion	Reduce the dose in cases of hepatic disease CNS toxicity
	Amoxicillin+Clavulanic Acid	8.75–25 mg/kg q8h IV **or** q24h IM/SC **or** 12.5–25 mg/kg q8–12h PO	Possible adverse effects include nausea, diarrhea and skin rashes

1.2-4. Differential diagnosis for acute gastroenteritis

Acute gastroenteritis cases in companion animals are often unspecific and self-limiting. Most times, the underlying causes are not identified. After ruling out any extra-GI causes like pancreatitis, hepatic, renal, or endocrine disease, and foreign bodies or other obstruction causes, gastroenteritis' etiology may be infectious, dietary, drugs or toxins, or an acute hemorrhagic diarrhea syndrome (AHDS).[1,7]

1.2-4.1. Infectious causes

Several agents can affect the gastrointestinal tract and cause acute gastroenteritis: viruses, bacteria, protozoa, parasites, and fungi.[7]

Canine *parvovirus* type-2 (CPV-2) is one of the most common viral diseases in dogs and is fully assessed further in this review. It is the most prevalent gastrointestinal virus, and it is highly contagious and life-threatening.[47] Canine c*oronavirus* and r*otavirus* are two other viruses known for causing acute gastroenteritis in dogs.[5] Typical clinical gastrointestinal manifestations of these viruses tend to be milder than CPV infections, which can probably be explained by the fact that these affect the epithelial cells on the villi while CPV infects the crypts.[7,47]

Canine *coronavirus* (CCoV) is a positive-sense RNA virus that is part of the family *Coronaviridae*, which contains different *coronavirus* that can infect multiple species like humans, cattle, cats, dogs, and so on.[47] CCoV infections are generally associated with mild and self-limiting enteritis with low mortality and high morbidity, particularly in puppies[48–50]. However, reports have found a pantropic strain of CCoV which was linked to a fatal systemic enteric disease that resembled CPV infections.[51–54] It is, thus, considered a somewhat significant pathogen in dog populations.[55] Transmission is oronasal, and the incubation period ranges from one to four days. The pathogenesis of CCoV infection is similar to other enteric pathogens: it replicates in the intestinal villi's epithelial cells, leading to villous atrophy resulting in malabsorption and diarrhea. Unlike CPV, it rarely causes villous necrosis and hemorrhage.[47,56]

The *rotavirus* is a less prevalent virus in dogs, capable of causing mild and self-limiting signs of gastroenteritis in puppies younger than three months. It is part of the family *Reoviridae* and is a double-stranded RNA and non-enveloped virus. Rotaviruses are recognized as enteric pathogens in many animal species and humans.[47,57,58] Like the previous viruses, transmission is oronasal through contact with contaminated feces. The incubation period ranges from 16 to 24 hours, and shedding lasts from two to 10 days. The clinical signs are similar to the other enteric viruses as well, being anorexia, vomiting and diarrhea that can occasionally be bloody.[57]

A fourth virus that can cause gastrointestinal signs is the canine distemper virus (CDV). This virus causes acute to subacute systemic disease with high mortality rates in dogs and other carnivores.[57] It is an enveloped, single-stranded RNA virus that belongs to the family *Paramyxoviridae* and genus *Morbilivirus*. The transmission is oronasal, and the virus starts replicating in lymphoid tissue right away, resulting in severe immunosuppression. The incubation period can range from one to four weeks or more and, in cases of weak immune response, by six to nine days after infection, CDV spreads to the epithelial cells of most organs by cell-mediated viremia. The intestinal signs, combined with respiratory and dermatological signs, can occur ten days after infection due to the epithelial localization of the virus. When severe systemic signs appear, the mortality rate is very high.[57] After 20 days, neurological signs start to appear.[59] Given the multisystemic nature of this infection, diagnosing CDV based solely on clinical signs can be

challenging. This variety of signs does, however, differentiate CDV infection from another viral enteritis, and definitive diagnosis can be achieved by molecular assays like PCR.[59,60]

Moreover, there have been reports of isolation of a few other viruses from feces of dogs with diarrhea that include *Norovirus*[61,62]*, Astrovirus*[63]*, Sapovirus*[62,64]*, Kobuvirus*[65]*,* and *Circovirus*[66–68], among others. Since there are only a small amount of reports for each virus, further studies are required to assess the real pathogenic potential of these agents.[69]

The etiology of acute, nonviral infectious gastroenteritis is not very well understood in dogs.[70] Bacterial gastroenteritis can be caused by enteropathogens like *Clostridium difficile*, *Clostridium perfingens*, *Salmonella spp*, *Campylobacter jejuni,* and *Escherichia coli*.[43]

Clostridium difficile is a fastidious, gram-positive, spore-forming anaerobic bacillus that has been linked to enteric disease and has major clinical importance in humans.[71] Apart from a few studies documenting the presence of C. *difficile* in dogs with diarrhea, there's little knowledge about its pathogenicity in puppies and adult dogs.[39,72–74] C. *difficile* strains produce up to 5 toxins, but only 2 of these toxins - toxin A (TcdA) and B(TcdB) - have been studied thoroughly. Toxin A is an enterotoxin, and both of them have cytotoxic activity.[74] Clinical signs associated with C. *difficile* infection (CDI) in dogs vary from subclinical to potentially fatal acute hemorrhagic disease, and infected dogs often show signs of both small and large intestine involvement.[39,75] The gold standard assay for its diagnosis is the cell culture cytotoxicity assay (CTA) that detects TcdB activity in feces, but it is costly and time-consuming and thus not readily available.[76] Instead, it is recommended to use a combination of toxin testing by ELISA (that detects both TcdA and TcdB) and organism detection by culture, antigen ELISA, or RT-PCR in the diagnosis of CDI in dogs.[43]

Clostridium perfingens, on the other hand, is a broadly ubiquitous and highly diverse, gram-positive anaerobic bacillus. It is one of the most widespread pathogens, and it is part of the normal microbiota of GI tracts of animals and humans.[43] There are five biotypes of C. *perfingens*: A to E. This division is based on the presence or absence of major toxin genes (α, β, ε, and ι).[72] There are about ten other toxin genes that biotypes can express[77], including C. *perfingens* enterotoxin (CPE) and beta 2 (β2) toxin, which are the ones that gather the most information about their potential role in disease.[43,72] Enterotoxigenic C. *perfingens* type A has been associated with canine acute and chronic small and large intestine diarrhea, AHDS, and human food poisoning.[39,75,78–80] It is the most prevalent type to have the enterotoxin gene (*cpe*).[81] Nonetheless, the pathogenesis of C. *perfingens*-associated diarrhea is not fully understood. The prevalence of C. *perfingens* in healthy and diarrheic dogs is similar (80%), although detection of CPE is more common in the latter.[74,75] This could be explained by the presence of β2 toxin, but its role in disease is not well known either.[43] Clinical signs are nonspecific and can vary in severity, ranging from mild and self-limiting diarrhea to life-threatening and hemorrhagic.[39] Diagnosis of C. *perfingens*-associated diarrhea would ideally be made by detection of CPE using ELISA accompanied by a PCR to detect enterotoxigenic strains.[75]

Salmonella spp. are also pathogens for dogs but mainly cause subclinical infections, and their pathogenesis raises many questions still. They belong to the family *Enterobacteriaceae*, have numerous serovars, and are gram-negative, facultative anaerobic, motile, and spore-forming.[43,82] The prevalence of this pathogen appears to be similar in healthy and diarrheic dogs (0 – 3,6%).[39,83–85] However, the prevalence is much higher in dogs fed with raw food diets (about 30%).[86] Clinical signs most often have an acute onset, with fever, lethargy, and anorexia followed by vomiting, abdominal pain, and diarrhea. The diarrhea is usually mucoid and watery but can also be hemorrhagic in very severe cases. There can be clinical signs of sepsis.[43] However, these are highly variable, and thus salmonellosis should be suspected in cases of both acute and chronic gastroenteritis in dogs.[82] Additionally, it highly depends on host immunity status. Most dogs that shed *Salmonella* don't even have any clinical signs, and it represents a disease of very high zoonotic importance since almost all *Salmonella* serotypes can infect both humans and animals.[43,72] Diagnosis of canine salmonellosis is typically acquired by isolation of the organism by culture or PCR in conjunction with clinical signs and risk factors.[43]

Campylobacter spp. are gram-negative, motile bacillus. There are many species of *Campylobacter,* but many of them are thought to be non-pathogenic. *C.jejuni* is the most prevalent pathogenic species.[43] No correlation has been found between the presence of C.*jejuni* in the feces and disease in most previous studies[87,88], except for one that recorded two times the prevalence rate of this pathogen in the feces of young dogs with diarrhea than in those of healthy dogs.[89] In many cases, dogs will be healthy carriers of this pathogen, and when clinical signs are present, they can vary from mild and self-limiting to watery or mucoid bloody diarrhea associated with anorexia, vomiting and fever.[43]

Escherichia coli are gram-negative bacillus that belong to the family *Enterobacteriaceae* and are part of the normal intestinal microflora.[43] There are seven pathotypes that include enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (verotoxigenic - EHEC), necrotoxigenic (NTEC), enteroinvasive (EIEC), adherent-invasive (AIEC), and enteroaggregative (EAEC). The role of these strains is poorly defined, and many of them have been isolated from healthy dogs. However, in the presence of virulent factors and impaired immunity, they can be associated with gastroenteritis, as have been in several studies.[70,90–92] AIEC strain has been associated with granulomatous colitis in Boxers, but this condition has a more chronic presentation.[43,72] The diagnosis of E.*coli* infection is made mainly by isolation of the pathogen and detection of the pathological genes.[72]

Fungal infections may also cause GI signs. Histoplasmosis is the most common fungal pathogen to cause signs of gastroenteritis in dogs and may provoke protein-losing enteropathies. Driven by the pathogen *Histoplasma capsulatum,* it also provokes pyrexia, anorexia, lethargy, and weight loss in affected dogs. *Pythium* spp. can also cause a similar disease but often presents with a palpable abdominal mass and grants a worst prognosis.[7,26]

Lastly, parasitic infections represent another cause of gastroenteritis in dogs. Most dogs only present mild clinical signs, but severe infections can cause significant inflammation of the gastrointestinal tract, with consequent vomiting and diarrhea. This is especially common with severe hookworm (*Ancylostoma caninum, Uncinaria stenocephala*) infestations.[7] Additionally, they may predispose to other diseases, which is why regular anthelminthic treatment is essential for the health of young animals. Several anthelmintics are available, including fenbendazole, mebendazole, milbemycin oxime, selamectin, pyrantel, and many others.[93] These parasites can be hookworms (mentioned above), roundworms (ascarids – *Toxocara canis*, *Toxascaris leonine*), whipworms (*Trichuris vulpis*), and tapeworms (e.g., *Dipylidium caninum*). Hookworms, specifically A. *caninum*, can be responsible for acute anemia, bloody diarrhea and death in small puppies. Roundworms are very common amongst dogs younger than one year-old and can cause an intussusception or intestinal blockage in large infestations.[93,94] The diagnosis is based on the identification of eggs in feces through floatation techniques.[94]

Protozoa are also parasites that can cause gastroenteritis. Amongst them are *Giardia, Isospora* and *Cryptosporidium.* These are typically considered minor pathogens in dogs, but *Giardia* can be clinically significant.[93,95]

