PARASITIC INFECTIONS IN FINGER-SUCKING SCHOOL AGE CHILDREN

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Abstract: Prevalence of parasites, acquired by the fecal-oral route, was recorded in 80% of primary school children with a finger-sucking habit, which was higher than that in nonfinger-sucking children. About 85% of the children did not wash their hands after defecation. The toilet facility available to the children also affected the infection pattern in finger-sucking children who used pit latrines recording higher prevalence of parasites.

Key Words: parasites, infections, finger-sucking, children

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Finger sucking (FS) is a common habit among many children. It is common with children <2 years of age and may signal hunger, fatigue, sleep, teething, and shyness.[1] School age children are often the group that has the highest parasitic infection rate as well as the highest worm burden, which contribute greatly to the contamination of the environment.[2] The sucking habit of children may be one of the key means of completing the fecal-oral life cycle of some intestinal parasites. Availability of safe water for drinking and washing hand is important for promoting health in schools.[2] Effective hand washing includes the use of warm water, soap, and a clean dry towel. Ukoli[3] reported that the use of drugs for the treatment of fecal-orally transmitted parasites is limited if the conditions promoting transmission are not removed. Parasitic infections in children can promote malnutrition and retard the growth of the children. It can affect the weight and height of these children through impaired digestion, malabsorption, and poor growth rate.[4,5] The effect of parasitic infection on cognitive function in children has been reported.[6] This study investigates whether FS is a risk factor for parasitic infection among school children.

MATERIALS AND METHODS

Study Area. The study was conducted in 6 randomly selected primary schools in Abeokuta, Ogun State Capital in the southwest of Nigeria. Abeokuta is an urban settlement that is densely populated consisting of civil servants and traders. The town has social amenities such as electricity and pipe-borne water. The majority of residents belong to the Yoruba ethnic group.

Ethical Clearance and Informed Consent. The written approval was obtained from the ethical committee of the Local Government Area, whereas the school authorities, parents, and pupils gave verbal and written informed consent to participate.

Sampling Method. Semi-structured questionnaires were given to selected pupils from randomly selected schools (using a balloting method). All FS pupils from each school were enrolled and, for each FS child enrolled in the study, a nonfinger-sucking (NFS) child living under similar conditions was also enrolled. Information was also obtained from parents and teachers on the age and FS habit of pupils, toilet facilities at home and school, academic performance of pupils, previous treatment for fecal-oral parasitic infections, access to good water, and eating habits of the pupils. Height and weight of each pupil were obtained using meter rule and weighing scale, respectively.

Specimen Collection. Enrolled pupils were directed to defecate into the clean papers, and small portions of the fresh stool samples were collected in well labeled universal tubes in paper bags and taken to the laboratory for investigation.

Examination of Stool Samples. Physical observation of stool samples was carried out immediately after receipt in the laboratory. Presence of blood stains, mucus, and stool consistency were observed.

Direct wet examination of stool sample in saline solution was done using a compound microscope to observe the mobility of the parasites. Formal ether concentration technique was also used for the concentration of the parasites in each stool sample.

Data Analysis. Data obtained were analyzed using Epi6-info version 6.04[7] (CDC, Atlanta GA).

RESULTS

We enrolled 100 randomly selected pupils, including 50 FS pupils and 50 NFS pupils. Five different fecal-orally transmitted parasites were observed in the study of which *Entamoeba histolytica* (33%) was the most prevalent parasite; others were *Ascaris lumbricoides* (23%), *Enterobius vermicularis* (17%), *Trichuris trichiura* (14%), and *Giardia duodenalis* (12.8%) (Table 1).

The frequency of fecal-orally transmissible infection among FS pupils was 94% (47 pupils) as compared with 66% (33 pupils) of NFS pupils ($P < 0.05$). Infection was significantly higher among female (82%) than male (46%) pupils in the NFS group; however, there was no significant difference between sex and infection among children who sucked their fingers.

Treatment of infection as a measure of controlling fecal-orally transmitted parasites had no beneficial effect on finger suckers. The prevalence of infection among finger suckers who had previously treated for helminth infection and pupils who had not been treated for helminth infection was similar. The NFS group exhibited some level of reduced prevalence among previously treated pupils as compared with the untreated pupils.

The type of toilet facilities was also observed to influence distribution of infections. FS significantly increased the frequency of infection among those using a pit latrine (97%) as compared with NFS children using a pit latrine (70%) ($P = 0.019$); however, there was no significant difference between FS (90%) and NFS (59%) children using the water closet ($P = 0.22$).

DISCUSSION

In this study on the risk factors associated with FS in the transmission of intestinal parasites, the highest prevalence of infection was found for *Entamoeba histolytica* (33%), whereas *G. duodenalis* (13%) had the lowest prevalence. A similar study among school children in the eastern part of Nigeria reported 4.9% prevalence of infection with *A. lumbricoides*, 2.5% with hookworm, and 0.7% with *T. trichiura*.[8] Although *G. duodenalis* is prevalent in children, the present study is also in agreement with that of Houmsou et al[9] who reported the lowest prevalence of infection with *G. duodenalis* in the middle belt region of Nigeria. In various previous studies carried out in Abeokuta, a high prevalence of intestinal helminths, especially *A. lumbricoides* was reported among school children.[10,11] *T. trichiura* infections is known to have similar conditions influencing its endemicity and that of *A. lumbricoides*.[12]

A higher frequency of infection was observed among FS pupils (94%) than NFS pupils (66%). FS creates a route of transmission for these parasites and has contributed to the observed high prevalence of infection. There have been reported cases of indiscriminate defecation leading to fecal contamination

TABLE 1. Parasite Distribution by Species and Pupil Gender Among Finger-sucking and Nonfinger-sucking Children

Parasite	Population Occurrence N (%)	Finger-sucking			Nonfinger-sucking		
		Male	Female	Total	Male	Female	Total
Entamoeba histolytica	44 (33)	14 (35)	16 (38)	30 (37)	6 (26)	8 (29)	14 (28)
Ascaris lumbricoides	30 (23)	10 (25)	9 (21)	19 (23)	4 (17)	7 (25)	11 (22)
Enterobius vermicularis	23 (17)	6 (15)	7 (17)	13 (16)	4 (17)	6 (21)	10 (20)
Trichuris Trichiura	19 (14)	4 (10)	6 (14)	10 (12)	4 (17)	5 (18)	9 (18)
Giardia duodenalis	17 (13)	6 (15)	4 (10)	10 (12)	5 (22)	2 (7)	7 (14)
Total	133	40	42	82 (62)	23	28	51 (38)

of the environment in the study community.[13,14] School age children have also been known to be more exposed to the risks of being infected with these fecal oral parasites because of their poor level of personal hygiene, coupled with the fact that they involve themselves in activities that facilitate contact with the soil where the ova and cysts of these parasites are found. The toilet facilities available to most of these school children have also been reported to be poorly used and lack regular water supply.[11]

There was no significant difference in sex regarding parasite infection among FS pupils, but a higher percentage of females (82%) were infected than males (46%) among NFS pupils. This is similar to the result of Ekpenyong and Eyo study,[8] in which prevalence of infection was significantly more common in females than males.

History of previous treatment of intestinal parasites had no effect on the prevalence of infection among FS pupils as observed in this present study. All finger suckers who had been previously treated for these parasites and those who had not been treated were positive for infection. This shows that the FS habit exposed these pupils to reinfection, despite previous treatment of the parasites. An earlier study in Abeokuta and other towns in the state using a Monrate tool predicted a 3-month reinfection period for helminth in school children,[10,15] FS would probably increase the reinfection period because of the continuous hand-to-mouth activities of FS children.

These results strengthen the need for education of parents and their children on the risks associated with FS, especially in areas where there is a high level of fecal contamination. The need to improve sanitary conditions is also to be emphasized.

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THE BURDEN OF INFECTIONS BY PARAINFLUENZA VIRUS IN HOSPITALIZED CHILDREN IN SPAIN

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Abstract: We designed a prospective study to describe the clinical impact of the parainfluenza viruse (PIV) types detected in hospitalized children with respiratory tract infections from September 2008 to August 2010 in Spain. PIV infections were a significant proportion of viral respiratory detections (11.8% of cases). PIV types 3 and 4 were most commonly detected. There were clinical differences between PIV and respiratory syncytial virus infections.

Key Words: parainfluenza virus, respiratory tract infections, hospitalized children

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Parainfluenza viruses (PIVs) are responsible for a significant proportion of respiratory tract infections in children. The rate of PIV detections is variable depending on the pathology (upper or lower tract infections) and whether one simulates ambulatory or

hospitalized children. A few articles have focused on this virus in the last years.[1–3]

Human PIVs are divided into following 2 genera: respirovirus (types 1 and 3) and rubulavirus (types 2 and 4). PIV types 1 and 2 are associated with laryngotracheobronchitis and type 3 with lower tract infections such as bronchiolitis, recurrent wheezing, and pneumonias. Type 4, although less frequent, is associated with lower respiratory infections in infants.[4] There are few data about this type of PIV.

We designed a prospective study with the objective of describing the clinical impact of the different PIV types detected in hospitalized children with respiratory tract infections in Spain. To clarify whether PIV infections have specific characteristics, clinical and epidemiologic features were compared with respiratory syncytial virus (RSV) infections, the most prevalent respiratory virus in the same population.

MATERIALS AND METHODS

Clinical Assessment. The study population comprised of all children less than 14 years old with a respiratory tract disease admitted to the secondary public hospital Severo Ochoa (Leganés, Madrid), between September 2008 and August 2010. The study was approved by The Medical Ethics Committee. Informed consent was obtained from parents or legal guardians. All patients were evaluated by an attending physician. Clinical characteristics of patients with PIV detection were analyzed. During the hospital stay, and as part of the study, a physician filled out a study-questionnaire with the clinical data.

Upper respiratory tract infection was diagnosed in patients with rhinorrhea and/or cough, no signs of wheezing, dyspnea, crackles or bronchodilator use, with or without fever. Asthma was diagnosed on the basis of the National Asthma Education and Prevention Program guidelines.[5] All other episodes of acute expiratory wheezing were considered to be recurrent wheezing. Acute expiratory wheezing was considered to be bronchiolitis when it occurred for the first time in children younger than 2 years. Laryngotracheobronchitis was associated with inspiratory dyspnea and wheezing and laryngitis with inspiratory dyspnea without wheezing. Cases with both focal infiltrates and consolidation in chest radiographs were, in the absence of wheezing, classified as pneumonia.

Virus Detection. Specimens from patients consisted of nasopharyngeal aspirates (NPA) taken from each patient at admission (Monday through Friday). Each specimen (1 for each patient) was sent for virologic investigation to the Influenza and Respiratory Virus Laboratory at the National Microbiology Center (ISCIII, Madrid, Spain). Specimens were processed within 24 hours after collection. Upon receipt of NPAs, 3 aliquots were prepared and stored at −70°C. The reception and the NPA sample aliquoting areas were separate from those defined as working areas.

Polymerase Chain Reaction Methods for Detection of 16 Respiratory Viruses. Three reverse transcription (RT)-nested polymerase chain reaction (PCR) assays were performed to detect the 16 respiratory viruses. In these assays, RT and first amplification round were carried out in a single tube using the Qiagen OneStep RT-PCR kit (Qiagen). Influenza A, B, and C viruses were detected by using previously described primer sets only to amplify influenza viruses in a multiplex PCR assay.[6] A second multiplex PCR was used to detect PIVs 1 to 4, human coronaviruses 229E and OC43, enteroviruses (EV), and rhinoviruses (RV).[7] Presence of RSV-A and -B types, hMPV, HBoV, and adenoviruses (AD) were investigated by a third multiplex RT-nested PCR-bronchiolitis method.[8]

Statistical Analysis. Values were expressed as percentages for discrete variables, or as mean and standard deviation for continuous variables. Clinical characteristics of patients with infections associated with PIV were compared with those associated with infection by RSV. Clinical characteristics and laboratory variables were compared using the Student t test, the Mann-Whitney U test, the χ^2 test, and Fisher exact test. A 2-sided value of $P < 0.05$ was considered statistically significant. Results were adjusted to age. All analyses were performed using the Statistical Package for the Social Sciences, version 13.0 (SPSS Inc., Chicago, IL).

RESULTS

Patient Characteristics and Screening of Viruses. The study population consisted of 1106 hospitalized children less than 14 years old. A total of 916 patients were analyzed and 190 patients were excluded either because of lack of NPA samples or because they refused to participate. One NPA sample was included in the study from each patient and positive results were obtained in a total of 740 NPA samples (80.8% of the 916 tested). Out of positive samples, 540 were single virus infections (73%) and 200 children had dual or multiple viral infections (27%). Specific viruses detected and identified in the total population of 916 children are listed in Table 1, in descending order of frequency.

PIVs were detected in 82 patients, 11.8% of positive cases, 8.9% of the whole analyzed group. There were 47 males (57.3%), 57 had fever (69.5%), 43 had hypoxia (52.4%), and in 33 an infiltrate was present in chest radiographs (40.2%). Antibiotic therapy was prescribed for 24 patients (29.3%). Fourteen children had been born preterm (17.1%). The mean age of the group was 522 ± 586 days, and the stay in the hospital was 3.7 ± 1.2 days. Diagnoses in order of frequency were recurrent wheezing or asthma (45%), bronchiolitis (26%), pneumonia (14%), and laryngitis (5%). PIV type 1 was detected in 12 cases (14.6%), PIV type 2 in 8 cases (9.8%), PIV type 3 in 47 cases (57.3%), and PIV type 4 in 15 cases (18.3%). Thirty-six patients had associated coinfection with other viruses (43%), mainly rhinovirus and adenovirus. Two patients were admitted to the intensive care unit suffering pneumonia with pleural effusion.

Clinical Findings Associated With the Presence of the 4 PIV Types. PIV was detected in 82 patients, of whom 46 had only PIV. Table, Supplemental Digital Content 1, http://links.lww.com/INF/A767, shows clinical data about PIV 3 and 4 infections, because they were the most prevalent groups in our population.

We observed an increased proportion of single infections associated with PIVs in relation with other viruses. In a previously published article of our group,[9] during 2005 to 2007 single PIV infections were 4.8% of the total viral infections in the same population, and in the present study the proportion was 11.8%. We observed an increase of the proportion of PIV 3 and 4 infections during the period of study. Notably, 55% of the cases were detected in the last year, 2010 ($P = 0.06$).

TABLE 1. Frequency of Viruses Detected in 916 Children Hospitalized for Respiratory Tract Infections*

Total Virus (n = 970)	N (%)	Single Infections (n = 540)
Respiratory syncytial virus	261 (26.9%)	170 (31.6%)
Rhinovirus group	282 (29%)	160 (29.6%)
Adenovirus group	90 (9.3%)	36 (6.6%)
Parainfluenza virus	82 (8.4%)	46 (8.5%)
Human bocavirus	77 (7.9%)	17 (3.1%)
Human metapneumovirus	66 (6.8%)	45 (8.3%)
Influenza virus	66 (6.8%)	55 (10.2%)
Enterovirus	24 (2.5%)	8 (1.5%)
Coronavirus	22 (2.3%)	2 (1.5%)

*Patients, 916; single infections, 540; multiple infections, 200; and negatives, 176.

