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ORIGINAL ARTICLE

Recent clinical relevance of mono-genital colonization/infection by *Ureaplasma parvum*

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Abstract

Ureaplasma parvum is the most prevalent genital mycoplasma in women of childbearing age. There is debate around the relevance of its presence in male or female genitals for disease development and as a cofactor. The objective of this study was to determine the prevalence of colonization/infection by *U. parvum* and its possible relationship with reproductive tract infections. We retrospectively analyzed the presence of *U. parvum* in patients referred by specialist clinicians for suspicion of genitourinary tract infection. *U. parvum* was detected in 23.8% of samples, significantly more frequently in females (39.9%) than in males (6%). Among the males, *U. parvum* was found alone in 68.4% of episodes, with Ct ≤ 30. Among the females, *U. parvum* was detected in 88.6% of cases, with Ct ≤ 30, including 22 cases with premature rupture of membranes and 6 cases with threat of preterm labor. Co-infection was significantly more frequent in females (62.6%) than in males (31.6%). Given the high prevalence of *U. parvum* as sole isolate in males and females with genitourinary symptoms, it should be considered in the diagnosis and treatment of genital infections, although its pathogenic role in some diseases has not been fully elucidated.

Keywords Genital infection · *Ureaplasma parvum* · Emerging infection

Background

Mycoplasma spp. and *Ureaplasma* spp. belong to the *Mollicutes* class, microorganisms mainly characterized by the absence of a cell wall. Globally, they are known as genital mycoplasmas because of the associated clinical symptoms [1]. *Ureaplasma urealyticum* was first isolated in 1954, 17 years after the isolation of *Mycoplasma hominis* [2]. Other species were subsequently differentiated with the advance of molecular biology techniques, including *Mycoplasma genitalium* and *Ureaplasma parvum*. The species *U. urealyticum* was first considered to contain two closely related "Biovars," composed of 14 individual serovars based on inhibition by polyclonal rabbit serum, which were eventually separated into two separate species; confusingly, however, one of them retained the original name for the entire species [3].

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Ureaplasma spp. have been isolated in 30–80% of vaginal/endocervical samples from sexually active women [2, 4]. A PCR study by Leli et al. (2018) reported that *U. parvum*, alone or combined with other *Ureaplasma* species, was the most prevalent mycoplasma in women of childbearing age, with a prevalence of 38.3% [4]. A previous study in our setting found that it was by far the most frequent genital mycoplasma, detected in a large number of patients, especially females, receiving specialist care for genital tract infections [5, 6]. However, the pathogenic role of *Ureaplasma* spp. remains unclear, and there is debate around the relevance of their presence for disease development and their role as cofactor in both males [7] and females [4]. Their proliferation appears to be favored by sexual hormones [8], explaining the increased presence of *Ureaplasma* spp. during pregnancy. These microorganisms are frequently detected in the vaginal exudate of women with full-term or preterm premature rupture of membranes or with threat of preterm labor, incompetent cervix, chorioamnionitis, or idiopathic vaginal bleeding [9, 10]; however, their pathogenicity is not well established. An association of *U. parvum* with the induction of preterm birth and chronic lung disease in neonates < 26 weeks of gestation is well established in humans. Furthermore, *U. parvum* is almost exclusively used rather than *U. urealyticum* in animal models substantiating the link to preterm birth [3]. The most prominent study was by Novy et al. (2009) [11], whose experimental infection of pregnant macaques with Serovar 1 *U. parvum*-induced contractions consistent with preterm birth in all animals within 16 days of post-intrauterine inoculation. They subsequently reported the induction of preterm birth by ascending infection with Serovar 3 *U. parvum* after experimental vaginal inoculation [12], finding that damage to the cervical epithelia significantly increased ascending infection and preterm birth by up to 28%. More recently, however, the link between *U. parvum* and other clinical symptoms/sequelae has been called into question, and an association with *U. urealyticum* was found to be stronger in some male patients [7].

The introduction of PCR techniques into clinical diagnostics permitted the differentiation of *Ureaplasma* spp. between *U. urealyticum* and *U. parvum)*, changing the epidemiology of these microorganisms [4] and allowing the re-evaluation of *U. parvum* in patients.

The objectives of this study were to determine the prevalence of colonization/infection by *U. parvum* in patients with suspicion of genital tract infection and to evaluate its possible relationship with these infections.

