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Changes in the epidemiological pattern of *Mycoplasma pneumoniae* infections in Denmark

A 30 years survey

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SUMMARY

A seroepidemiological survey has shown a remarkable shift in the previously reported regular cyclic pattern of *Mycoplasma pneumoniae* epidemics which culminated every 4·5 years during the period 1958–74. The last of four regular epidemics occurred in 1972. It was followed by 'premature' epidemics in 1975 and 1977/8 which inaugurated a change from an epidemic to an endemic pattern of the infection in Denmark. Over the following 9 years (1978–86) there has been an irregular but significant decrease in the annual number of seropositive samples with the usual high incidence during winter seasons. This endemic period terminated in the fourth trimester of 1987 with the development of a new epidemic. The hypothesis is advanced that a sixfold increase of children in day care may have influenced the change from an epidemic to an endemic situation which after 9 years led to a new epidemic of *M. pneumoniae* infection.

INTRODUCTION

Mycoplasma pneumoniae is a common cause of lower respiratory tract disease in humans. In most countries the disease is prevalent all year round, with a relatively high incidence during winter. Longitudinal studies indicate that the incidence varies markedly from year to year (Chanock *et al.* 1967; Evans, Allen & Sueltmann, 1967; Foy *et al.* 1979; Glezen *et al.* 1971; Joosting *et al.* 1976).

In a previous serological investigation of the incidence of *M. pneumoniae* infection in Denmark during the 17-year period 1958–74 we found that four epidemics had occurred, which culminated at regular intervals of 4·5 years (Lind & Bentzon, 1976). The present serological investigation was carried out over the following 13 years until the end of February 1988. From this it appears that there has been a change of the epidemiological pattern of *M. pneumoniae* infections in this country, which took place over the years 1972–8. Since then the infection had been endemic with a trend of decreasing incidence until the fourth trimester of 1987 when a new epidemic started.

SAMPLE AND METHODS

During the 30 years of the study all blood specimens were received at the same central laboratory serving all hospitals and general practitioners in Denmark. The main purpose for the submission of specimens was to confirm the diagnosis of what was first called cold agglutinin positive primary atypical pneumonia or Eaton Agent pneumonia. (Eaton, Meiklejohn & Herick, 1944; Chanock *et al.* 1961). Later on, as knowledge of the disease increased, more specific serological tests in the diagnosis of *M. pneumoniae* infection became available (Clyde, 1979).

In the years January 1958 to November 1973 all specimens were tested by cold agglutinin (CA) and *Streptococcus* MG agglutinin titrations (Lind, 1968), and CA positive sera were stored at −20 °C for further study. As described previously (Lind, 1971; Lind & Bentzon, 1976) frozen sera, representative of each month, were randomly selected and tested for antibodies to *M. pneumoniae*. Briefly, the majority of sera from 1958 to September 1969 were tested by an indirect immunofluorescence (IF) test. In the following years this test was supplemented first by an indirect haemagglutination (IHA) test and later by a complement fixation (CF) test which has been used almost exclusively since January 1970, and exclusively since 1973 (Lind & Bentzon, 1976).

Through the 30 years from 1958–87 the population has increased in a linear fashion from 4·5 to 5·1 million inhabitants.

Serological methods

The CF test for antibodies to *M. pneumoniae* was performed according to the method of Kenny & Grayston (1965), slightly modified as described in detail in an appendix to a previous article (Lind & Bentzon, 1976). Since June 1985 the test has been performed in a microtitre system equilibrated to give results with reference sera similar to the 'macro'-method, and using the same titre of $\geqslant 64$ as the criterion for a positive result. In each set-up two pools of patients' sera, one with a high titre and one with a medium high titre, together with a negative serum, were included. The Mac strain, isolated in 1944 (Eaton, Meiklejohn & Herick, 1944), was used throughout the entire period for the preparation of a chloroform–methanol extracted glycolipid antigen. This strain was processed between the 35th and 60th passages. There was no correlation between number of passages and titre of antigen.

Throughout all 30 years the CA test was carried out on all serum samples, before 1969 as described by Lind, Mansa & Olesen (1963), and since 1970 by a slightly modified procedure (Lind & Bentzon, 1976). In each test the two pools of patients' sera, both positive with respect to CA, and the negative serum, have been included as controls.

