Weaning diarrhea in puppies

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Introduction

Gastrointestinal diseases are some of the most frequent problems reported in dogs (1-3), with puppies being at higher risk of diarrhea than adult animals; about 10-25% of all puppies will have digestive problems at some point in the first year of life (4,5). The aim of this article is to review the factors which can affect a puppy's digestive health and to discuss procedures that will help both manage and prevent this problem.

Weaning: a critical stage

Weaning is a critical stage for puppies. From a digestive viewpoint, moving from milk to solid food brings modifications in the digestive mucosal architecture (increased depth of the intestinal crypts), in the transport of nutrients, in enzyme activity (reduced lactase activity and increased amylase and lipase activity), and in the intestinal flora (reduced aerobic bacteria). At the same time, puppies go through an immunity gap when they are refractory to vaccination due to the persistence of maternal antibodies (6) but are susceptible to infections, notably gastrointestinal ones. In addition, separating a puppy from its dam induces considerable stress, which can impact the metabolism, immune system and intestinal function. All these phenomena can explain the higher prevalence of diarrhea in puppies compared to adults.

KEY POINTS

- **Weaning diarrhea is a complex phenomenon with multi-factorial origins. Various infectious and non-infectious causes may simultaneously, and in synergy, damage the health of the gastrointestinal tract.**
- **Type-2 canine parvovirus is one of the main agents involved in weaning diarrhea. Although it can cause severe systemic signs, the virus may simply alter the stool quality without impacting on general health.**
- **Prevention of weaning diarrhea requires both medical prophylaxis and implementation of management protocols designed to maintain health.**

Weaning diarrhea – the risks

Weaning diarrhea is both a problem for puppies and a risk to public health. Diarrhea may reduce growth rates and increase the risk of mortality (7) — gastrointestinal problems can be the prime cause of death in dogs under a year of age (8) — and it is essential to treat all animals presenting with a digestive disorder rapidly and effectively. Moreover, digestive complaints also represent a public health risk; some of the infectious agents excreted by diarrheal puppies are potentially zoonotic, *e.g.*, *Giardia duodenalis* and *Toxocara canis* (9). The veterinarian's role in both preventing and treating these diarrheas is therefore crucial.

Defining diarrhea

Beyond a subjective analysis of what might classify as a "soft stool", the first difficulty is to define what an abnormal stool actually is. Stool quality can be evaluated using a "puppy fecal score", a visual 13-point scale ***(Figure 1)*** where 1 = liquid stool and 13 = formed and very dry stool (7). This scale differs from that used for adults. Physiological variations must be taken into account to define an abnormal fecal score.

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Fecal scoring system
for puppies

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Liquid feces

1 Feces completely liquid

2 Liquid feces associated with soft feces (liquid feces represent the main fraction of feces)

3 Liquid feces associated with soft feces (soft feces represent the main fraction of feces)

Soft unformed feces

4 Pasty, shapeless feces

5 Pasty unformed feces. The cylindrical shape of the feces tends to be lost due to the high water content.

6 Feces mainly unformed with a small formed fraction

Soft formed feces

7 Pasty feces, formed but very soft. Cylindrically shaped without any ridges observed

8 Formed but very soft feces. Cylindrically shaped with presence of ridges

9 Formed but very soft feces. Cylindrically shaped separated into pellets

Formed, dry but not hard feces

10 Cylindrically shaped feces, slightly sticky, separated into pellets

11 Cylindrically shaped feces, dry appearance, separated into pellets, can be easily deformed

12 Cylindrically shaped feces, dry appearance, separated into pellets, can be deformed with some difficulty

Formed hard feces

13 Formed, dry and hard feces

Fecal scoring system adapted from: Grellet A, Feugier F, Chastant-Maillard S, et al. Validation of a fecal scoring scale in puppies during the weaning period. Prev Vet Med 2012;106(3-4):315-323.

