An epidemic of *Mycoplasma pneumoniae* in Manitoba: A serological report

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L SEKLA, W STACKIW, G EIBISCH, D KOLTON. An epidemic of *Mycoplasma pneumoniae* in Manitoba: A serological report. Can J Infect Dis 1993;4(1):43-46.

OBJECTIVES: To report an epidemic of *Mycoplasma pneumoniae* in Manitoba and to discuss the limitations of the serodiagnostic tests used. **DESIGN:** A retrospective analysis of the results of a province-wide serological testing for respiratory infections caused by *M pneumoniae*, using a complement fixation test and an indirect immunofluorescent antibody test for the detection of immunoglobulin (Ig) M antibodies. **MATERIAL:** From April 1, 1987, to March 31, 1991, 12,804 sera were tested and a serological diagnosis of recent *M pneumoniae* infections were established in 509 (3.97%). From April 1 to September 30, 1991, an additional 2088 persons were tested; the 158 (7.5%) recent cases of *M pneumoniae* were subjected to analysis. **RESULTS:** Compared with the previous three years, an increase in the number of recent cases of *M pneumoniae* was first noticed in July 1990 which persisted until September 1991. Of 856 single sera tested, 59 (6.8%) were recent *M pneumoniae* infections and 56 (96.1%) of these were positive for IgM antibodies. Of the 616 persons who submitted paired sera, 99 (16%) were recent infections, but only 46 (46.4%) had IgM antibodies. Primary infections (ie, positive for IgM antibodies) were detected in 102 (64.5%) and reinfections (ie, positive complement fixation test only) in the remaining 56 persons with recent *M pneumoniae* infections. Primary infections were detected more frequently in the 'under 16' than in the 'over 16' year age group (75% versus 55.8% of the recent cases of *M pneumoniae* in each age group). Reinfections were more common in the older age group. Of the 158 recent cases of *M pneumoniae*, 30.3% had a pneumonia; of these, 21 (55.2%) were under the age of 16 years. **DISCUSSION:** *M pneumoniae* is an important cause of morbidity. Serological tests are used for the diagnosis despite their limitations. The detection of IgM antibodies in acute serum establishes a diagnosis of primary *M pneumoniae*; however, their absence does not exclude *M pneumoniae*. A second (convalescent) blood test is required to diagnose all primary infections. To diagnose all reinfections, paired sera should be tested by complement fixation. **SUMMARY:** Manitoba experienced an epidemic of *M pneumoniae* in 1990-91. Properly selected serological tests can provide a specific and rapid diagnosis.

Key Words: Epidemic, Manitoba, *Mycoplasma*, Serological

Épidémie de *Mycoplasma pneumoniae* au Manitoba: rapport sérologique

OBJECTIFS: Faire rapport sur l'épidémie de *Mycoplasma pneumoniae* au Manitoba et discuter des limites des analyses sérodiagnostiques utilisées. **MODÈLE:** Analyse rétrospective des résultats d'un dépistage sérologique à l'échelle provinciale pour les infections respiratoires à *M. pneumoniae* à l'aide d'un test de fixation du complément et d'un test indirect d'immunofluorescence pour la détection d'anticorps IgM (IFA-IgM). **MATÉRIEL:** Du 1^{er} avril 1987 au 31 mars 1991, 12 804 échantillons de sérum ont été analysés et un diagnostic sérologique d'infection récente à *M. pneumoniae* a été établi chez 509 sujets (3,97 %). Entre

