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ORIGINAL ARTICLE

High prevalence of *Chlamydia pneumoniae* infection in an asymptomatic Jordanian population

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Background/Purpose: The bacterium *Chlamydia pneumoniae* is associated with respiratory diseases and nonrespiratory illnesses like atherosclerosis. This study aims to investigate the seroprevalence of immunoglobulin G (IgG) against *C. pneumoniae* in an asymptomatic population in Jordan and to analyze the immunity state in relation to age and sex.
Methods: Serum samples were collected from 588 apparently healthy individuals aged 2—86 years. Using the microimmunofluorescence (MIF) test, seropositivity was defined as an anti-*C. pneumoniae* IgG titer $\geq$1:16. Titers from 1:16 to 1:256 were considered indicative for a past infection, whereas 1:512 was considered diagnostic of an acute infection.
Results: The overall prevalence of *C. pneumoniae* was 54.9%. The mean seropositivity in males was slightly higher than females. The seroprevalence of infection was relatively low in children aged 2—9 years, and steadily increased to reach a plateau of 66.7% at around 30—39 years of age, which remained stable in later years. Recent infection was indicated in 14.3% of study subjects. The seropositivity was highest in males, and more frequent in adults than in children and teenagers.
Conclusion: A high seroprevalence of *C. pneumoniae* in the asymptomatic population suggests that infection with this pathogen is common in Jordan. Higher seropositivity in males compared to females was observed. The primary infection is acquired during the first four decades of life, and in older ages high antibody levels are likely maintained by reinfection or persistent infection.
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Introduction

Chlamydia pneumoniae, recognized as a new species in 1989,[1] causes lower and upper respiratory tract infections in humans and causes about 10% of the community-acquired pneumonia cases worldwide.[1,2] Persistent *C. pneumoniae* infections have been implicated in several chronic diseases, such as asthma, chronic obstructive pulmonary disease, and cough.[3–5] This pathogen has attracted increasing attention due to its association with chronic atherosclerosis and coronary heart disease,[3,6,7] as shown by seroepidemiology and presence of the bacterium in atherosclerotic plaques.

C. pneumoniae is transmitted from person to person by nasal droplets. Seroepidemiological analyses have shown that respiratory tract infections caused by *C. pneumoniae* occur throughout the world.[7] Outbreaks of infection have been reported in families, at schools, military bases, and work places.[1,8–10] It is estimated that all people are infected by the age of 20 years and acquire at least one *C. pneumoniae* infection during their lifetime.[11,12] It has also been suggested that asymptomatic infections or respiratory infections with mild symptoms are common.[1] About 40–86% of asymptomatic adults in different parts of the world have antibodies to *C. pneumoniae*.[8,13–25]

Cultivation of *C. pneumoniae* in a cell culture system is technically complex and, therefore, it is not routinely used for detection of the pathogen in clinical specimens. However, more rapid diagnostic techniques such as the polymerase chain reaction (PCR), antigen immunoassay, and direct immunofluorescence testing are more frequently used.[16,26] More importantly, the indirect microimmunofluorescence (MIF) test is specific for the pathogen and is still considered as the standard method for *C. pneumoniae* detection in sera specimens.[27–30]

Little is known about either the prevalence of asymptomatic *C. pneumoniae* infection or the association between this agent and respiratory and chronic infections in Jordan. A single study investigated the seropositivity for *C. pneumoniae* in male blood donors aged 18–50 years in Jordan.[31] The present study expanded the population-based survey and aimed, using the MIF serologic test, at evaluating the seroprevalence of asymptomatic *C. pneumoniae* infection among apparently healthy Jordanian subjects from both sexes and of various ages.

