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Original Article

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Molecular Detection of *Chlamydia trachomatis* among Children in Jarmai Village - Gadarif State-Sudan

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Abstract

Background: Trachoma is the leading infectious cause of blindness and it caused by ocular infection with the bacterium *Chlamydia trachomatis* (Ct). While the majority of the global disease burden is found in sub-Saharan Africa, the Middle East, Central and South America, Asia, Australia, and the Pacific Islands bear a considerable burden. The World Health Organization and its partners are aiming to eliminate trachoma as a public health problem by 2020.

Objective: This study aimed to determine the prevalence of active trachoma and *Chlamydia trachomatis* among children in Jarmai village - Gadarif State, Sudan.

Methods: A population-based prevalence study was conducted in Jarmai village, Alrahad locality, Gadarif State, during the period from Nov 2016 to Nov 2017. A total of 509 children, whose ages ranged between 1 and 9 years old, were surveyed. Each eye was examined for trachoma follicles and trachoma inflammatory intense, (TF, and TI). Swab samples were collected from children, clinically diagnosed as suffering active trachoma, for the DNA analysis. Collections were done from the tarsal conjunctival surface with a Dacron polyester swab and with UTM media. DNA was, then, extracted and amplified by molecular technique with Touchdown protocol and primers for *C. trachomatis* outer membrane protein complex B, (omcB).

Result: The overall result of the 509 participants was as follows: 63 (12.4 %) were diagnosed for active trachoma, 7.0 (11.11 %) of them had follicles in the upper tarsals (TF), and showed 9 (14.29 %) had trachoma inflammatory intense (TI) and 47 (74.60 %) had the both signs of trachoma follicles and trachoma inflammatory intense (TF and TI). The prevalence of *Chlamydia trachomatis* in all the children in the village was 1.8%, and from the 63 children were, those who were clinically diagnosed as active trachoma patients i.e. 9, (14.9%).

Conclusions: This study revealed that the clinical signs of active trachoma can persist even when ocular *Chlamydia trachomatis*, were not detected. This may be due to long lasting clinical sign or there might be another causative agent other than *Clamydia Trachomatis.*

Keywords : Trachoma , *Chlamydia. trachomatis*, PCR, Prevalence, Jarmai village.

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Introduction

The World Health Organization (WHO) defines blindness as visual acuity in the better eye of less than 3/60 with available refractive correction, which predicts the inability to walk safely without assistance. The best published estimate suggests that 5.9 million people in the world fulfill this criterion because of trachoma, which makes it responsible for about 15% of all cases of blindness[(1)]. In addition to those already blind, an estimated 600 million people live in areas of Africa, the Middle East, Central and South America, Asia, Australia, and the Pacific Islands, where trachoma is endemic [(1,2)]. The accuracy of these estimates is questionable, however [(3)]. There is a pressing need for further research on the global distribution and prevalence of disease [(4)].

Trachoma, is a neglected tropical disease, caused by conjunctival infection with particular serovars of *Chlamydia trachomatis*. The bacteria are spread by direct contact with ocular and nasal discharges from infected individuals, by contact with fomites, (inanimate objects that carry infectious agents, such as towels or washcloths), and by eye-seeking flies, particularly *Musca sorbens* that by repeated and/or prolonged episodes, may cause ocular infection by the bacterium *Chlamydia trachomatis*. [(5)].

The disease disproportionately affects individuals living in rural and resource-poor settings, and children are the primary carriers of ocular *C. trachomatis* infection and active disease [(6)].

The presence of follicles and inflammation in the upper tarsal conjunctiva, known as active trachoma, is characteristic of childhood infection. Following years of repeated infection, the upper tarsal conjunctiva may become so severely scarred that the eyelashes turn inwards, rub on

the eyeball and cause corneal opacity and blindness[7].

The year 2020 is the target date for the global elimination of trachoma as a public health problem. As the first step to achieving this goal, mapping is needed to assess the endemicity of trachoma and determine the need for interventions [8].

The World Health Organization (WHO) advocates the implementation of the SAFE strategy (Surgery for trichiasis, Antibiotics for active infection, Facial cleanliness to prevent disease transmission and Environmental improvement to increase access to water and sanitation) for trachoma elimination. Mass Drug Administration (MDA) of azithromycin aims to clear infection from communities such that trachoma ceases to be a public health concern [9].

