

# Prevalence of *Chlamydia trachomatis* Infection in Thai Men with Pyospermia

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**Objective:** To demonstrate the prevalence of *Chlamydia trachomatis* infection in the semen of Thai men with pyospermia.

**Materials and Methods:** A randomized controlled trial examining the effect of coenzyme Q10 in the seminal fluid of Thai men with pyospermia, tested for *C. trachomatis* infection using a polymerase chain reaction, was carried out. The participants that underwent semen analysis at the Siriraj Infertility Clinic between March 2014 and February 2015 were included. Pyospermia was referred to a white blood cell count (WBC) of greater than 1 million per 1 mL of seminal fluid according to World Health organization criteria 2010.

**Results:** One out of eighty-four specimens tested positive for *C. trachomatis* (1.2%). The mean age of the present participants was 36.6±5.7 years. Four men had symptomatic genital infections, while the participant who had a positive test for chlamydia experienced no symptoms. The positive specimen had a WBC count of  $1.5 \times 10^6$ /mL.

**Conclusion:** The prevalence of *C. trachomatis* infection in semen of Thai men with pyospermia was 1.2%. *C. trachomatis* infection can be asymptomatic.

**Keywords:** *Chlamydia trachomatis*, Pyospermia

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Pyospermia, an abnormal finding on semen analysis, is referred to a white blood cell count (WBC) of greater than 1 million per 1 mL of semen according to World Health organization (WHO) criteria 2010<sup>(1)</sup>. The prevalence of pyospermia in infertile men varies from 3% to 23% and has been associated with clinical and subclinical genital tract infection or inflammation<sup>(2-4)</sup>. Pyospermia could be caused by bacterial or viral infections such as *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, human immunodeficiency virus (HIV), cytomegalovirus (CMV), human papilloma virus (HPV), or some other causes including varicocele,

smoking, alcohol consumption, and autoimmune diseases<sup>(1,4-6)</sup>. An abnormally high WBC in semen could cause negative impact on the number of spermatozoa, sperm motility, sperm morphology, and deficient sperm function<sup>(2,3,5)</sup>.

Chlamydia is one of the most common sexually transmitted infections (STIs)<sup>(6-8)</sup>. It can infect female genital tract and causes pelvic inflammatory disease (PID), which might lead to many consequences including female infertility. Chlamydia can also affect male and causes scarring in male reproductive tract<sup>(9)</sup>. Chlamydia can cause decreased seminal fluid volume, low semen concentration, reduced motility, and abnormal sperm morphology, which induces male infertility<sup>(9,10)</sup>. The prevalence of the presence of chlamydia in semen in various population were reported to be between 2% and 40%<sup>(6,7,9,10)</sup>. Comparing with fertile men, the prevalence of chlamydial infection was higher in infertile men. Among infertile population, men with chlamydial infection tended to have pyospermia on semen analysis more than those without infection<sup>(7,11)</sup>. Therefore, the present study

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aims to demonstrate the prevalence of *C. trachomatis* infection in Thai men with pyospermia.

## Materials and Methods

As being a part of the randomized controlled trial examining the effect of coenzyme Q10 in treating Thai men with pyospermia, the seminal fluid was tested for *C. trachomatis* infection using polymerase chain reaction (PCR). The ethical certificate of approval was provided by the Siriraj Institutional Review Board, Mahidol University (COA Si133/2014).

### Participants

All male partners of the infertile couples, who attended Siriraj Infertility Clinic, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital between March 2014 and February 2015, underwent semen analysis at the Clinic. Those with pyospermia, WBC of more than  $1 \times 10^6/\text{mL}$  of seminal fluid<sup>(1)</sup>, were invited into the study.

### Semen analysis

Semen samples were obtained from male participants by masturbation after three to five days of sexual abstinence. The ejaculated semen was collected in a sterile plastic container at the Clinic. After being incubated at 37°C for 30 minutes, all specimens of seminal fluid were analyzed and confirmed by using computer-assisted sperm analysis (CASA) (Hamilton-Thorne Bioscience, USA). The semen parameters were reported as volume (mL), sperm concentration ( $\times 10^6/\text{mL}$ ), motility (%), normal morphology (%), and WBC ( $\times 10^6/\text{mL}$ ). Pyospermia was determined by counting all round cells in the counting chamber, at least 200 round cells were counted. If the number of round cells was more than  $1 \times 10^6/\text{mL}$ , peroxidase staining was used to differentiate leukocyte from other round cells.