Materials and methods

Study population and serum collection

A total of 588 subjects attending the outpatient clinics at the Hospital of the University of Jordan, Amman, Jordan, were enrolled in this study. They visited the clinics between December 2008 and May 2009 for various reasons. Individuals were excluded from the study if they had symptoms of respiratory disease or received antibiotics within the last 3 months preceding enrollment. Study subjects were aged 2–86 years (mean age 36 years) and consisted of 199 males (mean age 35.8 years) and 389 females (mean age 36.2 years). According to their ages, they were divided into eight age groups: 2–9 years ($n = 38$); 10–19 years ($n = 61$); 20–29 years ($n = 142$); 30–39 years ($n = 99$); 40–49 years ($n = 94$); 50–59 years ($n = 84$); 60–69 years ($n = 54$); and ≥70 years ($n = 16$). Informed consent was obtained from all study subjects. This study was approved by the Scientific Research Committee at the Department of Biological Sciences and the Hospital of the University of Jordan.

Aliquots of blood samples drawn for necessary laboratory tests were obtained for this study. A single serum specimen from each individual was prepared and stored at $-20°C$ until tested.

Antigen preparation

The *C. pneumoniae* strain VR1310, generously provided by Thomas F. Meyer (Max Planck Institute for Infection Biology, Berlin, Germany) was used for antigen preparation in the serologic testing method MIF. Antigens were whole elementary bodies prepared as described previously,[30] with minor modifications. Briefly, *C. pneumoniae* were grown in cell culture, purified by centrifugation, and treated with 0.05% formalin. Before dotting onto wells of immunofluorescence slides, antigens were mixed in 0.5% yolk sac. The dotted slides were dried and fixed with acetone for 15 minutes at room temperature and then stored at $-70°C$ until used.

Serologic assay

The MIF test is based on the indirect detection of human IgG antibodies against *C. pneumoniae* using labeled anti-human IgG antibodies.[30] IgG antibody in sera was first screened at 1:16, a dilution considered a marker for *C. pneumoniae* positivity in this study. Positive and negative controls were also applied in each run at 1:16. Slides were incubated in a humid chamber at 37°C for 1 hour. After washing with PBS to remove unbound serum antibodies, each antigen spot was overlaid with fluorescein-labeled goat anti-human IgG antibody (Bio-Rad, Hercules, CA, USA) and incubated as before. Slides were then washed, dried, mounted, and examined using an epifluorescence microscope (Nikon, Tokyo, Japan) at 400× magnification. If sera were reactive in MIF at 1:16, they were further tested at serial twofold dilutions (from 1:16 to 1:512) for IgG antibody titer determination. A chlamydial IgG antibody titer of 1:16 to 1:256 was considered indicative of past or previous infection and a titer of 1:512 was defined as a marker for acute or recent infection.[1,11,13,16,20,26,32]

Statistical analysis

The statistical significance of the data obtained for sex and age groups was determined using the Chi-square test. A p value <0.05 was considered statistically significant.

Results

The prevalence of *C. pneumoniae* antibodies in Jordan was evaluated using sera collected from a total of 588 asymptomatic Jordanian nationals (199 males and 389 females). The mean ages of males and females were 35.8 years and

Table 1 Prevalence of anti-*C. pneumoniae* IgG antibodies in a healthy Jordanian population by age and sex

Age group (y)	Males			Females			Both sexes		
	No. examined	No. positive[a]	% Positivity	No. examined	No. positive[a]	% Positivity	No. examined	No. positive[a]	% Positivity
2—9*	28	8	28.6	10	1	10.0	38	9	23.7
10—19	27	10	37.0	34	9	26.5	61	19	31.1
20—29	33	19	57.6	109	52	47.7	142	71	50.0
30—39*	21	15	71.4	78	51	65.4	99	66	66.7
40—49*	25	14	56.0	69	49	71.0	94	63	67.0
50—59	32	20	62.5	52	30	57.7	84	50	59.5
60—69*	23	18	78.3	31	16	51.6	54	34	63.0
≥70*	10	7	70.0	6	4	66.7	16	11	68.8
Total	199	111	55.8	389	212	54.5	588	323	54.9

[a] IgG ≥ 1:16.
*Statistically significant difference between males and females ($p < 0.05$).

