This report demonstrates an apparently paradoxical inverse relationship between a positive RDT result and severity of illness among patients with pandemic (H1N1) 2009. This observation cannot be explained by differences in the time to access to medical care, performance of RDT (*7*), or prior antiviral therapy. Variants of pandemic (H1N1) 2009 virus may preferentially infect the lower respiratory tract in certain hosts (*8*). Invasive properties of pandemic (H1N1) 2009 virus and severity of illness may be more closely related to heterogeneity in host immunity than to viral load (*9*). US Centers for Disease Control and Prevention guidance advises that "hospitalized patients with suspected influenza should receive immediate empiric antiviral treatment…, a negative RIDT or DFA test result does not exclude influenza virus infection…" (*10*). Moreover, this guidance also recommends that collection of lower respiratory tract specimens may be useful for reverse transcription–PCR testing to improve diagnosis for patients suspected of having severe lower respiratory tract disease caused by pandemic (H1N1) 2009 virus. The current findings strongly support this recommendation, particularly for severely ill patients.

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# Human *Brucella canis* Infections Diagnosed by Blood Culture

**To the Editor:** Brucellosis is a worldwide zoonosis caused by *Brucella* spp. The 4 species known to infect humans are *B. melitensis*, *B. suis*, *B. abortus*, and *B. canis* (*1*). Since 1999, 11 cases in Japan have been reported. Although no bacteria were isolated, serum antibody detection indicated that 4 were caused by *B. melitensis* or *B. abortus* acquired abroad and the other 7 by *B. canis* (*2*). Of these 7 patients, 2 were presumed to have received their infection from dogs, and the sources of infection for the other 5 are unclear. We report 2 cases of *B. canis* infection diagnosed by blood culture.

Patient 1 was a 71-year-old male pet shop manager with hypertension. He came to Chubu Rosai Hospital, Nagoya, Japan, on August 9, 2008, after having fever and fatigue for 3 weeks, which were nonresponsive to third-generation cephalosporins. At the time of admission, his temperature was 37.8°C, but physical examination findings were unremarkable. On day 2, gram-negative coccobacilli were detected in a culture of blood collected at the time of admission. Ceftriaxone (1 g 1×/d) was administered, but fever persisted. On day 5, coccobacilli were growing poorly on culture media. Because the patient's history indicated the possibility of a zoonotic disease, doxycycline (100 mg 2×/d) was administered. Thereafter, the patient's fever and generalized symptoms resolved. The blood specimen and isolated bacteria were sent to the National Institute of Infectious Disease, *B. canis* was identified by combinatorial PCR (*3*). Serum tube agglutination test indicated an antibody titer against *B. canis* of 1,280 (Table). On day 10, streptomycin (1 g 1×/d) was added to the treatment regimen. On day 33, the patient was discharged; his laboratory

values were almost within reference limits, and he continued taking doxycycline for 6 weeks and streptomycin for 2 weeks.

Patient 2, a previously healthy 44-year-old co-worker of patient 1, exhibited similar signs and symptoms—fever and general fatigue—that started around the same time as for patient 1 (3 weeks before August 9, 2008). Physical examination findings at that time were unremarkable. Blood tests indicated moderate liver dysfunction. Treatment with fosfomycin was not effective. On August 19, the day after the diagnosis of brucellosis was made for patient 1, patient 2 came to Chubu Rosai Hospital, where *B. canis* was identified from blood culture. Serum antibody titer was 320 (Table). This patient was treated with doxycycline (100 mg 2×/d) plus rifampin (600 mg 1×/d) for 6 weeks. All signs, symptoms, and liver dysfunction resolved.

Neither patient had an immune disorder. About 2 months before illness onset they had each handled, without protection, the placenta of an aborted dog fetus. Negative antibody results were obtained for other persons at risk for infection: laboratory workers who were exposed to the patients' specimens, the patients' families, and a veterinarian who had been stuck by a needle when collecting blood from pet shop dogs to examine for antibody against *B. canis.* We prescribed doxycycline plus rifampin for 3 laboratory workers because brucellosis is among the most commonly reported laboratory-acquired bacterial infections and because postexposure prophylaxis is recommended for persons at high risk for exposure (*4*).

Several days after identification of *B. canis* for patient 1, the dogs in the pet shop (37 dogs, 23 adults and their 14 puppies) were examined for antibody against *B. canis* by using the microplate agglutination test (*5*) and for the *B. canis*–specific gene by combinatorial PCR (*3*). A total of 6 dogs were positive for antibody (titers 320–5,120) and the specific gene; 5 were positive for antibody only (titers 320–5,120), and 4 were positive for the specific gene only. Only adult dogs had positive results. Blood cultures were positive for 6 dogs that were antibody positive. Dogs that were determined by any method to be infected and their puppies (with negative test results) were euthanized. Since January 2008, a total of 8 puppies from the infected dogs had been sold; they were located, tested, and found to not have antibody against *B. canis*. The local government reported this information to the Ministry of Health, Labour and Welfare, Japan, and the ministry shared the information with related organizations.

