**ORIGINAL RESEARCH ARTICLE**

# *Chlamydia Trachomatis* infection in infertile men from Cotonou, Benin

DOI: 10.29063/ajrh2025/v29i4.8

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## Abstract

Male infertility accounts for 20-30% cases of infertility, and may be due to hormonal, genetic, toxic or infectious factors. Male urogenital infections caused by *Chlamydia trachomatis* (CT) are potential causes of male infertility. This study determined the prevalence of *Chlamydia trachomatis* infection among infertile men in Cotonou. The descriptive cross-sectional study used Real time PCR (RT PCR) to detect *Chlamydia trachomatis* infection on the first urine stream from 90 participants who did sperm analysis for infertility problems between March 2023 and December 2023. The mean age of the patients was 35.31 ± 7.00 years. Most were cases of primary infertility (62.22%), with an average duration of 5 years. The results showed *Chlamydia trachomatis* genome detection with RT PCR in 3.33% of subjects. *Chlamydia trachomatis*-positive subjects exhibited leukospermia and low sperm motility. We conclude that *Chlamydia trachomatis* infection is common and can lead to serious complications, including male infertility. Serological diagnosis lacks specificity and in case of suspected infection, RT-PCR testing is strongly recommended for the detection of CT. *(Afr J Reprod Health 2025; 29 [4]: 89-95).*

**Keywords:** Male infertility; spermogram; infection; Chlamydia trachomatis

## Résumé

L'Infertilité masculine représente 20 à 30 % des cas d'infertilité et peut être due à des facteurs hormonaux, génétiques, toxiques ou infectieux. Les infections urogénitales masculines causées par *Chlamydia trachomatis* (CT) sont des causes potentielles d'infertilité masculine. Cette étude a déterminé la prévalence de l'infection à *Chlamydia trachomatis* chez les hommes infertiles à Cotonou. Il s'agissait d'une étude transversale descriptive qui a utilisé la technique de PCR en temps réel (RT PCR) pour détecter l'infection à Chlamydia trachomatis sur le premier jet d'urine de 90 participants qui ont effectué un spermogramme pour exploration de l'infertilité entre mars 2023 et décembre 2023. L'âge moyen des patients était de 35,31 ± 7,00 ans. La plupart étaient des cas d'infertilité primaire (62,22%), avec une durée moyenne de 5 ans. Les résultats ont montré une détection du génome de Chlamydia trachomatis par RT PCR chez 3,33% des sujets. Les sujets positifs à *Chlamydia trachomatis* présentaient une leucospermie et une faible mobilité des spermatozoïdes. Nous concluons que l'infection à Chlamydia trachomatis est fréquente et peut entraîner de graves complications, y compris l'infertilité masculine. Le diagnostic sérologique manque de spécificité et, en cas de suspicion d'infection, le test RT-PCR est fortement recommandé pour la détection de CT.
. *(Afr J Reprod Health 2024; 29 [2]: 89-95).*

**Mots-clés:** Infertilité masculine ; spermogramme ; infection ; Chlamydia trachomatis

## Introduction

The World Health Organization defines infertility as the inability of a couple to conceive after at least 12 months of regular and frequent sexual intercourse without the use of any contraceptive methods.[1]It affects 15 to 25% of the global population, which is about 150 to 180 million people. Male infertility is frequently associated with abnormalities in the spermatic parameters.[2] It represents a significant public health problem, with a prevalence of 2.5% to 4.8% in sub-Saharan Africa, according to Argarwal *et al*. in 2015.[3] Male infertility can be caused by numerous factors, including hormonal, genetic, and toxic factors, with 35% of cases attributed to infectious and

inflammatory factors.[4] *Chlamydia trachomatis* is responsible for most widespread bacterial sexually transmitted infections (STI) in industrialized countries.[5,6] It is also found in developing countries, particularly in Africa. Diagnosis is often performed using serological tests, which have low sensitivity and specificity. The *Chlamydia trachomatis* infection is often asymptomatic, and its detection requires modern diagnostic methods.[7]The association between *Chlamydia trachomatis* infection and male infertility is a subject of diverse opinions. In Benin, few studies have been conducted on the prevalence of *Chlamydia trachomatis* infection, and diagnostic methods remain limited.[8] This study determined the prevalence of *Chlamydia trachomatis* in infertile men at Cotonou and to highlight its association with male infertility.

