CALF DIARRHOEA **DIAGNOSTICS**

Bernie Vaatstra, of Gribbles Veterinary, discusses the 'three Ps' of broad-spectrum investigation of calf diarrhoea.

CALF DIARRHOEA IS a frustrating and economically important disease that is responsible for more than half of all calf losses to weaning.

Outbreaks are often multifactorial, involving interactions between infectious agents, calf and dam nutrition, environment, husbandry and calf immunity. Successful treatment and prevention, therefore, require knowledge of the status of as many of these factors as possible.

Investigating calf diarrhoea outbreaks requires a broad-spectrum approach, beginning with a thorough history-taking and clinical examination. Age and origin of the calves, herd vaccination status, colostrum management, environment, physical condition and hydration status are important parts of the puzzle. In severe outbreaks where calves are dying or moribund, postmortem examination of one or more freshly dead or euthanased calves is extremely valuable.

In addition to the on-farm investigation, a range of laboratory testing options can help to further define the problem. It is tempting simply to hone in on faecal pathogen detection, but while this is a key diagnostic aid, there are other tests that contribute to the successful management and prevention of outbreaks. Diagnostic tests can therefore be categorised according to the three Ps: pathogen testing, evaluation of predisposing factors, and characterisation of pathophysiologic changes.

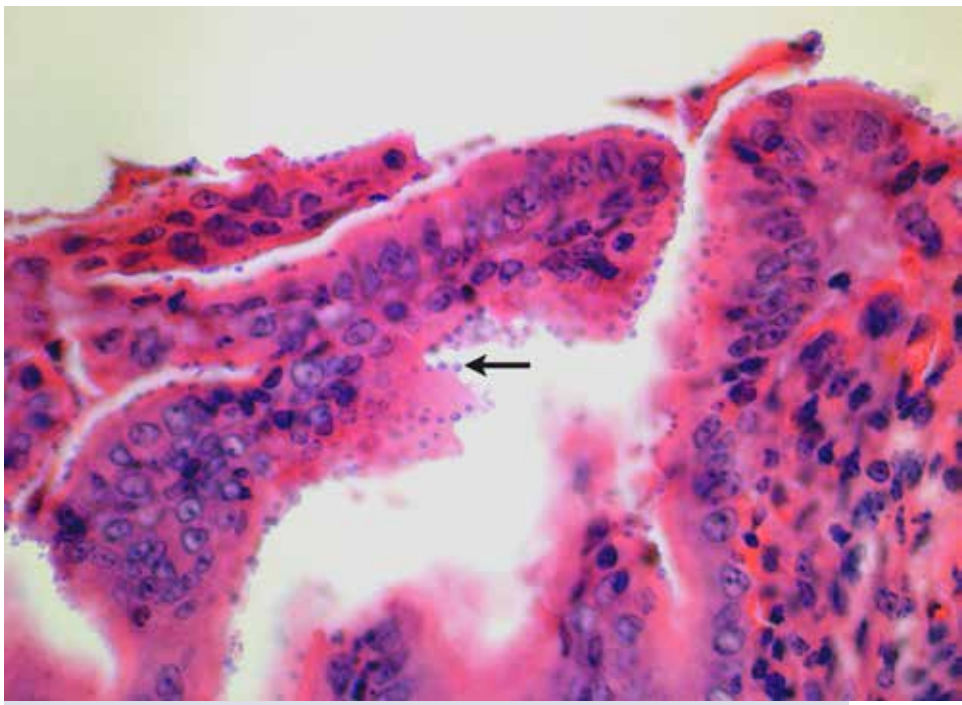

FIGURE 1: Cryptosporidia (arrow) lining up along the apical surfaces of intestinal villous enterocytes. H&E 1,000x. Photomicrograph courtesy of Fraser Hill.

