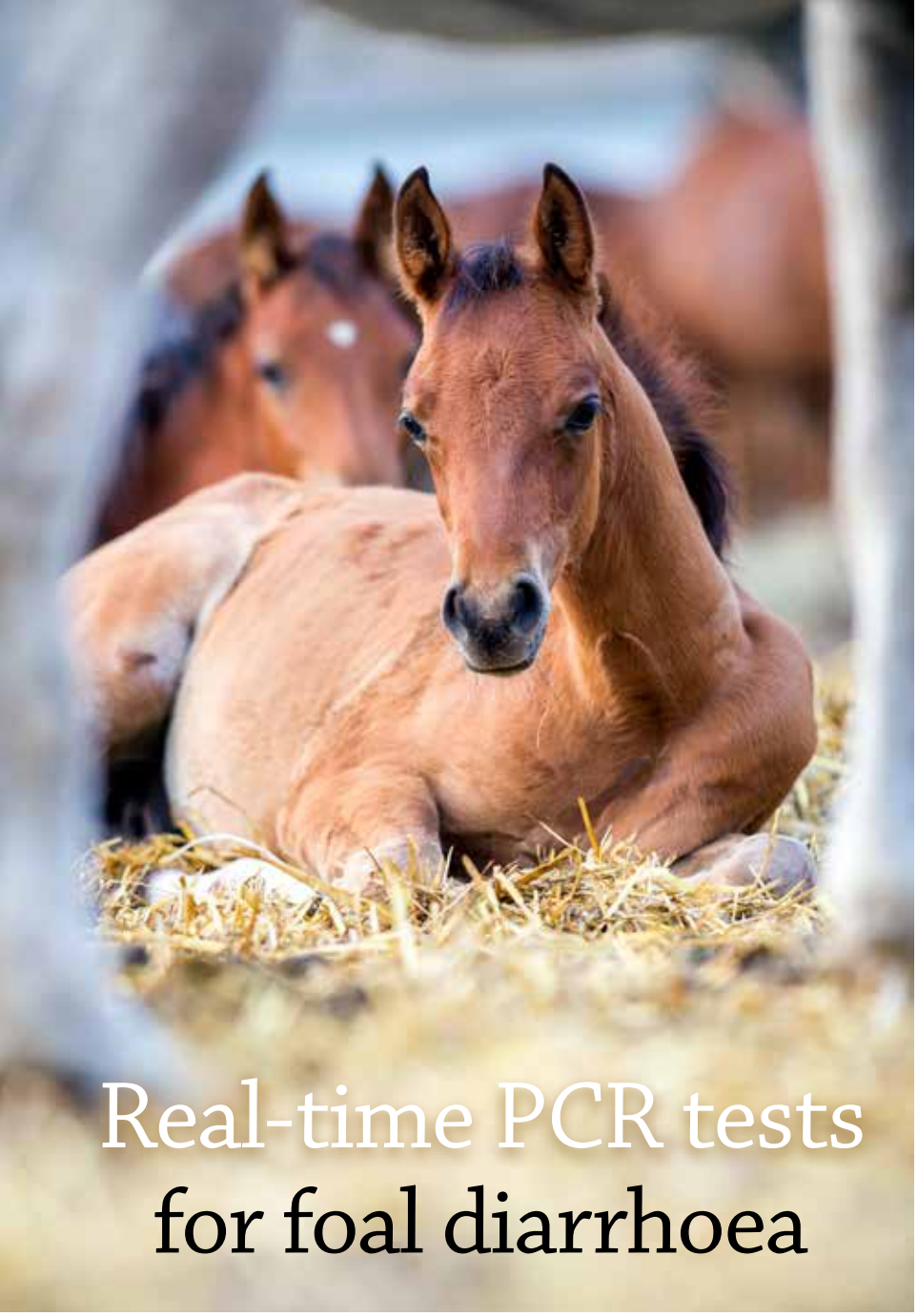

Real-time PCR tests for foal diarrhoea

Alice Fraser of SVS Laboratories, Hamilton, outlines the advantages of using real-time PCR tests to diagnose the cause of foal diarrhoea.

PROMPT AND DEFINITIVE diagnoses are important to the equine clinician when dealing with foal diarrhoea cases to enable early decisions on treatment, care and case management and establish biosecurity measures to prevent disease spread. Enteric infections may be caused by multiple pathogens, so it is useful to be able to perform a diagnostic panel of the clinically suspected organisms. This is now possible with cost-effective, real-time polymerase chain reaction (RT-PCR) tests. In addition, some RT-PCR tests are able to detect genome sequences encoding for virulence factors, enabling distinction from organisms that exist in the normal range of faecal microbiota or in the environment.

PCR BASICS (FOR THOSE WHO AREN'T MOLECULAR SCIENTISTS)

RT-PCR tests have taken over from the original, more time-consuming PCR tests. RT-PCR (or quantitative PCR) means that rather than having to wait to detect a result at the end of the full DNA amplification procedure, we can monitor the result throughout the test by generating fluorescence during the target genome amplification. Several techniques produce measurable fluorescence. A commonly utilised method is based on Taqman® probes that bind to specific genome sequences on a positive DNA test, which are bracketed by the primers added to drive the reaction. The Taqman probes are hydrolysed during amplification, causing the separation of their fluorophore label and inciting detectable fluorescence.

The RT-PCR test including fluorescent detection is performed by one machine, thereby decreasing the previous issues of DNA contamination. As well as speed, the RT-PCR tests have the benefit of giving a quantitative result by the number of cycles taken for a given positive result.

