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A novel *mba*-based Real time PCR approach for genotyping of *Ureaplasma parvum* validated in a cohort of Mongolian mothers and offspring

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ABSTRACT

The role of *Ureaplasma parvum* in abnormal outcomes of human pregnancy has been discussed controversially in the past. Of the 14 known ureaplasma serovars, the *Ureaplasma parvum* serovars 1, 3, 6 and 14, have been found to derive from smaller genomes. Serovars 3 and 6 have been described more often to cause complications in pregnancy. To elucidate the serovar distribution in *U. parvum* positive specimens of 200 Mongolian mothers and their offspring, a new set of *mba*-targeting PCRs was developed enabling a fast and reliable serovar differentiation by melting peak analysis in a Real time PCR approach or by conventional agarose gel electrophoresis. 92% maternal and 55% neonatal samples were retrospectively genotyped and a dominance of serovars 3 and 6 was detected while serovar 14 was almost absent. Transmission from mothers to newborns was detected in 83% of *U. parvum* positive neonates exhibiting serovar patterns identical to their mothers. No statistically significant correlation between a distinct serovar and pregnancy outcome could be detected. However, neonatal colonization with serovar 1 declined with progressing pregnancy suggesting that a higher ureaplasma load shortened pregnancy and thereby had a potential negative effect on offspring health. Our novel *mba*-based Real time PCR approach, which can also be used in conventional PCR and gel electrophoretic analysis, provides the proof of principle that the four *U. parvum* serovars 1, 3, 6 and 14 can be differentially detected and quantified. A larger scale study outside the scope of this work should be conducted to clarify the impact of serovar 1 on pregnancy outcome.

1. Introduction

The pathogenic role of ureaplasmas in human pregnancy has been controversially discussed (Donders et al., 2017). The colonization rate of the urogenital tract is generally high, even in healthy women, and ureaplasma-positive pregnant women without signs of infection are rarely treated with antibiotics. With improved molecular biological methodology it became easier to distinguish *U. parvum* from *U. urealyticum* (Cao et al., 2007; Cunningham et al., 2013; Mobius et al., 2012; Vancutsem et al., 2011; Xiao et al., 2010; Xu et al., 2016) and to genotype both species. For the differentiation of *U. parvum* serovars 1, 3, 6 and 14 and *U. urealyticum* serovars 2, 4, 5 and 7–13, the *mba* gene, which encodes the multiple banded antigen, was often targeted (Kong et al., 2000a, b; Payne et al., 2014; Tang et al., 2011; Xiao et al., 2011a). Although the repetitive region of the *mba* 3′-end leads to size variants, which are known to modulate the host immune response (Sweeney et al., 2017), the conserved 5′-end of *mba* enabled serovar differentiation. Thus, *U. parvum* serovar 6 was significantly more often isolated from women belonging to a small study group who delivered preterm offspring (Knox and Timms, 1998; Payne et al., 2016). *U. parvum* serovars 3 and 6 were detected as predominant ureaplasma genotypes colonizing the respiratory tract of preterm infants (gestational age < 33 weeks), but no correlation of a certain serovar to the severity of bronchopulmonary dysplasia could be found (Sung et al., 2011).

In our former study of 200 Mongolian mothers and offspring (gestational age: 35–40 weeks) (Otgonjargala et al., 2017), ureaplasmas were found to be the most prevalent pathogen within the tested cohort (positive in 90.5% of the vaginal swabs of women and 47.5% of the oral swabs of the newborns). Colonization of the mothers with *U. urealyticum* was associated with transmission to newborns, but no significant correlation could be found between maternal colonization with *U. parvum* and transmission to the neonates. The findings suggested that neonatal colonization with *U. parvum*, but not *U. urealyticum* was

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Table 1
Primers used.

Primer	Sequence (5'-xxx-3')	Amplicon	Gene / Acc.-no.	Reference
Up-F	TTGCAATCTTTATATGTTTTCGTTA			(Tang et al., 2011)
Up-R	ACATGAAGCTGCTATAGCAACTAT	236 bp		(Tang et al., 2011)
SV1-F	AAAACTGAAGAACCAAAAGAAAATGTT			*this study*
SV1-R	TGTTCTTTACCTGGTTGTTGT	68 bp	AF056983	*this study*
SV3-F	ATTACTGTAGAAATTATGTAAGATTACC			*this study*
SV3-R	CCAGCTCCAACTAAGGTAACA	173 bp	AF222894	*this study*
SV6-F	AATAAATCTTAGTGTTCATATTTTTTACTAG			*this study*
SV6-R	TCCAGCTCCAACTAAGGTAACT	150 bp	AF056984	*this study*
SV14-F	ATAAATCTTAGTGTTCATATTTTTTACAT			*this study*
SV14-R	ACTAAGGTAACACCTAATGTCATAGCTA	138 bp	AF056982	*this study*