Clinical Differences Between PIV and RSV Infections. Clinical characteristics of PIV single virus infections were compared with RSV single infections in the same period (n = 170). Data are shown in Table, Supplemental Digital Content 1, http://links.lww.com/INF/A767. Patients with RSV infection had hypoxia more frequently (73% vs. 50%, $P = 0.01$) and during more days (2.9 vs. 1.95, $P = 0.003$). Bronchiolitis was more frequent in RSV group (58% vs. 28%, $P = 0.001$). Children with RSV infection were younger than those in the PIV groups (348 ± 447 vs. 626 ± 725 days, $P = 0.05$). Five patients diagnosed as bronchiolitis associated with RSV infection (4 of them with single infection), needed intensive care admission. All of them were less than 2 months of age. When results were adjusted to age (we stratified at 12 months), fever ($P = 0.05$) and hypoxia ($P = 0.02$) were more frequent in RSV group. Pneumonia was more frequent in PIV group ($P = 0.02$), and children up to 12 months received antibiotherapy more frequent in this group ($P = 0.08$). In the group of infants (<12 months), prematurity was more frequent in PIV group.

Circulation of RSV was maximal in December and the peak of PIVs circulation depended of the specific type but was statistically significant in March ($P = 0.001$).

DISCUSSION

PIV types 1 to 4 infections have a significant prevalence in hospitalized children in Spain, accounting for 11.8% of the viral infections. PIV 3 and 4 were the most important types in our population, and the main associated diagnoses were asthma or bronchiolitis.

A major strength of this study is the use of 3 multiplex RT-PCR assays, with a very high sensitivity and specificity for a complete range of respiratory viruses over 2 full calendar years. Influenza A/H1N1pdm was included in the second year. Except for CoV-NL63 and HKU1 and the recently identified polyomavirus KU, and WU, the rest of the respiratory viruses were successfully detected and identified. These technologies allow us to attribute the presence/absence for each of the 17 different viruses or group of viruses (RV, ADV, and EV). This fact, also explains the high percentage of viral agents identified (80.8%), and allows us to know the real burden of PIV infections in hospitalized children. In this context, including the influenza A/H1N1pdm pandemic peak, PIV infections were 11.8% of the positive cases (8.6% of the total group). Influenza infections were only 6.8% of the total group. There are few data about the relative burden of PIV infections. Other studies analyzed only some types of PIV,[10] and included outpatient[1] and hospitalized children.[3] Between 2006 and 2008, Farichock et al[1] while studying daycare toddlers found that PIV infections were 12% of the total respiratory viral infections, but these authors did not study human bocavirus, and did not specify how many children needed hospitalization. In our experience, between 2005 and 2007[9] in a similar population, we found that PIV infections were only 4.8% of total viral compared with 8.5% in the present study. This difference could be explained by annual variations.

Although, virus detection is common in NPA of children without respiratory diseases, our group published a study in 116 healthy children, and PIV was detected only in 1 patient.[11]

Clinical characteristics of PIV infections differed among types. PIV 1 and 2 were less frequent in hospitalized children than PIV 3 and 4. PIV 3 was the most frequent type identified in hospitalized children in previous studies[1,3] and mainly associated with lower respiratory tract infections (recurrent wheezing and asthma) in agreement with our data. PIV 4 is probably underestimated in the literature because few studies included the specific detection of this PIV type. Nevertheless, when PIV 4 was looked for, it was identified in an important proportion of cases and it was associated with lower respiratory tract infections mainly in infants.[1,4] Comparing PIV infections with those due to RSV several significant differences were observed. RSV patients were younger than PIV children, and the most frequent clinical diagnosis was bronchiolitis. Hypoxia was more frequent and prolonged in RSV children. These data support the idea than RSV infections are more severe than those where a PIV is detected. The younger age of the patients could be an important risk factor. Nevertheless, the proportion of cases needing intensive care unit was similar in both infections.

PIV 1 and 2 were mainly detected in autumn (October) and PIV 3 and 4 in spring with an incidence peak in March. These data are in agreement with previous studies.[3] RSV infections occurred mainly in December and January.

We conclude that PIV infections are a significant proportion of viral respiratory detections in hospitalized children.

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A TWICE DAILY POSACONAZOLE DOSING ALGORITHM FOR CHILDREN WITH CHRONIC GRANULOMATOUS DISEASE

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Abstract: Posaconazole (PSZ) may be an attractive alternative for antifungal prophylaxis in children with chronic granulomatous disease. Expe-

rience with PSZ in pediatric patients is limited, and no specific dose recommendations exist. A twice daily dosing algorithm based on allometric scaling (body-weight based) for PSZ results in adequate exposure and appears to be safe in children with chronic granulomatous disease.

Key Words: CGD, children, posaconazole, pharmacokinetics, prophylaxis

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Chronic granulomatous disease (CGD) is a rare primary immunodeficiency in which phagocytes fail to generate the microbicidal reactive oxidant superoxide anion and its metabolites due to mutations in any of the 4 structural components of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[1] Clinically, as a result of the defect in the key innate host defense pathway, CGD patients suffer from recurrent life-threatening bacterial and fungal infections. Itraconazole (ITZ) is used as a first line prophylactic agent in CGD patients,[1] but there are several disadvantages: breakthrough infections have been observed,[2] it has shown erratic absorption and patients dislike the taste of the solution.

Posaconazole (PSZ; Noxafil, Schering Corporation, Kenilworth, NJ [now Merck & Co., Inc]) may be a better alternative for prophylaxis. PSZ has a less potent drug-drug interaction profile on CYP3A4 than some other azole drugs such as ITZ. However, the major variability of PSZ exposure mainly due to unpredictable drug absorption may be a challenge[3] (for instance by pH changes due to simultaneous administration of proton pump inhibitors).

Prophylactic use of PSZ in children has not been reported and no dosage recommendations are available for the pediatric population. Treatment of children with PSZ has been described in literature, but its use is off-label. Data derived from an open-label study and a multicenter retrospective survey in which PSZ was used as salvage therapy, showed that PSZ was safe and well tolerated in children 3 to 17 years of age.[4,5] In CGD patients (9–36 years) receiving salvage treatment with PSZ for an invasive fungal infection, 7 of 8 patients showed a complete response.[6]

Since a twice daily regimen has been shown to be efficacious in the curative setting of antifungal treatment, this frequency was preferred for prophylaxis over a 3 times daily dosing algorithm since adherence of young children may improve with less frequent intake of a drug.

The objective of our study is to determine whether this algorithm for PSZ prophylaxis is safe and well tolerated and results in adequate exposure in children with CGD.

METHODS

Approval was obtained from the Committee on Research involving Human Subjects of the Radboud University Nijmegen Medical Centre and the Academic Medical Centre in Amsterdam. The trial was conducted in compliance with the Declaration of Helsinki during 2009 and 2010 (Clinicaltrials.gov ID: NCT00799071). Informed consent was obtained from all parents and patients older than 12 years.

In this open-label, nonrandomized, multicenter, phase II, dose-finding trial, we aimed to include 20 CGD patients aged 2 to 16 years, to evaluate 16 patients. Any ITZ prophylaxis was stopped upon inclusion in the trial. An allometric dosing algorithm based on bodyweight (Kleiber potency index 0.75) was chosen for PSZ. Patients received PSZ oral suspension 40 mg/mL during 30 days with the following dosages: 10 to 14 kg: 120 mg; 15 to 19 kg: 160 mg; 20 to 24 kg: 200 mg; 25 to 29 kg: 220 mg; 30 to 34 kg: 260 mg; 35 to 39 kg: 280 mg; ≥40 kg: 300 mg. All doses were given BID with a meal (without dietary recommendations) and with a preferred interval of 12 hours. The patient and the parents or legal representative were provided with a diary to record the intake of PSZ.

Blood samples were drawn in the morning after 10, 20, and 30 days. A PSZ trough plasma concentration (C_{trough}) of 0.5 mg/L was pursued for adequate prophylaxis.[7] If the PSZ C_{trough} was lower, the dose was doubled and accompanied by repeated dietary advice. The dose was lowered by 50% if C_{trough} was >3.0 mg/L.

Safety was clinically assessed by using the Clinical Toxicity Grades (ACTG) scoring system at 4 predefined time points. Patients were to report side effects to the physician during the treatment period.

PSZ plasma concentrations were determined by a validated high performance liquid chromatography (HPLC) assay with fluorescence detection at the laboratory of the Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, The Netherlands. The assay is externally validated by an international proficiency testing program.[8] Patients could choose to continue PSZ after termination of the trial.

SPSS 16.01 (SPSS Inc., Chicago, IL) was used for statistical testing. A repeated measures ANOVA was used to compare PSZ C_{trough} on day 10, 20, and 30. A Mann-Whitney *U* test was used to test for differences in PSZ C_{trough} on day 10 for patients with and without prior ITZ prophylaxis.

RESULTS

Twelve patients (9 boys) were included and completed the study. The demographic characteristics and the PSZ C_{trough} on days 10, 20, and 30 are presented in Table 1 and in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A836.

A statistically significant difference was noted between the mean PSZ C_{trough} on days 10, 20, and 30 (repeated measures ANOVA, $P < 0.001$). Post hoc analysis revealed that the mean PSZ C_{trough} on day 30 was significantly lower than on day 10 and 20 ($P = 0.002$ and 0.004, respectively). One PSZ C_{trough} was above 3.0 mg/L (patient 11 on day 20). The dose was reduced by 50% and on day 30 the C_{trough} was 2.6 mg/L. Patient 7 had a C_{trough} <0.5 mg/L on day 30 (no deviation of adherence was reported in the patients diary). Median PSZ C_{trough} on day 10 for patients with or without ITZ prophylaxis prior to the trial was 1.90 and 1.50 mg/L, respectively (Mann-Whitney *U* test, $P = 0.432$).

TABLE 1. Demographic Characteristics and Posaconazole Trough Concentrations on Day 10, 20, and 30

Patient	Gender	Subtype CGD	Age (yr)	Weight (kg)	Twice Daily Dose PSZ, mg (mg/kg)	PSZ C_{trough} (mg/L)		
						Day 10	Day 20	Day 30
6	Male	AR (p47)	3.5	15	160 (10.6)	1.0	1.0	0.8
2*	Male	AR (p47)	4.8	22	200 (9.1)	2.1	2.4	1.9
4*	Male	AR (p47)	8.2	26	220 (8.5)	2.3	2.1	1.9
5*	Male	AR (p47)	8.2	27	220 (8.1)	1.9	1.7	1.7
12	Female	AR (p47)	8.3	28	220 (7.9)	2.1	2.7	2.2
9	Male	XL (p91)	9.7	29	220 (7.6)	1.3	1.4	1.0
8	Male	AR (p47)	12.8	35	280 (8.0)	1.5	2.5	1.4
11	Female	AR (p47)	11.7	37	Day 1–20: 280 (7.6) Day 20–29: 140 (3.8)	2.2	3.5	2.6
1*	Male	XL (p91)	14.9	39	280 (7.2)	1.8	1.8	1.6
7	Male	AR (p47)	15.6	47	300 (6.4)	0.8	0.6	0.3
3	Female	AR (p47)	11.4	49	300 (6.1)	1.8	1.7	1.5
10*	Male	AR (p47)	15.4	72	300 (4.2)	0.8	—†	—†
Mean						1.63	1.95	1.54
Median			10.6	32		1.80	1.80	1.60
IQR						1.23–2.10	1.55–2.45	1.20–1.90

*Indicates ITZ prophylaxis prior to trial.

†Patient refused to have blood samples taken on day 20 and 30.

Out of specification concentrations are underlined.

AR indicates autosomal recessive; XL, X-linked; PSZ, posaconazole; C_{trough}, trough plasma concentration.

Four patients had adverse events judged to be possibly or probably related to PSZ: skin rash (n = 2), stomach ache (n = 2), headache (n = 1), nausea (n = 1), and vomiting (n = 1). Both skin rashes started during treatment and both patients had had no prior azole exposure. One skin rash, 1 headache and 1 stomach ache were grade 2, all other adverse events were grade 1. In 1 patient the rash resolved during treatment. In all patients the adverse events did not lead to cessation of therapy.

No serious adverse events were reported. The following laboratory abnormalities were observed, each in one patient: elevation of alkaline phosphatase (grade 1) and aspartate aminotransaminase (grade 1), decreased albumin (grade 2), and mild leukocytosis.

No breakthrough fungal infections occurred during the trial. All 5 children who used once daily ITZ prophylaxis before the study chose to continue with the twice daily regimen of PSZ after completion of the trial.

DISCUSSION

The results show that an allometric approach is suitable for designing a dosing algorithm for PSZ prophylaxis in children with CGD, regardless of their age. PSZ was safe and well tolerated. All children using once daily ITZ (n = 5) before the study, chose to continue with PSZ after the trial, mostly because PSZ tasted better and caused less nausea. Patient 7 had a PSZ C_{trough} <0.5 mg/L on day 30. This may have been caused by temporary reduced adherence, as previous plasma concentrations were adequate. Although only one dose intervention was necessary, the data are still too limited to apply the dosing algorithm without determining PSZ C_{trough}. Increasing data suggests that therapeutic drug monitoring (TDM) is warranted during PSZ treatment,[9] because of large intra- and interindividual pharmacokinetic differences as a result of food intake, gastric pH, and mucosal damage.[9]

The mean PSZ C_{trough} was lower on day 30 than on day 10 and 20. A possible explanation could be an increased clearance of PSZ, as observed previously in mice.[10] Another explanation could be intake of PSZ with smaller amounts of food during the trial. Irrespective of the responsible mechanism, the small differences are regarded clinically irrelevant. TDM was pursued in all 5 patients who continued on PSZ after the trial showing no further decline in plasma concentrations over time. We tested for an influence of prior exposure to ITZ to rule out an effect on PSZ due to for instance inhibition of the P-glycoprotein drug transporter.

The European Medicines Agency registered prophylactic dose of PSZ in adults is 200 mg 3 times daily. A twice daily schedule was chosen to improve adherence and because combining the daily dose of PSZ in a single administration would probably lead to insufficient exposure because of saturable absorption. A twice daily dosing regimen is feasible also due to the long terminal half-life of PSZ.

To limit the number of blood samplings, C_{trough} was preferred over a full pharmacokinetic curve for exploratory purposes. The most suitable plasma concentration for prophylaxis and treatment with PSZ remains a point of discussion. At the time of study initiation, Cornely et al[7] reported on the superiority of PSZ to fluconazole or ITZ in the prophylaxis of invasive fungal infections in adult patients with neutropenia, with mean plasma PSZ concentration of 0.583 ± 0.381 mg/L. In line with these findings a target C_{trough} of 0.5 mg/L was chosen. In a recent report,[9] the FDA recommends a higher target value of an average PSZ plasma concentration of 0.7 mg/L. As for 11 patients in this trial, the PSZ C_{trough} (and thus also the average PSZ plasma concentration) was >0.7 mg/L, the current FDA recommendation has no influence on the interpretation of the results of this trial. There is no evidence to support the 3 mg/L upper limit but this target was chosen empirically to prevent unnecessary high drug exposure with possible subsequent toxicity.

