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ORIGINAL ARTICLE

High transient colonization by *Pneumocystis jirovecii* between mothers and newborn

Cristian Vera[1,2] · **Yudy A. Aguilar**[1] · **Lázaro A. Vélez**[1,3] · **Zulma Vanessa Rueda**[4]

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Abstract The aim of the study was to explore the frequency and dynamics of acquisition and colonization of *Pneumocystis jirovecii* among neonates, as well as the epidemiological and genotypic characteristics in mother-child binomial. In a prospective enrolled cohort of women in their third trimester of pregnancy, nasopharyngeal swabs (NPS) and clinical and epidemiological data were collected at four different times: 17 days, 2nd, 4th, and 6th month of life of the newborn. *P. jirovecii* was detected by nested-PCR for the *mtLSU-rRNA* gene in each NPS; the genotypes were determined amplifying four genes. Forty-three pairs and 301 NPS were included. During the third trimester, 16.3% of pregnant women were colonized. The rate of colonization in mothers at delivery was 16, 6, 16, and 5% and in their children 28, 43, 42, and 25%, respectively. Within pregnant women, 53% remained negative throughout follow-up, and among these, 91% of their children were positive in at least one of their samples. In both, mothers and children, the most frequent genotype of *P. jirovecii* was 1.

Conclusion: The frequency of colonization by *P. jirovecii* was higher in newborns than in their respective progenitors. Colonization of both mothers and children is transitory; however, the mother of the newborn is not necessarily the source of primary infection.

What is Known:
- *We did not find studies comparing P. jirovecii colonization between mothers and children simultaneously, yet the frequency of colonization by serologic and molecular methods in pregnant women has been reported.*

What is New:
- *According to our findings, 3/4 of the children had transient colonization during the first 6 months of life, in only half in the mothers, without proof of mother-to-child transmission or* vice versa.

Keywords *Pneumocystis jirovecii* · Primary infection · Colonization · Mother · Neonate

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✉ Cristian Vera
cristian.vera.marin@hotmail.com

Yudy A. Aguilar
yudyagui@yahoo.com

Lázaro A. Vélez
clamona@une.net.co

Zulma Vanessa Rueda
zulmaruedav@gmail.com

[1] Grupo investigador de Problemas en Enfermedades Infecciosas (GRIPE), Universidad de Antioquia, Medellín, Colombia

[2] Facultad Nacional de Salud Pública, Universidad de Antioquia, Medellín, Colombia

[3] Sección de Enfermedades Infecciosas, Hospital Universitario San Vicente Fundación, Medellín, Colombia

[4] Facultad de Medicina, Universidad Pontificia Bolivariana, Medellín, Colombia

Introduction

Pneumocystis jirovecii is an important cause of pneumonia, called *Pneumocystis pneumonia* (PcP), in immunocompromised patients [1–3]. It is known that this fungus can also colonize the lung of its host, and predisposing factors for colonization may be a weakened or immature immune system, as well as gestation [4, 5]. During gestation, the maternal immune system undergoes changes that predispose to the asymptomatic acquisition of *P. jirovecii*. These changes

consist of possible decrease in the Th1-type cellular response and a proportional increase in the Th2 humoral response, in addition to the increase of certain cytokines involved in the innate and humoral response, such as IL-4, IL-6, and IL-10 [6]. However, the role of the colonized mother as a possible source of infection to the newborn is not elucidated, as well as, the timing of first infection with *P. jirovecii*, and the persistence and duration of colonization.

Two hypotheses have been proposed: one suggests a de novo infection, in which susceptible human host is exposed to *P. jirovecii* infection and that infection can be acquired at any point in life. This hypothesis is supported by recurrent pneumonia in patients infected with HIV-AIDS, where they found different genotypes of *P. jirovecii* in each episode [7–9]. The other hypothesis consists of asymptomatic acquisition of *P. jirovecii* at some point in life, which has been postulated to occur during childhood. *P. jirovecii* remains latent and can become activated in the context of immune compromise with resulting increased fungal burden and ensuing pneumonia. This hypothesis has been debated and questioned (Yinnakis y Boswell), because most researchers believe in de novo infection where the first contact of the newborn with the fungus occurs at the time of birth, where the mother would be the most likely source of *P. jirovecii* infection [10–12]. This finding is supported by a serological response and fungal DNA findings in the first months of life of newborns [13–15].