Caution is necessary when basing diagnosis on serum tube agglutination test because *B. canis* has rough surface antigen and does not cross-react with *B. abortus* antigen (smooth *Brucella* spp.), which is usually used to diagnose brucellosis (*1*). Furthermore, because brucellosis is relatively rare and signs and symptoms are nonspecific, the number of cases reported is thought to be underestimated (*6*–*8*). A recent report showed that 2.5% of dogs in Japan have antibody against *B. canis,* but adult dogs are rarely seriously ill despite this generalized systemic infection (*5*,*9*). Thus, if a febrile person has signs and symptoms of unknown cause and a history of close contact with dogs, brucellosis should be considered and appropriate action to prevent spread of infection should be taken.

Table. Laboratory data for 2 patients infected with *Brucella canis*, Japan, 2008

| Patient no. | Isolation of *B. canis* by blood culture | *B. canis* titer (date of sample collection)* | *B. abortus* titer (date of sample collection)* |
|---|---|---|---|
| 1 | + | 1,280 (Aug 11)<br>1,280 (Sep 30)<br>320(Nov 4) | <40 (Aug 11) |
| 2 | + | 320 (Aug 19)<br>320 (Oct 7)<br>160 (Nov 11) | <40 (Aug 19) |

*Titers determined by serum tube agglutination test.

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# Oseltamivir-Resistant Pandemic (H1N1) 2009 in Patient with Impaired Immune System

**To the Editor:** We detail the development of oseltamivir-resistant pandemic (H1N1) 2009 in a chronically immunocompromised patient and the pitfalls encountered when treating such patients with neuraminidase inhibitors. On August 6, 2009, a 56-year-old man was seen in the emergency room of a local hospital with a 24-hour history of fever, myalgia, coryzal symptoms, and cough. He was on day 3 of a postexposure course of oseltamivir (75 mg 1×/d); influenza A had been presumptively diagnosed for his wife after she had similar symptoms.

The patient's medical history showed grade IVB nodular sclerosing Hodgkin lymphoma, which had been diagnosed in 2001. Lymphoma was initially treated with chemotherapy, but relapse required autologous peripheral stem cell transplantation in July 2005. Further relapses in 2006 and 2007 were treated with radiotherapy and chemotherapy, respectively, before the patient underwent an allogeneic peripheral stem cell transplantation in July 2008. This treatment was complicated by graft-versus-host disease, and the patient required ongoing immunosuppression.

When hospitalized, the patient was being treated with cyclosporine A (50 mg/d) and prednisolone (20 mg/d). Physical examination showed a temperature of 39°C and wheezing from the left lung. Initial tests showed a neutrophil count of $2.02 \times 10^9$/L, a lymphocyte count of $0.87 \times 10^9$/L, and a C-reactive protein level of 33 mg/L. He was started on piperacillin–tazobactam and gentamicin, and oseltamivir was increased to the treatment dose of 75 mg 2×/d. A nasopharyngeal aspirate collected on August 7 contained pandemic (H1N1) 2009 viral RNA by real-time PCR for generic influenza A (*1*) and capillary sequencing for subtype H1N1 (testing by Micropathology Ltd, Coventry, UK). By August 9, the patient was still febrile, and zanamivir (10 mg 2×/d) was started. Oseltamivir was given for a total of 7 d and zanamivir for 3 d.

Nose and throat swabs taken on August 21 still contained pandemic (H1N1) 2009 viral RNA. Real-time PCR and pyrosequencing demonstrated a histidine-to-tyrosine substitution (H275Y) in the neuraminidase gene associated with oseltamivir resistance (Respiratory Virus Unit, Centre for Infections, Health Protection Agency; methods not in public domain). A mixture of wild-type and resistant virus was present (A. Lackenby, pers. comm.). The sample from August 7 did not contain this mutation, suggesting a de novo H275Y substitution secondary to oseltamivir use.

The patient improved and was discharged on August 23 but returned for treatment on September 7 with worsening fever and cough. Nose and throat swabs obtained on September 11 were PCR negative, but follow-up samples on September 25 and October 1 contained detectable pandemic (H1N1) 2009 viral RNA. Because virus isolation was not performed, true infectivity remains unresolved, but intermittent detection suggests ongoing replication, such as that seen in other immunocompromised patients (*2*,*3*).

By February 3, 2010, a total of 225 cases of oseltamivir-resistant pandemic (H1N1) 2009 had been identified worldwide; a high proportion of cases were in immunocompromised persons (*4*). A minority of these mutations were detected in treatment-naive patients. Immunocompromised, particularly lymphopenic, patients shed virus for prolonged periods leading to longer treatment courses and viral shedding reviving on termination of treatment. Viral shedding for up to 18 months has been reported for seasonal influenza, which has important implications for infection control (*5*). Our patient demonstrated that a single PCR-negative test does not reliably determine the end of viral shedding, which continued despite co-treatment with 2 neuraminidase inhibitors. Neuraminidase inhibitors interfere with the release of progeny influenza virus from their infected host cells. Effective treatment depends partially on immune system destruction of the foci of infection (*6*), or potential persistent viral particles can be released as soon as oseltamivir therapy is stopped. The low genetic barrier to oseltamivir means that resistance is a likely consequence of monotherapy in immunocompromised patients.

Concern about oseltamivir resistance has led to issuance of additional guidelines, especially in light of the transmission of resistant virus between immunocompromised patients on hospital wards in the United States and Wales (*7*,*8*). This finding suggests that immunocompromised patients should be treated with oseltamivir and zanamivir, or with zanamivir alone, for a