### Molecular diagnosis of *C. trachomatis*

Nucleic acid of *C. trachomatis* was tested by genesig® Standard Kit real time (RT) PCR for *C. trachomatis* (Primerdesign Ltd., Southampton, United Kingdom) as described by the manufacturer's protocol. Briefly, a 20  $\mu\text{l}$  reaction was prepared with 10  $\mu\text{l}$  of oasis™ 2x qPCR MasterMix, 1  $\mu\text{l}$  of *C. trachomatis* primer/probe mix (BROWN), 4  $\mu\text{l}$  of RNase/DNase free water and 5  $\mu\text{l}$  of DNA template. The RT-PCR was performed and analyzed by comparing with positive and negative controls using a standard curve method.

**Table 1.** Baseline characteristics and semen parameters of 84 Thai men with pyospermia

Variables	Mean $\pm$ SD
Age (years)	36.61 $\pm$ 5.71
BMI (kg/m <sup>2</sup> )	24.90 $\pm$ 4.30
Positive for HBsAg; n (%)	4 (4.5)
Semen analysis	
Total volume (mL)	2.81 $\pm$ 1.24
Sperm concentration (/10 <sup>6</sup> )	48.68 $\pm$ 36.03
Sperm motility (/10 <sup>6</sup> )	44.81 $\pm$ 10.40
Normal morphology (%)	10.75 $\pm$ 5.58
WBC count (/10 <sup>6</sup> ); median (Q25, Q75)	1.5 (1.2, 2.5)

BMI=body mass index; HBs Ag=hepatitis B surface antigen; SD=standard deviation; WBC=white blood cell

### Statistical analysis

IBM SPSS Statistics software, version 20.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. Descriptive statistics was used to describe participants' characteristics, were presented as number [n (%)], mean  $\pm$  standard deviation (SD), and a median and interquartile range (Q25, Q75) as appropriate for type of data.

## Results

Eighty-four Thai men with pyospermia were included during the study period. One specimen was tested positive for *C. trachomatis* (1.2%). Baseline characteristics and semen parameters are shown in Table 1. The mean age was 36.6 $\pm$ 5.7 years (min-max 26 to 52 years). Eight participants had underlying medical diseases and included two with hypertension, two with dyslipidemia, one with diabetes mellitus, two with allergic rhinitis, and one with remitted acute lymphoblastic leukemia (ALL). Seven of them required medications including lipid lowering drugs, anti-diabetic drugs, antihypertensive drugs, aspirin, and antihistamines. None of the participant had syphilis nor HIV infection. Four men had symptomatic genital infections, while the participant who had a positive test for *C. trachomatis* experienced no symptoms. The positive specimen had a WBC count of  $1.5 \times 10^6/\text{mL}$  while other semen parameters were within normal ranges. None of the female sex partners currently had abnormal vaginal discharge or any signs of PID.

## Discussion

One out of 84 seminal specimens of Thai men with pyospermia was tested positive for *C.*

*trachomatis* (1.2%) whereas previous studies showed a wide range, at 1% to 40%<sup>(8,10,12-15)</sup>. This finding could be explained by a variety of diagnostic methods. The study that used chlamydial antibodies<sup>(6)</sup> reported a higher prevalence than those detected by using PCR for DNA detection<sup>(7,10)</sup>. This implies that *C. trachomatis* antibodies in semen represent previous infection. However, its association with pyospermia remains to be explored.

The low prevalence of *C. trachomatis* infection in the present study (1.2%) supports the multifactorial etiologies of pyospermia. The authors did not test for other etiologic pathogens, including bacteria and virus<sup>(7,16)</sup>. This appears to be the principal limitation of the study. Microorganisms directly affect the male reproductive tract and have negative impact on sperms, causing agglutination that leads to decreased sperm motility, diminishing the ability of acrosome reaction. Moreover, the inflammatory reaction response to the infection are also the cause of the production of reactive oxygen species, which were then followed by sperm damage<sup>(17)</sup>. Unlike previous studies that *C. trachomatis* infection was related to impaired semen analysis<sup>(2,8)</sup>, the participant who had positive result had normal sperm parameters.

## Conclusion

The prevalence of *C. trachomatis* infection in semen of Thai men with pyospermia was 1.2%. *C. trachomatis* infection could be asymptomatic.

## What is already known on this topic?

*C. trachomatis*, which is one of the most common STI, can be asymptomatic or mildly symptomatic. Pyospermia, which is one of the major causes of male infertility, can be badly affected by *C. trachomatis* infection. According to previous studies, the prevalence can be found in up to 10%. Therefore, the further study of the prevalence of *C. trachomatis* should be investigated, particularly among Thai men.

## What this study adds?

The prevalence of *C. trachomatis* in Thai men with pyospermia was 1.2% (1/84), which is rather low compared with previous studies. Most of the female sex partners were asymptomatic and having no symptoms and signs of PID. Therefore, the pyospermia in Thai men may not indicate STIs, especially *C. trachomatis*.

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## Conflicts of interest

All authors declare no personal or professional conflicts of interest, and no financial support.

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