As a test for day-to-day reproducibility of both the CF and the CA test, the results of tests on positive reference sera from four periods were randomly chosen, one from 1976–7, the others from 1984–5. From each period consecutive test results were analysed, representing a total of 327 test days. The standard deviation for both positive reference sera was between 0·53 and 0·60 dilution step in the CF test, and between 0·62 and 0·77 in the CA test. A test to monitor for trends over each period showed no significant fall or rise of titres.

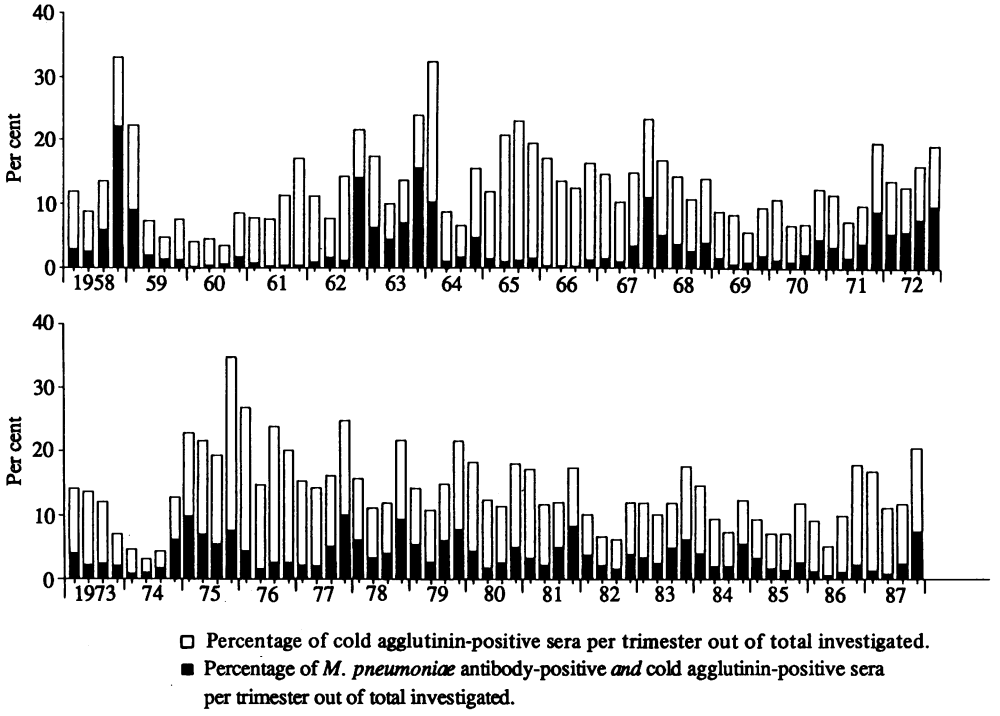

Fig. 1. The columns show samples of serum with a positive cold agglutinin (CA) test, and the black part of the columns are those with a positive CA test plus a positive anti-*M. pneumoniae* test only, both given as percentages of all sera investigated in each trimester in the period 1958–87.

Statistical methods

The periodicity in the frequency of seropositive samples from the period 1958–74 was investigated by means of a multiple regression analysis as described earlier (Lind & Bentzon, 1976). For other periods a two-way analysis of variance model was found to give a better fit. Such an analysis was therefore used to test the variations from year to year and the seasonal variations.

RESULTS

The annual number of blood specimens received increased from approximately 4000 in 1958 to over 20000 in 1987. The increase has been stepwise, and each major step has coincided with an increase in number of specimens showing a positive anti-*M. pneumoniae* test plus a positive cold agglutinin test, indicating epidemics of *M. pneumoniae* infection (see discussion).

In Fig. 1 the columns show samples of serum with a positive CA test, and the black part of the columns are those with a positive CA test plus a positive anti-*M. pneumoniae* test, both given as percentages of all sera investigated in each trimester in the period 1958–87. The frequency of seropositive samples from the period 1958–74 corresponds well with four major waves (Lind & Bentzon, 1976). Two of these which culminated in 1963 and in 1972, respectively, also show

Table 1. *Annual number of serum samples tested for cold agglutinins (CA) and* M. pneumoniae *(MP) antibodies over a period of 30 years*

