

## **Unusual oropharyngeal asymptomatic manifestations caused by atypical pathogens detected by PCR into altered ecosystems of an infertile couple**

### **ABSTRACT**

The young couple casually arrived at our observation presented two different and painless altered ecosystems. The female partner exhibited inflamed lingual surface, while the man manifested several genital ulcerated ulceration areas. Microbiological analysis of samples collected from several sites belonging to each ecosystem revealed a polymorphic growth with different bacterial concentrations, indicating a latent dismicrobic ecosystem, as also confirmed by altered pH values. Interestingly, microbiological analysis for *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Chlamydia trachomatis* gave different results in both ecosystems, highlighting that each partner was differently infected by the same "silent infections". The frequency of Ear Nose and Throat (ENT) chronic morbidity for these atypical infections increases, in the couple, the probability that mycoplasmosis and chlamydiasis serve as a reservoir of infection and/or reinfection for the urogenital tract. Although it is well known that several common bacteria and "atypical pathogens" -like mycoplasmas and *Chlamydia C. trachomatis* - can colonize both oral and urogenital ecosystems, rarely happens that they can clinically manifest their presence by unusual clinical manifestations we observed in different ecosystems investigated, hiding the unique true problem of infertility in the young couple. In this regard, our results showed that only starting from an accurate anamnesis, perpetuating the holistic vision of "Human Being", using an adequate sampling modality, adopting a new procedure to quantify microbial populations into asymptomatic ecosystems, and introducing the constant use of PCR analysis, it will be possible to disclose similar precocious problems of couple infertility, in genetically predisposed subjects genetically predisposed.

**Keywords:** young couple infertility; atypical pathogens; *Chlamydia trachomatis*; *Mycoplasma spp.*; *Ureaplasma spp.*; altered ecosystems; PCR analysis.

## 1. INTRODUCTION

A young caucasian couple (woman: 26 years old; man: 30 years old) manifested different inflamed ecosystems together with a referred infertility problem. At the urological visit, the male partner declared the presence of inflamed areas on the glans that periodically peeled without any particular pain (Figure 1A). Previous local treatment with gentamicin-betamethasone had not given any significant stable improvement. Furthermore, after sexual activity the patient referred for a sudden burning into meatus with acute pain (Figure 1B) that spontaneously regressed in 2-3 days (Figure 1B). This clinical manifestation resulted to be ever more persistent during the first emission of the morning. Urine culture carried out during these clinical manifestations resulted negative, and urine analysis showed no erythrocytes and leucocytes in the sediment. Since about 2-3 months he presented ulcerated areas/sulcerations on both sides of glans and on scrotum since about 3 months (Figures 1A, 1C). No other particular symptomsatology was referred for other ecosystems.

The female partner showed a furry tongue with intense viscous salivation highly suggestive for a clinically altered lingual ecosystem (Figure 2). No particular sign or pharyngeal alteration could be observed in both partners, although the male partner referred for infrequent pharyngitis episodes during the year. On demand, the couple confirmed a frequent oral intercourse in their sexual ménage.

From clinical history - collected by an Ear Nose and Throat (ENT) specialist team together with an expert of "atypical infections" - emerged that the infertility of the young couple represented, at that moment, the sole early and important reversible problem.

## 2. MATERIALS AND METHODS

Patients were studied according to the declaration of Helsinki, obtaining written informed consent from each partner. No personal hygiene was carried out by both partners before microbial investigations.

**2.1. Prostatic and urological evaluation.** According to National Institutes of Health guidelines (NIH CPPSI: Chronic Pelvic Pain Symptom Index) [1], the patient was classified as belonging to category IV of asymptomatic inflammatory prostatitis. Trans-rectal ultrasound scan imaging showed few disreactive fibrous-calcific parenchymal areas into the prostatic gland, suggestive for chronic inflammation probably secondary to an infection. No evidences for lymph node swelling or discharge were observed. A blood sample was taken for Prostatic Specific Antigen (PSA), Venereal Disease Research Laboratory (VDRL), and *Treponema pallidum* Haemoagglutination Assay (TPHA) measurements. First void urine (FVU), seminal fluid (SF) - collected by masturbation after four days of sexual abstinence - and urine sample emitted after prostatic massage (UAMP) were collected and immediately processed for detecting the presence of bacteria, yeasts, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and Human Papilloma Virus (HPV) [2].

Briefly, 1.0 µL each of FVU, SF, and UAMP was plated onto chromIDCPS agar (bioMérieux) and Columbia CNA agar + 5% sheep blood (bioMérieux), and incubated at 37°C for allowing growth of common bacterial and fungal pathogens. Remaining FVU and UAMP was centrifuged (5.000 rpm, 5 min, 4°C), and the pellet resuspended at 1.0 mL with supernatant for molecular analyses.