PATHOGEN TESTING

Laboratory techniques used to target specific calf scour pathogens include faecal antigen immunoassay, culture, polymerase chain reaction (PCR), parasitology and histopathology. These tests may be applied to faecal samples taken from sick calves or colonic contents retrieved from postmortem calves. It is important to note that the detection of an organism does not necessarily prove causation. Results of pathogen testing always need to be interpreted in light of the clinical signs, and preferably alongside appropriate gross and histopathological changes.

Faecal immunoassay

Rotavirus and cryptosporidium are the most frequently detected calf scour pathogens in calves up to around five weeks old. Rapid faecal antigen enzyme-linked immunosorbent assay (ELISA) testing provides convenient, sensitive and specific detection of these agents. Antigen ELISA testing is also available for enterotoxigenic *E. coli* expressing

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TABLE 1:

Percentages of different pathogens detected in <one-week-old calf scour panels submitted to Gribbles Veterinary, Palmerston North, from August 2017 to August 2018.

Pathogen	Rate of detection
Rotavirus	50%
Cryptosporidium	47%
***Salmonella* spp.**	19%
***E. coli* K99**	11%
Coronavirus	0%
Cases with multiple pathogens detected	37%
Cases with no pathogens detected	23%

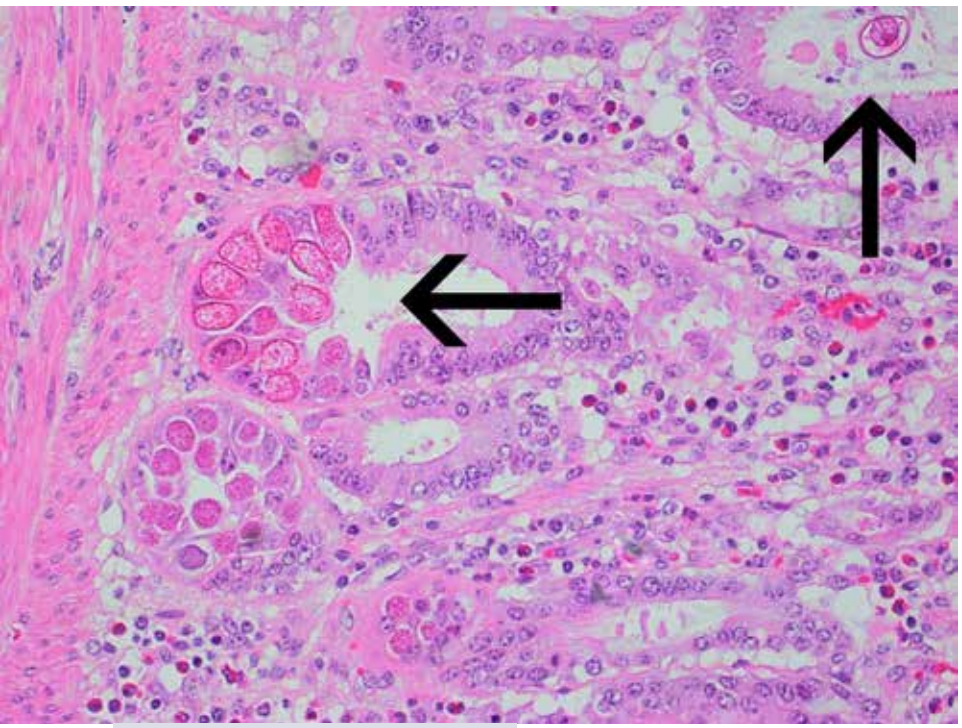


FIGURE 2: Coccidial gametocytes (horizontal arrow) and oocyst (vertical arrow) within colonic crypts. H&E 400x

K99 antigen, usually diagnosed in young calves less than one week old, and coronavirus, an infrequent cause of scours up to about five weeks old.