Year	Total number investigated	CA positive		Anti-MP positive plus CA positive		Anti-MP positive		Anti-MP pos. plus CA pos. in % of all anti-MP positive
		Number	% of all sera	Number	% of all sera	Number	% of all sera	
1958	4395	822	18·7	432c	9·83	—	—	—
1959	4219	524	12·4	178c	4·19	—	—	—
1960	3771	197	5·2	25c	0·66	—	—	—
1961	3854	438	11·4	16c	0·42	—	—	—
1962	4597	644	14·0	222c	4·83	—	—	—
1963	5947	1023	17·2	530c	8·91	—	—	—
1964	6247	1156	18·5	331c	5·30	—	—	—
1965	5932	1110	18·7	77c	1·30	—	—	—
1966	6387	977	15·3	34c	0·53	—	—	—
1967	6704	1126	16·8	330c	4·92	—	—	—
1968	7819	1137	14·5	322c	4·12	—	—	—
1969	7103	600	8·4	92c	1·30	—	—	—
1970	6317	611	9·7	140c	2·22	—	—	—
1971	7423	985	13·2	377c	5·08	—	—	—
1972	9396	1491	15·9	690c	7·34	—	—	—
1973	9309	1122	11·8	259c	2·78	—	—	—
1974	9195	601	6·5	232	2·52	540	5·87	43·0
1975	12209	3060	25·1	928	7·60	1436	11·76	64·6
1976	13372	2934	21·9	383	2·86	721	5·39	53·1
1977	12250	2283	18·6	653	5·33	945	7·71	69·1
1978	17266	2712	15·7	1031	5·97	1722	9·97	59·9
1979	16262	2566	15·8	910	5·60	1483	9·12	61·4
1980	17354	2727	15·7	615	3·54	1211	6·98	50·8
1981	15872	2411	15·2	759	4·78	1321	8·32	57·5
1982	17785	1598	9·0	512	2·88	1187	6·67	43·1
1983	18559	2397	12·9	762	4·11	1304	7·03	58·4
1984	16923	1945	11·5	599	3·54	1122	6·63	53·4
1985	18716	1738	9·3	453	2·42	1062	5·67	42·7
1986	19211	2063	10·7	253	1·30	573	2·98	44·2
1987	20143	3282	16·3	710	3·52	1166	5·78	60·9

c, Calculated, see Lind & Bentzon (1976)

depressions due to the annual low incidence in the summer season. The intervals between the top of the waves are regularly 4·5 years each in this 17-year period (Fig. 1). The three low-incidence intervals between these first four waves are 14 trimesters each. The following fifth wave, however, culminated in 1975, only 3 years after the last peak, with a low-incidence interval of only six trimesters. The next and last low-incidence interval was of five trimesters, terminated by a new early wave which had its maximum in the winter 1977–8. Since then each winter season has shown a relatively high incidence.

Analysis of the 9 years from 1978 to 1986 demonstrated only minor variations in the shape of the annual curves, but there were no significant changes in incidence from year to year (Fig. 1). While the annual number of sera investigated

in these 9 years has varied around 17550 (range 15872–19211) with an increasing trend, the annual number of CF plus CA positive samples has shown an irregular decrease from 1031 in 1978 to 253 in 1986, and in this last year the lowest number of positive sera since 1974 when 232 were detected (Table 1).

Towards the end of the next year, 1987, a new epidemic developed, indicated by an almost fivefold increase in number of CF/CA positive specimens from 52 in September to 255 in December. In the same period the total number of CF positive specimens (irrespective of CA titres) increased from 88 in September to 384 in December, and in February, 1988, the figure rose to 506 (not shown in Fig. 1 or Table 1). This is the highest monthly number of antibody positive specimens recorded in the 30-year period. An increase of this order of magnitude has only been observed at the beginning of previous epidemics. This epidemic is the first since that of 1977–8.

The number of anti-*M. pneumoniae* plus CA positive sera made up about half of the total anti-*M. pneumoniae* positive sera. In the period 1974–87 the average was 54·4% (range 42·7–69·1%). Generally the ratio was high when the total number of anti-*M. pneumoniae* positive sera was high (Table 1).

If a curve showing the number of CF plus CA positive sera each month were to be superimposed on a curve showing the total number of CF positive sera (irrespective of CA test result) each month, the two curves would run closely in parallel and the peaks of the waves would coincide exactly.

All these data are based on results from testing single serum samples. Since more samples are generally received from seropositive than from seronegative patients (Lind & Bentzon, 1976), it is possible that high incidence waves of positive sera may exaggerate the reflection of positive cases, but the time when the curves peak is not likely to be much displaced from the culmination of outbreaks.

