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## **Pneumocystis primary infection in non-immunosuppressed infants in Lima, Peru**

Theresa Ochoa, Beatriz Bustamante, Coralith Garcia, Edgar Neyra, Karina Mendoza, Enrique Calderón, Solene Le Gal, Robert Miller, Carolina Ponce, Gilles Nevez, et al.

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1 *Pneumocystis* primary infection in non-immunosuppressed infants in Lima, Peru.

2

3 Short title. *Pneumocystis* primary infection in Peru

4

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30

31 **Key words:** *Pneumocystis jirovecii*; pulmonary colonization, primary infection; infants, Peru,

32

33 **Objectives.** To provide original data on *Pneumocystis* primary infection in non-  
34 immunosuppressed infants from Peru.

35 **Methods.** A cross sectional study was performed. Infants less than seven months old, without  
36 any underlying medical conditions attending the “well baby” outpatient clinic at one hospital  
37 in Lima, Peru were prospectively enrolled during a 15-month period from November 2016 to  
38 February 2018. All had a nasopharyngeal aspirate (NPA) for detection of *P. jirovecii* DNA  
39 using a PCR assay, regardless of respiratory symptoms. *P. jirovecii* DNA detection was  
40 considered to represent pulmonary colonization contemporaneous with *Pneumocystis* primary  
41 infection. Associations between infants’ clinical and demographic characteristics and results  
42 of *P. jirovecii* DNA detection were analyzed.

43 **Results.** *P. jirovecii* DNA was detected in 45 of 146 infants (30.8%) and detection was not  
44 associated with concurrent respiratory symptoms in 40 of 45 infants. Infants with *P. jirovecii*  
45 had a lower mean age when compared to infants not colonized ( $p < 0.05$ ). The highest  
46 frequency of *P. jirovecii* was observed in 2-3-month-old infants ( $p < 0.01$ ) and in the cooler  
47 winter and spring seasons ( $p < 0.01$ ). Multivariable analysis showed that infants living in a  
48 home with  $\leq 1$  bedroom were more likely to be colonized; Odds Ratio =3.03 (95%CI 1.31-  
49 7.00;  $p = 0.01$ ).

50 **Conclusion.** *Pneumocystis* primary infection in this single site in Lima, Peru, was most  
51 frequently observed in 2-3-month-old infants, in winter and spring seasons, and with higher  
52 detection rates being associated with household conditions favoring close inter-individual  
53 contacts and potential transmission of *P. jirovecii*.

54

55

## 56 **Introduction**

57 The primary infection with *Pneumocystis jirovecii* appears to be highly common during the  
58 first year of life in non-immunosuppressed infants. Indeed, in the early 1970s sero-  
59 epidemiological surveys suggested that first contacts with the fungus contemporaneously  
60 occurred with primary infection in 90% of humans during infancy [1, 2]. Initially, primary  
61 infection occurring in this context, was thought to be essentially asymptomatic [1, 2]. In the  
62 early 2000s, a study conducted in Chile showed that primary infection in non-  
63 immunosuppressed infants can be asymptomatic but also symptomatic [3]. *P. jirovecii* was  
64 detectable using a PCR assay in nasopharyngeal aspirate (NPA) samples from symptomatic  
65 infants developing primary infection with a frequency of 32% [3]. These low burdens of *P.*  
66 *jirovecii* detected using PCR were considered to reflect pulmonary colonization rather than  
67 overt *Pneumocystis* Pneumonia (PCP) since the infants recovered in the absence of specific  
68 anti-*Pneumocystis* treatment. Another study conducted in France showed that primary  
69 infection in non-immunosuppressed infants can be symptomatic and may or may not be  
70 associated with viral or bacterial infections, *P. jirovecii* being also detectable in NPAs using a  
71 PCR assay [4]. More recently, in Denmark, it was reported that primary infection in non-  
72 immunosuppressed infants frequently occurred in the context of a self-limiting upper  
73 respiratory tract infection (URTI) [5]. Data on primary *Pneumocystis* infection in non-  
74 immunosuppressed infants living in emerging countries from South America are scarce. In  
75 this context, the general objective of the present study was to provide original data on primary  
76 *Pneumocystis* infection in Peru, a upper-middle-income country of South America  
77 characterized by a Gross Domestic Product per capita of 6,710 US \$ in 2017. The specific  
78 objectives were to detect *P. jirovecii* in a cohort of *a priori* healthy infants, i.e. non-  
79 immunosuppressed infants at risk for primary infection considering their age, and to correlate  
80 *P. jirovecii* presence with putative specific clinical and demographic characteristics.

