



## Short Communication

## Detection of *Borrelia crociduræ* in a vaginal swab after miscarriage, rural Senegal, Western Africa

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## ABSTRACT

Tick-borne relapsing fever (TBRF) borreliae are one of the main causes of fever in rural Africa and can cause miscarriages. This article reports *Borrelia crociduræ* as a probable cause of spontaneous miscarriage, which was detected through vaginal self-sampling. This appears to be the first such report. © 2019 Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The over-diagnosis of malaria in endemic areas overshadows the presence of other pathogens that induce fever, such as bacteria (Gwer et al., 2007). In Senegal, several studies have already reported the presence of DNA from pathogenic bacteria in the blood of patients with non-malarial fever, including the DNA of tick-borne relapsing fever (TBRF) borreliae (Mediannikov et al., 2014; Parola et al. 2011; Talagrand-Reboul et al., 2018). TBRF borreliae are risk factors for pregnant women and can cause miscarriages (Giakoumelou et al., 2016). *Borrelia* sp. are known to readily penetrate different biological barriers. This is explained by their helical shape and their motility, and is facilitated by the use of the host protease.

In recent years, reliable and effective vaginal self-sampling has been used in the diagnosis of several infections, including human papillomavirus (HPV) and bacteria causing sexually transmitted infections (STI) (Lagier et al., 2017). For this study, a project was first conducted to assess the feasibility of taking vaginal samples in rural areas of Senegal for the detection of the carriage of certain bacteria and HPV; the results have been reported previously (Fall et al., 2019). The present study on miscarriages, which began in March 2016, involved the self-sampling women who had a

miscarriage or other pregnancy problems and was performed to determine whether pathogens are responsible for abortions or other pregnancy problems in rural areas of Senegal. This study is novel in reporting what appears to be the first detection of *Borrelia crociduræ* in vaginal self-sampling in such clinical involvement.

The study was performed in health posts in Dielmo, Ndiop, and Niakhar (Diohine, Toucar, Ngayohème) in Senegal, West Africa (Fall et al., 2019). Women who had a miscarriage performed a vaginal self-sampling (Transwab, M40 compliant, for bacterial detection). Informed consent was obtained from the patients. Ethical authorization for this study was granted by the National Ethics Committee of Senegal (n°83/MSAS/DPRS/CNFRS). The microorganisms, including those associated with STIs and those known to cause abortion, were tested by qPCR and will be the subject of a separate publication. When a sample was positive for one gene, a second qPCR targeting another gene was performed (Table 1). To check the quality of the DNA extracts, the human albumin gene was tested, as described previously (Fall et al., 2019).

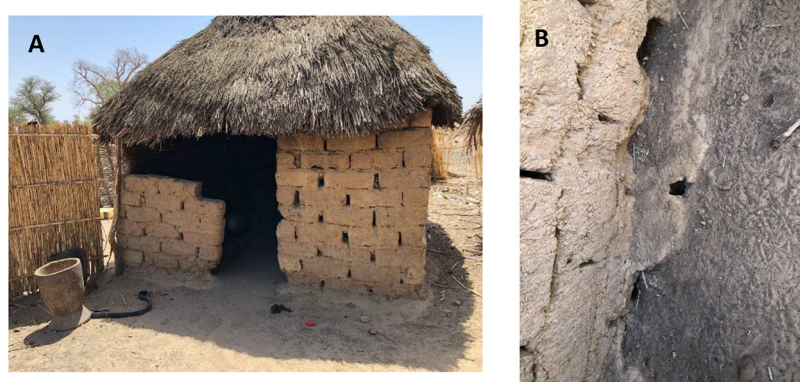
From March 2016 to July 2017, a total 132 vaginal samples from controls (women living in the same geographic area and with no history of complicated pregnancy) and 26 vaginal samples from patients were tested. All samples were of high quality (100% positive for human albumin). Using qPCR, three vaginal samples from patients were positive for *Borrelia* sp. 16S; none of the control samples were positive. However, only one patient sample was confirmed using the qPCR targeting the ITS4 (intergenic spacer) locus. A standard PCR targeting the ITS locus confirmed the diagnosis (Table 1). Sequencing of the ITS locus amplicons (539 base pairs, GenBank accession number LT984797) revealed 100%

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**Table 1**  
Molecular biology tools used for the identification of *Borrelia crocidurae*.

