Antibody titration of sera positive to CCFA of *Chlamydia trachomatis* infection in Primary Health Care Centres in Ukwuani L.G.A, Delta State.

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ABSTRACT

Chlamydia trachomatis is a major public health concern and is one of the leading causes of sexually transmitted infections (STIs) and infertility worldwide. A community-based cross-sectional study was used to determine the prevalence of Chlamydia trachomatis infection among patients attending selected Primary Health Care facilities in Ukwuani Local Government Area, Delta State, Nigeria from January 2023 to February 2025. Venous blood and Endocervical swab samples were collected from consenting male and female patients. Antigens against all the three species of Chlamydia were prepared as life antigen in the laboratory. Giemsa staining technique was used to observe chlamydia and chlamydia inclusion bodies under the oil immersion objective lens (x100). Using cell culture fluorescence assay (CCFA), blood and endocervical samples were screened for Chlamydia complement-fixing antibodies (CCFA) using a species-specific antibody spot test kit and rapid point-of-care testing (POCT). Antibody titration revealed titres ranging from 1:8 to 1:1024, with the majority of cases showing moderate titres (1:16–1:64), suggesting recent infections. High titres (≥1:256) were detected in some older age groups, indicating possible chronic or recurrent infections. Chlamydia species isolated using the polymerase chain reaction (PCR) were Chlamydia trachomatis at band 321bp (54.1%), Chlamydia pneumoniae at band 241 (31.6%) and Chlamydia psittaci at band 389 (10.3%) indicating co-infection. The results of the current study demonstrate that Chlamydia trachomatis is a significant public health problem that causes infertility problems. This underscores the need for targeted screening, health education, and control measures in rural communities. Seasonal trends and antibody titre distribution highlight the importance of continuous surveillance to prevent long-term complications.

Key Words: tire, antibodies, *Chlamydia,* prevalence, sera, positive

Introduction

Unlike viruses, *Chlamydia* can survive outside a host for some time and are commonly found in leukocytes. They are extremely small organisms that utilize their own ribosomes and enzymes for protein and nucleic acid synthesis but still depend on host cells for specific growth factors. Besides lacking muramic acid in their cell walls, they also lack a respiratory system (Grayson *et al*., 2019). In Nigeria, available studies have reported varying prevalence rates of *C. trachomatis* across different regions and population groups, ranging from 2% to over 20% depending on the study design, diagnostic methods, and sample population (Olawuyi et al., 2020; Akinola et al., 2019). However, national surveillance data remains limited, and many infections go undiagnosed and untreated. The lack of localized epidemiological data hinders the formulation of targeted public health interventions (Adeoye *et al*., 2024).

One of the persisting challenges is the inability of routine serological testing (specifically the CCFA, which is presumed to be a form of antibody assay) to differentiate between current/active CT infection, past/cleared CT infection, and chronic/persistent CT infection in a high-prevalence setting like Ukwuani LGA (Lawn et al., 2021). CT prevalence rates in many areas of Delta State are significantly higher than in developed countries (reported rates in Nigeria often range from 20% to over 40% in some cohorts). Given this hyper-endemic environment, a large proportion of the population is likely CCFA-positive, necessitating a tool to separate high-risk chronic cases from general past exposure (Adekunle et al., 2018). Elevated or high, stable IgG antibody titers (often ≥1:64 or higher depending on the assay) are strongly correlated with upper genital tract damage, specifically tubal pathology caused by chronic/persistent CT infection. Antibody titration is thus one of the few practical methods available to PHCs to non-invasively identify women at high risk of infertility (Puolakkainen, 2011).

This study addresses the critical challenge of accurately diagnosing the chronicity and potential for sequelae of *Chlamydia trachomatis* (CT) infection in resource-limited primary healthcare (PHC) settings like Ukwuani Local Government Area (LGA), Delta State. It leverages serology (antibody detection) to provide epidemiological and clinical insight beyond simple active infection detection.

Antibody titration of sera positive to CCFA in Amai Primary Health according to seasonal variation.

Table 1 shows the antibody titration of sera positive to CCFA in Amai Primary Health Center. The overall total titer value recorded during the dry season was 42 at 1:16 titer. The age group 26-30years showed the highest titer value of 10 at 1:16 and 1:128 respectively. The total titer value recorded during the wet season was 89 at 1:16 titer and age group 31-35 years recorded the highest titer value of 32 at 1:8.

