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The seroepidemiology of *Chlamydiae* in Finland over the period 1971 to 1987

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SUMMARY

The seroepidemiology of chlamydial infections in the Finnish population was studied by analysing the prevalence of chlamydial complement fixing (CF) antibodies in patients sera sent for virus serological screening tests over 17 years from 1971 to 1987. The total number of sera studied was over 160000. In the early 1970s, the prevalence of chlamydial CF antibodies (CF titres $\geqslant 8$) was low (less than 2%), but later the proportion of seropositive cases rose, and in 1976, 18% of sera contained antibodies. In 1984, the seropositivity rate was over 31%. The prevalence of high chlamydial CF titres (titres $\geqslant 64$) also showed annual variation. In general, under 1% of sera contained chlamydial CF antibodies in high titre, but in 1979 and 1984, distinct peaks occurred when 1·3% and 1·4% of sera, respectively, had titres $\geqslant 64$. The age-related antibody positivity rate showed a decline during early infancy, an increase in childhood and adolescence, and a stable level in adulthood when approximately 20% of the sera contained antibodies. The chlamydial antigen used in this survey was genus-specific, i.e. it detects antibodies against all chlamydial species. Epidemiological data support the hypothesis that infections due to a novel chlamydial species, TWAR chlamydia, are the most likely explanation for the relatively frequent occurrence of chlamydial CF antibodies and for the variation in CF antibody prevalence.

INTRODUCTION

Infections due to chlamydia are very common. *Chlamydia trachomatis* is the cause of trachoma, a blinding disease affecting mainly individuals living in developing countries. In developed countries, *C. trachomatis* is one of the most important causes of sexually transmitted diseases the sequalae of which includes infertility. *Chlamydia psittaci*, another species of the genus, occurs widely in animals (Storz, 1971), but can also infect humans. In humans, *C. psittaci* infections may manifest as pneumonia (ornithosis) but placentitis and abortion have also been reported (Wong *et al.* 1985). In recent years, a novel chlamydial species called TWAR chlamydia has been discovered to be an important pathogen in human

A preliminary report based on the data was presented to the First Meeting of the European Society for Chlamydia Research, 30 May–1 June, 1988, Bologna, Italy (Puolakkainen, M., Ukkonen, P. & Saikku, P. Prevalence of complement fixing (CF) antibodies against *Chlamydiae*. In *Proceedings of the European Society for Chlamydia Research*, **1**, Bologna, Italy, 1988.

infections. This agent has been associated with respiratory infections in teenagers and young adults (Saikku *et al.* 1985; Grayston *et al.* 1986) and in military recruits (Kleemola *et al.* 1988), and is estimated to cause 5–10% of all pneumonias (Grayston *et al.* 1986; Marrie *et al.* 1987).

Although serology is most commonly used in the diagnosis of respiratory chlamydial infections, its role in the diagnosis of chlamydial infections in general has been disputed. The complement fixation (CF) test is genus-specific, i.e. it detects antibodies elicited in infections due to all chlamydial species, but its sensitivity may be poor. More specific tests based on the micro-immunofluorescence method (micro-IF) developed by Wang & Grayston (1970) do exist, but difficulties in antigen supply and in performing these tests reliably have hampered their wider use in diagnosis. Despite its minor role in the diagnosis of sexually transmitted chlamydial infections, serology has proved useful in epidemiological studies of chlamydial infections (Wang & Grayston, 1982). We present here data on the prevalence of chlamydial CF antibodies in the sera from patients with suspected viral illnesses obtained from the routine diagnostic laboratory of our institute. The results on an exceptionally large number of sera could be analysed, because all the data produced in our diagnostic laboratory have been stored by computer since 1970.

MATERIALS AND METHODS

Complement fixation test

The complement fixation (CF) test was performed in microtitre plates (Ukkonen *et al.* 1984). An ether-acetone extracted genus-specific antigen from *C. trachomatis*, serotype D (kindly provided by Dr Mordhorst, Statens Serum Institute, Copenhagen, Denmark) was used up to July 1983, and thereafter a commercially available chlamydial genus-specific antigen (Ornithosis antigen for CFT, Behringwerke AG, Marburg, West Germany) was used.