# Methods

### *Study site*

This study was conducted in Cotonou, the economic capital of Benin Republic. Participants were recruited at the Centre for Research in Human Reproduction and Demography (CERRHUD) and in the Laboratory of Histology, Reproductive Biology, Cytogenetics and Medical Genetics (LHBRCGM). The molecular analyses were performed in LHBRCGM at the Faculty of Health Sciences (FSS) at Cotonou.

### *Study design*

This was a descriptive cross-sectional study with an analytical aim. It was conducted on men admitted for a spermogram test as part of an infertility assessment in both laboratories from March to December 2023.

### *Study population*

The study population consisted of infertile men admitted to the two centres during the study period.

### *Inclusion criteria*

Participants were men who had undergone a spermogram and had given their written consent after a diagnosis of infertility by the clinicians .

### *Exclusion criteria*

This study excluded men who: (1) did not give their consent; and (2) were not available or unable to provide biological samples; and 3) men undergoing antibacterial treatment

### *Sample size*

The sample size was determined by Schwartz's formula; knowing that the prevalence of male infertility is 4%, the expected number of participants was 59.

### *Data collection*

Data were collected through a survey form with a unique identification number for patient confidentiality. First urine sample was collected by the patient at home and sent to the LHBRCGM.

### *Detection of Chlamydia trachomatis (CT)*

The samples were centrifuged and cell pellet obtained was used for DNA extraction using the Qiagen kit following manufacture's instruction. A Thermo Scientific Evolution 60S UV-Visible spectrophotometer was used to determine the DNA quality and concentration. DNA extracted was amplified by real-time PCR (RT-PCR) using the TaqMan method following the protocol described by Eboigbodin *et al* .[9]

### *Statistical analyses*

The data were entered, processed, and analyzed using Microsoft Word and Excel 2019, Epi Info version 7.2.6.0. The statistical test, Chi-square or Fisher's exact test, was used as appropriate, with a significance level of 5%, to make associations between the variables.

### *Ethical considerations*

Ethical approval was obtained from the Research Ethics Committee of the Institute of Biomedical Science and Applications (CER-ISBA) in Cotonou under approval number N°178 of 30/10/2023. Patient identities were not disclosed. This study was conducted as part of academic work and adhered strictly to Good Clinical Practice (GCP) guidelines.

Written informed consent was obtained from all participants, and confidentiality was rigorously maintained throughout data collection. Information collected was processed anonymously. The results of the analyses were communicated to the attending physician for the benefit of the patients

## Results

### *1.1 Epidemiological-Clinical parameters*

A total of 90 patients were included in this study. The mean age was 35.3 ± 7.0 years, ranging from 24 to 61 years. The most represented group was in the range of 30-39 years old, comprising 64.4% of the participants (Figure 1). Married patients comprised 75.6% of the sample, most having cohabited for less than five years. The majority of couples reported at least two sexual intercourses per week. Primary infertility was observed in 62.2% (n=56) of the patients.

### *1.2 Results of the spermogram of the study samples*

The spermogram was performed on all patients according to WHO standards to evaluate spermatic parameters. Tables I and II summarize the spermogram results for all the patients.

### 1.3 Detection of *Chlamydia trachomatis* genome by RT PCR

Out of the 90 patients in the study, CT was detected in 3 patients (3.3%). Figure 3 shows the prevalence of *Chlamydia trachomatis* in infertile men in Cotonou. For the RT PCR results, If the Ct (Cycle threshold) value of the sample was below the detection threshold, the sample was considered CT negative (CT-); if the CT value was ≤40 and the amplification curve was a typical "S" curve, the sample was considered CT positive (CT+). However, if the amplification curve was not a typical "S" type curve, the sample was considered CT−, as shown in Figures 3 and 4.

### *1.4 Spermogram data of CT+ patients*

All CT+ patients exhibited leukocytospermia, asthenozoospermia, and low sperm motility ( Table III).