Initially, the trial would encompass 16 patients. An interim analysis was planned after 8 patients to prevent treating too many patients with inadequate doses; no stopping criterion was defined if PSZ exposure was adequate in all patients. After the evaluation of the data for 12 patients, we concluded that the dosing algorithm was suitable for children with CGD. It was decided not to burden any further patients with this trial.

It remains to be proven whether the current algorithm can be used in other pediatric patient populations. We believe that even in patients known to have lower exposure to PSZ due to aspects such as mucositis, poor oral intake, etc, this dosing algorithm can be used as an initial starting regimen, and be further guided by TDM to ensure whether adequate exposure is attained.

Although no invasive fungal infections were observed, the trial design does not allow for any conclusions concerning the

superiority of PSZ with respect to ITZ, because of short duration of treatment and the limited number of patients.

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RESPIRATORY SYNCYTIAL VIRUS- AND INFLUENZA VIRUS-ASSOCIATED HOSPITALIZATIONS IN INFANTS LESS THAN 12 MONTHS OF AGE

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Abstract: Infants hospitalized because of respiratory syncytial virus (RSV) infection (n = 388) were significantly younger, had longer hospital stays, had a more severe course of disease, and required supplemental oxygen more often with longer duration of treatment as compared with those with influenza virus (n = 37) infection. Seasonal distribution varied, with RSV-associated hospitalizations peaking in January and influenza virus-associated hospitalizations in February. Congenital heart disease was more commonly a risk factor in infants with RSV infection.

Key Words: respiratory syncytial virus, influenza virus, hospitalization

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Acute respiratory tract infections due to respiratory syncytial virus (RSV) and influenza virus cause significant morbidity in young children.[1–3] Average annual hospitalization rates attributable to RSV infection have been reported to be 17 per 1000 children within 6 months and 3 per 1000 children aged below 5 years.[4] Healthy children less than 1 year of age are reported to have hospitalization rates due to influenza virus infection similar to those for adults at high risk,[2] with the average annual hospitalization rate being reported as 0.9 per 1000 children.[5] Aims of this retrospective cohort study were (1) to compare the severity of RSV versus influenza virus-associated respiratory tract infections, requiring hospitalization in infants aged <12 months and (2) to analyze associated risk factors.

Patient charts were identified using a search for International Classification of Diseases, 10th Revision codes including J12.1, J20.5, J21.0, and B97.4 for RSV infection; J10.1, J11.1 for influenza virus infection; and J20.8, J20.9, and J31.0 for general diagnoses of respiratory tract infections. Data were collected between October and May for the years 2004 to 2009 by reviewing the medical charts using the local electronic data system (Open Medocs—Medical Documentation System) that documents every episode of a patient's admission to the Department of Pediatrics, a tertiary care center, of the Medical University of Graz in Southern Austria. There are no written admission criteria regulating hospitalization for cases of suspected RSV or influenza infection at our pediatric department. Therefore, the decision is based on a physician's assessment of the severity of disease. In general, tachypnea/dyspnea, increased work of breathing, feeding difficulties, or low oxygen saturation <92% are criteria for hospitalization.

Infants were included if RSV or influenza virus infection was proven by enzyme-linked immunosorbent assay antigen testing or immunofluorescence technique, and the age was less than 12 months. Infants were excluded if RSV or influenza virus infection was acquired nosocomially or medical charts did not prove the diagnosis coded by the International Classification of Diseases.

RSV was detected by a rapid RSV ELISA test (Directigen EZ RSV Test, Becton Dickinson, Sparks, MD, 66.7%–87.2% sensitivity, 85.5%–91.6% specificity compared with culture) from nasopharyngeal aspirates. Influenza virus was detected by a rapid influenza A and B test (Actim influenza A&B Test, Medix Biochemica, Kauniainen, Finland) from nasopharyngeal aspirates.

Severity of respiratory tract infection was classified according to the lower respiratory illness (LRI) score [5] with 1 being an upper respiratory tract infection, 2–4 being a mild, moderate, or severe (with oxygen requirement) lower respiratory tract infection (LRTI), respectively, and 5 being LRTI with the need for mechanical ventilation. The highest score during hospitalization was taken for each infant.

Data collected included LRI score, days of hospitalization due to respiratory illness, days of oxygen requirement, days of respiratory support (either continuous positive airway pressure or mechanical ventilation), risk factors including presence of siblings, crowding (≥4 persons in one household), history of prematurity, and underlying diseases including bronchopulmonary dysplasia, congenital heart disease (CHD), immunodeficiency, neuromuscu-

TABLE 1. Risk Factor Evaluation in 388 Infants With RSV Compared to 37 Infants With Influenza Virus-associated Hospitalization

Risk Factors	RSV n = 388	Influenza Virus n = 37	*P*
Presence of any associated risk factor	223 (57.5)	24 (64.9)	0.193
Presence of siblings	188 (48.5)	21 (56.8)	0.168
No. siblings	1.15 ± 0.99 (0–5)	1 ± 0.65 (0–3)	0.445
Prematurity*	62 (16.0)	5 (13.5)	0.347
Gestational age (in wk)	38.0 ± 2.6 (24–42)	37.4 ± 3.7 (27–41)	0.174
Crowding (≥4 persons in one household)	185 (47.7)	21 (56.8)	0.146
Persons per household	4.2 ± 1.1 (3–9)	4.0 ± 0.6 (3–6)	0.153
BPD	0 (0)	0 (0)	1.000
CHD	50 (12.9)	1 (2.7)	0.034
Neuromuscular disease	0 (0)	0 (0)	1.000
Immunodeficiency	2 (0.5)	0 (0)	0.331
Anomalies of the airways or lungs	2 (0.5)	1 (2.7)	0.065
Multiple birth	14 (3.6)	3 (8.1)	0.091

Data are presented as number (%) or mean ± SD (range).
*All infants born before 37 wk of gestational age.
BPD indicates bronchopulmonary dysplasia; CHD, congenital heart disease.

lar disease, and anomalies of the airways or lungs. Length of hospital stay, duration of oxygen requirement, or days on mechanical ventilation were calculated as 1 day if duration was below 24 hours and as 2 days if duration was 25 hours or more, and so on.

The study was approved by the local ethics committee (number 20–328 ex 08/09). Statistical analysis was performed by using the χ^2 and the Yate's corrected χ^2 tests, as appropriate, for categorical data and the *t* test and Fisher exact tests as appropriate for numerical data. Descriptive statistical analysis included number and percentage for categorical and mean with standard deviation and range for numerical data. A $P < 0.05$ was considered to be significant.

During the study period, a total of 433 infants were hospitalized, 388 due to RSV (89.6%), 37 due to influenza virus infection (8.6%), and 8 infants tested positive for both the viruses (1.8%). In all 31 infants tested influenza A (83.8%) positive, 5 influenza B (13.5%), and 1 was positive (2.7%) for both the types. The seasonal distribution revealed RSV-associated hospitalizations peaking in January and influenza virus-associated hospitalizations peaking in February (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A785). The number of hospitalized infants during 5 consecutive cold seasons was 113, 25, 55, 53, and 142 for RSV and 7, 1, 10, 8, and 11 for influenza cases, respectively (8 cases with mixed viral infections excluded).

The clinical course of infants with RSV compared with influenza virus-associated hospitalization differed significantly regarding length of stay (7.5 ± 4.3 vs. 5.9 ± 3.4, $P = 0.013$), frequency of need for supplemental oxygen (45% vs. 2.7%, $P < 0.001$), duration of treatment with supplemental oxygen (5.1 ± 3.7 vs. 0.1 ± 0.8 days, $P < 0.001$), and severity of respiratory tract infection evaluated by LRI scores (mean, 3.0 vs. 1.6, $P < 0.001$). There were no significant differences between intensive care unit (ICU) admission rates (6.2% vs. 0%, $P = 0.060$), the need for respiratory support (2.8% vs. 0%, $P = 0.150$), length of stay in ICU (10.4 ± 7.1 vs. 0 days, $P = 0.098$), and days on mechanical ventilation (9.4 ± 7.1 vs. 0, $P = 0.206$). Infants with RSV infection were of significantly younger age (mean, 2.8 ± 2 vs. 4.2 ± 2.8 months, $P < 0.001$). There was no difference in gender distribution between the 2 groups (male, 50% vs. 57%, $P = 0.208$).

There were associated risk factors in 223 (57%) infants with RSV compared with 24 (65%) infants with influenza virus infection. CHD was more commonly a risk factor in infants with RSV infection (12.9% vs. 2.7%, $P = 0.034$), further results are depictured in Table 1.

In this retrospective study, we found that infants hospitalized for RSV infection were of significantly younger age, had longer hospital admissions, a more severe course of disease measured by LRI score, and required supplemental oxygen more often with longer duration of treatment compared with infants with influenza virus infection. The overall admission rate to ICU and the need for mechanical ventilation were low in both the groups. The seasonal distribution showed a peak in January for RSV-associated hospitalizations and a peak in February for influenza-associated hospitalizations.

A comparison with other studies concerning the severity of respiratory tract infections was limited due to different methods of severity assessment including LRI score, length of hospital stay, ICU admission, duration of intensive care therapy, or the requirement for supplemental oxygen, but findings for RSV cases were comparable to previous prospective findings.[6]

In infants hospitalized for RSV infection, age at admission was comparable to a Danish study with a median age of 2.7 months,[7] whereas others reported on median ages between 3.5 and 6 months.[6,8–10] A recent study on the burden of influenza in children by Poehling et al[5] reported on nearly half of the hospitalized children being <6 months of age.

An average length of hospital admission for RSV and influenza infection of 4 to 7 days has been reported throughout Europe[8,11,12] compared with 2 days in the United States[1,4,13] and the United Kingdom.[14] The difference in duration of in-patient treatment in different countries has already been documented by Behrendt et al.[15] A shorter average duration of hospital admissions in cases of influenza infection has also been reported by other authors.[10,12] The frequency of need for supplemental oxygen in RSV cases was similar to the rates of 38% and 43% reported by others, respectively,[16,17] and remarkably higher rates were reported from the United States[1] and Switzerland.[11] Several studies focusing on frequency of oxygen treatment in influenza-virus-associated LRTI[1,17,18] reported on rates between 30% and 42%, which is in contrast to our significantly lower rates. The study of Poehling et al[5] revealed that infants aged below 6 months required supplemental oxygen less often (13%) compared with older infants aged 6 to 59 months (32%). The New Vaccine Surveillance Network re-

ported on a comparable rate of 4.3% of ICU admissions associated with RSV LRTI and no infants with influenza virus infection being admitted to the ICU. Mechanical ventilation was rarely needed, and our data was compared with studies from Norway,[8] Switzerland,[11] and Israel.[17] Associated risk factor analysis revealed a lower CHD rate ranging from 1.4% to 2.4% in RSV cases from several studies[8,9,11] but remained similar after exclusion of persisting foramen ovale.

In conclusion, we found a significantly higher burden of RSV compared with influenza virus disease in hospitalized infants aged below 12 months by means of clinical severity score, duration of hospitalization, and need for oxygen therapy.

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WANING IMMUNITY TO VARICELLA IN INFANTS OF HUMAN IMMUNODEFICIENCY VIRUS-SEROPOSITIVE AND -SERONEGATIVE MOTHERS

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Abstract: Immunity to varicella in HIV-exposed and -unexposed infants born to unvaccinated mothers, acquiring protective antibodies at birth declined to nonprotective (<1:8) levels by 5 months of age. Therefore, infants become susceptible to varicella before 12 months, which is the recommended time for varicella immunizations in the United States. Vaccination of susceptible HIV-seronegative women in the postpartum period may be important to consider.

Key Words: varicella zoster virus, passively acquired antibodies, HIV status

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The incidence of varicella in the United States has significantly decreased as a result of universal varicella vaccination administered at 12 months of age since 1995[1] and a second dose at 4 to 5 years of age introduced in year 2006.[2] Although varicella outbreaks continue to be reported in vaccinated populations in the United States even after receiving 2 doses,[3] disease severities are milder than in primary varicella in susceptible individuals. As overall disease incidence declines, the risk for exposure to varicella zoster virus (VZV) decreases, leading to susceptible children in adolescence and adulthood.

Varicella can cause significant morbidity and mortality, particularly in young and immunocompromised populations.[4,5] Therefore, it is not surprising that young infants and HIV-seropositive individuals suffer more complications with this infection.[6–9] A recent study has suggested that even HIV-exposed but uninfected infants can suffer higher morbidities when infected with this virus, reflecting a clinical immunodeficiency.[10]

Varicella during pregnancy is not common, presumably due to immunity in most women of childbearing age.[11] Infants in their first few weeks of life are thought to be protected by passive IgG antibodies from their mothers. However, varicella infection during the first and third trimesters of pregnancy can be complicated by congenital varicella syndrome and pneumonitis or meningitis.[11–13]

Duration of passive immunities to VZV during infancy has not been well studied. A recent study from France has documented significant decline of these antibodies during infancy,[14] however, it did not include HIV-exposed infants. Therefore, we studied immunity levels to VZV between unvaccinated HIV-seropositive and -seronegative mothers, cord blood of their infants, and duration of passive immunities in the first 6 months of infants' lives to determine whether disparities existed between the 2 groups.

METHODS

Study Population. The protocol was reviewed and approved by the Institutional Review Board at Meharry Medical College (MMC), and informed written consents were obtained from each pregnant

woman. The study was conducted between June 2000 and August 2001.

Pregnant Women. Fifteen HIV-seropositive pregnant women attending obstetric clinics at MMC and Metro-Nashville General Hospital were enrolled in the study during their first, second, or third trimester of pregnancy. Age of the subjects, HIV testing results, antiretroviral therapy, T cell counts, and viral loads were obtained from the medical records of the patients. Twenty-nine HIV-seronegative pregnant women attending the same clinic (matched for age) served as the control group. None of the pregnant women received intravenous immunoglobulin or varicella vaccination in their life time. Pregnant women were followed prospectively up to their deliveries.

Infants. Cord bloods from infants were obtained at the time of delivery. Gestational ages of the newborn infants were noted from their medical records. Infants were followed up to a mean of 5 months of age when repeat blood samples were obtained.

All cord bloods were mother–infant pairs. All infants were born at term. Twelve of 15 (80%) HIV-exposed and 13 of 29 (45%) HIV-unexposed (control) infants were available for follow-up at 3 to 6 months of age. None of the infants had contracted varicella during the study period.

Sample Size Calculation. The sample size required to achieve statistically significant results was based on a previous study.[15] A sample size of 14 in each group would have 80% power to detect a difference in means of 1.6 in antibody levels to varicella and a standard deviation of 1.165 using a 2-group *t* test with a 0.01 2-sided significance level.

Determination of Antibody Titers. Antibody titers against VZV were performed by indirect fluorescent antibody at Specialty Laboratories, Santa Monica, California. Baseline and follow-up antibody levels were evaluated in both HIV-exposed and -unexposed cord bloods and peripheral bloods of infants at a mean of 5 months of age. Correlates of immunity were defined as antibody titer >1:8 indirect fluorescent antibody.