81

82 **Methods**

83 Hospital settings and infant population.

84 The free National Health System hospital network in Lima, Peru is divided into areas with  
85 outpatient clinics that serve populations living in these geographical locations. The outpatient  
86 clinics at Cayetano Heredia Hospital provide care to an infant population that lives in a  
87 densely populated neighborhood.

88 Criteria for enrollment of infants

89 Infants less than seven months of age, attending outpatient clinics for well-baby checks were  
90 prospectively enrolled in this cross-sectional study during a 15 month-period from November  
91 2016 to February 2018. They had an NPA for *P. jirovecii* detection regardless of respiratory  
92 symptoms. Infants were placed into one of six groups based on age: one month, two months,  
93 three months, four months, five months, and six months. Infants' clinical and demographic  
94 data were collected using a case report form. Demographic data consisted of age, sex, type of  
95 birth and birth weight. At enrollment, information about respiratory symptoms that had  
96 occurred during the previous two weeks, including the day of enrollment, were collected.  
97 Parents were subsequently contacted by telephone 15 days later to ask about occurrence of  
98 any respiratory symptoms following the clinic visit.

99 Infants with significant chronic disease (e.g. HIV infection, primary immunodeficiency,  
100 congenital heart disease, Down syndrome), previous hospitalization (except that related to the  
101 birth period and who were discharged  $\leq 3$  days after birth), mothers  $< 18$  years of age, or  
102 infants whose parents could not be contacted for the 15 days follow up, were not enrolled.

103 Sampling and *P. jirovecii* detection.

104 One NPA specimen was collected per infant. Specimens were transported to the clinical  
105 mycology laboratory at Universidad Peruana Cayetano Heredia in Lima in a cooler with ice  
106 packs. DNA was extracted using a commercial kit following manufacturer's instructions  
107 (QIAamp® DNA Mini Kit, Qiagen). *P. jirovecii* DNA detection was performed using a  
108 nested-PCR assay that amplifies the mitochondrial large subunit rRNA gene. The primers and  
109 methods were described elsewhere [3, 6, 7]. Each specimen was also examined with a PCR  
110 assay targeting the  $\beta$ -globin gene to estimate cellular quality of specimens and the presence of  
111 potential inhibitors [8]. To avoid contamination, each step of the PCR assay was performed in  
112 different areas of the laboratory with different sets of micropipettes. To monitor for possible  
113 contamination, negative controls were included in each experiment and PCR round. A  
114 positive control (*P. jirovecii* DNA positive) was added in each PCR experiment. Each  
115 positive NPA specimen was controlled in a second experiment.

116 Possible influence of climatic conditions.

117 Weather data for Lima, Peru between November 2016 and February 2018 were obtained from  
118 [www.worldweatheronline.com](http://www.worldweatheronline.com)

119 Data analyses.

120 Data analyses were performed with Stata version 15 (StataCorp, College Station, USA). A  
121 descriptive analysis was carried out where frequency measurements of variables were  
122 estimated according to the main dependent variable, i.e. *P. jirovecii* DNA detected, or not,  
123 using the  $\chi^2$  test or Fisher exact test when appropriate. Student T test was used to compare  
124 quantitative variables.

125 Association between variables and *P. jirovecii* DNA detection results was estimated by Odds  
126 Ratios (OR) with 95% confidence intervals (95% CI), in bivariable and multivariable logistic  
127 regression models. A two-sided p value <0.05 was considered significant.