VIRAL INFECTIONS

Rotavirus is a major cause of diarrhoea in neonates of humans, mammals and avians, with nine species (A to L) having been identified worldwide. Equine rotavirus (ERV) is a Group A rotavirus that is ubiquitous throughout the global equine population and is a major cause of diarrhoea in foals up to three months of age. There are a significant number of genome similarities between the rotaviruses affecting different species. An RT-PCR test for ERV targets the ERV-specific, non-structural protein gene 3 (NSP3), giving the test a high sensitivity and specificity. ERV is rarely found in the faeces of healthy foals (Slovis et al., 2014) and occurs as either a monoinfection or co-infection in foals with diarrhoea.

Equine coronavirus (EqCo), typical of coronavirus in most species, is of questionable significance in foal diarrhoea. Like ERV, currently RT-PCR tests for EqCo do not detect virulence factors, so results must take into account other factors such

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RT-PCR TESTS GIVE THE GREAT ADVANTAGES OF SPEED AND ACCURACY, AND THEREFORE EFFICIENCY, **IN DIAGNOSES OF FOAL DIARRHOEA.**

as clinical signs, other laboratory results, etc. In a study of cases of foal diarrhoea by Slovis et al. (2014), the incidence of EqCo in foals with gastro-intestinal disease was similar to that in healthy foals, and when detected in foals with diarrhoea it was usually as a co-infection (particularly with *Cryptosporidium* and/or *Salmonella*). The incidence of EqCo as a cause of diarrhoea in New Zealand is still unclear and will remain so if it is not widely tested.

CLOSTRIDIAL DISEASES

Infections by *C. perfringens* and *C. difficile* are considered the most common enteric clostridial diseases of horses. Both *C. perfringens* A and C have been reported in association with enterocolitis. However, a review by Uzal and Diab (2015) notes that since type A can be found in the intestines of most healthy horses, its pathogenic role is still in question. It may be that some strains of type A carry virulence factors not present in commensal strains.

C. difficile is an important cause of diarrhoea and enterocolitis in both adult horses and foals. Major risk factors include hospitalisation and antimicrobial therapy, although there are reports of related disease in horses without these risk factors (Diab et al., 2013). Toxins A and B (TcdA and TcdB) are responsible for the pathological changes that cause the clinical signs of disease (Weese et al., 2001). RT-PCR tests can detect the genome sequences encoding for these toxins, giving evidence of potential toxin production, thereby differentiating from non-toxigenic strains. The zoonotic potential of *C. difficile* is becoming more apparent.

C. perfringens type C causes disease mostly in neonatal foals, although cases in older foals occur occasionally. Since *C. perfringens* can be isolated from the faeces of normal horses and from horses with colitis of other aetiologies, direct or even quantitative culture is not useful in diagnosing this disease. *C. perfringens* type C produces toxins A and B (CPA, CPB and CPB2) with CPB toxin being the main virulence factor. Toxin identification or identifying the genome for toxin production is required for definitive diagnosis. An RT-PCR test is available for the CPB toxin, which, if present, designates it as a virulent form.

RHODOCOCCUS EQUI

Rhodococcus equi is a gram-positive coccobacillus whose natural habitat is the soil (particularly where contaminated by faeces from livestock/horses). It is associated with pneumonia and less commonly diarrhoea in foals aged mostly under four months. Important virulence factors on some strains of *R. equi* allow the bacterium to survive and replicate within phagocytic cells. These factors are in the form of a plasmid that encodes a virulence-associated surface-expressed protein (vapA). Two separate RT-PCR tests detect the *R. equi* cholesterol oxidase gene, which is universally conserved in *R. equi* (both virulent and environmental forms) and the vapA gene. Hence, environmental isolates can be differentiated from the clinically relevant equine virulent strains.

SALMONELLOSIS

Salmonellosis in horses is most commonly associated with infection with *Salmonella typhimurium*. Stress and antibiotic treatment are the main predisposing factors for the onset of clinical salmonellosis to occur, particularly when antibiotic-resistant strains are present in the small intestine. Culture on enrichment media is required for full bacterial typing and sensitivities. A generic *Salmonella* RT-PCR test can be run while awaiting cultures.

LAWSONIA INTRACELLULARIS

Lawsonia intracellularis is a pathogen affecting older foals, particularly during the weaning or post-weaning period. It is a disease of the small intestine that causes a proliferative enteropathy leading to weight loss, hypoproteinaemia and, in some cases, diarrhoea. Cultures of this organism from faeces are not possible (tissue culture required), but RT-PCR is an excellent method of identifying the pathogen. The RT-PCR targets a non-plasmid metabolic gene.

CRYPTOSPORIDIOSIS

Cryptosporidium parvum causes diarrhoea in immunologically normal and immunosuppressed foals between five days and six weeks of age (most commonly, under four weeks). It can occur as a monoinfection or coinfection. Diagnosis is traditionally by microscopic demonstration of oocysts in faecal smears stained with modified Ziehl-Neelson, or via an ELISA test. In addition, an RT-PCR test is a highly sensitive method for detecting *Cryptosporidium spp*.

SUMMARY

While bacterial cultures, ELISA tests and faecal examinations still have their place for certain aspects of these diseases, RT-PCR tests give the great advantages of speed and accuracy, and therefore efficiency, in diagnoses of foal diarrhoea. VS

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