Gastrointestinal Pathogenicity of Adenoviruses and Reoviruses Isolated from Broiler Chickens in Alabama

Stephen D. Lenz, Frederic J. Hoerr, Alfred C. Ellis, Maria A. Toivio-Kinnucan and Maria Yu

J VET Diagn Invest 1998 10: 145
DOI: 10.1177/104063879801000205

The online version of this article can be found at:
http://vdi.sagepub.com/content/10/2/145

Published by:
SAGE
http://www.sagepublications.com

On behalf of:

Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc.

Additional services and information for Journal of Veterinary Diagnostic Investigation can be found at:

Email Alerts: http://vdi.sagepub.com/cgi/alerts
Subscriptions: http://vdi.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav
Citations: http://vdi.sagepub.com/content/10/2/145.refs.html

>> Version of Record - Apr 1, 1998
What is This?
Gastrointestinal pathogenicity of adenoviruses and reoviruses isolated from broiler chickens in Alabama

Stephen D. Lenz, Frederic J. Hoerr, Alfred C. Ellis, Maria A. Toivio-Kinnucan, Maria Yu

Abstract. Adenoviruses and reoviruses isolated from commercial broiler chickens were evaluated for gastrointestinal pathogenicity in specific-pathogen-free Leghorn chickens. The viruses were originally isolated from either the proventriculus or a gastrointestinal pool of tissues of broiler chickens with proventriculitis or enteritis. Isolates were cloned by terminal dilution. Day-old chickens were inoculated by oral and ocular routes with undiluted tissue culture fluids (titers of $10^3$–$10^4 \text{ TCID}_50$/ml) and then examined at necropsy on days 5, 10, and 15 postinoculation. Chickens in all virus groups (but not the control group) developed wet, unformed fecal droppings that persisted for the duration of the study. Mild lesions occurred in reovirus-inoculated chickens and included hyperplasia of lymphocyte aggregates in various organs and mild gizzard erosions. Chickens inoculated with adenovirus isolates developed marked gizzard erosions and necrotizing pancreatitis as well as mild proventriculitis. Intranuclear viral inclusion bodies occurred in gizzard epithelium and pancreatic acinar cells at the sites of lesions. Lymphocytic atrophy occurred in the bursa of Fabricius. Respective viruses were reisolated from proventriculus and duodenum collected from chickens of each group; no viruses were isolated from controls. Under the conditions of this study, adenovirus isolates were more pathogenic than the reovirus isolates in the digestive system.

Digestive system signs and lesions often are observed in broiler chickens submitted for laboratory evaluation because of impaired weight gain and feed conversion, feed passage, and watery feces. Various lesions have been identified in the proventriculus, gizzard, intestines, pancreas, and liver in the course of these investigations. Adenoviruses and reoviruses are commonly isolated from the affected viscera, particularly from the proventriculus and duodenum in birds with lesions in these organs.

Digestive tract disease in broiler chickens in the southeastern United States has several causes and clinical and pathologic expressions. Reoviruses have a causative link with digestive syndromes, but mainly these syndromes are caused by certain reovirus strains of high pathogenicity. The role of adenoviruses in digestive disease of chickens is equivocal, which has made it difficult to interpret virus isolation results from chickens with digestive system signs and lesions.

The purpose of this study was to extend routine diagnostic investigation of digestive tract disease by attempting to assess the pathogenic potential of viral isolates in susceptible chickens.

Materials and methods

Selection and preparation of field isolates. Reoviruses and adenoviruses were isolated from the proventriculus alone or from a tissue pool that included proventriculus or intestine (Table 1) of broiler chickens with gastrointestinal disease, which included some or all of the following histologic lesions. In the proventriculus, glands had necrosis of ductular and acinar epithelium accompanied by epithelial metaplasia and hyperplasia and lymphocytic inflammation. The mucosal lining had lymphocytic inflammation and hyperplastic mucosal epithelium. The gizzard lesions were fibrillation and uneven detachment of collagen with inflammation of the underlying mucosa. Small intestinal lesions included villus atrophy, crypt epithelial cell hyperplasia, and lymphoplasmacytic inflammation of the lamina propria. Hepatic lesions included Kupffer cell hypertrophy, mild bile duct proliferation, periductular fibrosis and inflammation, and multifocal periportal and midzonal inflammation. Pancreas had hyperplasia of lymphocytic aggregates. Individual virus isolates were propagated on primary chick embryo kidney cell cultures derived from specific-pathogen-free (SPF) eggs and cloned by terminal dilution performed for 3 serial passages.

