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Journal Name:	British Microbiology Research Journal
Manuscript Number:	2014_BMRJ_13082
Title of the Manuscript:	Unusual oropharyngeal asymptomatic manifestations caused by atypical pathogens detected by PCR into altered ecosystems of an infertile couple
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<u>Compulsory</u> REVISION comments		
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments	The manuscript entitled "Unusual asymptomatic oropharyngeal Manifestations Caused by atypical pathogens detected by PCR into altered agricultural ecosystems of an infertile couple" is a clinical case of an infertile couple showing oropharyngeal and genital infection. During bacteriological and molecular analysis were identified several bacteria, including <i>Mycoplasma hominis</i>, <i>Ureaplasma urealyticum</i> and <i>Chlamydia trachomatis</i>, all they considered as sexually transmitted pathogens. Although, the manuscript is interesting requires of a big discussion about the results obtained.	



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	I consider that the title of the manuscript is inadequate because it is not unusual that there are no clinical manifestations during an oropharyngeal infection by atypical pathogens. The detection of these pathogens in genitals as well in pharynx is not strange since it has been reported in several articles. This manuscript would be interesting, if the authors could identify the Chlamydia trachomatis serovars obtained. The presence of ulcers would suggest that it is a serovar of lymphogranuloma venereum. However, in the manuscript it has not considered this possibility.	▪ We agree with Reviewer that C. trachomatis and mycoplasma were previously found in pharynx but, at the best of our knowledge, the clinical evidences we observed in the couple arrived at our observation (Figure 2) were not yet published in literature. Therefore, “unusual” doesn’t refer to the “absence of clinical manifestations” but to the “original clinical manifestations” they caused. ▪ We agree with Reviewer but, unfortunately, we didn’t store the strain. However, lack of lymphonodal swelling and discharge was probably not suggestive of LGV serovar. In this regard, in the “Mats and Meths” section of the revised version of the manuscript we added this evidence (Line 61).
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	In the case of <i>Mycoplasma</i> and <i>Ureaplasma</i> identification. These bacteria are considered in some cases as normal flora of the genitals. However, “in vitro” models of infection have <u>shown</u> that these bacteria produce <u>morphological</u> alterations and damage to sperm. In this manuscript there is not new information about infection with this pathogen. Notwithstanding the foregoing, the microbiological isolation of <i>Corynebacterium ulcerans</i> is very interesting. I believe that the major contribution of this research is the isolation of <i>C. ulcerans</i>. Due to the above, I suggest	▪ We think that this comment comes from an “old/classic vision” of Medicine, as we reported in the “Discussion” section. In fact, several published evidences by our and other groups clearly showed that long incubation period of mycoplasma infection – as is the case we described in our manuscript - can be detrimental in genetically predisposed subjects (references 2, 10, 23-25 of the revised version). To support this, reference 25 has been added in the revised version of the manuscript. ▪ We do not agree with Reviewer on the need to change manuscript’s article and, consequently, to distort its significance. Here are the reasons. Although there is growing
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	the following title for this manuscript: "Transmission of <i>Corynebacterium ulcerans</i> through of an oral-genital contact." <i>C. ulcerans</i> is part of the normal flora of animals (cause mastitis in cows) and is considered as zoonotic diseases. Although rarely this pathogen causes pharyngitis, there are some strains produce toxins that can be confused with clinical manifestations of <i>C. diphtheria</i>. Furthermore, the <i>C. ulcerans</i> toxin can produce extra-pharyngeal infections that may depend on the immune status of the patient. For example Sing et al., reported the isolation of this pathogen in deep skin ulcerative lesions. Also, <i>Corynebacterium propinquum</i> (Abdolrasouli A, Roushan A. <i>Corynebacterium propinquum</i> associated with acute, nongonococcal urethritis. Sex Transm Dis. 2013 Oct;40(10):829-31. doi: 10.1097/OLQ.000000000000027.; Berger A, Lensing	evidence that <i>C. ulcerans</i> is able, per se, to cause pharyngitis (or extra-pharyngitis infections), we think that this was not our case. <i>C. ulcerans</i> has been in fact cultured together with those we believe to be the true etiologic agents (<i>Mycoplasma</i>, <i>Ureaplasma</i>, and <i>C. trachomatis</i>). This means that <i>C. ulcerans</i> might synergistically interacts with <i>Mycoplasmas</i> and <i>C. trachomatis</i> to cause host's damage. For example, by increasing pH values at the site of infection thus decreasing cellular redox state. In the revised version of the manuscript we referred at these topics in "Discussion and Conclusions" section (Lines 205-206; Lines 237-242). Furthermore, 2 new references (21, 22) were added to introduce <i>C. ulcerans</i>.
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	C, Konrad R, Huber I, Hogardt M, Sing A. Sexually transmitted diphtheria. Sex Transm Infect. 2013 Mar;89(2):100-1. doi: 10.1136/sextrans-2011-050418. Epub 2012 May 23.) and <i>C. diphtheriae</i> have been isolated of individuals with non-gonococcal urethritis, so the detection of <i>C. ulcerans</i> would not be surprising to detect it in the genitals.	With regard to toxigenic ability of <i>C. ulcerans</i> strain, we didn't see any evidence of a diphtheria-like thick pseudomembrane (as shown in Figure 2), thus probably suggesting the strain wasn't toxigenic. The Reviewer cited some studies in support of non diphtheria Corynebacteria as a possible new cause of nosocomial infections capable of colonizing several human tissues. However, these studies were carried out without searching for <i>C. trachomatis</i> or <i>Mycoplasma</i> that might be the main etiological pathogens. In this regard, we recently (ref #8: Gallenga PE, et al. Molecular approach by PCR is the best method to detect the presence of <i>Chlamydia trachomatis</i> and to define the true agent of ocular bacterial
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	Furthermore, this manuscript would be the first report of transmission from person to person of <i>C. ulcerans</i> and this is very interesting by the possibility of a sexual transmission with this pathogen.	inflammation. Int J Immunopathol Pharmacol. 2011;24:285-296.) found that 70.8% of conjunctival swabs were positive for <i>Corynebacteria</i> spp, <i>C. ulcerans</i> included, although all of <i>Corynebacterium</i> species were co-cultured with <i>C. trachomatis</i>, as assessed by PCR, thus confirming the scarce role of this bacterium in determining conjunctival lesions, also considering that these bacteria are frequently found in healthy conjunctivas. ▪ Unfortunately, our results do not provide demonstration for an interpersonal transmission of <i>C. ulcerans</i> since strains from both male and female partners would have been subjected to genetic fingerprinting (i.e. by using PFGE or MLST techniques) to
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	The authors describe that patients accepted and signed off informed consent.	demonstrate they were clonally related. In the revised version of the manuscript we referred at this topic (Lines 243-245).
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