INTRODUCTION

Fowl adenoviruses (FAdV) belong to the family Adenoviridae, genus Aviadenovirus, and are classified into 5 groups (A–E) and also into 12 serotypes (Hess, 2000). The FAdV are endemic worldwide and can be isolated from birds, including poultry and wild species (Adair and Fitzgerald, 2008). The pathogenesis of FAdV infection is affected by the serotypes or genotypes involved (McFerran, 1997). Although most avian adenoviruses are considered opportunistic pathogens and do not produce clinical signs when inoculated into birds by themselves, some are considered primary pathogens, such as the ones responsible for inclusion body hepatitis, hydropenicardium syndrome, respiratory disease, necrotizing pancreatitis, and adenoviral gizzard erosion in chickens and other birds (McFerran et al., 1971; Jones and Georgiou, 1984; Anjum et al., 1989).

These FAdV are routinely diagnosed by virus isolation in embryonated chicken eggs or cell culture, by electron microscopy, or, more recently, by PCR (Raue and Hess, 1998). Use of PCR followed by restriction enzyme digestion of the products allows the differentiation of field isolates to species and presumptive serotypes (Meulemans et al., 2001). This method has been more recently supported by sequencing data (Meulemans et al., 2004).

Natural cases of adenoviral gizzard erosion in chickens were reported first in 1993 (Borzemska et al., 1993; Tanimura et al., 1993) and FAdV serotype 1 (FAdV-1)-induced gizzard erosions in chickens have been recently reported in many countries, such as United Kingdom, Italy, Poland, Germany, and Japan (Okuda et al., 2001; Marek et al., 2010). In Korea, outbreaks of a disease clinically affecting primarily the digestive tract, including the proventriculus and the gizzard, have continuously been recorded in chicken flocks by field veterinarians. However, isolation and identification of FAdV-1 from chickens has not been reported yet.

In the present study, we isolated and identified FAdV-1 from chickens with outbreaks of gizzard erosion. Furthermore, to reproduce the disease under experimental conditions, we used the FAdV-1 isolate (K181 strain) to inoculate in specific-pathogen-free (SPF) chickens via the oral or intramuscular route.
MATERIALS AND METHODS

Case History

In June 2010, 150-d-old commercial layer chicks, with signs of gizzard erosion and mortality, were submitted for diagnosis to the College of Veterinary Medicine, Konkuk University. The percentage of chickens showing signs of dullness, anorexia, and emaciation in the flock was relatively high (1–2%) and the mortality was approximately 0.2% per week. The gross lesions observed during the postmortem examination consisted of dilated proventriculus and gizzard with bloody fluids, gastric perforation, gizzard erosion, and ulceration. Furthermore, the koilin layer of the gizzard showed black areas of erosion.

Virus Isolation and Propagation

Gizzard samples were frozen and thawed twice and macerated in PBS containing 1% gentamycin (80 mg/ml). The homogenate was incubated at room temperature for 1 h. The homogenate was then centrifuged for 10 min at 890 × g and the supernatant was collected. The supernatant was filtered using a 0.45-μL syringe filter and inoculated into chicken embryo liver cells (CELiC) prepared with 15-d-old SPF chicken embryos.

DNA Extraction, PCR, and DNA Sequencing

Viral DNA was extracted from CELiC by use of Viral Gene-spin (iNtRON Biotechnology, Korea). Then, PCR amplification of an 897-bp fragment containing the L1 loop of the hexon gene was performed with hexon A (5′-CAARTTCAGRCAGACGGT-3′) and hexon B (5′-TATGTAGMCSCGGACATCAT-3′) primers following a procedure described elsewhere (Meulemans et al., 2001). The amplified product was visualized using 1% agarose gel electrophoresis followed by ethidium bromide staining. The amplified DNA product was purified using a gel extraction kit (Elpis Biotech, Korea) and the purified product was ligated to a pGEM-T Vector (Promega, Fitchburg, WI). Nucleotide sequencing was performed with a BigDye Terminator v3.1 cycle sequencing kit and the product was analyzed on the ABI PRISM 3730xl genetic analyzer (Applied Biosystems, Foster City, CA). The nucleotide sequence for the hexon gene acquired in this study has been submitted to GenBank under accession number JN181575.

Hexon Gene Phylogenetic and Sequence Analysis

The nucleotide sequence of the hexon gene of the FAdV-1 isolate was compiled using Bioedit v7.0.9.0 (Hall, 1999). The 16 FAdV reference strains were retrieved from the GenBank database. The nucleotide sequence analysis of the hexon gene was performed by the Clustal W multiple alignment method (Thompson et al., 1994). The phylogenetic tree was constructed with the neighbor-joining method with 1,000 bootstrap replicates using MEGA version 4 (Tamura et al., 2007).