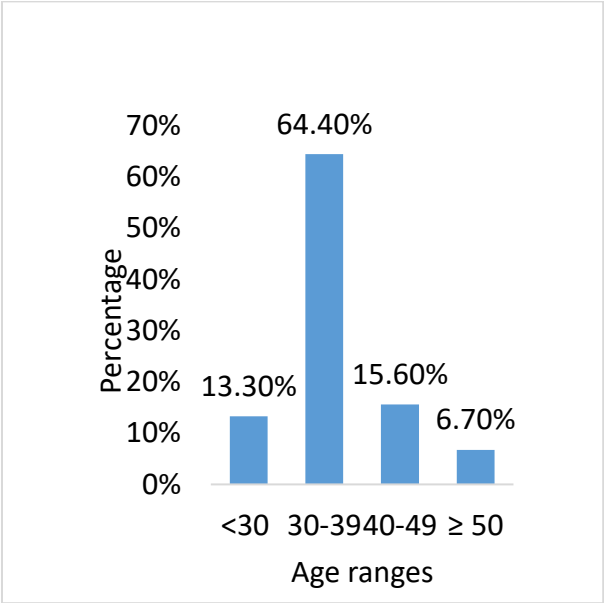


**Figure 1:** Age distribution of patients

**Table 1 :** Distribution of Samples based on macroscopic Parameters of Spermogram

| | Number (n=90) | Percentage (%) |
|---|---|---|
| Volume | | |
| Hypospermia | 17 | 18,89 |
| Normal | 71 | 78,89 |
| Hyperspermia | 2 | 2,22 |
| Viscosity | | |
| Normal | 88 | 97,78 |
| Abnormal | 2 | 2,22 |
| Ph | | |
| <7.2 | 4 | 4,44 |
| 7,2-8 | 84 | 93,33 |
| >8 | 2 | 2,22 |

## Discussion

The mean age of the participants was 35.31 ± 7.00 years, with an age range of 25 to 61 years. The most represented age group was 30–39, comprising 64.44% of participants. Our findings align with data in the literature.[7,8,11] In Benin, societal evolution has led to marriage typically occurring in the third decade of life, after which concerns about fertility often arise. In Benin and broader African society, conception is generally expected after marriage.

**Table 2:** Distribution of samples based on microscopic parameters of Spermogram

| Parameter | Number (n=90) | Percentage (%) |
|---|---|---|
| Normal | 56 | 62,22 |
| Azoospermia | 3 | 3,33 |
| Oligospermia | 14 | 15,56 |
| Polyspermia | 17 | 18,89 |
| Mobility | | |
| Asthenospermia | 85 | 94,44 |
| Normal | 2 | 2,22 |
| Not applicable | 3 | 3,33 |
| Vitality | | |
| Necrospermia | 82 | 91,11 |
| Normal | 5 | 5,56 |
| Not applicable | 3 | 3,33 |
| Morphology | | |
| Treratospermia | 73 | 81,11 |
| Normal | 14 | 15,56 |
| Not applicable | 3 | 3,33 |
| Concentration of round cells | | |
| $< 10^6$/ml | 68 | 73,12 |
| $> 10^6$/ml | 22 | 23,66 |

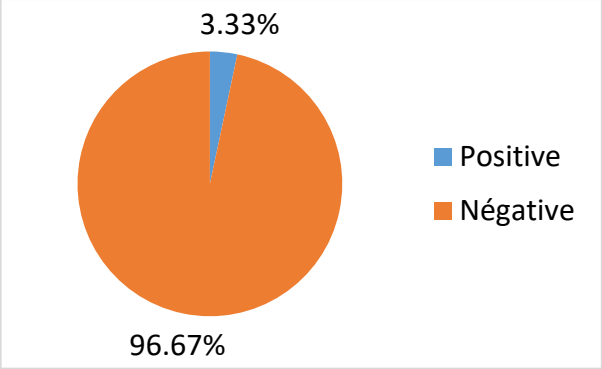


**Figure 2:** Distribution of Patients based on PCR Result

In 94.4% of the cases, patients exhibited asthenozoospermia (motility below 40% at the first hour). Motility percentage is a key parameter that partly guides therapeutic decisions, as only sperm with normal progressive motility can reach and fertilize the oocyte.[6] It is essential to investigate any history of sperm infections, infections of the accessory glands, and the presence of bacteria, as these factors can lead to reduced motility and flagellar abnormalities.

In our study, 91.11% of cases presented necrozoospermia, while only 5.56% showed normal sperm vitality. A study by Kbirou *et al.* reported a lower necrozoospermia rate of 3%, which contrasts with our findings.[10] However, our results are more comparable to those of Ndiaga *et al.* (2020) in Senegal who reported a necrozoospermia rate of 60%.[11]

The high rate of necrozoospermia in our study may be attributed to the prevalence of sexually transmitted infections (STIs). Typically, only common pathogens (e.g., *Neisseria gonorrhoeae, Escherichia coli, Staphylococci,* and *Streptococci*) are routinely screened in sperm cultures. The potential role of intracellular pathogens, such as *Chlamydia trachomatis*, is often overlooked. It would be beneficial to systematically screen for these pathogens in cases of altered sperm parameters, such as asthenozoospermia or necrozoospermia, given their significant prevalence.