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le premier avril et le 30 septembre 1991, 2 088 autres personnes ont subi les analyses. Les 158 cas (7,5 %) d'infection à *M. pneumoniae* récente ont été soumis à des analyses. **RÉSULTATS:** Par comparaison avec les trois années précédentes, une augmentation du nombre de cas d'infection récente à *M. pneumoniae* a d'abord été notée en juillet 1990, puis a persisté jusqu'à septembre 1991. Sur 856 spécimens sériques testés, 59 (6,8 %) étaient des cas d'infection récente à *M. pneumoniae* et 56 (96,1 %) de ces derniers étaient positifs à l'égard des anticorps anti-IgM. Des 616 personnes ayant soumis des paires de sérum, 99 (16 %) présentaient des infections récentes, mais 46 seulement (46,4 %) étaient porteuses d'anticorps anti-IgM. Des infections primaires, (c'est-à-dire positivité à l'égard des anticorps anti-IgM) ont été décelées chez 102 (64,5 %) et les cas de réinfection (c'est-à-dire positivité uniquement au test de fixation du complément) dans les 56 cas restants atteints d'infection récente à *M. pneumoniae*. Les infections primaires ont été décelées plus fréquemment dans le groupe des moins de 16 ans que dans celui des plus de 16 ans (75 % contre 55,8 % des cas d'infection récente à *M. pneumoniae* dans chaque groupe d'âge). Les réinfections s'observaient plus fréquemment dans le groupe d'âge plus avancé. Des 158 cas d'infection récente à *M. pneumoniae*, 30,3 % avaient une pneumonie; 21 d'entre eux (55,2 %) étaient âgés de moins de 16 ans. **Discussion:** *M. pneumoniae* est une cause importante de morbidité. Les analyses sérologiques sont utilisées pour son diagnostic, en dépit de leurs limites. La détection de l'anticorps anti-IgM dans les paires de sérum confirme le diagnostic d'infection primaire à *M. pneumoniae*. Cependant son absence ne permet pas d'écarter l'hypothèse de sa présence. Une deuxième analyse sanguine (durant la convalescence) est requise pour diagnostiquer toutes les infections primaires. Pour diagnostiquer toutes les réinfections, les paires de sérum doivent être analysées par méthode de fixation du complément. **RÉSUMÉ:** Le Manitoba a présenté une épidémie de *Mycoplasma pneumoniae* en 1990-91. Des analyses sérologiques adéquatement sélectionnées peuvent offrir un diagnostic rapide et précis.

MYCOPLASMA PNEUMONIAE INFECTIONS ARE COMMON, occur worldwide and range in severity from asymptomatic to mild respiratory infections, atypical pneumonia and extrapulmonary complications. If diagnosed early, *M pneumoniae* infections respond well to treatment with tetracycline or erythromycin. There is no pathognomonic clinical feature. The specific diagnosis of *M pneumoniae* requires laboratory testing but there is no 'gold' standard as cultivation rarely is attempted (1). Traditionally, the diagnosis of *M pneumoniae* has relied on the complement fixation test (2) with the *M pneumoniae* antigen included in a battery of tests for respiratory viruses. For proper interpretation of results, the complement fixation test requires the simultaneous testing of acute and convalescent sera, thus delaying the diagnosis. Tests for cold agglutinins lack sensitivity and specificity (3). Recently, a number of tests for the detection of specific immunoglobulin (Ig) M antibodies have been developed, eg, indirect fluorescent antibody (IFA-IgM). The specific IgM response has been studied (4); the detection of IgM antibodies in a single acute serum establishes an etiological diagnosis and thus allows treatment to be started earlier.

The present report on the serodiagnosis of *M pneumoniae* in Manitoba shows that an epidemic was experienced in 1990-91. The limitations of serological testing are discussed.

METHODOLOGY

In Manitoba, the Cadham Provincial Laboratory is the sole provider of serodiagnostic testing for *M pneumoniae* and respiratory viruses, using a complement fixation test. In addition, an IFA test for the detection of IgM antibodies has been performed since 1987. The antigen used in the complement fixation test is an ether extract of mycoplasma organisms (Microbix Biosystems

Inc) and the test is performed as standardized by the Centers for Disease Control (2), usually on paired sera. For the IFA-IgM test, a whole organism antigen (Behring Diagnostics, Behringwerke AG, Marburg, Germany) is spotted on the slide and the conjugate is a sheep antihuman IgM (Wellcome Diagnostics, Dartford, Kent, United Kingdom). All positive sera are absorbed by Gull sorb (Gull Laboratories, Inc, Utah) to ensure the removal of all IgG antibodies and thus increase test specificity. The test is performed as previously described (5) on single, as well as on paired, sera. Criteria for the diagnosis of a recent *M pneumoniae* infection include a seroconversion, a fourfold rise in complement fixation antibodies, static high (at least 1:128) complement fixation antibody levels or the presence of IgM antibodies.

Results of complement fixation and IFA-IgM tests were compared. In persons with serological indication of a recent *M pneumoniae* infection, the number of sera required to establish the diagnosis, the age of the patient (younger or older than 16 years) and available clinical information (ie, pneumonia or upper respiratory tract infection) were recorded.

