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EDITORIAL

Although he did not actually use the term, Charles Darwin is generally regarded as the scientist who first recognized the concept of "survival of the fittest", arguing that animals which are best adapted to their environment are most likely to flourish, whilst those with a trait or characteristic which leaves them disadvantaged are liable to perish. If asked to draw up a list of qualities that would seem to be desirable for survival, precocity – the term used to describe species in which the young are relatively mature and mobile shortly after arriving in this world – would seem to be a prerequisite. Birth is essential for life to continue, but it can be a precarious start to an existence, and the sooner the young of any particular species can run, swim or fly, the better they may be at escaping from potential predators – surely a major plus point in the game of survival.

So to the casual observer it might seem that precocial animals have an in-built advantage in the evolutionary tree, and yet many domesticated species are anything but at birth, being very much dependent on others. The opposite of a precocious species is an altricial species – from the Latin *alere*, meaning "to nurse, to rear, or to nourish" – and is typified by puppies and kittens, who are entirely reliant on their dams for warmth, nourishment and protection in the first few weeks of life; indeed, even at two or three months of age they are still susceptible to many potential problems.

And to return to the Darwinian theme, it may be tempting to claim that *Veterinary Focus* is well adapted to the world today, having evolved to succeed where other clinical journals have sometimes failed. In any event, this issue certainly plays its part in supporting altricial species, in that the knowledge it contains will help us as clinicians to look after our youngest patients – because even the fittest need help to survive sometimes.

Ewan McNeill – Editor-in-chief

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HOW I APPROACH...

A young puppy with a heart murmur

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Introduction

Puppy murmurs are a common clinical finding for many practitioners. They are typically detected during routine primary vaccination appointments and are therefore found as "incidental" murmurs, although occasionally some will be found after clinical signs of heart disease are identified. Owners can be very distressed by the diagnosis of a puppy murmur, and the guidance and reassurance of their veterinarian is essential. Knowledge of the differential diagnosis, and the significance and approach for each type of murmur, is needed to help better guide appropriate management in each case. Referral to a cardiologist should always be considered and offered to the owner if a clinically significant murmur is identified.

KEY POINTS

- **Puppy murmurs are often found, but can vary greatly in their significance.**
- **Understanding cardiac anatomy and physiology will assist with localization and timing of murmurs.**
- **Assessment and accurate description of the murmur will enable creation of a differential diagnosis list.**
- **Early identification and management of many congenital cardiac abnormalities result in a better long-term outcome for the patient.**
- **Referral to a cardiologist is warranted with any cardiac murmur to allow for accurate diagnosis using echocardiography.**

What is a murmur?

Murmurs are sound waves created by vibrations caused by turbulent or regurgitant blood flow in the heart or nearby vasculature (the chamber walls, valves and walls of blood vessels). Blood must move at high velocity to produce turbulence or a regurgitation which creates a murmur loud enough to detect. The key is the difference in pressure across two chambers; for example, a mitral regurgitation forces blood into the low pressure left atrium (approximately 10 mmHg) from the high pressure left ventricle (approximately 120 mmHg) in systole, therefore moving blood with an overall pressure difference of 110 mmHg and causing local tissue vibrations which translate into a detectable sound.

It is important to note that shunting of blood through defects may not always cause a murmur. Movement of blood between two low pressure systems will not create a murmur loud enough to hear, for example with an atrial septal defect. Furthermore, some shunts will originate left to right, with the high-pressure differential between the systemic and pulmonary circulations. However, over time chronic overload of the right side can increase right-sided pressures until they equate and then surpass left-sided pressures, resulting in a right to left shunt; this may translate into a dramatic worsening of clinical signs but with loss or significant reduction in the murmur.

What is important in the history and signalment?

Even in routine health check/vaccination appointments, a thorough history must be taken. Should you suspect a

murmur, pay particular attention to the cardiovascular history, including exercise tolerance, resting respiratory rate/effort and any coughing. Check if the puppy has been wormed appropriately for lungworm and heartworm prophylaxis (in countries where this is prevalent). Note if there have been any other concerns with the littermates or whether either of the parents were reported as having heart disease or a murmur. Importantly, note the breed; signalment can be useful in guiding you towards a differential diagnosis, although the rules are not absolute.

How do I diagnose a murmur in a puppy?

Auscultation is a skill learnt during veterinary training, but is honed with practice. Auscultation of puppies can be challenging; they can be wriggly, noisy and very non-compliant, and it may be necessary to try and calm the puppy to enable better examination. Options include lifting the animal from the table and cradling in your arms with the stethoscope placed on the chest ***(Figure 1),*** or giving the puppy a brief feed and then allowing it to relax with the owners before attempting again (as they often then fall asleep).

Understanding cardiac anatomy and physiology can assist with localization and timing of murmurs. Auscultation should include listening to several sites on the thoracic wall (apical and basilar cardiac regions, left and right); again this may be challenging with small puppies. Small-headed stethoscopes (pediatric, neonatal) can enable better localization of sounds and auscultation with both the diaphragm and bell head of the stethoscope may enable clearer detection of a wider range of sound frequencies.

How do I describe a murmur?

If a murmur is identified it is essential to grade, localize and time the murmur wherever possible. This will lay the foundation for your differential diagnosis list. Murmurs are classically described using a grading system of 1-6 ***(Table 1),*** which defines the "loudness" of the murmur in relation to the normal heart sounds. Importantly, grade 5 and 6 murmurs have a concurrent palpable "thrill". Palpation of the thoracic wall must be performed in all cases ***(Figure 2).*** This can be done by placing the flat palms onto the puppy's chest, cranio-ventrally (as though you were about to lift it up) and paying particular attention to the dorsal axillary region.

Murmurs are then further described by their point of maximal intensity; this is the location where the murmur is loudest, or the grade is highest. Typically, this is identified as either left or right and then apical or basilar. Murmurs can also be described using timing within the cardiac cycle, *i.e.* systolic, diastolic or both (known as continuous). The character of a murmur can be added (*e.g.*, soft, harsh, blowing, plateau), although this can be more subjective to define. Finally, radiation of the murmur should be noted; this can be challenging in cases where it is necessary to differentiate between a radiating murmur and a puppy with two separate murmurs. Using these assessments, a final description can be built which can be used to limit differential diagnoses: for example, a classic mitral regurgitation murmur could be described as "grade 3/6 left apical, holosystolic murmur, with radiation cranially and across to the right apex".

Figure 1. Auscultation of murmurs in puppies can be challenging; cradling a puppy can help calm it and enable more accurate assessment.

What other aspects of the physical examination are important?

Murmur assessment is just one aspect of a full and thorough cardiovascular clinical examination. Mucous membrane color should be noted. In a normal healthy puppy

these will be pink, with a brief capillary refill time (less than 2 seconds). Look for cyanosis and include assessment of mucous membranes at both cranial (gingiva) and caudal (vulva or prepuce) sites. Right to left shunting cardiac abnormalities will cause cyanosis, and dependent on the location of the shunt differential cyanosis may be seen (see below).

Respiratory rate/effort and pulmonary auscultation should be noted. Again, with a lively, wriggly puppy it can be more difficult to ascertain, but taking time to allow the puppy to relax (and preferably fall asleep) can allow for better assessment.

Assessment of the pulses should be made routinely, ideally whilst also auscultating the heart to ensure pulses are matching with heart rate. The character of the pulses should be noted, paying particular attention to weak pulses and hyperdynamic, "bounding" pulses (see below).

Assessment of the abdomen should be made to look for organomegaly or evidence of ascites (ballottement should be performed) which can be indicative of right-sided heart failure ***(Figure 3).*** Assess the neck to look for jugular distension, and performing a hepatojugular reflux test (gentle compression of the abdomen whilst assessing for jugular distension) can help this.

Heart rate and rhythm should also be noted, and the details documented. Rhythm is important to assess, and any doubts over this can be followed up with an electrocardiograph (ECG) to confirm presence of a sinus rhythm or to diagnose arrhythmias (see below).

Table 1. Guide for murmur grading assessment.

Grade	Description
1	Intermittent, hard to hear, quieter than heart sounds, very focal
2	Constant, hard to hear, quieter than heart sounds, focal
3	As loud as heart sounds, easy to hear, can be focal
4	Louder than heart sounds, easy to hear, radiates
5	As 4 with palpable thrill
6	As 5 but murmur can be heard with stethoscope away from chest

Figure 2. Palpation for a thrill should be performed in all puppies, with particular attention paid to the dorsal axillary region.

What other diagnostics can I perform?

History and physical examination will be the mainstay of your initial diagnostics, but further investigations include blood pressure measurement, thoracic radiography and an ECG. Referral to a cardiologist for accurate echocardiography, particularly in more complex congenital abnormalities, is recommended.

How do I make a differential diagnosis?

The following is a summary of the most likely differential diagnoses based on murmur description and localization, but the list is not exhaustive, and occasionally murmurs will not follow the "rules". Brief notes regarding treatment are included where appropriate. Finally, very rare complex congenital abnormalities can occur, which can mimic more typical murmurs, and you should always be mindful of that.

Left apex

The left apex defines the region of the mitral valve. At

this location blood moves from the left atrium across the valve into the left ventricle during diastole. During systole the mitral valve closes and blood is ejected from the left ventricle out through the aorta.

Systolic murmurs occur due to regurgitation across the mitral valve. In a puppy, the defect is likely to be congenital and therefore mitral valve dysplasia is considered to be the main differential. Prevalence is similar between purebred and crossbreed dogs (1), although English Bull Terriers and German Shepherd dogs are predisposed (2, 3). Cases of mitral dysplasia may be more likely to show clinical signs (4) when compared to other congenital abnormalities, but this will relate to age at diagnosis, with younger dogs less prone to show signs.

Diastolic murmurs at the left apex are rare and difficult to appreciate. These are filling murmurs, related to the movement of blood from the left atrium to the left ventricle, and are consistent with mitral valve stenosis. This can be an extension of severe mitral valve dysplasia, but can be a defect in its own right, with a narrowed mitral valve orifice causing dramatic increases in left atrial pressures (5). Again English Bull Terriers are thought to be predisposed, and also Newfoundlands. Outcome in these cases is very poor, with a reduced life span (typically around 2-3 years) (6).

In general, when faced with mitral dysplasia and stenosis, the overall treatment will be management of heart failure when or if this develops in the longer term. Arrhythmias such as atrial fibrillation may also develop and therefore rate management control with anti-arrhythmic therapy may be indicated.

Left base

This location covers the valve annulus of both the pulmonary and aortic arteries. Again murmur timing should enable assessment of outflow versus regurgitant murmurs. Systolic murmurs over this region are consistent with turbulence in blood exiting across the valve annulus, leading to a suspicion of aortic or pulmonic stenosis.

Pulmonic stenosis (PS) is the most common congenital heart disease found in dogs (accounting for 32% of all congenital cardiac problems in a recent study (7)) and classically identified as a harsh, ejection-type (crescendo-decrescendo) murmur. PS is caused by either fused (type A) or dysplastic (type B) valves, with or without a hypoplastic annulus (8). It is impossible to differentiate the two types based on auscultation alone, and therefore referral

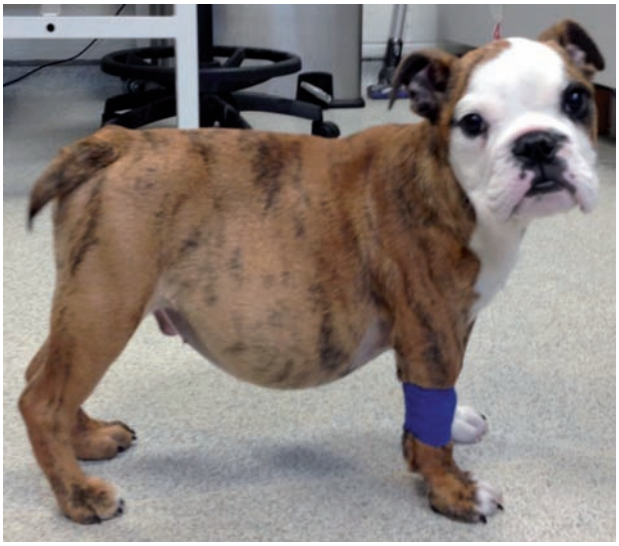


Figure 3. Ascites in an English Bulldog with severe pulmonic stenosis and tricuspid dysplasia.

for echocardiography is essential. Pulmonic stenosis can be graded as mild, moderate or severe, with a worse long-term outcome for those in the latter category (9). Murmur grade can assist in differentiating the degree of severity, but it is defined objectively by pressure gradient across the stenosis via echocardiography. Breed predispositions include Boxers, Bulldogs (English and French) and Staffordshire Bull Terriers (7). Balloon valvuloplasty can be highly successful in type A PS, improving the long-term outcome, but the outlook for severe cases without valvuloplasty is poor (10). Other treatments may include beta-blockers (*e.g.*, atenolol) but these should be discontinued once heart failure occurs. Pulmonic stenosis can also be associated with coronary artery aberrancy (typically in brachycephalic breeds) and angiography must be performed in these cases prior to balloon valvuloplasty to ascertain the coronary anatomy.

Tetralogy of Fallot (pulmonic stenosis, right ventricular hypertrophy, ventricular septal defect and overriding aorta) can present with a murmur of pulmonic stenosis. This is a rare condition (approximately 1% of congenital abnormalities (7)) and should not be diagnosed without echocardiography; cases typically have a guarded long-term prognosis.

Aortic stenosis is identified when there is an increased velocity in blood flow exiting the aorta, caused by obstruction just below the valve (subaortic stenosis, or SAS), at the valve (aortic stenosis), or above the valve (supravalvular stenosis). In dogs, SAS is by far the most

common form and accounts for approximately 20% of all congenital cardiac defects (7). SAS is found more commonly in purebred dogs, with Newfoundlands, Boxers, Bull Terriers, Rottweilers, Golden Retrievers, Dogue de Bordeaux, Irish Terriers and Bouvier des Flandres predisposed (1,7). SAS is defined as mild, moderate or severe based on echocardiographic assessment of pressure differentials across the aortic obstruction. Outcomes in mild cases are good, with a normal predicted lifespan. However, severe aortic stenosis is associated with a poor long-term prognosis (estimated median survival 19 months) and increased risk of sudden death (11). Treatment includes beta-blockers, but again these must be withdrawn should signs of heart failure occur, and whilst this class of drug make physiological sense, there is no evidence to suggest it improves long-term outcome in severe cases (12). Interventional therapy with a cutting balloon valvuloplasty has been described (13), but long-term outcomes following this procedure have not been documented. SAS severity can alter with age, therefore final grading of the condition is typically performed once the patient is skeletally mature (14). However, this should not delay early referral to a cardiologist to confirm diagnosis as timely introduction of beta blockade may be necessary.

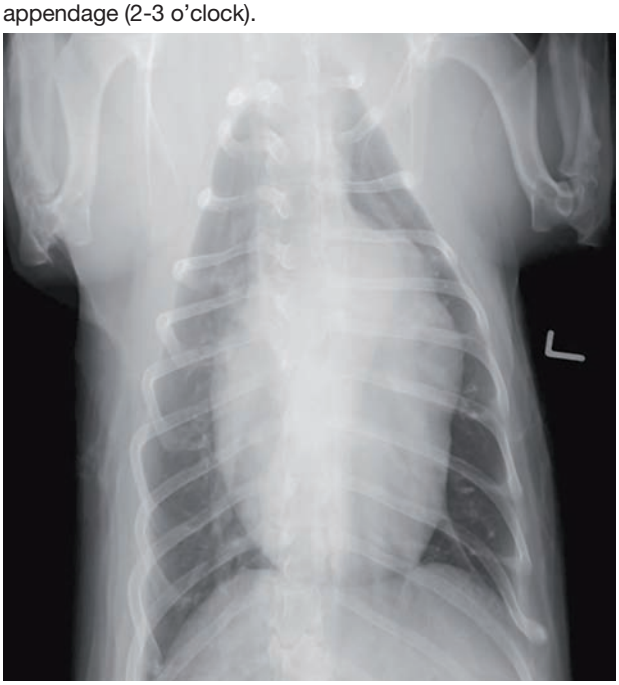
Diastolic murmurs over the left base are consistent with aortic and pulmonic insufficiency. These are rare and difficult to determine. Increased pulmonary pressures, consistent with pulmonary hypertension can cause an audible pulmonary regurgitation murmur if the severity degree is high enough. In this situation the puppy should be investigated for causes of pulmonary hypertension, including parasitic disease. Aortic insufficiency is rare, and may be associated with aortic valvular dysplasia, endocarditis (very rare) or systemic diastolic hypertension.

A continuous murmur at the left heart base is pathognomonic for a patent ductus arteriosus (PDA). This accounts for approximately 20% of congenital cardiac abnormalities (7) with females predisposed (15) and an over-representation of German Shepherd Dogs (7,16). It is important to recognize this murmur, as a significant proportion of these patients can be effectively "cured" by surgery to close the defect. Definitive diagnosis requires echocardiography, although murmur character, bounding pulses and thoracic radiography can be highly suggestive, the latter typically demonstrating a "three knuckle sign" on dorsoventral views consistent with dilation of the ascending aorta, proximal pulmonary artery and the left auricle ***(Figure 4).*** Puppies can initially be asymptomatic, but significant left-sided volume overload occurs over the longer term, leading to dilation and remodeling of the left side of the heart and increased filling pressures. Ultimately, cases will typically go into left-sided congestive heart failure, and long-term prognosis for a PDA is poor without closure. Right to left shunting can also occur, and can usually be characterized by the loss of the previously detected loud murmur and decompensation in clinical signs, with differential cyanosis, pulmonary hypertension and polycythemia. Closure of the PDA is advised and can be done interventionally using specially designed implants by a cardiologist. An alternative is surgical ligation of the ductus via thoracotomy, which can be performed in animals too small to gain access via the vascular system.

Right apex

This identifies the location of the tricuspid valve, and murmurs associated with this region are related to passage of blood from the right atrium to the right ventricle. Typically, these are systolic, regurgitant murmurs and are associated with tricuspid valve dysplasia. This condition accounts for approximately 3% of all canine congenital heart disease cases, with Labrador Retrievers being over-represented (7). In the long term, cases of tricuspid dysplasia can progress to right-sided heart failure, and therefore early recognition of this defect allows for better

Figure 4. Dorsoventral thoracic radiograph of a puppy with a PDA demonstrating the classic "three knuckle" sign, with dilation at the level of the aorta (12-1 o'clock), the main pulmonary artery (1-2 o'clock) and the left auricular appendage (2-3 o'clock).



case management. Diastolic murmurs are not typically detectable due to the low-pressure differentials across this valve in diastole and are therefore a rare finding.

