Enteric Adenovirus Infection in Pigs

S.E. Sanford and D.M. Hoover*

ABSTRACT

Intranuclear adenovirus inclusion bodies were identified in intestinal epithelium by histological, electron microscopical or cytological examination of 43 conventionally raised pigs submitted for diagnostic evaluation from 38 farms between August 1981 and January 1983. The affected pigs were usually less than three weeks old, but ranged from five days to 24 weeks of age. The inclusion bodies occurred in the epithelium of ordinary intestinal villi, or more commonly, in the epithelium covering the blunt villi located over Peyer's patches in the ileum. Fifty-three percent of these adenovirus infected pigs had diarrhea. In most of these pigs with diarrhea (19 of 23) however, agents previously established as causes of porcine enteric disease were also identified. Of the pigs with enterocyte inclusion bodies but without diarrhea, the adenovirus infection appeared incidental to the major disease processes in the pigs.

These findings suggest that this adenovirus is an asymptomatic and probably incidental infection in conventional farm-raised pigs.

Key words: Adenovirus, intranuclear inclusion, diarrhea, pig.

RÉSUMÉ

En se servant de l'histopathologie, de la microscopie électronique et de la cytologie, les auteurs identifièrent des inclusions intranucléaires imputables à un adénovirus, dans l'épithélium intestinal de 43 porcs élevés de façon conventionnelle, dans 38 porcheries, et soumis au laboratoire de diagnostic, entre le mois d'août 1981 et celui de janvier 1983. Les porcs affectés étaient ordinairement âgés de trois semaines, quoique leur âge variait de cinq jours à 24 semaines. Les inclusions se retrouvaient dans l'épithélium des villosités intestinales ordinaires, mais surtout dans celui des villosités émoussées qui surplombent les plaques de Peyer de l'iléon. Cinquante-trois pourcent des porcs infectés par l'adénovirus présentaient de la diarrhée; chez 19 de ces 23 porcs, on identifia aussi des agents préalablement reconnus comme causes de maladies entériques porcines. Chez les porcs qui arboraient des inclusions dans leurs entérocytes, sans manifester de diarrhée, l'infection à adénovirus ne sembla qu'accompagner des maladies plus graves.

Il semble par conséquent que la présence de cet adénovirus dans l'intestin des porcs élevés de façon conventionnelle représente une infection asymptomatique et apparemment bénigne.

Mots clés: adénovirus, inclusion intranucléaire, diarrhée, porc.

INTRODUCTION

A porcine adenovirus was first isolated in 1964 from a pig with diarrhea (1). Adenoviruses have since been recovered from various organs of swine and from swine feces (2). These adenoviruses have been associated with encephalitis (2, 3), pneumonia (3, 4) and enteritis (2) in pigs, although their pathogenicity remained uncertain. Experimental adenovirus infection in pigs caused lesions in the brain, lung, kidney and intestines (2, 3, 4). In 1968, Fugiwara *et al* described adenovirus-like, intranuclear inclusion bodies in epithelial cells of the distal small intestine of two conventionally raised pigs with diarrhea (5). Coussement *et al* (6) recently reported similar inclusions in a three week old diarrheic pig from a Belgian breeding farm. They also experimentally infected pigs with a porcine adenovirus (strain 6618, serotype 3) resulting in diarrhea and intranuclear inclusion bodies in villus epithelium.

This report describes spontaneous enteric adenovirus infection in 43 pigs submitted to our laboratory for routine diagnostic investigation between August 1981 and January 1983.

MATERIALS AND METHODS

Animals considered in this study included all pigs submitted alive to the Huron Park Veterinary Services Laboratory for routine diagnostic investigation between August 1981 and January 1983 — a total of 979 animals. Necropsy examinations were performed on all pigs. Routine histology, cytology, bacteriology and virology evaluation were done as required to

*Veterinary Laboratory Services Branch, Ontario Ministry of Agriculture and Food, Huron Park, Ontario N0M 1Y0 (Sanford) and Veterinary Laboratory Services Branch, Ontario Ministry of Agriculture and Food, Guelph, Ontario N1H 6R8 (Hoover).

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establish the cause of disease. In addition to routine evaluation, sections of jejunum, ileum and colon from each pig were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm, stained with hematoxylin and eosin (H & E) and examined by light microscopy. Mucosal scrapings from the jejunum and ileum of all scouring neonatal pigs were smeared on glass slides, air-dried and stained with a modified Wright's stain (Camco Quik Stain, American Scientific Products, McGaw Park, Illinois), primarily for coccidial identification. Contents of ileum and colon from 35 scouring pigs (eight of which had intranuclear inclusion bodies in enterocytes) were negatively stained with 3% phosphotungstic acid and examined by direct electron microscopy for viral particles (7).

Formalin-fixed sections of ileum from eight of the pigs with intranuclear inclusion bodies in enterocytes were prepared for electron microscopy by postfixing in 1% osmium tetroxide, then dehydrating with acetone and embedding in Epon. Semi-thin (1 μm) sections were stained with methylene blue and examined by light microscopy. Ultra-thin sections of selected areas were stained with lead citrate and uranyl acetate and examined in a transmission electron microscope.