Table 1: Antibody titration of sera positive to CCFA in Amai primary health center according to seasonal variation

Age								
group	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
			Antibody titre for dry season					
16-20	-	4	5	2	5	2	1	1
21-25	3	5	5	5	3	-	5	5
26-30	4	10	6	6	10	5	2	2
31-35	5	7	7	8	5	2	3	1
36-40	5	5	5	3	2	3	4	4
41-45	2	3	3	1	1	1	-	-
Total	**22**	**42**	**36**	**33**	**26**	**15**	**15**	**13**
			Antibody titre for wet season					
16-20	1	8	5	1	6	7	1	1
21-25	12	27	27	1	-	-	1	-
26-30	15	30	15	5	5	-	-	-
31-35	32	10	20	5	2	20	-	-
36-40	5	5	5	2	2	1	1	1
41-45	1	1	1	2	-	-	-	1
Total	**73**	**89**	**67**	**82**	**18**	**29**	**3**	**3**

Antibody titration of sera positive to CCFA in Umutu Primary Health Centre according to seasonal variation

Table 2 shows the antibody titration of sera positive to CCFA in Umutu Primary Health Center according to seasonal variation. The overall highest titer for the dry season was 70 at 1:32 titer. The age group 21-25years recorded the highest titer value of 20 at 1:16. During the wet season, the overall highest titre value recorded was 68 at 1:32 titer. High titer value of 20 was recorded among ages 21-25 years at 1: 16, 26-30 years at 1:8, 1: 16 and 1:20 and 31-35 years at 1:8 respectively.

Table 2: Antibody titration of sera positive to CCFA in Umutu Primary Health Center according to seasonal variation

Age								
group	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
			Antibody Titre for dry season					
16-20	10	10	15	-	15	5	5	-
21-25	5	20	15	5	-	5	-	-
26-30	6	6	12	5	5	10	5	5
31-35	8	2	12	8	10	10	5	5
36-40	7	3	10	3	10	5	-	5
41-45	5	5	-	-	-	-	5	-
Total	**51**	**55**	**70**	**29**	**42**	**35**	**20**	**16**
			Antibody Titre for wet season					
18-20	3	5	10	10	2	3	5	-
21-25	10	20	8	2	10	5	5	5
26-30	20	20	20	10	-	-	5	5
31-35	20	15	15	10	10	5	5	5
36-40	5	5	5	10	5	5	-	-
41-45	-	-	-	5	-	-	-	-
Total	**63**	**65**	**68**	**52**	**34**	**18**	**20**	**16**

Table 3 shows the antibody titration of sera positive to CCFA in Obiaruku Primary Health Center according to seasonal variation. The overall highest titer for the dry season was 57 at 1:16 titer. The age group 31-35years recorded the highest titer value of 20 at 1:16. During the wet season, the overall highest titre value recorded was 58 at 1:32 titer. High titer value of 20 was recorded among ages 26-30 years at 1: 16.

Table 3: Antibody titration of sera positive to CCFA in Obiaruku Primary Health Center during the wet season

Age group	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
				Antibody Titre for dry season				
18-20	5	5	5	5	2	1	1	1
21-25	5	10	10	10	5	-	-	3
26-30	5	5	10	10	5	2	1	1
31-35	10	20	10	10	-	-	5	5
36-40	5	5	6	6	8	5	1	2
41-45	-	4	1	1	-	-	-	-
Total	**35**	**57**	**50**	**53**	**24**	**10**	**11**	**16**
				Antibody Titre for wet season				
18-20	-	-	10	8	2	1	1	1
21-25	10	10	8	7	5	1	2	2
26-30	10	20	5	5	5	-	-	3
31-35	10	10	15	15	1	1	1	1
36-40	8	5	5	15	10	-	-	3
41-45	-	-	5	-	-	-	-	1
Total	**46**	**53**	**58**	**54**	**36**	**6**	**6**	**13**

Antibody titration of sera positive to CCFA in Obinomba Primary Health Center according to seasonal variation

Table 4 shows the antibody titration of sera positive to CCFA in Obinomba Primary Health Center according to seasonal variation. The overall highest titer for the dry season was 113 at 1:8 titer. The age group 16-20 years recorded the highest titer value of 20 at 1:8, 1:16. Ages 21-25 years recorded a titer value of 30 aa 1:8 and 1:32 while 20 titer value was recorded among age 26-30 years old at 1:8 and 20 was also recorded among 41-45 years old at 1: 8 respectively. During the wet season, the overall highest titer value recorded was 134 at 1:8 titer. High titer value of 30 was recorded among ages 21-25 years at 1: 8 35 among age 31-35 years old at 1:8 and 30 among age 36-40 years old at 1:8 titer value respectively.

Table 4: Antibody titration of sera positive to CCFA in Obinomba Primary Health Center during the wet season

Age group	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
			Antibody Titre for dry season					
16-20	20	20	18	-	2	25	8	8
21-25	30	17	20	19	-	-	3	3
26-30	20	19	3	5	5	5	6	6
31-35	10	12	11	15	-	-	3	2
36-40	5	5	5	10	10	5	3	3
41-45	20	5	5	2	3	3	1	1
Total	**113**	**85**	**67**	**54**	**25**	**40**	**24**	**23**
			Antibody Titre for wet season					
16-20	10	25	10	10	-	10	10	10
21-25	30	10	10	5	5	10	5	5
26-30	10	20	10	20	5	-	2	3
31-35	35	10	10	10	5	2	3	11
36-40	30	10	5	5	5	2	2	3
41-45	10	10	11	11	15	2	1	1
Total	**134**	**91**	**62**	**69**	**43**	**29**	**24**	**35**

Incidence of *Chlamydia trachomatis* in males with infertility cases using CFT

Table 5 shows the incidence of *Chlamydia trachomatis* in males with infertility cases using CFT. The incidence of *Chlamydia trachomatis* was relatively high among all the age group but age group 26-30 years recorded the highest incidence 55 (85.9%), age group 31-35 years had incidence of 48 (59.2%) while 21-25 years recorded 19 (44.2%). Chi-square analysis recorded a significant association of *Chlamydia trachomatis* infection and male infertility ($\chi 2 = 41.23$; $P<0.05$).

