

# Using PCR Assay for Detection and Subtyping of *Ureaplasma Parvum* in Women with Recurrent Abortion

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The objects of this study concerted to investigate the occurrence of *Ureaplasma parvum* in women with recurrent abortion and to determine the distribution of *U. parvum* serovars (1, 3, 6, 14) in women with recurrent abortion by conventional PCR technique. In total, 130 samples included vaginal bleeding, vaginal swab, and urine, were collected from women with recurrent abortion and 40 samples included vaginal swab and urine from control women without recurrent abortion. Through the study, two types of media were used, *Ureaplasma* broth (IH Broth) and *Ureaplasma* agar (IH Agar). The positive isolates for *Ureaplasma* spp. were investigated by conventional PCR technique for identification of *U. parvum* and subtyping to their serovars (1, 3, 6, 14). The results revealed the *U. parvum* was identified in 29.6% from patient group and 11% from the control group. *U. parvum* isolates were further subtyped by using PCR, the results showed the serovar 3 was the most frequent isolate in proportion (42.8%), whereas serovar 1 (28.5%), serovar 6 (14.2%), and serovar 14 (14.2%) in patient group but in the control group only serovar 1 was isolated in rate (11%). These results evidently indicate that *U. parvum* may be an important etiologic agent for recurrent abortion.

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*Reviews in Medical Microbiology* 2017, **28**: 26 – 29

**Keywords:** IH medium, PCR, recurrent abortion, serovars, subtyping, *Ureaplasma parvum*

## Introduction

*Ureaplasma* spp. is the most prevalent, possibly pathogenic bacteria isolated from the urogenital tract of both men and women [1]. *Ureaplasma* spp. are also frequently associated with preterm birth and other adverse pregnancy outcomes and *Ureaplasma* spp. are colonies isolated in female genitourinary tract sometimes these microorganisms do not evaluate as infectious agents [2]. Detection of *Ureaplasma* is possible by the characteristic growth on appropriate media and urease activity, but species identification of *U. urealyticum* and *U. parvum* must be demonstrated by molecular methods [3]. Differentiation between *U. parvum* and *U. urealyticum* is very important, especially for correct interpretation of laboratory results and evaluation of pathogenicity [4]. *Ureaplasma* spp. do not have cell wall, are fastidious and mostly referred to as no cultivable organisms [2]. Genital tract infections with *Ureaplasma* caused approximately 50% of preterm labor and recurrent abortion [1]. Most of pregnancies produced infant with low weight at birth, so increase risk of recurrent abortion (14 weeks). Also, 60% of mortality among infants with no anatomic or chromosomal defects is low birth weight [5]. *U. parvum* has been linked with adverse

pregnancy outcomes such as late abortion and early preterm birth. *Ureaplasma* spp. are the microorganisms most frequently isolated from amniotic fluid or placenta in women who deliver preterm between 23 and 32-weeks pregnant [4]. *U. parvum* are involved in a variety of infections in genitourinary tract infections of humans [3,6]. Identified *U. parvum* in 57% of healthy non pregnant women and the organism was far more prevalent than any of the other genital mycoplasmas, *Chlamydia* spp., or viruses [7]. The proposed mechanisms for infectious causes of recurrent abortion include: direct infection of the uterus, fetus, or placenta; placental insufficiency; chronic endometritis or endocervicitis; amnionitis; infected intrauterine device. [8]. *Ureaplasma* can be detected in the cervix or vagina of 40–80% of sexually mature asymptomatic women [9]. *U. parvum* may play important role in pregnancy and eliciting conditions associated with prematurity [10]. The main aim of this study is to investigate the occurrence of *U. parvum* in women with recurrent abortion and to determine the distribution of *U. parvum* serovars (1, 3, 6, 14) in women with recurrent abortion by conventional PCR technique.

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Received: 4 October 2016; revised: 18 November 2016; accepted: 21 November 2016

DOI:10.1097/MRM.0000000000000095

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**Materials and methods**

**The bacterial isolates**

In total, 130 samples included vaginal bleeding, vaginal swab, and urine, were collected from women with recurrent abortion and 40 samples included vaginal swab and urine from control women without recurrent abortion. All specimens were cultured in IH broth, which consists of PPLO broth, trypton soya broth, yeast extract powder, distilled water, and supplements [11]. Then make a subculture to IH agar, which consists of PPLO agar, trypton soya broth, MgSO<sub>4</sub>.H<sub>2</sub>O, yeast extract powder, agar-agar, distilled water, and supplements [11]. The *Ureaplasma* spp. isolates were identified by examination of colonial morphology on IH agar media as dark golden-brown or rich, deep brown, and granular appearance because of accumulation of magnesium oxide inside and outside the colony [12].