1.2-4.2. Dietary-related

Dietary problems are probably the most common causes of acute vomiting and diarrhea. These may be due to food indiscretions, intolerances, food poisoning or hypersensitivity. Food indiscretion problems are often related to scavenging and eating foreign materials or garbage, which are very common in dogs. These may lead to GI trauma or osmotic diarrhea because of the presence of indigestible substances in the GI tract. Garbage ingestion can also expose the mucosa to bacterial toxins, leading to food poisoning from the ingestion of spoiled food.[5,7] Usually, dietary indiscretion leads to acute vomiting, diarrhea and anorexia. The history is of major importance in these cases since the owner might be aware of any exposure, and the diagnosis is presumptive. The prognosis in these cases is excellent, and the dog usually recovers in 24 to 72 hours.[7]

Dietary intolerances and hypersensitivities are adverse reactions to otherwise harmless foods. These include food allergies/hypersensitivity (with an immunologic basis) and food intolerances (with a non-immunologic basis).[96] Food allergies are aberrant immune responses to commonly tolerated foods, and they can be IgE-mediated, cell-mediated, or mixed. IgE-mediated responses are said to be the most common and dangerous type of food reaction.[96,97] In patients with mainly gastrointestinal signs, food allergies can be easily mistaken for food intolerance. However, the first are generally associated with cutaneous disease, contrarily to the latter. For the diagnosis, the elimination of all possible differentials is mandatory. The reduction of clinical signs after a food

elimination test and reoccurrence when the patient is fed the previous food is the gold standard test to diagnose food allergies.[96]

Rapid dietary changes are also a very prevalent cause of acute signs of gastroenteritis, especially in puppies.[5]

1.2-4.3. Toxin or drug-related

There's a variety of toxins (e.g., insecticides, pesticides) that, when ingested, can provoke signs like vomiting and diarrhea in dogs.[5] In addition, various drugs may induce gastrointestinal alterations. Examples of these are non-steroidal anti-inflammatory drugs (NSAIDs) and chemotherapeutics.[6]

NSAIDs can cause anorexia, nausea, vomiting, abdominal pain, and diarrhea - signs compatible with gastrointestinal erosion and ulceration, which are common after administration of these drugs, especially when administered incorrectly. GI perforation and peritonitis are the most severe signs. The toxic dose of NSAIDs varies a lot between agents. It is influenced by concurrent use of NSAIDs and other medications (such as corticosteroids) or underlying diseases (especially hepatic, GI or renal).[98]

Chemotherapeutics are usually well tolerated by dogs but can sometimes cause GI side effects like vomiting, diarrhea, and decreased appetite. A few reasons may be in the origin of these effects: the chemotherapy drug may give a direct stimulatory effect to the CNS vomiting center or chemoreceptor trigger zone (CRTZ), which will result in vomiting during or right after treatment; or it could provoke gastric inflammation and damage, which can induce secondary effects three to five days post-treatment.[99]

1.2-4.4. Acute Hemorrhagic Diarrhea Syndrome

Formerly known as hemorrhagic gastroenteritis or HGE, AHDS is an idiopathic disease that provokes the acute onset of vomiting that progresses to hematemesis, anorexia, lethargy, and bloody diarrhea.[94,100,101] A raised intestinal permeability results in blood, fluid, and protein losses in otherwise healthy dogs. This occurs in young to middle-aged small breed dogs (Yorkshire terrier, Maltese, Miniature Pinscher, and Miniature Schnauzer), especially in the winter.[101] Different possible etiologies have been discussed for this syndrome, including type 1 hypersensitivity reactions to food or bacterial endotoxins, enterotoxigenic *Clostridium* strains (C. *perfingens* enterotoxin and C. *difficile* toxins A and B), and more recently a pore-forming netF toxin produced by C. *perfingens*, which was mentioned in a study by Sindern *et al.*(2019)[102] as the cause for the necrotizing lesions in the intestines of many dogs with AHDS.[94] This syndrome is a diagnosis of exclusion, which means other possible differentials must be ruled out through history assessment (e.g., intoxication, alterations in diet, NSAIDs administration), physical

examination, CBC (e.g., parvoviral infection – dogs with AHDS typically have a marked hemoconcentration (PCV > 60%) and don't have leucopenia), serum biochemistry (e.g., pancreatitis, hepatic diseases, endocrine diseases), coagulation times, diagnostic imaging (e.g., foreign objects, intussusception, neoplasia), and fecal examination (e.g., parasitism).[101]

1.3- Leucopenia and gastroenteritis

The blood leucocytes are essential components of the innate and adaptive immune systems. Originally produced in the bone marrow from hematopoietic stem cells, their total count can be affected by infections, inflammation, autoimmune diseases, parasitic infestations, tissue lesions, and hormones.[103]

To evaluate the number of circulating leucocytes in the blood, clinicians perform a leucogram or total white blood cell count (WBC), part of the complete blood cell count. In healthy dogs, the WBC count can vary between 5000 and 14100 cells/µL. In puppies, however, this count is usually higher.[104] Apart from quantifying the total number of leucocytes, the leucogram also includes a differential WBC count, which includes the different types of leucocytes.[104,105] Typically, neutrophils are the most common white blood cells in circulation, followed by lymphocytes. Monocytes and eosinophils are seen less frequently, and basophils are very rarely seen. Consequently, leucopenia (low leucocyte count) is mostly associated with a decreased neutrophil count, called neutropenia.[103,106]

A variety of etiologies can be in the source of neutropenia, yet they're all related to increased use or decreased production of neutrophils. It can also be caused by immune-mediated responses that can lead to neutrophil destruction.[107]

To understand the mechanisms of neutropenia, one must be familiar with neutrophil production. Neutrophils are produced through granulopoiesis, a complex process that consists of the differentiation and maturation of pluripotent hematopoietic stem cells (HSC) into a series of more differentiated and committed cells (progenitor cells (multipotential, common myeloid and granulocyte), myeloblasts, progranulocytes, myelocytes, band neutrophils and segmented neutrophils). This process is regulated by inflammatory mediators such as transcription factors (PU.1, C/EBP-α, β and ε) and cytokines: stem cell factor (SCF), interleukine-3 (IL-3) and interleukine-6 (IL-6), granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF), among others.[108,109] Part of the mature neutrophils stay in the bone marrow storage pool, and the rest get released into the blood circulation.[107] This release is regulated by GM-CDF, G-CSF, tumor necrosis factors α and β (TNFα/β), complement 5a (C5a) and interleukine-1 (IL-1).[103,108,110]

The neutrophils that get released have two different destinations: the marginal neutrophil pool (MNP), where they adhere to the vascular endothelium, and the circulating neutrophil pool (CNP),

where they, as the name suggests, circulate through the blood. These last ones are the ones that are counted in the leucogram.[104] Figure 4 is a simple illustration of this process.

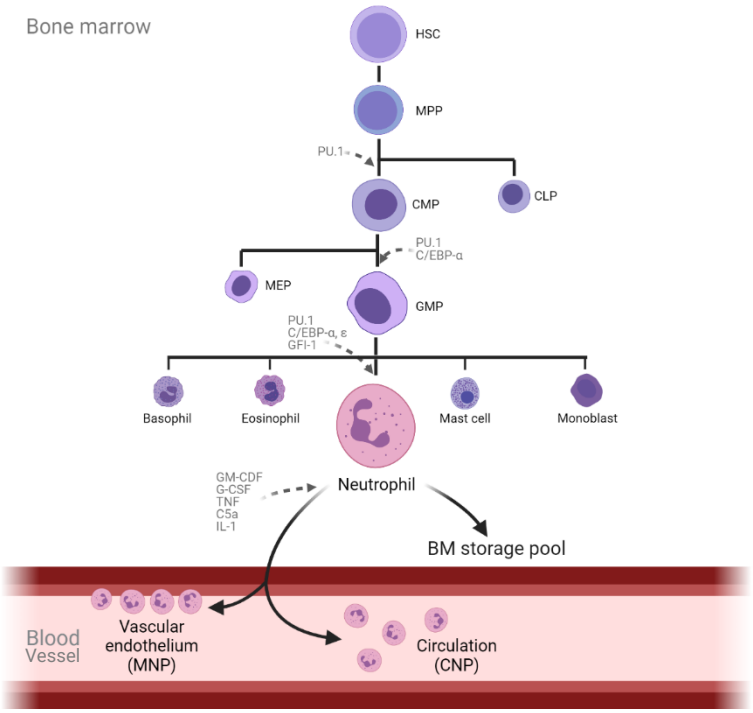

Figure 4- Neutrophil production. HSC- hematopoietic stem cells; MPP- multipotential progenitors; CMP- common myeloid progenitors; CLP- common lymphoid progenitors; GMP- granulocyte/monocyte progenitor; MEP- megakaryocyte/erythroid progenitor.
GMPs undergo multiple maturation processes and turn into myeloblasts, promyelocytes (progranulocytes), myelocytes and band neutrophils before they become mature segmented neutrophils. These processes are regulated mainly by G-CSF.[108]
(Original figure created with BioRender.com)

Leucopenia can also be caused by lymphopenia, although much less commonly.[103]

Despite seldom providing a definitive diagnosis alone, the leucogram is very useful in limiting the differential diagnosis list and predicting disease prognosis.[104] This is particularly true in young dogs with history and clinical signs compatible with acute gastroenteritis, as very few primary GI disorders cause leucopenia. In fact, in these cases, leucopenia is only usually associated with viral infections (parvovirus and less frequently coronavirus), ehrlichiosis, some toxins and sepsis (which can be caused by any of these infections, plus severe parasitic infestation as well).[103]

The presence of leucopenia (and neutropenia) is generally associated with parvoviral infection, as documented in a study by Brown *et al*.(2001)[111], where neutropenia was related to parvovirus in 123 out of 124 dogs.

1.4- Parvoviral infection as the main cause of acute gastroenteritis with associated leucopenia

1.4-1. The canine parvovirus type 2

Canine *parvovirus* (CPV) represents one of the most common causes of morbidity and mortality in puppies and juvenile dogs.[112,113] In dogs with infectious diarrhea, it's the most prevalent virus.[47,114–118]

The canine parvoviral disease, caused by CPV-2, was first reported as a new disease in 1978 and rapidly evolved into a pandemic that reached the entire world in just six months, having since become endemic globally.[119,120] There was a canine *parvovirus* disease described earlier that was caused by canine parvovirus 1 (CPV-1), or Minute Virus of Canines (MVC) from the genus *Bocavirus*, but it has significant genetic differences and is thought to have much lower pathogenic potencial.[119,121]

CPV-2 is a single-stranded DNA virus that is part of the family *Parvoviridae*, subfamily *Parvovirinae* and genus *Protoparvovirus*. The virion is nonenveloped, small, and has developed three main antigenic variants: CPV-2a and 2b in the early 1980s and CPV-2c in the 2000s.[119,122–125] These variants don't, however, seem to vary much in pathogenicity.[122] The virus is ubiquitous and, as is the case with all parvoviruses, incredibly stable, surviving in the environment for over a year.[119,126] It is also resistant to extremes of heat and pH.[119]

Its capsid is icosahedral and contains viral protein 1 (VP-1) and VP-2, and that's what makes the virus capable of binding to the host cell transferrin receptor (TfR), which is how it infects cells.[127,128] The VP-2, the main capsid protein (54 copies versus six copies of VP-1), is also the major antigenic protein and the one that defines viral tropism and host range.[129,130]

Parvoviral enteritis typically occurs in puppies younger than six months but can also cause severe disease in adults with insufficient immunity.[121,123,131] Natural infection by CPV-2 has also been reported in other *Canidae* members such as wolves and coyotes, and its variants may infect felids under experimental or natural conditions as well.[47,132]

Despite occurring mostly because of oronasal exposure to contaminated feces in the environment (fecal-oral route), the infection can also be caused by insects and rodents that serve as mechanical vectors for the virus, or fomites, that are an important mode of transmission too.[121,133] While very rare, transplacental infection has also been reported.[134] Infected animals shed parvovirus through their feces, urine, saliva, vomitus and nasal secretions.[133,135]

As mentioned before, infection happens by binding of the virus to the TfR on the host cell's membrane.[128,129] After binding, virions enter the cell by endocytosis and travel to the nucleus, where viral genome replication occurs. CPV-2 requires a mitotically active cell in the S phase to replicate. It needs to hijack elements of the host cell's DNA replication machinery, such as DNA polymerase (that is expressed in the S phase), to complete its own replication.[136,137] This might

explain why parvoviral infections are usually much more severe in puppies and young dogs than in adults since there are fewer dividing cells in the latter.[137]

The incubation period lies typically between four to fourteen days in the CPV-2 strains (2a, 2b and 2c), but there are several cases when the dogs never develop clinical signs and thus have a subclinical infection, especially if there are still maternally derived antibodies (MDA) in circulation.[123,138] These don't, however, fully protect puppies against parvoviral infection, as reported in a study by Decaro *et al.* (2005)[138], contradicting what was previously believed.