Therefore, the objective of this study was to explore the frequency of colonization of *Pneumocystis jirovecii* in mothers and their offspring, the time the neonate acquired the fungus, and changes in colonization during the first 6 months of life, as well as the epidemiological and genotypic features in a cohort of mothers and their newborns (mother-child binomial).

Materials and methods

Type of study

Descriptive prospective.

Study population and sample size.

Through a non-probabilistic and convenience sampling, women over 18 years old attending an antenatal clinic were screened in the last trimester of pregnancy (28–40 weeks of gestation) along with their newborn children (mother-child binomial).

Selection criteria

Inclusion: (i) women over 18 years who were in the last trimester of pregnancy (week 28–40) (ii) that agreed to participate in the study as well as their newborn children (when they are born) and (iii) voluntarily signed informed consent.

Exclusion: (i) if high-risk pregnancy for maternal mortality, preterm birth or stillbirths were considered (ii) any other situation that would prevent progenitor-offspring interaction in the first 6 months of life.

Patient recruitment and socio-demographic data

The health care provider established the initial contact with pregnant women, who were subsequently contacted by telephone to schedule an appointment and explain the project; informed consent was obtained from all individual participants included in the study with clinical, demographic, and epidemiological variables. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Mother-child binomial follow-up

After recruiting the mother in the third trimester of pregnancy, postpartum follow-up was conducted for up to 6 months to each mother-child pair (1st week, month and a half, 3rd, and 6th month postpartum).

Variables

Outcome variable was defined as the state of *P. jirovecii* colonization in each participant. Additionally, in each visit, data on clinical and epidemiological variables, contacts with respiratory symptomatic people, mother and child interaction time, most frequent place, and contacts of the binomial, together with the number of persons/room of the newborn were recorded.

Collection and processing of NPS

During each visit, each participant was taken for a nasopharyngeal swab (NPS) sample with a nylon swab (*floked*, *FLOQSwabs*™, *COPAN's*); each NPS was stored in a cryovial containing 0.9% saline. Subsequently, NPS were transported and refrigerated at 4 °C within a maximum period of 12 h at our laboratory, following international protocols of time, transport and packaging of biological material for sample processing.

DNA extraction and amplification

Each NPS sample underwent DNA extraction using the DNeasy Blood & Tissue Qiagen® kit, as follows briefly: each NPS was vortexed for 45 s, and incubation with buffer lysis was carried out for 1 h at 37 °C. The remaining steps of the

extraction were performed in accordance to the manufacturer's recommendations. Subsequently, DNA obtained was quantified using NanoDrop™ 1000 (Thermo, EEUU) and pre-concentrated for 30 min at room temperature through Savant SpeedVac®, using the concentrator model SPD111V (Netherlands) for further analysis by a nested-PCR, amplifying a fragment of the *mtLSU-rRNA* gene of 346 and 246 bp in the first and second rounds, respectively. This nested-PCR has a documented sensitivity of 100% [16, 17]. Colonization was defined as a positive nested-PCR in NPS without pneumonia.

External controls and contamination prevention

For each round of DNA extraction, a negative control was performed. Also, during each round of nested-PCR and qPCR, a positive control of a bronchoalveolar lavage sample of a patient with PcP confirmed by toluidine blue O (TBO) staining and a negative control consisting of ultrapure water was used. For each DNA extract that was negative by nested-PCR for the *mtLSU-rRNA* gene, the PCR amplification was performed by conventional PCR of a 496 bp fragment of the constitutive gene Glyceraldehyde-3 phosphate dehydrogenase (GADPH) to rule out the presence of possible inhibitors. To avoid cross-contamination in DNA extraction, master-mix preparation, amplification, and further preparation of the products of the second round of nested-PCR were performed in separate work areas, with different micro pipettes for each step, with new and sterile material, and with the use of biosecurity booth.