Large breed puppies (> 25 kg at adulthood) produce stools that are softer than those produced by smaller-breed puppies, and young puppies (aged 4-5 weeks) will produce stools significantly softer than older puppies. The fecal score threshold defining a pathological stool will thus vary with an animal's breed size and age, but can be defined as ≤ 5 for large-breed puppies, ≤ 6 for small-breed puppies at 4-5 weeks of age, and ≤ 7 for small-breed puppies aged 6-8 weeks (7).

A systemic approach to the problem

Weaning diarrhea is a complex phenomenon, for several reasons. Firstly, puppies are frequently infected by different agents ***(Table 1)*** but the presence of an enteropathogen is not always associated with signs of a gastrointestinal problem. In fact, 18-54% of dogs can excrete parasites or viruses without developing clinical signs (5,10,11).

Secondly, any given enteropathogen does not always induce the same clinical signs in all puppies. The pathogenicity of an infectious agent and its clinical impact will depend on the age and immune status of the puppy, as well as the strain of the enteropathogen (12,13). For example, canine parvovirus (CPV) is classically regarded as an agent that causes diarrhea in puppies leading to severe systemic signs (vomiting, anorexia, prostration, dehydration) and even death in some cases. However, in some puppies the virus may only alter the stool quality without affecting the animal's general condition, or there may be no clinical signs whatsoever (5). Similarly, coronavirus can cause a variety of clinical signs, and a new strain of this virus has been recently identified (pantropic coronavirus) which seems to cause a much more severe clinical disease, including death in some cases. Coccidiosis can also cause enteric disorders, but to varying degrees; *Cystoisospora ohioensis* complex may produce digestive disturbances in very young animals (< 7 days of age) but does not affect puppies at weaning, whilst *C. canis* mainly induces clinical signs in puppies at weaning and, more particularly, after stress (*e.g.*, at rehoming) (14).

Thirdly, co-infections and interactions between enteropathogens are frequent. One study on 316 puppies with diarrhea revealed that 75% of them had more than one infectious agent ***(Figure 2)*** (5). Some of these infectious agents can interact and amplify the severity of the clinical signs; *e.g.*, coronavirus will aggravate the clinical signs during co-infection with type 2 CPV (15).

Finally, new enteropathogens are regularly identified. Various canine gastrointestinal viruses and parasites have recently been isolated (*e.g.*, astrovirus (16), norovirus (17)

Table 1. Various studies have identified the main gastro-intestinal infectious agents in puppies and the prevalence of each agent (5,21,22).

Pathogenic agents	Age of population studied	Number of puppies in study	Prevalence (%)
Type-2 canine parvovirus	5-8 weeks of age	266	14.7
Canine coronavirus	5-8 weeks of age	266	20.3
Toxocara canis	5-8 weeks of age	266	22.2
	Various*	143	12
	< 3 months of age	2661	12
***Cystoisospora ohioensis* complex**	5-8 weeks of age	266	25.6
	< 3 months of age	2661	15.6
Cystoisospora canis	5-8 weeks of age	266	13.2
	< 3 months of age	2661	11.8
Cystoisospora spp.	Various*	143	9
Giardia duodenalis	5-8 weeks of age	266	41
	Various*	143	34
	< 3 months of age	2661	37.5
Cryptosporidium parvum	5-8 weeks of age	266	25.9

*Pet shop puppies, hence variable age range

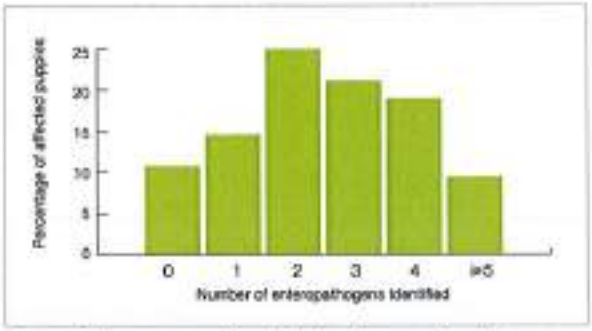


Figure 2. Frequency of co-infections in puppies with diarrhea around weaning.

and trichomonads (18,19)). Despite their strong prevalence in puppies (between 5 and 23 % depending on the pathogen and the origin of the animals), their role in weaning diarrhea has yet to be clearly established (16,18,20) and the majority of studies that have looked at these infectious agents do not take possible co-infections into account.