1.4-2. Pathogenesis

As stated earlier, CPV-2 targets active and rapidly multiplying cells to replicate in and successfully cause infection. This is a key factor in this virus' pathogenesis.[127,137]

It all begins with oral or oronasal infection. After 18 to 24 hours, the virus replicates in the lymphoid tissue of the oropharynx, thymus, and mesenteric lymph nodes. This replication causes tissue necrosis. One to five days after infection, the virus is spread systemically through viremia, causing damage to fast-dividing cells in the gastrointestinal tract, lymph nodes and bone marrow.[47,133,139] It has also been isolated from the lungs, spleen, kidneys, liver, brain and myocardium.[121,140,141]

An infected patient sheds the virus for a few days before the onset of clinical signs, and this shedding usually declines after about seven days.[121]

In the GI tract, the affected cells include the epithelium of the tongue, oral and esophageal mucosa, and the small intestine, specifically the germinal epithelial cells of the intestinal crypts within the jejunum and ileum, which are the primary site of replication.[47,121,135] This causes epithelium destruction and villous collapse.[119,140] Consequently, normal enterocyte turnover is compromised, and the villi become short and atrophic, which leads to malabsorption and increased permeability, and causes severe enteritis (Figure 5).[47,133,135,140] There is an intensified risk for secondary bacterial infections by gram-negative and anaerobic microflora, which can lead to additional complications due to bacterial translocation, like bacteremia, endotoxemia and disseminated intravascular coagulation (DIC).[47,121]

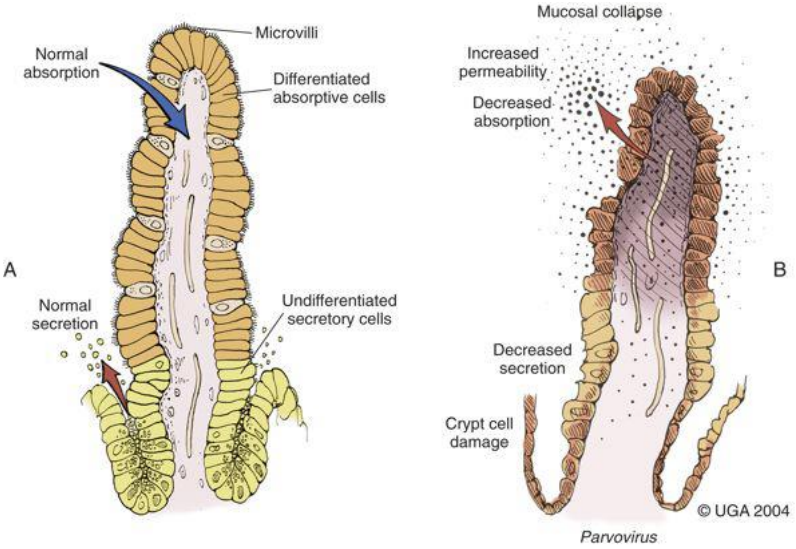

Figure 5 – Normal structure of a healthy intestinal mucosa (A) versus parvovirus-infected intestine showing villus destruction and collapse (B) (Reproduced from Infectious Diseases of the Dog and Cat, 2012)[47]

The infection in the thymus results in the destruction and collapse of the thymic cortex and germinal centers, which, along with the destruction of leucocyte precursors in the bone marrow and lymphoid cells in circulation, leads to severe leucopenia, particularly neutropenia and lymphopenia.[121,133,142] Neutropenia also happens due to the sequestration of neutrophils in damaged gastrointestinal tissue.[121,143]

This leucocyte shortage leads to immunosuppression which, in dogs with bacteremia due to bacterial translocation, can result in septic shock, systemic inflammatory response system (SIRS), multiorgan failure, and death if left untreated.[133] In fact, infected dogs can die as quickly as 24 hours after the onset of clinical signs, especially if they're young.[127] There have been reports of generalized infection in neonatal puppies, with hemorrhage and necrosis in the brain, liver, lungs, lymphoid tissues and GI tract.[144]

Additionally, CPV-2 has also been associated with myocarditis in young puppies (*in utero* infection or up until six weeks of age). This can be explained by the rapid proliferation of myocytes in the first weeks after birth.[47,119,145] The virus will infect the cardiomyocytes and trigger necrotizing myocarditis, which results in damage, inflammation and fibrosis of the myocardium.[145] The most dramatic manifestation of this process is the sudden death of the infected pups. However, it can also cause chronic and progressive cardiac injuries that lead to congestive heart failure and death, months or even years later.[145–147]

The pathogenesis of CPV is affected by several factors that include the dog's age, breed, and immunity status. The route of exposure, viral dose, and strain virulence are of great importance too. These factors influence the severity of the disease and clinical signs in each infected animal.[121,127]

1.4-3. Clinical Findings

As stated before, the virus usually targets three main tissues: the GI tract, bone marrow and myocardium. Still, it can affect the nervous system and skin as well.[47] Secondary infections and thrombosis due to hypercoagulability can also happen and will influence the severity of the disease, as we see on pups with intestinal helminths, protozoa, and enteric bacteria such as *Clostridium perfringens*, *Campylobacter* spp., and *Salmonella* spp.[121,148,149]

The response to infection differs tremendously from case to case, ranging from asymptomatic or subclinical infection to very severe and fatal disease. Many puppies with maternally derived antibodies, as mentioned, have subclinical or unapparent infections since these protect them from disease but not from infection.[47,140]

The most common clinical signs of parvoviral enteritis are vomiting, diarrhea, lethargy and anorexia.[133] The sudden onset of a liquid, foul-smelling, and often bloody diarrhea in puppies is strongly suggestive of parvoviral infection but not diagnostic. In contrast, CPV can also cause soft and mucoid diarrhea.[119,133] Vomiting is typically very severe.[127]

The physical examination typically reveals fever, dehydration, weakness, and abdominal pain.[47,140] Abdominal palpation, apart from discomfort, may occasionally reveal an abdominal mass effect because of intestinal intussusception. Moreover, mucosal paleness and delayed capillary refill are usually observed. Hypothermia, although rare, can be found as well. In more dramatic cases, tachycardia or bradycardia and weak or absent pulse are associated with septic shock.[121] Hypovolemic shock can also occur quickly due to massive gastrointestinal fluid losses and cause these same alterations.[133]

Neurological signs like seizures or tremors can appear due to hypoxia secondary to myocarditis, hypoglycemia, intracranial hemorrhage, or thrombosis, that happen during the disease process, sepsis, or acid-base electrolyte unbalances. These signs are, however, very uncommon, and, in these cases, possible co-infection with CDV should also be considered.[121,150]

Furthermore, there have been two reports of erythema multiforme in dogs with parvoviral infection, in which dogs had generalized cutaneous and mucosal ulcerations and swelling of foot pads, pressure points, mouth and genital mucosa.[151,152] There have been cases of ulcerative glossitis in some infected pups as well.[121]

Tachypnea and increased lung sounds are common in puppies with viral myocarditis - due to congestive heart failure. Nevertheless, the incidence of this form of infection has decreased since neonatal pups are now protected by MDAs because of the widespread vaccination of adult dogs. Myocarditis is mostly found on pups that do not nurse sufficiently or are born from unexposed or unvaccinated bitches.[123,140,153]

Lastly, parvoviral infection has been associated with a predisposition for asymptomatic urinary tract infections, being detected within 25% of infected pups. This may be due to fecal

contamination of the external genitalia, combined with neutropenia. When left untreated, these subclinical infections may lead to chronic urinary infections.[47]

1.4-4. Diagnosis

When a puppy or young dog is presented with the clinical signs described earlier (sudden onset of liquid diarrhea with a fetid smell and vomiting), it is often indicative of parvoviral infection.[121,127] However, it is not diagnostic since these signs can be caused by other agents such as *Coronavirus, Adenovirus, Rotavirus* and others, as mentioned before.[47,154]

Basic clinical laboratory testing should be performed, namely CBC, serum biochemistry, and coagulation tests. In the CBC, the most common findings are leucopenia, neutropenia and lymphopenia, as previously explained.[121] Leucopenia is thought to be proportional to disease severity, although not present in all cases.[155] Anemia can be present due to gastrointestinal blood loss, but it is not a consistent feature of infection. Thrombocytosis and thrombocytopenia can occur as well, although less frequently.[121]

The most frequent findings in the serum biochemistry panel are hypoproteinemia and hypoalbuminemia, secondary to gastrointestinal loss of protein, and hypoglycemia. In fact, in a study by Castro *et al.*(2001)[51], 100% of infected puppies were hypoproteinemic and hypoglycemic.[51] There can be electrolyte imbalances such as hyponatremia, hypokalemia, and hypochloremia as well.[121] Prerenal azotemia can sometimes occur due to severe dehydration and, in some cases, sepsis will cause hyperbilirubinemia and increased alkaline phosphatase.[155]

Coagulation tests may reveal abnormalities that lead to hypercoagulability, namely a prolonged activated partial thromboplastin time (PTT) and prothrombin time (PT), decreased antithrombin activity, and increased fibrinogen concentrations.[156,157]

Additionally, it has been found that the canine pancreatic-specific lipase (cPLI) may be elevated in dogs with CPV infection. It doesn't, however, seem to correlate with disease outcome.[158]

Diagnostic imaging is very nonspecific in cases of CPV infection. Nonetheless, abdominal ultrasonography can help rule out other possible causes of vomiting and diarrhea like gastrointestinal foreign bodies, obstruction, or intussusception.[133] Moreover, it has been reported that the degree of abnormalities detected in abdominal ultrasonography is positively correlated with the severity of the disease.[159]

Suspected clinical cases should be confirmed by laboratory tests. There are various methods available for diagnosing CPV infection, and most of them require antemortem feces or oropharyngeal swabs, or intestinal samples from necropsies.[133] Blood samples can be used to identify viremia in later stages of infection, as it can be long lasting.[123,160]

