This study was supported by a grant from the Japan Science and Technology Agency, Japan.

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DOI: 10.3201/eid1408.070391

References


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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article’s publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have one Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Rarity of Influenza A Virus in Spring Shorebirds, Southern Alaska

To the Editor: Knowledge of avian influenza (AI) virus and its host epidemiology and ecology is essential for effective monitoring and mitigation (1). Applicability of global and continental-scale models will be key for expanding this knowledge base. Research in the North Pacific area, eastern United States, suggests an ecologic and epidemiologic viewpoint of AI virus in wild birds in which shorebirds (family Scolopacidae) are predominant hosts in spring; however, research in Alberta, Canada, suggests that waterfowl are such in autumn (2,3). AI virus surveillance in Europe (4) suggests that the spring aspect of this scenario does not apply there. To increase knowledge of AI transport among shorebirds in spring in the North Pacific, we conducted AI virus surveillance during the springs of 2006 and 2007 at the Copper River Delta area of Alaska. Millions of birds congregate at this location in the spring, resulting in the highest spring shorebird concentrations in the New World (5). We also sampled gulls (Laridae), which are common and heretofore unsurveyed for AI in this ecosystem.

In 2006 and 2007, 1,050 shorebirds (Western Sandpiper, Calidris mauri, and Least Sandpiper, C. minuta) and 770 Glaucous-winged Gulls (Larus glaucescens) were sampled during peak spring migration at Hartney Bay, Cordova, Alaska (60°28′N 146°8′W; Table). Fresh fecal samples were obtained from tidal flats within <1 to 90 min after identified flocks were dispersed, and samples were placed in sterile medium (brain heart infusion buffer with 10,000 U/mL penicillin G, 1 mg/mL gentamicin, and 20 μg/mL amphotericin B) and either kept cool (<1 week) before transport to Fairbanks (2006) or placed into liquid nitrogen within 2 h of collection.
result for this gene indicates the presence of matrix (M) gene. A positive test for virus in the Delaware Bay area (3% in spring) was substantially lower (1%–2% in 2007) than 95% in each year. In gulls, probability of detecting infection rates >1% was 99% with 99% probability (95% probability of detecting rates 1%–2% or higher in each year). In gulls, probability of detecting infection rates >1% across both years of the study (≥6% in 2006 and ≥1%–2% in 2007) was 95%.

Virus prevalence in spring shorebirds in Alaska was substantially lower than prevalence in spring shorebirds in the Delaware Bay area (3%) and more similar to prevalence in spring shorebirds in Europe (4%). Our shorebird samples (1,050) were fewer than those in other studies (3; 4,266 samples from 4 species over 16 years, and 4; 3,159 samples from 47 species over 8 years, with 35% from spring), representing 25% and 33% of those studies, respectively. Our study covered only 2 years, but it would detect AI virus infections in shorebirds at rates ≥1%–2% within each year with 95% probability and at rates ≥0.9% across years with 99% probability. Thus, the prevalence rate among Copper River Delta shorebirds in our study is lower than that found in the 16-year Delaware Bay study (3). In the Delaware Bay area, 4 shorebird species were sampled: 3 Calidris and 1 Arenaria (3). Precise statistics are unavailable, but the average 16-year prevalence rate was 14.2%, fluctuating annually from ±2% to ±38% (3).

In Europe AI viruses were absent among spring shorebirds (4). Differences in prevalence rates found among studies may be influenced by species sampled, sampling procedures, and seasonal timing (4). However, with >1,000 spring shorebird samples, results suggest that differences might exist between the world’s major migration systems (3,4).

Our results corroborate other recent results (10) suggesting that AI prevalence rates among shorebirds at Delaware Bay are not typical within North America. Present evidence indicates (this study; 3,10) that the role of shorebirds in AI virus ecology and epidemiology is heterogeneous within North America and within a genus (Calidris). These findings confirm that knowledge of how AI viruses cycle in wild bird hosts remains incomplete at continental and family-level taxonomic scales. Only further surveillance can fill these knowledge gaps.

Acknowledgments
We thank C. Pruett and J. Maley for assisting with field work and D.E. Stallknecht and an anonymous reviewer for providing helpful comments.