SF was incubated at 37°C for 15 min to speed up its fluidification. Sample was then observed for the presence of leucocytes and sperm agglutination phenomenon, and finally diluted in sterile saline for measuring pH. Fifty microliters of SF were used for *Mycoplasma* detection by *Mycoplasma* IST2 (bioMérieux).

Urethra was also sampled for cellular material by introducing a sharp Dacron swab into urinary meatus for 1 cm and turning it once. Sample was dispersed by stirring in 1.0 mL of concentrated urine, and this suspension was used for culture and molecular analyses.

**2.2. Genital ulcer evaluation.** The cutaneous ulcer observed on scrotum (genital ulcer, GU) was sampled by scraping. Sample was dispersed into 1.0 mL of sterile saline and assayed for the presence of three major agents of genital ulcer disease (*T. pallidum*, *Haemophilus ducreyi*, *C. trachomatis*), and *N. gonorrhoeae* by both culture and molecular analyses, as previously reported [2-4].

**2.3. Urethral and cervicovaginal evaluation.** Urethral and cervical scraping were carried out in the female partner. A 30 mL aliquot of FVU was collected for desquamating cells, while other 20 mL of urine to remove living urethral cells. Urethral scraping was performed by Dacron swab introducing it for 2.5 cm and turning twice. Cervical scraping was carried out by Cytoc-brush (Cytoc Corp., Massachusetts, USA).

The suspension was microscopically observed for assessing cellular adequacy for molecular assay. Only samples showing at least 3-5 epithelial cells/hpf were considered.

Secretion around the vaginal fornix was sampled thrice by cotton swab, and samples were then pooled into 1.0 mL sterile saline. This suspension was assayed for pH, and observed for leucocytes counting and the presence of *Mobiluncus*, *Lactobacilli* and *Trichomonas vaginalis*. An aliquot was Gram stained to evaluate the presence of "clue cells" and microbial morphotypes. One microliter of the same suspension was plated onto chromIDCPS - for isolation, enumeration and immediate identification of *E. coli*, *Proteus* and KESC (*Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*) - and onto Columbia CNA agar for Gram-positive cocci.

**2.4. Lingual and pharyngeal evaluation.** Samples obtained by lingual and pharyngeal scraping were suspended into 1.0 mL sterile saline, then immediately evaluated for the oropharyngeal ecosystems by culture analysis for saprophytic flora, common pathogens and atypical ones (*C. trachomatis*, *M. hominis*, and *U. urealyticum*). One microliter of this suspension was plated onto chromIDCPS and Columbia CNA agar 5% sheep blood agar, then incubated under both aerobic and anaerobic atmosphere. Cultures were monitored daily up to 72h for total colony counting. Total microbial charge (TMC), expressed as colony-forming unit per milliliter (CFU/mL), was calculated by adding the number of colonies developed onto chromIDCPS agar to that anaerobically grown onto Columbia CNA agar. The relative abundance of each species was reported as percentage of TMC (considered as 100%). Since the oropharyngeal ecosystem is directly exposed to external environment, we could accept that this ecosystem present a TMC  $>10^7$  CFU/mL. By this way, the threshold of pathogenicity for a monoresident pathogen could be considered as  $\geq 10^6$  CFU/mL. The presence of mycoplasma was detected as above described. An aliquot of 0.1 mL of the initial suspension was used for pH measurement of each ecosystem investigated, while 0.5 mL was used for molecular assays.

**2.5. Molecular assays.** Each sample was centrifuged (5,000 rpm, 5 min, 4°C), then the pellet was suspended in 0.025 M buffer phosphate (pH 7.0) + proteinase K, and incubated at 56°C for overnight. DNA was precipitated by absolute ethanol, filtered, washed and finally eluted. *H. ducreyi*-DNA, HPV-DNA, *Neisseria N. gonorrhoeae*-DNA (Ng-DNA), and *C. trachomatis*-DNA (Ct-DNA) detection was carried out by PCR (BioAesissrl, Jesi, Italy), as previously described [2, 5]. Molecular analysis was performed in duplicate.

### 3. RESULTS

**3.1. Serologic evaluations.** VDRL test was negative for man, but positive for woman; both partners were negative at TPHA evaluation. Total PSA was 0.87 ng/mL (95%-ile of health population:  $0.60 \pm 0.35$ ).