Right base

This identifies the right ventricular wall, and a murmur in this region is typically a left to right shunting ventricular septal defect (VSD). VSD murmurs demonstrate an interesting paradox; the louder they are, the smaller and less clinically significant the defect. Very small defects (restrictive VSDs) allow a small volume of blood to pass through at high velocity, thus causing a loud murmur. Alternatively, a wide VSD allows a large volume of blood to pass and can equate left and right ventricular pressures, thus moving the blood at a slower velocity and causing a much quieter murmur. Clinical signs of a VSD can vary dependent upon the degree of defect; small restrictive VSDs can remain asymptomatic, whereas large VSDs have severe volume overload and progression to heart failure. VSDs are seen in approximately 7.5% of congenital cardiac cases and commonly occur in conjunction with another defect such as pulmonic stenosis (7).

Again, for tricuspid dysplasia and VSD, the overall treatment will be management of heart failure when or if this develops in the longer term. Arrhythmias such as atrial fibrillation may also develop and therefore rate management control with anti-arrhythmic therapy may be indicated.

"Innocent" murmurs

It is worth pointing out that a large proportion of puppies can present with an "innocent" murmur. These are typically low grade (< 3/6), early systolic and with a "musical" quality, localized on the left apical or basilar regions. They are not related to structural heart disease, and are thought to occur due to changes in blood viscosity. Essentially, these murmurs should disappear over time, usually by approximately 20 weeks of age (17).

Conclusion

In summary, puppy murmurs are a common clinical finding, typically presenting incidentally initially, rather than with overt clinical signs. Identifying and describing the murmur will enable better assessment of possible differential diagnoses, and therefore diagnostics and management. In the majority of congenital heart diseases, early identification allows for better long-term outcomes, with some conditions being potentially curable. In other cases there is no real evidence to suggest that any preemptive medical therapy will delay onset of heart failure, but it is generally recommended that owners are made aware of the severity of the heart condition, and this may be guided by referral to a cardiologist and having echocardiography. However, close monitoring for progression to failure by monitoring exercise tolerance and resting respiratory rate and effort is the main short-term objective if a heart condition is not treated at first diagnosis.

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Selected skin disorders of puppies

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Dr Kennis received his DVM from Michigan State University in 1989 and completed a residency in 1993. In 1996 he started at Texas A&M University as an instructor and earned an M.S. degree in the field of immunology. He moved to Auburn University in 2005 and currently holds the rank of Professor. A diplomate of the ACVD, and past president of the AAVD, he has presented continuing education seminars at state, national and international levels and has received several teaching awards. His research interests include food allergy, endocrine alopecia, and feline skin infections.

Introduction

There are many skin disorders that are recognized in puppies, with a variety of etiologies. These include infectious causes, congenital and/or heritable disorders, and autoimmune problems, and specific treatment depends upon an accurate diagnosis. This paper identifies a selected number of skin disorders that are relevant to a worldwide audience, and each problem will be presented in a clinical-based format of signalment, history, clinical signs, differential diagnoses, diagnostic methodology and treatment options.

KEY POINTS

- **Direct impression skin cytology is an important diagnostic tool whenever papules, pustules, crusts or scale are present.**
- **The presence of lymphadenopathy, pyrexia, and anorexia differentiates juvenile cellulitis from impetigo or bacterial folliculitis.**
- **Primary causes of scale are associated with ichthyosis. Golden Retrievers have a unique presentation of ichthyosis which seems to be more prevalent than other forms.**
- **Scale as a clinical sign in puppies may be associated with myriad causes including nutrition, allergies, parasites and infection.**

Impetigo

Impetigo, or puppy pyoderma, is a problem that occurs in very young puppies before puberty. One or several puppies within a litter may be affected, and the lesions appear very quickly, so there is little formative history pertaining to prior treatment. It has been noted that impetigo may be associated with poor nutrition, ectoparasitism, or endoparasitism, but the problem may also be idiopathic. Clinically, an infected puppy presents with pustules, which can range from few to many. The pustules are usually within the glabrous skin of the ventral abdomen, inguinal, and axillary regions, but may also be present elsewhere on the body ***(Figure 1).*** The pustules are usually not associated with the hair follicles (as is common for adult dogs with bacterial folliculitis) and they tend to rupture easily, leaving a small crust or potentially an epidermal collarette. The owner may be helpful in describing the pustules if none are present at the time of presentation. Affected puppies are usually not bothered by the presence of these lesions, and in general they are neither pruritic nor painful. The presence of pruritus would be suggestive of folliculitis due to bacteria or dermatophyte infection. There is usually no enlargement of regional lymph nodes and the puppies are typically afebrile. Additional clinical signs may be present if parasites or nutritional deficiencies are present.

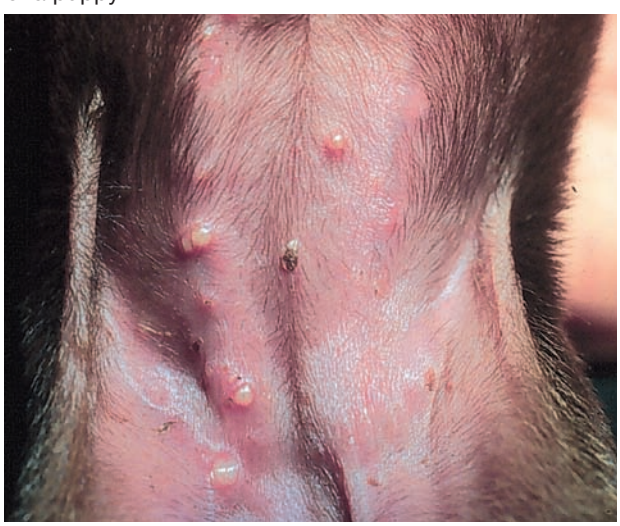
The primary differential diagnoses that should be considered for the presence of pustules include infectious causes such as bacteria, demodicosis, and dermatophytosis. Immune-mediated differentials include juvenile cellulitis (see later) and pemphigus foliaceus; pemphigus would be considered very uncommon in a young dog, but the clinical signs are very similar. Ectoparasitism is

an important differential diagnosis; in particular fire ants (*Solenopsis invicta*) are common in the southern United States and regionally across several continents, and bites from these insects lead to the formation of pustules.

Direct impression skin cytology is the preferred diagnostic technique. A glass slide may be used to gently rupture the pustule before smearing the contents onto the slide. Alternatively, the pustule may be ruptured with a small needle, but care should be taken not to cause bleeding. If only crusts are present, they must be carefully lifted and the slide should be applied to the skin surface. The slides should be air dried before staining with a modified Wright's stain and evaluated, first with the 10X microscope objective and then 100X oil immersion. Numerous cocci bacteria (usually *Staphylococcus spp.*) will be seen in a predominantly neutrophilic inflammatory response in routine cases of impetigo. If acantholytic cells are present, the suspicion of pemphigus will be raised. If fire ants are responsible, bacteria are rarely identified, and — depending upon the stage of the pustule post venom inoculation — only necrotic debris may be seen. Later stages of fire ant bites may reveal a mixed inflammatory cell response with many eosinophils. It would also be prudent to perform a deep skin scraping to check for *Demodex* mites, and a fungal culture to rule out dermatophytosis. Biopsy or bacterial culture is rarely needed for these cases, but a fecal floatation test is recommended to evaluate for concurrent endoparasitism.

Mild cases may spontaneously resolve. Bathing the puppy with a 2-4% chlorhexidine-based shampoo twice weekly until remission is usually adequate; benzoyl peroxide-based shampoos are effective but tend to be too harsh for puppy skin. Individual lesions may be treated with topical chlorhexidine solution or mupirocin ointment twice daily. Severe cases may require oral antibiotics to achieve a remission; an antibiotic with a spectrum of activity against *Staphylococcus spp.* should be selected, but first or third generation cephalosporins, amoxicillin with clavulanate, or clindamycin are good empirical choices. Amoxicillin/ampicillin, fluorinated quinolones, and tetracycline antibiotics should be avoided for myriad reasons. Systemic therapy rarely needs to exceed 14 days unless there is concurrent bacterial folliculitis.

Figure 1. Multiple flaccid pustules on the abdomen of a puppy.



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The prognosis is very good and relapses are uncommon. It is important to identify and treat any underlying conditions which may have been predisposing factors for impetigo. The role of nutrition is very important; feeding a complete and balanced diet formulated for puppies is essential, and probiotics have been suggested to help normalize gut flora and improve immune responses, especially if endoparasitism is identified.

Juvenile cellulitis

Juvenile cellulitis, also known as juvenile pyoderma, juvenile sterile granulomatous dermatitis, or puppy strangles, is a disorder of unknown etiology. It mainly affects puppies less than 4 months of age and is rarely seen in older dogs. There is no breed or sex predilection, although some authors speculate that certain breeds (Gordon Setter, Dachshund, Golden Retriever) may be over-represented. There does not appear to be an infectious cause, even though more than one puppy in a litter may be affected. There has been no conclusive data to support the role of vaccinations in the development of this disorder.

The progression of the disease is somewhat variable but tends to have a pattern to it. Swelling of the face, especially the muzzle and periorbital regions, is first noted. During the early onset of disease, pustules may be identified in the concave aspect of the ear pinnae and may extend down the vertical canal. The pustules rupture quickly, leaving behind crusted lesions ***(Figure 2).*** Similar lesions may be seen on the face including the periorbital, chin, and muzzle regions ***(Figure 3),*** but in some cases pustules are not seen. There is a progression of alopecia, skin induration, and later erosion and ulceration of the affected areas, with the muzzle and chin being most severely affected ***(Figure 4).*** The periorbital regions of the face are similarly affected, and the facial lesions tend

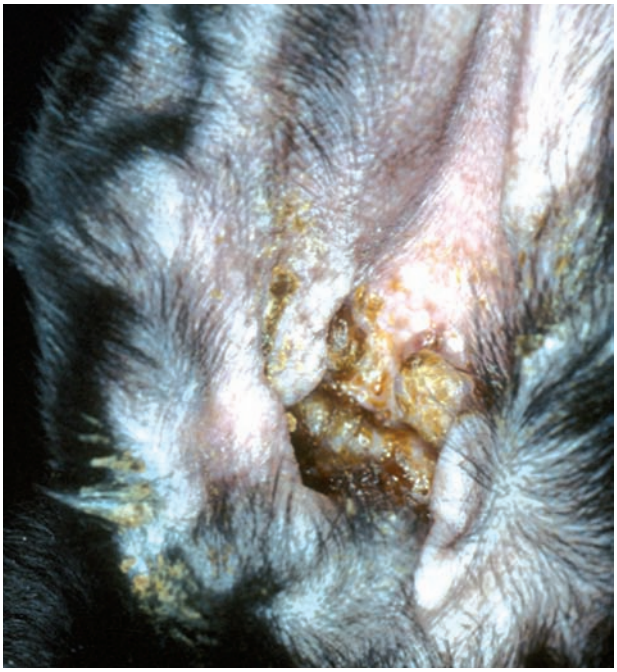

Figure 2. Crusts in the ear of a puppy associated with juvenile cellulitis.

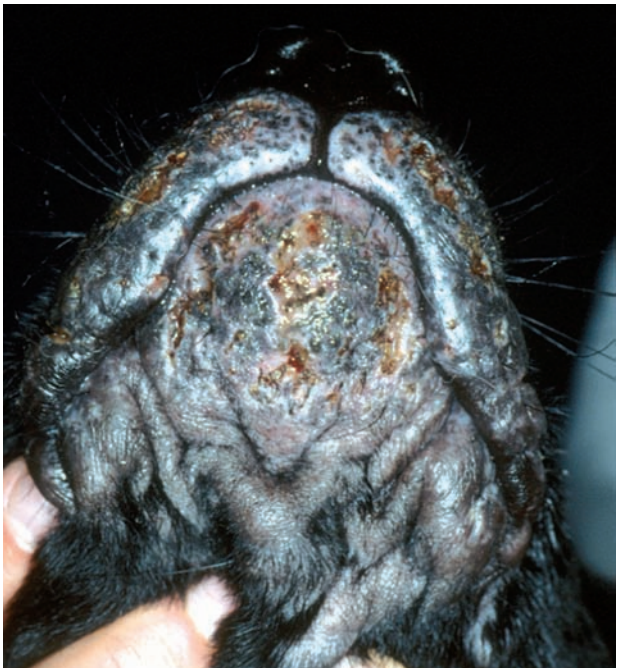

Figure 3. Severe cellulitis on the chin of a puppy.

to be painful. The pinnae may become thickened and warm to the touch, and a variety of lesions can develop; secondary otitis may also occur. As the lesions progress there is usually regional lymph node involvement; the mandibular lymph nodes tend to become symmetrically enlarged and may ulcerate through the skin surface, and pre-scapular and inguinal lymph nodes may also be affected. Sterile panniculitis may be seen within the inguinal and perianal regions, and draining tracts may develop ***(Figure 5).*** These dogs are almost always febrile, inappetent and inactive. Progression of the skin lesions leads to hypo- or hyper-pigmentation. The deep inflammatory reaction (cellulitis) tends to damage the hair follicles, leading to scarring within the affected regions of the face, chin and muzzle.

The primary differential diagnoses that should be considered for the presence of pustules include infectious causes such as bacteria (impetigo or bacterial folliculitis), demodicosis, and dermatophytosis. Immune-mediated differentials include pemphigus foliaceus, lupus-like reactions, vasculitis, and adverse drug eruptions. Neoplasia, especially lymphoma, should be considered due to the rapid progression and lymph node involvement.

A tentative diagnosis can be made based upon signalment and clinical findings. It is important to rule out the aforementioned differential diagnoses, as more than one problem may be present. Direct impression skin samples should be collected to evaluate for bacteria, deep skin scrapings taken to check for demodicosis, and hair samples collected and evaluated for fungal culture. Stained direct impression skin samples will reveal a mixed pyogranulomatous inflammatory response; bacteria are not usually identified. Lymph node aspirates and biopsies should be cytologically evaluated to rule out lymphoma and samples may be collected to confirm juvenile cellulitis. Most cases are diagnosed based on clinical signs and exclusion of the aforementioned diseases. Biopsy with histopathology and bacterial culture would be recommended for refractory cases or those that present at an atypical age.

The treatment of choice is oral prednisone or prednisolone, with a dose considered to be in the “immune suppression” range (1.5-2 mg/kg/day in divided doses). Anti-inflammatory doses (*i.e.*, 0.5-1 mg/kg/day) are inadequate to achieve a remission. Dexamethasone at 0.2 mg/kg/day may be used if the initial response to oral prednisone is inadequate, but injectable steroids should be avoided due to the unpredictability in the duration of effect. Rapid clinical improvement is usually seen within a few days of starting oral steroids; a speedy drop in temperature and an improvement in appetite demonstrating

the correct treatment is being administered. The full steroid dosage should be continued until a remission of the skin lesions is seen, which can take a week or more. The dosage should be tapered gradually and stopped when there is no longer improvement in clinical signs; therapy should not continue any longer than necessary. Relapse is uncommon unless medication is stopped too quickly. The concurrent use of antibiotics is controversial; there is usually an absence of bacterial skin disease, but severe cases may have ulcerated lymph nodes or skin lesions, predisposing them to secondary bacterial infection. The administration of glucocorticoids will suppress both innate and acquired immunity, and the author prefers to recommend oral antibiotics with a spectrum of activity against *Staphylococcus spp.* (as mentioned above in the discussion on impetigo), for the same duration of time that the puppy is receiving glucocorticoid medication. Warm compresses may be applied if panniculitis is present. The lesions on the face are usually very painful so topical therapy should probably be avoided.

The prognosis for resolution is very good, but unfortunately scarring and concomitant alopecia of the most severely affected regions is common, and hyper- or hypo-pigmentation may be a post inflammatory effect. There are no data to support a heritable cause or that the condition is a predisposing factor for additional immune-mediated disorders as an adult dog.

Scaling

Increased scale is a common clinical finding in puppies. The scale may be mild to moderate, dry or greasy, tightly or loosely adherent, localized or generalized. Differentiating primary causes from secondary causes of scaling is of utmost importance in determining a prognosis for resolution.

Primary causes of scale are associated with a group of diseases called ichthyosis or “fish scale disease”. These are both heritable and congenital, and clinical signs are often noted at a very young age, although occasionally signs may not be appreciated until the animal is older. Various molecular defects have been identified in the development of the *stratum corneum* of affected animals. Several breeds have been identified as being susceptible to this condition, including Jack Russel Terriers, Soft-coated Wheaten Terriers, West Highland White Terriers, Cavalier King Charles Spaniels, American Bulldogs and Golden Retrievers, although this is not an exclusive list. There is tremendous variability in the clinical presentation of these breeds including the severity

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Figure 4. Crusts and erosions on the face of a puppy with juvenile cellulitis.

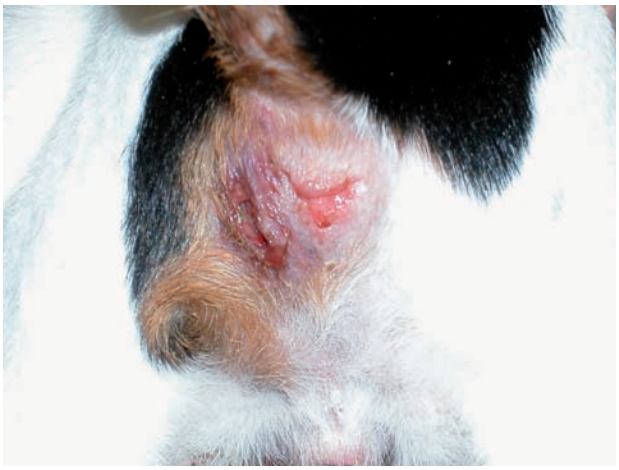

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Figure 5. Panniculitis and draining lesions from a puppy with juvenile cellulitis.

and adherence of the scale, and a full review of these is beyond the scope of this article.

