

## Seroprevalence of anti-*Mycoplasma pneumoniae* antibodies in otherwise healthy children

### Seroprevalencia de anticuerpos anti-*Mycoplasma pneumoniae* en niños menores de 12 años

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#### What do we know about the subject matter of this study?

*Mycoplasma pneumoniae* infection can occur in children with both pulmonary and extra-pulmonary manifestations. Such manifestations have been reported in numerous studies, using specific laboratory tests.

#### What does this study contribute to what is already known?

This study provides information on the presence of antibodies against *Mycoplasma pneumoniae* in healthy children from an early age, showing that this infection is not exclusive to adolescents or young adults.

#### Abstract

*Mycoplasma pneumoniae* (*Mypn*) infection could be occurring at an earlier age due to social phenomena such as attending daycare centers more frequently and earlier than decades ago. **Objective:** to estimate the prevalence of anti-*Mypn* antibodies in children aged 0-12 years, and to explore whether age, attendance to daycare center/school, overcrowding or the presence of children aged below 12 years in the households increase the risk of seropositivity. **Patients and Method:** Cross-sectional study including healthy children aged 0-12 years which required blood draws for routine laboratory tests. In all cases, the aforementioned variables were recorded and anti-*Mypn* IgG was determined by enzyme immunoassay. The association between predictors and seropositivity was assessed in a logistic regression model. **Results:** We included 232 patients (average age  $56.4 \pm 40.0$  months). 56.9% attended a daycare center/school, 63.8% co-habited with children under 12 years old, and 15.9%

#### Keywords:

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lived in overcrowded households. The prevalence of anti-*Mypn* antibodies was 14.6%. There were no significant differences between seropositive and seronegative children regarding age ( $63.1 \pm 40.7$  vs.  $55.4 \pm 41.3$  months), school/day-care attendance (64.7% vs. 55.5%), overcrowding (14.7% vs. 14.9%), or co-habiting with children (64.7% vs. 63.6%). Age was not an independent predictor of seropositivity in the multivariate model. **Conclusion:** The prevalence of anti-*Mypn* antibodies in children was 14.6% and age was not a predictor of seropositivity.

## Introduction

Acute respiratory infections are a prevalent cause of morbidity and mortality in childhood<sup>1</sup>, where *Mycoplasma pneumoniae* (*Mypn*) is one of the pathogens frequently involved in these conditions<sup>2</sup>. *Mypn* can cause pneumonia<sup>3</sup>, even highly severe<sup>4</sup>.

Prevalence data on *Mypn* infection are variable and can be influenced not only by local epidemiology but also by the method used to identify the infection. Therefore, the presence of IgG antibodies has been reported in 50% of children under 12 years, and at least 80% of adults<sup>5</sup>. In Barakaldo, Spain, there is 37.5% of prevalence in children aged between 1 and 5 years<sup>6</sup>; in Diyarbakir, Turkey, it is 10%<sup>7</sup>; and in Buenos Aires, Argentina, it reaches 6.9% in children under 5<sup>8</sup>.

While *Mypn* infection was considered typical of school-age children, adolescents and/or young adults for many years, the new evidence points out that it may occur at younger ages. This could be related to social changes, such as attending day-care centers more frequently and at younger ages<sup>9</sup>.

Although our current treatment schemes are appropriate to address community-acquired pneumonia<sup>10-12</sup>, there is a need for monitoring epidemiological changes that may require re-evaluation.

Moreover, in Buenos Aires, previous data from our institution<sup>8,13</sup> allow us to objectively compare the seroprevalence of anti-*Mypn* antibodies throughout 25 years, providing useful information to monitor the epidemiology of this microorganism.

Therefore, the objectives of this study are to estimate the prevalence of anti-*Mypn* antibodies in children aged between 0 and 12 years and to explore whether age, attendance at a day-care center/school, overcrowding, or living with children under 12 years of age increase the risk of seropositivity for *Mypn*.

