

# PCR TESTING

## AT BEAUFORT COTTAGE LABORATORIES

During the last 12 months we have developed the additional specialised facilities, installed the equipment, reagents and staff and have developed the expertise for running polymerase chain reaction (PCR) tests at Beaufort Cottage Laboratories. All our PCR assays use direct quantitative technology with internal controls to ensure the validity of each assay.

PCR tests are now used in medical and forensic science laboratories for the detection of specific DNA and RNA. Very small amounts, whether viable or non-viable, are 'amplified', i.e. replicated to produce measurable quantities for reliable detection. This now allows us to detect the DNA or RNA of specific pathogenic organisms, i.e. some of the causes of important equine infectious diseases, more accurately and efficiently than by traditional microbiological culture.

We can now offer PCR testing services for the following equine pathogens:

### ***Taylorella equigenitalis* (CEMO)**

During the 2009 breeding season, we ran a field trial of a newly available direct quantitative PCR assay (Qiagen, UK) for *T. equigenitalis* (CEMO), the cause of Contagious Equine Metritis (CEM), a well-recognised cause of equine venereal disease. This test was initially developed by the Veterinary Laboratories Agency (VLA) and is used by them for the confirmation of positive isolates. We tested over 2000 of the mare clitoral swabs and a smaller number of mare uterine and stallion penile swabs that we had received for routine pre-season screening as recommended by the Horserace Betting Levy Board's (HBLB) Code of Practice and found that none were positive for CEMO either by traditional bacterial culture or by PCR test. This means that we found no 'false positive' results in over 2000 tests. We tested the swabs that we received from the HBLB for our bi-annual quality assurance (QA) tests and detected



Lorraine Palmer in our PCR laboratory

the CEMO positive swabs with 100% accuracy, i.e. we found no 'false negative' results. This has given us confidence that this PCR test is robust enough to be used as an alternative to routine bacterial culture for CEMO screening. At the June 2009 annual HBLB Code of Practice committee meeting, delegates agreed that PCR testing for routine screening for CEMO (as an alternative to microaerophilic culture) will be acceptable for the 2010 season and the 2010 Code of Practice will reflect this.

The advantages of the PCR test for CEMO include:

1. Quicker results – microaerophilic culture for CEMO takes at least 7 days to confirm a negative result, whereas a PCR result can be obtained within 24-48 hours. This will overcome many of the delays associated with CEMO culture to the advantage of the mare 'walking in' system and to the artificial insemination (AI) industry. It will be possible for CEMO PCR results to be available alongside the traditional aerobic culture results for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.
2. More accurate results – traditional microaerophilic culture for CEMO cannot detect non-viable bacteria, which have died during transport delays or which have been killed by exposure to sunlight or inappropriate temperatures. We detected non-viable CEMO DNA in swabs from a non-UK (EU) stallion. We had cultured CEMO

from these swabs 6 months previously and had stored them at +4°C rendering the organisms non-viable. Also, PCR tests are not subject to the problems of bacterial overgrowth that have been reported to obscure CEMO growing under conditions of conventional microaerophilic culture. We can therefore have confidence that a negative CEMO PCR test is a true negative.

3. Differentiation from similar organisms - microaerophilic culture for CEMO cannot differentiate *T. equigenitalis* from *Taylorella asinigenitalis* (found in donkeys in North America and some non-UK EU countries) but PCR test can. In addition, the PCR test can differentiate between CEMO and environmental non-pathogenic bacteria, which are sometimes cultured under microaerophilic conditions from equine genital swabs.

In the UK, the isolation of CEMO is notifiable by law under the Infectious Diseases of Horses Act (1987) and any positive CEMO PCR test result is similarly notifiable to Defra, who will then require further investigation by repeat swabbing and microaerophilic culture and PCR testing at the VLA. PCR testing is not yet internationally accepted for equine export testing.

#### ***Rhodococcus equi* ('summer pneumonia', 'rattles')**

We have direct PCR assays for the virulence-associated protein A (VapA) containing and non-virulent (does not contain the VapA protein) strains of *R. equi*. The VapA strain causes life-threatening bronchopneumonia, enteric lymphadenitis and/or osteomyelitis in foals. The organism is difficult to treat and requires prolonged medication with specific antibiotic combinations. The earlier a positive diagnosis is made and specific treatment is applied, the better are the chances for success. Conversely, the earlier that a reliably negative diagnosis is made, more appropriate antibiotic therapy can be applied. The PCR test may be performed on nasal discharge or nasopharyngeal swab and tracheobronchial aspirate and/or washing samples. Faecal samples can be examined but consistent faecal shedding of the organism is less reliable.