A screening test for viral antibodies performed in our department was done by CF test with 16–18 antigens, which also included the chlamydial genus-specific antigen. Data obtained from this routine screening have been stored by computer since 1970. Data for 1983 were not available in the computer files. The age-specific frequencies of viral and mycoplasmal antibodies in the same material for the years 1971–8 have already been published (Pönkä & Ukkonen, 1983; Ukkonen *et al.* 1984).

Patients

Sera from 5000 to 14000 patients have been studied annually, and the total number of sera studied from 1971 to 1982 and from 1984 to 1987 was 162401. A random sample, test results from 1245 sera, was collected from the manual files of the year 1983. These limited data were only used for calculation of the overall antibody positivity (Fig. 1). The percentage of serologically determined recent infections caused by any identified agent was very low in this material (less than 3%), and thus > 97% of the patients could be considered as 'normal' population with respect to a particular agent (Ukkonen *et al.* 1984). The age distribution of the individuals whose sera had been included in this serological survey is shown in

Table 1. *Age distribution of individuals whose sera were tested by CF screening in 1971–82 and 1984–7*

Age	No. tested	(%)
< 1 months	3811	2·3
1–3	2046	1·3
4–6	1379	0·8
7–11	2505	1·5
12–23	5307	3·3
2–3 years	5900	3·6
4–6	7155	4·4
7–10	8282	5·1
11–20	18058	11·1
21–30	26579	16·4
31–40	24886	15·3
41–50	17757	10·9
51–60	16278	10·0
61–70	12397	7·6
> 71	10125	6·2
Total	162401	

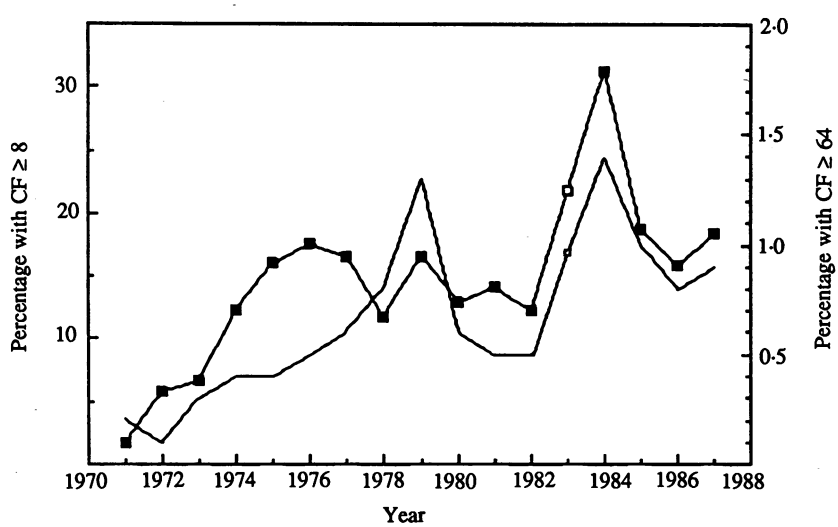

Fig. 1. Prevalence of chlamydial CF antibodies (-■-, titres ⩾ 8) and high titres (—, titres ⩾ 64) in sera sent for virus serological screening in 1971–87. The figures for 1983 (open symbols) are based on a small proportion of sera studied in 1983.

Table 1. Almost one quarter of the study population were children under 10 years old and one half was under 30.

RESULTS

In the years 1971–82 and 1984–7, 162401 sera were screened with the CF test for chlamydial antibodies. Fig. 1 shows the prevalence of chlamydial CF antibodies by year. In 1971, less than 2% of sera studied were antibody positive (CF titres ⩾ 8). In the early 70s the number of seropositives rose, and in 1976, 18% of the sera had antibody titres ⩾ 8. Later, the antibody positivity rate was between 12