Sequencing and genotyping of PCR products

Amplified products by the nested-PCR were sent to an outside laboratory for sequencing. Subsequently, editing and quality control of sequences with the Bioedit® (version 7.2.6.1, copyright© 1999 Caredata.com, Inc. http://www.mbio.ncsu.edu/BioEdit/bioedit.html) program was performed and compared in the ClustalW® (copyright© EMBL-EBI 2016. http://www.ebi.ac.uk/Tools/msa/clustalw2/) program with published reference sequences in databases. The genotypes obtained for each mother (or their respective partner) and her infant were compared with each other between those obtained from the mother with her newborn or, if it was the case, with their respective partner. An epidemiological link between amplified *P. jirovecii* from the mother and her child was considered if both samples had the same genotype.

Statistical analyses

Data analysis was performed using SPSS software version 21.0 and STATA version 12. Absolute and relative frequencies of PCR results and other dichotomous variables obtained from mothers and newborns were calculated. For age, weight, height, and days of symptoms, means or medians (Me) were determined (as appropriate). A Chi-square test was performed to explore possible statistical differences between groups of newborn colonized and not colonized by *P. jirovecii*. It was considered that there was statistical difference when the value of $p < 0.05$.

Ethical considerations

The research protocol was approved by the Bioethics Committee of the School of Medicine (approval certificate no. 015) of Universidad de Antioquia. Individuals who met inclusion criteria were clearly informed about the objectives of the study, the voluntary nature of participation by the mother-child, and the right of each participant to refuse or withdraw of the same. Mothers who agreed to participate signed the informed consent.

Results

Characteristics of the mother-child binomials

Between May 27, 2015, and August 4, 2016, 46 mothers were surveyed in the last trimester of gestation (32.7 ± 3.1 weeks); three were excluded for the following reasons: (i) loss collection data form and contact details, (ii) stillbirth, and (iii) failure to respond to phone call attempts made by the physician. Overall, 43 pregnant women were included (Table 1).

During the study, none of the mothers or their children received any inhaled or systemic immunosuppressive therapy in the last 3 months before to the inclusion in the study (including one HIV-infected mother) nor had any hematologic cancer or chronic respiratory disease. Similarly, none of these women smoked cigarettes. At the time of enrollment, 39.5% (17/43) of the mothers had some form of respiratory symptoms. Neither mother nor infant had pneumonia or required hospitalizations for respiratory causes during the study (Table 2).

Pairs follow-up

Among the pairs enrolled, 43 were followed at the first visit (Me 17 days, IQR 8–34), 35 in the second (Me 76 days, IQR 56–101), 31 in the third (Me 127 days, IQR 99–176), and 20 in the fourth postpartum visit (Me 183 days, IQR 174–200). The reasons for withdrawal or lost to follow-up of 23 pairs during follow-up were (i) 17% (4/23) residency change without informing the researchers, (ii) 17% (4/23) does not respond or rejected calls, (iii) 17% (4/23) unavailability for further visits, (iv) 13% (3/23) went to live abroad, (v) 13% (3/23) had disagreement with the husband, (vi) 13% (3/23) voluntary withdrawl, and (vii) 9% (2/23) had shown displeasure with NPS.