Chickens. Leghorn chickens hatched from SPF eggs were used to ensure an absence of maternal reovirus and adenovirus antibodies (adenovirus-free broiler chickens were not available). Eggs were incubated and hatched at the Poultry Science Biocontainment Facility, Auburn University. Upon hatching, the chickens were randomly assigned to treatment groups and kept in negative-pressure plexiglass isolators with filtered air that were maintained at 27 C. Food and water were provided ad libitum.

Mycotoxin assays. Food for all of the experimental groups was supplied from a single batch. On day 7 of the experiment, a composite sample of food from all of the units was collected. The composite sample was assayed for aflatoxins, zearalenone, and deoxynivalenol.

Tissue collection. Tissues were processed routinely for...
Table 1. Titters of undiluted tissue culture fluids of reovirus and adenovirus isolates inoculated into 1-day-old SPF Leghorn chickens.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Field isolate no.</th>
<th>Diagnosis</th>
<th>Tissue of origin</th>
<th>Virus</th>
<th>Titer (TCID&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92D-2787</td>
<td>proventriculitis, enteritis</td>
<td>liver/proventriculus/ pancreas</td>
<td>reovirus</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>92D-358</td>
<td>proventriculitis</td>
<td>proventriculus</td>
<td>reovirus</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>91D-10963</td>
<td>proventriculitis</td>
<td>proventriculus/liver/ lung</td>
<td>reovirus</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>91D-9824B</td>
<td>enteritis</td>
<td>duodenum/pancreas</td>
<td>reovirus</td>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>92D-356</td>
<td>proventriculitis</td>
<td>proventriculus</td>
<td>adenovirus</td>
<td>10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>92D-1048</td>
<td>. . .</td>
<td>tissue pool</td>
<td>adenovirus</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>91D-9823A</td>
<td>enteritis</td>
<td>proventriculus</td>
<td>adenovirus</td>
<td>10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 2. Distribution and severity* of microscopic lesions in SPF Leghorn chickens following inoculation with reovirus or adenovirus isolates.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Field isolate no.</th>
<th>Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Proventriculus</td>
</tr>
</tbody>
</table>

Reoviruses
1  91D-2787  –  +  –  –  –
2  92D-358  +  +  +  –  –
3  91D-10963  +  –  –  –  –
4  91D-9824B  –  +  –  –  –

Adenoviruses
5  92D-356  –  +++  +++  –  +
6  92D-1048  +  ++  +  –  +
7  91D-9823A  +  +++  –  –  –

Control (8) . . .  –  –  –  –  –

* – = no lesion; + = mild lesion; ++ = moderate lesion; +++ = marked lesion.

Histopathology. Samples of proventriculus and duodenum were collected aseptically, pooled by treatment group, and stored in sterile plastic bags at 0°C until processed for virus isolation. Blood was collected by cardiac puncture, and the serum was harvested and frozen until assayed. Sections of proventriculus, gizzard, duodenum, jejunum, cecum, colon, pancreas, kidney, liver, bursa, lung, heart, and spleen were collected for histopathology.

Serology. Seroconversion to adenovirus was evaluated by agar gel immunodiffusion using CELO virus antigen. Serology for reovirus was measured by enzyme-linked immunosorbent assay (ELISA) and by 2 agar gel immunodiffusion tests, which used the C08 or 1133 strains of reovirus as antigen. In addition, serology was performed for Newcastle disease virus, infectious bronchitis virus, and infectious bursal disease virus by ELISA.

Virus isolation. For virus isolation, tissue homogenates were inoculated onto chicken kidney cells and observed for 7 days for cytopathic effect (CPE). If CPE was observed, adenoviruses were directly identified by an indirect immunofluorescence test using a chicken anti-adenovirus primary antibody and a fluorescein isothiocyanate (FITC)-labeled rabbit anti-chicken secondary antibody. Reoviruses were similarly identified using chicken anti-reovirus primary antibody and a FITC-labeled rabbit anti-chicken secondary antibody. If CPE was not observed after 7 days, 2 blind passages were performed and cells were examined for CPE.

Experimental design. On the day of hatching, chickens were randomly assigned to 7 treatment groups (10 chickens/group) and 1 control group (15 chickens). Chickens in each treatment group were placed into individual isolation units. Individual chickens of a treatment group were weighed and inoculated by both oral and ocular routes. Each chick was inoculated with a standard volume (0.1 ml) of undiluted tissue culture fluid (0.08 ml orally and 0.02 ml into the conjunctival sac) to maximize the possibility of developing lesions. Chickens were inoculated while in the negative pressure isolator to prevent cross-contamination between groups. All chickens were observed twice daily for clinical signs. On days 5 and 10 postinoculation (PI), 3 chickens from each treatment group and the control group were randomly selected, blood samples were obtained, and the chickens were euthanized by cervical dislocation and examined at necropsy. The remaining chickens in all groups were examined on day 15 PI.