**3.2. Oral and pharyngeal ecosystems.** Culture and molecular analyses of different samples collected from the male partner showed that common microbial flora was differently distributed. Lingual scraping revealed the presence of *C. ulcerans* only ( $1.0 \times 10^6$  CFU/mL), while pharyngeal scraping revealed positivity for *S. faecalis*, *C. ulcerans*, *E. coli*, and *K. pneumoniae* (20%, 40%, 30%, and 10% of TMC, respectively; TMC:  $0.5 \times 10^6$  CFU/mL), (Table 1). Mycoplasmas were present at low concentrations ( $10^3$  CCU/mL), although differently distributed. Particularly, *U. urealyticum* was present in both lingual and pharyngeal scraping samples, while *M. hominis* was observed in lingual scraping samples only (Table 1). The pH value measured in the lingual ecosystem of female partner resulted to be significantly higher than the physiologic one measured in the male partner (pH: 8.9 vs 6.2, respectively).

Pharyngitis episode is one of the most common clinical evidence in patients at the consulting room of the ENT clinic [5, 6]. Although female partner appeared in good health and without clinical pharyngeal history, the characteristic oral ecosystem observed led us to investigate the pharynx too. Ecosystems showed different microbial pattern for saprophytes and common opportunistic pathogens (Table 2). In particular, lingual scraping revealed the presence of *C. ulcerans* only ( $6.0 \times 10^5$  CFU/mL), contrarily to the polymicrobial growth observed on pharyngeal mucosae, consisting of *S. milleri*, *S. faecalis*, *C. ulcerans*, *K. pneumoniae* (10%, 20%, 50%, and 20% of TMC, respectively; TMC:  $>10^7$  CFU/mL). The two ecosystems differed as well when compared for atypical bacteria. In fact, only the lingual one showed the presence of *M. hominis* and *U. urealyticum* (both at  $>10^4$  CCU/mL), and *C. trachomatis*. With regard to common microbial patterns, no significant differences were observed between different samples collected from the pharyngeal ecosystem. Pharyngeal swab resulted to be positive for *S. milleri*, *C. ulcerans*, *E. coli*, and *K. pneumoniae* (30%, 20%, 20%, and 30% of TMC, respectively; TMC:  $>10^7$  CFU/mL), but negative for sexual common atypical pathogens (*C. trachomatis*, *M. hominis*, and *U. urealyticum*). Pharyngeal scraping sample was positive for *S. milleri*, *S. faecalis*, *C. ulcerans*, and *K. pneumoniae* (10%, 20%, 50%, and 20% of TMC, respectively; TMC:  $>10^7$  CFU/mL). Pharyngeal scraping revealed the presence of *M. hominis* and *U. urealyticum* (both at  $10^3$  CCU/mL), although it was negative for *C. trachomatis*.

**3.3. Urogenital ecosystem.** The male urogenital ecosystem, assayed by both culture and molecular analyses, showed a polymicrobial resident flora in each of different sites investigated (Table 1). FVU resulted to be positive for *S. epidermidis*, *S. faecalis*, and *C. ulcerans* (20%, 40%, and 40% of TMC, respectively; TMC:  $3.7 \times 10^4$  CFU/mL). *M. hominis* and *U. urealyticum* were both present at  $10^4$  CCU/mL, while PCR resulted to be positive for *C. trachomatis* only. Microbiological evaluation of genital ulcer revealed the presence of *S. epidermidis*, *E. coli*, *K. pneumoniae*, and *C. ulcerans* (40%, 20%, 10%, and 30% of TMC, respectively; TMC:  $2.3 \times 10^4$  CFU/mL), while *M. hominis* and *U. urealyticum* were both at  $10^3$  CCU/mL. PCR was positive for *C. trachomatis* only (Table 1). SF showed a pH value of 8.7 (reference range: 7.2-8.0), while microscopic observation revealed 70-120 leucocytes/hpf, some of which degenerated. Nemasperms concentration was higher than normal ( $72.7 \times 10^6$  nemasperms/mL; reference range:  $>20 \times 10^6$  nemasperms/mL), while vitality was reduced to 30% (reference range: 50-80%). A remarkable sperm agglutination phenomenon was also observed in 60-70% of total nemasperms. SF was positive for *S. faecalis*, *S. epidermidis*, and *C. ulcerans* (40%, 20%, and 40% of TMC; TMC:  $7.0 \times 10^4$  CFU/mL) (Table 1). *C. trachomatis* was also detected, as well as *M. hominis* and *U. urealyticum* (both at  $>10^4$  CCU/mL) (Table 1). UAPM was positive for *M. hominis* and *U. urealyticum* (both at  $>10^4$  CCU/mL), as well as for *C. trachomatis*.