## Patients and Method

Cross-sectional study, including healthy children aged between 0 and 12 years who required blood sampling for pre-surgical studies of planned surgery at the *Hospital General de Niños Pedro de Elizalde* during the

study period, and whose mother, father or legal guardian signed informed consent to participate in the study (and child's assent, where applicable). We excluded children with acute infectious condition, any known chronic or acute pathology, or previously known immune disorders.

All patients in the waiting room of the hospital's blood collection service were called consecutively, identifying those with pre-surgical routines for planned surgeries such as inguinal hernias, tonsillar hypertrophy, phimosis, cryptorchidism, among others. The parent or guardian of each child was invited to participate, signing the respective informed consent.

One of the researchers interviewed the caregiver in order to record information related to the variables under study. Patients were included between 23/08/2018 and 27/02/2019 until reaching the calculated sample size.

## Variables

The following variables were established:

- **Prediction variable:** Age in months (calculated based on birth date and blood sampling date). In the logistic regression model, this variable was considered as dichotomous (< 5 years and  $\geq$  5 years).
- **Primary outcome variable:** Serology (IgG) for *Mypn* (considered positive or negative according to the cut-off value described in the section related to the diagnostic test).
- **Variables to be monitored:**
  - Attendance to day-care center/school (attendance of 4 hours a day for at least 3 times a week, in the last 2 months).
  - Overcrowding (more than 3 people per room other than the bathroom and kitchen).
  - Living with children under 12 years of age (the presence of cohabitants from this age group was recorded).

## Laboratory procedure

From the blood sample collected by venipuncture for the requested pre-surgical studies, at least 1.5 mL of blood was taken. The resulting serum was divided into two aliquots, one was at least 0.3 mL. The samples were stored at  $-20^{\circ}\text{C}$  until processing.

The determination of anti-*Mypn* antibodies (IgG) was carried out through enzyme immunoassay (EIA), using Vircell SL equipment. (Granada, Spain).

The optical density (OD) was measured with an EIA reader at 450 nm, along with the bichromatic measurement at a 620 nm reference wavelength. Each assay uses a positive control, a negative one, and a cut-off. The OD of the controls must be in the following ranges: positive control > 0.9; negative control < 0.5; cut-off > 0.55 and < 1.5 (otherwise the test is ruled out). Antibody index = (OD of sample/serum OD cut off) x 10: < 9 negative; 9 - 11 uncertain; > 11 positive. Kit reference: G1002. The manufacturer reports 98% of sensitivity and 97% of specificity ([http://peramed.com/peramed/docs/G1002\\_EN.pdf](http://peramed.com/peramed/docs/G1002_EN.pdf)).

According to the protocol, the results of the serology were reported to the patients on request, when available.

### Sample size determination

It was determined based on the known prevalence of anti-*Mypn* antibodies (IgG), for 2 different age ranges. Data were collected from a previous study, where it was observed that the prevalence of seropositivity was different for those over and under 5 years of age (7% and 20%, respectively) (10). Given the large variability of the topic in the literature, a confidence interval between 12% and 27%, respectively, was considered. To calculate the sample size, we considered approximately 1,000 potentially enrollable patients who visit the surgery service annually (data from the last 3 years), with an age distribution of 65% younger and 35% older than 5 years. Therefore, with a 95% confidence interval, 121 subjects aged 0-4 years and 92 aged 5-12 years are required. Assuming that up to 5% of serums may not be evaluable, we decided to incorporate 130 and 100 subjects, respectively.

### Ethical considerations

The study was carried out according to the standards of good clinical practice, the Declaration of Helsinki, and the regulations in force of the Government of Buenos Aires. The study was approved by the Research Ethics Committee and the Institutional Review Board of the Hospital and the project was registered in the Health Research Council of the Government of Buenos Aires. In all cases, the patients' guardian signed the informed consent to participate in the study and we ask those patients older than 7 years to give their assent. The confidentiality of the subjects' identity was guaranteed according to the procedures established in the protocol.