Table 1 Characteristics of pregnant mothers at the time of recruitment (third trimester of pregnancy)

Variable	Mothers		
	P. jirovecii positive (n = 20)	*P. jirovecii* negative (n = 23)	Total (n = 43)
Age, mean ± SD	28.7 ± 5.1	30.7 ± 5.9	29.7 ± 5.62
Week of pregnancy at recruitment, mean ± SD	33 ± 3.3	32.9 ± 3.3	32.9 ± 3.3
Weight, mean ± SD (kg)	69.3 ± 9.9	69.6 ± 10.5	69.5 ± 10.1
Height, mean ± SD (cm)	159 ± 6.7	161.3 ± 6.2	160 ± 6.5
Vascularization alteration or placental insufficiency, n (%)	1 (5)	3 (13)	4 (9.3)
Respiratory symptoms or signs at the time of inclusion, n (%)	8 (40)	9 (39.1)	17 (39.5)
Place where most of the days is spent (> 8 h), n (%)			
Home	7 (35)	6 (26.1)	13 (30.2)
College	1 (5)	2 (8.6)	3 (6.9)
Work	12 (60)	15 (65.2)	27 (62.8)
Contact with children < 6 years by more than 4 h/day/5 days a week, n (%)	8 (40)	6 (26.1)	14 (32.6)
Cohabiting with immunosuppressed person, n (%)	0 (0)	1 (4.3)	1 (2.3)
Health-related occupation, n (%)	4 (20)	3 (13)	7 (16.3)
HIV, n (%)	0 (0)	1 (4.3)	1 (2.3)

Nested-PCR *mtLSU-rRNA* and PcP colonization in mothers and infants

Three hundred one NPS samples (172 mothers and 129 children) were collected. During the last trimester of pregnancy, 16.3% (7/43) of the mothers were colonized. Overall, 46.5% (20/43) of the mothers and 74.4% (32/43) of their children were colonized by *P. jirovecii* at some point during follow-up ($p > 0.095$). Of the newborns, 23% (10/43) had up to two consecutively positive samples.

Table 2 Characteristics of newborns during the study

Variable	Newborns		
	P. jirovecii positive (n = 32)	*P. jirovecii* negative (n = 11)	Total (n = 43)
Sex			
Male, n (%)	18 (56.3)	5 (45.4)	23 (53.5)
Birth weight (mg), mean ± SD	3310 ± 320.5	3235 ± 439.9	3292 ± 350.7
Gestational age at birth, mean of weeks ± SD	40 ± 1.1	39 ± 1.3	40 ± 1.5
Height at birth (cm), mean ± SD	50.2 ± 1.7	50.8 ± 2.1	50.4 ± 1.8
Respiratory rate, Me (IQR)	48 (42–50)	42 (40–46)	44 (42–50)
Children with respiratory symptoms during follow-up, n (%)			
Cough	13 (40.6)	1 (9.1)	14 (32.5)
Fever	7 (21.9)	1 (9.1)	8 (18.6)
Nasal congestion	18 (56.2)	2 (18.2)	20 (46.5)
Rhinorrhea	15 (46.9)	4 (36.4)	19 (44.2)
Where spends most of the day, n (%)			
Home %(n)	32 (100)	11 (100)	43 (100)
With whom spends most of his time (> 8 h/day), n (%):			
Mother	29 (90.6)	10 (90.9)	39 (90.7)
Father and mother	3 (9.4)	1 (9.1)	4 (9.3)
Is in contact with children < 6 years for more than 4 h/day/5 days a week, n (%)	13 (40.6)	4 (36.4)	26 (60.5)
Receives breast milk, n (%)	31 (96.9)	11 (100)	42 (97.7)