A widely used assay for CPV detection is the fecal ELISA antigen test. These tests are available in practice for detection in fecal samples, and they can detect all CPV-2 variants.[123,134] They are usually very specific but limited in sensitivity in comparison to other methods. In fact, two studies

reported sensitivities no higher than 60% when compared to molecular assays or electron microscopy. They also confirmed the high specificity previously mentioned (close to 100%).[161,162] On samples containing higher loads of CPV, these tests had a sensitivity of 77% to 80% in a different study.[123] The low sensitivity of these tests can be explained by the relatively short period of fecal shedding in clinical cases: the incubation period of four to six days will complicate early detections, and after seven days, the shedding decreases again. Furthermore, this shedding can be intermittent, and binding of antibodies to CPV antigen in the intestinal lumen will sequestrate the CPV particles and cause false negatives.[157,163]

False positives are rare but may occur four to six days after vaccination with a modified-live CPV vaccine. Still, it would be a weak positive, whereas most dogs with natural infection show strong positive results.[47,164]

Thus, if the clinical suspicion of parvovirus infection persists after a negative ELISA antigen test, the result should be confirmed by PCR methods.[134] PCR can be performed on feces, oropharyngeal swabs, blood, and tissues to detect CPV DNA.[47,133] These assays have higher specificity and sensitivity than conventional methods of viral antigen determination in feces and, although more time consuming and labor-intensive, have been a reliable tool for CPV infection diagnosis.[47,123,134] Real-time PCR (RT-PCR) has a very high sensitivity, allowing viral detection in dogs shedding low titers of virus in the feces, provides an estimation of the viral load and can even differentiate between field and vaccine viral strains that can be detected in the feces by PCR up to 28 days following vaccination.[165,166]

Hemagglutination testing can also be performed since parvoviruses agglutinate erythrocytes.[47] However, despite being considered a rapid method of diagnosis, this test doesn't overcome the limitations of conventional tests and is poorly specific.[123,167]

Although possible for every variant, viral isolation can only be carried out in specialized laboratories and has low sensitivity.[160] Thus, it is not commonly used for diagnostic purposes despite being an important research tool.[121] The same goes for fecal electron microscopy, which is very time-consuming for diagnostic purposes hence why it is used mainly for research purposes.[47] Serology can also be used but mainly to document an immune response to CPV or to evaluate MDA titers in puppies (to access the need for vaccination) since a positive serology is not diagnostic for active CPV infection.[121,134]

Lastly, post-mortem necropsy samples may reveal segmented enteritis, intestinal crypt necrosis, shortened or obliterated villi, lymphoid tissue necrosis, myocarditis, pulmonary edema and alveolitis from secondary septicemia, and intranuclear viral inclusion bodies.[47]

1.4-5. Treatment, Prognosis and Prevention

The treatment of parvoviral infection is primarily supportive and symptomatic.[134] Its main goals are to restore the fluid and electrolyte balance by replenishing interstitial fluid losses, preventing

secondary infections, and maintaining hydration. This consists of fluid therapy, antibiotics, antiemetics, and corticosteroids.[47,133]

Intravenous (IV) fluid therapy is crucial and should be continued for as long as fluid losses, i.e., if vomiting, diarrhea, or both, persist. Ideally, a jugular catheter would be best to lessen the risk of contamination by vomitus and feces; however, it's not always possible, and thus, the peripheral catheter should always be replaced every 72h to minimize the chances of bacterial colonization.[133,168] In severely hypovolemic and dehydrated patients, it sometimes can be challenging to establish an intravenous access. If that access can't be achieved, an intraosseous catheter can be used with the same degree of success.[169–171] The initial rate and volume of infusion will depend on the patient's hydration status and if hypovolemia is present. IV fluid boluses may be needed in patients with hypovolemic shock signs like tachycardia, weak pulses, bradycardia, and delayed capillary refill time and should be repeated until these signs of hemodynamic compromise are no longer present.[133,172] Furthermore, fluid deficits should be replaced as soon as possible after presentation. After replenishing the deficits, the rate is reduced to a maintenance rate along with estimated ongoing losses. It is indicated to administer a balanced isotonic crystalloid solution, like Lactated Ringer's solution, and it can be supplemented with potassium and 2.5% glucose to treat hypokalemia and hypoglycemia, respectively, that are frequently present in CPV enteritis.[169]

Contrarily to what was previously believed, in terms of nutritional support, early enteral nutrition has been found to be beneficial when compared to the *nil per os* (NPO) strategy in CPV patients, as it provides nutrients required for faster repair of the intestinal mucosa and helps keep mucosal integrity, which lowers the risk of bacterial translocation.[133,173,174] In fact, Mohr *et al.*[175] showed that this strategy led to earlier clinical improvement and a decreased morbidity rate in dogs with CPV infection. Enteral nutrition can be provided through forced feedings with a syringe or by the placement of a feeding tube (nasogastric, nasoesophageal, esophagostomy, gastrostomy, or jejunal).[173]

In addition to fluid therapy and enteral nutrition, antiemetics are used to manage vomiting. Drugs like metoclopramide, prochlorperazine and maropitant can be used to control frequent and persistent vomiting.[169] Moreover, serotonin receptor antagonists like ondansetron or dolasetron have also been described as effective antiemetics and can be used in CPV enteritis as well. Still, the use of these drugs should be closely monitored since it can cause hypotension and does not always limit vomiting.[47]

Since the severe destruction of the intestinal epithelium facilitates bacterial translocation into the bloodstream and, combined with neutropenia, increases the risk of sepsis, it is advised to administer broad-spectrum antibiotics in patients with CPV enteritis.[133] Antimicrobial therapy may, in some cases, consist only of broad-spectrum single-agent therapy with anaerobic coverage, like ampicillin or metronidazole, or enteric coverage, such as enrofloxacin.[176] In cases of hemorrhagic diarrhea, however, the recommended practice is to use a combination of antimicrobial drugs,

specifically antimicrobial agents with action against anaerobic and gram-negative bacteria.[121,127] The typical combination of penicillin and an aminoglycoside ensures antimicrobial activity against the needed bacterial spectrum.[121] *Escherichia coli* and *Clostridium perfingens* appear to be the most prevalent bacteria.[47,148,149] Third-generation cephalosporins may be an alternative to aminoglycosides in cases where nephrotoxicity is a concern.[121] The use of quinolones is contraindicated in young dogs since it has been associated with cartilage damage in prolonged use.[169]

Considering puppies with CPV often have comorbidities such as gastrointestinal parasitism, antiparasitic treatment should be started as soon as the patient tolerates oral therapies and isn't vomiting.[133,169]

The role of blood products in the treatment of CPV enteritis is controversial.[169] As there are serious losses of blood, some puppies may develop severe anemia and, therefore, benefit from whole blood or packed red blood cells transfusions. Hypoproteinemia is a frequent consequence of CPV infection due to protein-losing enteropathy and, while a whole blood transfusion can improve it, plasma transfusions are more appropriate.[47,140] Plasma transfusions are thought to be beneficial as they theoretically provide immunoglobulins and oncotic support. However, antibody counts may not be sufficient to make a difference, and the volume that has to be infused to raise serum albumin levels is too large.[169] Plus, the animal usually develops its own antibody response within three days after the onset of clinical signs. For this reason, studies with hyperimmune plasma have been performed but its use has been questioned since the level of antibodies may be increased naturally.[47,121,177]

When edema is present due to hypoalbuminemia and plasma transfusions are unable to correct it, the use of synthetic colloids such as *hetastarch* has been mentioned. Despite having its clinical benefits, namely good anticoagulant proprieties (useful in hypercoagulation states), its use has been controversial due to being associated with acute kidney injury.[121,178]

Dogs with CPV often have abdominal pain as a result of severe enteritis.[126] Opioids can cause vomiting and promote ileus, while NSAIDs and alpha-2 agonists are contraindicated (NSAIDs because of effects like nephrotoxicity and gastrointestinal ulceration, and the alpha-2 agonists because of the vasoconstriction they cause, which may limit gastrointestinal perfusion).[133] Thus, appropriate analgesia can be provided by lidocaine, buprenorphine (opioid partial agonist) and butorphanol (opioid agonist-antagonist).[126,133,179] Maropitant, a neurokinin-1 receptor antagonist previously mentioned as an antiemetic (main action), can also provide some visceral analgesia, and therefore be helpful in pups with CPV enteritis.[126,180]

When dehydration is corrected, glucocorticoids may be beneficial in treating early sepsis or endotoxemia.[47] However, repeated doses are not advised, and some authors don't consider their benefits to be proven.[169]

Lastly, additional adjuvant therapies have been investigated for use in CPV enteritis.[133] Antiviral drugs like oseltamivir, a neuraminidase inhibitor used to treat human influenza, have been studied

in dogs with CPV but have shown no clear benefit for these patients.[181,182] Although one of these studies reported increased body weight and maintenance of leucocyte count in dogs that received oseltamivir[182], neither documented any improvements in morbidity, mortality or length of hospitalization.[181,182] There have been many studies about the role of interferons in the treatment of canine parvoviral enteritis, specifically the recombinant feline interferon-omega (rfIFN-ω), and all of them have shown a positive response to this therapy, with improvements in appetite, lower incidence of vomiting, fever and diarrhea and lower mortality rates.[183–186]

The usefulness of recombinant granulocyte colony stimulating factors (G-CSFs) has been investigated in multiple studies in dogs with parvovirosis.[187–192] Although one study with recombinant human G-CSF (hG-CSF) did show improvements in neutrophil counts[188], most documented no improvement in length of hospitalization, neutrophil counts, or survival.[189,190] On the other hand, increases in endogenous cG-CSF concentrations have been correlated with improved neutrophil counts in puppies with CPV.[187] Recombinant canine G-CSF (cG-CSF) has been shown to have a positive effect in WBC and neutrophil counts[191], as well as in monocyte and lymphocyte counts.[192] Length of hospitalization was also shorter in dogs treated with cG-CSF in one study, but survival times did not improve; they were even shorter in dogs that belonged to the treatment group.[191] Thus, this treatment option still requires further studies to evaluate its overall safety.

Equine endotoxin antiserum and recombinant bactericidal permeability-increasing (BPI) protein have also been used and have not caused any significant difference in clinical outcome.[193,194]

Studies have been conducted to attest to the efficacy of probiotics in the treatment of parvoviral enteritis.[195,196] The fecal microbiota has several benefits to the host's health: enterocyte nutrition, protective barrier functions, immune regulation, and gastrointestinal motility. In parvoviral enteritis, there is a disruption of this microbiota.[24,197] Therefore, probiotics may positively affect these patients, as seen in a study by Arslan et al. (2012)[195], where a significant improvement in appetite, degree of dehydration, and incidence of vomiting and diarrhea was noted. It was suggested that probiotics may have had an effect on increased survival rate and faster recovery as well.[195] A second study, however, showed no benefit regarding the length of hospitalization or survival rate.[196] Transfaunation and fecal transplants from a healthy host have also been investigated as a method of restoring fecal microbiota. Despite having found no improvement in survival, a 2018 study documented a faster resolution of diarrhea and shorter hospital stay in dogs that received a fecal transplantation of 10g of feces from a healthy dog.

