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Short communication

The detection of *Mycoplasma* (*formerly Eperythrozoon*) *wenyonii* by 16S rDNA PCR and denaturing gradient gel electrophoresis

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Abstract

Although the role of *Mycoplasma wenyonii* in disease is still subject to some debate, infections have been reported to result in parasitaemia, anaemia, scrotal and hind limb oedema, tachycardia, pyrexia, infertility, swollen teats, prefemoral lymphadenopathy and decreased milk production. Previously, diagnosis of *M. wenyonii* has been based on blood smears but is not specific for *M. wenyonii* and can be difficult to interpret. We have previously described the use of PCR and denaturing gradient gel electrophoresis (DGGE) for the detection and differentiation of *Mycoplasma* species. DGGE enables the rapid and specific identification of *Mycoplasma* species and is ideally suited to detecting both mixed infections and new and unusual species. In this study, we have used DGGE with universal primers to detect *M. wenyonii* DNA from blood samples. DGGE can be used on blood samples as a rapid and specific test for *M. wenyonii* and can also be used as a screening test for other blood borne pathogens.

Keywords: DGGE; *Mycoplasma wenyonii*; Eperythrozoon

1. Introduction

Mycoplasma wenyonii is a wall-less haemotrophic prokaryote previously classified as *Eperythrozoon wenyonii* in the order *Rickettsiales* but recently assigned to the *Mycoplasma* genus on the basis of 16S rDNA analysis (Neimark et al., 2001). *M. wenyonii* adheres to erythrocytes but may also be found in plasma unattached to erythrocytes (Neimark et al., 2001). Scanning electron microscopic analysis of *M. wenyonii* infected blood shows deformed erythrocytes with invaginations and the presence of either rod or coccoid shaped organisms embedded in the membrane of erthrocytes (Bretana et al., 2002;

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Neimark et al., 2001). Although the mode of transmission is unknown there is evidence that flies, lice and mosquitoes may serve as mechanical vectors and oral transmission also seems likely (Hofmann-Lehmann et al., 2004; Prullage et al., 1993; Smith et al., 1990). Although the precise role of *M. wenyonii* in disease is still controversial, infections caused by *M. wenyonii* have been reported to result in parasitaemia and anaemia with infected cattle rarely dying but on occasions showing acute clinical signs (Smith et al., 1990). Scrotal and hind limb oedema, tachycardia, pyrexia and infertility were reported in a Charolais bull in the USA that was chronically infected with *M. wenyonii* (Montes et al., 1994). In addition, dairy cows with swollen teats, oedema of the distal portion of the hind limbs, transient fever, prefemoral lymphadenopathy, rough coat, dramatically decreased milk production and subsequent infertility and weight loss have been reported (Smith et al., 1990). Recently, other as yet unclassified haemotrophic mycoplasmas have been discovered in cattle in Switzerland with severe anaemia that were co-infected with *M. wenyonii* and *Anaplasma marginal* (Hofmann-Lehmann et al., 2004), however, these organisms have not yet been found in the UK.

Previously, diagnosis of *M. wenyonii* has been based on blood smears but more recently a PCR has been described but is not in widespread use (Neimark and Kocan, 1997). The staining of blood smears is not specific for *M. wenyonii* and can be difficult to interpret with stain deposits sometimes mistaken for *M. wenyonii* organisms. We have previously described the use of PCR and denaturing gradient gel electrophoresis (DGGE) for the detection and differentiation of *Mycoplasma* species (McAuliffe et al., 2003, 2005). DGGE can theoretically detect single base mutations in DNA (Lerman and Beldjord, 1999; Fisher and Lerman, 1983). The method is based on the prevention of migration of DNA fragments following strand separation caused by chemical denaturants. DGGE enables the rapid and specific identification of *Mycoplasma* species and is ideally suited to detecting both mixed infections and new and unusual species. We have investigated the suitability of DGGE to detect *M. wenyonii* DNA from blood samples and have used DGGE and 16S rDNA sequencing to detect *M. wenyonii* in a dairy cow unit in which lactating cattle were affected with swollen, painful udders and hindlimb oedema. We propose that 16S rDNA PCR and DGGE is a rapid and specific test for *M. wenyonii* and the generic nature of the test means that it may also show promise as a screening test for other blood borne pathogens.

2. Materials and methods

2.1. Blood sampling

Blood samples were taken from three Friesian Holstein cows showing clinical signs. All blood samples were taken on the same day. No other animals in the group of 250 dairy cows exhibited clinical signs. Animals were housed in cubicles with bedding of shavings.

2.2. DNA extraction and PCR

DNA was extracted from 100 μl blood samples using a tissue DNA extraction kit (Sigma).

Amplification of the V3 region of the 16S RNA gene was performed according to the method of as described previously (McAuliffe et al., 2003) using universal bacterial primers GC-341F 5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG and 534R 5′-ATT ACC GCG GCT GCT GG. For the PCR, 1 μl of lysate was added as a template to 49 μl of a reaction mixture containing 10 mM Tris–HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 0.2 mM each deoxynucleoside triphosphate, and 0.5 U of Taqgold (Applied Biosystems). The cycling conditions were: denaturation at 94 °C for 5 min followed by 30 cycles of 95 °C for 1 min, 55 °C for 45 s and 72 °C for 1 min, a final extension step of 72 °C for 10 min and samples were kept at 4 °C until analysis. Aliquots were checked for correct amplification by electrophoresis on a 2% agarose gel followed by visualization with ethidium bromide under UV illumination.

2.3. DGGE

DGGE was performed using the Ingeny PhorU 2 × 2 apparatus (GRI Molecular Biology, Essex, UK).