We have therefore compared these data with the number of seropositive patients each month from January 1973 to December 1987. In this exercise seropositivity was defined by *either* a $\geqslant$ fourfold rise in titre in the anti-*M pneumoniae* CF test, *or* a CF titre of $\geqslant 512$, *or* a CF titre plus CA titre both of $\geqslant 64$. The curve showing the number of these patients each trimester is closely in parallel with that of the above-mentioned curve for serum specimens, with coincidence of their peaks.

DISCUSSION

The prerequisite for an estimation of the varying incidences of *M. pneumoniae* infection has been the investigation of CA positive sera for antibodies to *M. pneumoniae*. Since a rise in titre of the specific antibodies has not consistently been recorded in our patients we have had to rely on testing single sera. It has been well documented that while the specific antibodies may be demonstrated at a positive level for several months to more than a year after a *M. pneumoniae* infection, the occurrence of CA is of much shorter duration. Usually, CA appear transiently at a positive level from the second week till 1 or 2 months after onset of *M. pneumoniae* illness (Rytel, 1964; Fernald,, Clyde & Denny, 1967; Biberfeld, 1971). Therefore, the simultaneous occurrence of CA and the specific antibodies in a blood specimen indicates a current or recent *M. pneumoniae* infection of the

Table 2. *Number of children registered in day-care institutions and day care in private homes in Denmark 1961–86*

	Day nurseries*	Day-care institutions†	Day care in private homes	Total
1961	4·358	38·458	—	42·816
1965	4·690	44·079	—	48·769
1970	9·132	69·852	6·397	85·381
1975	17·088	121·719	23·840	162·647
1980	19·470	164·308	61·418	245·196
1986	21·615	183·040	61·779	266·434

* 0- to 2-year-old children.

† including kindergartens, recreation centres and age-integrated institutions (3- to 14-year-old children).

patient. Although CA as a non-specific manifestation may develop also during respiratory infections caused by other agents (e.g. adenovirus and influenza virus) their association with *M. pneumoniae* infection is much more common (Chanock *et al.* 1961; Mufson *et al.* 1962; Lind, Ravn & Møller, 1970).

It also appears from the above cited studies as from the present investigation (Table 1) that CA develop in only about half of patients with a positive test for *M. pneumoniae* antibodies, a proportion which apparently has not changed over the years. It therefore seems justified to assume that the major waves showing high incidences of sera with these concurrently positive tests closely depict epidemics of *M. pneumoniae* infection. For this reason and for the sake of creating a uniform serological basis for the entire longitudinal study, a positive CA test was chosen as an inclusion criterion for the whole material.

The present study has shown a remarkable shift in the previously reported regular pattern of epidemics culminating every 4·5 years during the period 1958–74. The 'premature' epidemics in 1975 and 1977–8 seem to have inaugurated a change from epidemic to endemic occurrence of *M. pneumoniae* infection in Denmark. Since the winter 1977–8, each winter has shown a relatively high incidence over each of the following 9 years till 1986 (Fig. 1 and Table 1). The shape of the annual seasonal waves in these years has shown minor variations, but the incidences have varied significantly from year to year with an obvious trend of decrease which ended in 1986 with the lowest incidence since 1974. This trend must be evaluated against the background of a slightly varying but significant increase in number of specimens received over these 9 years, during which a significant decrease in the percentage of seropositive samples has been observed (Table 1). Towards the end of 1987 a large new epidemic began, when the highest monthly number of positive specimens ever were recorded in this laboratory. Clearly a second remarkable shift in the epidemiological pattern had occurred.

The factors that govern the observed periodicity of *M. pneumoniae* epidemics or the remarkable changes therein are not known. The communicability and transmission of the infection and the immunity of the host have been extensively studied (Clyde, 1979; Denny, Clyde & Glezen, 1971) but their possible role in outbreaks or epidemics of the disease in a whole population is unresolved.

