Reproduction of Adenoviral Gizzard Erosion by the Horizontal Transmission of Fowl Adenovirus Serotype 1

Masaaki ONO1), Yo OKUDA1), Isao SHIBATA1), Shizuo SATO1) and Kosuke OKADA2)

1)JA Zen-noh (National Federation of Agricultural Co-operative Associations) Institute of Animal Health, 7 Ohja-machi, Sakura, Chiba 285–0043 and 2)Department of Veterinary Pathology, School of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka, Iwate 020–0043, Japan

(Received 14 March 2007/Accepted 5 June 2007)

ABSTRACT. The horizontal transmission ability of fowl adenovirus (FAV) serotype 1 99ZH strain, isolated from chickens exhibiting gizzard erosion, was investigated. Twelve 13-day-old specific pathogen-free chickens were inoculated orally with 10^6 TCID50/0.05 ml of the strain. An in-pen contact group (chickens in the same pen with inoculated chickens), hedge contact group (chickens in a pen connected with pens housing inoculated chickens), non-contact group (chickens in a separate pen placed at a distance of 70 cm from the connected pens), human exposure group (chickens in the next room and attended last every day) and negative control group were examined. Each group consisted of 11 or 12 uninoculated chickens. Gizzard lesions were grossly or histologically observed from 10 days after exposure (DAE) in the in-pen contact group, and from 15 DAE in the hedge contact and non-contact groups. The FAV gene was detected by polymerase chain reaction performed on cloacal swabs taken on 5 and 13 DAE from chickens in both contact groups, and on 20 and 26 DAE from those in the non-contact group. Serum neutralizing antibodies against FAV serotype 1 were detected in chickens from 13 DAE in the in-pen contact group, and from 15 DAE in the hedge contact and non-contact groups. We conclude that FAV-99ZH strain spreads rapidly through direct contact with inoculated chickens, and slowly through non-contact transmission, and that adenoviral gizzard erosion is reproduced by this horizontal transmission.

KEY WORDS: adenovirus, chicken, gizzard erosion, horizontal transmission.

Adenoviral gizzard erosion (AGE) in broiler chickens, which is caused by fowl adenovirus (FAV) infection, has been frequently observed in Japan. In most outbreaks, the affected broiler chickens exhibit no apparent clinical signs; AGE is confirmed by inspection at slaughterhouses [10, 17, 20]. The large quantities of condemned gizzard at slaughterhouses involve a considerable economic loss [16], and at least one study has examined mortality due to AGE in growing chickens in a broiler flock [2]. Most FAV isolates from AGE lesions has been reported to be serotype 1, however, serotype 8 has also been isolated on occasion [16, 20]. AGE-induced FAV serotype 1 strains may have a characteristic long fiber gene and can be distinguished from other strains through the detection of this gene by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis [11].

FAVs are transmitted in 2 ways: vertically from hen to progeny through the embryonated egg, and horizontally from chicken to chicken by direct fecal contact or aerial contact over short distances [7]. Outbreaks in broiler flocks obtained from seronegative hatcheries and differences in the prevalence of AGE lesions in outbreaks at broiler houses and farms suggest that horizontal transmission plays an important role in AGE outbreaks at broiler farms. AGE lesions have been reproduced in broilers and specific pathogen-free (SPF) White Leghorn chickens by oral infection with high doses (above 10^5 median tissue culture infectious dose (TCID50)/chicken or 10^7 plaque-forming units/chicken) of FAV serotype 1, isolated from gizzard erosions, at a high probability [9, 10, 12, 13]. However, titers of fecal virus excretion in the experimentally infected chickens were below 10^3 TCID50/0.05 ml [12, 13]. In our previous study, gizzard lesions appeared later and were milder in chickens inoculated at the lower dose (10^3 TCID50/chicken) than in those that received the higher dose (10^6 TCID50/chicken) [15].

In the present study, AGE lesions were reproduced by the horizontal transmission of FAV in a small model of broiler houses using pens on wood chips to examine the epidemiology of AGE outbreaks at broiler farms.

MATERIALS AND METHODS

Chickens: Seventy-one White Leghorn chickens hatched from SPF eggs were used for the present experiment to ensure the absence of maternal antibodies against FAV. The chickens were studied in accordance with a protocol approved by the Animal Care and Use Committee of the JA Zen-noh Institute of Animal Health; the experiment was performed in accordance with the Guidelines for the Care and Use of Laboratory Animals established by that committee. Throughout the experiment, the chickens were kept in isolated rooms in a negatively pressured house containing filtered air.