FVU collected from the female partner resulted positive for a polymicrobial bacterial growth, consisting of *S. faecalis*, *S. epidermidis*, and *C. ulcerans* (10%, 40%, and 50% of TMC, respectively; TMC:  $5.0 \times 10^4$  CFU/mL). Microscopic observation was negative for *T. vaginalis*. *M. hominis* was absent, while *U. urealyticum* was found at  $10^3$  CCU/mL. PCR was positive for *C. trachomatis* only.

The yellow-mucous secretion sampled from the cervicovaginal ecosystem showed a pH value of 5.7, higher than normal (physiological range: 3.8-4.5), while observation revealed a count of 50-60 leucocytes/hpf, some of which degenerated. TMC of this ecosystem was of about  $1.0 \times 10^6$  CFU/mL, lower than physiological value (from  $1.0 \times 10^{10}$  to  $1.0 \times 10^{12}$ , depending on age). *S. faecalis*, *C. ulcerans*, *E. coli*, and *K. pneumoniae* (10%, 70%, 10%, and 10% of TMC, respectively) were found in this ecosystem (Table 2). *L. acidophilus* concentration was significantly decreased ( $3.0 \times 10^5$  CFU/mL; physiological concentration:  $>1.0 \times 10^8$  CFU/mL). *C. trachomatis* was present, as well as *M. hominis* and *U. urealyticum* (both at  $>10^4$  CCU/mL) (Table 2).

#### 4. DISCUSSION AND CONCLUSIONS

Since initial anamnesis, it was highlighted the true problems of couple, even though the first clinical manifestations were reported into the different body sites under several clinical pictures.

In this study we investigated all the ecosystems involved in sexual activity of a young couple apparently presenting an a latent infertility problem only. In the male patient, lingual pH and  $PSA_{tot}$  values, added to negative VDRL, were highly suggestive for apparent physiological conditions, despite of oropharyngeal and urogenital asymptomatic clinical pictures. On the contrary, however, culture and molecular analysis of lingual ecosystem highlighted the exclusive presence of *C. ulcerans*, while molecular analysis revealed the presence of *M. hominis*, *U. urealyticum*, and *C. trachomatis*. This our consideration for lingual ecosystem is supported by the fact that if it were carried out a routine culture, *C. ulcerans* would have been the sole resident, thus perpetuating the old evidences of Medical Science. Compared to lingual one, the lower bacterial load we observed in male pharyngeal ecosystem. The bacterial load we observed in male pharyngeal ecosystem, lower than that observed in the lingual one, could reflect an apparent "physiologic status" at this pharyngeal site, as supported by lack of clinical picture. Furthermore, this evidence of polymicrobial growth in pharyngeal ecosystem is in accordance with methodological preparation and standardization of the samples we carried out. Quantitative microbiological analyses allowed us to ascribe to the higher mycoplasma lingual presences compared to pharyngeal site, the major clinical evidence of an asymptomatic condition of silent and/or low-grade inflammatory status. If lingual and pharyngeal weak evidence for *C. trachomatis* infection observed in male partner could explain asymptomatic conditions, on the other side it is possible to involve both the direct modality of lingual contagion and different cellular typology to colonize. In our previous studies [2, 6, 7, 9], we have never found these situations because the patients were affected by chronic oropharyngeal and conjunctival inflammation. Alternatively, it is possible to evoke for both patients both different genetic individual susceptibility [8], and the always possible "time difference" with respect to contagion.

The high pH value observed in the female lingual ecosystem and its clinical manifestation are indicative of the altered microbial ecosystem observed in the oral cavity (Figure 2). On the contrary, no demonstrated pharyngeal mucosae inflammation was noted. Considering microbiological findings obtained from lingual samples – that is the presence of *C. ulcerans*, the high mycoplasmas (*U. urealyticum*, and *M.*

200 *hominis*) concentrations and the presence of *C. trachomatis* - it is plausible to hypothesize that this site would  
201 be the first one to be colonized during sex oral behaviour [8], while *C. ulcerans* could be considered as a  
202 microbiological marker of the altered lingual ecosystem following urogenital contamination.