Microbiology

Salmonella enterica Typhimurium, and occasionally other serovars, may cause severe mucoid or haemorrhagic diarrhoea in calves of all ages. Diagnosis can be confirmed through culture in conjunction with appropriate clinical signs and pathology. Culture readily detects non-K99 strains of *E. coli* in normal and diarrhoeic calves, but usefulness is limited where strain typing is not available. Histopathology can be helpful for attaching and effacing strains. In older calves around weaning, *Yersinia pseudotuberculosis* is a consideration, and can also be detected using culture.

Table 1 shows the percentages of different diarrhoea pathogens detected using faecal immunoassay and culture in calves less than one week old. This panel uses faecal antigen testing to detect rotavirus, cryptosporidium, *E. coli* K99 and coronavirus, while salmonella is detected using culture. The data was taken from submissions to Gribbles Veterinary, Palmerston North, over a one-year period, from August 2017 to August 2018.

Parasitology

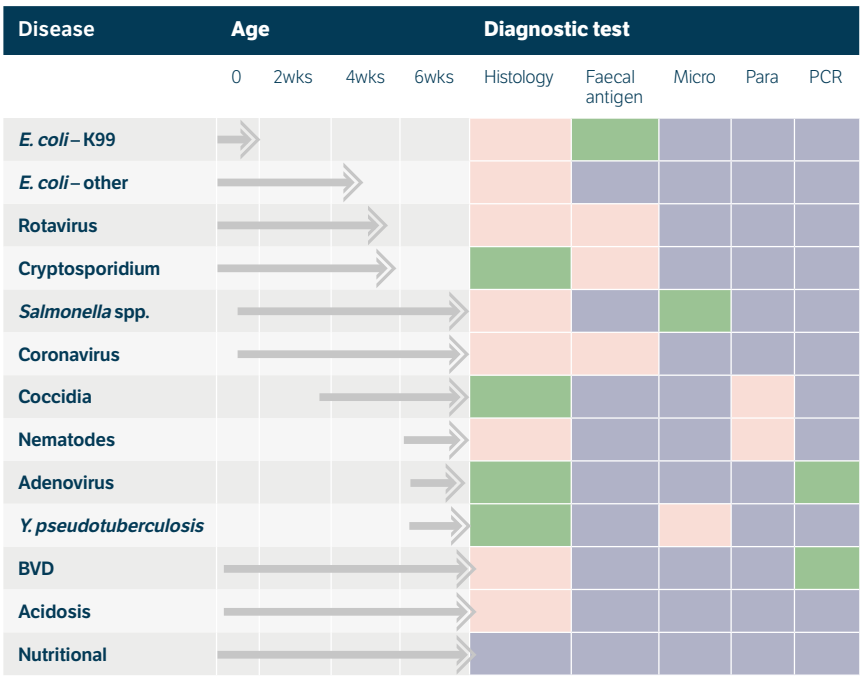
Calves from about four weeks old onward are susceptible to coccidiosis. Clinical signs of diarrhoea with or without blood are related to maturation of oocysts in the large intestine during days 18–19 of infection. Faecal oocysts are present in highest numbers during days 22–25 of infection; therefore, detection of only small numbers of oocysts does not rule out significant disease. Oocysts are readily detected using traditional parasitology methods. As calves approach weaning and increase the percentage of pasture in the diet, nematode faecal egg counts provide a rough guide to the level of parasite challenge, and may help inform parasite management.

PCR

Most of the calf diarrhoea pathogens are detected rapidly and reliably using immunoassays, microbiology or parasitology. Exceptions include attaching and effacing *E. coli* strains,

TABLE 2:

Age of onset and utility of diagnostic tests for common causes of calf diarrhoea. = test not useful or routinely available; = test may contribute to diagnosis in conjunction with other tests; = test may provide a definitive diagnosis.



BVD virus and bovine adenovirus. PCR may be useful for the detection of viral pathogens in certain circumstances.

BVD may have a role in neonatal scours through immunosuppression leading to increased susceptibility to other pathogens, or through transient or persistent infection. BVD PCR is recommended for testing calves <35 days old, while antigen ELISA can be used for older calves. Both techniques are available on both serum and skin samples.