The prognosis of parvoviral enteritis varies with the severity of the disease and the owners' ability to afford the treatment. Outpatient protocols are cheaper but have been proven to be less effective than in-hospital treatment.[199,200] However, they may be a reasonable alternative for dogs whose owners can't pursue hospitalization due to financial limitations.[200] Studies have reported that, with adequate care, 75% of CPV cases should respond to medical treatment.[127] Survival rates vary from 60% to 90%, depending on the study, type of therapy and individual response to

treatment.[153,201–203] It has been described that puppies that survive the first three to four days of disease typically make a rapid recovery.[47,121] More severe cases where sepsis or other complications are present require further hospitalization and more aggressive treatment.[121,204] Without treatment, the prognosis is poor, with a mortality rate of about 90%.[201]

Prevention of CPV is focused mainly on strict hygiene strategies and vaccinations.[133] Disinfection procedures are essential in the control or prevention of a parvovirus outbreak. These can be accomplished with diluted household bleach (dilution of 1:30) on tolerable surfaces, and exposure should last for at least 10 minutes. Likewise, utensils and bedding should be washed with added bleach.[205] In fact, a solution of sodium hypochlorite at 0.75% has been proven to be efficient against CPV in one minute, while at 0.37%, it can also inactivate the virus if the contact time is prolonged to 15 minutes.[206] Moreover, it is essential to isolate dogs that develop gastrointestinal illnesses in a separate housing from healthy pups in shelters. However, the only foolproof way for preventing infection is to isolate at-risk puppies from exposure to the virus until they're fully vaccinated because healthy and well-vaccinated dogs may also shed the virus.[133]

Adding to disinfection procedures, vaccination is widely available, and it is the most effective method of preventing CPV infection and disease. Puppies with access to colostrum have maternally derived passive immunity and are protected until eight to 12 weeks of age, which is when maternally derived antibodies (MDA) titers start to decrease, and they become more susceptible to infection.[127,207] MDAs may interfere with vaccine protection, so it is important to consider the timing when formulating a vaccination protocol.[133] Adding to disinfection procedures, vaccination is widely available, and it is the most effective method of preventing CPV infection and disease.

Although both live attenuated and inactivated vaccines are available, current vaccination guidelines recommend using live attenuated vaccines, which are safe and provide better immunity with a longer duration. The protocol should be started at around six to eight weeks old and then every three to four weeks until the patient is 16 weeks old.[47,133,208] In shelter or breeding kennel dogs, it is recommended to vaccinate as early as four weeks of age until 18 to 20 weeks of age.[47] The current vaccines provide protection against every known CPV variant and, without the interference of MDAs, this protection starts as early as three days post-vaccination.[209–212] A booster vaccination is recommended at one year of age, and then it should be repeated every three years.[133] Modified live CPV vaccines replicate in the intestinal tract, and lower quantities of virus are shed after vaccination. Usually, they shed up until ten days after vaccination, but a recent study has shown that shedding may last until 28 days after vaccination.[165,213]

Vaccination failures occur mainly due to MDA interference.[123] There's a window of susceptibility between 40 to 69 days of age when the MDAs interfere with the vaccine's ability to stimulate an effective immune response but do not protect against infection.[121] However, vaccination failures have also been documented in adult dogs many times.[214–217] To guarantee protection against CPV, serum antibody titers should be at least 1:80 after vaccination.[214]

In Europe, DHPPi, the multivalent vaccine that is most frequently used, provides immunity against canine distemper, canine parvovirus, canine adenoviruses, and canine parainfluenza.[208]

2- Plasma transfusions

2.1- Introduction to blood-component transfusions and types of plasma

For hundreds of years, blood transfusions have been used in both human and veterinary medicine.[218] In 1665, Richard Lower successfully collected blood from one dog and replaced it with another dog's blood.[219] Nevertheless, it has been over the last 60 years that veterinary transfusion medicine has seen its greatest advances as it has been a great area of research.[220–222] As it's grown, veterinary transfusion medicine has become more complex, and several blood components have been made widely available, thus decreasing the use of whole blood transfusions.[218,219,223,224]

Blood-component transfusions are an essential tool in small animal practice, being very frequently needed in emergency and critical care medicine.[223,225,226] There are several advantages in the use of blood components instead of whole blood as they allow for more specific treatments, reduced chances of transfusion reactions, longer storage time, and better and more economical usage of the blood – there is less waste because one single dose can be used for more than one patient.[220,227–229] Whole blood is separated into its components by centrifugation, usually done within eight hours from collection.[219,227] The final products can be packed red cells (RBC), fresh frozen plasma (FFP) or frozen plasma (FP), cryoprecipitate or cryopoor plasma and platelet-rich plasma or concentrate (rare in veterinary medicine[224]).[227] Other plasma products are available, like human serum albumin (HAS), canine albumin, and intravenous immunoglobulin (IVIG).[218,230]

FFP can be obtained by fractionation of fresh whole blood or plasmapheresis, which is a process where the blood is removed from the donor to a device that automatically separates it, later returning it to the donor after saving the wanted components.[229,231,232] The plasma must then be frozen within six to eight hours of collection at -30ºC to -20ºC of temperature.[223,230–232] Its components will be viable for a year when stored correctly, the main ones being clotting factors, albumin, and immunoglobulins.[231,233]

After a year of being stored, or if frozen after six to eight hours from blood collection, FFP is relabeled as frozen plasma.[223,231,234] Although being suggested by Walton *et al.* [235] in a 2014 study that fresh plasma stored and frozen up to 24 hours post collection can still be classified as FFP because its composition is not affected during that time, this article reported a few limitations, making further investigation essential to validate this statement.

The main difference between FP and FFP is that coagulation factors V and VIII are not preserved, as they are more labile than the rest, making FP unsuitable for patients with hemophilia A, for

instance.[223,231,236] However, it is still usable in patients with other conditions, such as hypoalbuminemia or other factors deficiencies.[230,232] FP can then be stored for an additional four years (a total of five years since blood collection).[223]

Fresh frozen plasma can also be divided into cryoprecipitate (CRYO) and cryopoor plasma (CPP) through partial thawing and separation of precipitate and supernatant.[223,224] Cryoprecipitate contains higher concentrations of fibrinogen, von Willebrand's factor (vWf), fibronectin and factor VIII than FFP.[223,237,238] In human medicine, CRYO is recommended for fibrinogen replacement, but that is yet to be proven beneficial in canine patients, as there are no clinical studies performed to date.[237] On the other hand, CPP is made of the remaining components in FFP and has been proven to have higher albumin concentrations.[237,239] A recent study suggests that cryopoor plasma may be a viable and less expensive treatment option for patients with hypoalbuminemia.[239]

2.2- Indications and administration of fresh frozen plasma

FFP is a source of clotting factors, albumin, immunoglobulins and alpha-macroglobulin.[228] In veterinary practice, it is mainly used in the treatment of coagulation disorders and hypoproteinemia, but also in acute pancreatitis and parvovirosis.[228,230,231,240]

Coagulation disorders can be inherited - hemophilia A and B (fVIII and fIX deficiencies, respectively) and von Willebrand's disease (vWf deficiency) - or acquired - for example, when caused by hepatic disease, DIC, or rodenticide intoxication.[223,228] Fresh frozen plasma is used for two main purposes: to stop active bleeding or to prevent it (in dogs with abnormal coagulation times and an invasive procedure planned, for instance),[234] and has been proven to be effective in decreasing coagulation times in animals with coagulopathies.[228,240,241] However, cryoprecipitate is more appropriate in patients with von Willebrand's disease and hemophilia A since the risk of an adverse reaction is lower.[242] According to a 2010 study by Snow *et al.* [240], coagulation disorders were the main reason for performing FFP transfusions at a veterinary teaching hospital from 2006 to 2008.

FFP is mostly administered in hypoalbuminemia cases to maintain vascular integrity and treat or prevent edema caused by low oncotic pressure.[230] Other than being responsible for 80% of colloid oncotic pressure (COP) and 50% of plasma protein concentration[243,244], albumin has other vital functions like binding to drugs to transport them and aiding in the removal of free radicals and bacterial toxins.[218,230,245] Despite having been used for decades, evidence of FFP efficiency in treating hypoalbuminemia is lacking.[231,240,246] Besides, each unit has a low albumin concentration, thus making the required dose to raise albumin serum concentration by 0,5g/dl at least 22,5ml/kg of plasma, which is a substantial volume that can both be cost-prohibitive and raise the risk of transfusion reactions and fluid overload.[218,230,240,247]

Another indication is acute pancreatitis, in which FFP can be used as a source of alpha-macroglobulin.[228] Initially, many studies associated alpha-macroglobulin levels with the severity of disease, making FFP transfusions recommended as a standard treatment for acute pancreatitis.[248,249] However, recent studies have shown no correlation between these and have failed to prove any benefit from the administration of plasma to the patient's outcome. One study even recorded an increased mortality rate in dogs that received a transfusion.[250] This practice has, therefore, been abandoned in human patients and is decreasing in veterinary practice as well.[251]

Lastly, in patients with parvoviral enteritis, plasma transfusions are typically used to provide oncotic support, coagulation factors, and most importantly passive immunization, through antiviral antibodies in immune plasma (plasma from donors that have been infected and recovered, or that have been recently vaccinated).[126,208,228] Theoretically, the antibodies delivered by the plasma would be able to bind with circulating parvovirus and decrease infection and their ability to replicate, but in studies where immune plasma was used, no obvious beneficial effects were noted, neither in length of hospitalization nor in the outcome of the patients.[122,231,252] However, in a 2020 study by Acciacca *et al.*[177], patients were treated with hyperimmune plasma at 10ml/kg within the first six hours of hospitalization, and markers of shock were improved within 24 hours (shock index and plasma lactate concentration). In contrast, as for the length of hospitalization and outcome, there were still no effects observed. It remains unknown whether FFP from a routinely vaccinated donor provides enough immunoglobulins to benefit dogs with parvoviral enteritis, endotoxemia or sepsis.[228] The use of FFP transfusions in patients with parvoviral enteritis remains, then, very controversial since there is no substantial evidence to support or refute its use.[231]

FFP is best administrated intravenously at the recommended dose of 10-20 ml/kg, though higher doses may be required, for example, to control bleeding.[223,227] The ideal rate of infusion depends on the patient's hydration status and general condition but should lie between 5 and 10 ml/kg/h.[220,227]

II- Study: The effects of plasma transfusion in the outcome of acute gastroenteritis in young dogs with leucopenia

1- Study introduction

Acute gastroenteritis is among the leading reasons for veterinary visits in young dogs. When associated with leucopenia, gastroenteritis is mostly due to viral infections, particularly parvoviral infection.[111]

In dogs with parvoviral enteritis, supportive therapy is the mainstay of treatment protocols, but plasma transfusions are very commonly used. There is no significant evidence to support their use, and, at the same time, there isn't any to refute it.[231] There's a big need for new studies to check the usefulness of this treatment option since it carries risks and can be quite expensive.[169,231] Moreover, factors that may influence the outcome or duration of hospitalization need further investigation since there's scarce literature about it.

1.1- Objectives

This study aimed to investigate the therapeutic value of plasma transfusions in young dogs with signs of severe gastroenteritis and leucopenia. Therefore, the main objective was to check the influence of the plasma on the patients' outcome and the number of days they were hospitalized, compared with patients who didn't get a transfusion. A secondary goal of this study was to check whether other predisposing and prognostic factors had any relation or influence on the hospitalization duration or outcome as well.

1.2- Materials and methods

1.2.1- Study population

The hospital database (Ivet Clinic) and physical archives were reviewed for young dogs (≤1 year of age) hospitalized with signs of acute gastroenteritis between 2014 and 2020.