In 2012, the World Health Organization (WHO) estimated that 21 million people had active (inflammatory) trachoma (trachomatous inflammation – follicular, TF and/or trachomatous inflammation – intense, TI)[10], and more than 7 million people had in-turned eyelashes (trachomatous trichiasis),TT, that could lead to corneal opacity and blindness[5]. Sub-Saharan Africa bears the largest burden of disease, with more than 80% of the cases of trachoma[5].

In Sudan Trachoma mapping is complete in the northern states except for the Darfur States. TF prevalence was above 10% in three districts and between 5% and 9% in 11 districts. TT prevalence among adults was above 1% in 20 districts (which included the three districts with TF prevalence .10%). The overall number of people with TT in the population was estimated to be 31,072[11].

Trachoma grading

Grading systems are used in an effort to standardize diagnosis in

field surveys and research studies. The WHO simplified system was designed as a cut-down version of the FPC (Follicle, Papillae and Cornea opacity) system, with which it was intended to coexist. Thylefors *et al*. considered the simplified scheme suitable for use by "less experienced observers" in "population based surveys or for the simple assessment of the disease at the community level". It provides considerably less information than the FPC scale [(3)]. However, the simplified system has enjoyed broad acceptance and is now widely used in research, community assessment, and program monitoring by both medical assistant and ophthalmologists. The system requires the examiner to assess an individual for the presence or absence of each of five signs. The WHO simplified system uses the following criteria: TF, trachomatous inflammation, follicular, the presence of five or more follicles at least 0.5 mm in diameter in the central part of the upper tarsal conjunctiva; TI, trachomatous inflammation, intense, pronounced inflammatory thickening of the upper tarsal conjunctiva obscuring more than half the normal deep tarsal vessels; TS, trachomatous conjunctival scarring, the presence of easily visible scars in the tarsal conjunctiva; TT, trachomatous trichiasis, and at least one eyelash rubs on the eyeball. [(3)].

Methods

Ethical approval

This study is in accordance with the declaration of NHREC Ethics Sudan Federal Ministry of Health, and Central Institutional review board, Al-Neelain University.

Oral informed consent was obtained from all study participants and their guardians. During survey, all infected children on the study village were treated with a single height-based dose of oral azithromycin in

accordance with WHO and national protocols, and Opticyclin ointment for the children had ocular infection other than trachoma.

Study area and Study design

A population-based prevalence study was conducted in Jarmai village –Alrahad locality, Gadarif State during the period from Nov 2016 to Nov 2017. According to trachoma map in 2010 surveys of trachoma prevalence in the northern states of Sudan TF prevalence was above 10% in three districts ,Gadarif state was one of these district [(10)] .

Trachoma clinical diagnosis:

A total of 509 children in the age between 1 to 9 years old were clinically examined which are represent all eligible children residents on the day of the survey in Jarmai village. Examination for trachoma signs was conducted by ophthalmic medical assistants trained in using the WHO simplified grading system[(10)]. Potential examiners underwent training to apply the simplified grading scheme led by an ophthalmologist experienced in trachoma grading. A reliability study was conducted using a set of standardized photographs.

Each eye was examined for trachoma follicles and trachoma inflammatory intense (TF, and TI). Both eyes were examined and findings for the worst affected eye recorded. Signs had to be clearly visible in accordance with the simplified grading system in order to be considered present. Alcohol-soaked cotton was used to clean the examiner's fingers between examinations. Individuals with signs of active trachoma (TF and/or TI) were offered free treatment with antibiotics according to WHO and national guidelines.

Sample Collection:

Conjunctival swab samples collected from children clinically diagnosed as active trachoma (TF and TI) for DNA analysis

collection were done from tarsal conjunctival surface with Dacron polyester swab and with UTM media (Fisher Scientific, UK) sampling was standardized as four horizontal passes across the conjunctiva, with a one-quarter turn between each pass. After collection, swab will be returned to transporting media and stored until used. The sample divided into two parts; a group covered by mass drug administrations, (MDA), with azithromycin, and another group not included in the MDA program. The patients with conjunctivitis or any other eye infections were excluded from investigation.

