Mycoplasma pneumoniae infections in schoolchildren of a tropical community

E. Campos[1]*, H. Bolaños[2], J. Serra[3], J. A. Ramírez[2], O. Barboza[3] and E. Jacobs[4]
[1] Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA)-DESAF, Tres Ríos, Costa Rica.
[2] Ministerio de Salud, San José, Costa Rica.
[3] Programa de Salud Comunitaria, Hospital sin Paredes, San Ramón, Costa Rica.
[4] Institute of Medical Microbiology and Hygiene, University of Freiburg, Germany.
* Corresponding author

Abstract: Information about *Mycoplasma pneumoniae* infections in tropical countries is scarce. This study reports the prevalence of anti-*M. pneumoniae* antibodies among schoolchildren from the tropical community of Palmares, Costa Rica. The serum samples were collected in July 1983 from 2021 children who represented 99% of the county's schoolchildren. Immunofluorescense (IFA) and in some cases ELISA serological methods were applied. IFA seropositivity was high (53%) and ranged from 22% to 79% depending on the district. Marked differences in the prevalence of antibodies in children attending schools of neighboring communities, suggest that the school may be epidemiologically important as a focus to spread mycoplasma infections among children. The presence of a low percentage of antibodies (IgG, IgM and IgA) against the 168 kd- *M. pneumoniae* antigen suggested that there were few cases of active or recent infections.

Key words: ELISA, IFA, *Mycoplasma pneumoniae* infections, schoolchildren, seroepidemiology, Costa Rica.

Mycoplasma pneumoniae has been well established as a major cause of acute respiratory infections (ARI) among children and young adults (Foy *et al.* 1971, Foy *et al.* 1979). The clinical manifestations caused by infection with this agent may include severe pneumonia (Biberfeld and Sterner 1969, Denny *et al.* 1971).

The ARI caused by *M. pneumoniae* are known to spread slowly in the community, affecting more frequently institutionalized individuals (Chanock *et al.* 1967, Evans *et al.* 1967). Additionally, 3 to 10% of the infected cases develop pneumonia (Foy *et al.* 1979).

The importance of *M. pneumoniae* infections in temperate countries has been well documented, but information from tropical and other countries with a low industrial level is virtually non-existent.

The World Health Organization review of ARI in the Americas reported that there are seven times more deaths due to respiratory diseases in Central America than in North America (PAHO 1980). This difference is partly due to nutritional problems, poverty, poor sanitation and fewer public health services in Central America. Even though in Costa Rica the ARI mortality in children over one year old has decreased significantly in the last 20 years, these infections are one of the most important causes of morbidity and hospital consultation (James 1972, Chavarría 1985).

The aim of this study was to determine the exposure to *M. pneumoniae* in children from a tropical community and to study the role of the school and the family in the transmission of these infections.

MATERIAL AND METHODS

Design: This work was part of a cross-sectional study conducted in Palmares County, Costa Rica, to evaluate the impact of a Community Health Program. Among other parameters, antibody prevalence against polio, measles, rubella, tetanus, hepatitis A and *M. pneumoniae* were used as biological indicators of the programe's effects on children's health. Palmares was also chosen because it had detailed individual health records, allowing us to recover information of epidemiological relevance.

With the writen consent of parents and collaboration of school teachers, 2021 sera samples were collected from schoolchildren, 5-12 years old, in July 1983. These represented 99% of the total schoolchildren's population for the county and included, in many cases, children that belonged to the same family nucleus. Serum specimens were obtained in the school by venipuncture and stored at -20°C until they were analyzed.

Palmares is a County located 53 Km from the Capital and is divided into seven districts which represent a variety of characteristics (Table 1, Fig. 1A).

