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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 Delaware Bay 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. minutilla*) 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

(2007). Samples were stored at –70° C; shipped frozen overnight to Athens, Georgia; and maintained frozen until analyzed.

Samples were screened by real-time reverse transcriptase–PCR (RT-PCR) for influenza A virus, and virus isolation was performed on samples that were positive. RNA was extracted by adding 250 μL of sample to 750 μL Trizol LS reagent (Invitrogen, Inc., Carlsbad, CA, USA). Samples were mixed and incubated at room temperature for 10 min. A total of 200 μL of chloroform was then added, incubation was continued for 5 min, and samples were centrifuged for 15 min at 12,000 × *g* at 4° C. Supernatant was removed, and 50 μL was extracted with the MagMax AI/ND viral RNA extraction kit (Ambion, Inc. Austin, TX, USA). RNA was tested for AI virus matrix (M) gene. A positive test result for this gene indicates the presence of any influenza viruses (*6*) when an internal positive control is used (*7*). Positive samples were processed for virus isolation in embryonated chicken eggs by standard methods (*8*). Real-time RT-PCR results were corroborated by processing 50 randomly selected negative samples for virus isolation with 3 egg passages.

Screening for AI virus was conducted on 1,820 samples (Table). Among these, 1 AI virus was identified (A/Glaucous-wingedGull/AK/4906A/2006; H16N?), reflecting an overall prevalence of 0.055% (0% in shorebirds and 0.13% in gulls).

Results of power analysis (*9*) suggested that our shorebird samples would detect infection rates >0.9% 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*).

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

Species	Sample size		
	2006	2007	Total
Western sandpiper (*Calidris mauri*)	500	300	800
Least sandpiper (*C. minutilla*)	0	250	250
Glaucous-winged gull (*Larus glaucescens*)	100	670	770
Totals	600	1,220	1,820

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 shorebirds sampled, 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.

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Isolation of *Brucella microti* from Soil

To the Editor: *Brucella microti* is a recently described *Brucella* species (*1*) that was isolated in 2000 from systemically 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′-ATACTCAGGC AGGATACCGC-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 (10^0–10^{-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 10^0 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-