203 With regard to the ~~second pharyngeal ecosystem of oral cavity, namely pharyngeal one~~, while swab  
204 samples suggested the absence of "atypical infections", *M. hominis* and *U. urealyticum* ~~was~~ were observed,  
205 although at low concentrations, in scraping samples, thus demonstrating the latter as the best sampling  
206 modality. Additionally, microbiological evaluation of the female pharyngeal ecosystem by means of different  
207 sampling modalities showed an noteworthy overlapping of bacterial species, thus suggesting the marginal role  
208 of these bacteria in triggering inflammation of the pharyngeal ecosystem. They might, however, contribute in  
209 altering, together with mycoplasmas, the nutritional state of colonized cells, thus favouring the development of  
210 the critical condition of inflammatory status [10,11], triggering the unbalance of cellular potential redox state  
211 [12]. The absence of chlamydia at this uninflamed site could be explained by both an initial phase of  
212 contagion from tongue and a chlamydial condition not detectable by PCR [13-15]. In this regard, it is  
213 noteworthy to remember that during renewal of chlamydial infection in a patient with asymptomatic chronic  
214 infection, each reticular body produces much more progeny from a single inclusion, assuring a new potential  
215 infecting load for contiguous tissue or its diffusion in intestinal habitat.

216 It is known that higher pH value, increased number of leucocytes/hpf, and sperm agglutination are some  
217 of causes of asymptomatic inflammatory prostatitis, often incidentally diagnosed during infertility or prostate  
218 cancer evaluation [16-19]. Together with an accurate anamnesis and prostatic investigation, adequate  
219 evaluations concerning SF demonstrated an altered fertility due to low vitality test and high agglutination  
220 phenomenon in the presence of an adequate sperm concentration.

221 The presence of asymptomatic recurrent genital ulcers, suggestive for chlamydial and/or mycoplasma  
222 infections, together with increasing trend of PSA value [20], were justified by microbiological evaluation of  
223 urogenital ecosystems. Considering culture and molecular analyses of FVU, GU, SF, and UAPM, it is  
224 highlighted that the positivity for mycoplasma infections are distributed differently on the several sources,  
225 indicating a major presence of these infections in parenchymal prostatic tissue compared to FVU, GU, and  
226 SF. In the same sources, chlamydial infection presented a different behaviour, being more positive in GU  
227 and UAPM, indicating in *C. trachomatis* the true aetiologic agent of ulcerated areas, while the parenchymal  
228 prostatic tissue represents a major reservoir of chlamydial infection [10].

229 In our operative conditions, the polymicrobial colonization found in each ecosystem was highly suggestive  
230 for considered saprophytic flora. Lastly, yellow-mucous secretion, high pH value, relevant presence of  
231 leucocytes/hpf, very low CFU/mL of *L. acidophilus* concentration, replaced with an abundant presence of *C.*  
232 *ulcerans*, characterized an altered female genital environment due to asymptomatic chronic mycoplasma  
233 and chlamydial infections. Although *C. ulcerans* has been increasingly isolated as an emerging zoonotic agent  
234 of diphtheria and other infections from companion animals [21, 22], we believe that in our case it has to be  
235 considered as an opportunist pathogen [5], a biomarker of altered oral, as well as ocular, ecosystems as  
236 already found in previous studies [6-8, 6-9]. The relevant presence of *C. ulcerans* we observed in the female  
237 patient might in fact indicate that it concurred to the pathogenic determinism by increasing pH level and,  
238 therefore, favouring the decrease of cellular redox state [5]. Furthermore, toxigenic *C. ulcerans* could have  
239 contribute in developing the evidence of a "patinated whitish tongue" with mucoid salivation we observed.

There is currently no direct evidence of person-to-person transmission of *C. ulcerans*. Although our findings rise the possibility of a sexual transmission, unfortunately strains from both partners were not subjected to genetic fingerprinting to demonstrate their clonal relatedness.

Furthermore, The female patient also showed VDRL positive test, indicating an early condition triggering autoimmune diseases [21-23]. We have recently reported that these atypical infections - being "silent and/or at low grade inflammatory" - might developed, in patient genetically susceptible, a VDRL positivity after decades from contagion, indicating the first biochemical marker of silent mycoplasma infection, developed itself in any ecosystem of the host body [2, 9, 10, 22-24, 25]. It is interesting to note that silent atypical chronic infections by *M. hominis*, *U. urealyticum*, and *C. trachomatis* determine several health problems on genital female reproductive system [23-26, 24], but one of which, such as the precocious infertility of a susceptible genetically young couple [10], was solved following the holistic vision of "Human Being" and carrying out a double sampling modality. Gavazzi G. et al. [25-28] stated that "ageing may be the cause of infection but infection can also be the cause of ageing", considering the holistic vision "Human Being" as the best way to approach the human problems before these become irreversible decline. Franceschi et al. described the "inflammaging" phenomenon [26, 27-29, 30], caused by innate immune system chronically stressed. Furthermore, Chang et al. [28-31] reported that chronic mycoplasma infections are responsible of subsequent risk of cancer among elderly adults. In a recent study, Gallenga et al. [29-32] demonstrated that the standardization of sampling methodology by scraping of living cells is crucial in establishing *C. trachomatis* presence in the ocular ecosystem during the early phase of conjunctivitis.

