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Mycoplasma Pneumoniae infections in the Maltese islands

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This article is based on a paper presented at the IVth Maltese Medical School Conference held at the Mediterranean Conference Centre on 10-14th March 1999.

ABSTRACT: *Mycoplasma pneumoniae* is a free living micro-organism which is classified in the family Mycoplasmataceae. It is primarily a human respiratory pathogen and the infection may vary from a mild pharyngitis to a pneumonia. *M. pneumoniae* infections are usually endemic in the community but periodic epidemics can also occur. These micro-organisms are sensitive to specific antibiotics such as tetracycline and erythromycin and a laboratory diagnosis using rapid methods is therefore important. Studies were carried out at the Virology Laboratory, St. Luke's Hospital involving 1,022 cases during the period 1995 to 1998. These were examined for specific anti-mycoplasmal IgM antibodies using an enzyme immunoassay (EIA) technique. One hundred and forty samples or 13.7% were found to be reactive. Compared to the traditional cultural methods, EIA techniques are very rapid laboratory diagnostic methods, the result being communicated to the clinician within a few hours. The differential diagnosis of the particular case can thus be resolved quickly and appropriate antibiotic therapy instituted without much delay for the maximum benefit to the patient.

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Introduction

Mycoplasma pneumoniae, also called Eaton's agent after the investigator who discovered it, is a free-living micro-organism which is classified in the genus Mycoplasma in the family Mycoplasmataceae [1]. It is a human pathogen and mainly causes respiratory diseases ranging from mild pharnygitis to tracheobronchitis and pneumonia[2]. The latter infection is usually insidious in onset with generalised malaise, pyrexia and headache. The symptoms usually increase within a couple of days and a hacking non-productive cough develops. Tiredness and marked weakness are also common. A maculopapular rash may appear in about 15% of cases. Muscle tenderness and arthralgias are common in all age groups. The infection is seen especially in older children and young adults, being milder and asymptomatic in pre-school children[3,4]. *M. pneumoniae* infections are endemic in the community but epidemics occur every 4-5 years. Non-respiratory manifestations caused by *M. pneumoniae* include mucocutaneous eruptions and haemolytic anaemia[5] the latter being possibly due to the development of 'cold agglutinins', pericarditis and myocarditis[6] and neurological involvement such as meningism, aseptic meningitis or meningoencephalitis[7].

M. pneumoniae forms pleomorphic filaments with an average diameter of about 0.5μm and lacks the peptidoglycan cell wall component characterisitic of bacteria. The absence of the peptidoglycan makes this micro-organism resistant to penicillins, cephalosporins and other antibiotics which interfere with the integrity of this structure. The cytoplasmic contents are enclosed in a well-developed plasma membrane. The major antigenic determinants are present in this membrane and are composed of glycolipids and proteins. Cross-reactivity of these antigens with human tissues and other micro-organisms occurs. *M. pneumoniae* is fastidious and grows slowly on complex enriched media giving rise to colonies which have a homogenous granular morphology unlike the "fried-egg" appearance of other my-

	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec
Reactive Samples	38	33	19	27
Non Reactive Samples	348	243	157	163
Total No. of samples tested	386	276	176	190

Table 1 - Total Mycoplasma samples according to season

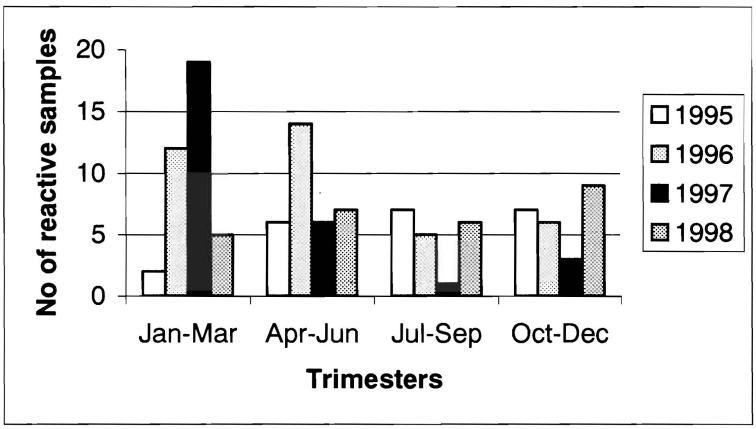

Fig 1 - Reactive Mycoplasma samples according to season (n=117)

coplasmas. The colonies are very small and details can only be seen with a colonial microscope. Unlike other species which are facultatively anaerobic, *M. pneumoniae* grows aerobically[8].