Analysis of sperm concentration per ejaculation showed that 62.22% of our patients had a normal count, while 15.56% exhibited oligospermia and 3.33% had azoospermia. These results do not align with data in the literature.[7,13] Sperm concentration can vary based on factors such as pellet volume examined, centrifugation speed, and duration.

Additionally, as concentration measurement is operator-dependent, calculation biases can occur when using a hemocytometer.

Leukocytes semen analysis has shown out of the 90 patients, 11 (12.22%) had a white blood cell count exceeding one million. These results are comparable to those of Lemkecher *et al.*,[12] who found high leukocyte levels in 15–20% of cases. The presence of leukocytes in semen typically indicates chronic inflammation of the genital tract and/or accessory glands. High leukocyte counts may suggest a genital infection commonly associated with reduced sperm count and progressive motility. In our study, 81.11% of patients showed teratospermia. This is in contrast to the study of Barry *et al.* in Morocco,[13] which reported 14% teratozoospermia.

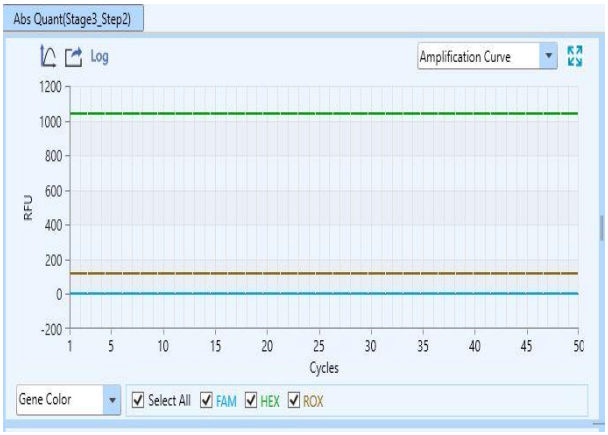


**Figure 3 :** Amplification curve of a CT- sample

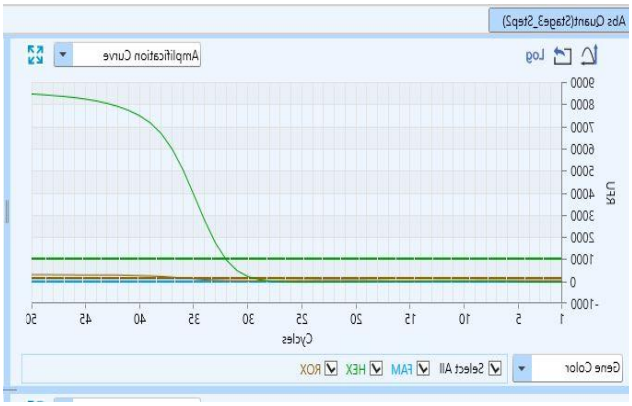


**Figure 4 :** Amplification curve of a CT+ sample

Morphologically abnormal spermatozoa have reduced fertilization potential, especially when multiple abnormalities are present. Certain morphological abnormalities and the multiple anomaly index have prognostic significance for pregnancy outcomes.[6,12] Notably, physical and chemical factors can impact the occurrence of these anomalies. Sperm morphology assessment can also be influenced by operator and technician variability. RT-PCR detected *Chlamydia trachomatis* (CT) in 3.33% of the study participants. This result aligns with Debonnet's (2020) study in France, which found a 1.4% prevalence by testing the first urine stream in men.[14] In China, a study conducted by Zhou *et al.* (2022) found a prevalence of 5.81%.[15] These findings could be explained by the influence of additional factors, such as sperm abnormalities, viral infections, medical history, and genetic anomalies. Furthermore, it is possible that some patients underwent treatment prior to sample collection in our study.