Methods

This cross-sectional descriptive study included all genital samples from symptomatic adults received by the Microbiology Laboratory of Virgen de las Nieves Hospital in Granada for "study of a possible infectious episode" between 1 April 2017 and 31 December 2018. No exclusion criteria were imposed. Samples were from patients receiving standard protocol treatment in a specialist clinical department [5, 6]. Briefly, the standard culture for bacteria and fungi in genital samples was performed (with subsequent microbiological evaluation in cases of mono-microbial growth of an opportunistic pathogen or the presence of a strict pathogen), with a PCR study using the BD MAX™ system (Becton Dickinson, Sparks, USA) for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* (BD MAX CT/GC/TV) and for *M. genitalium*, *M. hominis*, *U. parvum*, and *U. urealyticum* (BD MAX System, BioGX DNA, 350–011-A-MAX). Cultured microorganisms were identified by Maldi-Tof mass spectrometry (Bruker Daltonics, Billerica, USA) or by biochemical tests using the MicroScan system (Beckman Coulter, Madrid, Spain). In addition, a hybridization test (BD AFFIRM VPIII System, Becton Dickinson) with BD MicroProbe processor (Becton Dickinson) was applied to detect *T. vaginalis*, *G. vaginalis*, and *Candida* spp. in vaginal exudates from patients over the age of 14 years.

The BD MAX system is a combined extraction and amplification platform (from sample lysis to result) with an integrated sample processing control (SPC), applying real-time multiplex PCR with fluorescence and melting curve. Information is yielded on the threshold cycle (Ct), which is inversely proportional to the initial amount of DNA, with a margin of error < 5% according to the manufacturer. The limit of detection of *U. parvum* is 13.4 copies per reaction. The SPC is added to the test to facilitate the identification of specimens containing PCR amplification inhibitors and as a control for reagent integrity and the assay system as a whole. BioGX *Mycoplasma-Ureaplasma* test results can sometimes be inconclusive due to an invalid SPC or can be indeterminate/incomplete through instrument failure, and retesting is required. Less than 1% of the present samples had an initial SPC failure, and this problem was resolved by retesting.

Results were evaluated after excluding referrals for the study of infection by *Treponema pallidum*. The samples came from Emergency Departments and Departments of Obstetrics-Gynecology, Urology, and Infectious Diseases. Information was gathered on sample type, origin, clinical situation, results, and demographic data from the laboratory computer system and/or from the form ordering anonymized evaluation. We studied the possible relationship between a positive PCR result for *U. parvum* (Ct ≤ 30) as single microorganism and the clinical outcome reported by the attending physician. More information on the sensitivity of the PCR system for *U. parvum* is available from the manufacturer (https://drive.google.com/drive/folders/1KuQwlMsrmBlQx_3ftQHc4T3re1ZdO_tk) and from a study on the relationship

between quantitative PCR Ct values and microorganism concentrations [13]. Given that a CT value ≤ 30 indicates a higher concentration of the microorganism, only patients with this result were studied, with no data being available on patients with Ct values > 30.

No other clinical data were available to study the association of clinical factors with the presence of the microorganism. The anonymity of data was strictly preserved, considering infectious episodes (with inter-episode interval of ≥ 6 weeks) rather than patients.

We performed descriptive statistical analysis, calculating absolute and relative frequencies for qualitative variables. We applied Pearson's chi-square test and Fisher's exact test to analyze differences in the presence of the microorganism by sample type and sex. $p < 0.05$ was considered significant. The SPSS version 19 (IBM SPSS, Chicago, IL) was used for statistical analyses.

Results

The study included 1343 samples referred for microbiological diagnosis from 705 (52.5%) episodes in females and 638 (47.5%) in males. Table 1 displays the results obtained by sample type. *U. parvum* was detected in 23.8% of samples, more frequently in females (281 episodes; 39.9%) than in males (38 episodes, 6%) ($p < 0.001$). In males, the most frequently positive sample type was glans exudate (9.1%), followed by semen (7.9%) and urethral exudate (5.6%), with no statistically significant difference among them ($p = 0.077$). In females, the most frequently positive sample type was vaginal exudate (42.3%), followed by endocervical exudate (41.5%), placenta (30.8%), and genital ulcer exudate (29.2%), with no statistically significant difference among them ($p = 0.192$).

In the males, *U. parvum* was detected alone in 26 episodes (68.4%) and alongside another agent in 12 (31.6%). In the females, *U. parvum* was detected alone in 105 episodes (37.4%) episodes and alongside another agent (mainly *M. hominis* or *Gardnerella vaginalis*) in 176 (62.6%) ($p < 0.001$). Table 2 lists the 291 microorganisms that accompanied *U. parvum* in the 176 female episodes and the 21 that accompanied *U. parvum* in the 12 male episodes.