Bovine adenovirus type 10 is a differential for diarrhoea and deaths in slightly older calves, particularly around the time of weaning and afterwards. A qPCR assay is available for use on EDTA blood, fresh, or fixed tissue.

Histopathology

Histopathology can be of great value in calf scour outbreaks. Firstly, it may help to confirm the significance of any pathogens detected by demonstrating typical pathological features (for example intestinal villus atrophy with rotavirus and coronavirus, pseudomembranous enteritis with salmonellosis). Secondly, organisms or their footprints may be identified. Examples include *E. coli* lining up on enterocytes, *Yersinia* spp. colonies, cryptosporidia populating apical enterocytes (Figure 1), coccidial stages within crypts of the distal small intestine and large intestine (Figure 2), and adenoviral inclusion bodies (Figure 3). Thirdly, pathogens or conditions not detected using the usual methods may be suspected or detected using histopathology (attaching and effacing *E. coli*, rumen acidosis, adenovirus and peritonitis).

It is important to note that the value of histopathology may be substantially limited by tissue degradation as a result of autolysis. The delicate GI mucosa starts to deteriorate within minutes after death, and after as little as 30 minutes, changes such as villus atrophy may be missed. Organisms such as cryptosporidia and coliforms may also be lost from the mucosal surface. Therefore, it is best to take histological samples from a very recently dead calf, or even better, euthanase a moribund calf. Multiple sections of rumen, omasum, abomasum and small and large intestines should be collected immediately, partially opened, and immersed in an appropriate volume of 10% formalin, before proceeding to the rest of the postmortem. Preservation of the mucosa may be aided by injecting formalin into the lumen of the bowel sections collected. Table 2 demonstrates the utility of the different diagnostic tests used in the investigation of calf scours.

PREDISPOSING FACTORS

In addition to looking for specific pathogens, some of the underlying factors contributing to diarrhoea may be investigated. BVD virus has already been mentioned and may be tested as outlined above.

Failure of passive transfer is an important contributor to calf diarrhoea. While the gold standard test for assessing passive transfer is IgG immunoassay, there are two other tests that can be used as rapid and cost-effective proxies. Serum total protein (TP) is convenient in that calves 0–8 days old can be tested without knowing the exact age of the calves. A cut-off of >53g/L is used as evidence of adequate transfer of colostral immunoglobulin (Cuttance et al., 2017). However, it is important to note that calves with diarrhoea are likely to be dehydrated and have proportionately elevated TP.

In situations where calves are sick and scouring, serum gamma glutaryl transferase (GGT) may be a helpful indicator of colostral transfer (Thompson and Pauli, 1981).

