genes, polymerase genes, and the concatenated sequences, between North American and Guangxi isolates were 0.002 ± 0.002 SE, 0.034 ± 0.004 SE, and 0.019 ± 0.003 SE, respectively (Figure).

Our findings highlight the possibility that APV has been recently introduced by wild waterfowl in the Northern Hemisphere into domestic mallard ducks. Further study is needed to determine the pathogenicity of this virus on other commercial poultry species and its influence on the poultry industry and wildlife protection.

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Figure. Bayesian phylogeny of concatenated DNA sequences (981 bp, n = 62) from genes encoding 4b core and DNA polymerase proteins of avipoxviruses. Posterior probability values of the Bayesian trees (1,000 replicates) are indicated. Asterisks (*) indicate sequences obtained in this study. Avipoxvirus clades A–C and subclades are labeled according to the nomenclature of Jarmin et al. (6) and Gyuranecz et al. (7). Reference sequences used in this analysis are chosen from the study of Gyuranecz et al. (7). Scale bar indicates nucleotide substitutions per site.

References

- Tripathy DN, Reed WM. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, editors. Diseases of poultry. 11th ed. Ames (IA): Iowa State University Press; 2003. p. 253–69.
- Bolte AL, Meurer J, Kaleta EF. Avian host spectrum of avipoxviruses. Avian Pathol. 1999;28:415–32. http://dx.doi. org/10.1080/03079459994434
- Yu YM, Liu LR, Yu RL, Zhu KX. Pathologic examination of poxvirus infected tumor-like lesions in Chinese geese. Acta Veterinaria et Zootechnica Sinica. 1991;22:281–4.
- Zhang DL, Jia JY, Chen FW, Wei WR, Zhang CH, Wu LH. Serological investigation of 16 infectious diseases in rare birds in the Lhanzou area. Chin J Vet Med. 1996;22:22.
- Joklik WK. The purification of four strains of poxvirus. Virology. 1962;18:9–18. http://dx.doi.org/10.1016/0042-6822(62)90172-1
- Jarmin S, Manvell R, Gough RE, Laidlaw SM, Skinner MA. *Avipoxvirus* phylogenetics: identification of a PCR length polymorphism that discriminates between the two major clades. J Gen Virol. 2006;87:2191–201. http://dx.doi.org/10.1099/vir.0.81738-0
- Gyuranecz M, Foster JT, Dán Á, Ip HS, Egstad KF, Parker PG, et al. Worldwide phylogenetic relationship of avian poxviruses. J Virol. 2013;87:4938–51. http://dx.doi.org/10.1128/JVI.03183-12
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61:539–42. http://dx.doi.org/10.1093/sysbio/sys029
- Offerman K, Carulei O, Gous TA, Douglass N, Williamson AL. Phylogenetic and histological variation in avipoxviruses isolated in South Africa. J Gen Virol. 2013;94:2338–51. http://dx.doi.org/ 10.1099/vir.0.054049-0
- Tamura K, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. http://dx.doi.org/10.1093/molbev/msr121

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Orientia tsutsugamushi in Lung of Patient with Acute Respiratory Distress Syndrome, France, 2013

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To the Editor: Pulmonary involvement is a well-documented complication of scrub typhus caused by *Orientia tsutsugamushi* (1). Lung involvement manifests as bronchitis and interstitial pneumonitis of various grades that

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progress to acute respiratory distress syndrome (ARDS), a serious complication that occurs in $\approx 11\%$ of scrub typhus patients (2). The death rate among scrub typhus patients with ARDS can reach 25% (3). Older age, thrombocytopenia, and the presence of early pneumonitis have been proposed as risk factors for the development of ARDS in scrub typhus patients (3). We report the detection and culture of *O. tsutsugamushi* in a bronchoalveolar lavage specimen from a patient with scrub typhus–associated ARDS.

A 50-year-old woman from Lyon, France, was admitted to the hospital in November 2013 with fever (39°C), dizziness, diarrhea, dyspnea, and nonproductive cough. The woman, who had just returned from travel to a jungle in Laos, reported that the fever and diarrhea had begun immediately before her return home. Examination revealed that she had an oval eschar on her back and a faint maculopapular rash. Laboratory values showed elevated C-reactive protein and liver enzyme levels, lymphocytopenia, and thrombocytopenia. Extensive microbiological testing was done, including tests to rule out malaria, dengue, viral hepatitis, and leptospirosis; all results were negative. *Salmonella* sp. infection was suspected, and treatment with ofloxacin was started.