However, the Golden Retriever has a unique presentation of ichthyosis which seems to be more prevalent than other forms. This may be due to the observation that the clinical signs of increased scale in a puppy may be considered to be within normal expectations. Occasionally, clinical signs may not be present until later in life. The scale may be very fine or very large and frequently seen within the hair coat ***(Figure 6).*** The scale is usually not tightly adherent to the skin surface, and the color of the scale may vary from light to dark depending upon the pigmentation of the skin.

A skilled dermatopathologist should evaluate biopsy samples; the diagnosis is made on histopathology observation

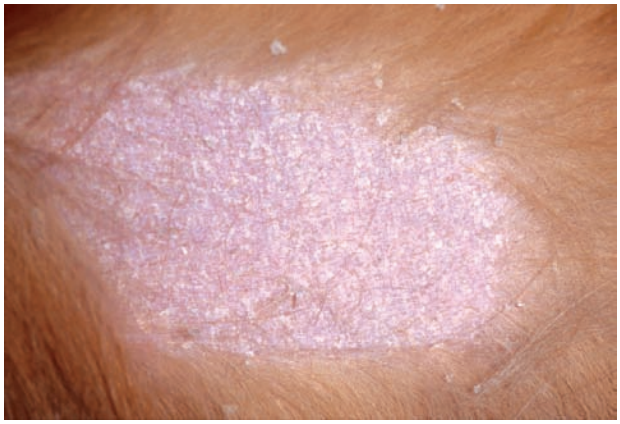


Figure 6. Fine scale associated with ichthyosis in a Golden Retriever dog.

of diffuse lamellar orthokeratosis and an absence of inflammation, but the changes may be subtle and can be missed by a pathologist not skilled in dermatopathology. There is a genetic test available in some countries to help assess carrier status in breeding animals, as this appears to be an autosomal recessive gene. There is no cure, but treatment is aimed at reducing the amount of visible scale present. Over-brushing or frequent bathing, especially with keratolytic shampoos, may exacerbate the problem. Bathing with a mild emollient hypoallergenic shampoo, followed by a moisturizing cream rinse or humectant is usually sufficient. Some products have been developed that aid in repairing the barrier function of the epidermis, and these may be useful as adjunctive therapy.

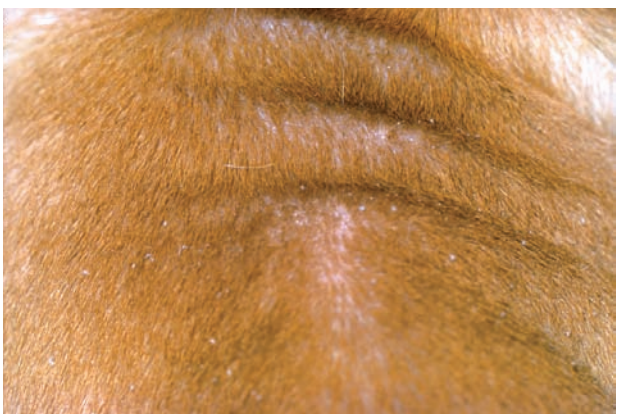
The concept of primary seborrhea is controversial. Seborrhea may be caused by myriad reasons and is often a secondary consequence. Studies have shown some Cocker Spaniels to have increased cell turnover time compared to other breeds, leading to the formation of scale. The scale may be dry (*seborrhea sicca*) or greasy (*seborrhea oleosa*). Many of these dogs will respond to therapy with vitamin A, but other factors may also contribute to the problem, including nutrition, allergies, ectoparasitism, environmental factors, infection, and endocrinopathies. All of these factors should be completely ruled out before claiming that the seborrhea is primary.

History is important when evaluating puppies with increased scale, as nutrition often plays a major role. Diets deficient in omega-6 series fatty acids will lead to a dull, dry hair coat with increased scale. Feeding a high quality puppy diet will lead to marked clinical improvement, although visible changes may not be seen for several weeks as it takes time for the fatty acids to be incorporated into the skin. Endoparasites may play a role in malabsorption of nutrients, and fecal floatation should be a routine test when evaluating a puppy with increased scale formation.

Allergies may be a cause of increased scale but most puppies do not develop allergies until a later age. The exception to this is food allergy, which may occur in puppies less than 6 months of age. Intestinal parasites may play a role in affecting the immune system, leading to the loss of tolerance to food sources. Food-allergic puppies may present with pruritus, gastrointestinal signs, and poor skin and hair coat, and urticaria is occasionally seen. The diagnosis is made by an elimination test diet. The author prefers hydrolyzed protein diets that are nutritionally balanced for all life stages over home-cooked diets, which may not be complete or balanced; this is especially important for puppies. Limited antigen diets may also be used if they are complete and balanced for all life stages (some are not). Any food trial should last at least 8 weeks before determining if the diet has influenced the clinical signs, and a challenge with the original diet should cause signs to return within one week. Offending food items should obviously be avoided. Personal experience has shown that puppies diagnosed with food allergies may develop allergies to additional food items later in life.

The presence of scale is often associated with folliculitis ***(Figure 7).*** Bacteria, *Demodex* mites and dermatophytosis are common culprits, and any puppy presenting with increased scale should have direct impression skin cytology, deep skin scraping, and fungal culture performed. Scale associated with folliculitis may be diffuse or associated with papules, pustules, or epidermal collarettes. Treatment should be aimed at resolving the cause of the

Figure 7. Scale and folliculitis associated with generalized demodicosis in a puppy.



folliculitis; bathing twice weekly with a keratolytic or emollient shampoo may speed resolution.

Malassezia may be a consequence or cause of increased scale. The yeast is frequently found in scaly lesions, especially if they are greasy. These organisms usually cause pruritus with consequent self trauma and inflammation, leading to an up-regulation of cell turnover time. These organisms are easily identified on direct impression skin samples stained with a modified Wright's stain. Alternatively, tape collected samples may be used for dry scaly lesions and hard-to-access areas such as the interdigitial regions, and again stained with a modified Wright's stain, avoiding the fixative step. The tape is then applied to a glass slide for microscopic evaluation, using the 100X oil immersion lens to identify the yeast organisms. Topical therapy with shampoos, sprays, or lotions containing one of the "azole" antifungal medications is usually recommended for puppies. Orally administered azole medications should be reserved for severe or refractory cases, and only given to puppies greater than 12 weeks of age. Topical lime sulfur solution may be used safely on puppies, applied weekly as a rinse until clinical remission is reached; an added feature of lime sulfur is that it is a very good antipruritic agent.

Conclusion

Puppies are prone to many different skin disorders. Although this paper has focused on certain dermatological problems that are more commonly seen in young animals, the clinician will appreciate that other conditions such as bacterial skin problems, demodicosis, and dermatophytosis are highly prevalent in puppies as well as adult dogs. It is therefore essential that a puppy with skin lesions should be approached in the same way as any other problem, and that specific treatment and a successful outcome will depend upon making an accurate diagnosis using a logical methodology that takes into account signalment, history and clinical signs, and involves appropriate diagnostic tests.

Further reading

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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

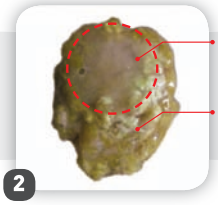
Beyond a subjective analysis of what might classify as a "soft stool", the first difficulty is to define what an abnormal stool actually is. Stool quality can be evaluated using a "puppy fecal score", a visual 13-point scale ***(Figure 1)*** where 1 = liquid stool and 13 = formed and very dry stool (7).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

Fecal scoring system
for puppies

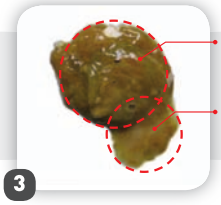
ROYAL CANIN®

Liquid feces

1 Feces completely liquid



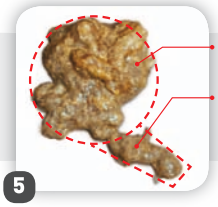
2 Liquid feces associated with soft feces (liquid feces represent the main fraction of feces)



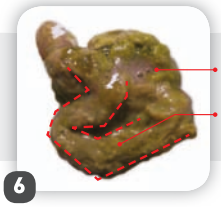
3 Liquid feces associated with soft feces (soft feces represent the main fraction of feces)

Soft unformed feces

4 Pasty, shapeless feces

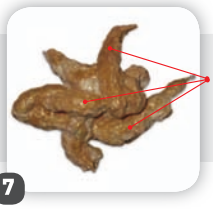


5 Pasty unformed feces. The cylindrical shape of the feces tends to be lost due to the high water content.



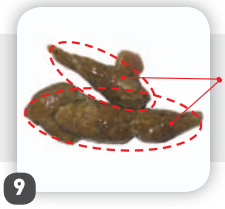
6 Feces mainly unformed with a small formed fraction

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

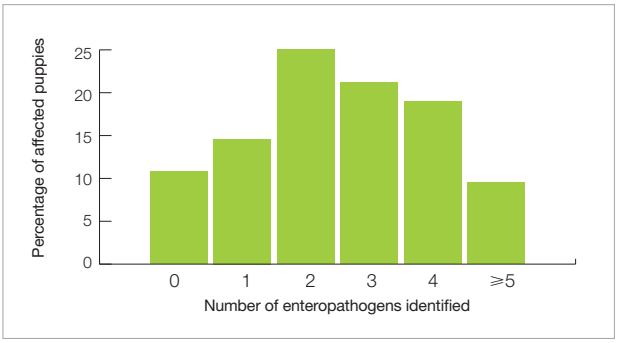


Figure 2. Frequency of co-infections in puppies with diarrhea around weaning.

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)

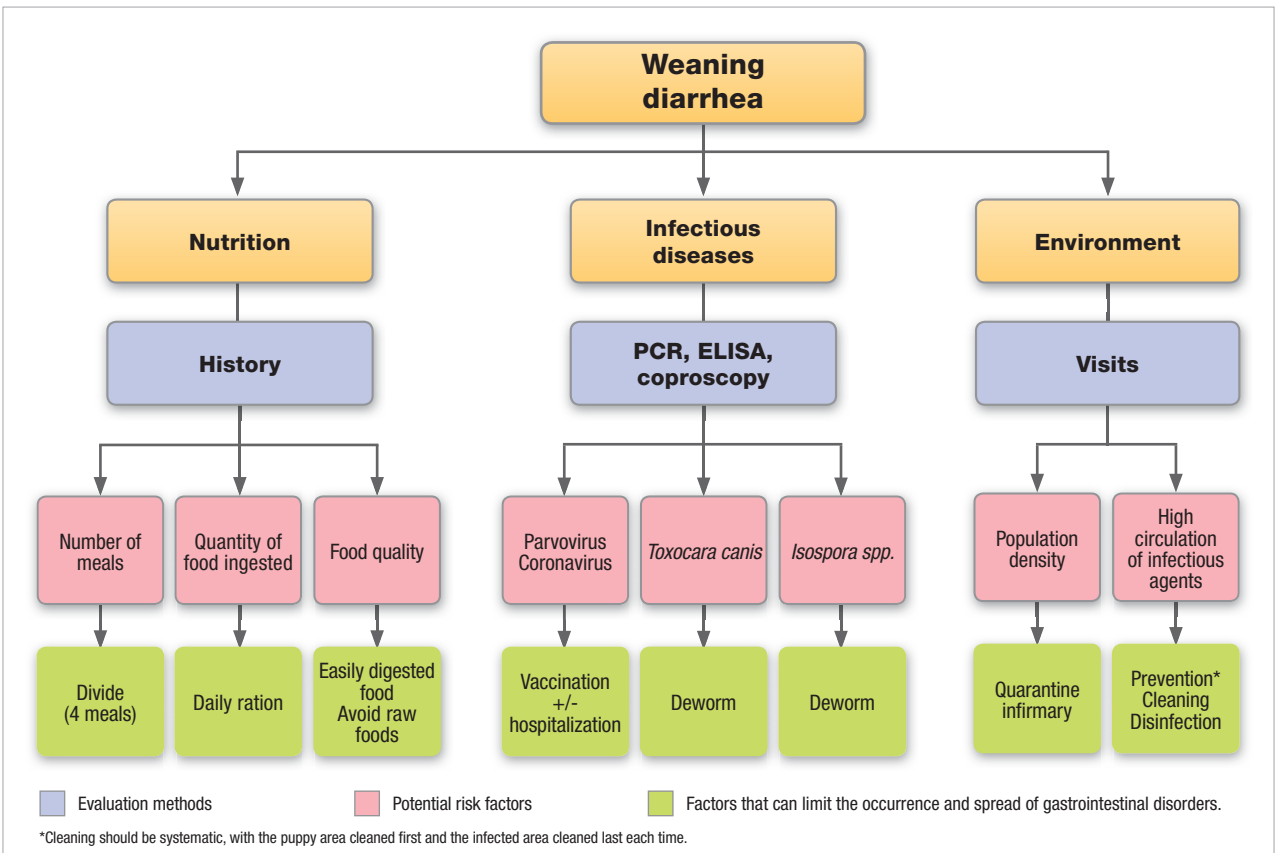
A multi-disciplinary approach is necessary, with evaluation of three major factors: nutrition, causal enteropathogen(s) and the environment ***(Figure 3).***

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).***

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).

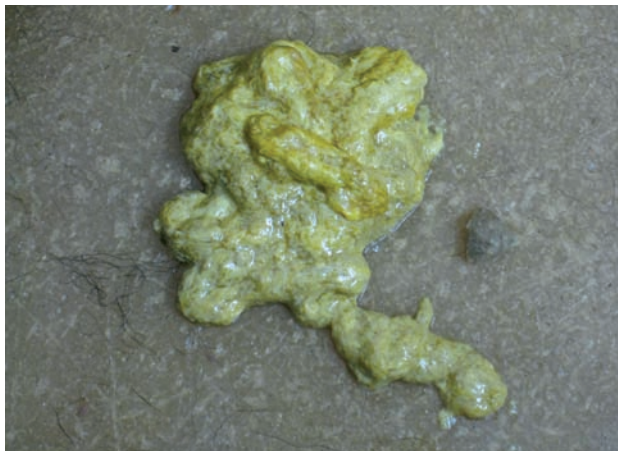


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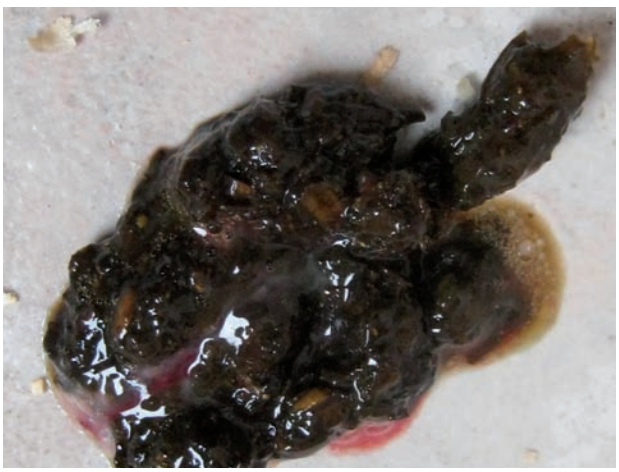
Figure 4. Yellowish feces with a high fat content may suggest infection with *Giardia*.

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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Occurrence of congenital conditions in puppies

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Introduction

The addition of a puppy into a household is a fun and exciting time for new pet owners. Young, playful pets present to the veterinary clinic for routine vaccinations and deworming, and most of these visits are routine and uneventful. However, on occasion the veterinarian may find something out of the ordinary, a congenital condition that may need to be managed or treated. In this report, commonly diagnosed congenital disorders are reviewed and an analysis of the temporal trends for these conditions is presented.

Methods of analysis

The health records of all dogs presented at Banfield Pet Hospital in the first and last years of a 5-year period (2010 and 2014) were screened to identify those that came in as a puppy where a congenital condition was recorded. Puppies were defined as being under 12 months of age on their first visit in each year – thus, a dog that presented in January 2014 at 8 months of age, but re-presented in September at 16 months old, was counted as a puppy for 2014. The congenital disorders diagnosed in puppies are listed in ***Table 1*** by organ system. The prevalence of the top 5 diagnoses and each organ system group for 2014 was identified, and the 2010 prevalence of each condition and system group provided for comparison. An analysis to evaluate observed temporal changes in disease prevalence was performed using a z-test to compare proportions (1).

Table 1. Congenital conditions diagnosed in 2014 at Banfield Pet Hospital.

Organ system category	Congenital conditions in this category
Cardiovascular	Aortic stenosis; Atrial septal defect; Cardiac septal defects; Factor VII deficiency; Hemophilia A, Factor VIII deficiency; Hemophilia B, Factor IX deficiency; Patent ductus arteriosus; Pulmonic stenosis; Tetralogy of Fallot; Ventricular septal defect; Von Willebrand's disease
Endocrine	Dwarfism; Growth hormone deficiency
Gastrointestinal	Cleft palate; Diaphragmatic hernia; Hiatus hernia; Megaesophagus; Megaesophagus, primary; Persistent aortic arch convolutions; Persistent right aortic arch; Pyloric stenosis; Vascular ring anomaly
Neurological	Cerebellar hypoplasia; Deafness, congenital; Hepatic encephalopathy; Hydrocephalus; Nystagmus, congenital; Portosystemic shunt
Reproductive	Cryptorchid (abdominal/inguinal/unspecified), pseudohermaphrodite

*Cryptorchid (non-specified) cases are animals found to be cryptorchid, but not diagnosed as either abdominal or inguinal.

Results

Almost 2.4 million dogs were seen in over 8 million visits at Banfield Pet Hospital in 2014, of which 540,183 (22.5%) were puppies. ***Table 2*** lists the 5 most common congenital disorders noted for this year; the top three diagnoses were cryptorchidism (with 38.3 to 120.9 cases per 10,000 dogs diagnosed with one of these three conditions) followed by congenital deafness and portosystemic shunts. These two conditions were noticeably much rarer, with less than 9 and 3 cases per 10,000 dogs respectively. The top 5 congenital conditions did not change in rank since 2010, although (with the exception of portosystemic shunts) the prevalence for each increased from 2010 to 2014. All of the changes in prevalence were found to be statistically significant.

Reproductive conditions were more commonly diagnosed than any other congenital disorder ***(Table 3).*** Neurological conditions were a distant second and gastrointestinal and cardiovascular conditions even further

third and fourth. The changes since 2010 were found to be statistically significant for the reproductive, gastrointestinal and endocrine categories.