Unlike some disorders which can be viewed simplistically (i.e., one agent = one disease) weaning diarrhea is a complex biological phenomenon and a "systemic" approach to this problem is essential. Essentially weaning diarrheas are influenced by a triad consisting of:

- The host (age, genetics, and local and systemic immunity)
- The pathogen (virulence, strain, dose)
- The environment (population density, stress, hygiene levels, temperature/humidity)

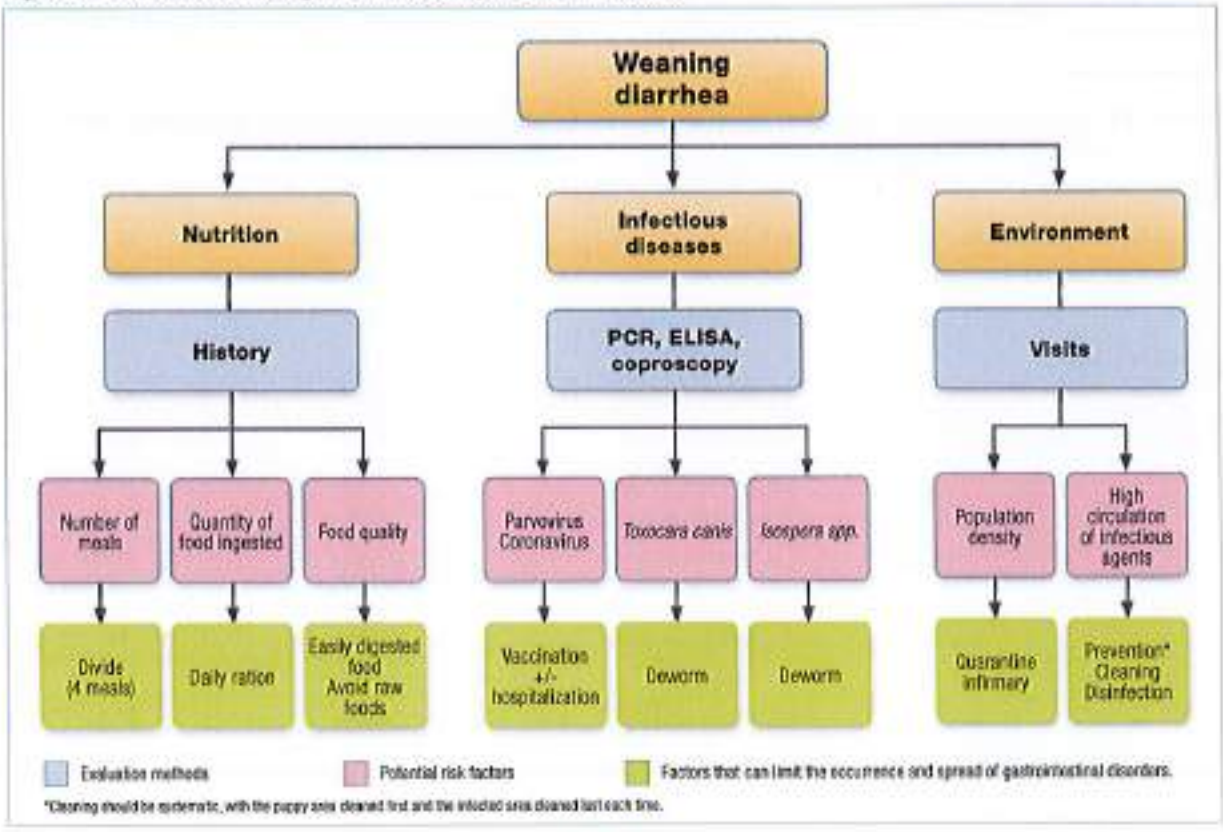
A multi-disciplinary approach is necessary, with evaluation of three major factors: nutrition, causal enteropathogen(s) and the environment ***(Figure 3)***.