Also, we have developed a specific quantitative enzyme-linked immunoabsorbent assay (ELISA),

which detects antibodies to *R. equi* VapA strain in blood (serum) samples and provides a measured titre. This will not prove active infection but will indicate the degree of exposure to the virulent antigen and in the presence of clinical signs a high titre may indicate the initiation of treatment with specific antibiotic combinations, at an early stage. Currently, we consider serum titres <1:1000 to indicate low-grade challenge, titres of 1:1000-1:5000 to indicate moderate challenge and titres of >1:5000 to indicate high challenge. Experience suggests that foals with active *R. equi* infections usually have titres >1:5000.

#### ***Streptococcus equi* ('strangles')**

We now offer a direct PCR assay for *Strep. equi*, which causes highly contagious local (notably submandibular and parotid) and sometimes generalised lymphadenitis in horses of all ages, which can sometimes be life threatening. The earlier a diagnosis is made, the earlier and more effective isolation and contact tracing will be, aiding limitation of disease transmission. For the infected horse(s), the earlier specific management and (if indicated) treatment is applied, the better are the chances for success. The PCR test may be performed on abscess pus, discharge or nasopharyngeal swabs, tracheobronchial or guttural pouch aspirates and/or washing samples.

A serological test measuring antibody titres to *Strep. equi* is available at the Animal Health Trust [www.aht.org.uk/bact\\_blood.html](http://www.aht.org.uk/bact_blood.html).

#### ***Streptococcus zooepidemicus***

We now offer a direct PCR assay for *Strep. zooepidemicus*, a very common opportunist equine pathogen, which causes sporadic infections of the skin, respiratory, genital and urinary tracts and joints of horses of all ages. It may cause cellulitis and abscesses in the jaw and throat area, and its early differentiation from *Strep. equi* can be very helpful in the management of the horse and its contacts. The PCR test may be performed on pus, discharge or nasopharyngeal swabs, tracheobronchial aspirates and/or washing samples. We will investigate its use for uterine fluid aspirates or washings, urine and synovial fluid samples.

### ***Lawsonia intracellularis* (proliferative enteropathy)**

Foals between the ages of 4 and 7 months, often weanlings, that develop sudden and marked depression, weight loss, subcutaneous oedema and sometimes diarrhoea, have been identified with proliferative enteropathy caused by *Lawsonia intracellularis* infection. This life-threatening infection causes thickening of the small intestinal mucosa, which leads to protein-losing enteropathy, has been identified in a number of other animal species, including the pig, dog and deer.

Initial routine blood sample analysis of clinically ill foals reveals high white blood cell counts, low serum protein but high inflammatory protein levels and low electrolyte (sodium, potassium and chloride) and calcium levels.

Early specific diagnosis is essential to guide specific treatment for recovery and we can examine faecal samples (Sterilin universal 30 ml container) from foals with clinical signs by specific direct qPCR test to detect *L. intracellularis* DNA. Faecal shedding of bacteria is variable and can be intermittent, leading to the possibility of false negative results, particularly where antibiotic treatment has already started.

### **Equine Herpesvirus-1 and -4**

We now offer direct PCR assays for EHV-1 and EHV-4, common causes of respiratory infection in young horses. EHV-1 is an important potentially epidemic cause of abortion in mares and a life-threatening cause of encephalomyelitis in adult horses. The PCR test may be performed on pus, discharge or nasopharyngeal swabs, tracheobronchial aspirates and/or washing samples, aborted foetal and placental tissues and cerebrospinal fluid (CSF) samples.

### **SAMPLING FOR PCR TESTS**

In order to have confidence in results it is vital that the most appropriate samples are submitted for testing, in relation to the stage of disease and

clinical signs shown. If in doubt please telephone to discuss a case with one of our laboratory clinicians before sampling.

### **PCR TESTING TAILORED TO CLINICAL SIGNS**

We can offer cost efficient PCR tests run in duplex or triplex combination to provide, for example, a 'respiratory panel' for foals with persistent 'snotty' noses. PCR test results for *R. equi*, *Strep. equi*, *Strep. zooepidemicus* and EHV-1, may be very helpful either by confirming infection with one of these pathogens, or by excluding them, providing managerial reassurance.

### **INTERPRETATION OF PCR TEST RESULTS**

As for all laboratory tests, results must be interpreted appropriately in relation to the case involved. Please provide an appropriate history with all samples referred to our laboratory and we will help interpret results on the report and, if required by telephone conversation.

### **COSTS**

Single, one-off PCR tests at our laboratory cost £25 plus VAT. A duplex test costs £40 and a triplex test costs £50. For batches of tests, e.g. for *T. equigenitalis* for HBLB Code of Practice screening, we are hoping to charge significantly less. We will announce this later in the year, i.e. before January 1st 2010.

### **REFERENCE**

Ousey, J.C., Palmer, L, Cash, R.S.G., Grimes, K.J., Fletcher, A.P., Barrelet, A, Foote, A.K, Manning, F.M. and Ricketts, S.W. (2009) An investigation into the suitability of a commercial real-time PCR assay to screen for *Taylorella equigenitalis* in routine prebreeding equine genital swabs. Equine Veterinary Journal, in press.