Immunologic and Virologic Studies. T-cell analyses were performed by flow cytometry at MMC for HIV-seronegative group and at Vanderbilt University Medical Center core laboratories, Nashville, TN, for HIV-seropositive and exposed subjects. HIV viral loads in HIV-seropositive pregnant women, and HIV DNA testing in their infants were performed by polymerase chain reaction at Vanderbilt University Medical Center.

Statistical Analysis. Prevalence of immunity to VZV between HIV and control groups and between cord and peripheral bloods of infants (matched pairs) at follow-up was compared by 2-tailed Fisher exact test. CD4 cell counts between the HIV and control mothers were compared by a 2-tailed *t* test. Immunologic (CD4 cell counts/mm^3) and virologic (viral load copies/mL) parameters were correlated with VZV antibody levels within the HIV group by Spearman correlation and linear regression tests. $P \leq 0.05$ was considered statistically significant. Software Intercooled stata version 8.0 was used for statistical analyses.

RESULTS

Pregnant Women and Infants. The mean (range) ages of pregnant women at the time of serologic assays were compared. None of the HIV-seropositive women were in the category of adult immunodeficiency syndrome (AIDS) based on a clinical history of opportunistic infections or CD4 counts less than 200 cells per mm^3. Of 15 (53%) HIV-seropositive pregnant women, 8 were on antiretroviral therapy at entry, and all (100%) were on combination therapy (zidovudine, dideoxyinosine, stavudine, lamivudine) at the time of delivery. All HIV-exposed infants tested negative for HIV by DNA polymerase chain reaction at birth, 1 month, and 6 months of age.

Immunity to Varicella in Mothers. Fifteen of 15 (100%) HIV-seropositive and 26 of 29 (90%) HIV-seronegative pregnant women were immune to VZV.

Immunity to Varicella in Infants. Protective immunity to varicella was compared in 14 of 15 (93%) HIV-exposed and 26 of 29 (90%) HIV-unexposed cord blood samples. Using infant paired data only, the protective immunity cord bloods of 11/12 (92%) HIV-exposed and 13/13 (100%) HIV-unexposed infants declined to nonprotective levels in 11/12 (92%) HIV-exposed and 10/13 (77%) HIV-unexposed infants (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A791).

Immunologic Parameters in Mothers. Mean CD4 counts were significantly ($P = 0.01$) lower in all HIV-seropositive (609/mm^3) mothers compared with the 20/29 (69%) HIV-seronegative (997/mm^3) mothers.

DISCUSSION

This manuscript describes a study of immunity to varicella in HIV-seropositive and -seronegative pregnant women and passive immunity in their infants. All 15 HIV-seropositive women on antiretroviral therapy had serologic evidence of immunity, with transplacental transfer in 14/15 (93%) women; 26 of 29 (90%) HIV-seronegative mothers were immune to VZV, and antibody was transferred across the placenta in all 26. At a mean age of 5 months, the passively acquired maternal antibody had disappeared in 11/12 (92%) HIV-exposed (none infected with HIV) and 10/13 (77%) HIV-unexposed infants. This decline in antibody was expected, given the half-life of IgG of 1 month. At the same time, the fact that babies born to HIV-seropositive mothers had passive protection against varicella equal to the babies for HIV-seronegative mothers is important. Both groups of mothers had antibody from natural infections rather than vaccine. Therefore, the findings in this report will not be applicable to pregnant women who have acquired their VZV immunity solely through varicella immunization, without ever having had community (wild-type) chicken pox. Because varicella vaccination is not universal except in North America (and a few other countries), the report remains relevant to children born throughout the world.

This study fills a gap in our knowledge about the duration of VZV antibody titers in infants after delivery to mothers known to have had both HIV infection and chickenpox. A recent study from France[14] has determined that their babies had high VZV titers at birth and low titers by 4 to 5 months of age. Therefore, the current study confirms the French study and expands the data to include HIV-seropositive subjects. Thus, the study provides important clinical information to neonatologists and pediatricians.

Our findings suggest that both HIV-exposed and -unexposed infants become susceptible to varicella infection well before the currently recommended time for routine VZV immunizations at 12 months in the United States. In addition, susceptible pregnant women will also be at risk for contracting varicella infection from these babies. We could not determine the impact of maternal clinical, immunologic, or virologic deterioration on VZV immunity levels within the HIV-seropositive mothers from this study. We recommend routine VZV screening during pregnancy of all women and vaccination of susceptible HIV-seronegative women before the hospital discharge. Health care providers involved in the care of HIV-seropositive mothers need to be aware and should consider prophylaxis with oral acyclovir or one dose of intravenous immunoglobulin for their patients within 96 hours of their infants' exposure to varicella outbreaks at day care centers. In addition, maintaining adequate numbers of CD4 counts and lower

viral loads with highly active antiretroviral therapy during pregnancy in HIV-seropositive women should be reinforced. Further prospective studies need to be conducted with a larger population to evaluate the effect of CD4 counts and viral loads on immunity levels in HIV-seropositive mothers.

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IMPACT OF HUMAN IMMUNODEFICIENCY VIRUS COINFECTION ON THE PROGRESSION OF MOTHER-TO-CHILD TRANSMITTED HEPATITIS C VIRUS INFECTION

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Abstract: Data on mother-to-child transmitted human immunodeficiency virus/hepatitis C virus (HIV/HCV) coinfection are scarce. A prospective observational study with a cohort of 70 HCV-infected children (13 of whom were HIV/HCV-coinfected; mean follow-up: 7.3 years) is presented. In our series, surrogate markers of disease progression (HCV viremia, maximum alanine aminotransferase values, and spontaneous HCV infection clearance) suggest that the evolution of liver disease in HIV/HCV-coinfected pediatric patients is more aggressive than it is in HCV-only infected children.

Key Words: coinfection, hepatitis C virus, human immunodeficiency virus, mother-to-child transmission

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Both human immunodeficiency virus (HIV) and hepatitis C virus (HCV) can be mother-to-child transmitted (MTCT). Reported HIV/HCV coinfection MTCT transmission rates range from 3.6% to 9.5%,[1,2] and it is estimated that 150 to 300 children are born to coinfected mothers in Western Europe each year.[3] Currently available prophylactic measures to prevent HIV MTCT have decreased the transmission rate of HIV below 2%, whereas the risk of HCV MTCT ranges from 5% to 23% in infants born to coinfected mothers.[3] New MTCT HIV/HCV coinfection is extremely rare and the number of coinfected children in Western Europe is small,[3] but the prevalence of this condition is increasing in developing countries.[4,5]

The data available on the natural course of liver disease in MTCT HIV/HCV coinfection are scarce, difficult to interpret because of the complex interactions between HCV, HIV and antiretroviral (ARV) drugs, and based on studies that show important methodologic limitations. We aimed to evaluate the influence of HIV coinfection on the progression of HCV-related hepatic disease in a cohort of MTCT-HCV-infected untreated children.

MATERIALS AND METHODS

We conducted a prospective observational study with a cohort of MTCT-HCV-infected pediatric patients, as defined in International Guidelines,[6] followed up in the outpatient clinic of a tertiary-care, pediatric hospital in Barcelona (Spain) since 1991. The study protocol was approved by the local ethics committee, and informed consent was required from the parents or legal guardians of the patients at enrollment.

As per protocol, at HCV infection diagnosis, epidemiologic data and medical history are collected. Quarterly visits include a clinical interview followed by a complete physical examination. The following laboratory determinations are performed at least every 6 months (at least every 3 months in HIV/HCV-coinfected patients): complete blood count, plasma alanine aminotransferase (ALT; normal values <40 IU/L), and HCV viremia (HCV-RNA; Cobas Amplicor HCV Monitor Test, version 2.0 and Amplicor HCV, Roche Molecular Systems, Basel, Switzerland from 1997 to 2005, limits 500 and 50 IU/mL, respectively; and Abbott RealTime HCV, Abbott Diagnostics, Chicago, IL since 2006, limit 30 IU/mL). HCV genotype is performed by real-time polymerase chain reaction (in house method[7]), usually at the time of diagnosis.

According to International Guidelines,[6,8–11] the following definitions regarding HCV infection evolution were used: (*a*) Clearance of HCV infection is defined as the situation in which the

2 most recent determinations of HCV RNA are negative, together with normal values of ALT in an asymptomatic patient; (*b*) In chronic asymptomatic infection, the HCV-infected patient has intermittent positive viremia and usually presents with normal ALT values and no symptoms; and (*c*) In chronic active infection, the child shows persistently positive HCV RNA determinations and elevated ALT, while usually remaining asymptomatic.

All children with at least 1 year of follow-up who had never received anti-HCV drugs were included (n = 70, 34% of whom followed up from birth). This group of patients was further divided into HCV-infected children and HIV/HCV-coinfected patients, and the evolution of HCV infection was compared between these 2 groups. In those children who had spontaneous clearance of HCV infection, data obtained after clearance were not further considered in the analysis.

In HIV/HCV-coinfected patients, the following HIV-related additional variables were collected: clinical CDC category,[12] nadir and current CD4 cell counts or percentages (flow cytometry, FACSCalibur; BD Biosciences, San Jose, CA), evolution of HIV plasma viral load (CA HIV Monitor; Roche, Basel, Switzerland; limit <50 copies/mL), and current and past use of ARVs and other hepatotoxic drugs.

Statistical Analysis. Categorical variables were described as percentages, and continuous variables as median values and ranges. To account for variation in the frequency of testing, summary variables for the proportion of positive polymerase chain reaction tests and elevated (>40 IU/L) ALT levels for each child were created.[6] Proportions of interest, along with their 95% confidence intervals, were estimated and compared using the χ^2 test or the Fisher exact test, as appropriate. Other nonparametric tests were used as indicated. Statistical significance was attributed to $P < 0.05$. The study was carried out using the SPSS 15.0 software.

RESULTS

As of January 2010, 92 MTCT-HCV-infected patients had been enrolled in the cohort. All children had been followed up for at least 1 year. After excluding patients who received interferon in the late 1990s, 57 HCV-infected patients and 13 HIV/HCV-coinfected patients were included in the study. The main characteristics of the 2 groups are summarized in Table 1.

Median age at initial assessment was 14 months for both groups. Overall, 19 HCV-infected patients (33.3%) and 5 HIV/HCV-coinfected patients (38.5%) were followed up from birth. The follow-up period was longer and the age at most recent follow-up was higher in HIV/HCV-coinfected children because they were born in earlier years of the study. Most patients remained asymptomatic during the follow-up period. HCV-related clinical signs or symptoms were uncommon, without differences between groups. The HCV genotype distribution was not different between the 2 groups.

Median (range) HCV viremia determinations in HCV-infected and HIV/HCV-coinfected patients were 7 (1–23) and 4 (1–20), respectively. Overall, 44 HCV-infected patients (77.2%) and 12 HIV/HCV-coinfected patients (92.3%) had positive HCV RNA in more than 75% of the determinations; the proportion of positive HCV viremia determinations was higher among HIV/HCV-coinfected children, although this difference did not reach statistical significance ($P = 0.07$).

In 22 HCV-infected patients (38.6%), ALT levels were abnormal in more than 75% of determinations, and only 4 patients in this group showed persistently normal ALT values. Five (38.5%) HIV/HCV-coinfected patients showed elevated ALT titers in more than 75% of determinations and no patient showed persistently normal ALT values. Maximum ALT levels were higher among coinfected children (178 vs. 99 IU/L, median values; $P = 0.03$).

TABLE 1. Main Characteristics of HVC-infected Patients and HIV/HCV-coinfected Patients

Characteristics	HCV-infected (n = 57)	HIV/HCV-coinfected (n = 13)	*P*
Females, n (%)	26 (46)	7 (54)	NS
Follow-up time (yr)	7.4 (1.3–21)	14 (4.1–20)	<0.0001
Current age (yr)	13.7 (5.3–23.4)	17.4 (13.5–25.2)	<0.0001
Children with HCV-related clinical signs or symptoms, n (%)	1* (2)	1† (8)	NS
HCV genotype, n (%)			
1a	18 (31.7)	3 (23.1)	NS
1b	11 (19.3)	1 (7.7)	
2	1 (1.7)	1 (7.7)	
3	7 (12.3)	1 (7.7)	
4	12 (21)	4 (30.7)	
Unknown	8 (14)	3 (23.1)	
No. ALT determinations	10 (3–23)	61 (16–93)	<0.0001
Proportion of elevated ALT determinations (>40 IU/L)	66.7 (0–100)	47.6 (6.2–98.5)	NS
Maximum ALT level (IU/L)	99 (14–655)	178 (47–782)	0.03
Proportion of positive HCV viremia determinations	100 (0–100)	100 (67–100)	0.07

Values are expressed as median (range) if not otherwise stated.

*A 14-year-old girl with epigastralgia and normal physical examination was diagnosed with genotype 1b HCV infection (ALT 104 IU/L, HCV RNA 376000 IU/mL).

†A 3-year-old vertically HIV-infected girl with nonsymptomatic hepatomegaly was diagnosed with genotype 4 HCV infection (ALT 80 IU/L, positive anti-HCV antibody, HCV RNA not available); she presented with decompensated liver disease at the age of 11 year, that lead to antiretrovirals (stavudine, didanosine, and nelfinavir) discontinuation because of hepatotoxicity. Her chronic hepatitis worsened to Class B of Child-Pugh Score at the age of 14 year, and later improved upon optimal immunologic and virologic response following the implementation of a new antiretroviral regimen (tenofovir, lamivudine, and nelfinavir).

ALT indicates alanine aminotransferase; NS, not significant; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

Spontaneous clearance of HCV infection occurred in 10 HCV-infected patients (17.5%, at a median age of 3.8 years), but not in any HIV/HCV-coinfected children. In 7 patients (3 of them HIV/HCV-coinfected), HCV infection followed a pattern of chronic asymptomatic infection. Finally, 43 HCV-infected patients (75.4%) and 10 (76.9%) HIV/HCV-coinfected patients showed a virologic and laboratory evolution consistent with chronic active infection. Prevalence rate of these 3 patterns was not different between HCV-infected and HIV/HCV-coinfected patients. One HIV/HCV-coinfected 11-year-old girl evolved to liver failure (Table 1).

Among HIV/HCV-coinfected patients, clinical status, HIV viral load, and CD4 cell counts did not correlate with ALT values or with HCV infection evolution patterns. Similarly, ARV treatments (time on therapy, number of highly active ARV therapy [HAART] regimens, and individual use of stavudine, didanosine, abacavir, or nevirapine) were not associated with maximum ALT levels or with proportions of abnormal ALT (data not shown).

