Avian Pneumovirus (APV) RNA from Wild and Sentinel Birds in the United States Has Genetic Homology with RNA from APV Isolates from Domestic Turkeys

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Avian pneumovirus (APV) is a newly emergent member of the Paramyxoviridae family of viruses belonging to the genus Metapneumovirus, which causes a highly contagious acute respiratory tract infection in turkeys characterized by coughing, sneezing, nasal discharges, tracheal rales, foamy conjunctivitis, and swollen sinuses (4). Uncomplicated cases have low mortality (2 to 5%), but infections accompanied by concurrent secondary infections can result in up to 25% mortality. The virus was first reported in South Africa in 1978, but it has since been isolated in Europe, Israel, Asia, and recently in the United States (1, 4). In the United States, APV was first isolated in commercial turkeys in 1997 in Colorado (APV/CO isolate) following a 10-month outbreak of an unusual upper respiratory tract infection in turkeys (6). The APV outbreak in Colorado was controlled, and severe disease has not been reported since 1997. However, the disease has emerged as a major problem in turkey flocks in Minnesota and neighboring states. For example in 1999, 37% of turkey flocks in Minnesota had APV outbreaks, resulting in economic losses estimated at $15 million. The losses are the result of poor weight gain in commercial turkeys, mortality, and processing plant condemnation due to airsacculitis. One interesting pattern noted in APV outbreaks in Minnesota is its seasonal trend of occurrence. Most outbreaks occur in two periods, March through May and October through November, suggesting that environmental factors may contribute to the disease. One of the suspected sources of the outbreaks is wild birds. With the isolation of other paramyxoviruses reported from wild birds (2, 5, 11), and APV RNA and antibodies were also detected in two different groups of sentinel ducks. Infectious APV was recovered from sentinel duck samples. The APV M gene isolated from the wild birds had over 96% predicted amino acid identity with APV/Minnesota 2A, which was isolated earlier from domestic turkeys showing respiratory illness, suggesting that wild birds may be involved in spreading APV infection.

Nasal turbinates or swabs were collected from wild ducks, geese, owls, sparrows, swallows, and starlings and from sentinel ducks placed next to turkey farms experiencing avian pneumovirus (APV) infections and were analyzed for APV genome and infectious particles. APV RNA was detected in samples examined from geese, sparrows, and starlings. APV RNA and antibodies were also detected in two different groups of sentinel ducks. APV in turkeys (10). Five microliters of the RT mixture was used for PCR at an annealing temperature of 51°C for 1 min for 35 cycles. APV-infected Vero cells were used as the positive control, while uninfected Vero cells were used as the negative control. Isolation of virus from all collected samples was also attempted. To isolate virus, samples were blindly cultured in chicken embryo fibroblasts for five passages and then five times in Vero cells (3). In the absence of detectable cytopathic changes, the fifth Vero cell passage was analyzed by immunofluorescence for viral antigens (7).

APV RNA was detected in sparrows, geese, swallows, and starlings (Table 1; Fig. 1). There was no viral RNA detected in two owl samples. In addition to captured birds, samples were collected from two groups of sentinel ducks caged in ponds neighboring two turkey farms experiencing APV outbreaks.

 FIG. 1. Detection of APV RNA in wild birds using M gene PCR. Total RNA was isolated from choanal swabs or turbinates, reverse transcribed to cDNA, and amplified by PCR using M gene-specific primers. Samples from sparrows (lane 3), geese (lane 4), and sentinel duck group 1 (lane 5) and group 2 (lane 6) were positive. APV-infected (lane 1) and uninfected (lane 2) Vero cells were used as positive and negative controls, respectively. The DNA molecular size marker is the lane M.
The ducks were APV negative at the time of introduction into the site. Choanal and tracheal swabs were collected from the sentinel ducks once a week for 9 weeks and analyzed for APV RNA, anti-APV antibodies, and virus isolation. Both the choanal and tracheal swabs from the sentinel ducks were positive for APV RNA at weeks 1 and 2 in group 1 ducks and weeks 8 and 9 in the group 2 ducks (Table 1; Fig. 1). Infectious virus was isolated from one sentinel duck nasal turbinate sample from group 1. Serum samples from the group 2 ducks tested positive for APV antibodies by enzyme-linked immunosorbent assay starting from week 4 after introduction. No clinical signs were observed in the ducks. Based on these results, we concluded that ducks in group 1 were exposed to APV within the first week after introduction because in experimental APV infection (H. J. Shin, D. A. Halvorson, D. P. Shaw, and K. V. Nagaraja, submitted for publication), APV RNA is detected 2 to 10 days after inoculation, whereas antibodies are detected after day 10 postinfection. As for group 2 ducks, they were most likely exposed to APV in week 7 after introduction into the cages.