On hospitalization day 5, the patient showed development of septic shock, renal failure, and ARDS. She was transferred to an intensive care unit, and treatment with ceftriaxone was started. On hospitalization day 6, a skin biopsy of the eschar (2 mm \times 5 mm) and blood, serum, cerebrospinal fluid (0.5 mL), and bronchoalveolar lavage (1 mL) samples were obtained and sent to the National Reference Center for Rickettsiae (Marseille, France) for analysis. Total genomic DNA was extracted (Biorobot EZ1 Workstation; QIAGEN, Courtaboeuf, France) from 200 mL of each sample and used as template in a real-time PCR, which used primers and probes targeting a 47-kDa outer membrane protein gene, as described (4). Blood, skin biopsy, and bronchoalveolar lavage samples were positive for O. tsutsugamushi; the cerebrospinal fluid sample was negative. The serum sample was positive for O. tsutsugamushi by indirect immunofluorescence assay (serotypes Gilliam, Kuroki, Sennetsu, and Kawasaki) (5) and positive for O. tsutsugamushi IgM. Oral doxycycline (200 mg/day) was started on hospital day 7; the fever resolved 4 days later.

For culture, the positive samples were directly inoculated into monolayers of L929 cells, as described (6). Cultures of blood and skin biopsy samples were negative, but *O. tsutsugamushi* was isolated from the bronchoalveolar lavage sample after 40 days of culture (Figure); 500 μ L of bronchoalveolar lavage fluid was used for culture. We performed PCR amplification and sequencing of the isolate, targeting a 372-bp fragment of the 56-kDa protein gene, and compared the sequences with *O. tsutsugamushi* 56-kDa protein–encoding gene sequences available in



Figure. *Orientia tsutsugamushi* (arrows) in culture of bronchoalveolar lavage fluid from a patient with acute respiratory distress syndrome (Diff-Quick stain, VWR International, France). Original magnification ×100. A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/21/2/14-0860-F1.htm).

GenBank (7). The sequences showed 99% similarity with strains Jin/2012 and Zhou/2013 (GenBank accession nos. KJ001159 and KJ001163, respectively), which were obtained from febrile patients in Zhejiang Province, China, and have not been linked to a reference serotype (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/21/3/14-0860-Techapp1.pdf). In light of the test results and the patient's recent travel to Laos, she was given a diagnosis of *O. tsutsugamushi* infection–associated ARDS.

Our isolation of *O. tsutsugamushi* in bronchoalveolar lavage fluid from a patient with scrub typhus shows that this bacterium can be present in such samples. We also showed that skin biopsy and bronchoalveolar lavage samples can be used for the diagnosis of scrub typhus. To be suitable for culture, samples must be collected as early as possible in the disease course. In this case, blood and skin biopsy samples were obtained late in the disease, which may explain why *O. tsutsugamushi* was not isolated from these samples. Endothelial cells are the target cells of *O. tsutsugamushi* in the lung (8), and it has been proposed that ARDS in scrub typhus is associated with a cytokine increase as part of the immune response to *O. tsutsugamushi* infection (9).

Rickettsial diseases are increasingly being diagnosed in international travelers: one report showed that 2% of imported fevers are caused by rickettsioses, and hospitalization was necessary for the 38% of *O. tsutsugamushi*–infected travelers (10). The diagnosis of rickettsial infections is challenging because many physicians are unfamiliar with these diseases. However, the diagnosis of scrub typhus in patients with ARDS is critical for initiating appropriate and timely doxycycline treatment. In the case reported here, a diagnosis of scrub typhus was not suspected even though the patient had compatible exposure and travel histories and clinical findings consistent with the disease. The delay in diagnosis led to a life-threatening condition for the patient. Physicians in areas where scrub typhus is nonendemic should have a high index of suspicion for rickettsial infections in patients with recent travel histories to areas where the disease is endemic and consider treatment with tetracyclines whenever rickettsial infection is suspected. Furthermore, the potential for aerosol transmission of *O. tsutsugamushi* from patients with scrub typhus–associated ARDS to health care workers should be evaluated.