Although mycoplasmas are considered to be bacteria, the laboratory diagnosis of this 'paraviral' is traditionally carried out in a virology laboratory. Besides cultural methods, the serological assays most frequently employed are the cold agglutination and complement fixation tests and enzyme immunoassays.

Materials and Methods

Studies were carried out at the Virology laboratory, St. Luke's Hospital. All requests for the detection of Mycoplasma IgM antibodies during the four-year period 1995 to 1998 were entered into a database. Three percent of the samples had to be excluded because they lacked essential data or the samples were haemolysed. Repeats were also excluded. One thousand and twenty-two samples were included and of these 52% were from male and the rest from female patients. Ninety-four percent of all referrals were from general hospitals (St. Luke's and Gozo General Hospital), 4% were from outpatients' clinics and only 2% were referred from the community (health centres and general practice). The majority of samples were obtained from patients suffering from respiratory disease or from cases of pyrexia of unknown origin. The patients' age varied from infants of a few months to elderly people in their eighties. The test chosen for the laboratory diagnosis was an enzyme immunoassay test for the detection of anti-mycoplasma IgM antibodies and adapted to microtitre plate technology employing the reverse EIA technique (Savyon Diagnostics Ltd, Israel. 1994).

Results

Out of 1,022 samples examined, 140 or 13.7% were found to be reactive to *M. pneumoniae*. Table 1 and Figures depict the reactive samples according to season, age group, sex and locality. The data in each Figure may not necessarily add to the total number of reactive samples because the data on some cards submitted to the laboratory with the samples was missing or the writing was illegible. Table 1 and Figure 1 show that out of 117 reactive samples, 38 were positive during the January-March period, 33 in the second period, 19 in the third and 27 during the October-December period. Figure 2 shows 99 reactive samples according to age groups. Twenty-five were in the 0 to 5 years age group, 41 in the 6 to 20 years age group while 33 were present in the group 21 years and over. Table 2 and Figure 3 show the number of reactive samples according to sex. Fifty-six out of 114 samples were from males while 58 were from females. Figure 4 shows locality distribution with three zones, North, Central and South, dividing the Maltese population into approximately three equal population zones. Twenty-six samples came from patients living in the North part of the islands including Gozo. Thirty-four samples came from the Central region while 33 reactive samples originated from the South.

As in similar studies[9] local *M. pneumoniae* infections were found to occur throughout the year with no consistent increased seasonal variation, although in our study, there was a slight increase in the number of reactive cases in the winter of 1997 (Fig. 1). Disease was more common in the younger age group (Fig. 2) and there were no overall differences between the sexes (Fig. 3) or between the different localities of the Maltese Islands (Fig. 4).

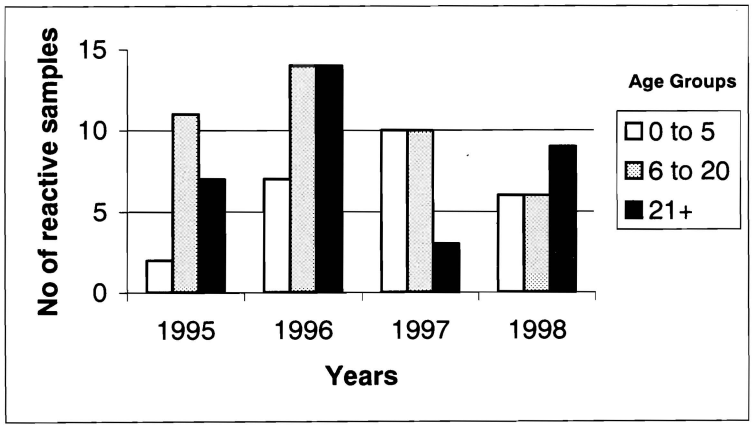

Fig 2 - Reactive Mycoplasma samples according to age group (n=99)

Discussion

Culture for *M. pneumoniae* can be carried out for the laboratory diagnosis of *M. pneumoniae* infection but growth is slow and the result can only be expected in about 2 to 6 weeks[10]. The cold agglutination test is carried out by reacting the patient's serum with human group O erythrocytes incubated at 4 °C. It appears that *M. pneumoniae* possesses a surface antigen which is similar to the I antigen of these red blood cell types. A positive cold agglutination assay is observed, on average, in