- 128 The study was approved by the Institutional Ethics Committees of Universidad Peruana
- 129 Cayetano Heredia and Hospital Cayetano Heredia (May 31 2016, registration code: 66639).
- 130 One or both parents signed the Informed Consent to allow their infant to participate.



131 **Results**

132 A hundred and forty-six infants were enrolled. Demographic and clinical data of infants  
133 examined are listed in Table 1. Infants' mean (SD) age was 3.7 months ( $\pm$  1.7 months), mean  
134 (SD) birth weight was 3.3 ( $\pm$  0.4 Kg), and 60.3% were exclusively breast-fed. *P. jirovecii*  
135 DNA was detected in 45 out of 146 infants, i.e. an overall prevalence of 30.8%. These 45  
136 infants were considered to be colonized by *P. jirovecii* contemporaneous with *Pneumocystis*  
137 primary infection because they were mostly asymptomatic and they improved despite the  
138 absence of specific treatment against *P. jirovecii*. The characteristics of infants colonized by  
139 *P. jirovecii* or not, did not differ apart from those related to infants co-sleeping with their  
140 parents [42/45 (93%) vs. 70/101 (69%),  $p < 0.01$ ], the number of bedrooms at home  $\leq 1$  [32/45  
141 (71%) vs. 50/101 (49.5 %),  $p < 0.05$ ], and the lower mean age of the infants [3.1 months vs. 4  
142 months,  $p < 0.05$ ]. Infants colonized by *P. jirovecii* had a lower rate of URTI, mainly a  
143 common cold, within the 15 days prior to and including the day of enrollment, compared to  
144 infants who were not colonized [5/45 (11.1%) vs. 27/101 (26.7%);  $p < 0.05$ ] however this  
145 difference was not observed at 15 days post clinic visit (Table 2). No infant had clinical signs  
146 compatible with lower respiratory tract infection (LRTI), such as wheezing or overt  
147 pneumonia (Table 2).

148 A significant difference in the proportion of colonized infants was observed by age group ( $p$   
149  $< 0.01$ ). The highest frequency of colonization was observed in 2-month-old and 3-month old  
150 infants 61% and 47%, respectively (Figure 1). A significant difference in the proportion of  
151 colonized infants by season of the year was also observed. Higher rates of colonization were  
152 observed in winter and spring, 50% and 36%, respectively (when average daily temperature  
153 was 21.3°C and 21°C, respectively) whereas lower rates of colonization were observed in  
154 summer and fall, 29 % and 8 %, respectively (when average daily average temperature was  
155 25.3°C and 26.3°C, respectively);  $p < 0.01$ . (Figure 2).

156 Using bivariable analysis, the ORs of colonization in infants living in a home with  $\leq 1$   
157 bedroom or co-sleeping with parents were 2.51 [(95% CI 1.18-5.33,  $p = 0.02$ )] and 3.4 [95%  
158 CI 0.94-12.30,  $p = 0.06$ ], respectively (Table 3). Using multivariable analysis, the only  
159 remaining significant factor associated with *P. jirovecii* colonization was having  $\leq 1$  bedroom  
160 at home; OR = 3.03 [(95% CI 1.31-7.00,  $p = 0.01$ )] (Table 3).