Nutritional evaluation

From a nutritional viewpoint, a complete case history will be necessary. It is particularly important to question the owner regarding:

- The foodstuffs consumed, in order to evaluate the quality (some cases of weaning diarrhea are linked to ingestion of raw meat contaminated by *Salmonella enterica* (23))
- The number of meals fed (dividing the food ration into 4 daily portions can reduce the risk of diarrhea for young puppies (5))
- The quantity of food fed (overfeeding should be avoided) and its quality (is it high digestibility?)

Figure 3. Evaluation and management of weaning diarrhea in puppies.



Evaluation of enteropathogens

It is also important to identify whether the animal is excreting one or more enteropathogens and in what quantity. The color of the animal's stool may help identify the pathogen(s) responsible for the diarrhea. For example, giardiasis will cause partial atrophy of the intestinal villi and a reduction in disaccharidase activity, leading to reduced food absorption and steatorrhea; the feces may be yellow in color ***(Figure 4)*** and coprophagia may be observed (the increased lipid content make the feces more palatable). An unformed stool containing mucous and blood may indicate coccidiosis ***(Figure 5)***, or parasites can be visible to the naked eye within the diarrhea ***(Figure 6)***.

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Figure 4. Yellowish feces with a high fat content may suggest infection with *Giardia*.

However, these differences do not permit a definitive diagnosis, and supplementary tests are necessary. Various options, including microscopy, ELISA and PCR, can be useful and should be employed according to the owner's financial means and the veterinarian's experience and clinical suspicions. Microscopic evaluation of feces is useful if parasites are suspected, but the test sample must be fresh and not overtly liquid (particularly when searching for protozoa). Because causal agents can be eliminated intermittently, the tests should be repeated over 3 consecutive days; a single negative test is of little value. Should a litter or group of puppies be affected, collective testing on pooled fecal samples can be performed, which limits false negative results linked to the pre-patent period and intermittent parasitic excretion. Various commercial kits are available to identify certain parasites (*e.g., Giardia spp.*) and these are relatively cheap, fast and do not require specific sample material. However, such tests only allow identification of one infectious agent at a time, which can be limiting when there are multiple enteropathogens present.

CPV should always be suspected with weaning diarrhea or sudden death in a puppy, and it is imperative to test for the virus regardless of an animal's vaccination status. ELISA tests are simple and fast, with high specificity but variable sensitivity (18-82% (24-26)) which is linked to the viral load excreted. False negative results are common with low viral excretion levels, and a negative result does not exclude parvovirus infection. There is also a risk of false positives if testing a few days after vaccination, although the result is usually less definitive than when testing an animal suffering from parvovirosis. Real-time PCR tests have better sensitivity and specificity and are the method of choice for CPV diagnosis, as they will distinguish post-vaccine excretion (low to very low viral load) from clinical disease (generally high to very high viral load).

Fecal bacterial culture is rarely helpful when evaluating weaning diarrhea. Indeed, the bacteria regarded as causative agents of diarrhea are frequently isolated in clinically healthy individuals. However, if a specific pathogenic bacteria is suspected, certain agents (such as *Salmonella spp.*, *Campylobacter jejuni*, *Clostridium perfringens*, and *C. difficile*) may be cultured.

Evaluation of the environment

When faced with a weaning diarrhea problem at a breeding establishment, it is essential to undertake a site visit. Note that if a group of dogs are involved, not all problems may be resolved with a single treatment, and it is sometimes better to target the contributory factors rather than the causative agent(s) directly. A site visit allows the veterinarian to understand the breeding establishment in its entirety, paying particular attention to:

Figure 5. An unformed stool containing mucous and blood may indicate coccidiosis.