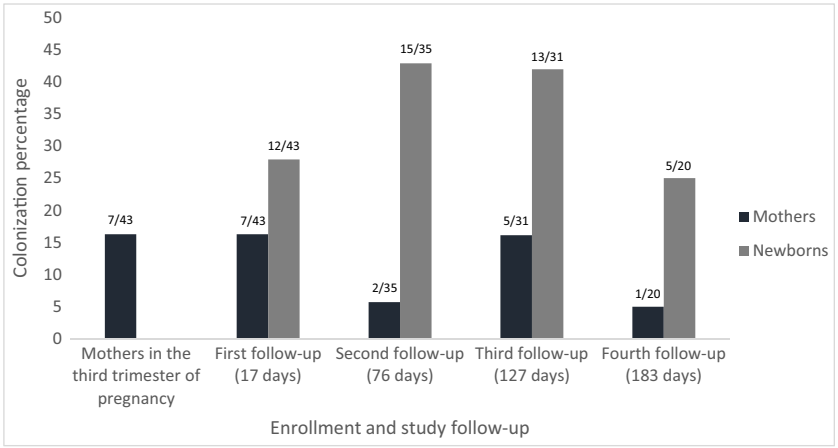

Fig. 1 Colonization by *P. jirovecii* detected in mothers and children during fifth and fourth follow-up visits, respectively. Of the 46 pairs studied at visit 1, 20 completed follow-up. The numbers in parentheses indicate medians

Figure 1 illustrates the frequency of colonization during mother and children follow-up. There were no statistical differences between cough ($p = 0.087$), fever ($p = 0.624$), nasal congestion ($p = 0.067$), rhinorrhea ($p = 0.8$), and any other variables between the colonized and non-colonized groups of the infants ($p > 0.05$).

Of the 43 pairs, 28% (12/43) of mothers and offspring were positive at some point during follow-up (Fig. 2). Of the 12 pairs, 41.7% (5/12) both mother and child were positive at the same time of the visit (binomial numbers 2, 4, 6, 8, and 10). In six pairs, colonization was evidenced by *P. jirovecii* in mothers, followed by their newborns (binomial numbers 1, 3, 7, 8, 11, and 12), while in four pairs, colonization was evident, first in newborns and then in their mothers (binomial numbers 4, 5, 6, and 9). The other 72% (31/43) were positive the mother (8/43) or the child (21/43). Of the 43 mothers, 23

Mother-child binomials	Enrollment	Follow-up visits 1	2	3	4	*mtLSU*-rRNA mother/child genotype	Plaussible Transmission
1						1 / 2	No relationship
2				N/A	N/A	Indeterminate* / 2	No relationship
3					N/A	1 / 1	Mother-Child
4						1 / 1 and 3**	Child-Mother
5						1 / 2	No relationship
6						1 / 1	No relationship
7						1 / 2	No relationship
8						1 / 1	Mother-Child
9						1 / 1	Child-Mother
10						3 / 3 and 1***	Mother-Child
11					N/A	3 / 3	Mother-Child
12						3 / 1	No relationship

Fig. 2 Colonization in 12 pairs during the study (mother-child binomials column), *mtLSU-rRNA* genotypes for mothers and children and a possible hypothetical directionality of the transmission are shown. Genotype 1 85C/248C. Genotype 2 85A/248C. Genotype 3 85T/248C. N/A not available. The blank boxes represent negative samples for *P. jirovecii* in mother and child. *Indeterminate: it was not possible to identify the nucleotide at position 85. **Mother: genotype 1, baby: genotype 1 at follow-up 2, and genotype 3 at follow-up 3. ***Mother: genotype 3 at follow-up 1, baby: genotype 3 at follow-up 1 and 3, genotype 1 at follow-up 4. ▒ indicates colonization in mothers. ■ indicates colonization in newborn. ▒ indicates colonization in both

remained negative throughout follow-up, and of these, 21 of their children were positive in at least one of their samples.

Nested-PCR for *mtSSU-rRNA* and genotypes found

Of the 62 NPS positive samples for the *mtLSU-rRNA* gene from 17 mothers and 23 infants at different follow-up times, four sequences were of low quality; hence, analysis included 58. The most frequent genotype in the mothers' group was 1 (85C/248C), followed by 3 (85T/248C) and 2 (85A/248C) in 70.6, 17.6, and 11.8%, respectively. In the group of newborns, the most common genotype was 1 (55.2%), followed by 2 (24.1%) and 3 (20.7%).

In couples where both (mother and child) were positive by nested-PCR, it was found that 50% (6/12) shared the same genotype (binomial numbers 3, 6, 8, 9, 10, and 11) (Fig. 2). During follow-up, three children had changes in *P. jirovecii* genotypes, where the mother of one of them was always negative.