**Molecular experiments**

Molecular experiments included the extraction of *Ureaplasma* DNA by using the Reagent Genomic DNA Kit (Geneaid, New Taipei, Taiwan). PCR identification of *U. parvum* was done according to Kong *et al.* [13,14] and master mix kit (BioNeer, Irvine, California). PCR was performed with primers specific for highly conserved regions in the 5<sup>0</sup> end of multiple band antigen gene of *U. parvum*. Primer for diagnosis of *U. parvum*, UMS-57/ UMA222, is shown in Table 1 [13,14]. The primers for detection of serovars UMS3S/UMA26, UMS14S/UMA314A, UMS-83/UMA1A, and UMS-54/UMA269

(BioNeer, Irvine, California) are shown in Table 2 [13,14] and were used for subtyping of *U. parvum* to amplify the repetitive of the multiple band antigen genes of *U. parvum* serovars.

**PCR technique**

The 20 ml amplification reaction mixtures contained 10 pmol of each primer, 5 ml of DNA template, and PCR water added to 20 ml for identification *U. parvum*. The PCR conditions were used as follows: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 1 min for 40 cycles, and final extension at 72°C for 5 min in a thermo cycler. The PCR positive isolates for *U. parvum* were further subtyped into serovars as described in Table 2. Briefly, the PCR conditions were used as follows: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 55–62°C for 30 sec, extension at 72°C for 1 min for 40 cycles. Amplified PCR products (12.5ul) were visualized under UV light after electrophoresis in 2% agarose gel which were stained with 0.5 mg/ml of ethidium bromide. A visible band of the appropriate size on UV transillumination was considered a positive result.

**Statistical analysis**

The data were analyzed using SPSS statistic software version 20 (IBM, Armonk, USA) for comparison of qualitative variables using *P*<0.05 and odd ratio. Association between *U. parvum* infection and recurrent abortion was statistically significant.

Table 1. PCR primer employed in the detection of *Ureaplasma parvum*.

organism	Primer (F) (R)	Sequence (5'- 3')	Size of amplified product (bp)	Target gene
<i>U. parvum</i>	UMS-57	F (TAA ATC TTA GTG TTC ATA TTT TTT AC -57)	326	5' Ends of MBA genes and upstream regions
	UMA222	R (GTA AGTGGA TTA AAT TCA ATG 222)		

MBA, multiple band antigen. Adapted with permission from [13,14].

Table 2. PCR primers employed for subtyping of *Ureaplasma parvum* in to serovars.

Organism	Primer (F)/(R)	Sequence (5'- 3')	Size of amplified product (bp)	Target gene
<i>U. parvum</i> Serovar 1	UMS-83 UMA1A	F (TTACT GTA GAA ATT ATG TAA GAT TGC) R (TTT CTT TTG GTT CTT CAG TTT TTG AAG)	578	MBA
<i>U. parvum</i> Serovar 3	UMS3S UMA269	F (TTA CTG TAG AAA TTA TGT AAG ATT ACC) R (AA CTA AAT GAC CTT TTT CAA GTG TAC)	400	MBA
<i>U. parvum</i> Serovar 6	UMS-54 UMA269	F (AAT CTT AGT GTT CAT ATT TTT TAC TAG) R (ACCA AAT GAC CTT TTG TAA CTA GAT)	370	MBA
<i>U. parvum</i> Serovar 14	UMS14S UMA314A	F (AAT TAC TGT AGA AAT TAT GTA AGA TTA AT) R (GTT GTT CTT TAC CTG GTT GTG TAG)	572	MBA

MBA, multiple band antigen; *U. parvum*, *Ureaplasma parvum*. Adapted with permission from [13,14].