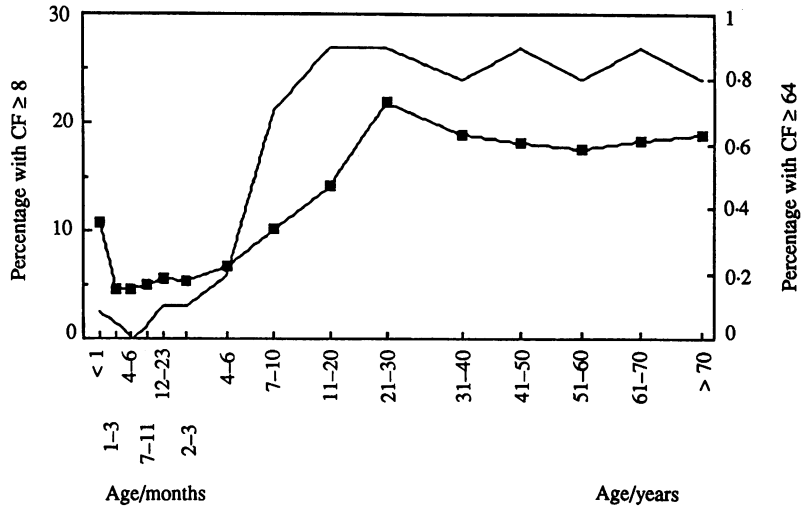

Fig. 2. Age-related frequency of chlamydial CF antibodies (-■-, titres ⩾ 8) and high titres (—, titres ⩾ 64) in sera sent for virus serological screening in 1971–82 and 1984–7.

and 22%, except in 1984 when over 31% of the sera contained chlamydial CF antibodies.

The prevalence of high CF titres to chlamydia (titres ⩾ 64) is also presented in Fig. 1. The number of high-titred sera rose from 0·1% to about 0·5% in the early 1970s. In 1979 and 1984, there were distinct peaks with high titres in 1·3% and 1·4% of the sera respectively. Since 1984, the prevalence of high titres has been 0·8–0·9%.

The age-related prevalence of chlamydial CF antibodies, calculated from pooled data from years 1971–82 and 1984–7, is shown in Fig. 2. In early infancy, about 10% of the study population had antibodies, apparently of maternal origin. The frequency declined rapidly, and the lowest rate (4·5%) was observed in the age group of 1–3 months. In childhood and adolescence, there was an increase in antibody positivity rate from 5 to 14%. In the age-group 21–30 years the proportion antibody positive had reached 22%, and the level remained quite stable thereafter.

High chlamydial CF titres were seldom found in infants and children under 6 years of age (less than 0·2%), but the prevalence increased rapidly after that age (Fig. 2). In adults, high titres were found in approximately 0·8% of the sera studied.

Annual variation occurring in the age-specific antibody prevalence can be seen in Fig. 3. All the antibody prevalence curves resembled each other in shape, but curves of years 1971–3 were in general at a lower level than those of years after 1973. In 1984, the age-specific antibody prevalence curve was at an exceptionally high level. The curves of years 1974–82 and 1985–7 were very similar in profile and level.