TABLE 1

Characteristics of Palmares and its districts, 1984*

District	Population density		Type of Community
	Inhabitants/km²	Children/school**	
Palmares Centro	3 487	598	urban
Zaragoza	512	54-214-201***	semi-urban
Buenos Aires	447	278	semi-urban
Santiago	249	180	rural
Candelaria	220	129-39***	rural
Esquipulas	331	154	semi-urban
La Granja	482	202	semi-urban

* According to the National Population Census, 1984.
** Only children between 5 and 12 years old were included.
*** Each value corresponds to different schools in the same district

Although most children attended the school in their own district, 237 (12%) traveled to neighboring districts to study. These were grouped according to the school they attended and the district of residence (Table 2). However, only those groups with more than ten individuals were considered for analysis.

LABORATORY METHODS

Organisms: *M. pneumoniae* strain FH, kindly provided by W. A. Clyde, Jr., University of North Carolina, was grown and harvested as previously described (Jacobs *et al.* 1985).

Indirect fluorescent assay (IFA): Indirect immunofluorescence against whole *M. pneumoniae* F.H. cells was done in the serum samples of 2021 schoolchildren with a modification of a technique previously described (Wreightit 1985). Slides for IFA testing were prepared by diluting whole *M. pneumoniae* FH antigen in PBS pH 7.2 to an OD = 0.1079. Then, 10 µl of the suspension was dropped in each well of the glass microscope slides and dried at 37°C for 30 minutes. For the test, dilutions of the sera from 1:4 to 1:128 were made in PBS pH 7.2 and 10 µl of each were applied to antigen-coated wells. A positive and negative control serum were included in each assay. After incubating in a moist chamber for 30 minutes at room temperature, the slides were flushed with PBS pH 7.2, immersed for 10 minutes in the same buffer and then dried. Ten microliters of a 1:30 dilution of FITC goat anti-human polyvalent immunoglobulins (Sigma F-6506) were applied to each well. After incubation at 37°C for 30 minutes at room temperature (in the dark), the slides were washed as described before, dried and a coverslip (0.2 mm) mounted with glycerol mounting fluid (10% glycerol

in PBS pH 7.2). The slides were examined in a fluorescence microscope under 40x magnification. The end point was determined as the last serum dilution in which the intensity of fluorescence was three times higher than the negative control (one drop of PBS instead of serum was added to the antigen well).

From previous experience in our laboratory, we chose a cut-off value of 1:8, which gave us a sensitivity of 100% and an specificity of 72%, as indicative of exposure to *M. pneumoniae*.

Enzyme linked inmunosorbent assay (ELISA): The quantification of serum antibodies (IgG, IgM and IgA) against the 168 kd-*M. pneumoniae* (P1-antigen) was performed in the first 837 samples collected by the ELISA method, following known procedures (Jacobs and Clad 1986, Jacobs *et al.* 1986). The cut-off value was 0.4 (OD = 405 nm) at sera dilutions of 1:100 for the IgM and IgA and 1:200 for the IgG. The ratio of absorbance reading of a high positive sample to that of a negative sample was at least 10:1.

Statistical methods: Significant differences were determined using a Z-test for contrast between proportions of independent samples. Odds ratio (OR) calculations were used to compare the prevalence of infection in children attending schools among the different districts (Dean *et al.* 1988).

RESULTS

The population consisted of 1015 males and 1006 females. Fifty three percent of all the children studied had an antibody titer $\geq$ 1:8 against *M. pneumoniae*, using the IFA test. The males showed a lower percentage of seropositivity than the females, 50% and 56% respectively, with an OR= 0.81 (CI= 0.68-0.96, $p \leq 0.05$), after adjusting for age and district.

Significant differences were also found among the percentage of positive children from different districts (Table 2). The children who lived and attended school in Palmares Centro had the highest prevalence of seropositives (79%), while Zaragoza had the lowest (21%).