References

- Kurup A, Issac A, Loh JP, Lee TB, Chua R, Bist P, et al. Scrub typhus with sepsis and acute respiratory distress syndrome. J Clin Microbiol. 2013;51:2787–90. http://dx.doi.org/10.1128/ JCM.00463-13
- Wang CC, Liu SF, Liu JW, Chung YH, Su MC, Lin MC. Acute respiratory distress syndrome in scrub typhus. Am J Trop Med Hyg. 2007;76:1148–52.
- Tsay RW, Chang FY. Acute respiratory distress syndrome in scrub typhus. QJM. 2002;95:126–8. http://dx.doi.org/10.1093/ qjmed/95.2.126
- Jiang J, Chan TC, Temenak JJ, Dasch GA, Ching WM, Richards AL. Development of a quantitative real-time polymerase chain reaction assay specific for *Orientia tsutsugamushi*. Am J Trop Med Hyg. 2004;70:351–6.
- Angelakis E, Munasinghe A, Yaddehige I, Liyanapathirana V, Thevanesam V, Bregliano A, et al. Detection of rickettsioses and Q fever in Sri Lanka. Am J Trop Med Hyg. 2012;86:711–2. http://dx.doi.org/10.4269/ajtmh.2012.11-0424
- Tantibhedhyangkul W, Angelakis E, Tongyoo N, Newton PN, Moore CE, Phetsouvanh R, et al. Intrinsic fluoroquinolone resistance in *Orientia tsutsugamushi*. Int J Antimicrob Agents. 2010;35:338–41. http://dx.doi.org/10.1016/ j.ijantimicag.2009.11.019
- Fournier PE, Siritantikorn S, Rolain JM, Suputtamongkol Y, Hoontrakul S, Charoenwat S, et al. Detection of new genotypes of *Orientia tsutsugamushi* infecting humans in Thailand. Clin Microbiol Infect. 2008;14:168–73. http://dx.doi.org/10.1111/ j.1469-0691.2007.01889.x
- Moron CG, Popov VL, Feng HM, Wear D, Walker DH. Identification of the target cells of *Orientia tsutsugamushi* in human cases of scrub typhus. Mod Pathol. 2001;14:752–9. http://dx.doi.org/10.1038/modpathol.3880385
- Tseng BY, Yang HH, Liou JH, Chen LK, Hsu YH. Immunohistochemical study of scrub typhus: a report of two cases. Kaohsiung J Med Sci. 2008;24:92–8. http://dx.doi.org/10.1016/ S1607-551X(08)70103-7
- Jensenius M, Davis X, von Sonnenburg F, Schwartz E, Keystone JS, Leder K, et al. Multicenter GeoSentinel analysis of rickettsial diseases in international travelers, 1996–2008. Emerg Infect Dis. 2009;15:1791–8. http://dx.doi.org/10.3201/ eid1511.090677

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Clustered Cases of *Oestrus ovis* Ophthalmomyiasis after 3-Week Festival, Marseille, France, 2013

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To the Editor: Ophthalmomyiasis is a zoonosis generally caused by *Oestrus ovis*, a fly that lays eggs on the eye of its host. The hatched larvae cause irritation, and left untreated, the infestation can lead to blindness (1). The disease is rare and is mainly reported as sporadic cases in pastoral areas where the population is in close contact with common reproductive hosts of the fly, such as sheep and goats in the Middle East (2), Southeast Asia (3,4), and the Mediterranean Basin (5,6). Only limited ophthalmomylasis outbreaks have been reported around the Mediterranean Sea (7). A century ago, the Provence region of southern France was a pastoral area, where twice a year, large herds of sheep migrated between the pastures in the mountains north of the region and the grassland plains in the southwest. These migrations were termed *transhumance*. In 2013, Marseille metropolis, the largest urban area in Provence, was chosen as the yearly "European Capital of Culture." In this context, from May 17 to June 9, a large-scale transhumance event took place, which featured the gathering of huge flocks of sheep (Figure) that had passed through many towns in the vicinity of Marseille. La transhumance culminated in a parade through downtown Marseille, where 600 horseback riders converged with flocks of 3,000 sheep and goats, and >300,000 spectators gathered.

From the last week of June through the third week of July, 4 cases of ophthalmomyiasis were reported in the area surrounding Marseille (Figure). Only 1 case had occurred in the region during the previous 5 years. The first case-patient was a 45-year-old female teacher, who lived and worked in Allauch, located in the immediate suburbs, 21 km (\approx 13 mi) east of Marseille. On June 25, while on the school playground, she described feeling a fly hit her right eye. In the evening, itching and irritation of the eye prompted her to seek referral to an ophthalmologic emergency center. Examination concluded the presence of small mobile larvae inside the eye, which were identified as *O. ovis* (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/2/14-0974-Techapp1.pdf).