	1995	1996	1997	1998
Non reactive samples	188	365	224	132
Reactive samples	22	37	28	27
Total samples tested	210	402	252	159
Males	105	210	114	81
Females	95	192	138	78

Table 2 - Total Mycoplasma samples according to sex

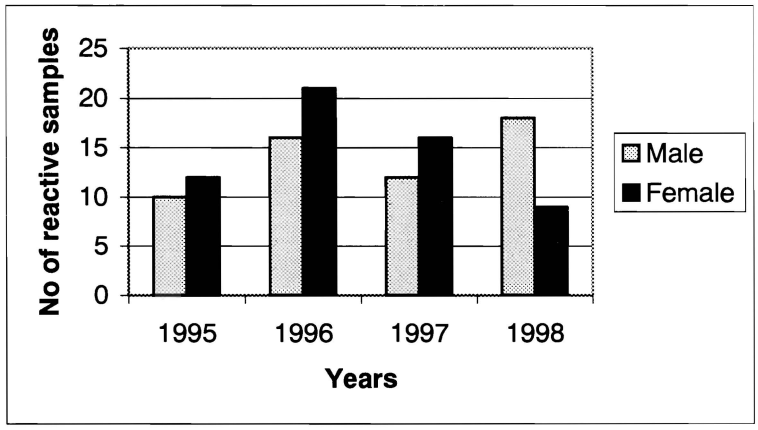

Fig 3 - Reactive Mycoplasma samples according to sex (n=114)

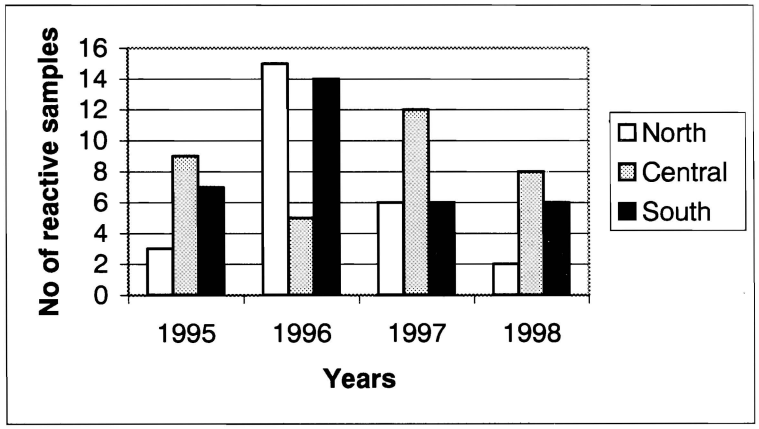

Fig 4 - Reactive Mycoplasma samples according to locality (n=93)

only 50% of patients with *M. pneumoniae* infection. The test is not specific for *M. pneumonaie* and cross-reactions occur with other infective agents e.g. Epstein-Barr virus and Adenovirus[11].

The complement fixation test utilises a lipid extract of the pathogen but this has been shown to cross-react with components of human tissue as well as bacterial glycolipids. It requires complicated reagent titrations which makes this test somewhat difficult to perform even by experienced laboratory workers. The test is usually significant if rising titres of antibodies can be demonstrated in two consecutive specimens taken at least 10 days apart and, therefore, introducing a delay in diagnosis.

The EIA test employed in our study is a rapid method of diagnosis, the result being transmitted to the remitting medical officer in a few hours. The test is also easier to perform than the complement fixation test and it shows higher sensitivity and specificity[12].

Although this study included all the referrals for Mycoplasma antibody tests, the total number of samples was small and, correspondingly, the number of positives was also small. It is therefore difficult to draw firm conclusions and perhaps a larger follow-up study is indicated.

Conclusion

A rapid method has been utilised to carry out the laboratory diagnosis of *M. pneumoniae* infections. Since this micro-organism is usually sensitive to antibiotics of the tetracycline and macrolide classes, it follows that appropriate therapy can be started without much delay and therefore to the maximum benefit of the patient. It also follows that clinicians should keep *Mycoplasma pneumoniae* in mind whenever the differential diagnosis of a respiratory infection is considered.

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