DISCUSSION

MTCT HCV infection spontaneously clears in up to 20% of cases. In the rest of the children, slow and progressive histologic injury occurs, although the natural course appears to be milder than in the adult patient and the development of advanced liver disease

is uncommon in children.[13] Most patients remain asymptomatic throughout childhood and adolescence.[6]

Very scarce data are available on the progression of HCV infection in HIV/HCV-coinfected children, and monitoring practices for these patients in Europe vary widely.[1,3] To date, only 5 studies[2,4,6,14,15] have reported data on the evolution of HIV/HCV coinfection in childhood, mostly focusing on HIV infection. In contrast, the effect of HIV/HCV coinfection on liver-related morbidity and mortality in adults has been extensively studied. In this population, HIV/HCV coinfection is associated with increased liver fibrosis progression, worsening of liver function, cirrhosis, hepatocellular carcinoma, and liver-related mortality.[16,17] In fact, viral hepatitis is among the leading non-AIDS-defining causes of death in the HIV-infected population in the HAART era.[18]

In HCV-only infected adults, HCV-related symptoms, serum aminotransferase titers, and viral factors correlate poorly with liver histology and disease progression. Conversely, in a multicenter prospective study including 266 vertically HCV-infected patients in Europe, hepatomegaly, viremia, and high ALT values were associated with active infection (although histologic data were not available), whereas low HCV RNA concentrations in the first year of life were predictive of spontaneous clearance later on.[6]

In HIV/HCV coinfection, higher levels of HCV viremia have been observed, correlating inversely with immunosuppression and having an impact on treatment response. In our study, a nonsignificant higher proportion of positive HCV viremia determinations was found in coinfected patients; similar results have been reported by other authors,[2,6] although statistical significance was never reached. Chronic active infection was the most common pattern of evolution in our cohort, affecting 75% of the patients, without differences between HCV-infected and HIV/HCV-coinfected children. Very similar figures were recently reported by Bortolotti et al in the largest pediatric observational study of chronic HCV infection to date.[13] However, as compared with what happened in 17% of the HCV-only infected children, we did not observe spontaneous virologic clearance in the setting of coinfection, a phenomenon that has very rarely been reported elsewhere.[6] Liver failure occurred only in an 11-year-old coinfected girl in our cohort; other cases of disease progression in pediatric patients have been reported,[13,19] mainly affecting children with MTCT-transmitted genotype 1 HCV infection. This questions the widely held opinion that the natural course of HCV infection in childhood is benign and raises the need to consider early pegylated interferon/ribavirin therapy in selected patients, including those with HIV/HCV coinfection.[20]

In our study, proportion of elevated ALT levels was not different between groups, but maximum ALT values were significantly higher among coinfected individuals. This has not been reported previously and may be partially explained by the synergistic hepatotoxic effect associated with several ARV drugs, as suggested by England et al in a large cohort study of parenterally coinfected Libyan children[15]; however, other authors have reported higher hepatic enzyme levels in coinfected patients when compared with HIV-only infected children, regardless of the use of ARV.[4,14] Small numbers probably prevented us from observing associations between liver disease markers and HIV infection-related variables that have been reported in adults.[21] Moreover, it should be noted that initial follow-up in most coinfected patients occurred in the pre-HAART era, with uncontrolled HIV replication and a higher risk of immunosuppression.

In summary, surrogate markers of disease progression (higher viremia and ALT levels and lack of spontaneous clearance) suggest that the evolution of liver disease in HIV/HCV-coinfected pediatric patients is more aggressive than it is in the HCV-only infected child. Larger studies with long-term data and histologic assessment are needed; until then, these patients should be monitored carefully, and early combined antiviral treatment should probably be considered.

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LINEZOLID AND LACTIC ACIDOSIS

A ROLE FOR LACTATE MONITORING WITH LONG-TERM LINEZOLID USE IN CHILDREN

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Abstract: Linezolid administration has been associated with lactic acidosis in adults; however, the same phenomenon has not been reported in children. Mitochondrial protein synthesis inhibition is a demonstrated mechanism for toxicity, which therefore may manifest as lactic acidosis. Three cases of linezolid-associated lactic acidosis in children are reported to reinforce the need for pediatric caregivers to be vigilant of this potential side effect.

Key Words: pediatric, lactic acidosis, linezolid, vancomycin-resistant *Enterococcus*, multidrug resistance, mitochondria

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Linezolid, an oxazolidinone antibiotic, is useful against multidrug-resistant Gram-positive bacteria by virtue of its unique suppression of mRNA translation in prokaryotic organisms. In particular, it is an agent of choice against vancomycin-resistant *Enterococcus faecium* (VRE). Several adult case reports describe lactic acidosis as an adverse effect of linezolid.[1–3] A putative cause of toxicity is inhibition of mitochondrial protein synthesis as demonstrated in cell models of linezolid exposure.[4–6] Possible reasons are physiologic homologies mitochondria share with prokaryotes. Given that lactate catabolism occurs primarily in the liver and kidneys, dysfunction of these organs could place a patient at risk for linezolid-induced lactic acidosis. To date, this adverse effect has not been reported in children regardless of hepatic or renal status. This article presents experiences of caregivers with 3 pediatric patients who received courses of linezolid complicated by lactic acidosis (Table 1). This highlights the need for the caregivers to be vigilant for its development.

TABLE 1. Summary of Cases

Age/Sex	Underlying Diagnosis	Pathogen Treated	Infection Site	Dose	Course	Lactate Peak	Parameters Near Lactate Peak					
							Lowest Pretransfusion Platelets	Lowest Pretransfusion Hgb	Bilirubin	AST	ALT	Creatinine
Case 1: 6 mo/male	Short bowel syndrome	VRE	Respiratory, bloodstream	75 mg (10 mg/kg) q.8h.	39 d	24.0 mMol/L	27×10^9/L	8.1 g/dL	28.5 mg/dL	2617 IU/L	685 IU/L	1.1 mg/dL
Case 2: 6 mo/female	CDG-1A	VRE	Respiratory	47 mg (10 mg/kg) q.8h.	31 d	38.1 mMol/L	60×10^9/L	7.9 g/dL	0.7 mg/dL	205 IU/L	101 IU/L	0.8 mg/dL
Case 3: 16 yr/male	Cryptogenic cirrhosis	VRE	Urinary tract	600 mg (18 mg/kg) q.12h.	7 d	30.0 mMol/L	44×10^9/L	7.3 g/dL	35.5 mg/dL	2749 IU/L	357 IU/L	2.6 mg/dL

VRE indicates vancomycin-resistant *Enterococcus*; CDG-1A, congenital disorders of glycosylation type 1A.

CASE 1

A 6-month-old boy with a history of prematurity at 25 weeks was admitted for small bowel and liver transplantation evaluation due to previous necrotizing enterocolitis and liver disease with coagulopathy. His medical history included polymicrobial peritonitis, methicillin-resistant *Staphylococcus aureus* (MRSA), VRE, and ventilator-dependent bronchopulmonary dysplasia. During his admission, he had multiple infections including respiratory tract *Citrobacter*, *Enterobacter,* and MRSA for which he was treated. Early in his admission, he received linezolid for surgical-site MRSA after repair of mucocutaneous fistulas and a history of VRE. After 4 days of therapy, he demonstrated no culture positivity or clinical signs of acidosis. Four weeks later, an ESBL-producing *Klebsiella pneumoniae*, catheter-associated

bloodstream infection necessitated ciprofloxacin and amikacin treatment that continued until 10 days after cultures cleared. However, he concomitantly developed a VRE bloodstream infection, which was treated with linezolid for 14 days. Cultures became negative, but a progressive metabolic acidosis developed with a pH of 7.37 on day 1 of therapy trending to 7.13 on day 13, lactate level measured at 4.9 mEq/L (reference ranges, 0.5–1.3 mEq/L) on day 13. Linezolid was discontinued as planned and his acidosis resolved.

As a diagnosis of linezolid-associated lactic acidosis was not clear, a third course of linezolid was administered 3 weeks later to treat sepsis symptoms and confirmed tracheal VRE. Coagulopathy from hepatic insufficiency led to unremitting slow mucosal bleeding, and plasma exchange was performed for multiple system organ failure. Despite exchange, his lactate level peaked at 6.1 mEq/L. He remained symptomatic from recalcitrant VRE. At 39 days after linezolid was started for his airway cultures, the patient clinically worsened with pressor-refractory shock and his lactate levels abruptly increased to 24 mEq/L. Linezolid therapy was discontinued and despite aggressive supportive care including continuous renal replacement therapy, the lactic acidosis improved only marginally and he died 2 days later.

This patient received 4 separate courses of linezolid totaling 53 days of treatment.

CASE 2

A 6-month-old girl with hepatic insufficiency and failure to thrive was admitted for a gastroenterology evaluation. Because of persistent respiratory failure, she was mechanically ventilated at arrival. She had protein-losing enteropathy with resultant hypogammaglobulinemia, hyponatremia, and chronic diarrhea. Her bloody stools and persistent protein losses resulted in hypoalbuminemia with interstitial fluid accumulation. Diuresis, octreotide, and albumin supplementation were provided for maintenance of her tenuous state. Early during her hospital stay, her lactate remained in the range of 0.9 to 1.6 mEq/L. This was in the light of significant inotropic support and adrenal replacement therapy. Eight weeks after her hospital stay, linezolid was added empirically because of prior VRE infection and suspected sepsis. Linezolid was continued when growth of VRE was observed from a respiratory specimen. Subsequently, she was diagnosed with congenital disorders of glycosylation type 1A both by serology and skin biopsy.

Four weeks after linezolid initiation, she had significant metabolic acidosis and was found to have a serum lactate of 7.9 mEq/L. In the last week of her life, her multiple organ system failure worsened, and despite increasing cardiovascular support, her levels of serum lactate did not improve. Blood oxygenation deteriorated and her lactates increased as high as 38.1 mEq/L. She manifested refractory hypotension and died soon after.

Her lactate value was 2.4 mEq/L that elevated 10 days after starting linezolid treatment. It increased to 7.9 mEq/L with the development of metabolic acidosis 27 days into linezolid therapy. D-lactate at 29 days into treatment was not detected with a concomitant whole blood lactate of 13.2 mEq/L, indicating that her hyperlactatemia was likely not from intestinal sources. Cumulatively, she received 31 days of linezolid treatment.

CASE 3

A 16-year-old boy with cryptogenic cirrhosis was admitted for poor weight gain and refractory ascites. A venogram demonstrated bilateral hepatic vein occlusion, and portal hypertension was documented with asymptomatic esophageal varices. His ascites was symptomatic and frequently required drainage during admission. He transiently required broad-spectrum antimicrobial coverage for spontaneous bacterial peritonitis but was otherwise maintained on spontaneous bacterial peritonitis prophylaxis. In his first month of admission, he developed *Candida* peritonitis with a MRSA bloodstream infection. These were treated with liposomal amphotericin B and vancomycin, respectively. He developed *Clostridium difficile* enteritis and *Enterobacter cloacae* peritonitis with resultant abdominal distention and respiratory insufficiency. Ongoing supportive management included intermittent drainage, diuretics, octreotide, directed antibiotic therapy, and mechanical ventilation.

During the next month of his stay, he had a period of relative stability, and underwent hepatic vein dilatation and stent placement. Approximately a week later, he developed depressed mental status and worsening respiratory distress. Linezolid was started for the growth of VRE in his urine with antifungal and broad-spectrum peritoneal coverage. His lactate concentration at this time was 0.9 mEq/L. He was soon intubated for worsening respiratory status and required inotropic support. By day 6 of linezolid therapy, his lactate level had increased to 8.7 mEq/L as he developed pressor-refractory shock. Despite continuous renal replacement therapy, his lactate concentration continued to rise being 28 mEq/L by day 7 of linezolid therapy. Given his rapidly deteriorating status, his family withdrew support.

DISCUSSION

Linezolid is one of few antimicrobials available for multidrug-resistant Gram-positive bacterial infections. Alternatives include daptomycin, quinupristin/dalfopristin, doxycycline, and chloramphenicol, but linezolid has a comparatively low-adverse-effect profile, and an oral formulation. Current approved indications for linezolid include treatment of VRE bacteremia, *E. faecalis*, nosocomial and community-acquired pneumonias caused by *S. aureus* or *Streptococcus pneumoniae*, and skin infections.

Lactic acidosis has emerged as a rare but significant adverse effect of linezolid in adults.[7] In 2003, Apodaca and Rakita[1] first described reversible lactic acidosis in a patient who received 11 weeks of linezolid for a *Nocardia* pneumonia. The patient's lactate peaked at 9.9 mMol/L but normalized in 2 weeks after cessation of linezolid. Subsequently, Palenzuela et al[2] described 3 patients who developed lactic acidosis after receiving extended courses of linezolid (40–84 days) with lactate values between 9.9 and 18.4 mMol/L. The investigators also noted mutations in the mitochondrial 16S rRNA in 2 individuals. Notably, bacterial 23S rRNA binds linezolid in cross-linking studies and shares conserved sequences with mammalian mitochondrial 16S rRNA, supporting the mechanism of linezolid-induced lactic acidosis as being likely related to structural homology between bacterial and mammalian mitochondrial rRNA.

A patient with liver dysfunction after receipt of a liver transplant has been noted to develop lactic acidosis within the first week of treatment as opposed to longer courses.[4] The authors hypothesized that hepatic insufficiency increased the risk for hyperlactemia and likewise parallels case 3 in this study. Hepatic dysfunction has also been implicated in development of thrombocytopenia while receiving linezolid, another adverse effect of long-term administration.[8] Moreover, renal insufficiency has also been implicated in delayed linezolid clearance,[9] and may have influenced toxicity onset in our patients as all had elevated serum creatinine. Consistent with cases involving hepatic dysfunction, the patients described in this series demonstrated onset of delayed hyperlactatemia with late, rapid escalation. This may reflect either a threshold at which mitochondrial inhibition causes aerobic metabolic failure, or when lactate production overwhelms its consumption. Of note, evidence exists suggesting linezolid impairs its own clearance over time,[10] possibly potentiating toxicity during prolonged courses.

In 2005, Soriano et al[5] found patients with linezolid-associated lactic acidosis to have depressed mitochondrial complex IV activity. The authors suggested that this reflects mitochondrial mRNA translation interference. This contrasted with relatively preserved function of complex II, which is encoded on nuclear DNA. This group further demonstrated that patients who developed lactic acidosis also had 51% of the respiratory chain activity seen in controls, lower mitochondrial mass, and decreased cytochrome c oxidase subunit II (COX-II) protein expression without a concomitant decrease in mitochondrial DNA content. COX-II function recovered after withdrawal of linezolid.[6]

The precaution section of the linezolid commercial package insert states that patients experience "repeated episodes of nausea and vomiting," when developing lactic acidosis. These did not occur in all of the cases noted in the literature, nor in the cases described in this study. Because pediatric patients may not express nausea clearly, it is prudent to follow lactate concentrations in pediatric patients receiving prolonged courses of linezolid or who have underlying hepatic or renal dysfunction. Future investigation is warranted into whether linezolid-related adverse phenomena such as lactic acidosis are associated with lower linezolid clearance in the setting of hepatic or renal dysfunction. This may include monitoring linezolid concentrations. This is of interest because linezolid dosage is not adjusted for patients' hepatic or renal function.

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CORTICOSTEROIDS IN THE TREATMENT OF SEVERE *NOCARDIA* PNEUMONIA IN CHRONIC GRANULOMATOUS DISEASE

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Abstract: *Nocardia* is 1 of the 5 main pathogens that infect chronic granulomatous disease patients. Despite aggressive antimicrobial therapy, medical treatment is not always successful and surgical resection of infected tissue has been intermittently required. We present 2 chronic granulomatous disease patients with severe *Nocardia* pneumonia whose pulmonary status worsened despite appropriate antimicrobials, but then improved clinically and radiographically with the addition of corticosteroids.