Virus: The virus strain used was FAV-99ZH, a strain belonging to FAV serotype 1 originally isolated from an...
outbreak of AGE in a commercial broiler flock in Japan [17].

Experimental design: The FAV-inoculated group (Group 1) consisted of 12 chickens inoculated orally at 13 days of age with 10^6 TCID_{50}/0.05 ml of FAV-99ZH. Ten of these chickens were placed in Pen 1 with 4 unoinoculated chickens and the other 2 were placed in Pen 2 with 8 unoinoculated chickens. The in-pen contact group (Group 2) consisted of the 12 uninoculated chickens in Pens 1 and 2. Pen 3, which contained 12 uninoculated chickens as the hedge contact group (Group 3), was sandwiched between Pens 1 and 2, and all chickens in these pens were able to have contact with each other through the hedge. Pen 4, which contained 12 uninoculated chickens as the non-contact group (Group 4), was placed at a distance of 70 cm from Pens 1–3. The connected pens (Pens 1–3) and Pen 4 were set up in parallel to the direction of air flow. Pens 5 and 6, which contained 12 and 11 uninoculated chickens as the human exposure group (Group 5) and negative control group (Group 6), respectively, were placed in separate rooms. Each pen measured 91 × 61 × 40 cm. Commercial clean wood chips for experimental animals were spread at approximately 5 cm thickness in all pens. Throughout the experiment, personnel attended first to the chickens in Pen 6, then to those in Pens 4, 3, 2, 1 and 5 in that order, changing into new clean silicon gloves and washing their boots carefully, but not otherwise changing their clothes when moving between rooms. The floors of the 3 rooms were washed every day with 2 w/v% glutaral. All uninoculated chickens were observed daily for clinical signs, and were euthanized by cervical dislocation for necropsy within 10–54 days after inoculation, as shown in Table 1. Two of the inoculated chickens were euthanized on 23 days postinoculation (DPI), and the remaining chickens were moved into another room in order to be used in another examination and were precluded from the present experiment. Cloacal swabs for the detection of the FAV gene by PCR and sera for the detection of serum antibody against FAV serotype 1 by a virus neutralization test were collected at weekly intervals until slaughter.

Pathology: After postmortem examination of the chickens, sections of the gizzard were removed. The tissues were fixed in 20% buffered neutral formalin. The paraffin-embedded tissues were sectioned at 4 µm, and stained with hematoxylin and eosin (H&E). Polyomavirus chain reaction: PCR was performed on the cloacal swabs to detect the FAV gene in our previous study [11] and in a study by Raue and Hess [18]. Serology: A virus neutralization test to detect serum antibody against FAV serotype 1 was performed on microculture plates with primary chicken kidney cell monolayers [6]. An antibody titer of 1:2 or above was regarded as positive.

RESULTS

The results of pathology are shown in Table 1. Some chickens in Groups 1–3 showed slight depression and anorexia from 4 to 14 DPI or days after exposure (DAE). Some chickens in Group 4 also showed slight depression and anorexia for a few days beginning on 14 DAE. No clinical signs were observed in any of the chickens in Groups 5 and 6. Gizzard erosion was observed grossly on 15 DAE in Groups 2 and 3, and on 27 DAE in Group 3. Histologically, gizzard lesions such as degeneration of the koilin layer and cellular infiltration of the lamina propria were observed on 23 DPI in Group 1, from 10 to 23 DAE in Group 2, from 15 to 27 DAE in Group 3, and on 15, 23 and 37 DAE in Group 4. Intranuclear inclusion bodies in epithelial cells were observed in 1 of 3 examined chickens on 15 DAE in Groups 2 and 3. No significant lesions were observed in the other chickens. The FAV gene was detected by PCR in the cloacal swabs taken from chickens on 5 DPI in Group 1, on 5 and 13 DAE in Groups 2 and 3, and on 20 and 26 DAE in Group 4 (Table 2), and serum neutralizing antibodies against FAV serotype 1 were detected in chickens from 5 DPI in Group 1, from 13 DAE in Groups 2 and 3, and from 26 DAE in Group 4 (Table 3). All chickens in Groups 5 and 6 remained serologically negative throughout the experiment.