Discussion

Our initial hypothesis was that the infections were transmitted from mothers to the newborn children. The design allowed us to have several samples to be collected from children, beginning in the first week of life and during the first 6 months, to assess the correlation with colonization of their mothers during the same period. Our findings suggest that mothers are not the only source of infection to their infants because the babies always had higher frequencies of positive samples, and because in 23 women that always were negative during follow-up, 91% of their babies had positive samples over time.

Our findings show a higher frequency of colonization by *P. jirovecii* in infants than in their mothers (74.4 vs. 46.5% $p > 0.095$). Higher frequency was consistently observed during all monitoring phases, especially between the second and third visit. We found no studies comparing *P. jirovecii* colonization between mothers and children simultaneously, yet the frequency of colonization by serologic and molecular methods in pregnant women has been reported. In a study conducted in Chile, the frequency of colonization by PCR on nasopharyngeal aspirates in pregnant women was higher than in those who were not pregnant (15.5 vs. 0%) [18], a frequency that is similar to our study (16.5%). Another study from Mexico determined the frequency of antibodies against *P. jirovecii* in a group of pregnant women and umbilical cord samples. They documented that 48% of analyzed sera of gravid women displayed anti *P. jirovecii* antibodies, as in 75% of samples taken from the umbilical cord [19]. The proportions of positivity found in our infants in the present study are higher than those reported in other studies in healthy children conducted in Cuba (29.4%), Chile (32.4%), España (25.7%), and France (24%) [13, 15, 20, 21]. The differences can be explained by the type of respiratory sample submitted in each survey (oropharyngeal wash, NPS, and tracheal aspirate) and the diagnostic method employed (serology, direct stains, and different types of PCRs), baseline clinical condition and geographic region [22–24]. In our study, nested and real-time PCR techniques were used for the detection of the mtLSU-rRNA gene of *P. jirovecii*, both of which had a sensitivity of 100% and a specificity of 97.7% [13]. All samples were taken following the same protocol for obtaining NPS samples and processed blindly, because the operator did not know the name of the participants. Our results were validated using a positive and negative control with each run. In addition, the negative samples were analyzed by another PCR to control potential inhibitors in the samples. Moreover, it should be noted that all NPS collected during our research were pre-concentrated to increase the probability of detecting *P. jirovecii* DNA by nested-PCR.

Although in our study, there were no statistical differences between the respiratory clinical findings between colonized and non-colonized infants, it is noteworthy that the positive infants for *P. jirovecii* had higher percentages of respiratory symptoms than the negative ones. We cannot conclude that the symptoms were related to *P. jirovecii* because we did not perform other microbiological tests to rule out other respiratory pathogens; a positive PCR for *P. jirovecii* in an immunocompetent host may indicate an asymptomatic presence or colonization, rather than an episode of mild respiratory disease, and because of the objective of our study, clinical and epidemiological information is insufficient to determine whether the respiratory episode was actually caused by *P. jirovecii* or other etiology such as allergic reaction or infection. Previous research described a possible relationship of *P. jirovecii* with respiratory infections, which can vary from mild colds to bronchiolitis, especially in children between the 2nd and 4th month of age [21, 22]. Vargas et al. Found *P. jirovecii* DNA by PCR in 32% of children between 1 month and 2 years of age with mild respiratory symptoms [13]. Recently, Rojas et al. reported significant increase of respiratory distress syndrome in preterm infants colonized with *P. jirovecii* compared to those that were negative (72 vs. 52%; $p = 0.04$, adjusted OR 2.75 (1.005–7.564)) [15]. Further studies should explore the causality between upper respiratory symptoms in newborns and *P. jirovecii*.