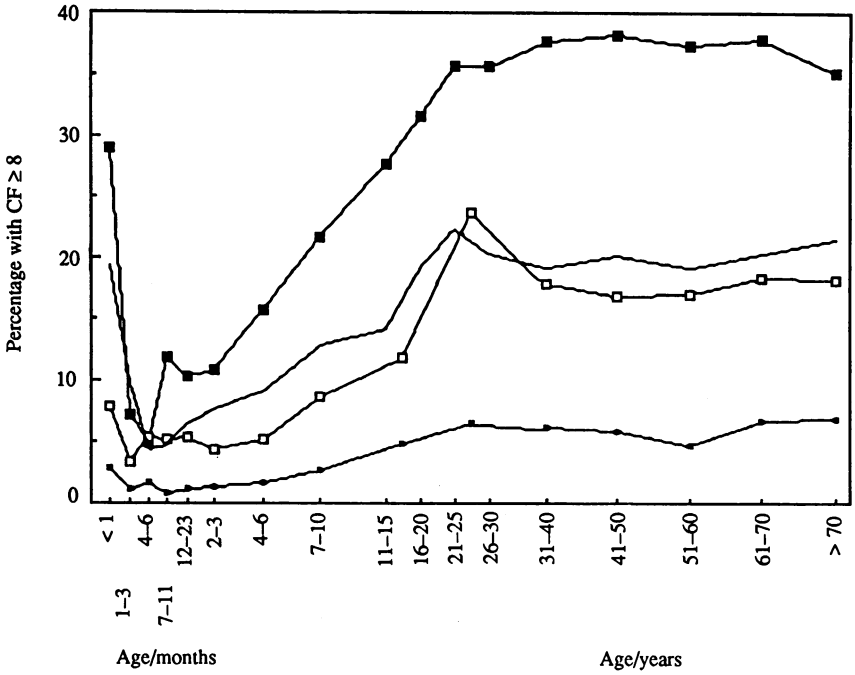

Fig. 3. Age-specific prevalence of CF antibodies to *Chlamydiae* in patients screened by virus serological survey in years 1971–82 and 1984–7. The curves represent following periods: ■1971–3, □ 1974–82, ■ 1984, — 1985–7.

DISCUSSION

The seroepidemiology of chlamydial infections in Finland was studied by analysing the prevalence of chlamydial CF antibodies in patient sera sent for virus serological screening. The study population can be considered to represent fairly well the general Finnish population for the following reasons. Our laboratory serves all types of hospitals and out-patient clinics covering about two-thirds of the whole population, and because of the nature of our public health care system, the patients included were not selected for socioeconomic reasons (Ukkonen *et al.* 1984). However, 80% of the serum specimens came from hospitals and only 20% from out-patient clinics, which may be a source for incorrect interpretations. The frequency of recent infections caused by any single agent included in the screening antigens was very low (less than 3%) (Ukkonen *et al.* 1984).

During the serological survey covering a period of 17 years (1971–87) the chlamydial antigen used in the CF test came from two sources. The change from the earlier non-commercial antigen to a commercial antigen in July 1983 was done only after careful comparative evaluation of the old and new antigens, and this change is an unlikely explanation for the changes observed in the antibody prevalence figures.

Chlamydial CF antibody titre levels usually decrease within a few months after an attack of acute ornithosis (Matthiesen & Volkert, 1956; Jansson, 1960), but they may sometimes remain elevated for years for unknown reasons (Dekking, 1962). Assuming that chlamydial CF titres usually decrease relatively soon after

an acute infection the age period showing increasing antibody prevalence should reflect the period of life when infections start to occur. The prevalence curves of this study showed that the time period of most rapid acquisition of antibodies was between 7 and 30 years. Because of the low prevalence of CF antibodies between 1971 and 1982, no difference in the seroconversions between sexually active as opposed to inactive age groups was observed. In later years it was shown, however, that the steep increase in the seropositivity rate started as early as in the age group of 4 to 6 years (Fig. 3, 1984), indicating that the infections responsible for this increase were evidently not sexually transmitted. Because of the relatively short duration of chlamydial CF antibodies, chlamydial infections must be occurring also in adults, although the antibody prevalence curve is not continuing to rise.

In this study, approximately 20% of the adult population had chlamydial antibodies detectable by CF. In an earlier study, CF titres $\geqslant 10$ were found in 14% of Finnish blood donors (Jansson, 1960), which is in agreement with our report.

It seems likely that infections due to *C. trachomatis* alone cannot account for the observed relatively high frequency as trachoma is a rarity in Finland (Vannas, 1970), and in the uncomplicated sexually transmitted form of the disease CF reactors are usually scarce (Schacter *et al.* 1979). For example, in chlamydial urethritis, only about 20% of the patients develop antibodies detectable by CF test (Pascienzy & Sommerville, 1966). Assuming 20% as an average CF antibody acquisition rate in mucosal *C. trachomatis* infections, and comparing it with the observed antibody prevalence of 20%, it is clear that other chlamydial infections or frequently recurring infections must contribute to the prevalence rate.