Key Words: chronic granulomatous disease, *Nocardia* pneumonia

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Chronic granulomatous disease (CGD) is a rare genetic disease of the phagocyte NADPH oxidase that impairs production of superoxide and its metabolites, allowing bacterial and fungal infections and exuberant granuloma formation. The organisms that characteristically cause infection in CGD are distinctive and important to recognize *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia* complex, *Nocardia* species, and *Aspergillus* species.[1,2] CGD is also characterized by significant granulomatous complications of the gastrointestinal and genitourinary tracts, which frequently require treatment with immunosuppressant agents, typically corticosteroids.[1,2]

Nocardia infections have been irregularly reported among different CGD cohorts, with surprisingly few identified outside of North America.[3] *Nocardia* species are found worldwide in water, soil, dust, and decomposing organic matter. We report 2 boys with X-linked CGD and severe multilobar *Nocardia* pneumonia whose intense inflammatory responses, despite antimicrobial therapy, responded to the addition of corticosteroids.

CASES

Patient 1, a 17-year-old white boy with X-linked CGD (missense mutation in $gp91^{phox}$), was hospitalized with 4 days of fever, mild cough, nausea, chills, and myalgias. Previous infections included cervical lymphadenitis at 4 and 8 years, *S. aureus* liver abscess at 6 years, and *Aspergillus fumigatus* pneumonia at 9 years. Pulmonary infiltrates without diagnosis occurred at 10 and 12 years. For CGD-related colitis, he received low-dose prednisone from 6 to 10 years of age. His last episode of colitis was at 15 years. Adherence to prophylaxis with trimethoprim/sulfamethoxazole (TMP/SMX), itraconazole, and interferon gamma was poor.

On admission, he was febrile (39°C) and chest computed tomography (CT) scan showed consolidations in the right middle and lower lungs with necrosis but without effusion (Fig. 1A). The remainder of the physical examination was normal, except for weight (47.4 kg, < third percentile for age), and height 163.2 cm (just above third percentile). Laboratory studies showed an elevated white blood cell count of 25,500 cells/mL, an elevated erythrocyte sedimentation rate of 61 mm/h, and an elevated C-reactive protein of 28.9 mg/L. *Nocardia farcinica* grew from needle aspirate of the lung as well as from sputum. Microbiologic diagnosis was confirmed by genomic sequencing.

He was treated initially with intravenous TMP/SMX and levofloxacin. His symptoms improved, but after about 2 weeks on treatment fever recurred and worsened (peaks around 40°C), despite intensified *Nocardia* coverage with linezolid, meropenem,

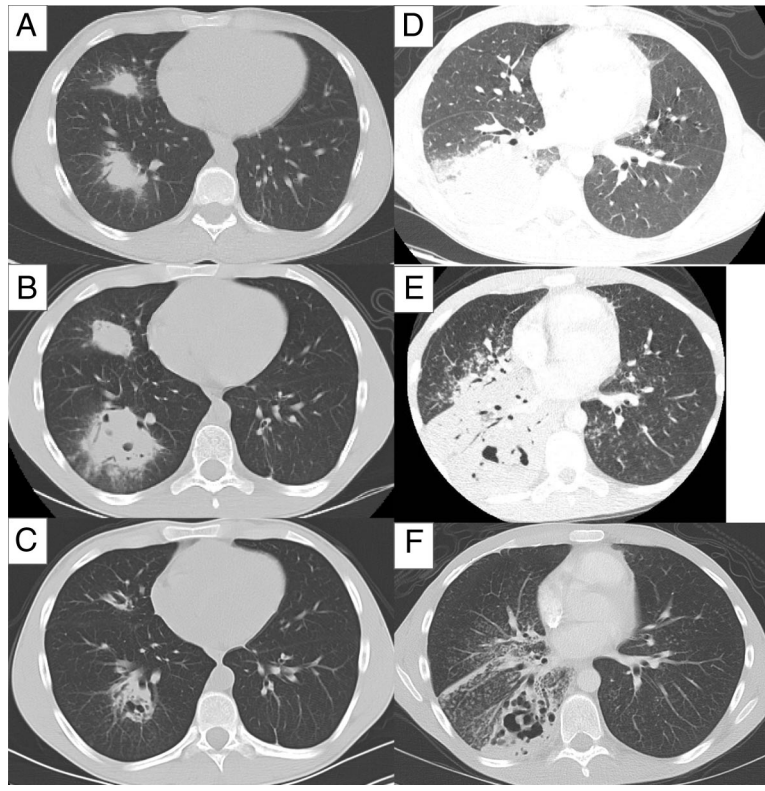

FIGURE 1. Chest CT findings at the initiation of antimicrobial therapy for patient 1 (A), 2 weeks after starting antibiotics (B), and 1 week after initiation of corticosteroids (C). Chest CT for patient 2 at the initiation of antimicrobial therapy (D), 3 weeks after starting antimicrobials (E), and 2 weeks after initiation of corticosteroids (F).

and amikacin. Voriconazole was added for possible occult coinfection with mold. Repeat CT chest showed marked worsening of the lesions with cavitation (Fig. 1B). Brain magnetic resonance imaging, echocardiogram, bone scan, and abdominal CT scan were unremarkable. Because his inflammation was profound and incapacitating and the only infection identified was the *Nocardia* for which he was receiving broad coverage, methylprednisolone 0.8 mg/kg/d was added intravenously. He had a rapid clinical improvement, along with decreases in white blood cell count and inflammatory markers.

After 1 week of intravenous corticosteroids, CT showed improvement (Fig. 1C). Corticosteroids were transitioned to oral prednisone and tapered to 0.6 mg/kg/d. After 10 days, fever recurred with worsened infiltrates. A repeat needle biopsy showed only chronic inflammation without organisms; all cultures were negative.

Intravenous methylprednisolone 0.8 mg/kg/d was restarted with rapid chest CT improvement. After a slower wean of intravenous corticosteroids over 4 weeks to 0.4 mg/kg/d, followed by transition to oral prednisone (0.4 mg/kg/d), he was discharged home. A slow taper of oral corticosteroids during the next 7 months, was accompanied by continued posaconazole, levofloxacin, and TMP/SMX. He had complete resolution and has had no relapse.

Patient 2 is a 21-year-old man with X-linked CGD receiving no prophylaxis who presented with fever and cough. Chest radiograph and CT scan showed multilobar pneumonia and enlarged mediastinal lymph nodes. Bronchoscopy cultures grew *Nocardia cyriacigeorgica*; intravenous imipenem and TMP/SMX were begun (Fig. 1D). Hypoxemia required intubation and ventilation. Linezolid and tobramycin were added to the TMP/SMX and imipenem based on antibiotic susceptibilities; voriconazole was added for possible mold coinfection. After extubation fevers returned and chest imaging showed increased consolidation in the right lung base, cavitations in the left upper lobe, miliary nodules, and infiltrates bilaterally (Fig. 1E). Intravenous methylprednisolone (0.7 mg/kg/d) was started; he defervesced in 24 hours and his chest radiograph improved.

On transfer to the NIH Clinical Center, oral TMP/SMX, linezolid (with pyridoxine), meropenem, and voriconazole were continued (Fig. 1F). Corticosteroids were weaned during 3 weeks, but at 0.25 mg/kg of prednisone, inflammatory markers increased and the infiltrates worsened. A fine needle lung biopsy showed no organisms and cultures were negative. Corticosteroids were increased to 0.34 mg/kg, and symptoms and inflammatory markers resolved. He was discharged after 2 months of hospitalization markedly improved to receive oral TMP/SMX, linezolid (with pyridoxine to prevent linezolid-induced neuropathy), and voriconazole. A slow taper of oral prednisone over several months was planned.

DISCUSSION

The immune dysregulation of CGD results in infection susceptibility and exuberant granuloma. Corticosteroids have been frequently used to control presumably noninfectious granulomatous complications, such as inflammatory gastrointestinal and genitourinary lesions.[1,2] Corticosteroids have also been used in addition to antimicrobials to treat refractory infections in CGD. For instance, fulminant mulch pneumonitis occurs after inhalation of *Aspergillus* in decaying organic matter, causing a diffuse, severe

hypersensitivity-like reaction with fever and hypoxemia.[4] Treatment with corticosteroids in addition to antifungals can be life saving. Other reports have included successful treatment of inoperable liver abscesses (one without a pathogen identified, others with *S. aureus*), combined *Burkholderia cepacia* and *Aspergillus* pneumonia, and a pneumonia with effusion with several bacterial isolates.[5–7]

Severe *Nocardia* pneumonia in CGD has often resulted in major pulmonary surgery.[3] In 1 report of 29 Nocardia infections in CGD, 7 (25%) required surgery in addition to antibiotics, either with lung resection, empyema drainage, or debridement of bone or skin. Pulmonary resection was an unattractive option for our patients with multilobar disease, and is a difficult option in CGD patients who have already had recurrent lung infections with associated damage and dysfunction.

Corticosteroids have been a beneficial adjunct to specific therapy in other (non-CGD) infections characterized by organ damage due to intense inflammation. Tuberculous meningitis, *Haemophilus influenzae* meningitis, and *Pneumocystis jiroveci* pneumonia are well-accepted examples.[8] In addition, corticosteroids have been advantageous in severe immune reconstitution syndromes, such as those seen after initiation of highly active antiretroviral therapy in human immunodeficiency virus. *Cryptococcus* and *Mycobacterium* species, which cause granulomatous inflammation, are frequent inducers of this syndrome, and corticosteroids may cause improvement when added to specific therapy.[9]

Because corticosteroids are capable of suppressing symptomatic inflammation, it is essential to be certain that all infections are adequately covered prior to steroid initiation. Corticosteroid treatment without optimal antimicrobial coverage can have disastrous consequences in CGD.[10,11] In our patients, repeat cultures were obtained before initiation of corticosteroids and antimicrobial coverage was maximized. In particular for *Nocardia* infections, species determination can guide antibiotic selection, as in vitro *Nocardia* susceptibility testing is often problematic. Coinfection with *Nocardia* and mold is common, so concomitant antifungal treatment is prudent. Disseminated infection may complicate severe *Nocardia* infection, and brain magnetic resonance imaging, should be performed even if neurologic symptoms are absent. Neither of our patients had extrapulmonary disease, so the use of adjunctive corticosteroids in that setting is unexplored.

Nocardia is one of the 5 typical infections in North American CGD and its treatment can be difficult despite appropriate antimicrobials. Neither of our patients was receiving appropriate prophylactic TMP/SMX at the time of infection, which might have prevented their infections. TMP/SMX prophylaxis has been shown to significantly decrease bacterial infections in CGD[12]; however, pulmonary *Nocardia* infections without dissemination have occurred despite prophylaxis.[3] These 2 cases of CGD *Nocardia* pneumonia had worsening symptoms on aggressive therapy and had dramatic improvement with the addition of corticosteroids, which required very slow tapers. Both patients avoided lung surgery and have returned to normal pulmonary function. Controlled prospective experience, including animal models, is necessary to determine the safe and proper roles for adjunctive corticosteroids in CGD.

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HYPERINFLAMMATORY PULMONARY DISEASE IN CHRONIC GRANULOMATOUS DISEASE

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The article by Freeman et al,[1] in this issue, on the use of corticosteroids in the treatment of severe *Nocardia* pneumonia in chronic granulomatous disease (CGD) points out a recurring problem in the diagnosis and treatment of patients with this genetically caused host defense abnormality. The primary defect in CGD, originally described by my mentor, Professor Paul G. Quie and associates,[2] was an abnormality of intracellular bacterial killing in the phagocytes of patients. This was subsequently found because of inability to generate adequate quantities of high-energy oxygen molecules and radicals, including hydrogen peroxide, hydroxyl radical, as well as myeloperoxidase-generated hypochlorite ion. These high-energy oxygen species not only contribute significantly to microbial killing but can also contribute to inflammation and tissue damage. Thus, it seems counterintuitive that these patients often develop processes at the sites of infection, particularly in the lungs and gastrointestinal tract, that are striking hyperinflammatory reactions, which can lead to serious compromise of the patient and even death.

Interestingly, some of the patients with milder forms of autosomal recessive disease, or the variant form of X-linked CGD, may present later in life with signs of inflammatory disorders only, rather than serious infections. We recently reported in this journal,[3] a 17-year-old male patient with variant X-linked CGD who initially presented with signs and symptoms of Crohn disease for which he received treatment but, apparently, the possibility of CGD did not enter into the differential

diagnosis. Subsequently, after suffering a life-threatening *Burkholderia cepacia* pneumonia, the presence of variant X-linked CGD was suggested by a broad, intermediate neutrophil oxidative burst dihydrorhodamine fluorescence pattern accompanied by the presence of a variant X-linked carrier dihydrorhodamine pattern in the carrier mother. Subsequent high-resolution melting and targeted sequencing revealed a T to A change at position c. 744 in exon 7 of the X chromosome encoding the 91 KD heavy chain of cytochrome b558.[3,4]

In other CGD patients, stomach outlet tract obstruction can be a prominent feature of the illness resulting in abdominal discomfort, bloating, weight loss, and associated complications. We have followed a family of 3 male patients, all of whom had severe X-linked CGD, each of whom developed severe gastric outlet tract obstruction. Two of the brothers died at the age of 10 to 12 years of disseminated *Aspergillus* infection before the use of interferon gamma or fungal prophylaxis for CGD patients. Presently, the third brother is in his 20s and has recently married after graduating from college assisted by an Immune Deficiency Foundation Scholarship. Interestingly, this third brother is able to control his outlet tract obstruction with nonsteroidal anti-inflammatory agents rather than having to resort to the use of corticosteroids, which has been shown to be effective in treating such obstruction.[5] Given the history of death due to disseminated aspergillosis in the 2 elder brothers, we were reluctant to use steroids in the third and youngest brother. Thus, inflammatory processes in the gastrointestinal tract may contribute significantly to the morbidity in CGD, a process which can clearly benefit from steroids and, in some cases, nonsteroidal anti-inflammatory medications.

In the cases of *Nocardia* pneumonia reported by Freeman et al,[1] severe pulmonary infection due to *Nocardia* developed along with profound inflammatory, incapacitating lung disease. The patients' treatment consisted of appropriate antibiotics for *Nocardia* in addition to voriconazole in one patient based on the suspicion that a fungal pathogen might also has been involved, as frequently happens in CGD. Unfortunately, lung disease continued to worsen until the patients were given intravenous methylprednisolone, which resulted in a prompt decrease in white blood cell counts and inflammatory markers and improved CT scans. The authors make a good case for using antibacterial therapy based on appropriate cultures as well as antifungal therapy, but then if symptoms, signs, and imaging studies progress, these suggest the need for anti-inflammatory therapy, including the use of corticosteroids.