DNA extraction

DNA was extracted from all samples using the G-spin Total DNA kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions.

PCR amplification with omcB gene primers:

Chlamydial DNA from clinical samples was amplified using a conventional PCR machine (SENSOQUEST, USA). Touchdown protocol was performed using Maxime PCR PreMix kit (iNtRON,Biotechnology,Korea) , and primers for *C. trachomatis* outer membrane protein complex B (omcB) (F/ GAC ACC AAA GCG AAA GAC) (R/ACT CAT GAA CCG GAG CAA CCT) [12]

Amplification was performed in 20µl reaction volumes containing 17µl by add H_2O, 1 µl of primer and 2µl of DNA template .The 106 bp fragment indicates the presence of *C. trachomatis* outer membrane protein complex B (omcB).

PCR Products Detection

All PCR products were subjected to electrophoresis on agarose gel along with a 100 bp DNA marker.

An amounts of 5 -µL of each PCR mixture separated in 1.5%

agarose gel, then stained with 3µ ethidium bromide and viewed under gel documentation system with a 100 bp DNA marker. A result was considered positive when a band of the size 106 bp was visible in the gel. Standard procedures for reducing contamination were strictly followed.

Results

In total, 509 eligible children were examined in this survey over the Jarmai village. The children participants, of age between 1 to 9 years old i.e. 63 (12.4 %) were clinically diagnosed as active trachoma (figure 2); of whom 7.0 (11.0%) had follicles in the upper tarsal (TF), 9 (14.0%) had trachoma inflammatory intense (TI) and 47 (75.0%) had the two signs of active trachoma, the follicles and trachoma inflammatory intense (TF and TI) (figure 3). Among those examined 26 (41%) were females, and 37 (59%) of those examined were males (table 1).

The prevalence of *Chlamydia trachomatis* among all children in the village was 1.8% and out of 63 children 9 (14.9%) were clinically diagnosed as active trachoma patients, (figure 4).