Discussion

Given the ease with which cryptorchidism is diagnosed, it is not surprising that this was the top condition identified. As Banfield hospitals are first opinion practices, it is possible that the other conditions listed in ***Table 1*** are under-diagnosed or under-recorded, as many require referral to a specialist for further diagnostic evaluation. In addition, the review was limited by the standardized list of conditions available in the record system, so if a diagnosis is made but the condition is not listed (or is listed under a different name), the veterinarian may not record the diagnosis appropriately. Given that this study was limited to those cases where a congenital disorder was noted within the first year of life, the calculations may under-estimate the true prevalence for some disorders, as they may not be detected or appropriately diagnosed until the pet is older – the chosen age limit was for ease of data extraction and to ensure diagnosis most likely reflected a congenital origin.

The changes in prevalence estimates may reflect increased or decreased recording of a diagnosis in the Banfield system (although there is no known reason why this should be so), or they could be attributed to improved diagnostic capabilities and/or changes in evaluating breeding quality by some breeders and pet owners. It would seem that the varying prevalence does indeed reflect a genuine alteration in the occurrence of these conditions in young dogs, although the underlying reasons are not apparent.

Table 2. Prevalence estimates for the top 5 congenital conditions found in puppies.

Diagnosis	2014		2010			
	No. of cases	No. of cases per 10,000	No. of cases	No. of cases per 10,000	Prevalence change	*p*-value
Cryptorchidism (non-specified)	6,531	120.9	5,060	92.8	+30.3%	< 0.0001
Cryptorchidism, inguinal	2,513	46.5	2,123	38.9	+19.5%	< 0.0001
Cryptorchidism, abdominal	2,071	38.3	1,881	34.5	+11.0%	0.0009
Deafness,c ongenital	447	8.3	295	5.4	+53.7%	< 0.0001
Portosystemics hunt	126	2.3	200	3.7	-37.8%	< 0.0001

Table 3. Prevalence estimates of the congenital conditions by organ system category.

Organ system category	2014		2010			
	No. of pets	No. of cases per 10,000	No. of pets	No. of cases per 10,000	% Change since 2010	*p*-value
Reproductive*	10,912	202.0	8,861	162.5	+24.3%	< 0.0001
Nervous	719	13.3	689	12.6	+5.6%	0.3270
Gastrointestinal	182	3.4	256	4.7	-27.7%	0.0006
Cardiovascular	141	2.6	150	2.8	-7.1%	0.6557
Endocrine	16	0.3	5	0.1	+200.0%	0.0154

* The total numbers for cryptorchid cases in **Table 2** is slightly more than the total number of dogs diagnosed with reproductive problems in **Table 3,** probably because in some instances a puppy was diagnosed initially as an abdominal cryptorchid, but the testicle descended to the inguinal region as the pup got older, or a non-specified cryptorchid was identified as being abdominal or inguinal at a subsequent visit.

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Anesthesia for cesarean section in the dog

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Introduction

The major goal in anesthesia for cesarean section (CS) is to minimize fetal effects of anesthetic drugs in order to minimize fetal respiratory, central nervous system and cardiovascular depression and deliver live, vigorous puppies. Of equal importance is to provide adequate analgesia to the dam and prevent anesthesia-related complications such as hypotension, hypoventilation, hypoxemia, hemorrhage and hypothermia, which will increase morbidity and mortality in both mother and puppies. The physiochemical properties which allow drugs to cross the blood-brain barrier also facilitate crossing of the placenta, therefore the assumption should be made (with very few exceptions) that anesthetics, analgesics and sedatives/tranquilizers all cross the placenta. Prolonged labor prior to delivery causes maternal physiologic compromise, resulting in fetal depression due to decreased placental perfusion, hypoxemia and acidosis. Maternal and puppy mortality is significantly increased during emergent versus planned CS (1,2). Timing and preparation are extremely important for puppy survival for both elective and emergency CS, and a thorough understanding of the maternal physiologic changes and the potential impact of anesthetic drugs is essential to optimize outcomes for both mother and fetus ***(Figure 1).***

KEY POINTS

- **The major goals for cesarean section are to deliver live, vigorous puppies while providing adequate analgesia to the dam.**
- **There is a higher anesthetic risk with cesarean section due to pregnancy-associated physiologic changes.**
- **Planning and preparation are important for both elective and emergent surgical scenarios.**
- **Optimized ventilation, oxygenation and perfusion in the dam should permit a "happy dam, happy baby" scenario.**
- **Neonatal resuscitation centers on stimulating respiration and supporting oxygenation and body temperature.**
- **Most commonly used analgesic drugs may be safely administered to lactating dams without adversely affecting the neonates.**

Maternal physiologic changes

Increased metabolic demands imposed by the fetus result in major physiologic changes during pregnancy and impact the anesthetic management of these patients ***(Figure 2).*** Much of the data describing these alterations have been obtained in humans and sheep, but should be comparable – if not greater in magnitude – in the dog, since birth weight as a percentage of maternal weight is significantly greater (3). Pregnancy-associated physiologic changes to the cardiac, pulmonary and gastrointestinal (GI) systems are summarized in ***Table 1.*** These result in greater anesthetic risk (due to decreased cardiac and respiratory reserve and a higher chance of vomiting/regurgitation with aspiration) as well as decreased anesthetic requirements (which may put them at risk for anesthetic overdose) (3,4).

Cardiovascular

The growing fetuses increase metabolic demand and maternal oxygen consumption. Increases in heart rate and stroke volume augment cardiac output by 30-40% to meet the demand (3-5), but result in decreased cardiac reserve.

Unlike other major organs, uterine blood flow is not auto-regulated (4). Uterine blood flow and placental perfusion are directly related to systemic blood pressure and inversely proportional to myometrial vascular resistance (3). Decreases in uterine blood flow will result in decreased fetal oxygen delivery. Pain, stress, hyperventilation and some drugs (*e.g.*, alpha-2 agonists) can all decrease cardiac output during labor and contribute to decreased uterine blood flow. Control of pain and anxiety are key components of successful patient management. Care must be taken to avoid cardiac depression from excessive doses of sedatives or anesthetics. In humans, the posterior vena cava and aorta can be compressed during dorsal recumbency which decreases venous return, cardiac output and uterine blood flow. Although less significant in dogs, the amount of time in dorsal recumbency should minimized (3,4).

Maternal blood volume during pregnancy increases by up to ~23% in dogs, along with red blood cells (RBCs) (6), with plasma volume increasing more than RBCs, leading to decreased packed cell volume. This pregnancy-associated anemia increases in magnitude in relation to the number of fetuses (7). Increased blood volume buffers against losses during parturition but can confound the use of packed cell volume as a measure of dehydration preoperatively; other clinical signs may need to be utilized. There is a greater risk of intra-operative hemorrhage due to the increased blood flow to the gravid uterus (20-40 times normal) and mammary glands (5). Intra-operative hemorrhage should be quantified and replaced with 3-4 times the amount in crystalloid solution (up to 10% loss of the total blood volume) to avoid the associated hypotension and decreased uterine blood flow. Colloid therapy should be added if hemorrhage reaches 20%. Hypotension may be treated with ephedrine (administered as a bolus, 0.03-0.1 mg/kg IV); this is the drug of choice in pregnant women since it increases blood pressure while maintaining uterine blood flow, whereas both dopamine and dobutamine decrease uterine blood flow (3,4).

Pulmonary

Tidal volume, respiratory rate and minute ventilation all increase, but functional residual capacity (FRC) decreases due to craniodorsal displacement of the abdominal organs and diaphragm by the gravid uterus (3-5). Decreased FRC leads to small airway closure and atelectasis. The combination of decreased FRC and increased oxygen consumption raises the risk of hypoxemia during periods of hypoventilation or apnea (*e.g.*, during anesthetic induction (3,4)). Pre-oxygenation prior to anesthetic induction is recommended to delay the onset of hypoxemia from approximately 60 seconds to up to five minutes if the patient is tolerant (8).

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Figure 1. Maintaining adequate ventilation, oxygenation and perfusion in the dam will optimize fetal outcome => "Happy dam, Happy baby".

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Figure 2. Pregnancy-associated physiologic changes to the cardiac, pulmonary and gastrointestinal systems result in greater anesthetic risk due to decreased cardiac and respiratory reserve and higher risk of vomiting/regurgitation and aspiration.

Gastrointestinal

Increased serum progesterone decreases lower esophageal sphincter tone, GI motility and gastric emptying, whilst cranial displacement of the stomach increases intra-gastric pressure; together these contribute to a greater risk of regurgitation and aspiration (3,4). Increased gastrin and gastric acid production lowers stomach pH and puts parturient patients at greater risk for aspiration pneumonitis and esophagitis (4). Prophylactic administration of metoclopramide or anti-emetics such as maropitant or ondansetron and/or H_2 receptor antagonists may help ameliorate these effects. Emergent patients are also likely to be inadequately fasted, and rapid IV induction followed by intubation (ensuring the cuff is properly inflated) is recommended.

Passive regurgitation may occur due to increased intra-gastric pressure, exacerbated by positive-pressure ventilation or manipulation of the viscera; if this occurs, esophageal suction and lavage should be performed and 4% sodium bicarbonate infused to increase the pH in the lower esophagus (9).

CNS

The minimum alveolar concentration of inhalant anesthetics decreases up to 40% in parturients (3,4). However, it may be offset by the omission of sedatives/tranquilizer premedication. Inhalant-sparing techniques such as incision blocks, epidural and parenteral analgesia will help avoid high inhalant concentration and reduce fetal cardiopulmonary and CNS depression.

General pharmacology and pregnancy

Placental transfer of drugs has been primarily studied in sheep and laboratory animals, and direct extrapolation to dogs may be misleading due to species differences in placentation, extent of placental metabolism and transport of drug across the placenta (4). However, in general, the same physiochemical properties that allow drugs to cross the blood brain barrier also facilitate their placental transfer. The safest assumption is that most, if not all, drugs cross the placenta and affect the fetus. Elective procedures requiring anesthesia are best avoided during the first trimester (20 days of gestation in dogs), when the fetuses are most vulnerable to teratogenic effects of drugs.

Simple diffusion is the most important mechanism of placental transfer of drugs. Properties favoring transfer include:

Table 1. Pregnancy-associated physiologic changes.

Cardiovascular	⇧Heart rate, stroke volume, cardiac output ⇩Vascular tone, arterial blood pressure ⇧Oxygen consumption ⇧Red blood cells, blood/plasma volume ⇩Packed cell volume/hemoglobin/plasma proteins
Respiratory	⇧Respiratory rate, tidal volume, minute ventilation ⇩Functional residual capacity
Gastrointestinal	⇩Lower esophageal sphincter tone ⇧Intra-gastric pressure/gastric emptying time ⇩GI motility, pH of gastric secretions ⇧Gastrin production
Central Nervous System	⇧Endorphins

- Molecular weight < 600 Da
- High lipid solubility
- Low degree of protein binding
- Non-ionization at maternal blood pH (3,4)

Most anesthetic drugs, with the exception of glycopyrrolate and neuromuscular blocking agents, have MW < 300 Da and are relatively lipid soluble, and thus readily cross the placenta.

Drug protein binding and degree of ionization is determined by its pKa and blood pH, which in turn can affect the distribution between the dam and fetus. As blood pH decreases, acidic drugs such as thiobarbiturates will be less ionized and the fraction of drug bound to protein is decreased, leading to greater clinical effect (3,4). Weakly basic drugs (opioids, local anesthetics) become more highly ionized, resulting in less effect on the dam and fetus (3,4). Redistribution of drugs out of the fetus back into the dam's circulation as maternal plasma levels decrease makes clinical estimation of fetal plasma concentrations difficult. Although ~50% of umbilical vein blood passes through the fetal liver, microsomal enzyme activity and metabolism are minimal (4).

Inhalant agents readily cross the placenta and should be titrated to the lowest level needed to achieve adequate anesthesia. Unlike halothane and methoxyflurane, which rely heavily on metabolism (~20-50% and 50-75% respectively) for elimination, isoflurane and sevoflurane are almost entirely eliminated through the respiratory system. Additionally, the low blood solubility results in rapid clearance from the newborn as long as they are breathing at delivery. It is important to avoid high inhalant concentrations to prevent neonatal respiratory depression and apnea. Isoflurane is associated with improved puppy survival at 7 days compared to methoxyflurane and there is no difference when compared to epidural anesthesia (1).

Anesthetic drugs

- **Anticholinergic agents** such as atropine and glycopyrrolate are primarily used to decrease vagal tone due to opioids or traction on the uterus, or to support fetal heart rate. Choice depends on the desire for placental transfer, since atropine crosses the placental barrier but glycopyrrolate does not. Glycopyrrolate will mitigate the increased vagal tone caused by mu agonist opioids and prevent maternal bradycardia and possible hypotension. It also increases gastric pH and may decrease the severity of chemical pneumonitis should regurgitation and aspiration occur in the dam (3). Fetal bradycardia (< 150 beats/min) indicates fetal distress and is one of the prime indicators for emergency CS (10). Fetal cardiac output is more dependent on heart rate than blood pressure. Atropine may be administered to the dam if the objective is to increase fetal heart rate, which may be low due to hypoxemia, fetal distress or mu agonist opioids. Increasing the fetal heart rate will increase myocardial oxygen consumption in the face of hypoxemia, which may lead to myocardial ischemia, so atropine use is controversial. However, it may support the heart rate long enough to allow delivery and proper resuscitation. It is important to optimize maternal oxygenation, cardiac output and blood pressure, and ensure good ventilation for the puppies after delivery.

- **Tranquilizers/sedatives** are typically avoided due to cardiorespiratory and CNS depression. Xylazine alone or in combination with ketamine is associated with higher fetal death (1). Medetomidine at low doses (< 20 µg/kg) does not increase uterine muscle activity or cause abortion (3), but both medetomidine and dexmedetomidine cause significant decreased cardiac output in the dam due to vasoconstriction and baroreceptor-mediated bradycardia, and drug label recommendations advise against their use in pregnant dogs. Benzodiazepines (diazepam and midazolam) can cause neonatal depression, lethargy, apnea and hypothermia, especially at higher doses. Low dose acepromazine may be an option for very stressed or anxious dogs to avoid decreased uterine blood flow. The drug crosses the placenta slowly due to its higher molecular weight and protein binding, and is not associated with increased maternal or neonatal mortality (2,4), but its α-adrenergic antagonism can cause vasodilation and therefore it should be avoided in dehydrated or compromised patients.

- **Opioids** include mu-receptor agonists such as morphine, hydromorphone, oxymorphone, fentanyl, methadone and meperidine. Buprenorphine is a partial mu agonist and butorphanol is a mu-receptor antagonist and a kappa agonist. These latter two drugs typically produce less sedation and respiratory depression than full mu agonists, but offer less potent analgesia.

Administering opioids to the dam will result in placental transfer, the extent of transfer differing depending on the drug. Less than 10% of buprenorphine is transferred to the fetus, whereas fentanyl (which is highly lipid soluble) crosses the placenta in high amounts, persisting long after maternal clearance (4). Of the commonly used mu agonists, morphine is the least lipid soluble and only 20-30% is non-ionized at normal plasma pH (4). It crosses the placenta less rapidly than more lipid soluble agonists such as fentanyl. The lipid solubility of hydromorphone lies between these two drugs. Neonates are significantly more sensitive to CNS and respiratory depression of opioids due to the immaturity of their CNS, increased permeability of the blood-brain barrier and end-organ sensitivity, and even small changes in ventilation may lead to hypoxemia and increased mortality. Respiratory and CNS depression in newly delivered neonates can be reversed with naloxone.

Anesthetic induction

The use of thiopental, ketamine, xylazine and methoxyflurane have been associated with increased puppy mortality and/or decreased puppy vigor at birth, and are best avoided (1,11,12). Epidural anesthesia/analgesia used alone for CS has minimal fetal effects but has various drawbacks; it is technically difficult, airway protection via intubation is impossible, and there is the risk of hindlimb paralysis, hypotension or urinary retention (if epidural opioids are used).

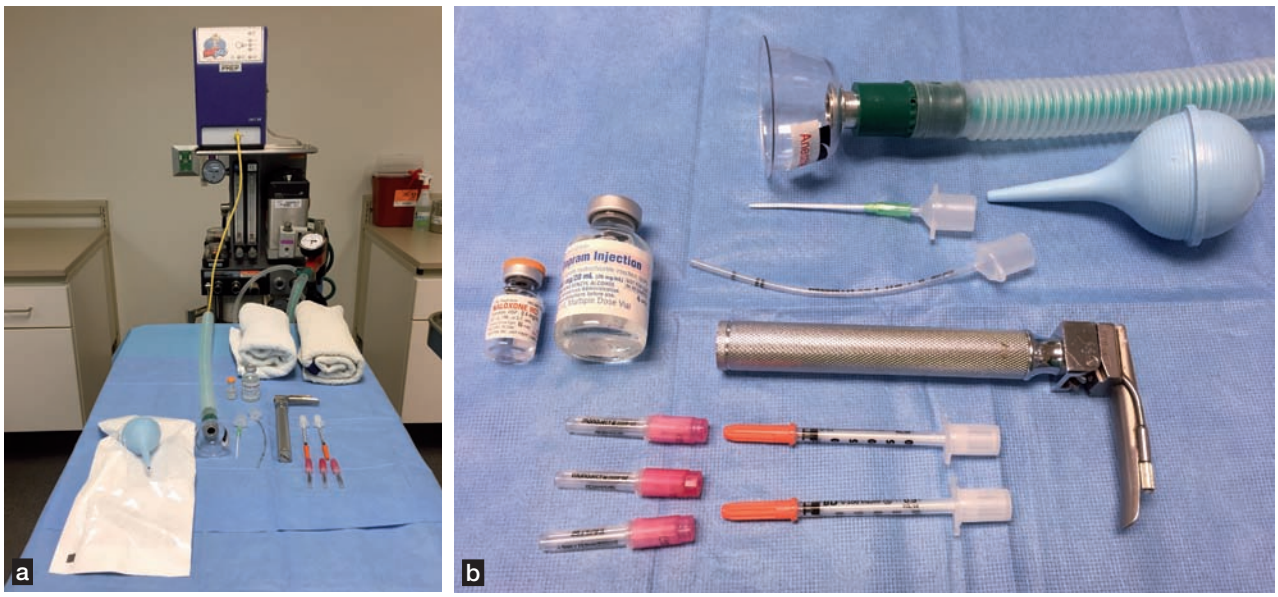

Figure 3 (a and b). Resuscitation equipment and drugs should be organized before induction of anesthesia.