TABLE 2

*Prevalence of anti-*M. pneumoniae *antibodies among children studying in their own district of residence and children who travel to a school in a neighboring district*

School	District of residence	Total IFA analysis*				P-1 Elisa Test**			
		Total children	% positive	Odds ratio (Conf. interv. 95%)		Total children	% positive		
							IgG	IgM	IgA
Palmares Cent.	Palmares Cent.	474	79	1.0		400	3	3	3
La Granja	Palmares Cent.	18	53	0.33 (0.12-0.96)*****		0	-	-	-
Zaragoza	Zaragoza	53-198-154***	21-22-24	1.0		0	-	-	-
Palmares Cent.	Zaragoza	12	56	4.97 (1.38-18.54)*****		0	-	-	-
Buenos Aires	Buenos Aires	274	31	1.0		0	-	-	-
Palmares Cent.	Buenos Aires	35	69	4.85 (2.15-11.11)*****		0	-	-	-
Santiago	Santiago	164	46	1.0		114	0	6	11
Zaragoza	Santiago	51	29	0.49 (0.24-1.02)*****		0	-	-	-
Candelaria	Santiago	11	62	2.08 (0.52-8.83)		0	-	-	-
Candelaria	Candelaria	108-36***	67-58			138	3	14	4
Esquipulas	Esquipulas	137	46	1.0		137	4	4	7
Palmares Cent.	Esquipulas	61	83	5.99 (2.67-13.76)*****		48	4	2	8
La Granja	La Granja	186	59	1.0		0	-	-	-
Palmares Cent.	La Granja	19	88	5.87 (1.25-37.94)*****		0	-	-	-
Other****		30	51			0	-	-	-
Total	Palmares County	2021	53			837			

   *   Total-antibodies $\geq$ 1:8.

  **   Was done in the first 837 samples collected. A positive result was considered when O.D.(405 nm) $\geq$ 0.4 in a dilution 1:200 for IgG and 1:100 for IgM and IgA. (-) Indicates not done.

 ***   Each value corresponds to different schools in the same district.

 ****   These children traveled in groups with less than 10 individuals. Therefore, they were included only for the estimation of Palmares County seropositivity.

***** $p \leq 0.05$.

In general, children who lived in a district of low prevalence of IFA-seropositives, and traveled to a community with high prevalence, showed a higher percentage of seropositives than the ones that attended school in their own district of residence (Fig. 1B).
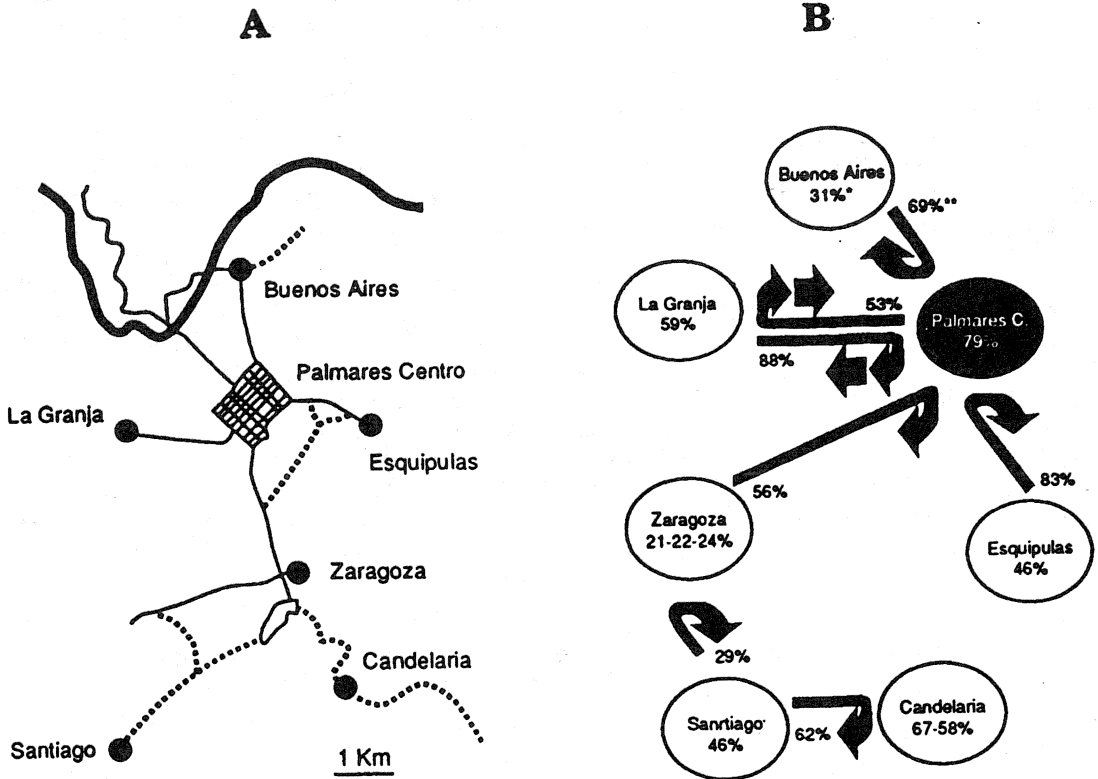
A

