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 PathoGenumics). 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).

Marco A. Montes-Cano,
Magali Chabe, María Fontillon-Alberdi, Carmen de la Horra, Nieves Respaldiza, Francisco J. Medrano, Jose M. Varela, Eduardo Dei-Cas, and Enrique J. Calderon

Author affiliations: Virgen del Rocio University Hospital, Seville, Spain (M.A. Montes-Cano, M. Fontillon-Alberdi, C. de la Horra, N. Respaldiza, F.J. Medrano, J.M. Varela, E.J. Calderon); Center for Biomedical Research in Epidemiology and Public Health, Seville (M.A. Montes-Cano, M. Fontillon-Alberdi, C. de la Horra, N. Respaldiza, F.J. Medrano, J.M. Varela, E.J. Calderon); and Lille Pasteur Institute, Lille, France (M.A. Montes-Cano, M. Chabe, E. Dei-Cas)

DOI: 10.3201/eid1501.080242

References

Address for correspondence: Marco A. Montes-Cano, Servicio de Immunologia, Hospital Universitario Virgen del Rocio, Avda Manuel Siurot s/n, 41013 Seville, Spain; email: mmontescano@hotmail.com

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 chickens, 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, clad 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) (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
collected on February 22, and a 4-fold increase in neutralizing antibody titers from 80 to 320 in paired blood specimens collected on February 25 and March 1, as assayed against autologous virus. This virus isolate was named A/Laos/Nong Khai 1/07(H5N1). Subsequent samples were collected on February 25 and March 7 (day of death). Results of RT-PCR were positive for the sample collected on February 25 only; virus isolation results were negative for both samples.

The virus was screened for a novel reassorted gene by a multiplex RT-PCR and 8 primer pairs specific for each genomic segment of genotype Z, clade 1 virus (3). All segments except the polymerase A (PA) segment were amplified, which indicated that the new virus was different from genotype Z viruses. The viral genome was sequenced and submitted to GenBank (accession nos. EU499372–EU499379 for hemagglutinin, nonstructural protein, matrix protein, nucleoprotein, PB1, PB2, neuraminidase, and PA genes, respectively). Phylogenetic analysis showed that this virus belonged to genotype V (online Appendix Figure, panel B) (4); phylogenetic analysis of the hemagglutinin gene (www.who.int/csr/disease/avian_influenza/smalltree.pdf) showed that it belonged to clade 2.3.4 (online Appendix Figure, panel A).

Protein sequence at the hemagglutinin cleavage site harbored many basic amino acids (RERR_RKR). One amino acid deletion and 1 amino acid change were found when compared with RERRRKKR, which is present in most avian influenza viruses (H5N1). There was no change in receptor binding site. This virus had glutamic acid at aa 627 in the PB2 protein, aspartic acid at aa position 92 in nonstructural protein 1, and 5 aa deletions at positions 80–84 in the nonstructural protein 1. Analysis of the neuraminidase gene showed a 20-aa deletion in the stalk protein; there was no mutation of histidine to tyrosine at aa position 274, a position shown to be the oseltamivir resistance marker in the neuraminidase 1 viral genome (5). Mutations in the matrix 2 gene showed that amantadine resistance was not present in our virus (6). Our in vitro assay (7) showed that this virus was sensitive to oseltamivir and amantadine.

Since 2003, genotype V influenza viruses (H5N1) have been reported in some East Asian countries. Genetic diversity in the hemagglutinin gene has classified those genotype V viruses into distinct clades. Viruses from avian species in South Korea in 2003 and Japan in 2004 (8,9) belong to clade 2.5, A/chicken/Shanxi/2/2006 isolate belonged to clade 7. Human cases in People’s Republic of China, i.e., A/China/GD01/06, A/Shenzhen/406H/06, A/Jiangsu/1/2007, and A/Jiangsu/2/2007, belong to clade 2.3.4, the same clade as A/chicken/Thailand/NP-172/2006 and the virus from our study.

Highly pathogenic avian influenza viruses (H5N1) that caused outbreaks in Thailand since 2004 belong to genotype Z, clade 1. Introduction of genotype V clade 2.3.4 virus, A/chicken/Shanxi/2/2006, to Nakhon Phanom Province occurred in November 2006 (10), the same year that clade 2.3.4 virus was introduced into Laos (online Appendix Figure, panel A). On the basis of hemagglutinin gene phylogeny, A/Laos/Nong Khai 1/2007 is closely related to A/chicken/Nongkhai/NIAH 400802/2007 and A/chicken/Thailand/NP-172/2006. Phylogenetic analysis suggested that viruses from these 2 countries shared the same origin. There was extensive movement across the Mekong River even before the bridge linking Nong Khai from Vientiane was opened. However, the route of transmission of genotype V viruses from east Asian to Southeast Asian countries could not be elucidated.

Acknowledgments

We thank the Lao Public Health Department, the Vientiane Municipal and Nong Khai Provincial Health Office, and Nong Khai Provincial Hospital for collaborative support of this study. The oseltamivir used in drug sensitivity assay was kindly provided by Roche, Ardenne, Belgium.

This study was supported by the National Biotechnology for Genetic Engineering, the Thailand Research Fund for Senior Research Scholar, and a Mahidol University research grant.

Pilaipan Puthavathana, Kantima Sangsiriwut, Achareeya Korkusol, Phisanu Pooruk, Prasert Auewarakul, Chakrarat Pittayawanganon, Derek Sutdan, Rungrueng Kitphati, Pathom Sawanpanyalert, Bounlay Phommasack, Khanthong Bounlu, and Kumnuan Ungchusak

Author affiliations: Mahidol University, Bangkok, Thailand (P. Puthavathana, K. Sangsiriwut, A. Korkusol, P. Pooruk, P. Auewarakul); Ministry of Public Health, Nonthaburi, Thailand (C. Pittayawanganon, D. Sutdan, R. Kitphati, P. Sawanpanyalert, K. Ungchusak); and Ministry of Health, Vientiane, Laos (B. Phommasack, K. Bounlu)

DOI: 10.3201/eid1501.080524

References

Fatal HIV Encephalitis in HIV-Seronegative Patients

To the Editor: Acute encephalitis is rarely seen in patients infected with HIV (1). In addition, HIV in patients who are seronegative is extremely rare, particularly in the setting of current screening ELISAs (2). We report a case of encephalitis and HIV in the same patient, which resulted in death.

A 44-year-old Caucasian woman sought treatment at our hospital with a 1-week history of fever, unsteady gait, and progressive confusion. Her medical history included hypothyroidism, depression, and chronic alcohol abuse. The patient’s first tests for HIV were negative at 19 and 12 months prior to admission during routine intake screening ELISAs (3). We report a case of encephalitis and HIV in the same patient, which resulted in death.

The patient’s first tests for HIV were negative at 19 and 12 months prior to admission during routine intake screening ELISAs (2). The patient’s first tests for HIV were negative at 19 and 12 months prior to admission during routine intake screening ELISAs (2). The patient’s first tests for HIV were negative at 19 and 12 months prior to admission during routine intake screening ELISAs (2). The patient’s first tests for HIV were negative at 19 and 12 months prior to admission during routine intake screening ELISAs (2). The patient’s first tests for HIV were negative at 19 and 12 months prior to admission during routine intake screening ELISAs (2). The patient’s first tests for HIV were negative at 19 and 12 months prior to admission during routine intake screening ELISAs (2). The patient’s first tests for HIV were negative at 19 and 12 months prior to admission during routine intake screening ELISAs (2).