DISCUSSION

Although some differences arise depending on the age of the chicken at time of inoculation and/or the titer of the inoculated virus, our previous studies have shown that gross lesions of the gizzard first appear on approximately 5 to 14 DPI, the virus is detected in feces in rectums from 3 to 10 DPI, and serum neutralization antibodies against FAV serotype 1 are present from 7 DPI [11, 13]. The results of the present study indicate that FAV-99ZH spreads rapidly to

<p>| Table 1. Results of the macroscopic and histologic evaluation of the spread of adenoviral gizzard erosion from chickens inoculated orally with FAV-99ZH to uninoculated chickens |
| --- | --- | --- | --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of chickens</th>
<th>10</th>
<th>15</th>
<th>23</th>
<th>27</th>
<th>37</th>
<th>46</th>
<th>54</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FAV-inoculated</td>
<td>12</td>
<td>NE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NE</td>
<td>0/2/2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>In-pen contact</td>
<td>12</td>
<td>0/1/1</td>
<td>2/3/3</td>
<td>0/1/1</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>0/0/7</td>
</tr>
<tr>
<td>3</td>
<td>Hedge contact</td>
<td>12</td>
<td>NE</td>
<td>2/2/3</td>
<td>0/1/2</td>
<td>1/1/1</td>
<td>0/0/1</td>
<td>0/0/1</td>
<td>0/0/4</td>
</tr>
<tr>
<td>4</td>
<td>Non-contact</td>
<td>12</td>
<td>NE</td>
<td>0/1/1</td>
<td>0/1/1</td>
<td>0/0/2</td>
<td>0/1/3</td>
<td>0/0/2</td>
<td>0/0/3</td>
</tr>
<tr>
<td>5</td>
<td>Human exposure</td>
<td>12</td>
<td>NE</td>
<td>0/0/1</td>
<td>NE</td>
<td>NE</td>
<td>0/0/1</td>
<td>NE</td>
<td>0/0/10</td>
</tr>
<tr>
<td>6</td>
<td>Negative controls</td>
<td>12</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>0/0/1</td>
<td>NE</td>
<td>NE</td>
<td>0/0/10</td>
</tr>
</tbody>
</table>

<sup>a</sup> No. of birds positive macroscopically/no. of birds positive histologically/no. of birds examined; NE=not examined; blank=10 of inoculated chickens were precluded from this experiment after 23 days postinoculation.
uninoculated chickens through direct contact with inoculated chickens. The uninoculated chickens in Group 2 were infected orally from the contaminated feces of the inoculated chickens (Group 1) within 7 DAE, and all Group 2 chickens tested positive for neutralizing antibodies on 13 DAE. The spread of the virus to the uninoculated chickens in Group 3 in the connected pen was a little slower than that to the uninoculated chickens in Group 2. Only 1 cloacal swab from a chicken in Group 3 was PCR positive on 5 DAE, swabs from most of the chickens in this group were PCR positive on 13 DAE, and all chickens in the group tested positive for neutralizing antibodies on 20 DAE. Thus, it seems that some chickens in Group 3 were infected orally within 7 DAE from the contaminated feces of the inoculated chickens, while others were infected later from the contaminated feces of the uninoculated chickens that were infected earlier.

Non-contact transmission of the virus was demonstrated in the present study. It seems likely that the virus was carried on dust particles in the air. The virus spread more slowly to Group 4, and the gizzard lesions were milder. It is possible that the severity of gizzard lesions and virus excretion increase later when chickens are infected with low-titer FAV-99ZH [12]. Only 1 cloacal swab from a chicken in Group 4 was PCR positive on 20 DAE, swabs from the majority of chickens in this group were PCR positive on 26 DAE, and all chickens tested positive for neutralizing antibodies on 41 DAE. It appears that most chickens in Group 4, like most in Group 3, were infected by contaminated feces from the uninoculated chickens in the same pen that had been infected earlier. The patterns of spread of the virus in the present study are similar to the results reported by Cook [5], who found that chick embryo lethal orphan (CELO) virus as the representative strain of FAV serotype 1 spread rapidly to uninoculated fowl in direct contact with inoculated chickens and spread slowly when chickens were kept in separate pens in the same room. On the other hand, Monreal [8] showed that CELO virus spread to uninoculated groups kept in the same room only when there was direct contact with the inoculated chickens. He was unable to demonstrate non-contact transmission of the virus, but suggests that it might occur if large numbers of chickens were used, thus increasing the concentration of the excreted virus.

Transmission through human contact was not observed in the present study; thus, washing boots and changing gloves may be effective in preventing the transmission of FAV. A previous study confirmed that biosecurity procedures such as handwashing, showering and donning new outerwear and gloves are effective in preventing the transmission of foot-and-mouth disease virus to pigs and sheep by personnel in contact with infected pigs [4].