**Results and Discussion**

The results showed the *Ureaplasma parvum* isolated in rate (29.6%) from women with recurrent abortion and (11%) from control as shown in Fig. 1 (*P* value <0.05 appeared highly significant). The results revealed positive isolates for *Ureaplasma parvum* by using UMS-57/UMA222 primer as shown in Fig. 2. The negative isolates for *U. parvum* may be because of the fact that *Ureaplasma* are divided into two species *U. parvum* and *U. urealyticum*, these two species cannot be identified by characteristic growth on appropriate media and only identified by molecular methods [13]. So the negative results may be *Ureaplasma urealyticum* rather than *Ureaplasma parvum* and the results appeared to be attributable to a higher proportion of women with recurrent abortion. It may be hormonal effects which could increase *Ureaplasma parvum* counts and thus the likelihood of detection during pregnancy. A previous study showed that there is *Ureaplasma parvum* in rate (20%) from women with recurrent abortion in China by using PCR technique [15]. Although *Ureaplasma parvum* was isolated in rate (25%) from women with symptoms of urethral, cervical discharge, genital pruritis, dysuria in India. [14]. However, some other studies detected this organism in high rate (approximately 79%) from pregnant women and women with sexually transmitted disease in Australia [13]. *Ureaplasma parvum* positive isolates were further subtyped into serovars 1, 3, 6, 14; the results revealed *Ureaplasma parvum* (biovar 2) serovar 3 was predominant among woman with recurrent abortion. As shown in Fig. 3, Fig. 4, Fig. 5, *Ureaplasma parvum* serovar 3 was isolated in proportion 42.8%, the most frequent isolate in women with recurrent abortion followed by serovar 1 in proportion 28.5%, whereas serovar 6 and 14 showed the same proportion (14.2%) detected it in patient group; however, in control group, *Ureaplasma parvum* was isolated only in serovar 1 in proportion 11%. Among the different serovars of *Ureaplasma parvum*, serovar 3 was the most frequent serovar detected in the patient group. Therefore, *Ureaplasma parvum* (biovar 2) serovar 3 was predominant among woman with recurrent abortion. We suggested the *Ureaplasma parvum* serovar 3 may be playing a role in recurrent abortion and prematurity. Also may be related to intra- amniotic inflammatory response to *Ureaplasma parvum* and that this is related not only to recurrent abortion but also to early onset sepsis in the baby. Although the difference in detection rates of the different serovars of *Ureaplasma parvum* was statistically significant, the predominance of serovar 3 was consistent with previous reports [14]. Another study detected *Ureaplasma parvum* serovar 3 is the most prevalent serovar detected in reproductive humans [16]. Another study isolated the complete genome sequence of *Ureaplasma parvum* serovar 3, clinical strain SV3F4, isolated from a Japanese patient who had an infectious abortion during the 13th gestational week in her previous

pregnancy [17]. Also Urszula *et al.* [3] Isolated *Ureaplasma parvum* serovar 3/14 in 86% of women with symptomatic genital tract infections. It is possible that the combination of variable serovar-specific genes of *Ureaplasma* with generally known virulence factors determines the development of pathological processes on the mucosal surface of the human genital tract. \*Statistical analysis includes the *P*-value <0.05 showed highly significant between patient group and control group according to isolation of *Ureaplasma parvum* serovars.

**Conclusion**

The results evidently indicate that *U. parvum* may be an important etiologic agent for recurrent abortion. And *U. parvum* serovar 3 was the most frequent serovar isolated in this study. It may play a role in recurrent abortion.

**Acknowledgements**

**Conflicts of interest**

There are no conflicts of interest.

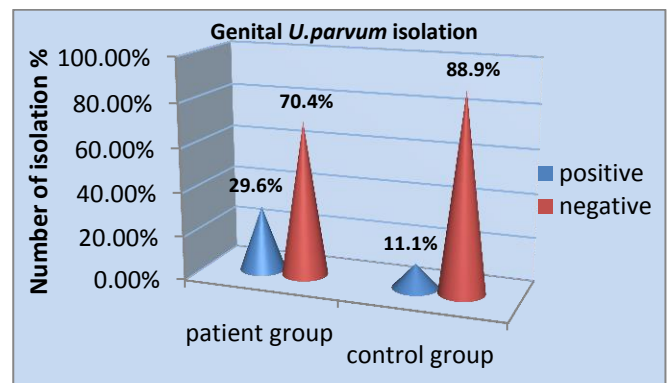


Fig. 1. Prevalence of *Ureaplasma parvum* among patient group and control group.