Results we obtained by adopting these two different sampling modalities, we also used in our previous studies [2, 9, 10, 22-24, 29-32], have borne out our beliefs regarding asymptomatic patients during initial chronic phase of infections. To correctly interpret this assertion it is necessary to highlight that this initial phase could last for decades. It is interesting to note as the two sampling modalities led to opposite results for *C. trachomatis* presence, nourishing once again the enigmatic behaviour of chlamydial infection. The failure of a laboratory diagnosis in clinically asymptomatic patients would underestimate these atypical and silent infections, keeping out of control by any control program of public health.

## ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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## ABBREVIATIONS

**ENT**, Ear Nose and Throat; **PSA**, prostatic specific antigen; **VDRL**, Venereal Disease Research Laboratory; **TPHA**, *Treponema pallidum* haemoagglutination assay; **FVU**, first void urine; **SF**, seminal fluid; **UAMP**, urine sample emitted after prostatic massage; **HPV**, human papilloma virus; **GU**, genital ulcer; **TMC**, total microbial charge; **CFU**, colony-forming unit; **Hd-DNA**, *Haemophilus ducreyi*-DNA; **Tp-DNA**, *T. pallidum*-DNA; **HPV-DNA**, human papilloma virus-DNA; **Ng-DNA**, *Neisseria gonorrhoeae*-DNA; **Ct-DNA**, *Chlamydia trachomatis*-DNA; **CCU**, color-changing unit.

| Microorganism         | Lingual scraping           | Pharyngeal scraping       | First voiding urine          | Genital ulcers            | Seminal fluid             | Urine after prostatic massage |
|-----------------------|----------------------------|---------------------------|------------------------------|---------------------------|---------------------------|-------------------------------|
| <i>S. pyogenes</i>    | absent                     | absent                    | ND                           | ND                        | ND                        | ND                            |
| <b>Streptococci</b>   | absent                     | <i>S. faecalis</i><br>20% | <i>S. faecalis</i><br>40%    | ND                        | <i>S. faecalis</i><br>40% | absent                        |
| <i>S. pneumoniae</i>  | ND                         | absent                    | ND                           | ND                        | ND                        | ND                            |
| <b>Staphylococci</b>  | absent                     | absent                    | <i>S. epidermidis</i><br>20% | <i>S. epidermidis</i> 40% | <i>S. epidermidis</i> 20% | absent                        |
| <b>Corynebacteria</b> | <i>C. ulcerans</i><br>100% | <i>C. ulcerans</i><br>40% | <i>C. ulcerans</i><br>40%    | <i>C. ulcerans</i><br>30% | <i>C. ulcerans</i><br>40% | absent                        |
| <i>E. coli</i>        | absent                     | 30%                       | absent                       | 20%                       | absent                    | absent                        |
| <i>K. pneumoniae</i>  | absent                     | 10%                       | absent                       | 10%                       | absent                    | absent                        |
| <b>Yeasts</b>         | absent                     | absent                    | absent                       | absent                    | absent                    | absent                        |
| <i>T. vaginalis</i>   | ND                         | ND                        | absent                       | ND                        | absent                    | absent                        |
| <i>M. hominis</i>     | 10 <sup>3</sup> CCU/mL     | absent                    | 10 <sup>4</sup> CCU/mL       | 10 <sup>3</sup> CCU/mL    | 10 <sup>4</sup> CCU/mL    | >10 <sup>4</sup> CCU/mL       |
| <i>U. urealyticum</i> | 10 <sup>3</sup> CCU/mL     | 10 <sup>3</sup> CCU/mL    | 10 <sup>4</sup> CCU/mL       | 10 <sup>3</sup> CCU/mL    | > 10 <sup>4</sup> CCU/mL  | >10 <sup>4</sup> CCU/mL       |
| <b>Hd-DNA</b>         | ND                         | ND                        | ND                           | absent                    | ND                        | ND                            |
| <b>Tp-DNA</b>         | ND                         | ND                        | ND                           | absent                    | ND                        | ND                            |
| <b>Ng-DNA</b>         | ND                         | ND                        | absent                       | ND                        | absent                    | absent                        |
| <b>Ct-DNA</b>         | weak present               | weak present              | present                      | highly present            | present                   | highly present                |
| <b>HPV-DNA</b>        | absent                     | ND                        | absent                       | ND                        | absent                    | ND                            |