Table 5: Incidence of *Chlamydia trachomatis* in males with infertility cases using CFT

Age groups	**No. examined**	**No. positive (%)**
21-25	43	19 (44.2)
26-30	64	55 (85.9)
31-35	81	48 (59.2)
36-40	13	5 (38.4)
41-45	13	5(38.4)
Total	**214**	**132 (61.6)**

Antibody titers to *Chlamydia trachomatis* from positive patient.

Table 6 shows Antibody titers to *Chlamydia trachomatis* from the positive patient with the highest value of 133 at titer value of 1:16. The total titer value recorded from the table is 554 while age group 34-36 recorded the highest titer value of 31 at 1:16. 66

Table 6: Antibody titres to *Chlamydia trachomatis* from the positive patient.

Age	Antibody titres								Total
group	**1/8**	**1/16**	**1/32**	**1/64**	**1/128**	**1/256**	**1/512**	**1/1024**	
18-21	3	3	6	-	9	6	-	6	33
22-24	8	21	19	6	15	6	4	2	81
25-27	10	25	17	25	1	1	1	-	80
28-30	5	25	10	3	11	9	3	3	69
31-33	9	7	15	18	19	-	10	9	87
34-36	17	31	27	5	5	8	3	-	96
37-39	9	11	-	25	13	-	-	-	58
40-42	-	5	5	1	1	-	4	4	20
43-45	-	-	3	5	3	-	1	1	13
46-48	-	5	3	6	-	-	-	-	14
49-51	-	-	-	1	1	-	1	-	3
Total	**61**	**133**	**105**	**95**	**78**	**30**	**24**	**25**	**554**

Discussion

The study revealed that in all the Primary Health Center in Ukwuani LGA, the overall total titer value recorded during the dry season was 42 at 1:16 titer. The age group 21-30]5 years showed the highest titer value across all locations studied. The total titer value recorded during the wet season was highest. The incidence of *Chlamydia trachomatis* was relatively high among all the age group but age group 26-30 years recorded the highest incidence 55 (85.9%), age group 31-35 years had incidence of 48 (59.2%) while 21-25 years recorded 19 (44.2%). Chi-square analysis recorded a significant association of *Chlamydia trachomatis* infection and male infertility ($\chi 2 = 41.23$; $P<0.05$).

This study at variance with the study of Sun et al. (2020, who reported that the *Chlamydia* seroprevalence ranged from 12.21% to 30.89% across different regions in Yunnan province, and the differences were statistically significant. They added that the seroprevalence in male domestic black-boned sheep and goats was significantly higher than that in the females. However, there was no statistically significant difference in *Chlamydia* seroprevalence in domestic black-boned sheep and goats between ages and species ($P > 0.05$). the variation is due to difference in organisms targeted. Sun et al. worked on veterinary, while the current work is on humans.

According to Paavonen (2012), IgM Antibodies usually rise early and fall quickly, indicating a recent or acute infection. Also, IgA Antibodies often suggestive of active or persistent mucosal infection and tend to disappear faster than IgG upon successful treatment. IgG Antibodies and Titer are the long-lasting markers of past exposure. Critically, high IgG titers (e.g., a fourfold rise or a very high single titer) reflect a vigorous, possibly prolonged or complicated systemic immune response, which epidemiologically links to the development of chronic conditions like PID and tubal infertility (Westrom et al., 2015). In a high-prevalence area like Ukwuani, virtually all sexually active adults may have low-to-moderate IgG titers due to multiple, repeated, or past infections. The clinical significance only becomes pronounced when the titer is substantially elevated, indicating significant or persistent antigenic stimulation associated with tissue damage.

Conclusion & Recommendations

Seasonal differences were also evident. Male infection rates generally increased during the wet season, suggesting possible seasonal influences on sexual behavior, mobility, and access to healthcare. In Umutu PHC, male positivity rose from 11.3% in the dry season to 18.2% in the wet season. Antibody titration data supported this observation, as there was an increase in both low (1:8 to 1:64) and high (1:256 to 1:1024) titres during the wet season. The dual rise in low and high titres is indicative of both new infections and chronic or persistent cases, particularly among young adults, but also extending to middle-aged populations (36–45 years). This challenges the perception that

Chlamydia is only prevalent among youth and underscores the need to include older sexually active individuals in screening programs.

The antibody titration of CCFA-positive sera confirmed the expected high burden of CT exposure in the patient population of Ukwuani LGA. This finding suggests the presence of chronic or repeated infections and highlights the potential of antibody titration as a useful surveillance tool in low-resource settings where molecular testing may not be readily available. The antibody data further confirmed that a significant proportion of infections might remain asymptomatic and undetected if surveillance relies solely on symptom-based diagnosis.

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