Results

Clinical signs
Brown, watery or mucoid droppings appeared in reovirus-inoculated chickens on day 1 PI (groups 1–
3). From day 2 PI on, brown mucoid droppings were observed from all reovirus or adenovirus groups but not from the sham-inoculated controls.

Serology

Seroconversion to adenovirus in inoculated chickens occurred at days 10 and 15 PI. Reovirus-inoculated chickens had not seroconverted to reovirus by day 15 PI. Control chickens were serologically negative for reovirus and adenovirus antibody at all 3 test periods. Chickens of all groups were serologically negative for Newcastle disease virus, infectious bronchitis virus, and infectious bursal disease virus by ELISA.

Virus isolation

Reovirus was reisolated from the proventriculus and the intestines of reovirus-inoculated chickens on days 5, 10, and 15 PI. Adenovirus was reisolated from the proventriculus and intestines of adenovirus-inoculated chickens on days 5, 10, and 15 PI.

Mycotoxin assays

Deoxynivalenol (0.98 ppm) was detected in the feed but aflatoxins or zearalenone were not (limits of detection; 20 ppb aflatoxins and 200 ppb zearalenone).

Pathology

Reoviruses. Dark green-brown, foamy cecal contents (cecal gas) were evident on day 5 PI (groups 3, 4). Raised 1–2-mm-diameter pale yellow foci were randomly scattered in the superficial mucosa of the proventriculus at day 15 PI (groups 2, 3). No gross lesions were observed in any reovirus-inoculated chickens on day 10 PI.

Mild histologic lesions occurred in the gizzard, proventriculus, and pancreas (Table 2). The gizzard had shallow microerosions with koilin fibrillation and focal hyperplasia of glandular epithelium. Hyperplasia of lymphocyte aggregates was the principal lesion in the submucosa of the proventriculus and the interstitium of the pancreas. The size and cellularity of these lymphocyte aggregates in reovirus-inoculated chickens were increased when compared with controls. Other tissues including liver had no significant lesions.

Adenoviruses. Foamy cecal contents were observed at day 5 PI in chickens of all three adenovirus-inoculated groups. On day 10 PI, some chickens had diffusely pale green livers with scattered small irregular yellow foci (groups 5, 7). On day 15 PI, scattered raised 1–2-mm yellow foci occurred in the mucosa of the proventriculus (groups 5, 6). The gizzard lining was rough and deeply fissured in chickens of all 3 adenovirus treatment groups on days 5, 10, and 15 PI.
Marked to severe histologic lesions occurred in the gizzard and pancreas, but only mild lesions occurred in the proventriculus (Table 2). Gizzard erosions were observed by day 5 PI as necrosis and erosion of the mucosa with fibrillation and detachment of the overlying koilin. The lamina propria and submucosa were expanded and infiltrated by increased numbers of lymphocytes, histiocytes, and heterophils (Fig. 1). Large (20–30 μm) hyperchromatic basophilic intranuclear inclusions occurred in epithelial cells (Fig. 2). Ultrastructurally, these inclusions consisted of large crystalline arrays of closely packed 70–90-nm icosahedral virus particles (Fig. 3) morphologically characteristic of adenoviruses. Virus particles were observed in degenerate and intact epithelial cells of the gizzard mucosa, but discrete crystalline arrays of virus particles were only seen in intact cells. By day 10 PI, the gizzard erosions were broader and deeper with a more intense inflammatory reaction, but intranuclear inclusions were not found. Hyperplasia of glandular epithelium indicated regeneration. At day 15 PI, erosions were observed in only 2 chickens, but gland hypertrophy (increased height) and epithelial hyperplasia were more pronounced and indicative of regeneration (Fig. 4).

On day 5 PI, the pancreas had small foci of acinar cell degeneration and necrosis in association with intranuclear inclusions, ultrastructurally the same as those observed in gizzard epithelium. Intranuclear inclusions were not observed in pancreata without acinar degeneration and necrosis. On days 10 and 15 PI, acini were focally effaced and interstitial tissues were expanded by dense infiltrates of histiocytes, lymphocytes, and plasma cells (Fig. 5). Intranuclear inclusions were not observed on days 10 and 15 PI.

On days 10 and 15 PI, the proventriculus had multifocal mucosal epithelium hypertrophy and hyperplasia, lymphoplasmacytic infiltrates in the lamina propria and submucosa, and hyperplasia of submucosal lymphocyte aggregates.

Bursal atrophy was found in chickens of 2 groups on days 10 and 15 PI. The follicular cortex was thin, only 1 or 2 cell layers thick as compared with the
Discussion

Adenoviruses and reoviruses typified by the isolates in this study are readily isolated from broiler chickens with signs and lesions of digestive system disease. Under the conditions described herein, each of the adenoviruses elicited considerably more severe digestive system lesions than did any of the reoviruses. The difference was especially pronounced in the gizzard.