To determine whether the APV genome detected in wild birds is similar to that of isolates responsible for the APV disease in turkeys, we isolated the APV M gene from ducks, geese, and sparrows and compared the predicted amino acid sequences. The isolation was accomplished by using 5′ primer (5′-ATGGAGTCTATCTACTCTTAGTGA-3′) starting from position 14 and 3′ primer (5′-GTAATAATATACAGCACTG-3′) starting from position 823 of the M gene (9). The M genes isolated (825 bp) from geese, ducks, sparrows, and turkeys (APV/Minnesota 2A strain) were subcloned into the pGEM-T-EASY vector (Promega, Madison, Wis.) and sequenced. Three independent clones of either gene were sequenced from each bird. Predicted amino acid sequence analysis revealed that the virus isolated from the wild birds shared over 96% identity (Fig. 2) with the APV/Minnesota 2A turkey isolates (GenBank accession numbers AF26673, AF26674, and AF26675).

Our data demonstrate the presence of RNA and antibodies to APV in wild birds, with viral genes that have close genetic identity with virus circulating in neighboring turkey flocks. In addition, we have isolated infectious APV from sentinel ducks placed next to an APV-infected turkey farm. These findings suggest that wild birds may act as reservoirs of APV and may

### TABLE 1. RT-PCR results for nasal turbinates from wild birds

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of birds</th>
<th>No. positive/no. testeda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sparrows</td>
<td>45</td>
<td>3/5</td>
</tr>
<tr>
<td>Geese</td>
<td>30</td>
<td>2/3</td>
</tr>
<tr>
<td>Swallows</td>
<td>24</td>
<td>1/2</td>
</tr>
<tr>
<td>Swallows</td>
<td>27</td>
<td>2/6</td>
</tr>
<tr>
<td>Owls</td>
<td>2</td>
<td>0/2</td>
</tr>
<tr>
<td>Wild ducks</td>
<td>28</td>
<td>0/2</td>
</tr>
<tr>
<td>Sentinel ducks</td>
<td>40</td>
<td>2/2</td>
</tr>
</tbody>
</table>

*Each PCR sample consisted of pooled swabs from 5 to 20 birds, except samples from owls, which were individually tested.

![FIG. 2. Comparative amino acid sequence alignment of matrix gene proteins of APV from turkeys and wild birds. The U.S. APV isolates from turkeys in Colorado (Turkey/CO) and Minnesota (Turkey/MN) were compared with isolates from ducks, geese, and sparrows. Predicted amino acid sequence differences are denoted by the single-letter code with the consensus sequence above. The GenBank accession numbers for these sequences are AF26673, AF26674, and AF26675.](http://jcm.asm.org/)

![Phylogenetic tree showing the relationship between the matrix gene of APV isolates from different species. The tree is rooted with the consensus sequence of the matrix gene from APV, and the branches are labeled with the species name. The GenBank accession numbers for the sequences are AF26673, AF26674, and AF26675.](http://jcm.asm.org/)
explain the periodic pattern of APV outbreaks that has not been attributable to management practices. Newcastle disease virus (5, 11) and avian paramyxovirus type 1 (2, 11) have been isolated from wild birds. In Europe, an epizootic of Newcastle disease in poultry that involved England, Scotland, and Northern Ireland was attributed to migratory birds that spread the virus either mechanically or biologically (2). As reported previously for the turkey viruses, the APV isolates detected in the birds in the United States can be distinguished phylogenetically from the European subgroup A and B isolates (8, 13) and are classified as members of subgroup C (6, 9). In the United States, it is difficult to attribute the spread of APV within the north-central states to bird migration alone, since Canada to the north and states in the south have not reported APV outbreaks. However, it is important to note that the state of Minnesota, which currently has the highest incidence of APV, has many lakes, providing water sanctuary for many wild birds. This may provide an ideal environment for maintaining APV in nature. Our immediate research is focused on isolating APV from wild birds and determining whether these birds can develop APV clinical disease following experimental or natural infection.

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REFERENCES