161

## 162 **Discussion**

163 This study provides original data on *Pneumocystis* primary infection among apparently  
164 healthy infants in Lima, Peru. *P. jirovecii* presence and its detection in infants less than 7  
165 months-old was considered to be related to first contacts with the fungus and consequently, to  
166 be contemporary with primary infection. This definition of primary infection could be  
167 discussed because putative *P. jirovecii* reinfection could not be strictly ruled out in some  
168 cases. Nonetheless, it is noteworthy that *P. jirovecii* was mainly detected in young infants [2-  
169 month-old (61%) and 3-month-old (47%)], and less frequently in older infants. *P. jirovecii*  
170 appears highly prevalent in infants mostly asymptomatic, its overall rate being 30.8%. This  
171 rate is similar to that previously observed in Chile (32%) [3] but differs significantly to those  
172 previously observed in France (from 18.2 % to 25.3%) [4, 9] and Denmark (16%) [5]. There  
173 is a higher prevalence in South America than in Europe ( $P < 0.001$ ,  $\chi^2$  test) [9]. Several  
174 hypotheses could be considered to explain these results, including differences in study design  
175 (prospective vs. retrospective), in infant populations (asymptomatic vs. symptomatic), in  
176 socioeconomical characteristics (middle income countries vs. high income countries), in  
177 methods of *P. jirovecii* detection (nested-PCR assay vs. real-time PCR assay), and in  
178 geographic and climatic characteristics (tropical/hot temperate climate vs. cool/temperate  
179 climate).

180 The highest rate of *P. jirovecii* detection in infants in Peru was at 3 months (IQR, 2-4 months)  
181 whereas it was 5 months (IQR, 4-6 months) and 3 months 24 days (IQR, 75-113 days) in  
182 France [4, 9], 5 months (IQR, 3.7–9.3 months) in Chile [3] and 3 months (IQR, 73–112 days)  
183 in Denmark [5]. Comparing the results of these different studies must be done cautiously,  
184 nonetheless the values of median and IQR ages of positive infants are similar. These common  
185 characteristics suggest that first contacts with *P. jirovecii*, and consequently primary infection

186 in infants, is a common process occurring at the same time in life, regardless the geographical  
187 location.

188 This study was not designed to establish correlations between *P. jirovecii* detection and  
189 putative specific symptoms. Indeed, it was conducted in an outpatient clinic for well-baby  
190 checks and consequently most of enrolled infants were asymptomatic (114 out of 146 infants:  
191 78%). *P. jirovecii* was detected in 45 infants of whom 40 did not have symptoms. Moreover,  
192 it is noteworthy that at enrolment, infants with URTI were less likely colonized than  
193 asymptomatic infants (table 2). The survey performed 15 days after the date of infants'  
194 enrollment confirmed absence of symptoms after *P. jirovecii* detection. These original results  
195 on primary infection represent an important advance in knowledge of *Pneumocystis*  
196 epidemiology. Indeed, in the study of Vargas and colleagues [3] it was suggested that primary  
197 infection could be asymptomatic but this observation was based on seroconversion against  
198 *Pneumocystis* antigens whereas only NPAs from symptomatic infants were examined for *P.*  
199 *jirovecii* detection. In Larsen and colleagues' study and Nevez and colleagues' study, no  
200 asymptomatic infants were enrolled and *P. jirovecii* detection was associated with URTI and  
201 LRTI respectively [5, 9]. In the early nineteen eighties, the association of *P. jirovecii* and  
202 pneumonitis in immunocompetent infants was suggested by Stagno and colleagues [10].  
203 However, they used *Pneumocystis* antigen detection in serum whereas *P. jirovecii* detection  
204 was positive in only one pulmonary sample [10].

205 Recently, it was shown that *P. jirovecii* was frequent in preterm infants in Europe [11].  
206 Preterm infants with *P. jirovecii* had a 2.7-fold higher probability of developing respiratory  
207 distress syndrome than infants without *P. jirovecii* [11]. Nonetheless, premature infants  
208 cannot be considered as healthy and strictly immunocompetent. This population differs from  
209 that we examined, likewise do the infant populations in who an unexplained association  
210 between *P. jirovecii* and sudden infant death syndrome (SIDS) that occurs at the first months

211 of life worldwide, has been observed. Indeed, *P. jirovecii* was detected in infants with SIDS  
212 in Santiago, Chile, Oxford, UK [12], Rochester, New York, New Haven, Connecticut [13],  
213 and San Diego, CA, USA [14]. Although the role of *P. jirovecii* as the causative infectious  
214 agent of SIDS could not be established, it was noteworthy that its presence in the lungs was  
215 associated with increased mucus and activity of chloride channel accessory1 (hCLCA1), the  
216 mucus precursor proteins [8, 15]. Whether these observations in deceased infants with  
217 putative primary infection may be applicable to primary infection in alive infants remains an  
218 open question.