RESULTS

The number of sera tested each year, the number considered recent *M pneumoniae* infections and the basis for such a classification are recorded in the Cadham Provincial Laboratory annual reports and summarized in Table 1 for April 1987 to March 1991. Of the 12,804 persons tested, 509 (3.97%) were considered recent infections; of these, only 288 (56.5%) were positive for IgM antibodies.

More cases were diagnosed in 1990-91 than in the entire previous three years. On average, one to eight recent cases of *M pneumoniae* were reported every

month, reflecting the endemic level of *M pneumoniae* in Manitoba; the number of recent *M pneumoniae* infections started to increase in July 1990, peaked through the winter months, dropped slightly from April to June 1991, but increased again to a higher than average level until the end of September 1991 (Figure 1).

Of the 2088 persons tested between April 1 and September 30, 1991, 158 (7.5%) were diagnosed as recent *M pneumoniae* infections; of these, 102 (64.5%) were positive for IgM antibodies. Of the 856 single sera tested, 59 (6.8%) were recent *M pneumoniae* infections; 56 (96.1%) were IgM-positive and three had high static complement fixation antibodies. Of the 616 persons submitting paired sera, 99 (16%) were recent infections; of these only 46 (46.4%) had IgM antibodies.

Three points were of particular interest: a seroconversion was detected by IFA-IgM in 22 and by complement fixation in 44 persons; static high complement fixation antibodies were found in 53 persons who were negative by IFA-IgM testing; and in 24 persons, IgM antibodies were detected in both blood samples submitted. Of the 158 recent cases of *M pneumoniae*, only 72 (45.5%) were under the age of 16 years and of these 54 (75%) were positive by IFA-IgM. Of the 86 recent cases of *M pneumoniae* who were 16 years of age or older, only 30 (34.8%) were IgM-positive.

The clinical information given on the requisition indicated an upper respiratory illness in 60 (38%) of the 158 recent cases; of these, 31 (51.6%) were under the age of 16 years. A pneumonia was specified in an additional 48 persons (ie, 30.3%), of whom 21 (55.2%) were under the age of 16 years.

DISCUSSION

In 1990-91, Manitoba experienced an increase in the number of recent *M pneumoniae* infections diagnosed by serological testing. As reviewed by Broughton (6), epidemic outbreaks occur at irregular intervals and can persist for several months, with the incidence of infection three to five times the level seen in other periods. Figure 1 clearly shows that an epidemic occurred in Manitoba in 1990-91 and persisted for over a year.

When it became obvious that Manitoba was experiencing an epidemic of *M pneumoniae*, questions were raised over the value of testing single versus paired sera, the age group more likely to be infected and the relevance of serological testing to the clinical diagnosis. In an attempt to address these issues, data collected from April 1 to September 30, 1991, were analyzed. Despite the limitations inherent in any retrospective study, this analysis revealed some useful information.

The presence of IgM antibodies indicates a primary infection while their absence, in a person with seroconversion or a fourfold rise in complement fixation antibody titres, points to a reinfection (7). Reinfections are common (8). In the present study, primary infections

TABLE 1

Number of cases of recent *Mycoplasma pneumoniae* infections diagnosed serologically on an annual basis from April 1, 1987 to March 31, 1991

Testing period: April 1 to March 31	Number of recent infections/ Number of sera tested	% positive
1987-88	101/2199	4.9%
1988-89	22/2653	0.8%
1989-90	87/3737	2.3%
1990-91	299/4215	7%
Total	509/12,804	3.97%

Figure 1) Number of cases of *Mycoplasma pneumoniae* infection in Manitoba detected each month from January 1, 1990 to August 31, 1991

were detected in 102 (64.5%) of the 158 recent cases and reinfections in the remaining 56 persons. In the under 16 years age group, 54 (75%) of the 72 recent infections were primary, while in the 16 years and older age group only 48 (55.8%) of the 86 cases had IgM antibodies. A significant decrease in the production of specific IgM antibodies has been previously reported in the older age group (4), suggesting that, in this group, reinfections are more common than primary infections. In the present study, reinfections were detected in 18 persons under the age of 16 years and in 38 persons 16 years or older, thus confirming the findings of Moule et al (4).