Propofol has a short half-life and rapid metabolism, including extra-hepatic metabolism, but it can cause cardiopulmonary depression depending on the dose and rate of administration. Propofol followed by isoflurane has puppy survival rates equivalent to epidural anesthesia and is associated with a positive effect on neonatal survival at 7 days (1). Propofol has similar fetal mortality rates to mask induction with isoflurane, but it allows IV induction and rapid control and protection of the airway (1,13).

Alphaxalone is available in many countries and has been shown to have a shorter terminal half-life compared to propofol (14,15). Two recent studies comparing propofol to alphaxalone for induction at CS found no significant difference in puppy mortality at 24 hours or up to 3 months after delivery, but both studies identified differences in "puppy vigor". Apgar scores and all four health vigor assessments (withdrawal, sucking, anogenital and flexion reflexes) were greater for puppies for up to 60 minutes post-delivery where the dams received alphaxalone (16,17).

Anesthetic technique

If labor has begun, the puppies are likely to be in some degree of distress and the dam may be exhausted and dehydrated; crystalloid fluids can be given and may be started pre-operatively and continued during surgery to save time. An IV catheter can be placed with the aid of a local block using an insulin syringe with 18 units of lidocaine and 2 units of sodium bicarbonate. Clipping and initial surgical preparation of the abdomen can be done prior to anesthetic induction.

As noted above, the increased risk for regurgitation and aspiration can be decreased using maropitant (1.0 mg/kg SC administered at least 30 minutes prior to opioid administration (18)). For more emergent situations, maropitant may be given (1.0 mg/kg IV over 5 minutes while monitoring blood pressure) and discomfort on injection can be decreased by diluting 50:50 with crystalloid solution. Alternatively, an opioid premedication which does not induce vomiting may be administered (*e.g.*, butorphanol at 0.2-0.3 mg/kg IM or IV). Sedatives, *e.g.*, acepromazine at 2.0-5.0 µg/kg IM or IV, should be reserved for highly stressed dams; however, remember that this drug has a long duration of action and cannot be reversed in either the dam or puppies.

Premedication with an opioid, either butorphanol or a full mu agonist should be administered even to quiet or debilitated dams as it decreases pain, stress and the induction and inhalant dose requirements. Using a low dose may lessen the respiratory and CNS depressant effects on the fetuses/neonates. Anti-cholinergics may be administered depending on the objective(s) as indicated above.

Lumbosacral epidural with either local anesthetic or opioid (alone or in combination) can be administered

Table 2. Neonatal resuscitation equipment list.

- Oxygen source
- Small tight-fitting mask
- Heat source (*e.g.*, circulating water blanket, hot air or electric blankets)
- Clean towels
- Neonatal bulb syringes
- 1mL or insulin syringes
- Small (25G) needles
- Dextrose
- Crystalloid fluids
- Monitoring equipment
- Intubation equipment:
  - Laryngoscope with small blade (size 0-1)
  - Endotracheal tubes (size 2.0-3.0 mm OD, 14G or 18G IV catheter)
- Resuscitation drugs:
  - Naloxone
  - Doxapram
  - Epinephrine

either before or directly after induction to provide anesthesia and analgesia. Lidocaine (2.0-3.0 mg/kg, 0.1-0.15 mL/kg of 20 mg/mL concentration)) will last for ~90 minutes, whilst bupivacaine (0.75-1.5 mg/kg, 0.1-0.2 mL/kg of 7.5 mg/mL concentration) lasts up to 4-6 hours. Both drugs will affect hindlimb motor function and may contribute to intra-operative hypotension through sympathetic blockade. Alternatively, preservative-free morphine may be used alone to provide analgesia (0.1-0.2 mg/kg); the onset of action may take up to 60 minutes, but motor function is not affected. Epidural opioids may cause urinary retention and – since early hospital discharge is advantageous for healthy dam and puppies – owners must monitor urine output for 24 hours after discharge. The combination of lidocaine (2.0 mg/kg) and morphine (0.1 mg/kg) provides rapid onset of anesthesia along with synergistic and long-lasting analgesia. Epidural drug administration requires much lower doses than the same drugs given parenterally and therefore decreases systemic effects in the dam and puppies. Alternatively, the epidural can be performed after the pups are delivered and the incision is closed to provide post-operative analgesia.

Pre-oxygenation of the dam (100 mL/kg/min by face mask for 3 minutes prior to induction) will prevent hypoxemia associated with hypoventilation and apnea at induction. Propofol or alphaxalone can be used for anesthesia induction, followed by maintenance with isoflurane or sevoflurane. Close monitoring of anesthetic depth is recommended to titrate inhalant to the lowest level that will provide adequate anesthesia in the dam. Monitoring of ECG, blood pressure, pulse oximetry and end-tidal carbon dioxide is recommended to ensure adequate oxygenation, ventilation and perfusion in the dam.

If local anesthetic epidural is not performed, a lidocaine block along the line of incision (at 2 mg/kg, diluted with sterile water if necessary to increase the volume) will provide intra-operative analgesia. Bupivacaine may be used (1.5-2.0 mg/kg) for an incisional block at the time of closure of the *linea alba* for longer post-operative analgesia. Lidocaine and bupivacaine mixtures have a decreased duration of action and are not recommended (19).

If butorphanol is used as a premedication, stronger opioid analgesics such as hydromorphone (0.05-0.1 mg/kg IV) or morphine (0.5-1.0 mg/kg slowly IV) can be administered once the puppies are delivered. Alternatively buprenorphine (0.01-0.02 mg/kg IV) will result in less sedation, bradycardia and respiratory depression than the full mu agonist, and also offers a much longer duration of action (4-10 hours in dogs), but the cost may be significantly higher. Milk transfer has not been analyzed for opioids, NSAIDs or local anesthetics in lactating dogs. However, breast milk transfer of opioids has been well studied in humans and although most opioids are excreted in low concentrations in human milk they do not pose a significant neonate risk unless large or repeated doses are administered (20). NSAID safety profiles have not been evaluated in puppies less than 4-6 weeks of age, but carprofen is poorly excreted and undetectable (< 25 ng/mL) in the milk of lactating dairy cattle (21).

Excretion of lidocaine and bupivacaine and/or their metabolites does occur but there is minimal effect on human neonates (22) and milk concentrations of ropivacaine are lower than for other local anesthetics (23). Although species differences may occur, based on current evidence it seems that most commonly used analgesics may be safely administered to lactating dams without adversely affecting the neonates.

Neonate resuscitation

Resuscitation equipment and drugs should be organized before induction of anesthesia ***(Table 2, Figure 3)*** with (ideally) one assistant per neonate delivered. Resuscitation should center on tactile stimulation of

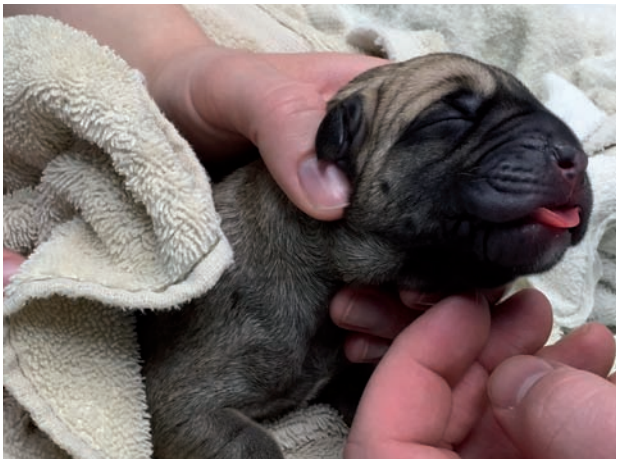

Figure 4. Rub the newborn pup vigorously with clean, warm towels to stimulate respiration.

Figure 5. Suction of fluid from mouth and pharynx can be accomplished with a neonatal bulb syringe.

Figure 6. The JenChung GV26 acupuncture point used to stimulate respiration; a 25G needle is inserted into the nasal philtrum until it contacts bone and then twisted.

respiration and maintaining or improving ventilation, oxygenation and body temperature. During the normal birth process through the pelvic canal, the neonate's chest is compressed, expelling fluid from the respiratory tract and stimulating the first breath through recoil of the chest wall. This does not occur during cesarean delivery, therefore immediately after delivery the fetal membranes should be removed, the umbilical cord clamped and severed, and the neonate rubbed vigorously with clean towels to encourage respiration ***(Figure 4).*** Stimulation of the perineal and umbilical areas, and rubbing of the hair in a backward direction, may also benefit respiration. At the same time, a suction bulb should be used to clear the mucous and fluid from the nose, mouth and pharynx ***(Figure 5).*** A proprietary aspiration/resuscitation device is available in some countries which clears the respiratory tract and stimulates the respiratory reflex. Acupuncture can be useful; a 25G needle inserted into the nasal philtrum until it contacts bone (the JenChung GV26 point) and then twisted can help stimulate respiration ***(Figure 6).*** The practice of "swinging" newborns to facilitate resuscitation or clearing of fluid from the respiratory tract is not recommended and increases the likelihood of injury (24).

Spontaneous breathing should be identified by observing the chest wall, listening for vocalization, or auscultation with a stethoscope. The two major causes of fetal depression are hypoxemia and drugs administered to the dam. Vigorous rubbing should continue along with oxygen supplementation and gentle chest compressions. Opioids administered to the dam should be reversed in the neonate with naloxone (0.002-0.02 mg/kg IV or 1-2 drops sublingually) after delivery if the pups are slow to begin breathing, moving and vocalizing. Neonatal heart rates should be ~220 bpm and can be counted by palpating a precordial pulse. Bradycardia usually indicates hypoxemia and should be treated by stimulating ventilation, supplemental oxygen, patient warming and mechanical stimulation as described above. Doxapram (1-2 drops sublingually) can be used as a respiratory stimulant, but it also increases cerebral oxygen consumption and should only be used with oxygen supplementation. There is no evidence against employing the drug in dogs, but its use has been discontinued in humans. If spontaneous breathing is still not observed, the pup should be intubated using a short-bladed laryngoscope and (depending on the size/breed) a flexible 14 or 18G IV catheter or 2.0-3.0 OD endotracheal tube; careful handling is necessary to avoid traumatizing the delicate neonatal tissues. Severe

bradycardia or asystole may be treated with epinephrine (0.1 µg/kg) diluted with 0.5 mL of crystalloid fluid, and administered though the umbilical vein (identified as the thin-walled vessel within the umbilical stump; umbilical arteries have thicker walls). Thermoregulation reflexes are underdeveloped in neonates, therefore as soon as the neonate is breathing, moving and vocalizing, they should be placed in a warmed incubator.

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Canine colostrum

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Introduction

The neonatal period is a major risk period in the dog, since approximately 20% of live-born puppies die before they are 21 days old; 70% of deaths are in the first week post-partum (1,2). Puppy survival within the early weeks is particularly dependent on colostrum, a specific secretion of the mammary gland produced during the first two days post-partum. Colostrum is both a source of nutrients and a source of immunoglobulins (Ig), as puppies are almost agammaglobulinemic at birth. The risk of neonatal mortality therefore depends on two factors: the quality of the transfer of passive immunity (evaluated by circulating IgG levels at 2 days of age) and the growth of the puppy between birth and 2 days old (at worst, weight loss should not be more than 4% of birth weight) (3,4). The immunity and energy supplied to the puppy by colostrum is therefore essential, but there is no guarantee that all puppies in a litter will receive sufficient colostrum; at two days of age, about 20% of puppies have a passive immunity deficit and 30% show insufficient early growth (3,4).

KEY POINTS

- **Colostrum is crucial for puppy survival, providing both immunoglobulins and nutrition for the newborn.**
- **The immunoglobulin concentration in colostrum in the first two days post-partum is five times greater than milk, but the levels drop very quickly with time.**
- **Colostrum's immunological quality varies from one dam to another, and also in the same dog between teats. The teats offering the highest-quality colostrum will also vary between bitches.**
- **Monitoring growth over the first two days of life is a good indicator for predicting puppy survival in the neonatal period.**
- **There is currently no complete (energy + immunity) substitute for canine colostrum.**

Colostrum formation and composition

Colostrum is the first mammary secretion produced after delivery (and is occasionally present before parturition), with the transition to milk occurring between day two and three of lactation ***(Table 1).*** The actual quantity of colostrum produced by a lactating bitch is unknown.

During gestation, the mammary tissue develops under the influence of estrogens and progesterone, and secretion – induced by prolactin – is only possible when progesterone levels drop. Some colostrum compounds are synthesized by the epithelial mammary cells (proteins, lactose, lipids) while others, such as immunoglobulins (Ig), white blood cells, hormones and certain growth factors, are taken from the maternal bloodstream. Macroscopically, colostrum is yellowish and more viscous than milk. Qualitatively speaking, it is distinguished from milk essentially by its high protein concentration (twice that of milk secreted two weeks post-partum, being especially rich in immunoglobulins), a slightly higher lipid concentration

Table 1. A comparison of colostrum and milk composition in the lactating bitch ((5) and unpublished data).

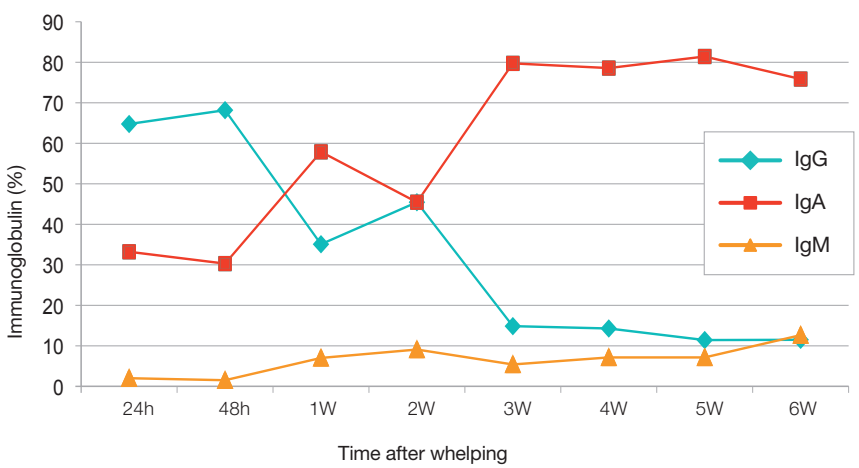
	Days of lactation				
	1	3	7	14	21
Nutrients	**Colostrum**	**Milk**	**Milk**	**Milk**	**Milk**
Proteins (g/L)	143.0	102.3	81.7	66.8	68.4
Immunoglobulin G (g/L)	23.8	*	5.9	0.6	0.6
Lipids (g/L)	132.2	137.2	132.1	118.5	112.5
Lactose (g/L)	16.6	29.3	35.4	39.9	39.4
Calcium (mg/L)	1,363	1,366	1,773	1,950	1,929
Phosphorus (mg/L)	935	914	1,166	1,175	1,359
Energy (kcal/L)	1,831	1,761	1,657	1,493	1,444

* value unknown

(10% more) and a lower carbohydrate content (half that of milk) (5,6). For various reasons (a paucity of studies, considerable variability between dogs, and disparity in analytical methods), measurement of key components of colostrum differ between studies; protein levels are typically between 4-14%, lipid levels between 6-13%, and carbohydrate levels between 1.7-2.3% (5,7,8 and unpublished data).

In addition to casein (60% of total proteins), immunoglobulins represent between 20-37% of colostral proteins (6,7,9,10). Three classes of immunoglobulin (IgG, IgM and IgA) are present in canine colostrum (with IgG, at 60-75% of the total, predominating) whilst IgE is undetectable. IgG in the colostrum is initially around 15-30 g/L but it falls very quickly, dropping to ~5 g/L on day 7 and less than 1 g/L on day 14 (unpublished data). The IgG concentration in milk is therefore 20 times less than that of colostrum. IgA represents 16-40% of colostral Ig but subsequently becomes the most common immunoglobulin in milk (7,10) ***(Figure 1).*** Most IgG comes from the mother's serum, although a small fraction is produced locally in the mammary gland (11). The mammary gland is responsible for concentrating IgG, such that colostral levels are typically 3 times higher than in the maternal bloodstream, although there is no relation between the colostral concentration of IgG and the maternal serum concentration (10,12). This selective concentration is under endocrine control, with the Ig stored in the mammary alveoli until its release after parturition (13). On the other hand, most IgA and IgM appear to be produced locally in the mammary gland by lymphocytes (13).

Figure 1. Immunoglobulin levels in the colostrum and milk; IgG, IgA and IgM were recorded from the mammary secretions of six Rottweiler dams (7).



Trypsin inhibitors are also found in the colostrum (but not in the milk), reducing degradation of colostral Ig and potentially increasing its absorption by the newborn (14). Colostrum also contains antimicrobial factors (such as lactoferrin and lysozyme), hormones (cortisol, thyroxine, insulin and growth hormone) and growth factors (*e.g.*, insulin-like growth factors, epidermal growth factor and nerve growth factor (15)). These are involved in the development and maturation of various organs such as the thyroid and intestines, as well as being vital for general puppy growth (see below).

Canine colostrum has high levels of two enzymes, gamma-glutamyl transferase and alkaline phosphatase, respectively 100 times and 10 times more concentrated than in maternal serum (16). These two chemicals are essentially absent from the circulating blood at birth, and so detection of these enzymes in a puppy's serum will confirm ingestion of colostrum (although the enzyme levels do not correlate to the IgG concentration).

Finally, canine colostrum also contains various cells including macrophages, neutrophils and lymphocytes. These cells are absorbed by the puppy before the intestinal barrier closes, and either enter the circulation, or play a role in cellular, humoral or local digestive immunity (17).