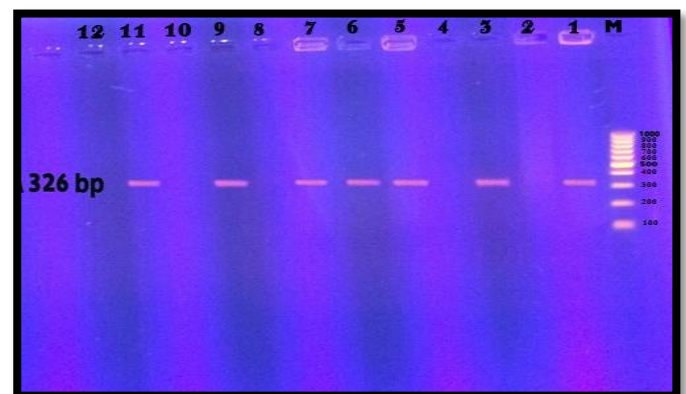


Fig. 2. Ethidium bromide-stained agarose gel showing PCR amplification product with (326 bp) primers for *Ureaplasma parvum*. M 100 bp standard size reference marker. Lanes (1, 3, 5, 6, 7, 9, and 11): *Ureaplasma parvum* positive results. Lanes (2, 4, 8, 10, and 12): *Ureaplasma parvum* negative samples.

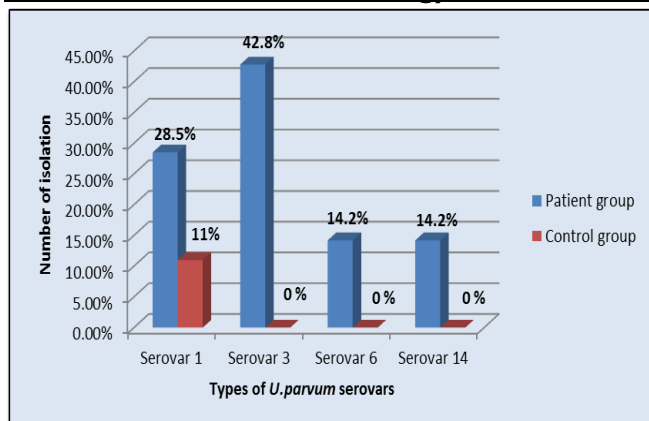


Fig. 3. Distribution of *Ureaplasma parvum* serovars among patient group and control group.

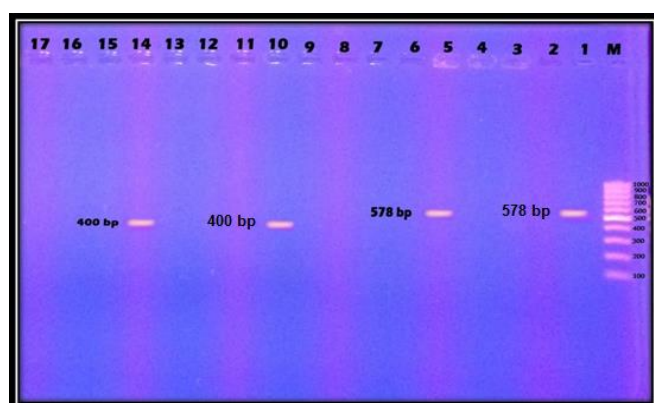


Fig. 4. Results of PCR amplification for identification of serovar 1 (578 bp) and serovar 3 (400 bp). M 100 bp standard size reference marker. Lane (1, 5): serovar 1 positive results. Lane (2, 3, 4, 6, 7, 8, 9): negative samples. Lane (10, 14): serovar 3 positive results. Lane (11, 12, 13, 15, 16, 17): negative samples.

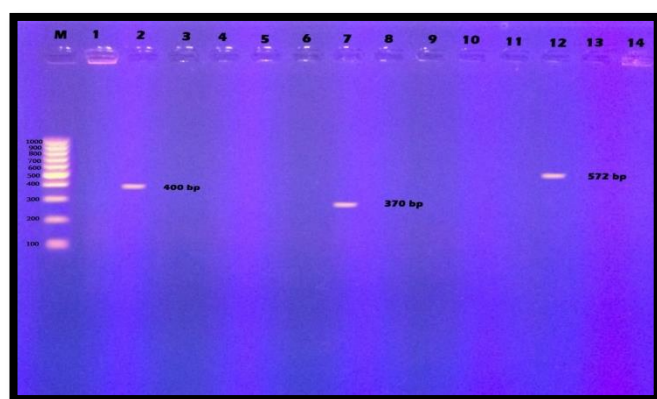


Fig.5. Results of PCR amplification for identification of serovar 3 (400 bp), serovar 6 (370 bp) and serovar 14 (572 bp). M 100 bp standard size reference marker. Lane (1, 3, 4, 5, 6, 8, 9, 10, 11, 13, 14): Negative samples. Lane 2 : serovar 3 positive results. Lane7: serovar 6 positive results. Lane12: serovar 14 positive results. (Agarose Con. 2% & Voltages 100).