The authors advance the hypothesis that one factor which may have contributed to the first change in the epidemiological pattern in this country is the number of children in day care institutions and day care in private homes. From 1961 to 1986 this number has increased more than sixfold. The increase was most steep in the years of change from an epidemic to an endemic situation (Table 2). The children are from 0 to 14 years old, but only a very limited number are above the age of 10. This means that today almost one quarter of this age group (which make up about 1 million out of the 5·1 million population) is in day care (Bertelsen, 1987). A natural consequence would be an increasing risk of transmission of *M. pneumoniae* infection in this highly exposed risk group and in the children's families. The resulting increasing number of individuals with acquired protective immunity would tend to reduce the number of infectious cases prevalent. However, pari passu with the termination of effective protective immunity, which may last between 1·5 and 10 years (Fernald, Collier & Clyde, 1975; Foy *et al.* 1977) the balance may have tipped, giving the relatively few remaining prevalent cases the opportunity to spread sufficiently to develop into the new epidemic of the winter 1987–8. Foy *et al.* estimated that after a time lapse of 3–5 years *M. pneumoniae* pneumonia occurs as frequently in patients formerly infected with *M. pneumoniae* as in the general population.

We have no information about the true incidence of *M. pneumoniae* infection in the population. Several factors may influence our data, some of which have been discussed previously (Lind & Bentzon, 1976). A major but unassessed factor is the interest among physicians in the diagnosis of *M. pneumoniae* infection which may have increased at least during the first two decades since the 1960s, when the aetiological significance of *M. pneumoniae* in primary atypical pneumonia became established (Clyde, 1979). This factor may influence the actual number of specimens sent for serodiagnosis, which again may increase when physicians are aware of an epidemic, resulting probably in a relative increase in seropositive results. However, it is difficult to conceive how this might influence the time when an epidemic will reach a maximum.

The CF test for *M. pneumoniae* antibodies which has been used since 1970 has been equilibrated by inclusion of pools of human reference sera to maintain its sensitivity, and the same hold true for the CA test.

Wild strains of *M. pneumoniae* isolated in this country between 1962 and 1985 showed the same profile when compared by sodium dodecyl sulphate polyacrylamide gel electrophoresis. In cross-over tests by Western Blotting with sera from the patients from whom the strains were isolated, no evidence of antigenic drift was found over this 23-year period (Søndergård-Andersen & Lind unpublished study, 1987). Similar observations were made in the USA over a 10-year period (Vu *et al.* 1987). However, we have no information about possible changes in virulence of wild strains.

Long-term studies of the infection in other populations have indicated a periodicity of 3–6 years for major outbreaks or epidemics (Chanock *et al.* 1967; Evans, Allen & Sueltmann, 1967; Glezen *et al.* 1971; Joosting *et al.* 1976; Foy *et al.* 1979; Denny, Clyde & Glezen, 1971). Niitu *et al.* (1982) has conducted a study over 22 years (1960–82) among pupils of the primary, middle, and high schools in Sendai, Japan, which showed a strictly 4-year periodicity.

Publications from parts of Europe since 1967 have described the following major outbreaks or epidemics of *M. pneumoniae* infection: in Czechoslovakia 1962–3 (Soběslavský *et al.* 1970), in Holland 1965–6 (Hers & Masurel, 1967), in Italy 1971–2 and 1975 (Martelli *et al.* 1976), and in the German Democratic Republic 1974–5 and 1979–80 (Ortel, Hermsdorf & Bender, 1979; Bretschneider, Witzleb & Witzleb, 1979; Ritter, Nicklisch & Lange, 1983; Handrick *et al.* 1987).

In laboratory reports on *M pneumoniae* infections in England and Wales 1970–86 a regular cyclic pattern with an epidemic culminating every 4 years was seen, the first one occurring in 1971–2 (Anonymous, 1986; Anonymous, 1987).

In Norway, case reports were few from 1967 to 1971. A small peak was seen in 1972 concurrently with a Danish peak. A sharp increase occurred in the third trimester of 1975, three trimesters later than in Denmark. Thereafter there were relatively high incidences in 1982 and 1983, and again in 1986 and 1987. However, since 1975 there has been no regular epidemiological pattern (Flugsrud, 1987).

According to Jansson, Essen & Tuuri (1971), Helsinki in Finland experience two outbreaks of *M. pneumoniae* pneumonia, one in the winter 1962–3, the other 1967–8. Monthly reports of serologically confirmed cases from different laboratories in Finland from 1971 to 1987 (Kleemola, 1987) show increases in the incidence in the winter 1972–3 and again in 1977–8. Between these two peaks there were no regular variations. From April 1978 till November 1981 the incidence was rather low. Since then the epidemiological pattern has resembled that in Denmark with varying high incidences in the winter seasons.

It is interesting that the regular cyclic pattern seen in England and Wales up to the end of 1986 has not occurred in Norway, Finland or Denmark over the 11 years since 1975.

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