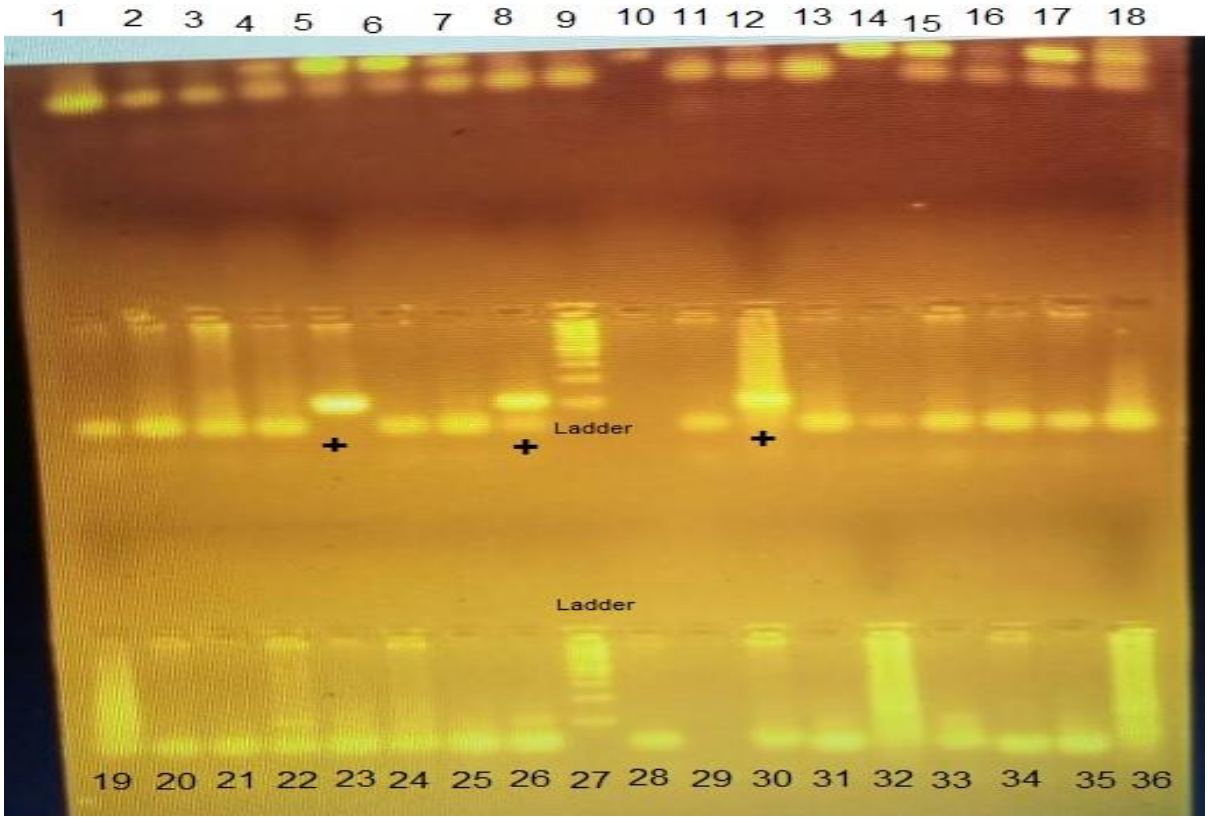

Figure 1 : PCR result of *Chlamydia trachomatis omcB* gene

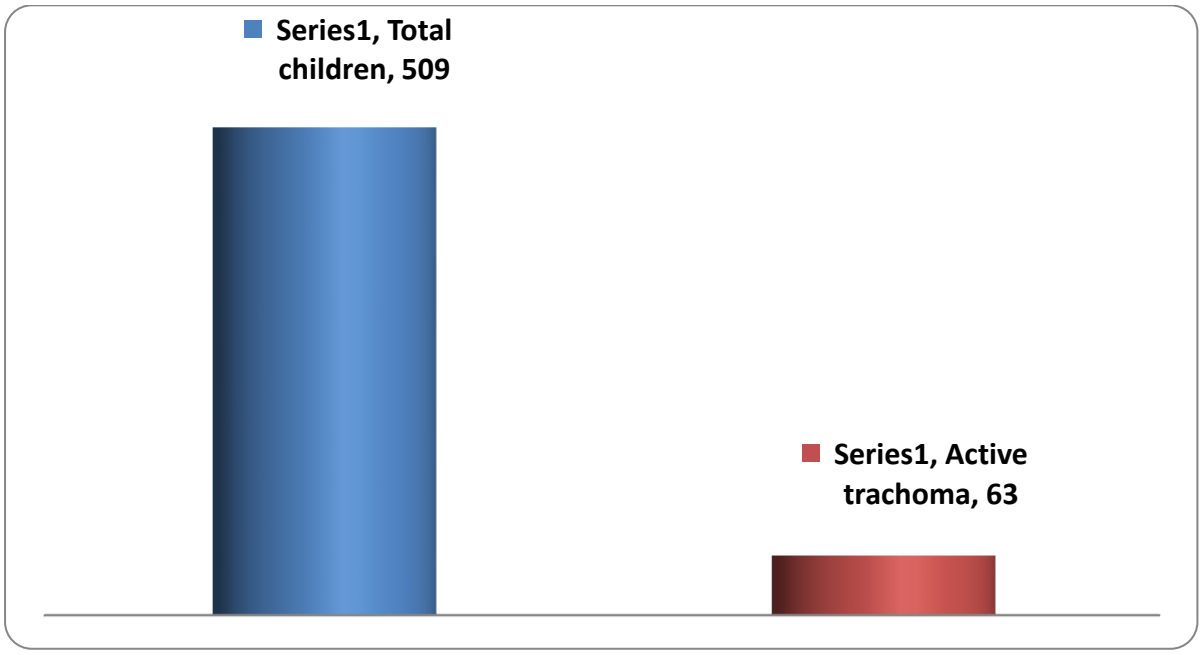

Figure 2 : The prevalence of active Trachoma among children of age 1–9 years old in Jarmai village