## References

- Kokkayil P, Dhawan B. *Ureaplasma* current perspectives. *Indian J Med Microbiol* 2015; **33**:205–214.
- Waites K, Xiao L, Paralanov V, Viscardi RM, Glass JI. Glass. **molecular methods for the detection of mycoplasma and Ureaplasma infections in humans.** *J Mol Diagn* 2012; **14**:437–450.
- Urszula K, Joanna E, Marek E, Mączyńska B, Sobieszcańska BM. **Colonization of the lower urogenital tract with U. parvum can cause asymptomatic infection of the upper reproductive system in women.** *Int J Gynecol Obstet* 2014; **289**:1129–1134.
- Larsen B, Hwang J. **Mycoplasma, Ureaplasma, and adverse pregnancy outcomes.** *Infect Dis Obstet Gynecol* 2011; **23**:138–141.
- Kataoka S, Yamada T, Chou K, Nishida R, Morikawa M, Minami M, et al. **Association between preterm birth and vaginal colonization by mycoplasmas in early pregnancy.** *J Clin Microbiol* 2006; **44**:51–55.
- Trembath A, Laughon M. **Predictors of bronchopulmonary dysplasia.** *J Clin Perinatol* 2013; **39**:585–601.
- Kacerovsky K, Pavlovsky M, Tosner J. **Preterm premature rupture of the membranes and genital mycoplasmas.** *Acta Medica* 2009; **52**:117–120.
- Holly B, Danny J. **Recurrent pregnancy loss: etiology, diagnosis, and therapy.** *Obstet Gynecol* 2009; **2**:76–83.
- Miralles R, Hodge R, McParland PC, Field DJ, Bell SC, Taylor DJ, et al. **Relationship between antenatal inflammation and antenatal infection identified by detection of microbial genes by polymerase chain reaction.** *Pediatr Res* 2005; **57**:570–577.
- Waites KB, Katz B, Schelonka RL. **Mycoplasma and Ureaplasma as neonatal pathogens.** *Clin Microbiol Rev* 2005; **18**:757–789.
- Al-Azawiy IH. **Cultural and molecular detection of mycoplasmal urogenital infection in woman.** *Int J Med Sci* 2013; **1**:25–29.
- Agbakoba NR, Adetosoye AI, Adesina OA, Adewole IF. **Isolation of vaginal pathogens along with genital mycoplasmas from asymptomatic gynaecology and antenatal clinic attendees.** *Am- Euras J Sci Res* 2008; **3**:195–198.
- Kong F, Ma Z, James G, Gordon S, Gilbert GL. **Species identification and subtyping of Ureaplasma parvum and Ureaplasma urealyticum using PCR-based assays.** *J Clin Microbiol* 2000; **38**:1175–1179.
- Kong F, Zhu X, Wang W, Zhou X, Gordon S, Gilbert GL. **Comparative analysis and serovar-specific identification of multiple banded antigen genes of Ureaplasma urealyticum.** *J Clin Microbiol* 1999; **37**:538–548.
- Dhawan B, Malhotr N, Sreenivas V, Rawre J, Khanna N, Chaudhry R, Mittal S. **Ureaplasma serovars & their antimicrobial susceptibility in patients of infertility & genital tract infections.** *Indian J Med Res* 2012; **136**:991–996.
- Knox C, Allan A, Allan M, Edirisinghe WR, Stenzel D, Lawrence FA, et al. **Ureaplasma parvum and Ureaplasma urealyticum are detected in semen after washing before assisted reproductive technology procedures.** *Fertil Steril* 2003; **80**:921–929.
- Ning H, Nakura Y, Motooka D, Nakamura S, Nishiumi F, Ishino S, et al. **Complete genome sequence of Ureaplasma parvum serovar 3 strain SV3F4, isolated in Japan.** *Genome Announc* 2014; **2**:254–256.