Roles of canine colostrum

Immunological protection

The endotheliochorial placenta in dogs is almost completely impermeable to large-sized molecules such as immunoglobulins. This explains why puppies are born with low circulating levels (around 0.3 g/L) of IgG, as opposed to 8-25 g/L in adult dogs (3,18,19). Colostral intake allows the acquisition of passive immunity, such that a neonate's IgG serum concentration will be in the order of 6 g/L 48 hours after ingesting colostrum; 85-95% of a puppy's circulating Ig is thus of colostral origin (20). The provision of Ig, which is potentiated by colostral anti-trypsins, is the most specific role for colostrum and is the determining factor for puppy survival (3), as most neonate mortality is due to infection (21). The colostral lactoferrin seems to play a marginal role in a puppy's immunity (22) while the role of immune cells contained in colostrum is still not well defined. For passive immunity to be acquired, puppies must receive colostrum within the first eight hours of life ***(Figure 2).*** This time frame is critical for two reasons:

- Firstly, colostral IgG decreases rapidly in the first few hours post-partum.
- Secondly, the rapid closure of the intestinal barrier; this is the point at which macromolecules (including IgG) can no longer cross the intestinal wall to enter the bloodstream, so that while a puppy absorbs ~ 40% of ingested colostral IgG at birth, only 20% is absorbed four hours after delivery and 9% twelve hours after delivery. 24 hours post-partum, absorption is nil (20).

The immunological quality of the colostrum, in terms of IgG concentration, is quite variable, both between female dogs and between teat pairs of the same female ***(Figure 3).*** In one study looking at the colostrum of 44 female dogs from 13 different breeds in a single breeding kennel, the IgG levels varied between females by a factor of 5; neither the dam's age or breed size, nor the litter size, appeared to influence the colostrum's immunological quality (12). The IgG concentration in 180 samples from different teat pairs varied between 0.8 and 61 g/L, with a variation coefficient of 42% between teat pairs of the same bitch (12). However, the teat pair producing the highest-quality colostrum varies from one animal to another, so there is no value in advising puppies should suckle from one particular teat. Nevertheless, the marked variation in immunological quality between dams (and between the teats of the same female) may mean that certain litters have an increased risk of neonatal mortality.

The colostrum supplies most of the IgG for systemic immunity, while IgA ensures local and digestive immunity, and in particular mucosal immunity. Colostral IgA is involved in local defense of the digestive tract and this role is continued with the ingestion of milk, which is rich in IgA. Other than the fraction absorbed into the bloodstream before closure of the intestinal barrier, IgA is

Figure 2. It is important to encourage early colostral intake – within the first eight hours of birth – for optimal transfer of passive immunity.

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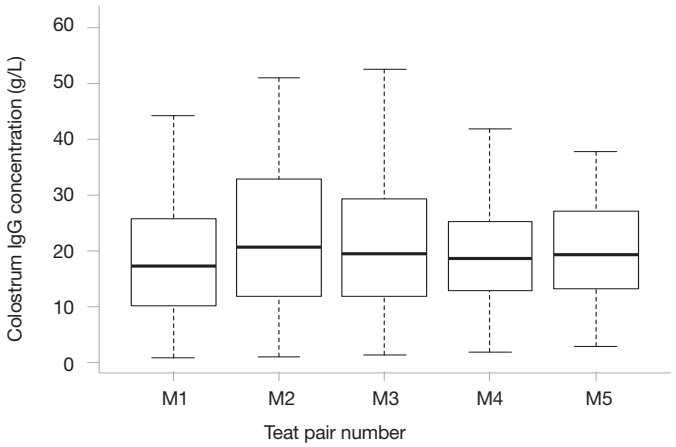
Figure 3. Immunological quality of colostrum according to number of teat pairs (12).

Box plot of the IgG concentration of the colostrum in 44 female dogs. Each box represents 1st and 3rd quartiles (25th and 75th percentiles, or 50% of the population studied). The bar in each box represents the median, and the whiskers represent the 1st and 9th decile (10th and 9th percentile). M1 indicates the axillary teat pair and M5 the inguinal pair. The medians are not significantly different – indicating only one pair number does not systematically produce colostrum of higher immunological quality – but the whiskers are very long, reflecting the large variability between the same teat pairs in different dogs.



distributed throughout both digestive and non-digestive mucosal sites (20,23).

Although transfer of maternal Ig helps reduce neonatal mortality, at the end of the pediatric period, when a puppy is 6-8 weeks of age, maternal immunity can interfere with puppy vaccination. The higher the IgG concentration acquired by two days of age, the higher it is during the pediatric period (24), and this increases the risk that a puppy may not be protected after vaccination. However, the interference is variable, being dependent on the individual animal, the immunogenicity of the vaccine, and the dose given.

Growth

Puppies have low reserves of adipose tissue at birth, and have limited glycogenolysis ability. The early energy supply from colostrum is therefore indispensable; growth is only possible if the energy supplied exceeds the puppy's maintenance requirements ***(Figure 4).***

The energy value of colostrum is at least 20% greater than milk, although the energy content can vary between dams (albeit within a fairly small range, by a factor of 1.6) and there can be slight differences between teat pairs of the same dog (a variation coefficient of around 8%, as opposed to 42% for the immunological value). Age, breed and litter size have not been shown to affect the energy value. 52% of the energy supplied by the colostrum is protein and 40% comes from lipids; variations in the energy value are principally explained by variations in the lipid levels (25).

Whilst the immunoglobulins and energy supplied from colostrum influence the risk of puppy mortality during the neonatal period (3,4) it is interesting to note that the immunological quality and energy value of colostrum are not correlated (28). In addition, the quantity of average colostrum that must be ingested for satisfactory immunity is 1.3 mL per 100 g of puppy body weight (assuming the puppy's IgG serum levels reach 2.3 g/L, with a digestive absorption rate of 40%, a hematocrit of 35%, and IgG levels in the colostrum of 20 g/L). In contrast, the average quantity of ingested colostrum required to cover energy needs is much higher, at 12 mL per 100 g of puppy body weight (energy need of 212 kcal/kg per day if the colostrum supplies 1800 kcal/L).

Figure 4. The growth of a puppy over the first two days of life has a direct impact on its chances of survival. Weight loss should not exceed 4% of birth weight.

Although it seems much more difficult to cover the energy need than the immunological need, a slightly higher proportion of puppies are deficient in energy (30%) than in Ig (20%) ***(Figure 5).*** Whilst the threshold levels of colostral IgG and energy required to control neonatal mortality have been determined in some species, they are currently unknown for the dog.

Organ development

In addition to growth, colostrum is also involved in the development and maturation of certain organs, in particular the digestive tract. This is linked to colostral hormones and growth factors. One study reported that puppies fed colostrum had gastrointestinal tracts 60-95% better developed when compared to puppies of the same body weight given a synthetic milk formulation (26), although other studies do not consistently observe this (27).

Induction of colostrum production and release

Sometimes a dam produces little or no colostrum at birth or immediately post-partum. This agalactia may be as a result of premature birth, cesarean section, endotoxemia or malnutrition, but it is most commonly due to post-partum stress, particularly in primiparous females. A quiet, calm area should always be provided at whelping, and in some cases medication may be recommended for anxious dams to encourage colostrum production ***(Table 2).***

Alternatives to colostrum

When the dam is absent or does not produce enough colostrum, it is essential to source a substitute in order to limit neonatal mortality. At the very least an energy supply must be provided for the puppies, but a supply of immunoglobulins is also desirable. The ideal scenario is to have another bitch who has given birth less than 2-3 days previously, and either make her adopt the puppies or draw colostrum from her to administer to the puppies. If an adoptive dam whelped more than 2-3 days previously, her milk will ensure sufficient energy supply (since the energy value of milk is only 20% less than that of colostrum), but the supply of IgG will be insufficient: milk contains only 1-2 g/L of IgG compared to 20 g/L in colostrum, so a puppy will require 13-26 mL of milk to supply the same quantity of IgG obtained from 1 mL of colostrum. Data is not available for puppies, but kittens fed with milk from a foster cat had no significant transfer of IgG (28). Formula milks are void of canine immunoglobulins and have an energy concentration of about 1 kcal/mL (*i.e.*, half that of colostrum) (29) and so again ensure a nutritional supply but no immunological supply.

Conversely, serum drawn from an adult dog will contain immunoglobulins, but at a concentration around 3 times less than that of colostrum, and will have only a limited energy value. Trials administering canine serum orally at birth to puppies deprived of colostrum showed an increase in circulating IgG, but at a much lower level than that obtained from standard colostrum (18,19).

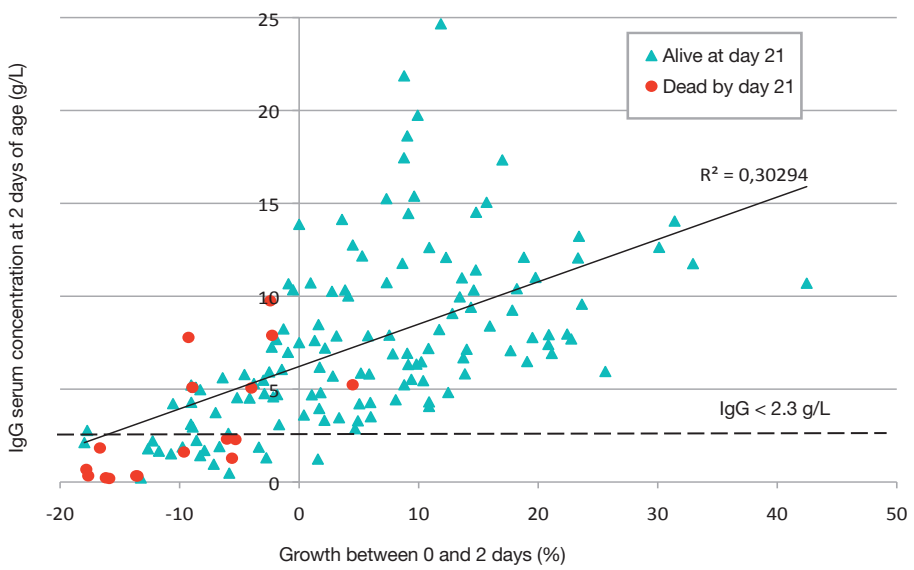


Figure 5. Growth and transfer of passive immunity are key indicators for puppy survival. The graph represents data from 149 puppies; 18 died before day 21 (red dots) and 131 were alive at day 21 (green triangles). The thresholds for survival during the two first days of life are weight loss of less than 4% of birthweight (approximated here to a nil growth) and a serum IgG level at the age of two days above 2.3 g/L.

Graph adapted from (3) and (4)

Table 2. Medical and other treatments indicated for agalactia in the bitch.

Medication	Effect	Dosage
Acepromazine	Tranquilization; favors release of prolactin and increases secretion of colostrum	0.1-0.2 mg/kg SC
Metoclopramide	Release of prolactin	0.1-0.2 mg/kg PO or SC q8h
Aglepristone	Reduces progesterone levels, and hence encourages prolactin release	15 mg/kg SC 59-60 days post-ovulation. Administration is only recommended 20-24h before cesarean section
Oxytocin	Local action stimulating the release of colostrum, but not its production	0.5-2 I.U. SC q2h
Fenugreek or fennel supplementation	Stimulates milk secretion but mechanism unknown	Oral administration; optimum dose unknown

However, one study (18) showed that oral administration of canine serum at birth to puppies deprived of colostrum did result in reasonable IgG levels. This suggests that, in some puppies at least, administering serum may ensure that the minimum protective IgG concentration (*i.e.*, 2.3 g/L) is reached.

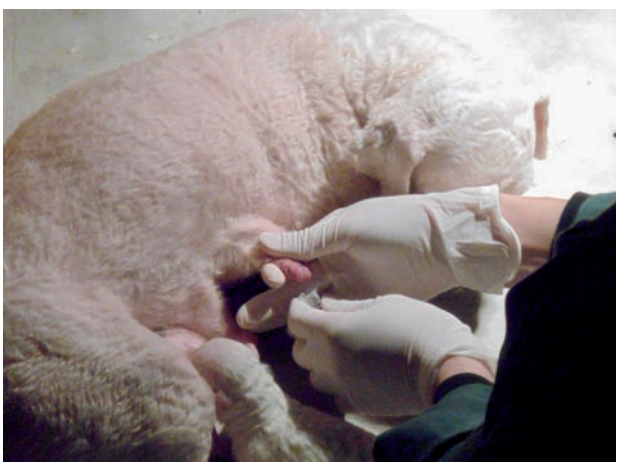
Currently bovine colostrum as a source of heterologous immunoglobulins is of interest, as it is easy to collect and readily available, but it has yet to be evaluated in puppies for its immunological or nutritional value. Another source of abundant Ig is IgY (from immunized hen eggs), with recent work showing that serum containing specific antibodies against canine pathogens (*E.coli* and CPV2) obtained from hyper-immunized eggs can be administered to puppies to provide immunity; the authors trialed administration of oral IgY to puppies before closure of the intestinal barrier and obtained promising results in terms of overall health, with improved growth over the first three weeks of life (unpublished data).

In the absence of an ideal substitute, the only solution currently available is to establish a colostrum bank, as currently practised for cattle and horses. Breeders can draw milk from a bitch on the second day post-partum (which ensures her own puppies have acquired passive immunity). Generally, milking a lactating bitch is easy; after cleaning the skin with a chlorhexidine-based soap the colostrum can be collected in small-volume plastic tubes and frozen ***(Figure 6).*** Small quantities of colostrum can then be thawed (at 37°C (98.6°F); under no circumstances should a microwave oven be used) as necessary and administered by bottle or feeding tube at a dose of 1.5 mL per 100 g of puppy body weight per day.

Conclusion

Canine colostrum is a secretion with a very particular composition designed to meet a puppy's specific needs – namely, provision of passive immunity, energy and certain factors required for organ growth and differentiation. The quantity of colostrum received may be a limiting factor in the survival of certain puppies of a litter, whilst the impact of maternal nutrition on the quantity and quality of colostrum produced remains to be explored. From a practical point of view the development of a colostral

Figure 6. If a dam cannot supply colostrum to her litter, colostrum can be drawn from another bitch between 24 and 48 hours after she has whelped, ensuring her own litter has received immunity but before the Ig levels have dropped.



substitute or supplement which can provide immunological support effective against canine pathogens as well as an energy supply would constitute a crucial advance in controlling neonatal mortality in puppies.

Acknowledgements

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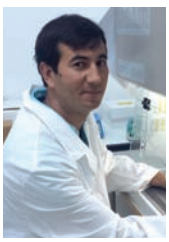
Canine parvovirus



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Introduction

Canine parvovirus (CPV) is a small, non-enveloped virus ***(Figure 1)*** consisting of a spherical capsid (composed of three proteins, VP1, VP2 and VP3) containing a linear, single-strand DNA molecule that encodes for two non-structural (NS1 and NS2) and two structural (VP1 and VP2) proteins. VP2 is the major capsid protein and is responsible for virus antigenicity (1,2). The nomenclature of the family *Parvoviridae* has been recently revised, with CPV being included in the unique species *Carnivore proto-parvovirus 1* along with feline panleukopenia virus (FPLV) and other related carnivore parvoviruses (3).

KEY POINTS

- **Canine parvovirus is the main cause of acute gastroenteritis in young puppies and is found worldwide.**
- **Three antigenic variants have completely replaced the original strain, with distribution varying according to geographic area.**
- **Typical clinical signs include vomiting, hemorrhagic diarrhea, and leukopenia; mortality rates can reach up to 60-70% in infected kennels and shelters.**
- **In-clinic assays for diagnosis are poorly sensitive, and additional testing using PCR-based methods may be required.**
- **Treatment is mainly supportive therapy, although several antiviral agents have been tested.**
- **Vaccination of puppies is still the most effective strategy to control infection, despite possible interference from maternally derived antibodies and suspected mismatching between vaccine viruses and field strains.**

CPV is the main cause of acute gastroenteritis in puppies between one and six months of age. Although recognized since the late 1970s, the virus still represents a major threat to young dogs due to the severity of clinical signs and interference with active immunization by maternally derived antibodies (MDA) that can impair a vaccination program (1,2). Another drawback for disease control is the circulation of field variants (CPV-2a, CPV-2b, CPV-2c) that are antigenically distinct from the original CPV-2 strain, which is still contained in most commercial vaccines. There are only a few amino acid changes between CPV-2 and its antigenic variants, but it has been suggested that vaccination may offer only partial protection which may expose vaccinated dogs to infection by field strains and can sometimes lead to onset of overt disease (4-6). The increased occurrence of the disease in adult dogs (4,5) and the ability of the antigenic variants to infect cats, inducing clinical signs identical to feline panleukopenia (7,8), are emerging issues that need to be confronted. This review will focus on the clinical, pathological and diagnostic aspects of CPV infection, with a brief overview of the current epidemiological situation in different countries and recommended vaccination protocols.

Epidemiology

The original CPV-2 strain emerged in the late 1970s, probably as an FPLV host variant through previous adaptation in an unknown wild carnivore species. In the early 1980s, the original virus was suddenly replaced by two antigenic variants, CPV-2a and CPV-2b, through 5 or 6 amino acid substitutions in the capsid protein VP2, and a third variant, CPV-2c, was reported in Italy in 2000 (9).

Currently the original CPV-2 strain, which is still present in most vaccine formulations, is no longer circulating in the field, whereas the three antigenic variants are variously distributed worldwide. In continental Europe the variants seem to co-circulate, with a prevalence of types 2a and

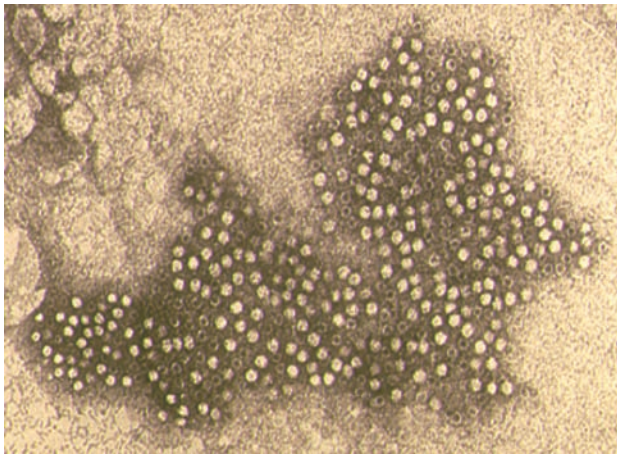

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Figure 1. Electron microscopy image of negatively stained parvovirus particles (X25,000 magnification).

2b in Portugal, France and Belgium, types 2a and 2c in Italy, type 2a in Eastern Europe, and type 2c in the Iberian Peninsula, with the three variants equally distributed in Germany. North and South America display a high frequency of CPV-2b/2c and CPV2a/2c, respectively; in Asia and isolated islands, such as the UK, Australia and Japan, types 2a and 2b predominate (1,2). The few reports from Africa indicate a co-circulation of the three strains in the north of the continent and a high frequency of CPV-2a and 2b in the south (10).