### Statistical processing

The prevalence of seropositivity for *Mypn* was determined for the whole population and each age

group (CI 95%). To examine possible factors associated with seropositivity for *Mypn*, we used the Chi-Square test for categorical variables and the Student's t-test in independent samples for continuous variables (after verifying goodness of fit for normality using the Kolmogorov-Smirnov test). Age (over and under 5 years) was assessed as a predictor of seropositivity through logistic regression in a model that included day-care-center/school attendance, overcrowding, and living with children as control variables. A significance level < 0.05 was considered. In addition, we evaluated, using the ROC curve (including the area under the curve -AUC- and its CI 95%), if there was a better age cut-off point to predict seropositivity. Data processing was performed with IBM SSPS Statistics 20.0 software.

## Results

We included 232 patients (129 < 5 years and 103 ≥ 5 years), with mean age 56.4 ± 40.0 months. One hundred and thirty-two patients attended day-care center/school (56.9%), 148 lived with children under 12 years of age (63.8%), and 37 patients lived in overcrowded households (15.9%). Anti-*Mypn* antibodies (IgG) were present in 14.6% (CI 95% 10.6-19.7) of our patients (table 1).

When stratified by age group, the proportion of seropositivity in the group under 5 years was 13.9%, and 15.5% in the group aged ≥ 5 years (OR: 1.1 CI 95% 0.5-2.3; p = 0.8). This age cut-off point for identifying seropositivity presented 47.1% of sensitivity (CI 95% 30.1-64.6), 56.1% of specificity (CI 95% 48.8-63.1), 15.3% of positive predictive value (CI 95% 9.4-24.3), 86.1% of negative predictive value (CI 95% 78.5-91.3), positive likelihood ratio 1.1 (CI 95% 0.7-1.5), and negative likelihood ratio 0.9 (CI 95% 0.6-1.3).

The seropositive children did not show significant differences with the seronegative ones regarding the mean age (63.1 ± 40.7 vs. 55.4 ± 41.3 months; p = 0.5), day-care center/school attendance (64.7% vs. 55.5%; p = 0.3), overcrowding (14.7% vs. 14.9%; p = 0.5), or living with children under 12 years of age (64.7% vs. 63.6%; p = 0.5).

In the multivariate analysis, after controlling by day-care center/school attendance, cohabitants under 12 years of age, and overcrowding, older age also does not appear as an independent predictor of seropositivity for *Mypn* (table 2).

In order to assess if there was a better age cut-off point to predict seropositivity for *Mypn*, we used the ROC curve which also showed very low predictive ability (aucROC = 0.53; CI 95% 0.4-0.6).

**Table 1. Characteristics of the population in which IgG antibodies to *Mycoplasma pneumoniae* were screened**

	Ig G Mypn Positive (n = 34)	Ig G Mypn Negative (n = 198)	p
Age (months)	63.1 ± 40.7	55.4 ± 41.3	NS
Day-care center/school attendance (%)	64.7	55.5	NS
Overcrowding (%)	14.7	14.9	NS
Living with children aged under 12 years (%)	64.7	63.6	NS

**Table 2. Multivariate analysis including potential predictors and seropositivity to *Mycoplasma pneumoniae***

	Significance	OR	95% CI	
			Inferior	Superior
Day-care center/school attendance	0.2	1.7	0.6	4.7
Overcrowding	0.6	0.7	0.2	2.3
Living with children aged under 12 years	0.4	1.1	0.7	1.7
Age	0.6	0.7	0.3	2.1
Constant	0.001	0.1		

## Discussion

*Mycoplasma pneumoniae* is a common cause of respiratory infections in childhood, so one would expect a significant number of healthy children to have specific antibodies (IgG). In our study, there was 14.6% of prevalence of anti-*Mypn* antibodies in children aged between 0 and 12 years.