219 The fact that *P. jirovecii* may have a pathogenic effect in infants with asymptomatic primary  
220 infection is prompted by recent data showing that subclinical *Pneumocystis* primary infection  
221 in rats was associated with a progression of Th2 cell-type inflammation and airway  
222 remodeling [16]. To sum up, primary infection in non-immunosuppressed or apparently  
223 healthy infants can be symptomatic contemporaneous with self-limited URTI or LRTI, or  
224 asymptomatic but in this later case *P. jirovecii* could not be entirely nonpathogenic. For  
225 obvious ethical reasons, this hypothesis could not be tested in our infant population which  
226 could not undergo invasive lung tissue sampling.

227 The sources from which infants acquired *P. jirovecii* are still unclear. Although an  
228 environmental reservoir cannot be strictly ruled out considering the report of *P. jirovecii* DNA  
229 detection in the air from the countryside [7], no environmental niche has clearly been  
230 identified. By contrast, there are several populations of patients who may contribute to the  
231 human reservoir of *P. jirovecii*. Interindividual transmission of *Pneumocystis* sp. has clearly  
232 been established in rodent models [17, 18] and is highly probable in humans (review in [19]).  
233 It is known that pregnancy which is associated with physiological changes in the immune  
234 system [20] may predispose to pulmonary colonization with *P. jirovecii* [21]. The mothers  
235 may have transmitted the fungus by airborne route to their newborns or infants, this mode of

236 transmission being suggested in humans [22] and demonstrated for *P. oryctolagi* in rabbits  
237 [23]. Likewise, the transplacental transmission of *P. oryctolagi* in rabbits has been  
238 demonstrated [23] and suspected for *P. jirovecii* in humans. Montes-Cano and colleagues  
239 detected *P. jirovecii* DNA in 11 lungs of miscarriage fetuses and 8 placenta samples [24].  
240 Szydlowicz and colleagues recently discussed transplacental transmission, specifically in  
241 newborns [25]. In the present study, no newborns, i.e. infants younger than one month, were  
242 enrolled. However, the mothers may not necessarily be the source of *P. jirovecii*. Indeed, in  
243 Spencer and colleagues' study, 5 out of 41 women and 5 out of 60 infants were colonized by  
244 *P. jirovecii* but none of the colonized women or children were members of the same family  
245 [26]. In another recent investigation of *P. jirovecii* detection and genotyping in sample pairs  
246 from mothers and newborns, the newborns were more frequently colonized than their mothers  
247 (74.4% vs. 46.5%,  $p < 0.01$ ) and no perfect matches of *P. jirovecii* genotypes in  
248 mothers/newborns sample pairs were observed [27]. Moreover, in a previous study, we  
249 detected *P. jirovecii* in only 5 out of 92 pregnant women near term (5.43%) while it was not  
250 detected in their newborns nor in their placentas [28]. Thus, other human sources of *P.*  
251 *jirovecii* including acquisition in households may be possible. For example, putative *P.*  
252 *jirovecii* transmission between a grand-father and his grand-son has been described by Rivero  
253 and colleagues [29]. The higher rate of *P. jirovecii* that we observed in infants living in a  
254 dwelling with only one or no bedroom suggests that *P. jirovecii* may have been acquired  
255 under overcrowded conditions within the family. Unfortunately, in this study, we could not  
256 investigate *P. jirovecii* acquisition by infants from their family members.