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- The owner and the breeding methods employed
- The animals and their environment (*e.g.*, what animals are kept, the housing employed, the diet offered)
- The management of the animals (reproduction, puppy husbandry)
- The overall sanitary conditions

Management of weaning diarrhea

Due to the numerous factors influencing digestive health, a global approach is recommended to manage and treat weaning diarrhea. A few examples serve to illustrate this:

Situation 1: the puppy with diarrhea but no systemic signs

It is frequently recommended that puppies should be starved for 24-48 hours before progressively reintroducing small quantities of food over 3-7 days. Even if this protocol has never been been scientifically tested, the approach is commonly accepted. However, studies have shown that enteral feeding during an acute diarrheic episode will help maintain the integrity of an animal's digestive tract, limiting destruction of the intestinal villi, intestinal permeability and bacterial translocation. Puppies suffering from parvovirosis that are given early enteral feeding show quicker weight gain and better recovery of normal appetite and stool quality compared to puppies fasted until vomiting ceases (27). Some authors recommend minimal enteral feeding (offering 25% of a dog's daily maintenance energy needs using a highly digestible food) with a view to limiting exacerbation of the diarrhea while ensuring the beneficial effects of enteral feeding, but ultimately the decision to opt for enteral feeding is at the veterinarian's discretion.

Figure 6. Parasites such as roundworms may be visible to the naked eye in some cases of diarrhea.

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For any parasitic infestation, the animal should be treated appropriately and also groomed to reduce the environmental parasite load. Cleaning of the environment and use of a quaternary ammonium disinfectant is recommended. Antibiotic therapy when there is diarrhea but no other clinical signs is controversial but should really only be considered when the intestinal mucosa is severely damaged (*i.e.*, obvious blood in the stool), if there is a systemic inflammatory reaction (fever and leukocytosis), and/or an abnormal fecal culture.

Situation 2: the puppy with diarrhea and other clinical signs

In this situation, the measures outlined above should be implemented, but the animal must also be hospitalized. The risk of dehydration and hypovolemia is considerable, and fluid (preferably IV) therapy is essential. When there is profuse diarrhea, the puppy may also be hypoglycemic secondary to profound malnutrition, hypermetabolism, inadequate liver function and/or sepsis. In severely affected patients, an initial intravenous fluid bolus of an isotonic crystalloid solution can be given followed by a continuous rate infusion. Calculating the volume to be administered must take into account the puppy's fluid deficit, the maintenance needs, and the losses induced by continuing vomiting and diarrhea. Hypokalemia is a risk; even if the animal has normal potassium levels on hospitalization, the levels should be rechecked a few hours after beginning fluid therapy and corrected if necessary. Note that potassium-rich fluids must not be given by bolus; any potassium infusion must not exceed 0.5 mEq/kg/h (28).

Situation 3: the puppy in a breeding kennel

In this situation it is important to both manage the animal's diarrhea as necessary (as outlined above) but also implement plans in order to minimize the risk to other animals. This requires both medical and hygiene measures.

Medical treatment consists of administering worming products and vaccinations. Deworming will depend on the parasite agents present in the breeding establishment. An annual microscopic evaluation of pooled fecal samples (from 3-5 individual dogs) is invaluable, looking at three different populations: stud dogs and bitches in anestrus, the pregnant and nursing animals, and puppies at weaning (*i.e.*, at 4-8 weeks of age). Where there are several litters of different ages present simultaneously, two distinct pooled fecal examinations may be carried out; one sample from puppies aged 4-6 weeks

and another sample from puppies between 6-9 weeks of age. Anti-parasite treatment depends on the results, with the choice of drug based on the spectrum of action, the treatment duration, the frequency and ease of administration, and cost. In all cases, regular deworming against *Toxocara canis* is recommended as this parasite is highly prevalent. Puppies may be dewormed every fifteen days from 2 weeks of age until 2 months of age; then monthly until 6 months old, with the dam treated at the same time as the puppies.

The vaccination regime depends partly on the individual situation. If there are several animals housed together, the protocol should be adjusted as necessary where there is evidence of CPV infection. Studies have shown that a monovalent CPV vaccine given at 4 weeks of age produces seroconversion above the protective threshold in 80% of puppies (29), and therefore routine early vaccination of puppies may reduce the negative impact of this virus in breeding kennels.