Table 1 Distribution of samples analyzed and presence of *Ureaplasma parvum*

		PCR *N* (%)		Total
	Samples	Positive	Negative	
Males	Oral exudate	0 (0)	9 (100)	9
	Rectal exudate	1 (1.4)	70 (98.6)	71
	Glans exudate	6 (9.1)	60 (90.9)	66
	Genital ulcer exudate	0 (0)	10 (100)	10
	Urethral exudate	13 (5.6)	220 (94.4)	233
	Urethral urine	0 (0)	19 (100)	19
	Semen	18 (7.9)	209 (92.1)	227
	Post-ejaculation urine	0 (0)	3 (100)	3
	Total males	38 (6)	600 (94)	638
Females	Oral exudate	0 (0)	1 (100)	1
	Rectal exudate	3 (60)	2 (40)	5
	Endocervical exudate	179 (41.5)	252 (68.5)	431
	Vaginal exudate	69 (42.3)	94 (57.7)	163
	Genital ulcer exudate	14 (29.2)	34 (70.8)	48
	Endometrial biopsy	0 (0)	5 (100)	5
	Placenta	16 (30.8)	36 (69.2)	52
	Total females	281 (39.9)	424 (60.1)	705
	Total	319 (23.8)	1024 (76.2)	1343

Relationship of *U. parvum* detection with the clinical outcome of patients in mono-microbial episodes (Supplementary Material)

Among the males, *U. parvum* was found alone with Ct value ≤3 0 in 26 (68.4%) of the 38 episodes analyzed. It was not possible to obtain clinical information from 6 of these patients. Among the other 20, 7 (35%) had non-specific micturition syndrome, 6 (30%) exacerbated chronic prostatitis, and 3 (15%) orchitis, all detected in semen samples, and 2 (10%) had urethritis, and 2 (10%) balanitis, detected in urethral and

Table 2 Distribution of microorganisms detected alongside *Ureaplasma parvum*

Microorganisms	*N* (%)		
	Males	Females	Total
Gardnerella vaginalis	2 (3)	64 (97)	66
Chlamydia trachomatis	3 (15)	17 (85)	20
Neisseria gonorrhoeae	4 (26.7)	11 (73.3)	15
Mycoplasma hominis	2 (2.8)	69 (97.2)	71
Mycoplasma genitalium	1 (25)	3 (75)	4
Ureaplasma urealyticum	2 (6.1)	31 (93.9)	33
C. glucuronolyticum	2 (100)	0 (0)	2
Haemophilus spp.	3 (25)	9 (75)	12
Virus Herpes Simplex	0 (0)	10 (100)	10
Candida albicans	1 (2.3)	43 (97.7)	44
Non-*C. albicans* yeasts	0 (0)	8 (100)	8
Trichomonas vaginalis	1 (12.5)	7 (87.5)	8
Others	0 (0)	19* (100)	19
TOTAL	21 (6.7)	291 (93.3)	312

* *E. corrodens* 2; *Leptotrichia* 1; *Pasteurella* 1; *S. aureus* 6; *S. pyogenes* 2; *Rothia mucilaginosa* 1; *Actinomyces* 1; Anaerobes 5

glans exudates, respectively. One patient was empirically treated with azithromycin and ciprofloxacin, one with levofloxacin, one with amoxicillin-clavulanic acid, and one with clotrimazole. Six patients received targeted treatment with azithromycin, one with ciprofloxacin, and one with levofloxacin. No clinical data were available for the remaining 14 patients.

Among the females, *U. parvum* was detected alone in 105 episodes: 93 (88.6%) with Ct value ≤ 30 and 12 (11.4%) with Ct of 31–35. The episodes with Ct 31–35 were not considered in subsequent analyses. Clinical data were not available for 53 (57%) of the 93 episodes with Ct ≤ 30, and the most frequent diagnosis in the other 40 (43%) episodes was preterm premature rupture of membranes, which was reported in 22 (55%) patients with *U. parvum* in vaginal exudate. The birth was preterm in 14 (63.6%) of these 22 patients, and *U. parvum* was detected in placenta samples from 7 (50%) of them, including 4 with a previous diagnosis of chorioamnionitis. *U. parvum* was isolated in 6 (15%) women with threat of preterm labor, including 2 who had a preterm labor and 1 who expelled the fetus at 19 weeks after showing chorioamnionitis symptoms since admission and had a good outcome. These obstetric patients were empirically treated with ampicillin and azithromycin and the possible association of gentamycin, in accordance with the protocol of our center. *U. parvum* was also detected in 4 (10%) vaginal exudate samples from patients with puerperal fever of undefined etiology, who received out-patient treatment with amoxicillin-clavulanic acid and reported no complications.

