Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 118 (2006) 247–254
veterinary microbiology
www.elsevier.com/locate/vetmic

Development of a real time PCR for the detection of *Taylorella equigenitalis* directly from genital swabs and discrimination from *Taylorella asinigenitalis*

P.R. Wakeley [a,*], J. Errington [a], S. Hannon [a], H.I.J. Roest [b], T. Carson [a], B. Hunt [a], J. Sawyer [a], P. Heath [a]

[a] *Technology Transfer Unit, Biotechnology Department, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom*
[b] *Central Institute for Animal Disease Control-Lelystad, Lelystad, The Netherlands*

Received 14 December 2005; received in revised form 2 August 2006; accepted 7 August 2006

Abstract

A discriminatory real time PCR for the detection of *Taylorella equigenitalis*, the causative agent of contagious equine metritis (CEM), and the related species *T. asinigenitalis* was developed for the direct examination of genital swabs. The 112 bp amplicons produced from the two species were discriminated from each other using TaqMan® probes labelled with different fluorophores. The TaqMan® PCR was shown to be specific for the 16S ribosomal DNA of the two species of taylorella and did not cross-hybridise with the 16S ribosomal DNA of other bacteria tested. Direct amplification from genital swabs was shown to be equally sensitive to that of culture methods. Prevalence in a sample set from The Netherlands was shown to be equivalent to that demonstrated by culture. A companion real time PCR that amplified a fragment of the 16S rDNA gene of equine commensal bacteria was developed to ensure bacterial DNA was extracted from swab material supplied for testing. The use of a rapid and reliable real time PCR for the organism causing CEM should aid the control of this disease.

Keywords: Contagious equine metritis; *Taylorella equigenitalis*; *Taylorella asinigenitalis*; Direct PCR amplification

1. Introduction

Contagious equine metritis (CEM) was first described in the UK in 1977 (Crowhurst et al., 1979) and is caused by the Gram-negative bacterium *Taylorella equigenitalis* (Sugimoto et al., 1983). Clinical signs of CEM, which develop in only 30–40% of mares served by an infected stallion, may include vaginal discharge, infertility and early abortion.

* Corresponding author at: Technology Transfer Unit, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom. Tel.: +44 1932 357759; fax: +44 1932 357890.
E-mail address: p.wakeley@vla.defra.gsi.gov.uk (P.R. Wakeley).

0378-1135/$ – see front matter.
doi:10.1016/j.vetmic.2006.08.007

Asymptomatic mares are the most likely reservoir of infection (Powell, 1981). Infected stallions are asymptomatic and act as the principal source of infection as they mate with numerous mares and the carrier status may persist for many months or even years (Schluter et al., 1991).

Effective vaccines that protect against infection by *T. equigenitalis* are as yet not available (Fernie et al., 1980) and the principle tool against spread of infection is identification of the organism following culture (Timoney et al., 1982). The sampling of both stallions and mares at the start of the breeding season and adoption of a code of practice, published annually by the Horserace Betting Levy Board in the UK, has effectively eliminated the disease from thoroughbred horses in the UK with only the occasional outbreak, e.g. arising from importation and breeding with a warmblood stallion in 2002 (Jackson et al., 2002). *T. equigenitalis* is slow growing, microaerophilic, and may lose viability relative to commensals found in the urogenital membranes of horses despite transport to the laboratory in Amies charcoal medium (Swerczek, 1978). Several days of culture (3–10 days) are required in order to produce visible colonies and this is problematic not only because commensals overgrow the *T. equigenitalis* (Bleumink-Pluym et al., 1994) but also because bacteria with similar morphology and phenotypic characteristics (Moore et al., 2001) may be present. The genetically related bacterium, *T. asinigenitalis*, originally isolated from the urethral fossae of three donkey jacks, does not apparently elicit disease in mares (Jang et al., 2001) but may cause confusion when using culture techniques, due to colony morphology similarity with *T. equigenitalis*, or assays employing the polymerase chain reaction (PCR) due to the high degree of sequence homology between *T. equigenitalis* and *T. asinigenitalis* (Bleumink-Pluym et al., 1994; Moore et al., 2000).

Several different PCR methods for the detection of *T. equigenitalis* (Anzai et al., 1999; Bleumink-Pluym et al., 1994) and discrimination from *T. asinigenitalis* (Arata et al., 2001; Premanandh et al., 2003) have been developed. With the exception of the method of Premanandh et al. (2003), all these methods require agarose gel electrophoresis in order to analyse the amplicon produced.

This report describes the development and validation, against culture techniques of a novel real time TaqMan® PCR for the detection of *T. equigenitalis* directly from genital swabs and discrimination of the amplicons generated from the closely related species *T. asinigenitalis*. The technique was shown to be sensitive, specific and rapid, allowing, in some instances, detection of bacteria where routine culture methods had failed.

2. Materials and methods

2.1. Bacteria

Two well characterised laboratory strains of *T. equigenitalis* (NCTC 11184, NCTC 11225) described previously (Bleumink-Pluym et al., 1993) and three isolates of *T. asinigenitalis* UK-1, UK-2, and UCD-1 (ATCC 700933) representing two isolates from donkey jacks in Kentucky, USA and the type strain respectively (Jang et al., 2001) were used in this study. Bacterial lysates from 28 *T. equigenitalis* isolates, associated with previous UK outbreaks and 3 specific isolates from the 2002 outbreak in the UK were assayed. Purified DNA received from Japan, from *T. equigenitalis* strains Te-1, Te-184 and CEMO-13 were also tested

Bacterial lysates from 20 different species of bacteria were tested in order to determine the analytical specificity of the TaqMan® PCR: *Actinobacillus equuli* (NCTC 3365), *Actinobacillus pleuropneumoniae* (NCTC 12370), *Alcaligenes faecalis* (NCTC 11953), *Arcanobacterium pyogenes* (FD 2294), *Bacteroides ureolyticus* (FD2477), *Bordetella bronchiseptica* (NCTC 8750), *Enterococcus faecalis* (NCTC 12697), *Escherichia coli* (NCTC 10418), *Klebsiella pneumoniae* (NCTC 8172), *Mannheimia haemolytica* (NCTC 10208), *Oligella urethralis* (NCTC 4087), *Pasteurella multocida* (NCTC 10322), *Proteus mirabilis* (NCTC 11983), *Proteus vulgaris* (NCTC 4175), *Pseudomonas aeruginosa* (NCTC 10662), *Rhodococcus equi* (NCTC 1621), *Salmonella poona* (NCTC 4840), *Staphylococcus aureus* (NCTC 6571), *Staphylococcus intermedius* (NCTC 11048), *Streptococcus equi* (NCTC 9682), *Streptococcus zooepidemicus* (NCTC 6180) and *Yersinia enterocolitica* (NCTC 10460).

2.2. Genital swab samples

Genital swabs from 778 horses (413 stallions; 242 mares; 123 unknown sex), were tested by bacterial