

formula in which  $a$  is the first order error (5%):

$$n = \frac{\log a}{\log(1-p)}$$

Because of the change in sample size, the limit prevalence was redetermined by using the inverse of the formula above:

$$p = 1 - \sqrt[n]{a}$$

If at least 1 sample was positive for *M. bovis*, the prevalence of bovine TB among patients would be  $\geq 4.2\%$ . However, the prevalence of *M. bovis* was  $< 4.2\%$  and confirmed the low-level involvement of *M. bovis* in human TB in Mbarara district. These findings are consistent with previous work in Uganda's capital, Kampala, and in other African or Asian countries (2,8,9). The estimation of extrapulmonary cases among all TB cases (95% confidence interval 2%–15.2%) did not differ from the official estimate. We can add, using the second formula shown above, that among the 6 extrapulmonary TB cases, the prevalence of *M. bovis* is  $< 39.3\%$ . Our results come from a population in a highly rural area (91.5% of the population in Mbarara district) (7), where the high prevalence of animal TB has been reported.

These results could be explained by the patients' consumption habits, which reduce the risk for contamination. Even if bovine TB could also be found in other farm or wild animals, it seems to have a minor effect on public health. Zoonotic TB appeared to not be a major public health problem in Mbarara district. However, this finding could also result from underdiagnosis of extrapulmonary TB, from prevalence of *M. tuberculosis* being so high that in proportion *M. bovis* is a minor problem, or from rural populations' difficult access to TB diagnosis (directly observed therapy case detection rate in Uganda in 2005 was 37%) (1).

### Acknowledgments

We thank the Mbarara University of Science and Technology, the staff of Mbarara University Teaching Hospital, the National Tuberculosis and Leprosy Control Programme, and the French Embassy in Uganda.

This study was funded by the Agricultural Consultation and Sector Structuring Project (French Development Agency).

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DOI: 10.3201/eid1501.080487

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## Vertical Transmission of *Pneumocystis* *jirovecii* in Humans

**To the Editor:** Currently, animal and human studies favor an airborne transmission pattern for *Pneumocystis* pneumonia (1). However, the early age of acquisition of *Pneumocystis* spp. in different mammals, including humans, warrants study of vertical/transplacental transmission as an additional route of transmission of this stenoxenic microorganism.

Available studies on transplacental transmission of *Pneumocystis* spp. suggest that it varies among mammal species on the basis of the type of placenta (2). Transplacental transmission of *Pneumocystis* spp. has been demonstrated in rabbits (2,3), but it

seems not to occur in rats and mice that have severe combined immunodeficiency (1). In humans, transplacental transmission was first suggested by a few reports of *Pneumocystis* spp. pneumonia in neonates published before the AIDS epidemic (4). Recently, a controversial case of vertical transmission of *P. jirovecii* was reported: an infection in the lungs of a fetus of an HIV-positive mother with *Pneumocystis* spp. pneumonia (5). However, the study did not identify the organisms as *Pneumocystis*, and a subsequent fluorescein-labeled monoclonal antibody test yielded negative results (6).

The present study was conducted to evaluate transplacental transmission of *P. jirovecii* by molecular techniques. Placentas and lung tissues of aborted fetuses from immunocompetent women who had miscarriages were studied. To enhance specificity of the study, we used 2 genetic loci in *Pneumocystis* spp. DNA: the mitochondrial large subunit rRNA (mtLSU-rRNA) gene and the gene encoding for dihydropteroate synthase (DHPS). We analyzed 40 paraffin-embedded tissue blocks from the placentas and lungs of 20 fetuses at  $28 \pm 8$  weeks of gestation. The study was reviewed and approved by the ethical committee of University Hospital, Seville, Spain.

DNA was extracted from a mixture of five 10- $\mu$ m sections of each tissue block. Histologic sections were processed by using xylene and ethanol for paraffin removal and were then rehydrated. DNA was extracted by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions.

DNA amplification at the mtLSU-rRNA locus was conducted by using nested PCR as described (7). Samples identified as positive by this PCR were amplified by using primers DHPS-3 and DHPS-4 to detect the DHPS gene (7). To prevent false-positive results caused by contamination, pipettes with

filters were used at all stages. DNA extraction, preparation of the reaction mixture, PCR amplification, and detection were conducted in different areas under a laminar flow hood. Positive and negative controls were included in each reaction. All experiments were repeated at least twice.

*P. jirovecii* genotypes can be characterized by identifying polymorphisms at the mtLSU-rRNA locus (positions 85 and 248) and at the DHPS locus (positions 55 and 57). Amplicons from all samples that yielded positive PCR results for the 2 loci were sequenced directly from both ends by using a model ABI 377 automated sequencer and an ABI prism Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. The derived mtLSU-rRNA and DHPS gene sequences were compared with sequences available in databases by using the National Center for Biotechnology Information (Bethesda, MD, USA) BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The mtLSU-rRNA fragment was amplified from 11 lung and 8 placenta samples. Simultaneous DNA amplification of 2 loci of *P. jirovecii* was ob-

served in lung tissue samples from 7 (35%) of 20 fetuses and from 1 (5%) of 20 placenta samples. Sequencing of the mtLSU-rRNA gene showed 3 polymorphisms, and DHPS gene analysis showed only wild-type genotype in all samples (Table).

