BRIEF COMMUNICATION

Bronchointerstitial Pneumonia in Guinea Pigs Following Inoculation with H5N1 High Pathogenicity Avian Influenza Virus

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Abstract. The H5N1 high-pathogenicity avian influenza (HPAI) viruses have caused widespread disease of poultry in Asia, Africa, and the Middle East, and sporadic human infections. The guinea pig model has been used to study human H3N2 and H1N1 influenza viruses, but knowledge is lacking on H5N1 HPAI virus infections. Guinea pigs were inoculated intranasally or intragastrically with A/Vietnam/1203/04 (VN/04) or A/Muscovy duck/Vietnam/209/05 (MDk/VN/05) viruses. Mild listlessness was seen at 2 and 3 days postinoculation (DPI) in guinea pigs inoculated intranasally with VN/04 virus. At 5 DPI, the guinea pigs had bronchointerstitial pneumonia and virus was identified in bronchiolar epithelium and alveolar macrophages. Virus was isolated from the lungs but was lacking from other organs. Minimal lung lesions were seen in intranasal MDk/VN/06 group and virus was not detected, but serologic evidence of infection was observed. Intragastric exposure failed to produce infection or lesions with either virus. The localized respiratory disease in guinea pigs with H5N1 viruses was very similar to that of H3N2 and H1N1 influenza in humans and was less severe than reported for H5N1 human cases.

Key words: Avian influenza; H5N1; guinea pigs; pneumonia; susceptibility.

Avian influenza viruses of the H5N1 subtype have been responsible for the ongoing outbreak of high-pathogenicity avian influenza (HPAI) in poultry and wild birds of Asia, the Middle East, and Africa, and such viruses have sporadically caused fatal infections in humans and other mammals including Bengal tigers (Panthera tigris tigris), stone martens (Martes foina), and domestic cats (Felis catus) and dogs (Canis lupus familiaris).10 In mammalian animal models such as ferrets, mice, and cats, infections with H5N1 isolates have produced varying syndromes ranging from respiratory disease to severe systemic infection with high mortality after intranasal exposure or when fed infected chicks.2,9 Guinea pigs have been proposed as a model to study infection with human influenza A viruses.1,3,4 Human H3N2 and H1N1 influenza viruses were shown to replicate efficiently in the respiratory tract of guinea pigs without prior adaptation, and they produce pulmonary lesions. This study was undertaken to determine the susceptibility and pathogenesis of infection by H5N1 HPAI viruses in 8-week-old guinea pigs after upper respiratory or gastric exposure.

Female Hartley strain guinea pigs (Charles River Laboratories, Wilmington, MA) weighing 300-350 g were housed in high-efficiency particulate air-filtered isolation units maintained within a Biosafety Level 3 Agriculture animal laboratory facility. Animal experiments were conducted under approval of the Institutional Animal Care and Use Committee. Two H5N1 HPAI viruses were used: the human isolate A/Vietnam/1203/04 (VN/04) (Centers for Disease Control and Prevention, Atlanta, GA) and the domestic duck isolate A/Muscovy duck/Vietnam/209/05 (MDk/VN/05) (Dr. Nguyen Van Can, National Center for Veterinary Diagnosis, Hanoi, Vietnam). Virus stocks were propagated in 10-day-old embryonating chicken eggs, and the infected allantoic fluid was titered and reported as 50% egg infective dose (EID_{50}), calculated using the method of Reed-Muench.8 For each of the viruses, groups of 3 (intragastric, VN/04) to 4 (intranasal, VN/04; intranasal and intragastric, MDk/VN/05) guinea pigs were anesthetized (intramuscular, ketamine [20 mg/kg] and xylazine [2 mg/kg]) and inoculated intranasally (i.e., canula, 10^6 EID_{50} of virus in 1 ml divided between each nostril) or intragastrically (i.e., gavage catheter, 10^6 EID_{50} of virus in 2 ml). Two sham control guinea pigs were intranasally inoculated with 2 ml of sterile phosphate-buffered saline (PBS). At 5 days postinoculation (DPI), two guinea pigs from each group, except only one guinea pig from the VN/04 intragastric group, were euthanized, and duplicate sets of the following tissues were collected for virus isolation and histologic examination: nasal turbinates, tonsils, trachea, lungs, olfactory...
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intranasal or intragastric routes.