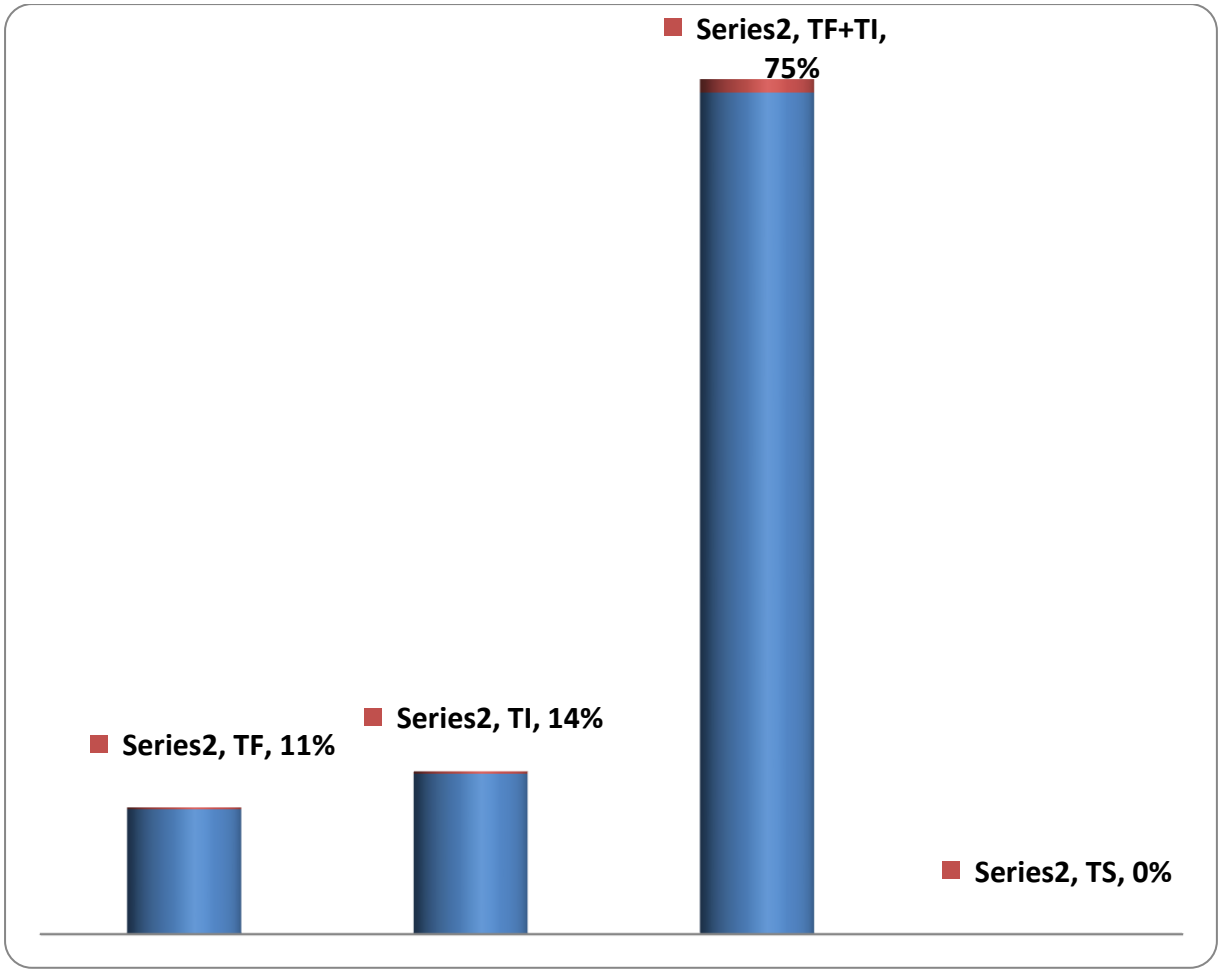

Figure 3 : The prevalence of Trachoma Follicular (TF),Ttrachoma Inflamatory Intense (TI) and TF+TI among children of age 1 – 9 years old in Jarmai village

Table 1 : The frequency of Trachoma Follicular (TF),Ttrachoma Inflamatory Intense (TI) and TF+TI in gender

		Trachoma grade			Total
		TF	TI	TF + TI	
Sex	Male	3	4	30	37
		5%	6%	48%	59%
	Female	4	5	17	26
		6%	8%	27%	41%
	Total	7	9	47	63
		11%	14%	75%	100%