**Table 1. Cultural and molecular analyses of specimens collected from different ecosystems of the male partner.** Percentage values show the relative abundance of each species in that ecosystem. ND, not detected, because clinically or scientifically not indicated. Hd, *H. ducreyi*; Tp, *T. pallidum*. Ng, *N. gonorrhoeae*; Ct, *C. trachomatis*; HPV, human papilloma virus.

| Microrganism          | Lingual scraping           | Pharyngeal swab           | Pharyngeal scraping                                   | First voiding urine          | Cervico-vaginal secretion  |
|-----------------------|----------------------------|---------------------------|-------------------------------------------------------|------------------------------|----------------------------|
| <i>S. pyogenes</i>    | absent                     | absent                    | absent                                                | ND                           | ND                         |
| Streptococci          | absent                     | <i>S. milleri</i><br>30%  | <i>S. milleri</i><br>30%<br><i>S. faecalis</i><br>20% | <i>S. faecalis</i><br>10%    | <i>S. faecalis</i><br>10%  |
| <i>S. pneumoniae</i>  | ND                         | absent                    | absent                                                | ND                           | ND                         |
| Staphylococci         | absent                     | absent                    | absent                                                | <i>S. epidermidis</i><br>40% | absent                     |
| Corynebacteria        | <i>C. ulcerans</i><br>100% | <i>C. ulcerans</i><br>20% | <i>C. ulcerans</i><br>50%                             | <i>C. ulcerans</i><br>50%    | <i>C. ulcerans</i><br>70%  |
| <i>E. coli</i>        | absent                     | 20%                       | absent                                                | absent                       | 10%                        |
| <i>K. pneumoniae</i>  | absent                     | 30%                       | 20%                                                   | absent                       | 10%                        |
| <i>G. vaginalis</i>   | absent                     | absent                    | absent                                                | absent                       | absent                     |
| Yeasts                | absent                     | absent                    | absent                                                | absent                       | absent                     |
| <i>L. acidophylus</i> | ND                         | ND                        | ND                                                    | ND                           | 3 x 10 <sup>5</sup> CFU/ml |
| <i>T. vaginalis</i>   | ND                         | absent                    | absent                                                | absent                       | absent                     |
| <i>M. hominis</i>     | > 10 <sup>4</sup> CCU/mL   | absent                    | 10 <sup>3</sup> CCU/mL                                | absent                       | > 10 <sup>4</sup> CCU/mL   |
| <i>U. urealyticum</i> | > 10 <sup>4</sup> CCU/mL   | absent                    | 10 <sup>3</sup> CCU/mL                                | > 10 <sup>4</sup> CCU/mL     | > 10 <sup>4</sup> CCU/mL   |
| Ng-DNA                | ND                         | ND                        | ND                                                    | ND                           | ND                         |
| Ct-DNA                | present                    | absent                    | absent                                                | present                      | present                    |
| HPV-DNA               | absent                     | ND                        | ND                                                    | absent                       | absent                     |

**Table 2. Cultural and molecular analyses of specimens collected from different ecosystems of the female partner.** Percentage values show the relative abundance of each species in that ecosystem. ND, not detected, because clinically or scientifically not indicated. Ng, *N. gonorrhoeae*; Ct, *C. trachomatis*; HPV, human papilloma virus.