Gizzard lesions caused by FAV were induced in uninoculated chickens through direct contact with inoculated chickens. The uninoculated chickens in Group 2 were infected orally from the contaminated feces of the inoculated chickens (Group 1) within 7 DAE, and all Group 2 chickens tested positive for neutralizing antibodies on 13 DAE. The spread of the virus to the uninoculated chickens in Group 3 in the connected pen was a little slower than that to the uninoculated chickens in Group 2. Only 1 cloacal swab from a chicken in Group 3 was PCR positive on 5 DAE, swabs from most of the chickens in this group were PCR positive on 13 DAE, and all chickens in the group tested positive for neutralizing antibodies on 20 DAE. Thus, it seems that some chickens in Group 3 were infected orally within 7 DAE from the contaminated feces of the inoculated chickens in the connected pens, while others were infected later from the contaminated feces of the uninoculated chickens that were infected earlier.

Non-contact transmission of the virus was demonstrated in the present study. It seems likely that the virus was carried on dust particles in the air. The virus spread more slowly to Group 4, and the gizzard lesions were milder. It is possible that the severity of gizzard lesions and virus excretion increase later when chickens are infected with low-titer FAV-99ZH [12]. Only 1 cloacal swab from a chicken in Group 4 was PCR positive on 20 DAE, swabs from the majority of chickens in this group were PCR positive on 26 DAE, and all chickens tested positive for neutralizing antibodies on 41 DAE. It appears that most chickens in Group 4, like most in Group 3, were infected by contaminated feces from the uninoculated chickens in the same pen that had been infected earlier. The patterns of spread of the virus in the present study are similar to the results reported by Cook [5], who found that chick embryo lethal orphan (CELO) virus as the representative strain of FAV serotype 1 spread rapidly to uninoculated fowl in direct contact with inoculated chickens and spread slowly when chickens were kept in separate pens in the same room. On the other hand, Monreal [8] showed that CELO virus spread to uninoculated groups kept in the same room only when there was direct contact with the inoculated chickens. He was unable to demonstrate non-contact transmission of the virus, but suggests that it might occur if large numbers of chickens were used, thus increasing the concentration of the excreted virus.

Transmission through human contact was not observed in the present study; thus, washing boots and changing gloves may be effective in preventing the transmission of FAV. A previous study confirmed that biosecurity procedures such as handwashing, showering and donning new outerwear and gloves are effective in preventing the transmission of foot-and-mouth disease virus to pigs and sheep by personnel in contact with infected pigs [4].

Gizzard lesions caused by FAV were induced in uninoculated chickens through direct contact with inoculated chickens or through airborne exposure to the virus. The gross and microscopic lesions in the gizzard were similar to those reported previously in chickens naturally or experimentally infected with AGE [2, 10, 12, 17]. The presence of characteristic intranuclear inclusion bodies in the epithelial cells of the gizzard confirmed that FAV was the cause of the gizzard lesions. Hydropericardium syn-
drome caused by FAV serotype 4 is known to occur in uninoculated exposed chickens through direct contact with inoculated chickens [1, 3]. Other researchers have reported severe growth depression in uninoculated chickens exposed by direct contact to inclusion body hepatitis-induced FAV serotype 8 [19]. To the best of our knowledge, the present study is the first in which AGE is dependent on the flock.

However, the results of the present study may offer insights into the question of why AGE is a sudden outbreak and ends with a short period of time at a broiler house or farm, and why the prevalence of AGE is dependent on the flock. When high-titer FAV-induced AGE exists in a broiler house, the virus spreads rapidly by direct contact and an outbreak may arise quickly. However, it is not easy for the virus to spread from flock to flock because its ability to be transmitted without contact is low. The virus may be introduced first by breeders by vertical transmission through embryonated eggs or young chicks, then it may lurk in litter in broiler houses, where it may infect a few chickens in a low-titer form. A high titer of the virus is then excreted in feces, and a large number of chickens in the broiler house are then infected at the same time by direct contact.

It would be exceedingly difficult to eliminate FAV from commercial flocks and to keep them free from the infection. However, the prevention of horizontal transmission by using partitions and enacting biosecurity procedures is very effective in containing an outbreak of AGE.

ACKNOWLEDGEMENT. The authors would like to thank Mr. Yoshihisa Nagata (Zen-noh Business Support Co., Ltd.) for his technical assistance.

REFERENCES