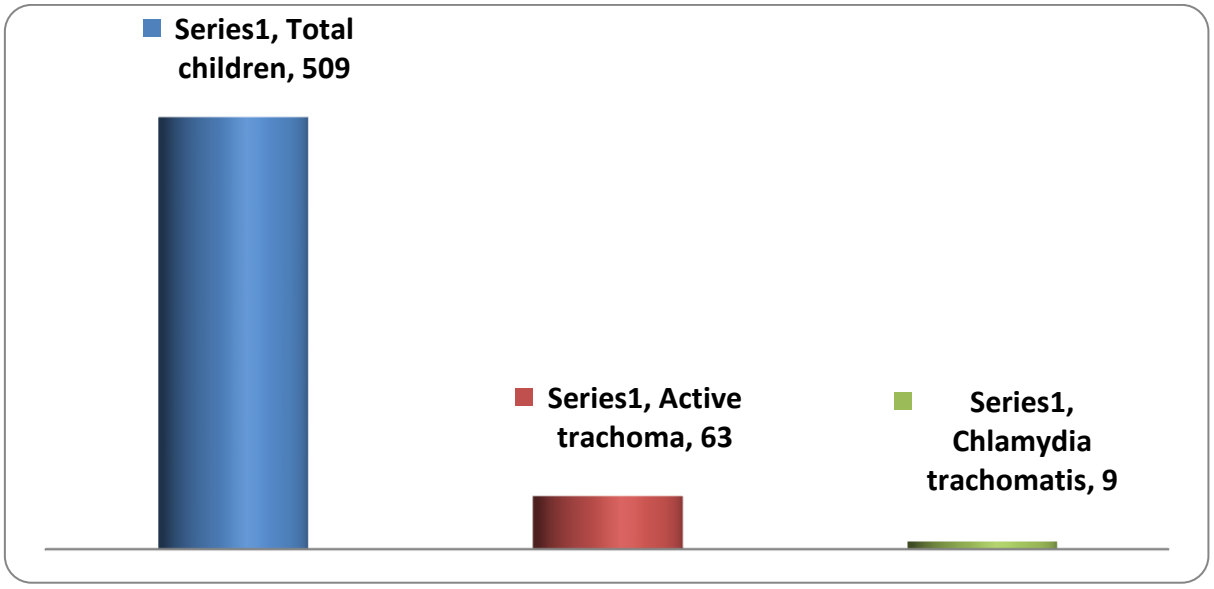

Figure 4 : The prevalence of Trachoma and *Chlamydia trachomatis* among children of age 1 – 9 years old in Jarmai village

Discussion:

The WHO led Global Alliance for the Elimination of Trachoma (GET2020) aims to elimination the disease as a public health problem in the world by 2020. The key trachoma control strategy is SAFE strategy, which is endorsed by WHO. The acronym stands for: "Surgery"; for patients with advanced disease, "Antibiotics"; namely azithromycin or tetracycline eye ointment for active trachoma, "Facial" cleanliness and "Environmental" improvement on water supply and sanitation[13,14].

Knowledge of trachoma prevalence at district level is particularly important in planning the national trachoma control program in order to achieve the goal of eliminating trachoma as a blinding disease by the year 2020. District level mapping of trachoma prevalence was carried out in the Northern States of Sudan 2006- 2010[11].

The total number of children in the village was 509 of ages between 1 – 9 years. All of them were clinically examined for ocular trachoma by

ophthalmic medical assistants trained in using the WHO simplified grading system. The prevalence of trachoma in Jarmai village was found to be 12.4%. A previous study of mapping trachoma made in Northern State of, Sudan, in Gadarif State where the village of our study is located, registered a prevalence of trachoma between 0.9 and 19.8[(11)].

The results of this population-based impact study demonstrated that the prevalence of active trachoma among children, of ages 1 to 9 years was greater than 10%; indicating that trachoma remains one of the major public health problems in that area of Sudan. and that coincides with the views reported from, South Sudan [(15)], countries in Pacific island [(16)]. India [(17)] and Ethiopia [(18)] have classified trachoma as an epidemic. The rate of active trachoma ranged from 25.2%–71.0% .

The prevalence of *Chlamydia trachomatis* children in entire village was 1.8% and out of 63 children, 9 (14.9%) were clinically diagnosed as afflicted by active trachoma , this may be due to a persisting clinical sign of trachoma or there may be due to hosting a causative agent other than *Clamydia. Trachomatis*.

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