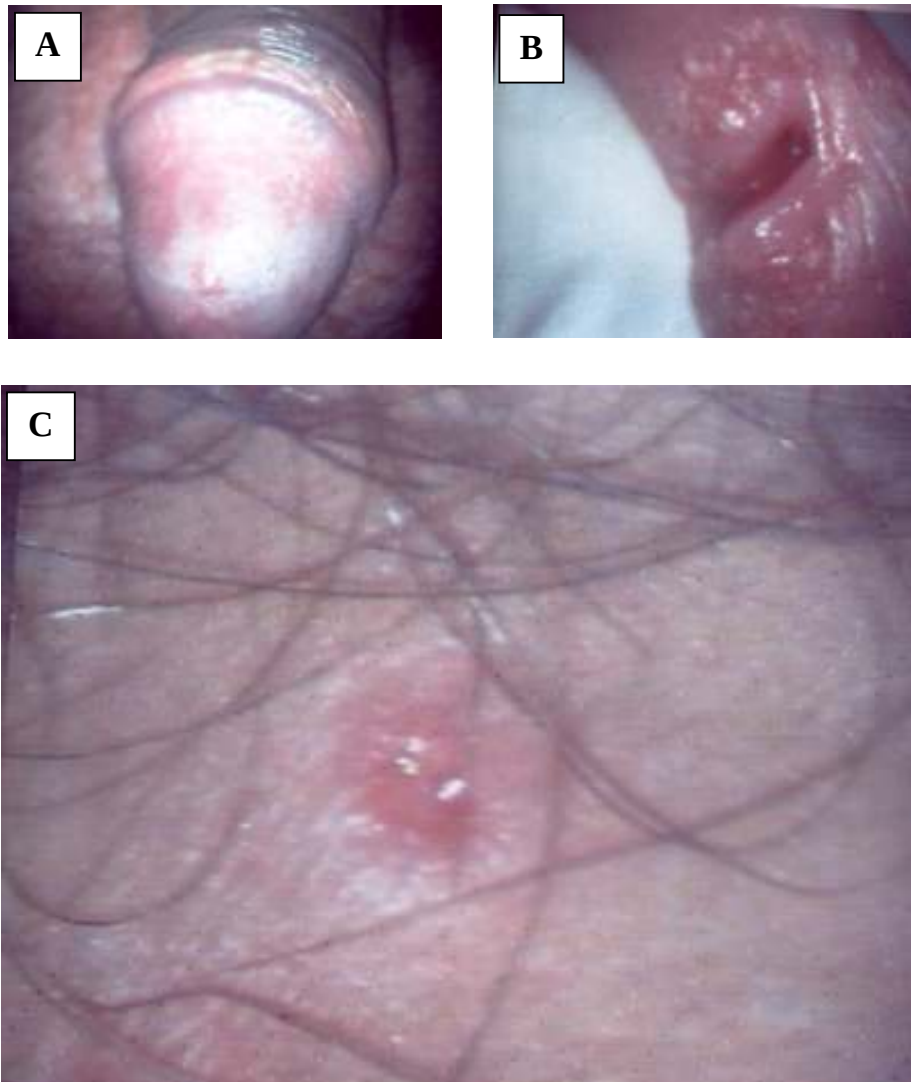


**Fig. 1. Macroscopic aspect of male genital tract. A)** Inflamed areas of glans. On the left, several lesions are observable. **B)** Urinary meatus presenting the inflamed areas sampled for cellular living material resulted strongly positive to *C. trachomatis*-DNA. **C)** Magnification of a scrotal inflamed area showing a cutaneous ulcer.

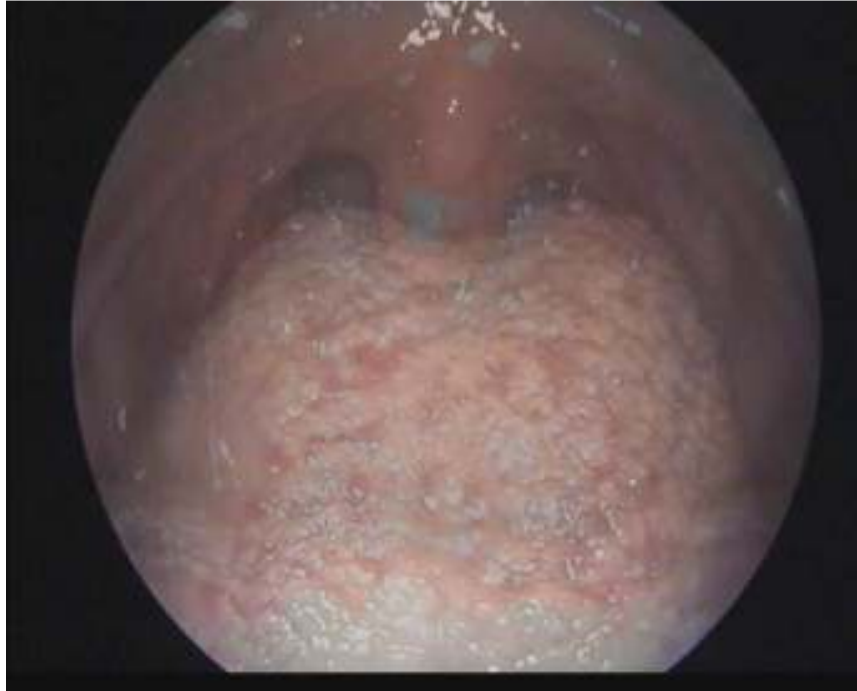


**Fig. 2.**Clinical aspect of the “patinated whitish tongue” with mucoid salivation. In the female patient, pharyngeal observation showed amucoid greyish secretion. Pharyngeal scraping was, therefore, performed to collect both secretion and cellular samples.