One issue that we have encountered on more than one occasion is the potential for antifungal therapy including amphotericin, and possibly azoles such as voriconazole, to cause an inflammatory lung disease in certain patients being treated for proven or possible fungal infections.[6,7] Amphotericin has been shown to activate Toll-like receptors 1 and 2 causing release of tumor necrosis factor alpha (TNFα), IL-6, and IL-8, likely resulting in an enhanced influx of leukocytes into the pulmonary tissue.[6] Even in CGD patients who have a decreased ability to release high-energy oxygen radicals, emptying of phagocyte granule contents, including numerous enzymes, cationic proteins, and permeability increasing factors could promote the development of severe inflammation in the lungs. In at least one patient of ours, with variant X-linked CGD who required assisted ventilation for *B. cepacia* pneumonia, discontinuation of amphotericin B initiated for a possible concomitant fungal infection led to a rapid clearing of the lung without the addition of corticosteroid therapy.[3]

Having been a part of the large international, randomized, double-blind study on the efficacy of interferon gamma (IFN-γ) in preventing serious infections in CGDs,[8] we feel fairly strongly that patients who develop severe infections should receive this medication on a continuous basis. Unfortunately, many patients fail to keep up their 3 times weekly subcutaneous injections, especially in their teenage years or older. Having lost 2 of the 3 brothers, who I mentioned previously, to disseminated aspergillosis and having observed a combination of antifungals along with increasing doses of IFN-γ cure the disease in CGD patients similar to the report by Bernhisel-Broadbent et al,[9] it seems appropriate to continue that therapy even in the face of severe pulmonary inflammation, as killing the organisms within phagocytic cells would seem to be a critical aspect of successful therapy. On one occasion in discussing the patient mentioned previously, who was also receiving amphotericin, with Dr. Steve Holland, the senior author on the paper by Freeman et al[1] and a leading authority in the treatment of CGD, it was suggested that we withdraw the IFN-γ therapy. Given the results in our previous patients and those reported in the literature in whom IFN-γ therapy was continued, along with results of the large double-blind study,[8] and the report by Bernhisel-Broadbent et al,[9] we elected to maintain the interferon and to stop the amphotericin in this individual with severe *B. cepacia* pneumonia. Fortunately, the severe progressive lung disease immediately began to reverse after the discontinuation of amphotericin, even in the face of continuing IFN-γ and without the addition of corticosteroids. Perhaps, in that case, we could have added corticosteroids as well, but elected not to and still had a successful outcome. In general, corticosteroids suppress the inflammatory response by inhibiting transcription of proinflammatory cytokines, which likely helped to reverse the inflammatory lung disease in the cases reported by Freeman et al.[1] However, one could possibly consider continuing IFN-γ therapy in the infected, steroid-treated CGD patient, allowing the production of nitric oxide from arginine by IFN-γ-induced nitric oxide synthetase in monocytes, macrophages, and even neutrophils, which might allow intracellular killing of the pathogen while not overly promoting pulmonary inflammation.

I feel that the article by Freeman et al[1] is important for pointing out the critical balance between halting progressing microbial infection and preventing potentially severe, damaging inflammation in CGD. One must not only always assess the medications that are administered to these patients and attempt to exclude the use of proinflammatory agents but also consider the use of an anti-inflammatory medication such as corticosteroids or perhaps even nonsteroidal anti-inflammatory agents or cytokine or cytokine receptor blocking reagents in the future. As pointed out by Dr. Robert Good, the senior author in Dr. Quie's seminal paper,[2] these "experiments in nature" can certainly teach us a great deal about the inflammatory response and host resistance in the normal as well as the immunocompromised host.

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SUCCESSFUL MANAGEMENT OF CHRONIC MULTIFOCAL Q FEVER OSTEOMYELITIS WITH ADJUVANT INTERFERON-GAMMA THERAPY

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Abstract: We present a 3-year-old girl who had chronic recurrent multifocal osteomyelitis caused by *Coxiella burnetii* despite long-term dual antibiotic therapy. Excellent clinical response was achieved and sustained when immunomodulatory therapy with interferon-γ was initiated. This is the case of a first child who was successfully treated with interferon-γ as adjuvant therapy for chronic multifocal Q fever osteomyelitis.

Key Words: chronic Q fever, coxiella burnetii, osteomyelitis, interferon gamma, polymerase chain reaction

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Q fever is a worldwide distributed zoonosis, caused by *Coxiella burnetii,* an obligate intracellular Gram-negative bacterium. Farm animals and pets are the reservoirs, and human beings acquire the infection by inhalation of contaminated aerosols and by consumption of unpasteurized dairy products. It is a rare disease in children and clinical presentation is variable; infection can be asymptomatic in a maximum of 60% of cases, acute (flu-like symptoms, pneumonia, and/or hepatitis) or chronic.[1] Chronic Q fever mainly presents as endocarditis and vascular infection; rare manifestations include chronic hepatitis, pericarditis, and osteoarticular infection.[2] Diagnosis is usually made by serologic tests; however, PCR of infected tissue can facilitate rapid diagnosis. The optimal choice and length of antimicrobial therapy is unclear. We report the case of a 3-year-old girl with recurrent multifocal osteoarticular *C. burnetii* infection who was successfully treated with interferon gamma (INF-γ) for chronic multifocal Q fever osteomyelitis because long-term dual antibiotic therapy with rifampin and ciprofloxacin was ineffective.

CASE REPORT

A previously healthy white girl of nonconsanguineous parentage, presented to a District General Hospital in September 2006, with a 2-month history of back pain. For the last 12 months, she had tenosynovitis of her left wrist and a pseudoparalysis of her left arm; subsequently in June 2006, she developed a skin lesion over the chest wall, which resulted in chronic abscess formation after drainage. She was born in Southern Spain and lived in a rural area; there was however history of direct contact with horses but not with other farm animals nor consumption of unpasteurized milk products.

At presentation, she was apyrexial and her physical examination was unremarkable. Radiographs and bone scintigraphy showed multiple destructive lesions in the left humerus, right femur, and the 10th dorsal vertebrae. A magnetic resonance imaging of the spine revealed a destructive vertebral lesion at T10 with extradural extension (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A777). Abdominal ultrasound and computed tomography of the thorax and abdomen were normal. A complete blood count and C-reactive protein were normal (0.5 mg/L), and erythrocyte sedimentation rate (ESR) was raised (55 mm/h). Mantoux test and serology for *Bartonella, Brucella, and Toxoplasma* species were negative. Histopathology of the humerus biopsy was normal; blood and biopsy cultures for bacteria including *Mycobacterium* species and fungi were negative.

A presumptive diagnosis of multifocal osteomyelitis was made, and parental cloxacillin plus clindamycin was given for 2 weeks followed by oral cefadroxil for 4 weeks with good clinical response. Six weeks after antibiotic therapy was stopped, the patient represented with fever, swelling, and tenderness of her right foot. A bone scan revealed increased activity in the right tarsus. At this point, the diagnosis of Q fever was made by serology showing elevated *C. burnetti* antiphase I (1/32000) and phase II (1/16000) IgG titer; antiphase I or II, Ig M being negative. Subsequently, PCR from tarsal biopsy was positive for *C. burnetii*. Immunologic investigations including neutrophil function tests, lymphocyte counts, and subsets were normal for age as was T cell response after PHA stimulation.

Antimicrobial therapy was initiated in February 2007 with rifampin and ciprofloxacin. Despite excellent drug compliance, the patient had recurrent osteomyelitis during the next 7 months, new lesions appearing in her right knee and wrist ganglion required repeated drainage. Her right foot needed surgical intervention including extensive bone curettage. Histopathologic analysis demonstrated noncaseating granulomas with chronic inflammation, and cultures were negative for atypical mycobacteria. A magnetic resonance imaging of the spine in June 2007 showed worsening pathology with vertebral osteomyelitis at T9–T11, and a large abscess with spinal cord stenosis without signs of myelopathy. As she improved clinically, her treatment was continued with dual antimicrobial therapy alone and antiphase I and antiphase-II IgG titer as well as her ESR began to decrease 7 months after start of treatment.

Because she continued to suffer from recurrent abscess formation of the sternum and right wrist between April 2008 and January 2009, adjuvant immunomodulatory therapy was considered. Before the initiation of INF-γ therapy (12.5 $\mu g/m^2$/3 times weekly) in January 2009, a defect of the interleukin-12 (IL-12) pathway including IL-12 p40 and IL-12 receptor beta 1 (IL-12Rβ1) and the INF-γ pathway, including INF-γ receptor 1 and 2 (INF-γR 1 and 2), was excluded. Immunologic studies were performed at Hospital Universitario Gran Canarias Doctor Negrin, Las Palmas, Spain, a referral center for investigations of primary immunodeficiencies. Within 3 months of antimicrobial plus immunomodulatory therapy, her lesions healed, inflammatory markers improved, and treatment response was further confirmed serologically. Treatment with INF-γ was continued for a total of 17 months (May 2010). Ciprofloxacin and rifampin were continued until June 2010 (a total of 40 months of dual therapy). Currently, at the age of 7.5 years without any treatment she continues to be in excellent clinical state, the inflammatory markers are normal (CRP: 0.5 mg/L,

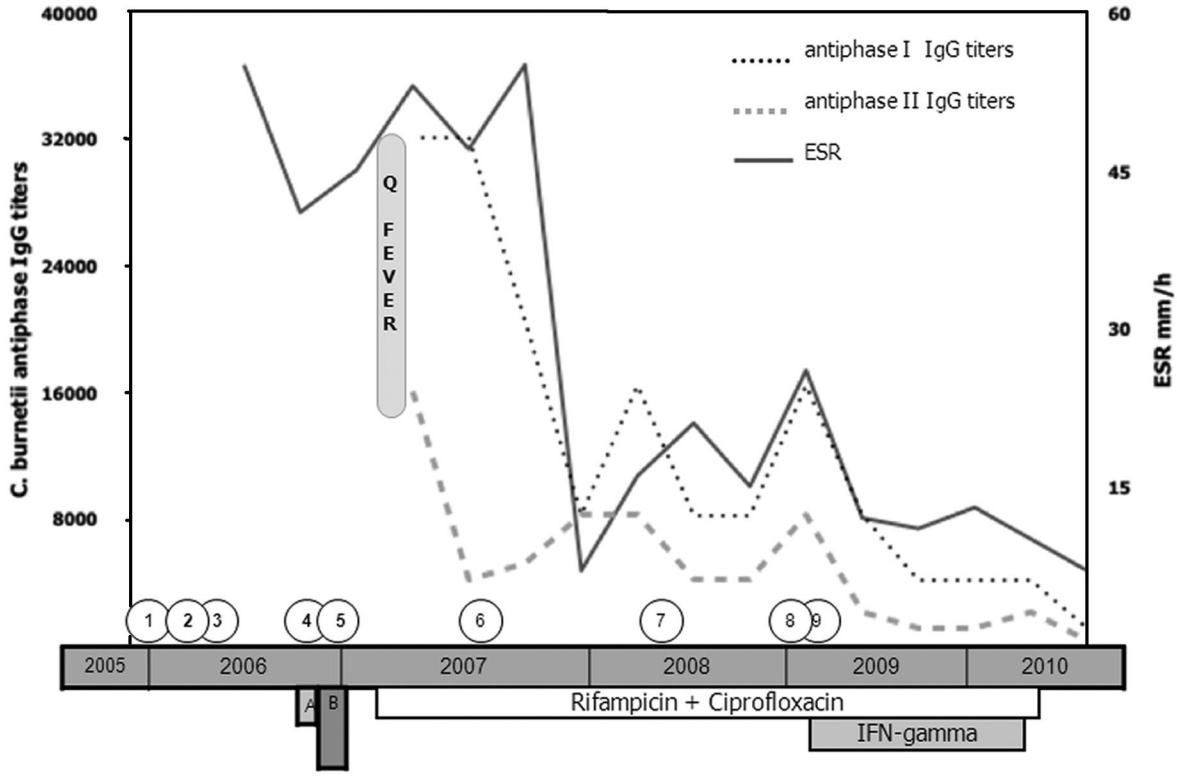

FIGURE 1. Summary of the clinical course in the patient with Q fever osteomyelitis.

ESR: 7 mm/h) and the *C. burnetti* antiphase I titer reduced to 1/1024. A summary of her clinical course is shown in Figure 1.

DISCUSSION

Q fever has been described rarely in children, possibly because the disease presents with fewer symptoms than in adults and therefore might be underdiagnosed. Q fever osteoarticular presentation is rare. To our knowledge, there have been only 19 published cases of Q fever osteomyelitis, 6 being children between 2 and 9 years of age.[3–5] Four of the 6 reported patients presented with chronic recurrent multifocal osteomyelitis (CRMO), including 2 children who developed spondylitis, as was the case with our patient.[4,5]

Differential diagnosis of CRMO with spinal involvement includes inflammatory CRMO and infectious multifocal osteomyelitis due to microorganisms that cause granulomatous bone lesions such as *Bartonella, Brucella*, *Francisella*, *Mycobacterium,* and *Nocardia* species. In contrast to inflammatory CRMO, soft-tissue involvement is typically seen in granulomatous multifocal osteomyelitis.[5,6] Our patient had chest wall and spinal abscesses and presented with tenosynovitis, a complication previously reported in Q fever osteomyelitis cases.[3–5] She lacked significant systemic symptoms and had only mild elevation of biologic markers despite extensive bone involvement, both of which are regarded as common features in Q fever osteomyelitis.

Diagnosis is made using serologic tests although PCR in affected tissues, particularly osteoarticular ones, is a useful tool for rapid diagnosis. Acute Q fever is diagnosed on the basis of an antiphase II IgG titer greater than 200 and IgM titer greater than 50; chronic Q fever has an antiphase I IgG titer greater than 800.[1,7] Despite specific therapy, a decrease in antibodies titer is often delayed, with some patients maintaining markedly raised values.[3,5] Our patient showed an antibody titer fall, which coincided with significant clinical improvement. Because the decline of antibody titer during treatment varies greatly, serologic test results are not useful in the decision for the optimum length of therapy. Q fever endocarditis should be treated with doxycycline and hydroxychloroquine for at least 18 months, but only limited data are available regarding treatment for Q fever osteomyelitis.[3,8] We did not use hydroxychloroquine for 2 reasons; concerns regarding its long-term effectiveness (personnel communication with Dr. Clare Nourse, Mater Children's Hospital, South Brisbane, Australia) as well as its side effects. Duration of antimicrobial treatment should be guided by clinical and serologic responses (until phase I IgG antibody titer is $<1/800$) and may need to be maintained for at least 18 to 36 months.[8] Our patient received dual antimicrobial therapy for 40 months.

A relapsing and multifocal clinical course with persistent recurrence is frequent, despite adequate treatment. The pathophysiology of this is not fully understood, but in an experimental mouse model, Andoh et al[9] demonstrated the importance of T cells for the clearance of intracellular *C. burnetii* with INF-γ as well as TNF-α playing a major role for the early control of this infection. Recombinant INF-γ therapy has been previously used successfully in the management of a 3-year-old boy who had Q-fever which did not respond to antibiotic therapy.[10] In contrast to this patient, our child had much more severe disease including multifocal osteomyelitis

with abscess formation. Full clinical response with INF-γ while receiving dual antimicrobial therapy was achieved within several months and therapy was maintained until all inflammatory markers as well as antiphase I and antiphase-II IgG titers stabilized. Our patient remains asymptomatic and in excellent clinical state 7 months after stopping antimicrobial and 8 months after INF-γ therapy.

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MEROPENEM/CLAVULANATE AND LINEZOLID TREATMENT FOR EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS

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Abstract: The combination of meropenem with clavulanate has high antimycobacterial activity in vitro against extensively drug-resistant *Mycobacterium tuberculosis* strains. We report the successful use of this combination in association with linezolid in the management of an advanced extensively drug-resistant tuberculosis disease with complex second-line drug resistance in a 14-year-old teenager.