This research was supported by US Department of Agriculture Service Center Agencies 58-6612-2-217 and 58-6612-6-244, and AI supplemental Current Research Information System 6612-32000-051-00D.

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DOI: 10.3201/ eid1408.080083

References

Table. Species and sample sizes of wild bird hosts screened for avian influenza virus, Cordova, Alaska, May 2006 and May 2007

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western sandpiper (Calidris mauri)</td>
<td>500</td>
</tr>
<tr>
<td>Least sandpiper (C. minutilla)</td>
<td>0</td>
</tr>
<tr>
<td>Glaucous-winged gull (Larus glaucescens)</td>
<td>100</td>
</tr>
<tr>
<td>Totals</td>
<td>600</td>
</tr>
</tbody>
</table>

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 14, No. 8, August 2008 1315
Isolation of Brucella microti from Soil

To the Editor: Brucella microti is a recently described Brucella species (1) that was isolated in 2000 from systematically infected common voles (Microtus arvalis) in South Moravia, Czech Republic. The organism is characterized by rapid growth on standard media and high metabolic activity, which is atypical for Brucella (2). The biochemical profile of B. microti is more similar to that of Ochrobactrum spp., of which most species are typical soil bacteria.

On the basis of the close phylogenetic relationship of Brucella spp. and Ochrobactrum spp. and the high metabolic activity of B. microti, we hypothesized that this Brucella species might also have a reservoir in soil. To test this hypothesis, we investigated 15 soil samples collected on December 11, 2007, from sites in the area where B. microti was isolated from common voles in 2000 (2). Ten of the samples were collected from the surface and at a depth of up to 5 cm near different mouse burrows 5 m apart. The remaining 5 samples were collected from an unaffected area without clinical cases of vole infection. The pH of soil samples ranged from 5.9 to 6.3. No frosts were recorded before the time of collection.

To specifically detect B. microti in soil samples, we have developed a PCR that targets a genomic island of 11 kb (H.C. Scholz et al., unpub. data) that is unique for B. microti. Briefly, primers Bmispec_f (5′-AGATACTGGAACATAGCCCG-3′) and Bmispec_r (5′-ATACTCAAGGCAGTACGGGC-3′) were used to amplify a 510-bp fragment of the genomic island. PCR conditions were denaturation at 94°C for 5 min, followed by 29 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Total DNA was prepared from 0.5 g of each soil sample by using the MO BIO Ultra Clean Soil DNA Kit (Dianova, Hamburg, Germany). DNA was eluted with 50 μL of double-deionized water of which 2 μL was used in PCRs. Template DNA of B. microti CCM 4915^T was used as a positive control. Type strains of all recognized Brucella species, 1 strain of each biovar of all species, and type strains of 11 Ochrobactrum species were used as negative controls.

In this PCR, 5 of 15 soil samples and the positive control were positive for the 510-bp fragment; other Brucella spp. and Ochrobactrum spp. were negative. Of the 5 positive samples, 3 were collected from surface soil collected near mouse burrows. However, the remaining 2 positive samples were collected from the unaffected and supposedly negative-control area.

For direct cultivation of Brucella spp. from soil, 2 g each of 2 selected PCR-positive samples with the highest amplification rate (both from the affected area) were thoroughly homogenized in 5 mL of phosphate-buffered saline (PBS), pH 7.2, in 50-mL tubes. Of a serial dilution in PBS (10^9-10^4), 100 μL was plated onto Brucella agar (Merck, Darmstadt, Germany) supplemented with 5% (vol/vol) sheep blood (Oxoid, Wesel, Germany) and Brucella selective supplement (Oxoid) and incubated at 37°C. Twenty suspicious colonies from the 10^9 dilution plate of 1 soil sample were subcultivated on Brucella selective agar. Two of the subcultivated bacteria (BMS 17 and BMS 20) reacted positively with monospecific anti-Brucella (M) serum. Both isolates were positive in the B. microti—specific PCR. Sequencing of the 510-bp fragments from both strains (GenBank accession nos. AM943814 and AM943815) and comparison with the known nucleotide sequence of B. microti showed 100% identity.

To confirm that strains BMS 17 and BMS 20 were B. microti, these strains were subjected to multilocus sequence analysis and multilocus variable number of tandem re-