257 A significant difference in the proportion of infants colonized or not colonized by *P. jirovecii*  
258 by season was observed ( $\chi^2$  test  $p < 0.01$ ). Higher rates of *P. jirovecii* in infants were  
259 observed in winter and spring whereas the lower rates were observed in summer and fall.  
260 These results suggesting seasonal variation could be related to a bias. The highest frequency

261 of *P. jirovecii* detection was observed in 2-month-old and 3-month-old infants and these two  
262 infant groups may have been over represented. The numbers of infants in the six groups of  
263 age (from one to six months) and in each month over the 15 months of the study were not  
264 strictly identical on the one hand and insufficient for a highly powerful analysis on the other  
265 hand.

266 Be that as it may, the higher rate of *P. jirovecii* detection in infants was effectively observed  
267 in the cooler months of the spring and the winter in Lima when the average temperature was  
268 around 21°C. This may be consistent with reports described elsewhere showing an association  
269 of PCP occurrence in immunosuppressed patients with average temperatures from 10°C to  
270 20°C rather than with the seasons themselves [30]. In Chile, the highest rate of *P. jirovecii*  
271 detection in autopsied infants was in winter, when the average temperatures were 16°C-18°C  
272 whereas the lowest rate was in the fall when the average temperatures were 20°C-28°C [31].  
273 However, the seasons and/or temperatures may not represent strictly independent factors.

274 Finally, we provide original data on *Pneumocystis* primary infection in Lima, Peru. We  
275 demonstrated using microbiological data, i.e. *P. jirovecii* detection in NPAs, that  
276 *Pneumocystis* primary infection could be asymptomatic, as it was previously established on  
277 serological data [3]. The higher detection rate was associated with household conditions  
278 favoring close inter-individual contacts and therefore potential transmission of *P. jirovecii*.  
279 Moreover, *Pneumocystis* primary infection was most frequently observed in infants 2-3  
280 months of age and during the cooler months of the winter and spring. Our overall results  
281 combined with the analysis of the literature show that *Pneumocystis* primary infection occurs  
282 in the first months of life worldwide in both high income and middle-income countries.

283

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288

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375 Table 1. Demographic and clinical characteristics of infants with or without *Pneumocystis*  
376 primary infection, Lima, Peru.

377

378 Table 2. Symptoms and putative respiratory diseases in infants attending “well baby” clinics  
379 with or without *Pneumocystis* primary infection, Lima, Peru.

380

381 Table 3. Bivariable and multivariable analyses of factors associated with diagnoses of  
382 *Pneumocystis* primary infection in infants, Lima, Peru.

383

384 Figure 1. Proportion of infants with or without *Pneumocystis* primary infection by age groups.

385 Foot notes. There was a significant difference in the proportion of infants with or without  
386 *Pneumocystis* primary infection by age groups ( $\chi^2$  test  $p < 0.01$ ).

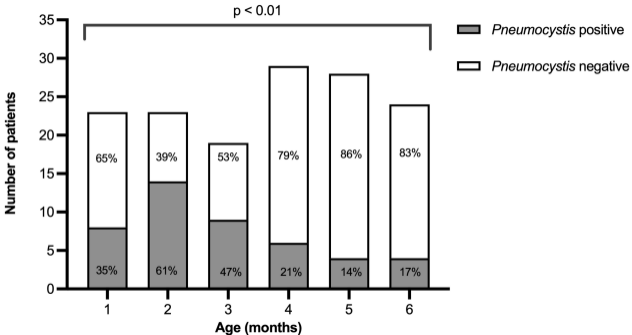
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388 Figure 2. Proportion of infants with or without *Pneumocystis* primary infection by season of  
389 the year.

390 Foot notes. A significant difference in the proportion of infants with or without primary  
391 infection by season was observed ( $\chi^2$  test  $p < 0.01$ ).