Key Words: extensively drug-resistant tuberculosis, meropenem, clavulanate, *Mycobacterium tuberculosis*, linezolid

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Extensively drug-resistant tuberculosis (XDR-TB) isolates are resistant to isoniazid, rifampin, any fluoroquinolone and at least one of the 3 second-line injectable drugs (amikacin, capreomycin, or kanamycin). The limited therapeutic arsenal available results in frequent treatment failures and a high mortality rate.[1] Treatment combining with 4 to 5 drugs has been effective in human immunodeficiency virus (HIV)-negative patients.[2] However, therapeutic options are poor for patients with XDR-strains with limited susceptibility. Linezolid, an antibiotic of the oxazolidinones class, has been used successfully in XDR-TB disease.[3,4] Meropenem and clavulanate, a beta lactam and beta lactamase combination has potent bactericidal antituberculous activity in vitro against XDR strains,[5] but no report has yet been made regarding the clinical efficacy of this combination in humans. We report for the first time the successful use of this combination in a young HIV-negative patient with an XDR-TB strain.

CASE REPORT

A 14-year-old girl, asylum seeker from Chechnya (Russian Federation), was admitted in our hospital with extensive pulmonary tuberculosis. Tuberculosis had been diagnosed 2 years before. She first received standard 4 drugs therapy (rifampin, isoniazide, ethambutol, and pyrazinamide) for 4 months. She relapsed 5 months later. The same therapy was restarted without improvement. She reported having also received moxifloxacin, cycloserine, and capreomycin. Her mother died 1 year before because of presumed multidrug-resistant TB. No other information regarding the mother's TB history and treatment was available.

On examination, she was acutely ill with persistent high fever, cough, anorexia, and failure to thrive. Her weight was 36 kg with a body mass index of 14 kg/m^2. Enzyme-linked immunosorbent assay for HIV was negative. Chest computed tomography showed extensive bilateral lesions with reticulonodular infiltrates in the right upper and middle lobes, cavitary lesions in the posterior segment of right upper lobe, and a huge cavitary lesion of the left lung with reticulonodular infiltrates in the residual lung parenchyma and almost complete destruction of the left lung. Sputum smears were positive for acid-fast bacilli and cultures confirmed *Mycobacterium tuberculosis*.

Enteral nutritional support by nasogastric tube and empiric second-line antituberculous drugs, based on her previous failing antituberculous treatment, were started: moxifloxacin 400 mg, amikacin 15 mg/kg, pyrazinamide 35 mg/kg, prothionamide 500 mg, and cycloserine 500 mg. Despite adequate directly observed therapy and nutritional support, no clinical improvement was noted and the cultures remained positive for 6 weeks. Second-line drug susceptibility testing showed resistance to isoniazide, rifampin, rifabutin, ofloxacin, ethambutol, pyrazinamide, amikacin, cycloserine, and prothionamide with susceptibility only to capreomycin, linezolid, and clarithromycin. Consequently, treatment was shifted to intramuscular capreomycin 15 mg/kg, linezolid 600 mg, clarithromycin 500 mg twice daily, intravenous (IV) meropenem 1.5 g thrice daily, and amoxicillin 1 g clavulanate 200 mg thrice daily. Pyrazinamide 35 mg/kg and cycloserine 500 mg were continued along with 250 mg of pyridoxine daily. Fever disappeared 4 weeks after initiation of this treatment and the first negative sputum smear was obtained after 8 weeks. Sputum culture conversion occurred after 11 weeks. Her general condition improved with a weight gain of 10 kg. A chest computed tomography scan performed 7 months after the initiation of the new treatment showed fibrocicatricial evolution of the left lung and decrease of the lesions in the right lung. Moderate signs of peripheral neuropathy were observed after 4 months of linezolid, for which the dosage of linezolid was reduced to 300 mg once daily. No other adverse events were observed. After 8 months of individualized second-line treatment, the patient was discharged from the hospital, with twice daily infusions of meropenem 2 g by an IV implanted device (Port-a-cath), oral amoxicillin-clavulanate 500 mg thrice daily, clarithromycin 500 mg twice daily, cycloserine 500 mg, pyrazinamide 35

mg/kg, and intramuscular capreomycin 15 mg/kg. Treatment was continued until 18 months after culture conversion. The patient underwent a successful left pneumonectomy 14 months after the start of the new treatment. No acid-fast bacilli were found on microscopic examination of the resected lung.

DISCUSSION

We describe for the first time the successful use of meropenem in combination with clavulanate along with linezolid in the management of XDR-TB. Association of a beta-lactam with a beta-lactamase has been reported in the management of multidrug-resistant TB, with conflicting results.[6,7] *M. tuberculosis* is intrinsically resistant to beta-lactams because of the presence of an extended spectrum beta-lactamase, BlaC. The beta-lactamase inhibitor, clavulanate, irreversibly inhibits in vitro the *M. tuberculosis* beta-lactamase.[8] Recently, meropenem, a potent beta-lactam antibiotic from the carbapenems class, was found to be a low-affinity substrate for the enzyme with hydrolysis 5 times slower than ampicillin.[5] The combination of meropenem with clavulanate is highly active in vitro against XDR *M. tuberculosis* strains, including nonreplicative ones, and is able to sterilize cultures in 14 days.[5] In the present case, despite advanced disease with bilateral involvement, profound malnutrition and previous exposure to second-line drugs, all factors associated with poor outcome,[9] we observed clinical improvement after 4 weeks (disappearance of fever) and culture sterilization in 11 weeks after the introduction of meropenem/clavulanate and linezolid. Linezolid has excellent in vitro and in vivo activity against *M. tuberculosis*. It has been used successfully in the treatment of XDR-TB with a similar resistance pattern.[3,4] Combination of meropenem/clavulanate and linezolid could have had a synergistic action to sterilize more efficiently and rapidly the lung lesions of our patient. The safety profile of meropenem is an advantage in comparison with other second-line anti-TB drugs. It is well tolerated in children and adults, with a low frequency of minor side effects.[10] Potential limitations for its wide use include high cost and the necessary IV use that requires the use of a long-lasting implanted device.

In conclusion, we report a case of XDR-TB of very limited sensitivity treated with the association of meropenem-clavulanate in combination with 2 active second-line drugs. Moreover, bacteriologic and clinical trials are needed to determine the true impact of meropenem-clavulanate in the clinical settings and the potential synergic effect of the association with linezolid.

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DISSEMINATED CONGENITAL *TOXOPLASMA* INFECTION WITH A TYPE II STRAIN

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Abstract: Disseminated congenital toxoplasmosis mimicking septic shock is unusual. We report a fatal case of disseminated congenital toxoplasmosis that was acquired after a third trimester maternal primary infection. The child had severe pneumonitis, purpura, and hepatitis. After 5 days of treatment, quantitative polymerase chain reaction analysis showed that parasite loads in the serum and in tracheal aspirates had decreased. The child died of refractory hypoxemia. Genotyping revealed a type II strain.

Key Words: Toxoplasma gondii, congenital toxoplasmosis, genotype

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The risk of mother-to-child transmission of toxoplasmosis increases later in the term when the pregnant mother acquires a primary infection, whereas the severity of fetal infection decreases. More than 80% of the congenital infections acquired in the third trimester are subclinical.[1] Cases of congenital disseminated toxoplasmosis are unusual and are associated with atypical *Toxoplasma* genotypes.[2] We report a case of disseminated congenital toxoplasmosis that was acquired after a third trimester maternal primary infection with a type II strain.

Case Report. The mother was a 37-year-old woman with no particular relevant history; she had 2 previous healthy children. In the beginning of the pregnancy, her HIV serology was negative. She was nonimmune for toxoplasmosis. The monthly serologic testing for toxoplasmosis remained negative until 27 weeks' gestation. Systematic serology performed at 33 1/2 weeks' gestation detected *Toxoplasma*-specific immunoglobulin G (200 IU/mL by HS agglutination) and immunoglobulin M (12/12 index by ISAGA). Fetal ultrasounds, including the final one carried out at 31 1/2 weeks' gestation, were normal. The mother was hospitalized at 34 weeks' gestation for spontaneous preterm labor, and she gave birth to a boy by vaginal delivery. His birth weight was 2600 g (50th percentile) and his head circumference was 33.5 cm (75th percentile). The Apgar score was 1 at 1 minute. After resuscitation, he was given exogenous surfactant and was transferred to the intensive care unit.

Upon admission, he had diffuse edema, purpura, hepatosplenomegaly, ascites, and severe respiratory distress syndrome that required ventilation with 80% oxygen. His chest radiograph showed nonconfluent mottled opacities. Echocardiography revealed persistent pulmonary hypertension. He had thrombocytopenia with a platelet count of 22,000/mm^3 and a leukocyte count of 26,000/mm^3. Initial treatment consisted of intermittent positive ventilation with inhaled nitric oxide and antimicrobial therapy (amoxicillin, cefotaxime, and amikacin).

The respiratory distress syndrome evolved with refractory hypoxemia and hypercapnia to persistent pulmonary hypertension that responded poorly to inhaled nitric oxide. Severe disseminated intravascular coagulation with macroscopic hematuria required 2 platelet transfusions and administration of freshly frozen plasma. Oliguric renal failure was treated by volume expansion with normal saline, dopamine, and hydrocortisone. An exchange transfusion was done on day 4 due to severe icterus (total bilirubin: 477 μmol/L; conjugated: 166 μmol/L). All bacterial cultures were negative.

Congenital *Toxoplasma* infection was established by the detection of specific immunoglobulin M in serum (12/12 index in ISAGA) and by positive results of the real-time quantitative polymerase chain reaction (PCR) using the 529-base pair fragment[3] on various specimens: serum (3800 parasites/mL), tracheal aspirates (2000 parasites/mL), urine (250 parasites/mL), and ascitic fluid (250 parasites/mL), collected on the third day of life. Head ultrasound showed diffuse echogenic areas. Ophthalmologic examination results were normal for the right eye but showed macular bleeding and edema in the left eye. Treatment with intravenous sulfamethoxazole (30 mg/kg/d) and trimethoprim (6 mg/kg/d) was begun on day 4. On day 9, quantitative PCR analysis of the serum and tracheal aspirates remained positive but were greatly reduced to $<$20 parasites/mL.

The infant died on day 10 because of severe refractory hypoxemia. Postmortem examination showed interstitial pneumonitis, myocarditis, focal myositis, and myelomeningoencephalitis without necrosis.

The *Toxoplasma* strain genotype was determined by the French National Reference Center for Toxoplasmosis, Pôle Souche (Limoges University). It was found to be a type II (BRC TgH 20059A).

Comment. Disseminated congenital toxoplasmosis mimics severe septic shock with multi-organ failure and differs from usual forms of congenital toxoplasmosis. Including ours, a total of 9 cases have been described.[2,4] Other than its rarity, the primary interest in this form of infection is to try to understand its pathogenesis for identifying fetuses that are at high risk for developing the disseminated congenital form.

The time of gestation when maternal infection is acquired is the main risk factor for the incidence and the severity of congenital infection. Congenital infections acquired after a third trimester maternal infection are subclinical in more than 80% of cases.[1] In contrast, all cases of congenital disseminated toxoplasmosis occurred after a primary maternal infection of the third trimester.[2,4] A relatively short period between maternal infection and birth seems necessary for this occurrence.

Among the 9 published cases of disseminated congenital toxoplasmosis, a favorable outcome was observed in 2 of the 3 cases that included prenatal treatment and in 2 of 6 cases that lacked prenatal treatment. In studies published $\geq$40 years ago, when prenatal treatment was not offered to pregnant women in France, systemic symptoms at birth were frequent (eg, pneumonia occurred in 8 and 41% of infants and splenomegaly in 56 and 90%).[5] By contrast, in recent large studies such as SYROCOT (Systematic Review on Congenital Toxoplasmosis), extraophthalmic or intracranial lesions were considered too rare to be taken into account in the analysis.[6] The decreasing frequency of systemic signs in European countries may reflect the widespread use of prenatal treatment in seroconverting pregnant women. However in the United States, although severe manifestations of toxoplasmosis are the general rule, septic shock, ARDS, and disseminated intravascular coagulation are rarely recognized as being due to congenital toxoplasmosis.

Parasite load may be another important factor that correlates with the reduced *Toxoplasma gondii* seroprevalence observed in many developed countries in recent decades.[1] The size of the parasite inoculum in cases of primary infection may have become smaller recently, resulting in infections that are less severe and have fewer systemic signs. Conversely, a massive maternal ingestion of parasites followed by the passage of large numbers of parasites through placenta could be a risk factor for the development of disseminated toxoplasmosis. In our case, quantitative PCR showed high parasite loads, with parasite concentrations to a maximum of 3800/mL. It has been shown that *Toxoplasma* concentrations higher than 100/mL in amniotic fluid are associated with poor outcomes for congenital toxoplasmosis following a maternal infection acquired before 20 weeks' gestation.[7]

Disseminated *Toxoplasma* infection is a severe complication for immunocompromised subjects, particularly in patients with AIDS or after a transplantation.[1] In immunocompetent adult subjects, disseminated forms of severe acquired toxoplasmosis have been recently reported. The isolated strains were highly virulent in mice and genotypic analysis showed them to be atypical.[8] Previous cases of disseminated congenital toxoplasmosis were also associated with an atypical strain genotype.[2] The type II strain found in our case is the one most frequently identified in congenital infections in Europe. To our knowledge, this is the first report of disseminated congenital toxoplasmosis that has been proven to be due to a type II strain. In the absence of an atypical virulent strain or an immunodeficiency, host genetic and epigenetic factors could have contributed. Polymorphisms in 2 genes implicated in juvenile retinal dystrophies have been associated with ocular disease or brain disease in children having congenital toxoplasmosis. More recently, polymorphisms at the gene that encodes a pro-inflammatory receptor on the macrophage cell surface (the purinergic P2X7 receptor) and at the gene ERAP1 of an endoplasmic protease (the endoplasmic reticulum-associated aminopeptidase, ERAAP) have been shown to influence susceptibility to congenital *T. gondii* infection.[9]

Usually, postnatal treatment for congenital toxoplasmosis consists of pyrimethamine combined with sulfadiazine.[1] As these drugs are not available intravenously and the illness severity of our case made the enteral route impossible, the infant was treated with trimethoprim and sulfamethoxazole. In vitro, trimethoprim is much less active than pyrimethamine on *T. gondii*,[10] but the clinical efficacies of trimethoprim plus sulfamethoxazole versus pyrimethamine plus sulfadiazine are similar in patients with AIDS.[1] The substantial decrease in parasite load, as measured by quantitative PCR analysis of the serum and tracheal aspirates after 5 days of treatment, suggests that the cause of death was not secondary to an uncontrolled toxoplasma infection but rather to its hemodynamic and respiratory consequences.

Disseminated congenital toxoplasmosis at birth is acquired after a maternal primary infection of the third trimester. Despite rapid postnatal administration of antiparasitic treatment, the disease is life-threatening. Disseminated forms are caused not only by atypical strains; type II strains can also be involved. The roles of

the maternal parasite load and of host-parasite interactions remain to be clarified.

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