36.2 years, respectively. Persons from each sex were grouped into eight age groups. Table 1 summarizes the presence of *C. pneumoniae* IgG antibody in study subjects according to age and sex. The differences in IgG seropositivity between males and females were statistically significant in the age groups 2—9 years, 30—39 years, 40—49 years, 60—69 years, and ≥70 years. After combining the sexes, the seroprevalence within the age groups was as follows: 23.7% in 2—9 years, 31.1% in 10—19 years, 50% in 20—29 years, 66.7% in 30—39 years, 67% in 40—49 years, 59.5% in 50—59 years, 63% in 60—69 years, and 68.8% in ≥70 years (Table 1).

The IgG detection rate in both sexes under the age of 10 years was the lowest for both males (28.6%) and females (10%) and increased notably in persons aged 10 years to 39 years (Table 1). When both sexes were combined, mean seropositivity for persons aged 2—9 years was 23.7% and steadily increased in the age groups 10—19 years (31.1%) and 20—29 years (50%) to reach a plateau of 66.7% in the age group 30—39 years, which remained almost stable to age ≥70 years (Table 1).

Overall, the seropositivity in all healthy individuals tested was 54.9% (323/588). The antibody detection rate was slightly higher in males (55.8%) than females (54.5%; $p < 0.05$). Data obtained in this study on individuals aged 2—18 years old are extrapolated in order to compare the prevalence of infection in children and teenagers with that in adult people older than 18 years. The overall prevalence of *C. pneumoniae* IgG in children and teenagers of both sexes (under 18 years old) was 26.4% (19/72), compared to 58.9% (304/516) in adults. In children and teenagers, *C. pneumoniae* antibodies were present in 12 out of 43 males (27.9%) and in seven out of 29 females (24.1%). IgG prevalence in males aging ≥18 years was 63.5% (99/156), appreciably higher than in females older than 18 years, who showed a 56.9% (205/360) prevalence.

Distribution of IgG titers for *C. pneumoniae* in the seropositive population (323 individuals) showed a relatively low (23/323, 7.1%) percentage of individuals with an endpoint titer, i.e., the highest positive dilution, of 1:16. Individuals with anti-*C. pneumoniae* IgG detectable at endpoint levels higher than 1:16 gradually increased and

reached 47 (14.6%), 55 (17%), 65 (20.1%), and 84 persons (26%) at titers 1:32, 1:64, 1:128, and 1:512, respectively. However, at 1:256, a sharp decrease in IgG titers occurred, with only 49 (15.2%) subjects testing positive (Fig. 1).

An IgG titer of ≥1:512 is considered serologic evidence of a recent infection with *C. pneumoniae* in this study. About 14.3% of individuals tested (84/588) had recent infections: 16.1% of males (32/199) and 13.4% of females (52/389). The prevalence of recent infection in adults (≥18 years old) was 14.9% (77/516), which was higher than rates detected in children and teenagers (9.7%; 7/72). Remarkably, recent infections were found in over a quarter (26%) of the total number of individuals tested positive for the IgG antibody (84/323 persons). Males with recent infection represented 28.8% (32/111) of the total number of seropositive males, whereas females represented 24.5% (52/212) of the total number of infected females ($p > 0.05$). When both sexes were combined, the prevalence of recent *C. pneumoniae* infection was highest in the age group 10—19 years and in ages over 60 years (Table 2).
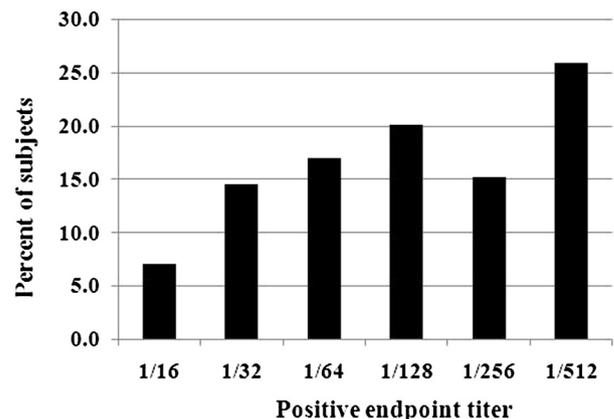
Figure 1. Distribution of endpoint titers of *C. pneumoniae* IgG antibody in the sera of 323 apparently healthy Jordanian subjects that showed seropositivity by MIF.