isolation negative.

geometric titers in parentheses.

for all four groups and were HPAI virus

glands, diaphragm, and skeletal muscle were examined for all four groups and were HPAI virus

bulbs, brain, heart, blood, spleen, stomach, small intestine, pancreas, large intestine, kidney, adrenal glands, diaphragm, and skeletal muscle. For virus

Titers were expressed as log TCID50 per tissue

for IMunohistochemically using as the primary antibody a

formalin and processed to 5 μm HE-stained sections for

histologically identified influenza viral antigen.8 Fast

counterstained with hematoxylin. Serologic evalu-

ation to determine infection was accomplished by the

standard hemagglutination inhibition test.

bulbs, brain, heart, blood, spleen, liver, stomach, pancreas, small intestine, large intestine, kidney, adrenal glands, diaphragm, and skeletal muscle. For virus

isolation and titration tissues were homogenized in

stere PBS to obtain 10% (w/v) homogenate tested by

standard procedures in Madin-Darby canine kidney

(MDCK) cells. Titers were expressed as log10 50% tissue

culture infecting dose (TCID50). The lower detection

limit was 101.5 TCID50/g of tissue. Similar tissue sample

sets were collected at 14 DPI in 10% neutral buffered

formalin and processed to 5 μm HE-stained sections for

histologic examination. Duplicate sections were stained

immunohistochemically using as the primary antibody a

mouse-derived monoclonal antibody (PI3C11) specific

for type A influenza virus nucleoprotein antigen.8 Fast

red was used as the substrate chromagen, and slides

were counterstained with hematoxylin. Serologic evalu-

ation to determine infection was accomplished by the

standard hemagglutination inhibition test.

Clinically, the four guinea pigs inoculated intranasally with VN/04 exhibited slight temporary lethargy between 2 and 3 DPI, and then recovered. No clinical

abnormality was observed in the other guinea pig

groups. VN/04 virus was isolated only from the

lungs of two intranasally inoculated guinea pigs at

5 DPI (Table 1), with titers of 102–75 and 103–75 TCID50/

g of tissue, respectively. No virus was isolated from any

of the others tissues. In addition, virus was not isolated from any tissues of the guinea pigs that had been

intranasally or intragastrically inoculated with MDK/

VN/05 virus, intragastrically exposed to VN/04, or

intranasally sham inoculated. However, guinea pigs

intranasally inoculated with VN/04 or MDK/VN/05 and

sampled at 14 DPI, seroconverted, while those that were

intragastrically or sham inoculated remained negative

for H5 antibodies (Table 1).

Mild congestion with slight edema of the lungs was

observed at 5 DPI in guinea pigs inoculated intranasally

with VN/04 virus. Histologically, moderate lymphocytic

bronchointerstitial pneumonia with mild to moderate

alveolitis characterized by peribronchial lymphocytic

infiltration with interstitial edema around the small

blood vessels (Fig. 1) as well as necrotic epithelium and

debris adhering to the surface, mainly of bronchioles

and less frequently in bronchi, were observed. Immu-

nohistochemically, influenza viral antigen was common-

ly identified in bronchiolar epithelium (Fig. 2). The

parenchyma was edematous; alveolar walls congested

adjacent to bronchioles; and alveolar lumens were

flooded with a mixture of fibrin, macrophages, and,

rarely, neutrophils (Fig. 3a). Lymphocytes and histo-

cytes were seen within alveolar walls, and macrophages

were identified within the lumens of terminal airways

(Fig. 3b). Rarely, influenza antigen was identified in

alveolar macrophages. In contrast, guinea pigs inoculated

intranasally with MDK/VN/05 virus exhibited minimal

bronchointerstitial pneumonia characterized by mild

lymphocytic infiltration around peribronchiolar areas

without demonstrating viral antigen in tissues, including

lungs. Lesions and viral antigen were lacking at 14 DPI in

guinea pigs intranasally inoculated with VN/04 and

MDK/VN/05 viruses. Guinea pigs inoculated intragastrically

with VN/04 or MDK/VN/05 viruses lacked macro-

scopic and microscopic lesion, and no viral antigen was

demonstrated in any tissues at 5 and 14 DPI.