CPV is able to infect domestic dogs, wolves and other wild carnivores, from which viruses intermediate between CPV-2 and CPV-2a have been frequently isolated (11). The original CPV-2 strain could infect feline cells *in vitro* but not *in vivo*; in contrast, the new antigenic variants can infect cats, inducing a disease indistinguishable from feline panleukopenia (7,8). Theoretically, there is no breed susceptibility to CPV infection. Large breeds, such as German Shepherds, Labrador Retrievers, Rottweilers, Alaskan Malamutes, and Doberman Pinchers, seem to be at increased risk, but this could be due to the fact that MDA levels decline more quickly in rapidly growing large-breed puppies than with smaller-sized dogs (1,2). In addition, although CPV infection and disease occur mainly in puppies less than 6 months of age, severe clinical signs in adult dogs, often associated with CPV-2c infection, are being reported with increased frequency (5,6).

The feces of infected puppies are the main source of the virus in the environment; the virus is exceptionally stable and can remain infectious for several weeks or even months. Naïve puppies are infected through the oronasal route by direct or indirect contact (1,2).

Pathogenesis

The target tissues for viral replication are the intestinal crypts and the lymphoid organs, but the virus can spread to all tissues. After penetration into the animal, CPV replicates primarily in the lymphoid tissue associated with the oropharynx, thymus, mesenteric lymph nodes and Peyer's patches, causing extensive necrosis before spreading to the bloodstream mainly by means of infected lymphocytes. Viremia is long lasting (the virus can be detected for up to 60 days by real-time PCR) and leads to colonization of the small intestine crypts, where active virus replication induces rapid lysis of the stem cells. The direct consequence is impaired turnover of the epithelium of the villous tips, leading to diarrhea. Virus shedding through the feces begins 2-3 days after infection and lasts up to 45-50 days, but high titers can only be recovered in the first 7-10 days. In newborn puppies (up to 2-3 weeks of age), CPV is able to reproduce in actively replicating myocardial cells, causing severe myocarditis, although this is currently only observed sporadically (1,2). In comparison with the original type 2, the antigenic variants display higher pathogenicity, a shorter incubation period (less than 4-5 days), more severe clinical signs, greater extent and duration of viral shedding, and lower amounts of virus needed to infect dogs (12). Concurrent infections with canine coronavirus (CCoV) may exacerbate clinical signs, with CPV and CCoV infecting the epithelium of the intestinal crypts and villous tips, respectively (1,2).

Clinical signs and pathology

As noted above, the incubation period for the original CPV-2 strain was up to 7 days, while the new variants usually require only 3-4 days before clinical signs appear. Depending on the age and immune status of an infected dog, CPV infection can cause different clinical forms, spanning from subclinical infections to acute gastroenteritis and (very rarely) myocarditis.

Subclinical infections

Subclinical infections usually occur in puppies with intermediate levels of MDA (hemagglutination inhibition antibody titers between 1:20 and 1:80) that protect against overt disease but not against infection. Differing MDA levels among puppies of the same litter may explain why some puppies can display severe clinical forms and others show few or no signs. Adult dogs may be also infected and show no or few clinical signs, due to the higher maturity of the intestinal mucosa. Sometimes only vague signs may be noted, *e.g.*, lethargy and loss of appetite for 2-3 days, along with transient moderate

leukopenia. Subclinical infections assume particular importance in kennels and animal shelters, where the presence of healthy but infected animals may favor the spreading of the virus to other puppies (1,2,12).

Gastroenteric form

This is the most frequently observed form induced by CPV infection in puppies between one and six months of age, although there are an increasing number of reports in adult dogs. After 3-4 days incubation, puppies develop anorexia, depression and vomiting, followed by diarrhea, leading to severe dehydration. Diarrhea is often, but not always, hemorrhagic, with feces being streaked or darkened by blood. Fever (40-41°C/104-105.8°F) may be present, but is not always seen. In contrast, leukopenia is frequently observed, with white blood cell (WBC) counts dropping below 2000-3000 cells/µL. Note that total WBC counts may be normal, with the virus-induced lymphopenia set against a concomitant neutrophilia from opportunistic bacterial infections. These bacteria frequently exacerbate the clinical course of the disease, inducing additional signs such as respiratory distress, leading to death. The degree of leukopenia is recognized as a prognostic factor; it has been reported that puppies with WBC counts below 1000 cells/µL are unlikely to survive. Death can occur as early as two days after the onset of clinical signs as a consequence of bacterial dissemination or disseminated intravascular coagulation. Mortality rates may vary greatly depending on the age and immunological status of the animal; adult dogs usually show less than 1% mortality (1,2).

Puppies dying from CPV enteritis are extremely dehydrated. At post-mortem examination gross lesions are evident in the gastroenteric tract, mainly involving the duodenum and subsequently the jejunum. The most common finding is hemorrhagic gastroenteritis ***(Figure 2);*** the intestinal wall is usually thickened and segmentally discolored, and the serosal surface can be dark red or purple and may be covered with fibrin. The gut can be completely empty or may contain dark (often bloody) material or hemorrhagic fluid. Mesenteric lymph nodes and Peyer's patches are enlarged and congested, often with hemorrhages scattered through the cortex and cut surface ***(Figure 3).*** Histopathologically, the small intestine is affected by multifocal crypt necrosis and intranuclear inclusion bodies, whereas extensive depletion of lymphocytes is seen in Peyer's patches, lymph nodes, spleen and thymus. Pulmonary edema and alveolitis can be observed when there are bacterial complications (1,2).

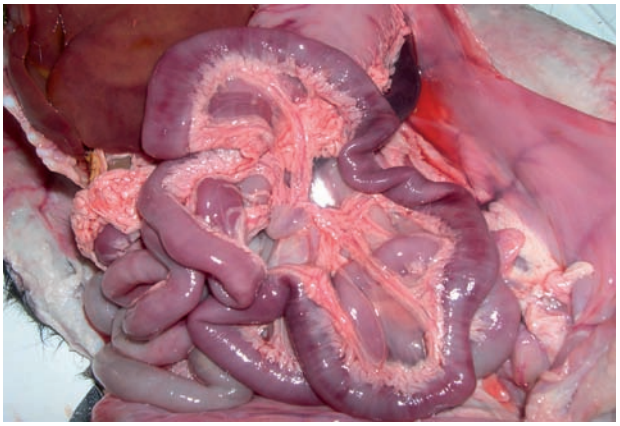

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Figure 2. Congested loops of small intestine in a puppy that died from CPV enteritis.

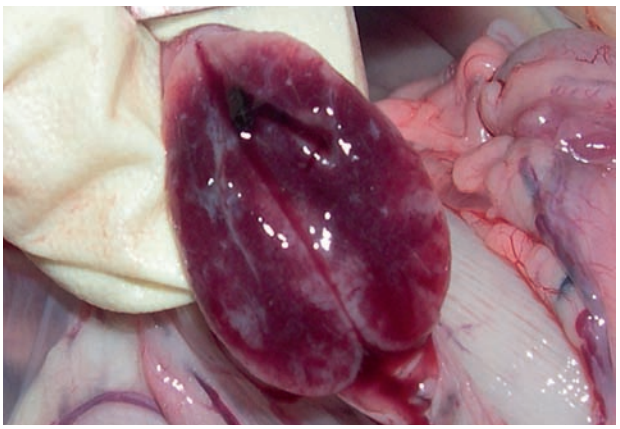

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Figure 3. The mesenteric lymph node from a puppy that died from CPV enteritis: note the hemorrhagic lymphadenitis.

Myocardial form

Acute myocarditis was a common finding during the first worldwide CPV epizootics when infection involved a naïve dog population, but currently this form is only sporadically observed in the field. In fact, CPV-induced myocarditis can occur only in puppies less than 3-4 weeks of age, when the myocardial syncytium is actively replicating and is susceptible to virus replication. Nowadays, however, since the majority of bitches have been vaccinated (or exposed to the virus) and have developed a strong immune response, nearly all puppies receive MDA from their dams which protects them from parvovirus infection during the first weeks of life.

CPV myocarditis is characterized by the sudden death of infected puppies; in some circumstances, death is preceded by gastroenteric signs and a short episode of dyspnea, crying and retching. Some animals may be clinically healthy, and cardiac pathology is evident only

on electrocardiography; in this situation the virus predisposes dogs to degenerative heart disease, and heart failure may develop weeks or months later. Puppies that recover from CPV myocarditis develop myocardial fibrosis. Dogs dying from the myocardial form are often in good condition and sometimes the only gross finding at post-mortem is pulmonary edema. In other cases, the affected heart exhibits flaccid walls and dilated chambers, with pale necrotic areas on the surface ***(Figure 4).*** Histopathologically, the myocardial lesions include non-suppurative myocarditis, multifocal infiltration of lymphocytes and plasma cells, and the presence of intranuclear inclusion bodies (1,2).

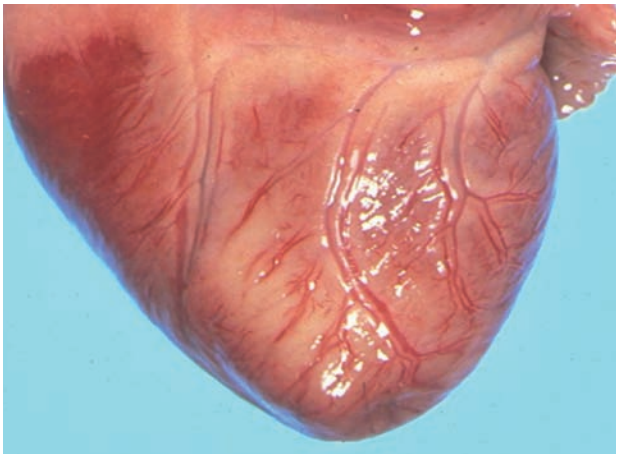

Figure 4. The heart of a puppy that died from CPV myocarditis: note the infarcted area.

Diagnostic approach

Diagnosis of CPV infection is often based simply on the presence of foul-smelling and bloody diarrhea, but it must be emphasized that other pathogens can induce similar findings and that CPV-related enteritis is frequently non-hemorrhagic. Therefore, a laboratory diagnosis is always needed to either confirm or rule out CPV infection (1,2).

Clinical diagnosis

The presence of vomiting and hemorrhagic diarrhea, in the presence of acute leukopenia, is highly suggestive of CPV infection. However, differential diagnoses include canine distemper, infectious canine hepatitis, enteric parasitosis and other alimentary disorders. CCoV usually causes non-hemorrhagic enteritis, but under certain circumstances this pathogen can cause hemorrhagic diarrhea, and hypervirulent strains (pantropic CCoV) have been associated with systemic disease and leukopenia (13).

Virological diagnosis

Direct detection of the virus can be carried out on the feces of ill dogs or on post-mortem tissues (gut, spleen, lymph nodes). In later stages of infection, blood is the most reliable sample due to the long-term viremia. The virus has been found at high levels in all tissues, including the brain, although maximal titers are reached in lymphoid tissues (14).

There are several in-clinic commercial assays for detection of CPV in the feces. These tests detect (with equal efficacy) the three antigenic variants and even the related FPL virus. However, they are poorly sensitive, missing up to 50-60% of CPV positive samples, especially in the later stages of infection when the amount of virus shed in the feces is low and/or high CPV antibody titers in the gut lumen suppresses viable virus production (15,16). Hemagglutination (HA) and virus isolation tests can be performed only in specialized laboratories, and do not present significantly higher sensitivity than in-clinic testing (17). In contrast, PCR-based methods that detect viral DNA are very sensitive and should be employed at least where there is a high suspicion of parvovirus but the puppy is negative with in-clinic testing (18). In addition, PCR assays have been developed to discriminate between the CPV variants (19), as well as between vaccine and field viruses (20-22), which can be useful if controversies arise between dog owners, veterinarians and vaccine companies when diarrhea occurs within a few days of CPV vaccination. In fact, commercially available vaccines contain modified-live viruses that replicate in the intestinal epithelium of vaccinated dogs; the virus is shed in the feces (albeit at low titers and for a shorter time period with respect to field strains (23)) and this can lead to detection of CPV in the feces of vaccinated dogs and misdiagnosis, when clinical signs are in fact from other enteric pathogens. Moreover, PCR assays are useful to rule out the suggestion that a vaccine virus has reverted to virulence if an animal develops acute gastroenteritis shortly after vaccination.

Serological diagnosis

Despite the existence of several assays, serological testing has no diagnostic value. In fact, specific serum antibodies may be unrelated to active CPV infection if the dog has been vaccinated or had previous exposure to the virus. However, serological assays are useful to assess a dog's immunological status with respect to CPV before and after vaccination, and by identifying the decline in MDA an assay can help calculate when a

puppy can be vaccinated without interference from MDA. Serological testing is also essential to assess whether a dog has responded to vaccination or not. The most commonly used serological test is hemagglutination-inhibition (HI), which requires specialized personnel and substrates, but only virus neutralization (VN) can detect protective antibodies, and this latter test has been extensively used to evaluate the cross-neutralization between vaccine and field viruses (1,12).

Therapeutic approach

Albeit supportive and non-specific, treatment is frequently beneficial in reducing mortality associated with CPV infection. The primary goal of therapy for CPV-induced enteritis is to restore fluids and electrolytic balance and prevent concurrent infection by opportunistic bacteria. Intravenous fluid therapy with Ringer's solution supplemented with glucose and potassium will counter hypoglycemia and hypokalemia. If electrolytes and serum blood glucose concentration cannot be routinely monitored, empirical supplementation of IV fluids with potassium chloride (20-40 mEq/L) and dextrose (2.5%-5%) is appropriate. Parenteral anti-emetic drugs (*e.g.*, chlorpromazine, acepromazine, prochlorperazine, metoclopramide, ondansetron, dolasetron and maropitant) can help reduce fluid loss and patient distress, thus facilitating enteral nutrition. However, note that α-adrenergic antagonists may exacerbate hypotension in hypovolemic puppies, whilst prokinetics may increase the risk of intussusception. Gastric protectants and H_2 blockers (cimetidine, ranitidine) may also be beneficial. Broad-spectrum antimicrobials should be administered to prevent or treat secondary infections. A combination of penicillin and aminoglycoside antibiotics represents the best approach to control the gram-negative aerobic and anaerobic bacterial infections that frequently complicate canine parvovirosis. Third-generation cephalosporins are preferred to nephrotoxic aminoglycosides in nephropathic patients, and quinolones should be avoided in growing dogs. If vomiting has subsided for 12-24 hours, withholding food and water in affected puppies is not recommended, since there is evidence for a faster recovery when animals are fed with easily digestible commercial or homemade food (24). Puppies with anorexia should be fed a suitable diet through naso-esophageal or nasogastric tubes. Whole-blood or plasma transfusion can help correct blood and protein losses from the severe enteritis (1,12). No specific drug has been demonstrated to be truly effective against CPV infection.

Administration of hyperimmune plasma or purified immunoglobulins may be beneficial as a prophylactic measure for puppies in contact with infected animals, but there is no evidence for its efficacy in ill puppies. In fact, by the time clinical signs appear, the virus has colonized the target tissues and antibody levels are already high. Molecules stimulating leukocyte production, such as recombinant human or canine granulocyte colony-stimulating factor, have been anecdotally reported to shorten the duration of hospitalization and increase survival rates, but further studies are needed to confirm their efficacy. In recent years, antiviral drugs have been tested for their efficacy against CPV infection; the anti-influenza drug oseltamivir may be beneficial, but further studies are needed. Research showed that recombinant feline interferon-ω reduced clinical signs and mortality only if treatment began very early after infection (1), a circumstance not reproducible in field conditions.

Management

Despite the long-term fecal shedding as demonstrated by testing (23), infectious virus is unlikely to be shed for more than 7-10 days. However, the exceptional resistance of the virus, due to the absence of an envelope, makes it hard to eradicate from the environment and it can persist for several weeks or months, leading to further spread of the infection. Strict isolation of affected puppies and extensive disinfection is mandatory. Fecal material should be removed as soon as possible, as it is the main source for environmental contamination. Most common disinfectants fail to inactivate CPV, but 5-10% sodium hypochlorite solutions have been proven to be effective. All tolerable surfaces should be exposed for at least 10 minutes to diluted bleach, particularly kennel boxes and hospital cages that are heavily contaminated by feces. Surfaces that do not tolerate bleach should be steam-cleaned (1).

Vaccination

MDA interference

The main issue for CPV vaccination is the MDA which protect puppies from infection by field strains but interfere with active immunization. MDA titers depend on the level of a dam's serum antibodies and on the amount of colostrum ingested by the puppies. Accordingly, puppies of the same bitch can have different MDA levels, and hence be susceptible to CPV infection (and active immunization) at different ages. Vaccinating puppies with high levels of MDA (HI titers >1:20) may result in lack of seroconversion due to destruction of the vaccine virus by colostral antibodies. Since only HI titers ≥ 1:80 are considered protective against infection by field strains, there is a period – the "window of susceptibility"

– usually lasting 2-3 weeks, during which puppies cannot be vaccinated but can be infected and develop disease.

To prevent interference with active immunization, vaccines should be administered to puppies only after MDA has waned (1,2). Different strategies have been recommended to overcome MDA interference, including high-titer vaccines and intranasal vaccination (25). Repeated intra-nasal administrations of CPV monovalent vaccines were effective in eradicating the virus from infected kennels (personal observation).

Guidelines from the World Small Animal Veterinary Association (26) recommend that a primary CPV vaccination course should not finish until 14-16 weeks of age, to ensure protection even in puppies with long-lasting MDA; the recommended protocol involves three CPV vaccine administrations in the first year of age and a booster after one year, followed by booster vaccinations every three years (1).

CPV-2 vaccines and cross-protection with the antigenic variants

Although the window of susceptibility is the main cause for active CPV circulation among vaccinated animals, there are also concerns about the complete efficacy of type 2-based vaccines against the newer antigenic variants (4-6). Most commercially available vaccines are prepared with the old CPV-2 strain that is no longer circulating in the field, and studies have proven the lack of full neutralization of CPV field strains by antibodies elicited against the vaccine virus. There are few licensed vaccines containing the CPV-2b variant, and it would be desirable to have formulations prepared with the new 2c variant, although the three variants are able to effectively cross-neutralize each other (4).

