COMPARATIVE TRIAL OF BACTOPROOF™ QPCR AND CONVENTIONAL CULTURE FOR MASTITIS TESTING.

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Identification of the pathogen involved is a key step in the control of mastitis; it should be used to help improving control measures, fine tune treatments and help with decision making for problem cows. Traditionally, this has been done by bacterial culture, but recently a Polymerase Chain reaction (PCR) test has been developed which allows a more rapid analysis for a range of bacteria. Additional advantages of PCR testing are that samples can be preserved immediately after collection and posted at ambient temperatures.

PCR routinely tests for Staph aureus, Coagulase Negative Staphylococci (CNS), Strep. uberis, Strep. dysgalactiae, Strep agalactiae, E. coli, C. bovis, Klebsiella, Enterococcus, Serratia marcescens, A. pyogenes and P. indolicus and the Beta-lactamase gene. Mycoplasma bovis can be identified using an additional PCR test.

MATERIALS AND METHODS

Duplicate milk samples were collected from 70 cases. These were from high cell count and clinical mastitis cases from vets or clients of the Shepton Veterinary Group. All samples tested using PCR and conventional bacteriology. For conventional bacteriology, clinical samples were frozen and sent as a batch while high cell count samples were posted fresh and sent with ice packs.

RESULTS

Overall PCR yielded a positive result in 64 of the 70 samples tested (91%). Culture gave a positive result in 52 (74%) of the samples tested. Contaminated cultures were not observed in this trial, perhaps reflecting the attention to sampling technique and transport conditions of the samples. In this study 54% of Staph aureus and 24% of CNS isolates had the β-lactamase gene present, penicillin resistance was not tested in culture.

There were differences between the results obtained using PCR and conventional culture (figure 1).
DISCUSSION

Conventional culture and PCR are different diagnostic techniques and in 40% of samples tested yielded substantially different results.

With a few exceptions PCR detected substantially more pathogens than conventional culture. No growth results are a significant problem in conventional culture and can be a factor in the reluctance of vets and farmers to test for mastitis pathogens. In culture negative samples which had positive results by PCR, over half of the bacteria detected were CNS; in the remaining samples *Strep. uberis*, *E. coli*, *Staph. aureus* and *C. bovis*. This highlights a major potential advantage of using DNA technology and supports the view that it may be more sensitive and accurate than bacteriology. It should be noted that in some cases conventional bacteriology identified pathogens which PCR testing cannot currently identify including yeasts, *Proteus* spp., *Bacillus* spp., *Pseudomonas* spp., and *Pasteurella* spp. Negative test results by either method should be interpreted with caution. The high rate of identification of β-lactamase gene for *Staph aureus* (56% from 13 cows) and moderate levels for CNS (24% from 42 cows) by PCR provided useful information for treatment decisions. Rapid identification of the β-lactamase gene responsible for penicillin resistance is a significant advantage compared to conventional culture.

Farmers liked the fact that they could get their PCR results back quickly and that PCR testing allowed easy sample despatch. The benefit of sampling non-responsive cows under treatment was also appreciated. The greater percentage of samples returning a positive result was also seen as important. Laboratory work is seen as being expensive, so the more positive samples returned the better. Although no diagnostic test is perfect for all situations, PCR technology for mastitis diagnosis has some significant advantages, and used correctly has the potential to improve mastitis control in the UK.