Various hygiene measures should also be implemented in order to limit the spread of infection and reduce the risk of recurrence. Specific, separate areas within a breeding kennel should be established and maintained; namely a maternity/nursery unit, a quarantine section for new arrivals, an area for adults, and an infirmary to isolate animals as soon as any signs of disease appear. It is essential to emphasize the importance of cleanliness and disinfection for each area and its equipment, and it is imperative to clearly differentiate between these two very distinct stages. Cleaning involves the use of chemicals or mechanical means (scrubbing or high pressure washing with a detergent) to remove organic materials. Most stains (excrement) are organic in nature and therefore acidic, so it is advisable to use an alkaline detergent six days out of seven, with an acid detergent employed once weekly in order to eliminate mineral (calcium) stains. Disinfectants should only be used once all surfaces have been cleaned and rinsed, because most disinfectants are inactivated by organic materials. The choice of product(s) depends on the infectious agent identified or suspected, the surface to be cleaned/disinfected, the ease with which a product can be applied, and its safety profile for personnel. The stability of a disinfectant is also important, as certain products such as sodium hypochlorite (household bleach) are unstable after dilution and an extemporaneous preparation is therefore advised for this kind of disinfectant. No product is ideal for all situations.

New techniques for evaluating digestive health

Biomarkers of digestive health

As noted above, weaning diarrhea results from a complex host/pathogen/environment interaction, and recent research has focused on various non-invasive gastrointestinal and blood markers, with the aim of evaluating how certain factors (*e.g.*, stress, infectious agents, diet alterations, changes in gut flora) may affect digestive health. Markers of intestinal permeability (α1-proteinase inhibitor), intestinal inflammation (fecal calprotectin and protein S100A12), enterocyte function (citrulline) and local immunity (immunoglobulin A) have all been evaluated in puppies, and initial studies are promising; altered levels of these markers have been found in puppies with digestive problems (notably CPV), but the results vary with the age and/or breed of the animal. The usefulness of these markers for diagnosis, prognosis and monitoring purposes in puppies with weaning diarrhea is still to be determined, but in future they may play a significant contribution in the approach to this problem.

Metagenomics and metabolomics

The digestive microbiome (intestinal flora) plays an important role in the health of individuals by stimulating the immune system, influencing the structure of the digestive tract, participating in defense against major pathogens, and contributing nutritional benefits to the host (such as the production of short-chain fatty acids). Studying the diversity of the bacterial microbiome is not easy, as a simple bacterial culture will not identify the full spectrum of microorganisms present in an animal's gastrointestinal tract. However, new techniques (mainly based on the sequencing of bacterial ribosomal RNA16S) allow identification of all the intestinal bacteria (microbiota) and better understanding of the complexity of the digestive flora.

In parallel with these studies, new research reports on the interaction between the microbiome and its host, analyzing the bacterial metabolites and those of the host in body fluids such as serum and urine. Known as metabolomics, this technique has identified various problems, including an intestinal dysbiosis associated with an alteration of the overall metabolic profile in adult dogs suffering from acute diarrhea (30), and a modification of the microbiome in dogs that are healthy carriers of *Giardia spp.* (31). Although such techniques are still in the research domain, in future microbiome analysis and metabolomics may be useful to evaluate the digestive health of puppies around weaning.

Conclusion

The quality of a dog's stool may be influenced by the characteristics of the animal itself (breed and age), the presence of enteropathogens (viruses, parasites, bacteria) and the diet (errors in dietary transition or food quality). Weaning diarrhea is thus a complex process resulting from the influence and interaction of different factors, and management of this problem demands a global approach encompassing nutritional, infectious and environmental aspects. Most importantly, prevention of diarrhea at weaning should always involve careful dietary control; highly digestible and rehydratable foods should be offered in order to ensure a harmonious transition between milk and solid food, and rationing to avoid diarrhea from overconsumption is important – the daily ration must be divided into typically four small meals to aid digestion.

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