Pathogenicity of FAdV Isolate in 7-d-old SPF Chickens

The FAdV-1 isolate designated as K181 strain was used in the following experiments. Ninety 1-wk-old SPF chicks were divided into 3 groups of 30 chicks each. Chicks in group 1 were challenged intramuscularly and chicks in group 2 were challenged orally with 10⁶ tissue culture infective dose 50 (TCID₅₀)/chick of the K181 strain. Chicks in group 3 were kept as the unchallenged negative control. The chicks were monitored daily for 21 d for signs of disease and mortality. At 7, 14, and 21 d postchallenge, 10 chicks from each group were humanely killed and a postmortem examination was conducted to look for gross lesions, and sections of liver, proventriculus, gizzard, and pancreas were collected from 10 chickens per group for reisolation and histopathological examination.

Histological Examination

Sections of liver, proventriculus, gizzard, and pancreas from chicks were fixed in 10% neutral-buffered formalin solution, routinely processed, and embedded in paraffin. Sections 5-mm thick were prepared from the paraffin blocks and stained with hematoxylin and eosin.

RESULTS

Sequencing and Phylogenetic Analysis

Cytopathic effect (CPE) characterized by the presence of round and refractile cells was observed after inoculation into CELiC and positive PCR amplification of the 897-bp fragment containing the L1 loop of the hexon gene in DNA isolated from the infected cells. By phylogenetic analysis of hexon gene sequences, the K181 strain was identified as genotype A/serotype 1, showing 99.3% identity with chicken embryo lethal orphan (CELO) strain (Figure 1).

Experimental Infection in SPF Chicks

Clinical Signs and Gross Lesions. No clinical signs of FAdV were observed in the chicks for the duration of the study. Grossly, focal gizzard lesions, such as a rough or cloudy koilin layer, were observed in chicks at 1, 2, and 3 wk postchallenge. These lesions were similar to the field cases. Significant gross lesions were not present in other organs or in the uninoculated control chicks. The incidence of gross lesions in the 1-wk-old SPF chicks infected orally was 30, 40, and 20% at 1, 2, and 3 wk postchallenge, respectively. The incidence of gross lesions in the 1-wk-old SPF chicks infected in-
tramuscularly was 20, 10, and 10% at 1, 2, and 3 wk postchallenge, respectively (Table 1).

**Virus Reisolation.** Reisolation results are summarized in Table 1. The FAdV was recovered from the proventriculus, gizzard, and liver samples of inoculated chicks only at 1 wk postchallenge. The FAdV was recovered from the proventriculus (10%) and the gizzard (50%) from the chickens orally, but not from liver. In the case of chicks infected intramuscularly, FAdV was recovered from the gizzard (40%) and the liver (20%), but not from the proventriculus. No FAdV was recovered from any sample from the chicks in the negative control group.

**Histology.** Table 2 shows the severity of the histological lesions from the chickens infected orally or intramuscularly. In chicks infected orally, the specific lesions in the gizzard included severe degeneration and necrosis of the glandular epitheliums and eosinophilic inclusion bodies (Figure 2). In contrast, in the chicks infected intramuscularly, the most unique lesions were observed in the liver and pancreas, and not the gizzard. These organs had individual hepatocytic necrosis.

![Figure 1. Fowl adenovirus (FAdV) phylogenetic tree of the L1 loop of the hexon gene. The tree is based on the sequence of the hexon gene from the FAdV isolate and the 16 reference strains. Clustal W alignment method for hexon nucleotide positions 34–858 corresponding to those of strain chicken embryo lethal orphan (CELO) was used. The FAdV isolate used in this study is identified in bold and underlined. Genotypes are indicated on the right.](Image)

**Table 1.** Fowl adenovirus serotype-1 reisolation from organs of challenged specific-pathogen-free chickens

<table>
<thead>
<tr>
<th>Group</th>
<th>Infection route</th>
<th>DPI</th>
<th>Liver</th>
<th>Proventriculus</th>
<th>Gizzard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intramuscular</td>
<td>7</td>
<td>2/10</td>
<td>0/10</td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0/10</td>
<td>0/10</td>
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<td></td>
<td>21</td>
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<tr>
<td>2</td>
<td>Oral</td>
<td>7</td>
<td>0/10</td>
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<td>14</td>
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<td></td>
<td>21</td>
<td>0/10</td>
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</tr>
</tbody>
</table>

*Days postinoculation.*
and mild-to-moderate inflammatory cellular infiltration throughout the parenchyma.