B

Fig. 1. A. Districts and main routes of communication in Palmares County, according to the National Census, 1984: (━) Highway, (—) Road, (---) Secondary road. B. Percent of seropositives (*) among individuals who attendent the school of its own district of residence, Candelaria and Zaragoza have two and three schools respectively; and (**) children who studied in schools of neighboring districts. The arrows indicate the way the children moved from one district to estudy in the neighboring school district.

In total, 1439 of the students belonged to families with two or more children included in the study, which allowed assessing family effect. The 2021 children were grouped according to district and number of family members participating in the study (Table 3). The prevalence of IFA antibodies in those groups was compared with the seropositivity of the corresponding district. Differences among the families with 1, 2, 3 or 4 children, within a district, were not significant, with the exception of the families with one schoolchildren from Palmares Centro, who had a higher percentage of positives than the families with two or more members (Table 3). Furthermore, differences between districts remained statistically signifi-

cant after adjusting by family groups, regardless of the number of children per family.

We observed that IgM and IgA positive titers (above 0.4 OD) were more frequent than IgG, in the 837 samples tested using ELISA (Table 2). The districts of Candelaria (14%) and Santiago (11%) showed the highest prevalences for IgM and IgA respectively.

DISCUSSION

There has been no systematic investigation in tropical countries on the occurrence of *M. pneumoniae* infections, and little is known about the prevalence of its antibodies and the relative burden of this agent on health in these areas.

TABLE 3

Comparison of the percentages of IFA seropositive children analyzed by district of residence and family nucleus conformed by 1, 2, 3 and 4 schoolchildren

District of residence	District %	Families with 1		Families with 2		Families with 3		Families with 4	
		No.*	% **	No.	%	No.	%	No.	%
Palmares Centro	79	178	85 ***	100	82	22	74	1	50
Zaragoza	22	139	27	85	24	17	22	6	17
Buenos Aires	31	93	32	55	31	15	44	1	50
Santiago	46	38	47	36	43	14	52	3	33
Candelaria	58	55	62	23	67	11	61	2	50
Esquipulas	46	46	46	40	51	16	49	1	25
La Granja	59	68	57	40	63	6	56	2	88
Total families		617		379		101		16	
Total children	49	617	52	758	52	303	52	64	45

*   Total number of families analyzed. Families with more than four schoolchildren were rare and therefore excluded.
**   Percent of seropositive schoolchildren.
***   p ≤ 0.05 when compared with seropositivity in the district.

In temperate countries, the frequency of *M. pneumoniae* infections is known to increase during winter months (Foy *et al.* 1979), while in the tropics, no specific studies on the seasonal variations of *M. pneumoniae* infections have been conducted. However, it has been observed that ARI increase during the rainy season (Monto and Johnson 1967). The samples for this study, were obtained during the rainy season of 1983. For that year, the Deparment of Statistics from the Ministry of Health, Costa Rica, reported a significant increase on ARI in relation to the previous three years, without identifying the etiology (División de Epidemiología, Ministerio de Salud de Costa Rica, 1983). Therefore, by coincidence, the sampling was performed during an ARI epidemic.