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393



$p < 0.01$

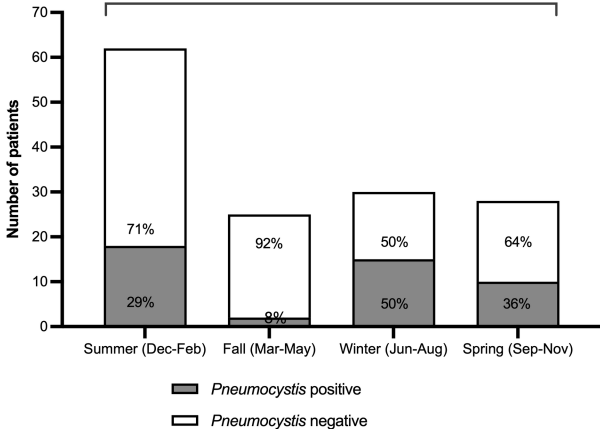


Table 1. Table 1. Demographic and clinical characteristics of infants with or without *Pneumocystis* primary infection, Lima, Peru.

	All infants (n = 146)	Colonized infants (n = 45)	Non-colonized infants (n = 101)	p value
Gender, female	76 (52.1%)	23 (51.1%)	53 (52.5%)	p = 0.88 <sup>a</sup>
Mean age, months (SD)	3.7 (± 1.7)	3.1 (± 1.6)	4 (± 1.7)	p < 0.05 <sup>b</sup>
Type of birth, C-section*	78 (53.4%)	26 (57.8%)	52 (51.5%)	p = 0.48 <sup>a</sup>
Birth weight (Kg)				
2.5 - <3.0	30 (20.6%)	10 (22.2%)	20 (19.8%)	p = 0.33 <sup>c</sup>
3.0 - <3.5	72 (49.3%)	26 (57.8%)	46 (45.5%)	
3.5 - <4.0	40 (27.4%)	8 (17.8%)	32 (31.7%)	
4.0 - 4.5	4 (2.7%)	1 (2.2%)	3 (3.0%)	
Exclusively breastfed	88 (60.3%)	30 (66.7%)	58 (57.4%)	p = 0.29 <sup>a</sup>
Parents smoking tobacco at home	10 (6.9%)	1 (2.2%)	9 (8.9%)	p = 0.17 <sup>c</sup>
Siblings at home	70 (48.0%)	18 (40.0%)	52 (51.5%)	p = 0.20 <sup>a</sup>
Co-sleeping with parents	112 (76.7%)	42 (93.3%)	70 (69.3%)	p < 0.01 <sup>a</sup>
Number of bedrooms ≤ 1	82 (56.2%)	32 (71.1%)	50 (49.5%)	p = 0.015 <sup>a</sup>
Waste disposal, indoor public network	120 (82.2%)	40 (88.9%)	80 (79.2%)	p = 0.15 <sup>a</sup>
Antibiotics since birth <sup>d</sup>	12 (8.2%)	2 (4.4%)	10 (9.9%)	p = 0.34 <sup>c</sup>
Antibiotics given in the last 15 days <sup>d</sup>	3 (2.1%)	0 (0%)	3 (3.0%)	p = 0.55 <sup>c</sup>
Symptoms in the last 15 days <sup>e</sup>				
Rhinorrhea	21(14.4%)	4 (8.9%)	17 (16.8%)	p = 0.20 <sup>a</sup>
Cough	10 (6.8%)	2 (4.4%)	8 (7.9%)	p = 0.72 <sup>c</sup>
Fever	6 (4.1%)	1 (2.2%)	5 (5.0%)	p = 0.66 <sup>c</sup>
Wheezing	3 (2.1%)	1 (2.2%)	2 (2.0%)	p = 1 <sup>c</sup>
Diarrhea	2 (1.4%)	0 (0%)	2 (2.0%)	p = 1 <sup>c</sup>
Subcostal retractions	1 (0.7%)	0 (0%)	1 (1.0%)	p = 1 <sup>c</sup>
Vomiting	1 (0.7%)	0 (0%)	1 (1.0%)	p = 1 <sup>c</sup>
Sibling and/or parents with symptoms	4 (2.7%)	0 (0%)	4 (4.0%)	p = 0.31 <sup>c</sup>

Key: \*C-section = Caesarean section; <sup>a</sup>  $\chi^2$  test, <sup>b</sup> Student T test, <sup>c</sup> Fisher exact test

<sup>d</sup> mostly beta-lactams or aminoglycosides; <sup>e</sup> including the day of enrolment

Table 2. Symptoms and putative respiratory diseases in infants attending “well baby” clinics with or without *Pneumocystis* primary infection, Lima, Peru.

