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Genetic variability of *Chlamydophila abortus* strains assessed by PCR-RFLP analysis of polymorphic membrane protein-encoding genes

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ABSTRACT

This study used PCR-RFLP to investigate the genetic variability of *pmp*-encoding genes from fifty-two *Chlamydophila abortus* (*C. abortus*) strains originating from abortion cases from various geographical regions and host species. Six primer pairs were used to PCR-amplify DNA fragments encoding eighteen pmps. PCR products were digested using four restriction endonucleases and Bayesian methodologies were used to compare RFLP profiles and assign strains to a RFLP genotype. Strains could be assigned to 2 genotypes in the region encoding *pmp18D*, 3 genotypes in the regions encoding *pmp1A-pmp2B*, *pmp3E-pmp6H* and *pmp11G-pmp15G*, 4 genotypes in the region encoding *pmp7G-pmp10G* and 5 genotypes in the region encoding *pmp16G-pmp17G*. In all regions, the majority of strains (88.4–96.1%) had the same genotype as the reference strain S26/3. No correlation could be made between genotype, host species or geographical origin except for the two variant Greek strains, LLG and POS, which formed a discrete genotype in all *pmp*-encoding regions except *pmp18D*. Relative rates of evolution calculated for each *pmp*-encoding gene locus suggest that differing selective pressures and functional constraints may exist on *C. abortus* polymorphic membrane proteins. These findings suggest that although intraspecies heterogeneity of pmp-encoding genes in *C. abortus* is low, the sequence heterogeneity should be an important consideration when using pmps as the basis for novel diagnostics or vaccine development.

1. Introduction

Ovine enzootic abortion (OEA) is an economically important disease accounting for approximately 43% of diagnosed abortions in sheep in the UK. The aetiological

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agent, *Chlamydophila abortus* (formerly *Chlamydia psittaci* serotype 1), can also cause abortion in cattle and pigs and poses a zoonotic risk to pregnant women (Longbottom and Coulter, 2003).

The genetic heterogeneity of *C. abortus* has been reported to be low, with methods based on the cross-reactivity of monoclonal antibodies, restriction fragment length polymorphism (RFLP) and the phylogenetic analysis of 16S rRNA genes, failing to differentiate strains based on host, pathotype or geographical origin (Denamur et al., 1991; Everett et al., 1999; Salinas et al., 1995). More recently, methods such as multiple loci VNTR analysis (MLVA) and multi locus sequence typing (MLST), have allowed the differentiation of *C. abortus* strains into novel genotypes (Laroucau et al., 2009; Pannekoek et al., 2010) that were partly related to geographical origin (Laroucau et al., 2009).

Genes encoding polymorphic membrane proteins (*pmps*) have been identified in all members of the family *Chlamydiaceae.* In *C. abortus* there are 18 *pmp*-encoding genes that are arranged as four loci composed of a single gene, two gene pairs and a large cluster of 13 genes (Thomson et al., 2005). Polymorphic membrane proteins have been implicated in chlamydial adhesion (Wehrl et al., 2004; Crane et al., 2006; Mölleken et al., 2010), tissue tropism (Stothard et al., 2003; Gomes et al., 2006) and the induction of immune responses (Mygind et al., 2004; Wehrl et al., 2004; Livingstone et al., 2005) making them potential diagnostic and vaccine targets (Longbottom and Livingstone, 2006; Sachse et al., 2009). The purpose of this study was to analyse the genetic diversity of *pmp*-encoding loci from *C. abortus* isolates using PCR-RFLP and determine whether strains could be distinguished by geographical origin or host species.

2. Materials and methods

2.1. Propagation of C. abortus and gDNA preparation

Fifty-two *C. abortus* strains isolated from various host species and geographical regions were used in this study (Table 1). Strains were propagated as previously described (Graham et al., 1995) and genomic DNA extracted using a Wizard Genomic DNA isolation kit (Promega).

2.2. PCR amplification of pmp-encoding loci

Oligonucleotide primers targeting the 18 *pmp*-encoding genes of *C. abortus* S26/3 [NC_004552] were designed using Lasergene 8 PrimerSelect software (DNASTAR). Three *pmp*-encoding loci encoding one or two genes in tandem were PCR-amplified as single fragments and are referred to in this study as BA (*pmp1B-pmp2A*), 16-17 (*pmp16G-pmp17G*) and 18D (*pmp18D*). The locus encoding 13 pmps was PCR-amplified as three fragments which are referred to in this study as EH (*pmp3E-pmp6H*), HG (*pmp6H-pmp10G*) and G (*pmp11G-pmp15G*).

PCR amplification mixes comprised 1× Expand PCR buffer 3 (Roche Applied Sciences), 500 μM dNTPs, 300 nm each primer (see Table 2), 0.075 U Expand Long Template Polymerase (Roche Applied Sciences), and 20–100 ng *C. abortus* gDNA made up to a 25 μl final volume using nuclease free water. PCR consisted of 1 cycle at 94 °C for 2 min followed by 10 cycles of 94 °C for 10 s, 51.9 °C for 30 s and 68 °C for 15 min. A second amplification stage consisted of 20 cycles of 94 °C for 10 s, 51.9 °C for 30 s and 68 °C for 15 min with a 20 s increment per cycle followed by one cycle of 68 °C for 7 min. Efficiency of amplification was assessed by agarose gel electrophoresis using 0.8% TAE-agarose gels containing 1× GelRed (Biotium Inc).

2.3. Restriction fragment length polymorphism (RFLP)

Restriction endonuclease (RE) digestions were performed using *Mbo*II (New England Biolabs), *Hae*III (New England Biolabs), *Rsa*I (Roche Applied Sciences) and *Alu*I (Roche Applied Sciences). Restriction endonuclease digestion reactions consisted of 1× buffer, 5 U of the appropriate RE and 5 μl of PCR-amplified DNA. Restriction endonuclease digests were incubated at 37 °C for 2 h. Five microlitres of each RE digest were electrophoresed on a

Table 1
C. abortus strains used for PCR-RFLP analysis.

Country of origin	Host species	Strain
France	Sheep	AB7
Germany	Cow	03DC34, 03DC36, C11/98, Z339
	Goat	532
	Pig	DC12
	Sheep	53, C1/98, C2/98, C7/98, C9/98, C10/98, C17/98, DC1, DC4, DC7
Greece	Goat	FAG, LLG
	Sheep	POS
Ireland	Sheep	C94/1, C94/2, C95/23, C95/27, VS88/576
Italy	Cow	LV350/93
Namibia	Goat	C21/98
Spain	Goat	Syva-1
	Sheep	AO-2, AO-3, AO-4, AO-7, Zar-2B, Zar-5
Tunisia	Goat	15
UK	Sheep	83/12, 86/30, 90/345, A22, H574, S3/3, S26/3, S82/3, S95/3, S124/3A, S152/3, S404/5, S507, T17, T19, T20, T28