The other two results that suggest that the mother is not the source of infection were (1) that in our study, 23 mothers remained negative during all visits, but 21 of their children had positive PCR in, at least, one sample and (2) different genotypes between mothers and infants. Those findings suggest that besides mothers, other possible sources of infection exist, including cohabitance with newborns. In our study, six mother-child binomials positive for nested-PCR, had the same *P. jirovecii* genotype. In 67% (4/6), genotype 1 was involved,

while the other two belonged to genotype 3. A local study conducted the first report of *P. jirovecii* genotyping in Colombia, based on single nucleotide polymorphisms in the *mtLSU-rRNA* gene from respiratory samples of immunosuppressed patients. The authors found genotypes 1 and 2 as the most frequent in oropharyngeal wash (5/13) and bronchoalveolar (4/11) samples, respectively [25]. Although our patients were immunocompetent, we cannot conclude that it is a real mother-child transmission or vice versa, since genotype 1 seems to be the most common in our environment; furthermore, genetic markers with greater sensitivity and power of discrimination are required, since the *DHPS* and *ITS* genes have only one copy in the *P. jirovecii* genome and fungal loads from samples were low.

Miller et al. described the apparent transmission of *P. jirovecii* among HIV-positive mothers with respiratory symptoms, who were found to have fungus by staining with methenamine silver in the bronchoalveolar lavage and in whom the same genotype was identified, concluding that the most probable transmission of the fungus in these binomials was by air due to the close contact between the mother and her son; however, they did not rule out exogenous or placental infection [26]. The possibility that *P. jirovecii* passes the placental barrier and is vertically transmitted between mother and child is feasible, but controversial [27–29]. In humans, in the early 1960s, the transplacental passage of *P. jirovecii* was suggested in a few reports of pneumonia in neonates published before the AIDS pandemic [27]. Montes-Cano et al. evaluated a possible passage of *P. jirovecii* via the transplacental route through PCR amplification of *mtLSU-rRNA* and *DHPS* genes detection in lung and placental tissue samples from 20 fetuses of 28 ± 8 week's gestation. The authors documented the presence of *P. jirovecii* DNA in 11 and 8 lung and placenta samples, respectively. In addition, they found both *P. jirovecii* loci in 35% (7/20) of the lung tissue samples and 5% (1/20) of the placenta samples [30]. In the same way, studies in rabbits demonstrated the transplacental transmission of *Pneumocystis oryctolagi* to the fetus from the first 10 days of pregnancy [31]. However, other studies in a murine model (in rats) concluded that the transmission of *Pneumocystis carinii* occurs immediately after birth, whereas the transplacental passage from the mother to the fetus of *P. carinii* is rare [29, 31, 32].

With regard to household contact transmission, Rivero et al. documented a case report in which they explored the possible transmission of *P. jirovecii* to an immunocompetent and HIV-negative 6-month-old girl with symptoms suggestive of PcP, where *P. jirovecii* was detected by PCR for the *mtLSU-rRNA* gene, in a nasopharyngeal aspiration sample. They investigated the possible source of infection and studied each cohabitant, using an oropharyngeal sample with the same PCR and genotyping. *P. jirovecii* genotype 1 (85C/248C) was found in samples from his grandparents, who had a history of rheumatoid arthritis and chronic bronchitis (the same genotype found in the patient), while samples of his parents and brother were negative. The authors concluded that grandparents were the possible sources of infection [33]. However, Hauser [34], in a letter to the editor, questioned those results, claiming that the allelic marker used to typify the fungus was not adequate, because of its small discriminative power, also arguing that the genotype found in both the patient and grandparents was the most prevalent in the geographical region where the family lives.