The data show that 53% of the schoolchildren had positive antibody titers against *M. pneumoniae* by the IFA technique. This prevalence is high and consistent with an epidemic of *M. pneumoniae*. Furthermore, when the information of different districts was analyzed separately, significative differences were observed: percentage of positives ranged from 21% in Zaragoza to 79% in Palmares Centro (Table 2). It is difficult to compare serologic data obtained by different procedures in several populations and at different times of the epidemic cycle; nevertheless our results ranged from as high as those reported by Brunner *et al.* 1977

(77%) and Pönkä and Ukkonen 1983 (77%) to as low as those obtained by Foy *et al.* 1971 (14%) and Golubjatnikov *et al.* 1975 (18%). The differences in the percent of seropositives observed among the Palmares's districts also suggests the coincidence of this study with an epidemic peak, which probably began in Palmares Centro, was spreading to the other communities, and yet had not affected some (like Zaragoza). This behavior is similar to the observation described by Foy *et al.* 1971, that the infection moved according to the supply of susceptibles, and the chance for effective contact causing minor localized epidemics. Surprisingly, in that year, epidemics caused by *M. pneumoniae* were also reported in several temperate countries (Ritter *et al.* 1983, Lind and Bentzon 1988).

The ELISA test was used to measure the titer of specific antibodies against *M. pneumoniae* P-1 protein. Since these antibodies increase and decrease more rapidly after infection (Jacobs *et al.* 1986), when compared with the IFA-total antibodies against the complete bacterial antigen (Hu *et al.* 1983), the presence of P-1 antibodies is associated with actual or very recent *M. pneumoniae* infection. Among the 837 children studied by this method, very few presented positive levels against P-1 protein, as expected, since they were attending school at the moment of sampling. The few that were found positive may have had a recent infection

or were in the course of a mild one, as observed by others (Foy *et al*. 1966, Biberfeld and Sterner 1969, Denny *et al*. 1971). The antibody responses, when positive, were mainly of the IgM and IgA type, as suggested by Chamberlain and Saeed 1983.

The districts of Candelaria and Santiago have the highest proportion of ELISA seropositive children (Table 2). Both have the smallest schools and are more rural and isolated (Table 1, Fig. 1). Furthermore, the two schools in Candelaria showed differences between them (58% and 67%), by IFA, in contrast to the behavior observed among the three schools of Zaragoza (21%, 22% and 24%). Again, the difference between the two schools of Candelaria may reflect an active epidemic process begining in that area and moving from Candelaria to Santiago. In this case, the children from Santiago who attended school in Candelaria could be taking the infection back to Santiago (Fig. 1).

In previous studies Foy *et al*. (1966) found that children 5-14 years old were the most common primary cases of infection in the family, acting as a reservoir and becoming the most important vector. In contrast to this observation, they considered that the " communicability " at school was low compared with the transmission within the family (Foy *et al*. 1971). In the present study, the antibody prevalence of children who travel to Palmares Centro School, from an outside district was compared with the antibody prevalence of the children attending their own district's school (Fig. 1). The risk of acquiring the infection was five times greater for children traveling to study in Palmares Centro, compared with the children studying in their own school district. The importance of the school in the transmission of the *M. pneumoniae* was also evident when children living in a high prevalence district traveled to study in schools of lower prevalence. In this case, the risk of acquiring the infection was lower for the children who traveled (Table 2, Fig 1B). Furthermore, there were no significant differences between the prevalence of *M. pneumoniae* in families with 1, 2, 3 or 4 siblings (Table 3).

Similar to temperate countries, for 5 to 12 year old children, females had a higher antibody prevalence (Golubjatnikov *et al*. 1975, Monto *et al*. 1975). The reasons are not known and deserve further investigation.

The results of this study suggest that in Palmares the school played an important transmission role by concentrating the susceptible individuals and increasing the risk of an outbreak that eventualy also hit families. This seems contrary to previous findings in countries with temperate climate (Foy *et al*. 1966). The reasons for these difference are not clear and should be subject for further studies.

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