CT+ patients in this study all exhibited leukocytospermia and reduced sperm motility, consistent with the study reported by Zhou *et al* (2022). in China, which showed that the proportion of men with a normal white blood cell count (<1 × $10^6$/ml) in the CT+ group (88.2%) was significantly lower ($P < 0.001$) than in the CT− group.[15] This difference suggests that an elevated presence of white blood cells is associated with *Chlamydia trachomatis* infection. However, Zhou's study also indicated that men in the CT+ group had higher rates of normal progressive motility (PR; ≥ 32%) (83.7%) and normal total motility (79.1%) compared to the CT− group, with respective rates of 78.4% and 74.5%.

The detection of *Chlamydia trachomatis* is associated with necrozoospermia, teratospermia, and low sperm vitality. Several studies indicated that sperm infections negatively impact fertility, particularly affecting the genital tract (scarring and obstruction of the epididymis, vas deferens, and ejaculatory ducts), seminal plasma, and spermatozoa (e.g., anti-sperm antibodies, agglutination, decreased motility, and increased abnormal forms).

By causing epididymitis, *Chlamydia trachomatis* can damage the canalicular system of the male genital tract, potentially leading to obstructive azoospermia or damaging the epithelial cells involved in spermatogenesis.[16-18]

According to Dupin *et al*it is recommended that the first urine stream to be collected at least two hours after the last urination, with a volume of 10–20 ml.[19] Greub *et al.* highlight that PCR testing on urine samples is particularly advantageous for screening asymptomatic populations due to its high specificity and sensitivity (90–98%), afforded by nucleic acid amplification. Other possible sample types include urethral or meatal swabs.[20]

In a study comparing self-collected urethral swabs and urine samples for diagnosing *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in men, superficial swabs from the urethral meatus exhibit a sensitivity comparable to both first-void urine samples and deeper urethral swabs (2–4 cm).[21] However, first-void urine collection is less painful than the other methods.

**Table 3 :** Spermogram profile of patients with Positive Chlamydia PCR result

| Variables | Case 1 | Case 2 | Case 3 |
|---|---|---|---|
| Identification ID | CT-AM 050 | CT-AM 040 | CT-AM 025 |
| Age | 34 | 35 | 35 |
| PCR Result | Positive | Positive | Positive |
| Volume | 4,8 | 2,3 | 2,3 |
| Ph | 7,8 | 7,5 | 7,5 |
| Viscosity | Normal | Normal | Normal |
| Mobility | Weak | Weak | Weak |
| Vitality | 45 | 45 | 42 |
| Total concentration of the ejaculate | 170.400.000 | 50.000.000 | 52.000.000 |
| Concentration of round cells | 3500000 | 5000000 | 6500000 |
| Normal form | 2 | 0 | 0 |
| Abnormal form | 98 | 100 | 100 |
| Number of years of living together | 5 | 3 | 6 |
| Frequency of sexual intercourse | 3/semaine | 2/semaine | 3/semaine |
| Type of infertility | Primaire | Primaire | Secondaire |
| Alcohol and tobacco | Parfois | Non | Parfois |
| Partner age | 30 | 27 | 34 |

Notter *et al.* have similarly highlighted the advantages of urine samples, noting they avoid the discomfort, burning, tingling sensations, and potential allergic reactions associated with using disinfectant gel during urethral swabs.[5] These factors guided our decision to use first-void urine samples in this study. Legkobyt *et al.* further demonstrated that molecular biology tests with gene amplification substantially enhance diagnostic sensitivity and specificity for *Chlamydia trachomatis* infections, recommending these methods over traditional techniques.[22] Since implementing these amplification tests, there has been a 30–50% increase in positive sample detection, with sensitivities exceeding 95%. This high sensitivity supports their use for low-microbial samples, such as urine or self-collected vaginal swabs, and in screening asymptomatic populations.[21-22]

This study was the first to investigate the molecular diagnosis of CT in infertile men. The main advantage of this study is the introduction and routine use of PCR for the diagnosis of this bacteria. This study is restricted to a few laboratories in Cotonou, and the data obtained here cannot be generalized to the entire Beninese population. This study deserves to be continued on a larger population of both infertile men and those suffering from sexually transmitted infections.

## Conclusion

Urogenital infection with *Chlamydia trachomatis* is the main cause of bacterial sexually transmitted infections, most often asymptomatic, it is more frequently detected in the male population. It is present in the population of infertile men in Cotonou, with a prevalence of 3.33%. The use of PCR allows for high specificity and sensitivity compared to other techniques and presents a sensitivity greater than 95% in low-microbial samples such as urine and in asymptomatic populations.

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