Gene	Targeted sequence
<i>Borrelia</i> sp. 16S	Bor_16S_3F: AGCCTTTAAAGCTTCGCTTGTAG, Bor_16S_3R: GCCTCCGTAGGAGTCTGG,
qPCR targeting ITS4	Bor_16S_3P: 6FAM-CCGGCTGAGAGGGTGAACGG Bor ITS4_F: GGCTTCGGGTCTACCACATCTA, Bor ITS4_R: CCGGGAGGGGAGTAAAATAG,
Standard PCR targeting the ITS locus	Bor ITS4_P: 6FAM-TGCAAAGGCACGCCATCACC ITS F: AGGGGGTGAAGTCGTAACAAG ITS R: GTCTGATAAACCTGAGGTCGGA



**Figure 1.** (A) Home of the woman suffering a miscarriage who lived near Niakhar, Senegal, West Africa, with detection of *Borrelia crocidurae* in her vaginal self-swab. (B) Crevices observed in the house.

sequence similarity with *B. crocidurae* (KF176340.1) isolated from *Ornithodoros sonrai*, the vector of borreliosis, collected in the same geographic area. This sample was collected from a woman who experienced a spontaneous abortion after 12 weeks of pregnancy. The difference between patients (1/26) and controls (0/132) was significant for *B. crocidurae*. The patient lived in Niakhar area and had a history of three spontaneous abortions. The 25 patients who tested negative for *B. crocidurae* had experienced an abortion at between 6 and 24 weeks of pregnancy (mean 20 weeks). The patient with the sample positive for *Borrelia* lived in an environment with animals (horse, calf, goat, donkey, and sheep) and had seen many rats in her house, but never ticks. Her house was built of bricks and the soil was non-cemented dirt. The floor and walls had some crevices (Figure 1). The 25 patients who had a miscarriage but with samples negative for *Borrelia* lived in the same geographical area, and no difference in the presence of animals close to home was observed.

This study suggests that vaginal self-sampling could be a valuable technique to detect *B. crocidurae*. The inclusion of a substantial number of controls living in the same geographical area and the use of stringent protocols confirm the accuracy of the results. The fact that the women were taking samples from the brick and cement dispensary and not at homes, and the fact that all controls were negative, excluded possible environmental contamination of the samples.

TBRF is transmitted to humans through the bites of soft ticks of the genus *Ornithodoros*. Wild rodents and insectivores are common reservoir hosts. TBRF is responsible for recurring fever associated with spirochetemia and is the main cause of non-malarial fever in Senegal (Talagrand-Reboul et al., 2018). Previous studies performed in Rwanda and Tanzania have demonstrated a 30% risk of pregnancy loss and a perinatal mortality rate of 15% in the case of TBRF (Talagrand-Reboul et al., 2018). With the inclusion of *B. crocidurae* infections, this study suggests that vaginal self-sampling is a method that can be adapted for the detection of infectious causes of spontaneous miscarriage in low-income

countries (Lagier et al., 2017). Certainty of a link between the vaginal detection of *B. crocidurae* and the causality of abortion will require further more in-depth studies. Nevertheless, this technique of sampling could be coupled with point-of-care laboratory diagnostics, which have previously demonstrated their efficacy in exploring the non-malarial causes of fever (Drancourt et al., 2016), and could be used to elucidate the infectious causes of miscarriage in low-income countries.

#### Author contributions

Study design: JCL, DR, CS; data collection: NSF, ND, JCL; data analysis: NSF, OM, FF, PP, JCL, CS, DR; writing: NSF, PP, DR, JCL.

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#### Ethical approval

Ethical authorization for this study was granted by the National Ethics Committee of Senegal (n°83/MSAS/DPRS/CNFRS).

#### Conflict of interest

None.

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