Our results provide molecular evidence of *P. jirovecii* transplacental transmission in humans. No available data on the development of *Pneumocystis* organisms in female genital organs was provided (8). In contrast, morphologic and molecular evidence of hematogenous dissemination of *P. jirovecii* from infected lungs has been provided by many authors (8). *Pneumocystis* DNA has been documented in blood or amniotic fluid samples from pregnant rabbit does (3), in which transplacental transmission of *Pneumocystis* spp. occurred. In humans, *P. jirovecii* colonization was observed in 5 (15.5%) of 33 pregnant women in their third trimester (9). These data suggest that physiologic immunodepression associated with pregnancy may favor *Pneumocystis* spp. colonization and mother-to-fetus transmission of the fungus by the hematogenous route. The transplacental route could enhance transmission of

Table. *Pneumocystis jirovecii* genotypes in samples identified as positive by nested PCR for 2 loci from fetal lung tissues and placenta samples\*

Case	Lung tissues		Placenta tissues	
	mtLSU-rRNA genotype	DHPS genotype	mtLSU-rRNA genotype	DHPS genotype
C1	3	1	—	—
C2	1	1	1	—
C3	3	1	—	—
C4	3	1	—	—
C5	2	1	1	—
C6	1	1	3	—
C7	1	1	—	—
C8	—	—	1	1
C9	3	—	—	—
C10	—	—	3	—
C11	3	—	1 and 3	—
C12	1 and 3	—	1	—
C13	—	—	1	—
C14	3	—	—	—
C15-C20	—	—	—	—

\*Genotype 1, polymorphism 85C/248C in mitochondrial large subunit (mtLSU)-rRNA gene and 55 Trh/57 Pro in dihydropteroate synthase (DHPS) gene; genotype 2, polymorphism in 85A/248C; genotype 3, polymorphism in 85T/248C.

*P. jirovecii* independent of environmental hazards.

Isolation of pathogens from an aborted fetus does not necessarily mean that they have caused the death of the fetus because many agents appear to pass through the fetal-placental unit and cause little damage. However, fungal infection is a major worldwide cause of abortion in cattle (10), and the surprising high prevalence of *P. jirovecii* infection found in dead fetuses in our study emphasizes the need to study the possible role of this fungal organism in human abortion.

Our findings could be of potential clinical importance and could open a new field of research, which should be explored. Further research should assess the scope of the problem and design rational preventive strategies, if necessary.

This study is part of the project “*Pneumocystis* Pathogenomics: Unravelling the Colonization-to-Disease Shift,” a Coordination Action supported by the European Commission (ERANET PathoGenoMics). This study was partially supported by the Spanish Ministry of Health (FIS 03/1743). M.A.M.-C. and C.d.l.H. were supported by the Spanish Ministry of Health (FIS CP-04/217 and FIS CM-04/146).

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DOI: 10.3201/eid1501.080242

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## Avian Influenza Virus (H5N1) in Human, Laos

**To the Editor:** The first avian influenza (H5N1) outbreak in poultry in Laos occurred in 2003 and subsided in March 2004 after massive killing of poultry to contain the disease. Extensive surveillance from July 2005 through January 2006 did not detect any influenza virus subtypes in chicken, ducks, quails, and pigs in live bird markets in the Vientiane, Champasak, and Savannakhet Provinces (1). Avian influenza virus (H5N1) was reintroduced into Laos in February 2006 but showed a lower incidence. Viruses isolated in this country in 2004 belonged to genotype Z, clade 1, and 2006 isolates belonged to clade 2.3.4 (online Appendix Figure, panel A, available from [www.cdc.gov/EID/content/15/1/127-appF.htm](http://www.cdc.gov/EID/content/15/1/127-appF.htm)) (1).

Avian influenza (H5N1) had not been reported in humans in Laos until February 27, 2007 (2). Our patient was a 15-year-old adolescent girl who lived in a suburb of Vientiane where an outbreak of influenza (H5N1) in poultry had been confirmed on February 7, 2007. Influenza-like symptoms developed in the patient on February 10. She was hospitalized in Vientiane with fever and respiratory symptoms on February 15. On February 17, her parents brought her to a private hospital in Nong Khai Province, Thailand. Oseltamivir was prescribed on February 19. On February 20, she was transferred to the Nong Khai Provincial Hospital because of rapid, progressive, severe pneumonia with acute respiratory distress syndrome. When we suspected avian influenza in this patient, clinical specimens were tested.

A diagnosis of infection with avian influenza (H5N1) was based on positive results obtained by reverse transcription-PCR (RT-PCR), viral isolation in MDCK cells inoculated with an endotracheal suction specimen