Among the gynecological patients, *U. parvum* was detected in endocervical exudates from 6 (15%) episodes of inflammatory pelvic disease, including 2 with a pelvic abscess that required surgical drainage; these patients were all empirically treated with ceftriaxone, doxycycline, and metronidazole, with the exception of one episode treated with clindamycin and gentamicin due to patient allergy. *U. parvum* was also detected in 2 (5%) episodes of recurrent vulvovaginitis and treated with oral doxycycline and dequalinium chloride ovules in one case and azithromycin in the other; outcome data are not available for these patients, who were followed in primary care with no further referrals, presumably due to their clinical improvement.

Discussion

Routine testing for *Ureaplasma* spp. in patients with uncomplicated genital tract disease has not been appropriate to date, because it is uncertain whether the detection of these microorganisms in the genital tract reflects normal colonization or infection. In 2018, the European STI Guidelines Editorial Board [14] recommended that genital mycoplasmas (except *M. genitalium*) should not be tested or treated, with possible exception of *U. urealyticum* when a high bacterial load is detected. However, these guidelines failed to consider pregnant women and preterm infants and the new diagnostic procedures available for quantification of the bacterial load in non-gonococcal infections [15, 16]. Therefore, a new clinical interpretation of laboratory results is necessary, and the current interpretative scenario may change.

The pathogenic role of *U. parvum* has not been fully elucidated. It is a frequent colonizer of the genitourinary tract and has been implicated in non-gonococcal urethritis, inflammatory pelvic disease, infertility, miscarriage, neonatal complications, and surgical wound infections, among others [10, 17–19]. However, there is a need for further clinical evidence of its participation in diseases, using the laboratory tests that are now available for its specific detection, facilitating the development of targeted treatments and reducing the empirical use of antibiotics.

The global prevalence of *U. parvum* 6 (15%) in these patients was 23.8%, within the reported range of 12 to 47% according to the study participants and their age and sexual activity [17, 18]. Its prevalence was significantly higher in the females (39.9% of samples) than in the males (6%) in the present study, as previously reported in mice [8], possibly because colonization by *Ureaplasma* spp. is favored by the presence of estradiol.

Among the females, *U. parvum* positivity was most frequently observed in vaginal exudate samples (42.3%), followed by samples of endocervical exudate (41.5%), placenta (30.8%), and genital ulcer (29.2%), with no statistically significant differences among them. Some studies have related the presence of *U. parvum* to vaginal microbiota disorders, e.g., bacterial vaginosis [17, 20]. In general, this microorganism can form a part of healthy urogenital microbiota, and studies are therefore needed to identify predictive markers of its invasiveness and pathogenicity. The bacterial load has been described as an important factor, with the detection of higher loads in samples from symptomatic women [21].

Culture studies were able to quantify concentrations of > 10^4 and relate them to clinical symptoms, but they did not differentiate between infection by *U. parvum* and *U. urelyticum*. The utility of quantitative PCR derives from the direct relationship between the fluorescent signal emitted and the amount of *U. parvum* DNA present. In this way, a larger initial number of copies of the target DNA are detected as an increase in fluorescence due to the accumulation of qPCR products. This explains the selection of patients whose samples had a Ct value ≤ 30. The present analysis only included samples with an elevated microbial load and no presence of other microorganisms. In the case of PCR, the cutoff Ct value should be 30 because higher values may indicate lower microbial concentrations that may be related to colonization. The epidemiology and clinical relevance of genital mycoplasmas can be better defined by taking advantage of the superior

sensitivity and specificity of new molecular diagnostic techniques and the possibility of semi-quantitative analysis [1, 4]. In the case of cervicitis, it is important to determine the etiology of the disease in order to prevent complications [22], and low bacterial loads can be clinically relevant. In the present patients, *U. parvum* was detected in 30.8% of placenta samples and is increasingly recognized as a cause of chorioamnionitis, spontaneous miscarriage, and bronchopulmonary dysplasia in the newborn [1, 19, 23–25]. *U. parvum* colonization in the newborn has been inversely related to gestational age at delivery [24], and colonization of the respiratory tract is considered a major risk factor for bronchopulmonary dysplasia in neonates [26]. We highlight the presence of the microorganism in 22 (55%) of our patients with preterm rupture of membranes, who most frequently received empirical treatment with ampicillin and azithromycin and the occasional association of gentamicin, with a good obstetric-perinatal outcome in all cases.