Two factors in the experimental design have influence on the interpretation of results. SPF Leghorns were used as an experimental host in lieu of SPF broilers because the source SPF broiler breeder flock had antibody to type I avian adenovirus. These antibodies could have interfered through either the transmission of maternal adenovirus antibody or the vertical transmission of virus by progeny, thus compromising the evaluation of gastrointestinal pathogenicity. We used the greatest titer of each respective virus in an attempt to evaluate the full pathogenic potential; inoculum titer was not equal between groups. The results must be interpreted cautiously; however, several findings in this study may apply to observations of the spontaneous disease.

Chickens in all virus groups, but not in the control group, developed abnormal fecal consistency that persisted for the duration of the study. Insufficient numbers of chickens were used to measure differences in weight gain. Despite differences in lesion distribution and severity, these results are indicative of the potential of each of these viruses to cause clinical signs of digestive dysfunction.

Gizzard erosions with intralesional adenovirus inclusions were the most consistent and prominent le-
sions in adenovirus-inoculated chickens. Adenoviruses were thought to have caused the gizzard erosions because basophilic intranuclear inclusions, ultrastructurally consistent with adenovirus, were only observed in the areas of gizzard erosion and not in unaffected portions of gizzard. Because no erosions occurred in control chickens fed the same feed, feed-related causes of gizzard erosions were excluded.

Adenoviruses have been associated with spontaneous erosive ventriculitis (gizzard erosions) in Leghorn and meat-type chickens and in quail. Gizzard erosions and ulcers and necrotizing pancreatitis occurred in SPF chickens inoculated with a type 8 avian adenovirus. The present findings indicate that the morphologic proof of adenovirus infection in the gizzard, inclusion bodies, occurs transiently. Spontaneous gizzard erosions are common in broiler chickens, but the cause is difficult to pinpoint because of the many potential causes, including histamine, fish meal, gizzerosine, mycotoxins (T-2, cyclopiazonic acid, tenuazonic acid), vitamin deficiencies, and bacterial complications. A low concentration of deoxynivalenol (DON, vomitoxin) was identified in poultry feed used in this study but was considered insignificant because no lesions developed in the control chickens fed the same feed. Increased relative gizzard weight was reported in Leghorn and broiler chickens administered 18 mg DON/kg of feed (18 ppm), but lesions were not described.

Proventriculitis with extensive glandular inflammation, as seen in field cases from Alabama, was not observed in this study. Mucosal changes, including epithelial and lymphocytic hyperplasia as in field cases, did occur in adenovirus-inoculated chickens. These lesions occurred later than those in the gizzard and lacked viral inclusions, which suggests that proventriculitis in adenovirus-inoculated chickens may develop secondary to gizzard erosion.

Necrotizing pancreatitis was observed in adenovirus-inoculated chickens, and the intrasheal inclusion bodies indicated a causal relationship. This lesion was identified only in the occasional case of fully developed inclusion body hepatitis, as has been reported in association with hypoglycemia and high mortality. Hyperplasia of lymphocytic aggregates in the pancreas is common in spontaneous disease and occurred in this study in all virus-treated groups.

Intestinal and hepatic lesions observed in field cases were not observed in experimental chickens, nor was inclusion body hepatitis encountered. Bursal atrophy was an inconsistent lesion in chickens inoculated with adenoviruses and was interpreted as a nonspecific lesion not directly attributable to adenovirus infection. Bursal atrophy with lymphocyte depletion has been reported in chickens subjected to various environmental stresses and infectious diseases and in chickens experimentally inoculated with adenoviruses and reoviruses.

It is common to isolate a reovirus from the digestive system in the absence of reovirus antibody detectable by ELISA. Because this result may reflect differences in the test antigen used in the ELISA and the intestinal isolate, intestinal and infectious tenosynovitis strains of reovirus were also used for immunodiffusion antigens. No antibody was detected as measured at 15 days PI, suggesting that the reovirus isolates were noninvasive and did not elicit a humoral immune response. These observations are consistent with each of these reovirus isolates possessing low pathogenicity. The hyperplasia of lymphocytic aggregates seen histologically in the digestive system may be a nonspecific response to the reovirus infection or may have no bearing on the humoral response. In contrast, each of the adenovirus isolates elicited a measurable humoral immune response.

Acknowledgements

This research was supported by Food Animal Health and Disease Research funds, Auburn University. We thank Dr. Joe Giambrone, Biocountainment Facility, Poultry Science Department, Auburn University, and Misako Hwang and Beth Landreth for their logistical and technical support.

Sources and manufacturers

a. Hyvac Laboratory Eggs Co., Gowrie, IA.
b. IDEXX, Westbrook, ME.

References