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A QUICK GUIDE TO...

Intensive care of newborn puppies

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Puppies are less well developed at birth than many other species, and high mortality rates are not uncommon in the first two weeks of life. A newborn puppy is at risk of hypoxia, and can develop hypothermia (due to its poor thermoregulatory function) and infection (due to an immature immune system). Other problems, such as dehydration and hypoglycemia, can also cause morbidity and mortality. Intensive care of the newborn must therefore be aimed at preventing the main trigger factors ***(Figure 1)*** and this short paper offers the clinician advice on how to maximize puppy survival.

Initial care

At birth, the dam will usually rupture the fetal membranes, cut the umbilical cord, and lick the puppy's chest to stimulate respiratory movements, before cleaning and drying the puppy. However, if the dam is negligent or inexperienced, human intervention is necessary. This involves using a clean, dry swab to remove all fluid from the nose and mouth, while simultaneously rubbing the chest to stimulate respiration. The puppy must be held horizontally on the palm of the hand, holding the head to protect it; sudden movements, or shaking or swinging the puppy, should be avoided. If necessary, amniotic fluid in the nasal passages can be removed by specialized suction pump, and the newborn must be dried to prevent hypothermia.

Neonatal clinical evaluation

An adapted Apgar score ***(Table 1)*** can be used for routine evaluation of the newborn and will indicate if the intensive care measures are proving effective.

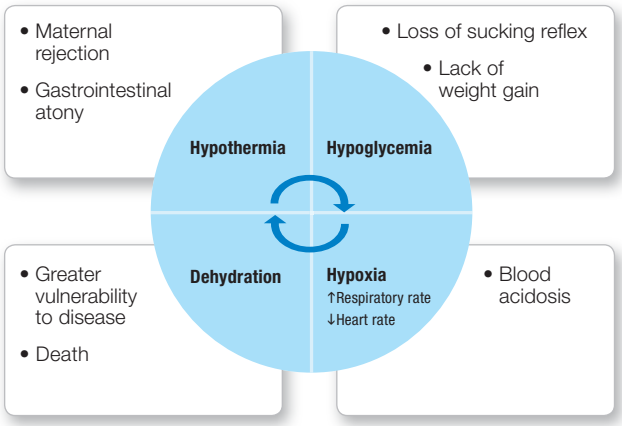


Figure 1. Main points of vulnerability in newborn puppies.

Heart rate and respiratory function can be assessed with a neonatal stethoscope ***(Figure 2)*** or by digital palpation of the heartbeat and observation of the respiratory effort. The muscle tone score is based on a puppy's ability to maintain an arch, or C-shape, of the thoracolumbar spine, and reflex irritability is the newborn's response to stimulus, either through movement or vocalization. The mucosal color can be evaluated by observation of the oronasal area ***(Figure 3).***

The Apgar score is a prognostic guide for neonatal survival, with the highest mortality levels in animals having a low score. To be considered healthy, the puppies must score 7 or above at five minutes post-partum; this is the

Table 1. Apgar score variables, adapted for the dog. Score each parameter in the left hand column from 0-2 as appropriate and summate the scores to give a total out of 10.

Parameter	Score 0	Score 1	Score 2
Heart rate	Absent	Bradycardia (< 200 bpm)	Normal (200-250 bpm)
Respiratory effort and rate	Absent	Irregular (< 15 rpm)	Regular and vocalization (15-40 rpm)
Muscle tone	Flaccid	Some flexion	Flexion
Reflex irritability	Absent	Some movement	Hyperactivity
Mucosal color	Cyanosis and pallor	Cyanosis	Pink

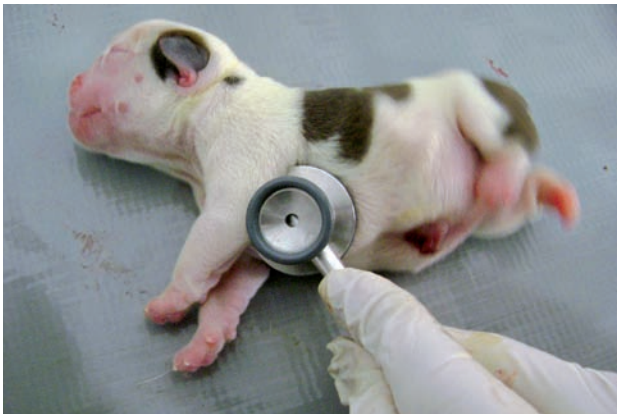


Figure 2. Evaluation of heart and respiratory rate by cardiopulmonary auscultation.

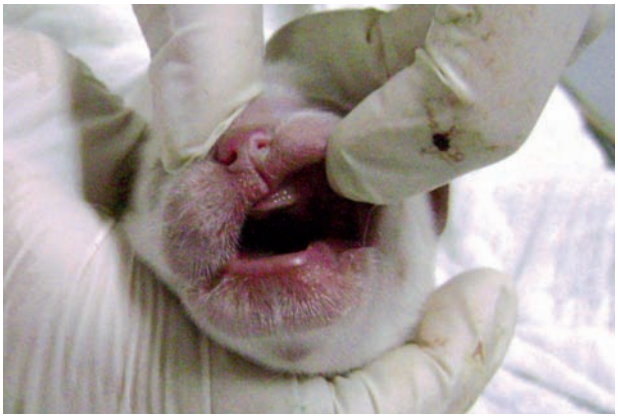


Figure 3. Pink coloration of the oronasal area in a newborn puppy.

critical time for reliable assessment, as some pups will have a lower score immediately after birth due to temporary suppression of vital functions. Neonates scoring between 4 and 7 require assistance and those with a score below 3 require emergency care.

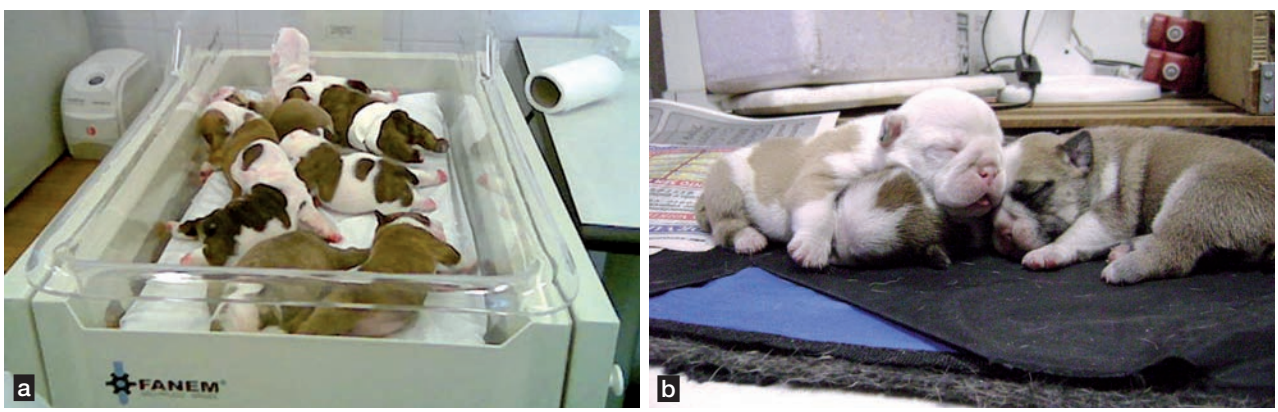
Adequate maintenance of body temperature is vital, as the thermoregulatory reflexes (vasoconstriction and shivering) are non-functional at birth. A puppy's body temperature should be 35-36ºC (95-96.8ºF) in the first week and 37-38ºC (98.6-100.4ºF) in the second and third weeks of life. Ambient temperatures below 27ºC (80.6ºF) cause hypothermia, while temperatures exceeding 33ºC (91.4ºF), along with high levels of relative humidity (85-90%), predispose to respiratory problems. Suckling helps a puppy to stay warm, as the dam's milk is 3-4ºC (37.4-39.2ºF) above body temperature.

If the bitch cannot keep the litter warm it is necessary to check each puppy's rectal temperature (using a small-diameter digital thermometer) at least once a day and provide an external heat source, either by incandescent lamps (20-40W) in the whelping box, or devices such as heat pads or warming bags ***(Figure 4).*** Ambient temperature must be monitored to prevent excessive heat, burning and dehydration.

Hypothermia adversely affects immunity, digestion and maternal care. With low temperatures a puppy loses its sucking reflex, resulting in reduced energy intake and general weakness. A hypothermic puppy must be warmed slowly (over 1 to 3 hours) to avoid peripheral vasodilation and hypoxia of vital organs; this should be followed by fluid therapy if necessary. Feeding must only be established after normothermia has been reached.

After ensuring respiration and thermal maintenance, each puppy should be examined for any birth defects such as hare-lip and cleft palate ***(Figure 5),*** umbilical hernia, anal atresia and skull disorders (*e.g.*, an open fontanelle).

Figure 4. Various forms of heating for neonates: a human incubator **(a)** and an electric heating mat **(b)**.



Body weight

Although many factors can affect birth weight (*e.g.*, age and health of the mother, placental effectiveness, litter size, and nutritional, infectious and environmental reasons), it is an important indicator for survival in most domestic species. Each puppy should be precisely identified and weighed regularly using digital scales ***(Figure 6).*** The birth weight can vary with breed and litter size but is typically between 100-200 g for a small breed puppy, 200-300 g for medium-sized breeds and 300-500 g for large breeds. Estimating the weight trend allows reliable monitoring of a puppy's development and may enable early detection of abnormalities. Body weight may drop in the first day of life (up to 10% of birth weight) due to dehydration, but after this point neonates should gain 5-10% of their birth weight daily, so that by day 15 the puppy will weigh around double its birth weight.

Natural or artificial lactation

During the first week of life, puppies will suckle every one or two hours and sleep the rest of the time. The mother licks them periodically to stimulate urination and defecation. If the mother is healthy and well nourished, her milk will satisfy the litter's needs for the first three to four weeks of life. However, if there is insufficient milk production (*e.g.*, following death of the dam, agalactia or mastitis) milk substitutes – either commercial or homemade – are necessary, with a formula that provides for the species requirements. Nevertheless, puppies given milk substitutes may not have the same growth rate when compared with those fed by natural lactation.

Milk substitutes may also be required for puppies with low body weight at birth (typically if at least 25% less than the average expected for that breed), for newborns that lose more than 10% of their initial weight in the first 24 hours of life, or where puppies do not double their birth weight within the first two weeks of life.

Neonate puppies use fat, rather than lactose, as an energy source, so a dam's milk has a high lipid content; cows' milk is unsuitable, as it is lactose-rich and low in fat and protein. The daily energy requirement of neonates is approximately 20-26 kcal/100 g body weight, but most commercial milk substitutes contain only 1 kcal/mL. Given that the maximum capacity of a neonate's stomach is approximately 4 mL/100 g of body weight, it is possible to estimate the daily requirements and feeding frequency required.

A milk substitute can be offered, either using an appropriately sized feeding bottle, or by orogastric tube,

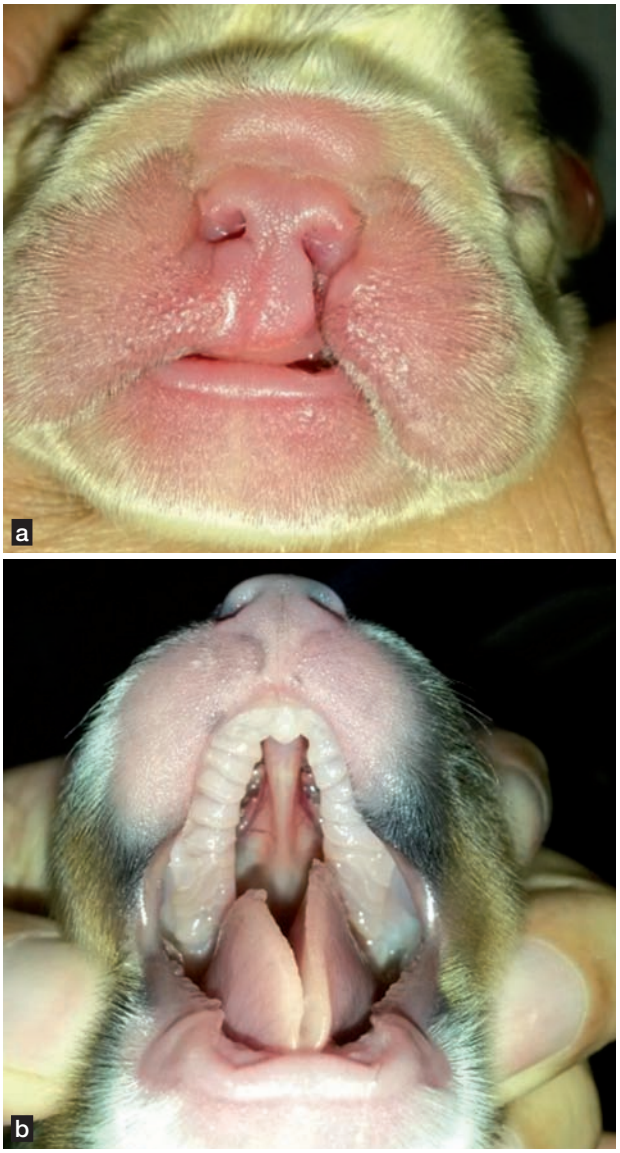


Figure 5. Evaluation of the neonate puppy's oral cavity to identify hare-lip **(a)** and cleft palate **(b)**.

depending on the puppy's health and if there is a vigorous sucking reflex. A feeding bottle stimulates the sucking reflex (and reduces the risk of aspiration) with the puppy held horizontally to maintain a near-natural feeding posture, without excessive stretching of the neck. Using an orogastric tube requires skill and risks intratracheal placement, and is more suitable if a large number of puppies require feeding, or if a puppy has poor sucking strength or inadequate weight gain. Monitoring is essential for puppies that require assisted feeding, to check for signs of overfeeding such as milk at the nostrils, regurgitation, abdominal discomfort and distension, and diarrhea; the latter sign can also be indicative of changes in the microbiota or even septicemia. Excessive

Figure 6. Weighing a puppy using digital scales calibrated in grams. Puppies should be weighed immediately after birth and at twelve hours, and then daily until 2 weeks of age, and then every three days until they are one month old.

feeding is considered to be one of the main causes of non-infectious diarrhea in puppies, but a weak sucking reflex, persistent whining, lethargy, and insufficient weight gain are indicative of inadequate milk intake.

Dehydration and hypoglycemia

80% of a puppy's body weight at birth is water; this, combined with other innate factors (*e.g.*, a relatively large body surface area, permeable skin, and poor renal function) contribute towards the risk of dehydration in neonates, but dehydration is normally linked to prematurity, diarrhea, pneumonia, high ambient temperature or inadequate nursing. Hydration status can be assessed by observing the urine; a sample may be obtained by gently massaging the foreskin or vulva with wet cotton wool. A yellowish color indicates dehydration, while dilute, translucent urine is normal. Dehydrated animals may also have dry, pale oral mucosae.

Rehydration via warmed (37°C/98.6°F) fluids may be necessary (60-180 mL/kg/day). Oral administration is preferable as long as intestinal function is normal and the animal is not hypothermic. However, the subcutaneous route is often used, while intravenous or intraosseous administration is more suitable for giving small volumes of fluid. The therapeutic challenges when treating neonates are considerable, and conservative administration rates and close monitoring during therapy may be beneficial. Signs of overhydration include a serous nasal discharge, ascites, tachypnea/dyspnea and pulmonary edema.

Dehydration may be accompanied by hypoglycemia. Because of low body fat reserves, limited gluconeogenesis and hepatic immaturity, a neonate must feed frequently in order to maintain normal glucose levels. Therefore, fasting for more than 2-3 hours may lead to hypoglycemia (< 35-40 mg/dL), which is manifested by lack of co-ordination, weakness or coma. Immediate treatment is essential, with slow administration of 5-10% dextrose solution into a jugular vein (at 2-4 mL/kg). If there is a poor response, additional doses can be given but blood glucose levels should be checked before administration due to the risk of hyperglycemia.

Immature immune system

Newborn puppies have an underdeveloped immune system and are completely dependent on the transfer of antibodies via the colostrum, which should be within a few hours of birth. There is considerable correlation between puppies with a low serum immunoglobulin level at two days of age and the neonatal mortality rate. Determination of serum alkaline phosphatase (AP) and gamma-glutamyl transferase (GGT) levels can confirm if a puppy has taken colostrum after birth (see article on page 32). For animals with low enzyme levels, it may be helpful to give serum or plasma from vaccinated adults of the same species, either orally if the puppy is less than 24 hours old, or subcutaneously in "bolus" form if older than this; note however it is important to test serum compatibility before administration.

Conclusion

Stimulated by the increasing emotional and financial value of pets, and the general vulnerability of animals in the post-partum period, specialized neonatology knowledge has developed greatly in recent years. Whilst proper handling of the parturient dam and her puppies is still the prime measure in combating neonatal problems, early identification of problems in young puppies allows for rapid intervention and application of appropriate intensive care therapies, leading to increased survival rates.

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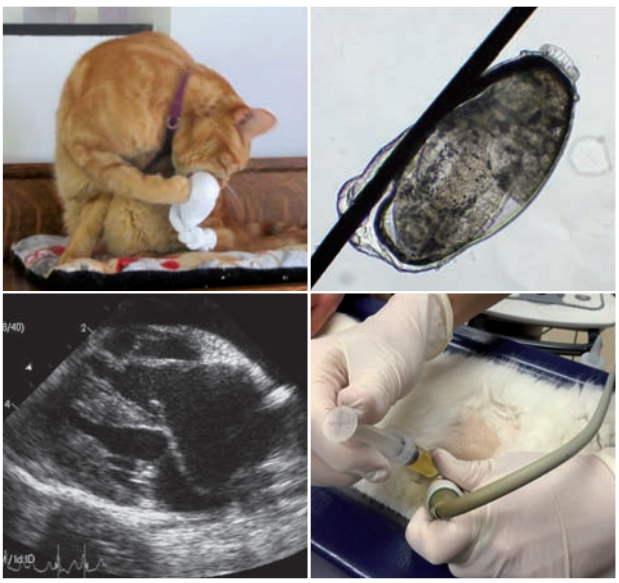

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In the next *Veterinary Focus*, we will look at different aspects of Feline medicine:

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  Maggie Scherk, US
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