A total of 46 dogs were included in this study. Breeds included 18 mixed breeds, three Labrador Retrievers, two Yorkshire Terriers, Spanish Mastiffs, Estrela Mountain Dogs, Cane Corsi, Miniature Pinschers, German Shepherds and Border Collies, and one of each of the following: Bull Terrier, Boxer, Dalmatian, Dobermann Pinscher, Jack Russel Terrier, Beagle, Pitbull, White Swiss Shepherd, Pekinese, Portuguese Pointer, and Epagneul Breton.

All the information collected regarding this dog population is incorporated into a table in Appendix I.

1.2.2- Study design

This was a retrospective study carried out in Centro Hospitalar Veterinário in Porto, Portugal. Inclusion criteria were:

-Age ≤ 1-year-old

-Clinical signs compatible with acute gastroenteritis (vomiting and diarrhea)

-Presence of leucopenia

After selection of all the cases that met the inclusion criteria, variables recorded for each patient included: age (in weeks); gender; breed; vaccination and deworming status; pulse quality; leucogram: neutrophil nadir; the presence of SIRS at admission; the presence of fever (pyrexia); plasma transfusion (yes or no); duration of hospitalization (DOH - in days); outcome (discharge or death/euthanasia).

Pulse quality

The pulse quality was assessed by evaluating the peripheral pulse, more precisely through the palpation of the metatarsal pulse in the physical exam. It was divided into three categories: weak, palpable, and strong. The pulse was considered weak when multiple exams revealed an absent metatarsal pulse. The ones that revealed palpable and weak pulses were put under the palpable category, and those with strong pulses were included in the strong category.

Fever

The patients were considered febrile when their rectal temperature was higher than 39.2ºC.[253]

Leucogram (neutrophil nadir)

The leucogram was obtained with blood collected from the external jugular vein with sterilized needles and syringes to tubes with ethylenediaminetetraacetic acid (EDTA) (Figure 6). The blood samples were analyzed for the hemogram in the *BC-2800VET-MINDRAY* (Figure 6). The patients had leucopenia when the WBC was lower than 6000 cells/µL.

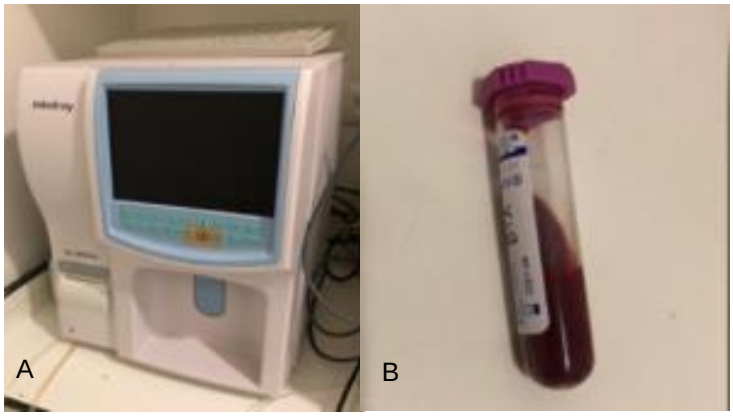

Figure 6 –Equipment used to perform the CBC in the population; A- hematology analyzer; B- EDTA tube with blood (original image)

SIRS at admission

To be diagnosed with SIRS at admission, patients must have met at least two criteria amongst the following (Table 3)[254]:

Table 3 - Criteria of SIRS[254]

Heart rate (beats/min)	>140
Respiratory rate (breaths/min)	>40
Body temperature	<37.2ºC or >39.2ºC
WBC	<5000 or >19500 or >5% bands

Plasma transfusions

Plasma transfusions were performed using fresh frozen plasma units from the Animal Blood Bank (BSA – Banco de Sangue Animal) (figure 7). The administration was done according to the instructions in the package, and dosage was calculated with the BSA online hemoCalculator (figure 7). In the population, some animals received more than one transfusion and, therefore, the total volume administrated per animal ranged from 5 to 22 ml/kg. There were, however, no objective or standardized criteria used in the decision to use a plasma transfusion or not.

The patients were closely monitored for any signs of transfusion reactions and, in this population, there were none.

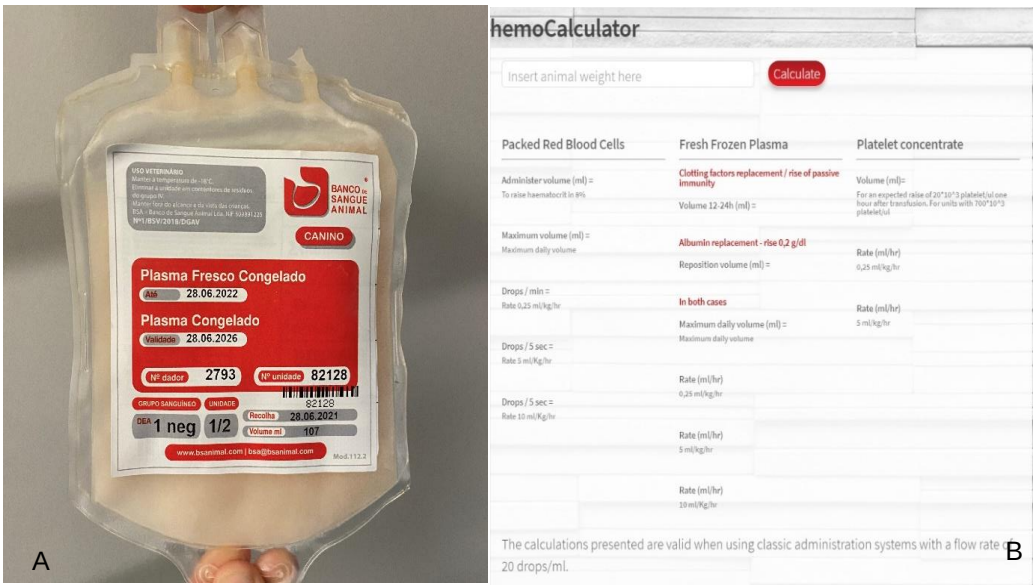

Figure 7 – A: Plasma unit (original picture); B: BSA hemoCalculator (http://bsanimal.co.uk/?lang=uk&page=calculadora&subpage=cao)

1.2.3- Statistical analysis

Excel software (Microsoft Corporation, Redmond, Washington, USA) and SPSS software (IBM, Armonk, New York, USA) were used to manage data and perform the statistical analysis.

Data analysis began with descriptive statistics. Kaplan-Meyer and Log Rank tests were conducted to compare two groups in a variable. Next, Chi-square and Fisher's exact tests were performed to evaluate the correlation between two categorical variables. Finally, Cox and Logistic Regression tests were performed to assess the correlation between all variables.

The statistical hypothesis H0 and H1 were defined as H0 – there is no significant correlation between the variables; H1 – there is a significant correlation between the variables. When the p-value was ≥ 0.05, H0 was not rejected, and when it was <0.05, H0 was rejected.

All relevant and statistically significant relations resulting from this analysis can be found in the following results.

2- Results

Duration of hospitalization and outcome

Overall, the study population had a survival rate of 89.1% (41 out of 46 patients) and a mean of 6.2 hospitalization days with a standard error of ± 0.431 days. Unfortunately, four patients died during hospitalization, and one was euthanized due to poor prognosis and condition.

Signalment: age, breed, and sex

The population was formed by dogs between the ages of four to 52 weeks. The median age was 15.5 weeks, and the mean was 20.2 weeks. The majority of the population had less than six months of age (33 out of 46). To verify the correlation between age and outcome, the population was divided into two groups: patients with 16 weeks (4 months) of age or younger; and patients older than 16 weeks. The results of this analysis are presented in Table 4. No statistically significant relationship was found between age and outcome (p-value = 0.155), nor was it found between age and duration of hospitalization (p-value = 0.448). However, the results suggest that older pups were more inclined to survive than younger ones (a 4.3% mortality rate versus 17.4% in younger pups). Survivors had a mean age of 20.9 weeks, and non-survivors had a mean age of 14 weeks.

Table 4 – Age: descriptive analysis

Age (weeks)	Total N	Discharge	Death		Hospitalization (days)	
		N	N	Percent	Mean	Median
≤16	23	19	4	17.4%	5.870 ± 0.858	5
>16	23	22	1	4.3%	6.462 ± 0.443	6
Overall	46	41	5	10.9%	6.214 ± 0.431	6

To analyze the relationship between breed and outcome (and DOH), the population was divided again into two groups: pure breeds and mixed breeds. One out of 18 mixed breed patients died, which presents a mortality rate of 5.6% in this group. In the pure breed patients' group, the mortality rate was 14.3%, having four out of 28 patients died. This rate was somewhat higher, but it was not statistically significant (p-value = 0.353). Likewise, there was no relation between breed and duration of hospitalization (p-value = 0.946), despite it being higher for pure breed patients as well (pure breeds were hospitalized for a mean of 6.5 days versus 5.9 days with mixed breed patients).

As for sex, twenty-six patients were female (56.52%) and 20 were male (43.48%). As shown in Figure 8, survival rates were nearly identical: the males had a slightly higher percentage of deaths (12%) than the females (10%). This difference was confirmed as not statistically significant by the analysis of correlation between the variables (p-value = 0.868).

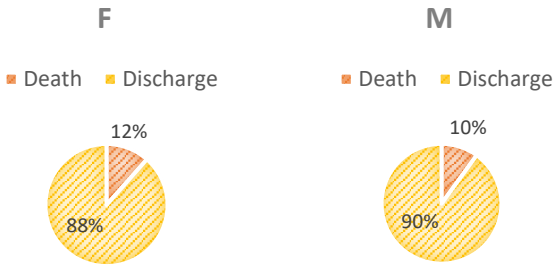

Figure 8 – Percentage of survivors and non survivors in each sex (F - female; M - male)

Although males had slightly higher hospitalization times (mean of 6.9 versus 5.7 in the females), it was not statistically significant either (p-value = 0.277).

Fever

In this study, it was observed that 25 patients were admitted with fever or developed fever at some point during the hospitalization (54.3%) and that 21 never did (45.65%).

The mean of hospitalization days in the group that developed fever was 6,589 days, while, in the group that didn't, it was 5.8 days. Still, it was concluded that there was no significant relationship between the occurrence of fever in patients and the duration of hospitalization (p-value = 0.423).

However, in the analysis of the relationship between fever and outcome, there was a statistically significant difference between groups (p-value = 0.030). In fact, this difference can be observed very clearly in Figure 9, even when comparing the values with the overall mortality rate. The mortality rate in the fever group was nearly twice as high as the whole population's (20% versus 10.9%), and every patient that died belonged to this group. In the non-febrile group, the survival rate was, thus, 100%.

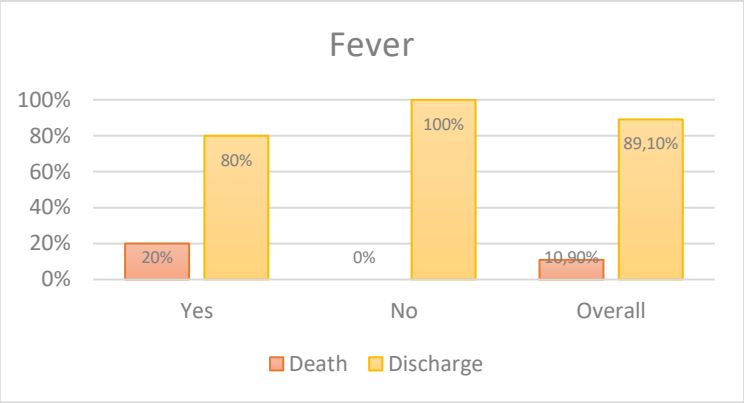

Figure 9 - Survival and mortality rates between groups and overall population

Neutrophil nadir

In the analysis of the neutrophil count nadir, two groups were formed: the patients with a neutrophil count of 1000 cells/μL or less were in one group (Group 1), and the patients with a higher neutrophil count were in the other (Group 2). Twenty-one dogs fit in the first group, and 25 fit in the second. Out of 21 members in the first group, four died, and out of 25 in the second, only one died. However, there was no significant relationship between the nadir of neutrophil count and the outcome (p-value = 0.102).