**DISCUSSION**

Although gizzard erosion associated with FAdV-1 infection has been described since 1993 (Borzemska et al., 1993; Tanimura et al., 1993), this is the first study isolating and characterizing FAdV from chickens in Korea. In this study, we isolated an FAdV-1 strain from layer chicken flock showing clinical signs of dullness, anorexia, and pale combs and wattles. Necropsy findings consisted of gastric perforation, gizzard erosion and ulceration, and bloody contents in the proventriculus and gizzard. Internal bleeding is probably responsible for the pale combs and wattles, and it is reasonable to assume that the FAdV-1 infection lead to gizzard erosion and ulceration with subsequent bleeding and death.

How FAdV-1 induces gizzard erosion in a host is not clear, but it has been reported that pathogenicity of FAdV differs between strains (Domanska-Blicharz et al., 2011). The CELO, a European reference FAdV-1 strain, does not induce gizzard erosions in chickens (Marek et al., 2010), but some strains can reproduce adenoviral gizzard erosions in SPF chickens and commercial layer chickens (Ono et al., 2004; Manarolla et al., 2009). In this study, experimental K181 strain infection in 1-wk-old SPF chickens resulted in gizzard erosions similar to those in the natural cases, indicating that the Korean FAdV-1 isolate is sufficiently pathogenic to cause clinical disease in chickens.

Various factors, such as the age of the bird, differences in challenge dose used for the experimental infection, and differences in the route of infection, can make the interpretation of onset and severity of clinical signs and lesions difficult (Ono et al., 2007). In these experiments, the route of infection caused significant differences in the presentation of the lesions. In chicks infected orally, the histological lesions were present in the proventriculus and gizzard, rather than the liver and pancreas. However, in the chickens infected intramuscularly, most unique prominent lesions were observed in the livers and pancreas (Table 2). These results indicate that gizzards were very susceptible to FAdV-1 infection through the natural route of infection, because

**Table 2.** Gross and histologic lesions of specific-pathogen-free chicks challenged with fowl adenovirus serotype-1

<table>
<thead>
<tr>
<th>Group</th>
<th>Infection route</th>
<th>DPI</th>
<th>Liver</th>
<th>Proventriculus</th>
<th>Gizzard</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
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<td>0/0/10</td>
<td>1/0/10</td>
<td>0/0/10</td>
</tr>
<tr>
<td>2</td>
<td>Oral</td>
<td>7</td>
<td>0/0/10</td>
<td>0/1/10</td>
<td>3/5†/10</td>
<td>0/0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0/0/10</td>
<td>0/0/10</td>
<td>4/2/10</td>
<td>0/0/10</td>
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<td></td>
<td></td>
<td>21</td>
<td>0/0/10</td>
<td>0/0/10</td>
<td>2/0/10</td>
<td>0/0/10</td>
</tr>
</tbody>
</table>

*DAYS postinoculation.*

*P < 0.05, by Fisher’s exact test, compared with group infected orally.

†P < 0.05, by Fisher’s exact test, compared with group infected intramuscularly.
50% of the gizzards had mucosal erosion or ulceration associated with moderate-to-severe inflammation and formation of inclusion bodies in the chief cells (Figure 2). Furthermore, histological lesions in the livers and pancreas of chicks infected intramuscularly indicate that chicks inoculated by this route developed a systemic infection.

The severity of clinical signs and gross lesions could not be reproduced to the same degree with the field observations. In general, immunosuppression caused by infection with chicken anemia virus or infectious bursal disease virus as well as various environmental stresses aid in producing diseases associated with FAdV (Fadly et al., 1976; Toro et al., 2000). However, in this case, because immunosuppressive pathogens were not isolated, we speculate that stress associated with farm conditions might have contributed to the outbreak of gizzard erosion. Furthermore, there are many other conditions that can produce gizzard erosions, such as histamine, gizzerosine, mycotoxins, and vitamin deficiencies, which can result in lesions that are indistinguishable from those resulting from FAdV-1 infection (Harry and Tucker, 1976; Giambrone et al., 1978; Okazaki et al., 1983). Therefore, we cannot exclude the possibility that the more severe clinical signs and lesions of the commercial birds might have been accentuated by some other contributing factors like the ones previously mentioned.

In conclusion, we reproduced FAdV serotype 1 infection in chicks and isolated FAdV-1 from chickens in Korea. Furthermore, the FAdV-1 isolate alone could induce clinical disease in SPF chicks infected experimentally. These observations support the possibility that FAdV-1 might be responsible for productivity losses in commercial flocks of chickens. Therefore, continued epidemiological surveillance is necessary for the prevention of FAdV-1 infection in commercial chicken flocks in Korea.

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REFERENCES