	At enrollment		On day15 of follow-up	
	Colonized infants (n = 45)	Non-colonized infants (n=101)	Colonized infants (n=45)	Non-colonized infants (n=101)
Asymptomatic	40 (88.9%) <sup>a</sup>	74 (73.3%) <sup>a</sup>	37 (82.2%)	77 (76.2%)
<u>Putative URTI</u>	5 (11.1%) <sup>a</sup>	27 (26.7%) <sup>a</sup>	8 (17.7%)	21 (20.8%)
Common cold	5 (11.1%)	26 (25.7%)	4 (8.9%)	7 (6.9%)
Pharyngitis	0	1 (1.0%)	4 (8.9%)	14 (13.9%)
Acute otitis media	0	0	0	0
Laryngotracheitis	0	0	0	0
<u>Putative LRTI</u>	0	0	0	3 (3.0%)
Bronchitis	0	0	0	2 (2.0%)
Bronchiolitis	0	0	0	1 (1.0%)
Asthma/bronchospasm	0	0	0	0
Pneumonia	0	0	0	0

Key : <sup>a</sup> p<0.05 (0.03) ( $\chi^2$  test) ; URTI =Upper respiratory tract infection ; LRTI =Lower respiratory tract infection



Table 3. Bivariable and multivariable analyses of factors associated with diagnoses of *Pneumocystis* primary infection in infants, Lima, Peru.

Variables	Bivariable analysis				Multivariable analysis			
	OR	95% CI		p value	OR	95% CI		p value
Gender								
Female	1				1			
Male	1.06	0.52	2.13	0.88	1.30	0.58	2.91	0.52
Type of birth								
Vaginal	1				1			
C-section	1.29	0.63	2.62	0.48	1.67	0.72	3.86	0.23
Birthweight (Kg)								
2.5 - <3	1				1			
3.0 - < 3.5	1.13	0.46	2.78	0.79	1.07	0.40	2.87	0.89
3.5 - < 4	0.50	0.17	1.48	0.21	0.47	0.14	1.55	0.21
4.0 - 4.5	0.67	0.06	7.25	0.74	1.25	0.07	22.26	0.88
Parents smoking tobacco at home								
No	1				1			
Yes	0.23	0.03	1.89	0.17	0.19	0.02	2.34	0.19
Siblings at home								
No	1				1			
Yes	0.63	0.31	1.28	0.20	1.02	0.45	2.32	0.96
Antibiotics used since birth								
No	1				1			
Yes	0.42	0.09	1.99	0.27	0.50	0.07	3.82	0.51
Wheezing								
No	1				1			
Yes	1.13	0.10	12.74	0.92	3.34	0.09	122.01	0.51
Cough								
No	1				1			
Yes	0.54	0.11	2.65	0.45	0.58	0.06	5.52	0.63
Fever								
No	1				1			
Yes	0.44	0.05	3.85	0.46	1.50	0.03	65.45	0.83
Rhinorrhea								
No	1				1			
Yes	0.48	0.15	1.52	0.21	0.70	0.16	3.13	0.64
Number of bedrooms								
≤1	2.51	1.18	5.33	0.02 <sup>a</sup>	3.03	1.31	7.00	0.01 <sup>a</sup>
>1	1				1			
Co-sleeping with parents								
No	1				1			
Yes	3.40	0.94	12.30	0.06	1.77	0.39	8.07	0.46

Key: C-section = Caesarean section; <sup>a</sup> Using bivariable and multivariable analysis, the number of bedrooms at home  $\leq 1$  remained the only significant factor associated with *Pneumocystis* primary infection.