A higher risk of preterm (< 32 weeks of gestation) or very preterm (< 28 weeks) birth has been associated with colonization by *U. parvum* Serovars 3 or 6 and, to a lesser extent, by serovar 1 [27, 28]. The microorganism was only identified at species level in the present study, highlighting its detection in a woman with chorioamnionitis who expelled the fetus at 19 weeks and had a satisfactory outcome.

U. parvum has also been related to idiopathic bleeding [29], which is frequent in pregnancy; however, no data were available on this event in the present series. We found the microorganism in an episode of vulvovaginal pruritus, which was resolved with azithromycin. Finally, *U. parvum* was also detected alone in 6 episodes of inflammatory pelvic disease, which were resolved with ceftriaxone, doxycycline, and metronidazole. These findings are consistent with the study of Oh et al. [29], in which *U. parvum* was the most frequently detected microorganism in patients with premature rupture of membranes, preterm labor, idiopathic bleeding, and clinical chorioamnionitis, with the addition of the patient with inflammatory pelvic disease in the present series.

In males, *Ureaplasma* spp. have largely been associated with non-gonococcal urethritis, although more frequently with *U. urealyticum* than with *U. parvum* [5]. In the present study, *U. parvum* was detected in only 6% of male samples, mainly from glans exudate and semen, and higher percentages have been reported by culture studies [30]. Depending on the study, the frequency of *Ureaplasma* spp. has ranged from 5 to 58% in the semen of infertile males and from 3 to 31% in the semen of fertile males [25]. Some authors have related infertility in men to the presence of these genital mycoplasmas, given their higher prevalence in the semen of individuals with reduced sperm concentration and motility [5, 25]. In the present study, non-specific micturition syndrome was diagnosed in some of the males infected with *U. parvum* alone, and this could be exacerbated chronic prostatitis, orchitis, urethritis, or balanitis. Two episodes of balanitis were empirically treated (with clotrimazole and amoxicillin-clavulanic acid, respectively), although there is no record of follow-up referrals, suggesting possible colonization by *U. parvum*. The targeted treatment of choice after the microbiology report was azithromycin or, alternatively, ciprofloxacin or levofloxacin, and there were no further requests were for sample diagnosis in any case. We highlight that successful treatment of *U. parvum* can be achieved with macrolides, fluoroquinolones, and tetracyclines but not with clotrimazole and amoxicillin-clavulanic acid [31]. This selective susceptibility is due to the absence of target cell walls (as in Ureaplasmas), the presence of anaerobic metabolism (Ureaplasmas are micro-aerobic), inherent resistance to clindamycin (23S rRNA polymorphism), or MIC values that are too high for treatment with some antibiotics (*U. parvum* MIC for gentamycin = 32 mg/L) [32].

As already noted, the pathogenic role of *U. parvum* in certain genitourinary diseases remains controversial. It is accompanied by strict pathogens in some cases and detected alone in others, when its presence in significant amounts may be clinically relevant as an opportunistic pathogen. Episodes of co-infection were significantly more frequent in females (62.6%) than in males (31.6%), making it more difficult to attribute clinical symptoms to this microorganism in female patients. Among the females, *U. parvum* was most frequently accompanied by *M. hominis* and *G. vaginalis*, followed by *Candida albicans*, suggesting a possible tendency for their synergetic action in the development of disease. Studies have been conducted on co-infection with *U. urealyticum* and other pathogens [33] but not on co-infection with *U. parvum*. Co-infection of *U. urealyticum* with *M. hominis* has been associated with a higher risk of premature rupture of membranes and other pregnancy complications [34].

The main strengths of this study are its focus on the clinical situation of the patient and its global analysis (in males and females) of a common microorganism that can be transmitted via sexual activities. The main weakness is that the presence of this bacteria was not studied in a symptom-free control group, although it is difficult to interpret comparisons between populations with and without clinical symptoms because this microbe behaves as an opportunistic pathogen. Hence, only scenarios in which the bacterial increase in number or reach normally sterile sites are clinically relevant.

In conclusion, the prevalence of *U. parvum* was elevated in both males and females in the present population of patients with suspicion of genital tract infection. These results suggest that it may be a potential cause of genital infection in the absence of other microorganisms, and its presence should be considered in patients with genital tract infection.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest

Ethical considerations The study complied with the principles of the Helsinki Declaration. The informed consent of patients was not required, because the biological material was used solely for the standard diagnosis of genital tract infection by attending physicians with no addition to routine procedures, in accordance with WHO ethical guidelines for health-related research in humans. There were no additional samplings or modifications to the laboratory diagnostic protocol. Permission to access and analyze the data was granted by the Clinical Microbiology Management Unit of our hospital.

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