In *C. psittaci* infections the CF antibodies appear more frequently than in *C. trachomatis* infections. However, *C. psittaci* infections with classical avian-to-human transmission are considered uncommon in Finland (E. Neuvonen VMD, State Veterinary Medical Institute, Helsinki, Finland, personal communication). In addition, occurrence of chlamydial CF antibody in Finnish bird ringers resembles closely that in Finnish blood donors (Saikku, 1987). Wreghitt & Taylor (1988) have noted a correlation between the number of human chlamydial respiratory tract infections in Britain and the number of psittacine birds imported into the country. Similar data from Sweden, USA, England and Wales have been reported (Reeve, Carter & Taylor, 1988). Import of psittacine birds into Finland has been severely restricted since the 1970s. Only birds belonging to families immigrating into Finland or birds for breeding purposes can be imported into Finland with the permission of the Ministry of Agriculture and Forestry. Thus, psittacosis contracted from psittacine birds is evidently not a significant source for human chlamydial CF antibodies in Finland.

Infections due to the novel chlamydial species, TWAR chlamydia, are another possible explanation for the unexpectedly high CF antibody prevalence. This agent causes respiratory infections with interhuman transmission (Saikku *et al.* 1985, Grayston *et al.* 1986). In fact, the first epidemic due the TWAR chlamydia was found with the aid of CF test (Saikku *et al.* 1985). According to studies based on serum samples from different parts of the world, up to one half of the adult population possesses TWAR IgG antibodies in titres $\geqslant 32$ when tested with the

micro-immunofluorescence (micro-IF) test (Wang & Grayston, 1986). In a Finnish survey, 36% of adult females and 47% of adult males had IgG TWAR antibody (Grayston *et al.* 1984; Wang & Grayston, 1986). In children under 10 years of age and in teenagers from Denmark and north-western USA the corresponding antibody prevalence rates were 4% and 28% respectively (Wang & Grayston, 1986). Consequently, the most common chlamydial CF antibody acquisition age coincides with that of the development of TWAR antibodies. Compared to corresponding IgG antibody prevalences detectable by *C. trachomatis*-specific micro-IF test, TWAR antibodies are more common in adolescence and adulthood. The same laboratory has reported a prevalence of IgG antibodies by *C. trachomatis* micro-IF to be 4% in children aged 1–17 years, 8% in adult males and 15% in adult females (Grayston *et al.* 1982).

More support for the role of TWAR infections as a possible contributor to the observed chlamydial CF antibody prevalence can be obtained by analysing the timing of Finnish TWAR epidemics and the changes in the prevalence of CF antibodies and high titres. In 1977, an epidemic of mild pneumonia with diagnostic or elevated titres against chlamydial genus-specific CF antigen occurred in the Oulu garrison (in northern Finland) (Saikku *et al.* 1985; Kleemola *et al.* 1988). In the following year, a similar type of epidemic was noted in the garrison of Kajaani, with some spread also among civilians (Saikku *et al.* 1985). Serological studies with the micro-IF test later implicated the TWAR agent as a causative agent in these epidemics (Saikku *et al.* 1985; Kleemola *et al.* 1988). High chlamydial CF titres, frequently observed in the early stages of the disease in young adults suffering from TWAR pneumonia (Kleemola *et al.* 1988), were noted in the general population more frequently in 1979. This might be due to a slow spread of the disease among civilians, or possibly to a selection of a more virulent strain(s) that circulated longer and wider in the population. Later, in 1985 and 1987 several pneumonia and respiratory tract infection epidemics in military establishments due to the TWAR chlamydia have been noted in Finland (Kleemola *et al.* 1988; Ekman *et al.* unpublished data).

TWAR epidemics have also been recorded in other Scandinavian countries (Mordhorst, Wang & Grayston, 1986). Epidemics, obviously due to TWAR infections, have occurred in Denmark in 1976, 1979 and 1982–3, and in Norway 1981–2 (Mordhorst, Wang & Grayston, 1986). During these epidemics, the majority of sera from patients having 'ornithosis' diagnosed by CF test contained, in fact, TWAR-specific antibodies determined by micro-IF.

Epidemiological data support a major role for TWAR chlamydia infections in causing the changes in chlamydial CF antibody positivity rate. More detailed studies are needed to clarify the proportion of various chlamydial species in explaining the observed CF reactions, and the clinical picture of TWAR chlamydia infections.

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