Both the complement fixation and IFA-IgM tests have their limitations. The specificity of the complement fixation test has been challenged; unspecific reactions are due mainly to the glycolipid antigen in current use. Specificity will be improved in the future when the 168 kDa antigen (9) becomes available commercially. Kenny et al (1) reported a sensitivity of 90% for the complement fixation when compared with cultures for *M pneumoniae*; in their comparison, low static complement fixation antibody was indicative of recent infections. In the present study, low static complement

fixations were not counted to avoid nonspecific reactions; thus the actual number of genuine cases of recent *M pneumoniae* was probably underestimated. Static high complement fixation antibodies (ie, 1:128 or less) were included as recent cases because complement fixation antibodies may be high at the time of acute illness and fourfold changes in titre may occur in a short time interval (6). The complement fixation test plays an important role in the serodiagnosis of *M pneumoniae* despite the need to collect a second blood sample two to three weeks after the first one; without the complement fixation test, 56 (35.4%) of 158 recent *M pneumoniae* cases would have been missed.

Sillis (7), reviewing the limitations of testing for IgM antibodies, reported that 40 of 50 persons (80%) with IgM antibodies detected by IFA were culture-positive and represented genuine primary infections. In primary infections, IgM antibodies appear seven to 14 days after the onset of illness, peak between days 10 and 30 and

disappear after 12 to 26 days (4). Using an IgM capture enzyme-linked immunosorbent assay (ELISA), Vikerfors *et al* (10) found that on day 8, 40% of their study cases of *M pneumoniae* had detectable IgM antibodies while on day 10 post onset of illness, 75% were positive for IgM antibodies. Therefore, while the detection of IgM antibodies in an acute serum established the diagnosis of *M pneumoniae*, their absence does not exclude *M pneumoniae*. A second blood test is required to diagnose all primary infections. Both tests (ie, complement fixation and a test for the detection of IgM antibodies) should be performed on the second blood sample to detect all reinfections as well as primary infections.

In summary, Manitoba has experienced an epidemic of *M pneumoniae* infections. Acute and convalescent sera must be tested by carefully selected serological tests to ensure the diagnosis of all recent primary infections as well as reinfections. *M pneumoniae* causes significant morbidity in all age groups.

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REFERENCES

1. Kenny G, Kaiser G, Cooney M, Foy H. Diagnosis of *Mycoplasma pneumoniae*: Sensitivities and specificities of serology with lipid antigen and isolation of the organism on soy peptone medium for identification of infections. *J Clin Micro* 1990;28:2087-93.
 2. Anonymous. US Department of Health, Education and Welfare Public Health Service. Standard diagnostic complement fixation methods and adaptation to microtests. Centres for Disease Control, Atlanta, 1974.
 3. Purcell R, Chanock R. Role of mycoplasmas in human respiratory disease. *Med Clin North Am* 1967;51:791-802.
 4. Moule J, Caul E, Wregghitt T. The specific IgM response to *Mycoplasma pneumoniae* infection interpretation and application to early diagnosis. *Epidemiol Infect* 1987;99:685-92.
 5. Carter J, Carter S. Acute phase indirect fluorescence antibody procedure for the diagnosis of *Mycoplasma pneumoniae* infection. *Ann Clin Lab Science* 1983;13:150-5.
 6. Broughton R. Infections due to *Mycoplasma pneumoniae* in childhood. *Pediat Infect Dis* 1986;5:71-85.
 7. Sillis M. The limitations of IgM assays in the serological diagnosis of *Mycoplasma pneumoniae* infections. *J Med Microbiol* 1990;33:253-8.
 8. Foy H, Kenny G, Sefi R. Second attacks of pneumonia due to *Mycoplasma pneumoniae*. *J Infect Dis* 1977;135:673-7.
 9. Jacobs E, Buchholz A, Kleinmann B, Bredt W. Use of adherence protein of *Mycoplasma pneumoniae* as antigen for ELISA. *Israel J Med Sci* 1987;23:709-12.
 10. Vikerfors T, Brodin G, Grandien M, Hirschberg L, Krook A, Pettersson C. Detection of specific IgM antibodies for the diagnosis of *Mycoplasma pneumoniae* infections: A clinical evaluation. *Scand J Infect Dis* 1988;20:601-10.
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