Table 2 Prevalence of high anti-*C. pneumoniae* IgG antibody, indicating possibly a recent infection (seropositivity at titer 1:512), in a seropositive Jordanian population

Age group (y)	Males		Females		Both sexes	
	Number [a]	% Positivity at 1:512	Number [a]	% Positivity at 1:512	Number [a]	% Positivity at 1:512
2—9	1/8	12.5	1/1	100	2/9	22.2
10—19	4/10	40.0	4/9	44.4	8/19	42.1
20—29	4/19	21.1	13/52	25.0	17/71	23.9
30—39	3/15	20.0	8/51	15.7	11/66	12.1
40—49	3/14	21.4	8/49	16.3	11/63	17.5
50—59	4/20	20.0	10/30	33.3	14/50	28.0
60—69	10/18	55.6	7/16	43.8	17/34	50.0
≥70	3/7	42.9	1/4	25.0	4/11	36.4
Total	32/111	28.8	52/212	24.5	84/323	26.0

[a] Number of persons showing seropositivity at 1:512/total number of seropositive cases.

Discussion

The prevalence of *C. pneumoniae* IgG antibody was assessed in a total of 588 apparently healthy Jordanian males and females, aged 2—86 years, using the MIF technique. Serology is the method of choice in routine clinical laboratories for diagnosis of *C. pneumoniae* infection.[11] In particular, MIF has been widely used to detect exposure rates to *C. pneumoniae*[28,29] and is considered the only currently acceptable serologic test.[27]

Most epidemiological studies published so far recommended the use of 1:16 as serologic evidence of *C. pneumoniae* infection.[1,11,15,20—22,24,26,27,32—34] A limited number have used 1:8,[35] 1:32,[13,16,18,19,36,37] or 1:64.[38] Nevertheless, studies performed worldwide have estimated exposure rates ranging from 40% to 86% in most adult populations, suggesting that *C. pneumoniae* infection is endemic in many countries and widely distributed. Here, the prevalence of *C. pneumoniae* infection was evaluated using the titer of 1:16. A high prevalence of *C. pneumoniae* IgG antibody (54.9%) was demonstrated in the Jordanian population. This prevalence is in agreement with exposure rates assessed in healthy populations worldwide; for instance, overall seropositivity in Turkey, Slovenia, Taiwan, Japan, and Korea is estimated at 41.5%, 41.6%, 55.8%, 52%, and 52%, respectively.[13,18,21,22,33] Prevalence of *C. pneumoniae* in Jordanian adults (58.9%) is moderate and comparable to estimates in other asymptomatic adult populations, which range from 40% to 86%. Exposure rates in adults are 48.4—60.9% in Japan,[18,24] 56—77.7% in Finland,[19,35,37] 49.4% in Slovenia,[21] 64.9% in Germany,[15] 64.5% in Turkey,[33] 75.2% in Hungary,[34] 72.3% in Taiwan,[22] 40—86% in USA,[8,16,20] 53% in Iceland,[14] 61.6% in Korea,[13] and 64% in Sudan.[23] The prevalence rate of *C. pneumoniae* in Jordanian children and teenagers (<18 years old), however, was markedly lower (26.4%), a rate also comparable to those previously reported for healthy children and teenagers in other countries such as Slovenia (with a detection rate of 26% in school age children) as well as Finland and Hungary (rural areas) with rates of 27% and 22.7%, respectively.[19,21,34] However, markedly lower or higher rates of prevalence in children in Turkey and Korea have also been reported.[22,33] Thus, asymptomatic *C.*

pneumoniae infection is common in Jordan, with seroprevalence rates comparable to other countries around the world.