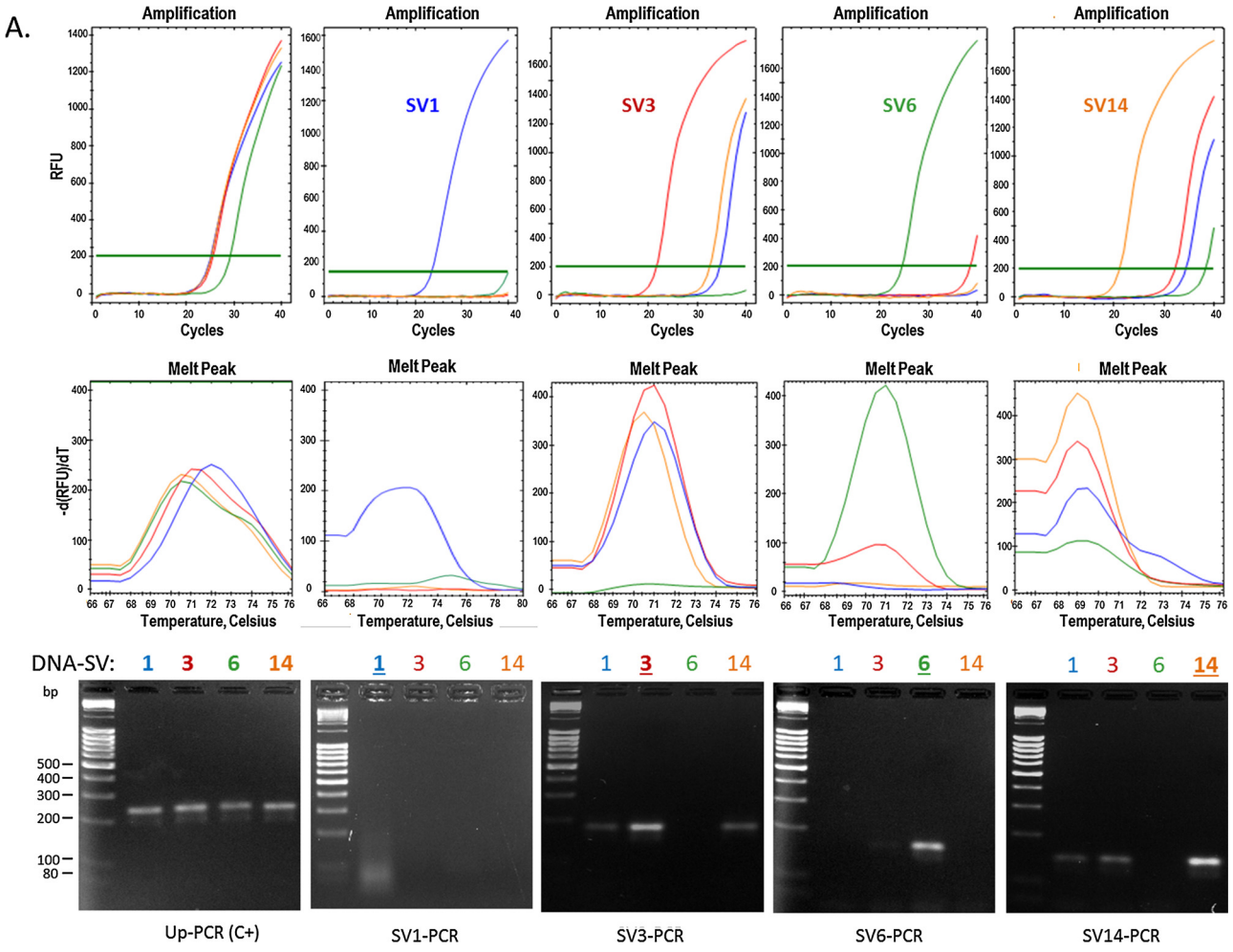

Fig. 1. Amplification and Melting peak plots of U. parvum (Real time) PCRs Up, SV1, SV3, SV6 and SV14. A., Real time PCRs were carried out in parallel with genomic DNA (25 ng/µl) of reference strains of serovars 1, 3, 6 and 14 (MacKenzie et al., 1996) in each PCR. The Up-PCR served as a positive control for presence of *U. parvum* DNA. Three of the four serovars (1, 3, 6 and 14) served as negative controls for each SV-PCR (e.g. in SV1- PCR DNA of serovars 3, 6 and 14 served as negative controls). Amplification curves, melting peaks and gel electrophoretically separated PCR products of each serovar-DNA are shown in the set of 5 PCRs. B. Respective results of Amplification, Melting peak plots and agarose gel electrophoresis are shown for selected maternal samples M5, M9, M11, M14 and M39.

associated with a reduced gestational age. This was in accordance to data of Prince et al. who found *U. parvum* to be associated with preterm delivery (Prince et al., 2016).

The present study was conducted to develop a novel PCR set designed to target the *mba* gene and to be suitable for Realtime PCR as well as conventional PCR with the aim of genotyping *U. parvum* strains. In a proof of principle study the frequency of individual *U. parvum* serovars was then elucidated in a cohort of Mongolian pregnant mothers and their offspring.

2. Materials and methods

2.1. Sample collection and questionnaire

DNA samples derived from vaginal swabs of 200 Mongolian mothers and oral swabs of their newborns were extracted using Blood & Cell Culture DNA Midi Kits (Qiagen, Hilden Germany) and stored at −20 °C until use in Real time PCR as outlined before (Otgonjargala et al., 2017). A questionnaire was filled out for each woman to obtain general information on the history, outcome and related risk factors of previous