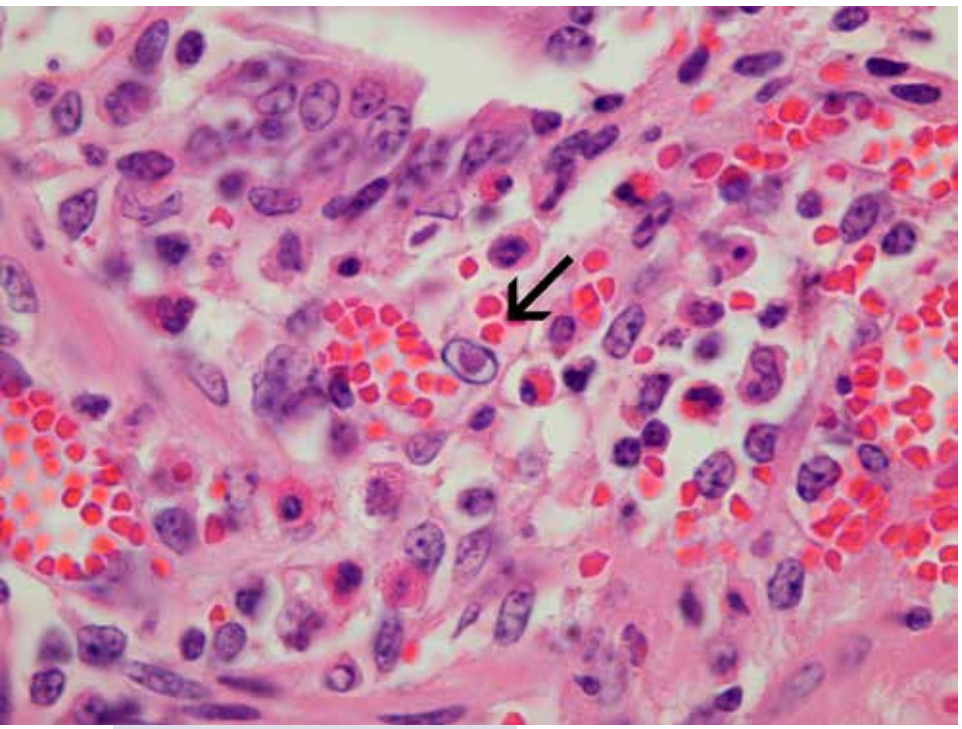


FIGURE 3: An intranuclear adenoviral inclusion body (arrow) within an endothelial cell in the intestinal mucosa. H&E 1,000x

The age of the calf is required in order to interpret the results. GGT levels indicate that sufficient colostrum was ingested, but do not give any information about the quality of the colostrum. Therefore GGT needs to be interpreted in light of colostrum storage practices and colostrum quality (eg, using a Brix refractometer).

Milk powder testing may be considered if there is a suspicion that nutritional factors are contributing to scours. Curd testing assesses the casein content and quality, while fat, protein and lactose testing can indicate how much energy is supplied by the fat fraction.

PATHOPHYSIOLOGY

As well as looking at aetiological and predisposing factors to calf diarrhoea, the successful treatment of clinical cases requires an understanding of the pathogenesis. Routine biochemistry and haematology can help evaluate some of the physiological changes taking place.

IN SITUATIONS WHERE CALVES ARE SICK AND SCOURING, SERUM GAMMA GLUTARYL TRANSFERASE (GGT) MAY BE A HELPFUL INDICATOR OF COLOSTRAL TRANSFER.

These include dehydration, manifested by clinical signs such as skin tenting and sunken eyes, and in blood work as haemoconcentration and hyperproteinaemia (unless complicated by haemorrhage). Metabolic acidosis develops due to loss of bicarbonate into the GI tract, L-lactic acid production in poorly perfused tissues and D-lactic acid production in the colon due to increased colonic fermentation. If unchecked, this process may lead to CNS depression, recumbency and coma. Serum bicarbonate can be used in addition to clinical signs to estimate levels of acidosis and base deficit in order to guide bicarbonate requirements. A recent *VetScript* article by Sandra Forsyth included a helpful table to estimate base deficit in calves in order to calculate bicarbonate requirements in fluid replacement therapy (Forsyth, 2018).

Electrolyte imbalances go along with dehydration, and need to be considered when calculating fluid replacement therapy. There may be a total body deficit of sodium, chloride and potassium due to intestinal fluid loss and decreased intake. However, serum biochemistry will often show hyponatremia, normochloremia and, sometimes, hyperkalemia. The latter occurs in metabolic acidosis, due to the exchange of extracellular hydrogen ions for intracellular potassium ions in order to buffer the extracellular fluid. Hyperkalemia results in cardiac arrhythmias. Other biochemical and haematology changes can include evidence of energy deficit (hypoglycaemia, ketosis) and inflammation (neutrophilia with left shift, hyperfibrinogenemia).

In conclusion, a thorough work-up of calf diarrhoea cases can be complicated and may require the evaluation of many diagnostic parameters, as well as clinical and environmental factors. However, putting all the pieces together in order to diagnose, treat and prevent scour outbreaks is highly satisfying and will improve animal and farmer welfare. VS

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