To further complicate things, our results showed that *P. jirovecii* colonization varies over time, in both mothers and newborns, since during follow-up samples went from positive to negative and vice versa. To date, there are no studies that systematically explore transient colonization with *P. jirovecii*; however, this colonization dynamics of children had already been suggested by Djawe et al. who, in a cohort study in 42 Chilean children between 2 and 24 months old, described the immune response in serum to *P. jirovecii* recombinant antigens: MsgA, MsgB, and MsgC through ELISA and the detection of fungal DNA in nasopharyngeal aspirate samples by PCR in those who suffered some type of respiratory symptoms. They found that serology and DNA detection varied in time during follow-up visits, making it difficult to correlate the results of both tests [35]. Other studies that have assessed *P. jirovecii's* seroprevalence in infants at different ages show that it increases with age. In Spain, it was found that 52% of infants at age 6 had been exposed to *P. jirovecii,* 66% at 10 years, and 80% at 13 years [36]. In Chile, on a two-year follow-up, Vargas and colleagues found that seroconversion occurred in 85% of healthy children aged between 1 week and 20 months [13]. These studies reveal that children conceivably become infected with *P. jirovecii* very early in life, and that their serological response lasts for years, without necessarily implying actual colonization. However, the biological reasons for changes in colonization by *P. jirovecii* over time have not been entirely elucidated. It is suggested that aspects related to the maturation of the immune system, as age advances, with switch from a TH2 to TH1 response in infancy may be related to changes in *P. jirovecii* colonization [37].

Some studies highlight the transitory nature of colonization. Sepkowitz et al. described, in an animal model, how when modifying the immune response of mice under experimental conditions and at different times, finding evidence of *Pneumocystis* through PCR and serology for up to 6 weeks and 5 months, respectively [38]. Among the reasons that may explain this phenomenon is that, as the host's immune system is re-established, the duration of the immune response may stay longer than PCR, even for months or years. In human adults, Durand-Joly et al. examined the presence of *P. jirovecii* and its variation over time in asymptomatic health workers who were in contact with patients who had PcP in a

hospital in France by nested-PCR for the *mtLSU-rRNA* gene in oropharyngeal wash samples. In their study, they found 10 potential healthy carriers of *P. jirovecii* in different units: seven in Hematology and 3 in Pediatrics Emergency. They also documented that the duration of colonization lasted between 3 and 10 weeks, and that there was no difference between the profession of workers, nor by sex or age. However, they emphasized that the differences between workers in different hospital units could suggest that *P. jirovecii* circulates more actively in environments frequented by immunosuppressed and pediatric patients than in immunocompetent adult services. Dependency of colonization and indeed clinical disease on host immune response is illustrated in the context of severe combined immune deficiency [39].

Our study had the following limitations: (i) 6 months monitoring failed to be completed in all binomials. It was a descriptive and convenience study because, in our country, the prevalence of *P. jirovecii* colonization in the maternal and child population is unknown. The lost to follow-up and withdrawals of participants could be attributed to the fact that these were healthy mothers and healthy children that do not require medical procedures; many of the newborns were the first born and their parents wanted to avoid subjecting them to uncomfortable procedure. Therefore, further studies are needed to verify our findings. However, we considered that these limitations did not invalidate the findings and two important things became clear: the mother is not necessarily the source of infection for infants and colonization varies over time; (ii) due to budget limitations, it was not possible to include all household contacts of the child in the study; this could have identified the potential source of infection.

In conclusion, our results suggest that the *P. jirovecii* colonization is transient; newborns are more frequently colonized than their mothers and that transmission likely depends on other possible sources that should be explored, including household contacts. This should be evaluated in further studies that include longer follow-up, larger sample sizes, and molecular high-resolution typing that will help to explain transmission dynamics and possible sources of *P. jirovecii* infection. The understanding of the dynamics of transmission of this fungus will allow understanding foci of infection and mechanisms of *P. jirovecii* acquisition and clearance by its host. This information will inform strategies for prevention and control measures for newborns and immunocompromised patients in other settings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Glossary

DHPS	dihydropteroate synthase
ITS 1 and 2	internal transcribed spacer 1 and 2
mtLSU-rRNA	large-subunit ribosomal RNA
mtSSU-rRNA	small subunit ribosomal RNA
Nested-PCR	nested polymerase chain reaction
NPS	nasopharyngeal swabs
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
TBO	toluidine blue O staining

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