On the other hand, there was a clear difference between the two groups regarding the duration of hospitalization, and it was proven to be statistically significant (p-value = 0.043). Thus, pups that belonged to the first group (nadir ≤ 1000 cells/μL) had to be hospitalized for a significantly longer period than those who belonged to the second group (a mean of 7.3 days of hospitalization versus only 5.4). This difference can easily also be seen in the graph in Figure 10. By analyzing this graph, we can conclude that, for example, 60% of pups from Group 1 had to stay hospitalized for six or more days, while for Group 2, this percentage was less than 40%. In the same way, 40% of the first group of pups stayed for eight or more days while only about 15% of the others did.

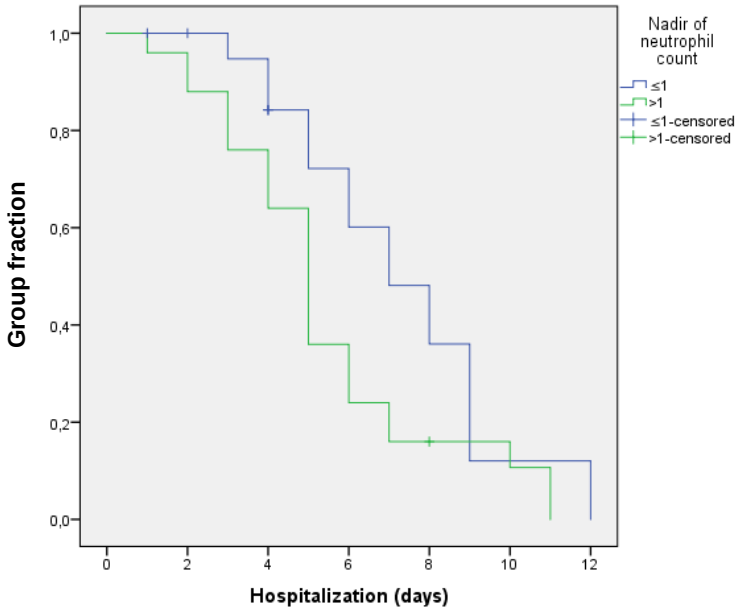

Figure 10 - Kaplan-Meier curve for neutrophil nadir: DOH distribution between groups (censored cases are the ones that died during treatment)

Pulse quality

The pulse variable had three categories: weak, palpable, and strong. Twenty-six pups fit in the weak pulse category, six in the palpable, and 14 in the strong. Despite having all deaths happen in the weak pulse category, the pulse quality had no significant influence on the outcome (p-value = 0.117).

However, it did significantly influence the hospitalization time (p-value < 0.001), as can be seen in the graph in Figure 11. Analyzing this graph, a clear difference can be seen, especially between the weak and strong categories, implying that the existence of weak and absent pulses resulted in longer hospitalization times. In fact, almost 50% of the dogs with weak pulses had to stay hospitalized for eight days or longer. In the strong pulses group, no dog was hospitalized for more than seven days. This difference is not as noticeable between the strong and palpable categories, but it is between the palpable and weak categories. Every dog with palpable pulses had already been discharged from the hospital by the 8-day mark as well.

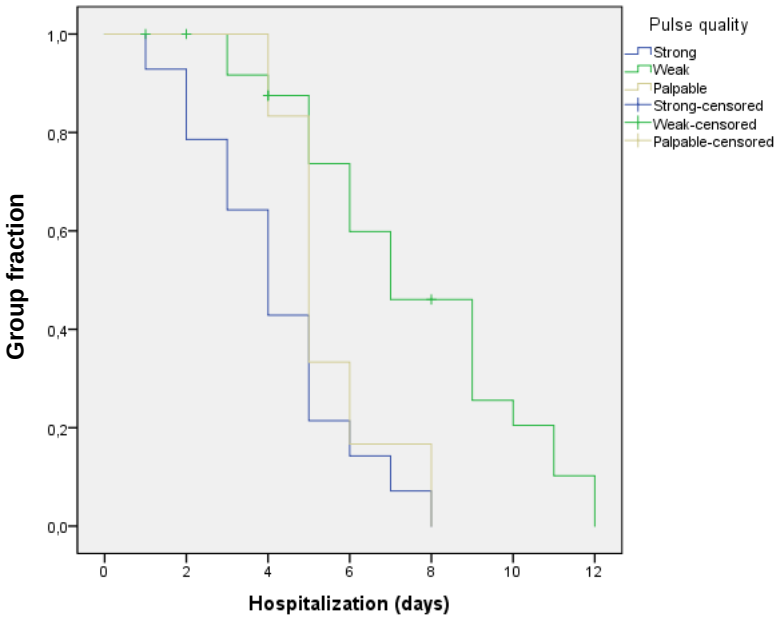

Figure 11 - Kaplan-Meier curve for pulse quality: DOH distribution between groups

SIRS at admission

When admitted into the hospital, 33 dogs met the criteria of SIRS and 13 didn't. Out of the 33 in the first group, only three didn't survive (9.1%), and amongst the other 13, 2 didn't survive (15.4%). This slight difference was found not to be statistically significant (p-value = 0.537).

Regarding the duration of hospitalization, pups that had SIRS at admission were hospitalized for a mean of 6.4 days and the ones that didn't were hospitalized for 5.7 days. There was also no statistically significant relationship between the presence of SIRS at admission and the hospitalization time.

Plasma transfusion

The population was divided into those submitted to plasma transfusions (A) and a control group (B), made up of those who did not receive any plasma. Group A had 18 dogs, and group B had 28. From Table 5, we gather that there were more deaths in group A than in group B – 4 (22.2%) versus 1 (3.6%). This difference was found to be statistically significant (p-value = 0.047).

Table 5 – Plasma: descriptive analysis

Plasma transfusion	Total N	Discharge	Death		Hospitalization (days)	
		N	N	Percent	Mean	Median
Yes (A)	18	27	4	22.2%	7.894 ± 0.616	9
No (B)	28	14	1	3.6%	5.339 ± 0.523	5
Overall	46	41	5	10.9%	6.214 ± 0.431	6

In a primary approach, it was assessed whether there were statistically significant differences between the time distribution functions of each group. Since the p-value was smaller than 0.05 (0.013), it can be concluded that there are. After verifying these differences, it's possible to determine what the actual difference is. From the analysis of the graph in Figure 12, we can conclude that, for instance, about 50% of the dogs in Group A were hospitalized for longer than nine days, while this time mark was only reached by 5% of the pups in Group B. We can also see that 60% of dogs in Group B were hospitalized for five days or longer, while this mark was reached by more than 80% of Group A pups.

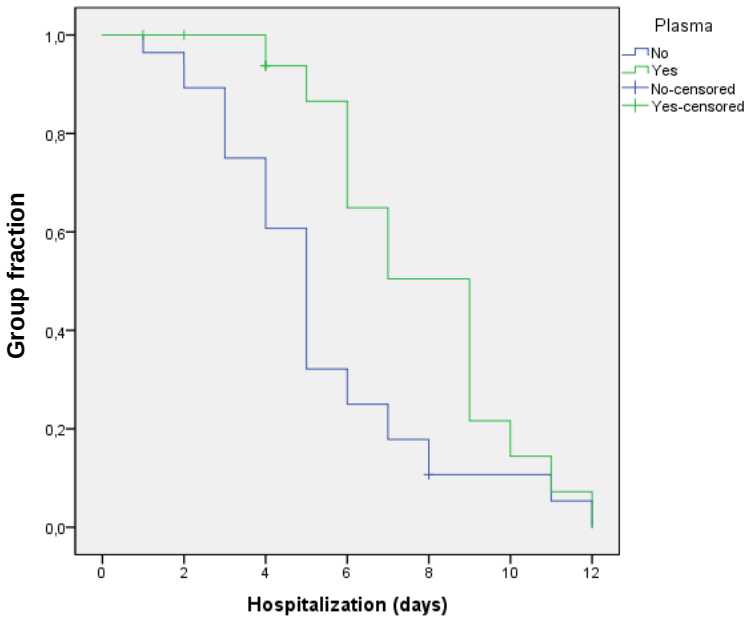

Figure 12 - Kaplan-Meier curve for plasma: DOH distribution between groups

Cox and logistic regression tests were performed to evaluate correlations between variables. These showed significant correlations between plasma transfusions and neutrophil nadir (p= 0.003); plasma transfusions and pulse quality (p< 0.001); and plasma transfusions and age (p= 0.002).

3- Discussion

In the present study, we evaluated the influence of plasma transfusions in the duration of hospitalization and outcome of young dogs with acute gastroenteritis, leucopenia, and a presumptive diagnosis of CPV infection. The impact on DOH and outcome of signalment and other potential prognostic and predisposing factors were also investigated. It is important to mention that an evident flaw in this study was the lack of a definite diagnosis of parvoviral infection. Thus, other studies on this subject should include at least a positive ELISA fecal antigen test to improve accuracy. Although there were not many statistically significant relationships between the examined variables, a few statistically relevant relationships and interesting findings are worth mentioning.

From the analysis of the age variable, it is evident that most of the population was younger than six months old (71.7%). This result is coherent with previous studies found in veterinary literature.[155,203,255] There was no statistically significant relationship found between age and outcome or DOH, which is supported by previous studies as well.[155,199,203] However, younger pups had more than double the death rate of older dogs. Interestingly, the results of a study by Horner *et al.* (1983)[256] also showed that pups younger than 12 weeks were less likely to survive than older ones.

Regarding the impact of breed, prior studies have revealed that Dobermann Pinschers and Rottweilers have higher odds of being infected.[47,203,257] One study even showed that Rottweilers are less likely to survive.[256] Contrarily, in the present study, we cannot make those conclusions since there were only two Dobermann Pinschers and no Rottweilers in the population. Likewise, two other studies from Germany and Greece corroborate these results.[155,258] Mixed breeds have been underrepresented in other publications, as they were in this study.[155,257]

There was a similar number of females and males, meaning no gender predisposition, and prior results support this.[155,259] In contrast, a recent study has found males to be more represented in parvovirus cases.[199]

No statistically significant impact was found from sex and breed in the outcome or DOH. The same has been reported in most studies, except for one by Pak *et al.* (1999).[155,199,203,260]

About fever as a predictor of outcome, it was found that its presence did have a statistically significant correlation with worst prognosis. In fact, the absence of fever was associated with a survival rate of 100%. It did not, however, have a significant effect on DOH. There's a dearth of studies evaluating the predicting value of fever in cases of CPV, and the only one the author has knowledge of showed opposite findings to the present results: puppies with fever had higher chances of survival.[155] Its authors couldn't give a clear explanation for these results.[155] In general, it is known that fever, as a part of the non-specific adaptive defense of the body, carries both benefits and risks.[253] There have been many studies, specifically in experimental settings and

human medicine, that support the results obtained by the study cited before, where fever improved the chances of survival and decreased the length of the disease.[261–263] In fact, fever has been shown to improve specific and non-specific immune responses. To an extent, neutrophil mobility and phagocytic abilities and lymphocyte proliferation are heightened by it. Nonetheless, the body temperature may get too high, and fever may become maladaptive as a result of overproduction of cytokines and other inflammatory mediators. This will lead to exaggerated inflammatory responses and can end in death.[253,262] Thus, it could explain this study's findings, since the patients who didn't survive may have had a more severe disease that provoked unsuited inflammatory responses and maladaptive fevers.