There is relatively little information on the seroprevalence of anti-*Mypn* antibodies in healthy children. The figures of our study are similar to that reported by Lezcano et al. in Buenos Aires (12.4%) in 2008<sup>8</sup> and to that referred by Tuuminen et al. in Finland, who reported a prevalence between 13 and 19% in children under 4 years of age<sup>14</sup>.

In a previous study, we reported that seroprevalence increased significantly with age, however, we could not corroborate it in this one. In such a study, we found that 6.9% of the children under 5 years of age and 24.7% of those over that age had anti-*Mypn* antibodies, a difference statistically significant<sup>8</sup>. In this study, the mean age showed no difference between seropositives and seronegatives, nor did the proportion of seropositives between the two age groups studied (0-4 and 5-12 years). This could be explained because seroconversion currently occurs at younger ages, as a result of social changes that are difficult to demonstrate<sup>9,15</sup>.

We evaluated three variables related to this phenomenon, attendance at day-care centers/schools, coha-

bitants under 12 years of age, and overcrowding, but we could not verify the change. Moreover, the proportion of subjects attending day-care centers/schools in the group under 5 years of age was similar between this study and that referred to by Lezcano et al. (56.9% and 57.6%, respectively).

A similar result was observed concerning cohabitants under 12 years of age (63.8% and 70.1%) and overcrowding (15.9% and 27.3%)<sup>8</sup>. This is consistent with a study carried out in Chile, which found that over 10 years (2003-2014) seropositivity for *Mypn* (IgM, probably secondary to acute infection by the microorganism) in children aged under 5 years increased from 8.6% to 30% and that this change was especially evident in the 2 to 5-year-old group (33%)<sup>16</sup>. It also could be that, an increase in *Mypn* resistance to macrolides could facilitate its circulation, affecting a younger population<sup>17</sup>, but there are no local reports that support this option.

There are reports that *Mycoplasma pneumoniae* infection has increased, making it a relevant issue. Although the most frequent age of presentation of pneumonia due to *Mypn* ranges from 6 to 10 years, almost 15% of cases of pneumonia in children under 3 years of age may be due to this microorganism<sup>18</sup>. A report from Vietnam found that more than 50% of *Mypn* cases of pneumonia can occur in children under 5 years of age<sup>19</sup>.

A limitation of our study is that it was not developed as a population-based study. However, the diver-

sity of patients that are usually seen at the institution<sup>20</sup> probably reflects, at least, the population of the Buenos Aires Metropolitan Area, where approximately one-third of the country's population resides<sup>21</sup>.

It could be argued that the sample size calculation caused some kind of bias by assuming the age of 5 years as the cut-off point for seropositivity. However, in a previous experience using the same diagnostic methodology, we found that this fact was a strong predictor of seropositivity<sup>8</sup>. Furthermore, the difference in the prevalence between both groups in our study is so small that, if we maintain the observed proportions, it will not be enough to quadruple the sample to find a statistically significant difference, proving that the lack of significance is not due to a power problem.

Despite potential limitations, this study provides updated data on one aspect of *Mycoplasma pneumoniae* behavior in the region, confirming childhood seroprevalence values. Although it may be appropriate to carefully evaluate the results, it is essential to continue surveillance to allow a proper understanding of the changes observed with higher precision, and to monitor epidemiological changes that could lead us to re-evaluate the community-acquired pneumonia current antibiotic treatment<sup>22</sup>.

In conclusion, in our study, the prevalence of anti-*Mypn* antibodies in children aged 0-12 years was 14.6%. We could not demonstrate that age would act as a predictor of it. The age of seroconversion may be lower than that observed in previous decades.

## Ethical Responsibilities

**Human Beings and animals protection:** Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

**Data confidentiality:** The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

**Rights to privacy and informed consent:** The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

## Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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