The prevalence of the *C. pneumoniae* antibody was 23.7% in children between 2 years old and 9 years old. Detection of the antibody increased rapidly in older children and teenagers (10—19 years old), indicating that the primary exposure to *C. pneumoniae* begins in children and young people. The seropositivity continued to increase in adults, and in the age group 30—39 years, it reached a plateau of 66.7%, which remained almost stable in older individuals. Collectively, the present study demonstrates that infection is acquired during the first four decades of life, and that the stable high rates of *C. pneumoniae* infection in elderly subjects might be the result of persistent infection or frequent reinfection.[9,39,40] Importantly, an obvious relationship between sex and the prevalence of the *C. pneumoniae* antibody was found. Prevalence rates were higher in males than in females in all age groups, except the 40—49 years group. This seemingly anomalous result might be due to the relatively low numbers of males investigated within this age bracket. The higher prevalence rates among males observed here is fully consistent with previous reports.[13,15,17,21,22,24,32,33,36—38] Previous work has attributed the higher prevalence in males to a greater proportion of men working outside the home than women.[39] However, this assumption cannot explain differences observed here and by Kese and coworkers,[21] between sexes of non-working subjects under the age of 18 years. Hence, further work is needed to clarify the underlying factor(s) responsible for sex-associated differences in the prevalence of *C. pneumoniae*.

In a previous study, the prevalence of *C. pneumoniae* antibodies in Jordanian blood-donating adult males aged 20—50 years was assessed using MIF.[31] The IgG seropositivity estimated in adult males was 63.3%.[31] This detection rate is almost equal to the IgG prevalence (63.5%) detected here in males aging ≥18 years. When extrapolated from the data presented in Table 1, the overall prevalence of *C. pneumoniae* IgG in adult males between 20 years and 50 years was 60.8%, a percentage that is still approximately comparable to that assessed previously for males within the same age range.[31] In addition, both studies indicate that

the primary infection is acquired during the first four decades of life.

Criteria for the definition of recent (acute or current) infection of *C. pneumoniae* using IgG class in MIF appear to be controversial. IgG titers ≥1:256[22] and more frequently ≥1:512[1,11,13,15,16,20,24,26,37] were defined as evidence for recent infection using one serum sample. Others considered IgG titers of ≥512 as indicative of a "possible" recent infection,[27] when single serum is used. Another criterion for recent infection is a fourfold titer rise in IgG levels between two consecutive sera.[27,35] Here, there were difficulties in obtaining paired serum samples from subjects examined. However, considering a titer of 1:512 was diagnostic of a recent infection allowed us to compare our results with the majority of previous reports. Surprisingly, acute infection was detected in 14.3% of apparently healthy Jordanian subjects aged between 2 years and 86 years. This rate is among the highest; for instance, prevalence rates of 3.7% and 4.5% have been reported in Korean and Taiwanese populations, respectively.[13,22] Elevated antibody titers observed in the present study indicate that some individuals had experienced recent subclinical (asymptomatic) infections. Nearly 15% of Jordanian adults showed serologic evidence of a recent infection. High rates were also demonstrated in healthy adults (about 18%) in Brooklyn, USA.[16,26] However, the prevalence of recent infection in Jordanian adults is considerably higher than those reported in asymptomatic Taiwanese (6.4%), Japanese (3.9%), and German (3.4%) adults.[15,22,24] Variations in serologic results may reflect differences in geographic areas, ages of subjects investigated, and the time periods in which serum samples were collected.

The present work is the first to demonstrate the seroepidemiologic profile of asymptomatic *C. pneumoniae* infection in the Jordanian population. The high overall prevalence of antibodies suggests that *C. pneumoniae* is endemic in Jordan. Further surveys are recommended to correlate the pathogen with different relevant clinical manifestations such as respiratory system and coronary heart diseases in Jordan.

Conflicts of interest

The author declares that he has no financial interests related to the material in the manuscript.

Acknowledgments

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