The neutrophil nadir was revealed to have a significant correlation with DOH, but not the outcome. In general, puppies with a nadir of 1000 cells/µL or less stayed hospitalized for almost two more days than those with higher counts. Leucopenia has been associated with poor prognosis in multiple previous studies.[264–266] This can be attributed to the consequent higher risk of developing opportunistic bacterial infections and septicemia.[267] Potgieter *et al.* (1981)[266] stated that leucopenia in parvoviral infections was mostly a result of severe neutropenia, which made him conclude that the neutrophils were the most important leucocyte to monitor. A study from 2007 confirmed these results.[268] On the other hand, there have been authors like Goddard *et al.* (2008)[267] that, like the present study, didn't find a correlation between the number of neutrophils and the outcome. Still, it was mentioned that the development of a degenerative left shift (neutropenia where immature neutrophils outnumber mature neutrophils) was associated with increased survival, and this could indicate that the bone marrow of survivors was less affected than that of non-survivors or that the disease onset was too acute for a bone marrow response in the non-surviving group.[267] In the current study, however, the presence or absence of a neutrophil left shift was not contemplated. On the other hand, the significant relationship between the lower neutrophil nadir and DOH can be explained by the fact that dogs with a lower neutrophil count had more severe disease and thus would inevitably be hospitalized longer than dogs with milder disease because they would take longer to recover. This has been seen in a study by Cohn *et al.* (1999)[187], where the neutrophil nadir coincided with the most severe clinical disease in puppies with parvoviral enteritis. However, the neutrophil's influence on DOH has not been reported or further explained in previous literature. A 2010 study by Kalli *et al.*[202] found that the presence of lymphopenia on admission increased DOH, but had no mention of the effect of neutropenia or neutrophil counts.

The assessment of the peripheral pulses revealed a significant relationship between weak pulse and DOH but no correlation with outcome. However, every patient that did not survive belonged to the weak pulse group, meaning they had many absent pulses. An absent metatarsal pulse is a highly specific indicator of hypotension[269,270], and hypotension has been indicated as a factor of poor prognosis in a recent study.[271] It is known that low systolic blood pressure negatively affects the body and is associated with shock syndrome, so these results are easily explained.[271–273] As for its correlation with DOH, there are no studies available that the author has knowledge of, only

a mention of it theoretically prolonging hospitalization time in a 2005 study by Mantione & Otto.[274] Notwithstanding, the present results are explained by disease severity in each group: it was clear that the strong pulses group had a milder infection than the other groups, and therefore would recover faster and more easily, leading to a shorter hospital stay.

The systemic inflammatory response syndrome consists of a complex systemic response to an infectious or non-infectious insult.[254,275] Despite most of the dogs in this study's population being diagnosed with SIRS at admission, this did not translate into a significant correlation between this diagnosis and either outcome or DOH. These results are not in line with previous studies that have found that dogs with SIRS at presentation have lower chances of survival.[202] In contrast, an older study showed no reliable relation between SIRS at admission and worst outcome but instead found one with the persistence of SIRS during hospitalization.[274] This discrepancy has been linked to the difference in criteria used in each study, having the most recent one used more strict criteria, increasing its specificity and predicting value.[202,276] The present study used slightly different criteria, but not less stringent than the one mentioned, so it is unlikely that this would be the reason for these results. Still, the results reported by Kalli *et al.* (2010)[202] would make more sense since the presence of SIRS represents an exaggerated and dysregulated response that can lead to adverse effects like immunosuppression and probably resulted of a worse insult.[277]

As for the primary focus of this study, plasma transfusions revealed significant correlations with both DOH and outcome. In fact, dogs belonging to Group A (the group that received a transfusion) died more and were hospitalized longer than those on Group B. As mentioned previously, plasma transfusions would theoretically be beneficial due to providing passive immunization, albumin and coagulation factors.[126,169,173] They have, thus, been recommended based on anecdotal evidence and the fact that immune plasma has been effective in treating other viral infections.[140,278] To the author's knowledge, there's a dearth of randomized, placebo-controlled prospective studies evaluating the therapeutic effect of fresh frozen plasma transfusions in dogs with CPV enteritis. Two studies with immune plasma failed to show improvements in outcome or DOH in treatment groups.[177,252] One of them, where hyperimmune plasma was used, did, however, show improvements in markers of shock in the initial parts of the treatment.[177] Additionally, an older study, where CPV-infected dogs were passively immunized with IV convalescent canine serum 24 hours after oral CPV inoculation, did show that the immunized group did not develop CPV clinical signs, lymphopenia, or fecal virus excretion, and had no evidence of intestinal CPV infection at necropsy.[279]

In this study, the results may have been compromised by its retrospective nature, the inability to standardize the severity of disease between both groups and the presumptive nature of the diagnosis. In fact, it doesn't make sense that fresh frozen plasma would worsen the patients' health condition unless it were because of transfusion reactions, which were not observed in any of the cases. In other words, these results may be explained by the fact that the patients treated with fresh frozen plasma may have had a more significant disease than the others. This might

even have been the reason for the option to treat with fresh frozen plasma in the first place. As seen with the regression tests, the administration of plasma transfusions was significantly correlated with the neutrophil nadir and the pulse quality, which may explain precisely these conclusions. The dogs that received plasma were in worse health conditions than those that didn't, and that's why they had worse outcomes and longer hospitalization times – in group A, 72% had a neutrophil nadir of 1000 cells/L or lower, versus only 17.8% in group B; the same way, 94% of the patients in group A had absent or weak pulses versus only 32% in group B. This reveals a significant limitation of this study as possible positive or negative effects of FFP, because of these differences, could not have been rightfully evaluated.

4- Conclusions

Parvoviral enteritis is still a disease of great importance in dog populations. Without treatment, parvoviral infection can reach up to 90% mortality rates. Supportive treatment is currently the mainstay of treatment for this condition, and it has been proven to be effective. However, alternative options are constantly being studied to raise the chances of survival for infected puppies.

This study has various limitations, including the fact that it is a retrospective study. There wasn't a definitive diagnosis of parvoviral enteritis for the whole population either, and the disease severity was not equivalent between treatment groups. It did, however, come to some interesting conclusions that support previous studies on this matter. It showed that lower neutrophil counts and worse pulse quality resulted in longer hospitalization times, and fever resulted in worse outcomes.

Regarding the primary goal, FFP transfusions were found to not bring any benefits in the treatment of this dog population; they were even associated with worst prognosis. However, dogs that received FFP were in worse health conditions, so further investigation with randomized and prospective studies is still required to validate its use.

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Appendix I

Dog population enrolled in the study.

Patient ID	*Sex*	*Age (weeks)*	*Breed*	*Plasma*	*Pulse quality*	*Neutrophil nadir (x10^3 cells/µL)*	*Fever*	*SIRS at admission*	*Duration of hospitalization (days)*	*Outcome*
1	M	12	Spanish Mastiff	No	Strong	1.0	No	Yes	4	Discharge
2	M	12	Spanish Mastiff	No	Palpable	1.0	Yes	Yes	8	Discharge
3	M	20	Mixed	No	Weak	0.2	No	No	7	Discharge
4	M	8	Mixed	Yes	Weak	1.3	No	Yes	6	Discharge
5	M	20	Bull Terrier	No	Strong	2.5	Yes	Yes	5	Discharge
6	M	24	Boxer	No	Weak	2.6	No	Yes	5	Discharge
7	F	8	Mixed	No	Strong	1.1	No	Yes	2	Discharge
8	F	28	Mixed	No	Weak	1.3	Yes	Yes	3	Discharge
9	F	20	Mixed	No	Strong	1.0	No	Yes	8	Discharge
10	M	12	Dalmatian	Yes	Weak	4.5	No	Yes	7	Discharge
11	M	4	Dobermann Pinscher	Yes	Weak	0.3	No	No	9	Discharge
12	F	16	Mixed	No	Palpable	2.4	No	No	5	Discharge
13	F	28	Jack Russel Terrier	No	Strong	1.8	Yes	Yes	7	Discharge
14	F	16	Mixed	No	Strong	4.4	Yes	No	3	Discharge
15	F	28	Mixed	No	Weak	1.5	Yes	Yes	8	Death
16	M	48	Mixed	No	Strong	1.2	No	Yes	4	Discharge
17	M	13	Mixed	No	Weak	0.4	No	Yes	12	Discharge
18	M	32	Pitbull	Yes	Weak	0.5	Yes	Yes	9	Discharge
19	F	8	Yorkshire Terrier	Yes	Weak	0.6	No	Yes	6	Discharge
20	F	8	Beagle	No	Weak	2.0	No	No	3	Discharge
21	F	48	German Shepherd	No	Strong	4.0	Yes	No	5	Discharge

22	F	16	Estrela Mountain Dog	No	Palpable	2.7	No	No	6	Discharge
23	F	10	White Suisse Shepherd	Yes	Weak	0.6	Yes	No	2	Death
24	F	12	Mixed	No	Strong	2.0	No	Yes	1	Discharge
25	F	36	Mixed	Yes	Weak	0.3	No	Yes	4	Discharge
26	M	20	Pekinese	No	Weak	1.8	Yes	Yes	5	Discharge
27	F	28	Cane Corso	Yes	Weak	1.4	Yes	Yes	10	Discharge
28	F	9	Labrador Retriever	Yes	Weak	1.9	Yes	Yes	6	Discharge
29	F	52	German Shepherd	No	Weak	3.6	Yes	No	5	Discharge
30	M	28	Mixed	No	Weak	3.9	Yes	Yes	11	Discharge
31	F	52	Mixed	No	Strong	0.9	Yes	No	5	Discharge
32	F	8	Labrador Retriever	Yes	Weak	0.1	Yes	Yes	4	Death
33	M	12	Cane Corso	Yes	Weak	0.4	Yes	Yes	1	Death
34	F	43	Miniature Pinscher	No	Strong	0.7	Yes	No	3	Discharge
35	M	8	Mixed	No	Palpable	1.3	Yes	Yes	4	Discharge
36	F	23	Mixed	No	Strong	1.3	No	Yes	2	Discharge
37	M	12	Miniature Pinscher	Yes	Weak	0.5	Yes	No	4	Death
38	M	12	Portuguese Pointer	Yes	Weak	0.5	No	Yes	9	Discharge
39	M	12	Border Collie	No	Strong	3.3	Yes	Yes	4	Discharge
40	F	21	Estrela Mountain Dog	Yes	Weak	1.3	Yes	No	11	Discharge
41	F	15	Epagneul Breton	Yes	Palpable	0.6	Yes	Yes	5	Discharge
42	M	8	Yorkshire Terrier	Yes	Weak	0.2	No	Yes	9	Discharge
43	F	12	Mixed	Yes	Weak	0.3	Yes	Yes	12	Discharge
44	F	52	Mixed	No	Palpable	4.0	Yes	Yes	5	Discharge
45	M	8	Labrador Retriever	No	Strong	0.9	No	Yes	6	Discharge
46	F	8	Border Collie	Yes	Weak	0.4	No	Yes	7	Discharge