Guinea pigs have several advantages as a mammalian

animal model for studying influenza disease, including

high susceptibility to infection with human influenza A

viruses;2 in addition, their lungs contain humanlike

bronchus-associated lymphoid tissue.1 Intranasal inoc-

ulation with human influenza A viruses, A/Hong Kong/

68 (H3N2),1,3,11 and A/England42/72 (H3N2),7 resulted

in virus replication within the lungs and interstitial

pneumonia but without overt clinical illness. Such

infections produced immunity to the respective influen-

za A viruses. By contrast, in our H5N1 HPAI virus

study, intranasal exposure to VN/04 virus produced a

limited, local lower respiratory tract infection with

minimal clinical signs and no mortality. Such infections

were associated with bronchiolitis and adjacent lympho-

cytic to histiocytic interstitial pneumonia. By con-

trast, intranasal inoculation with MDK/VN/05 virus

produced no virologic or immunohistochemical evi-

dence of infection, but minimal peribronchiolar inflam-

matory lesions and anti-H5 hemagglutinin antibodies

were observed, which suggests very limited virus

replication. However, the lack of virus replication and

lesions after gastric exposure to VN/04 or MDK/VN/05


<table>
<thead>
<tr>
<th>Virus</th>
<th>Inoculation Route</th>
<th>Virus Isolation from Lungs (5 DPI)*</th>
<th>Serology†</th>
</tr>
</thead>
<tbody>
<tr>
<td>VN/04</td>
<td>Intranasal</td>
<td>3.49†</td>
<td>2/2(16,16)</td>
</tr>
<tr>
<td>VN/04</td>
<td>Intragastric</td>
<td>-</td>
<td>0/2</td>
</tr>
<tr>
<td>MDK/VN/05</td>
<td>Intranasal</td>
<td>-</td>
<td>2/2(16,32)</td>
</tr>
<tr>
<td>MDK/VN/05</td>
<td>Intragastric</td>
<td>-</td>
<td>0/2</td>
</tr>
</tbody>
</table>

* Nasal turbinate, trachea, tonsils, brain, olfactory bulbs, heart, blood, liver, spleen, stomach, small intestine, pancreas, large intestine, kidney, adrenal glands, diaphragm, and skeletal muscle were examined for all four groups and were HPAI virus isolation negative.

† Values represent number hemagglutination inhibition-positive for the two guinea pigs euthanatized at 14 DPI; individual geometric titers in parentheses.

‡ Mean value is expressed as log10 TCID50 from two guinea pigs at 5 DPI; - = no virus detected.

Table 1. Viral isolation from lung and serology of guinea pigs inoculated with H5NI HPAI viruses via intranasal or intragastric routes.
suggests that inhalation or direct oropharyngeal mucus membrane contact is more likely to lead to infection than alimentary exposure at the same challenge dose in liquid medium, although alimentary exposure to higher doses of virus may have been responsible for H5N1 HPAI infections in domestic cats fed infected chicks.9

Interestingly, VN/04 virus produced mild clinical disease associated with respiratory infection and lesions in the guinea pig model, but this virus strain was isolated from a fatal human case. By contrast, this virus strain was highly pathogenic in ferret and mouse models, producing systemic, fatal disease associated with a broader tissue tropism for virus replication and lesion production, including high virus titers in multiple organs ranging from 10^5.5 to 10^6.0 TCID_{50}/g.2,12 However, no single animal model has exactly reproduced the human H5N1 infection and disease. Most fatal human cases have been associated with rapidly progressing fulminant primary viral pneumonia that often progressed to acute respiratory distress syndrome with virus replication in alveolar pneumocytes and macrophages.5 Most of the available post mortem examinations of humans have not supported viral replication or viral pathology in visceral organs except lungs and intestine. In the current study, the guinea pigs became infected with H5N1 HPAI viruses without production of clinical signs, but the
presence of mild specific lung pathology suggests that the guinea pig is worthy of further exploration as an animal model for studying nonlethal respiratory infections by some H5N1 HPAI viruses.

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References


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