RESULTS

HISTOPATHOLOGY

Of 979 pigs examined, 43 pigs (4.4%) from 38 farms had various numbers of intranuclear inclusion bodies in enterocytes of the distal small intestine. More than 50, ten to 50 and less than ten inclusion bodies were in single cross sections of the ileum of each of 16, 16 and 11 pigs respectively. Affected pigs ranged in age from five days to 24 weeks but nearly one half of the affected pigs were three weeks old or less and were still suckling (Fig. 1).

The affected enterocytes were usually present randomly along the sides and tips of ordinary intestinal villi of normal length or were

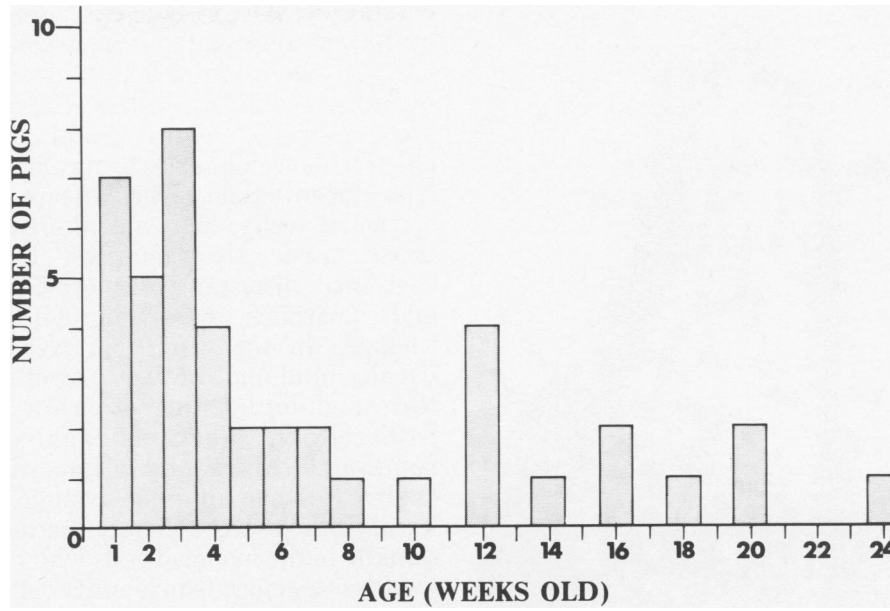

Fig. 1. Age distribution of the 43 pigs with enteric adenovirus infection.

along the apical border of the short blunt villi above Peyer's patches (Figs. 2 and 3). Affected enterocytes were often superficially positioned in the epithelium and appeared to be desquamating. Nuclei which contained inclusion bodies were large and round or oblong. The inclusion bodies were large, eosinophilic to amphophilic and usually separated by a clear halo from marginated chromatin lining the nuclear membrane, although in some cells the inclusion filled the entire nucleus (Fig. 3). The lamina propria above these Peyer's patches was infiltrated by varying numbers of lymphocytes, plasma cells and macrophages similar to that seen in most suckling and weaned pigs routinely examined by us.

CYTOLOGY

Mucosal smears from the ileum of two pigs had single, large, round intranuclear inclusion bodies in epithelial cells (Fig. 4). The morphology of these epithelial cells, corresponded to that seen in histological sections.

ULTRASTRUCTURAL FINDINGS

Affected enterocytes had greatly enlarged nuclei which contained granular electron dense material

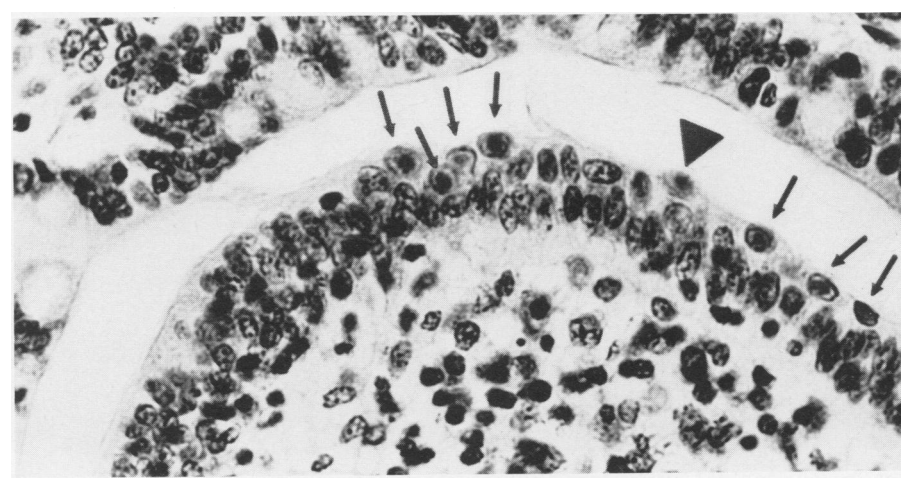

Fig. 2. Terminal ileum of a pig with natural enteric adenovirus infection. Numerous enterocytes have intranuclear inclusion bodies (arrows). One cell (arrowhead) appears to be sloughing into the intestinal lumen. H & E. X630.

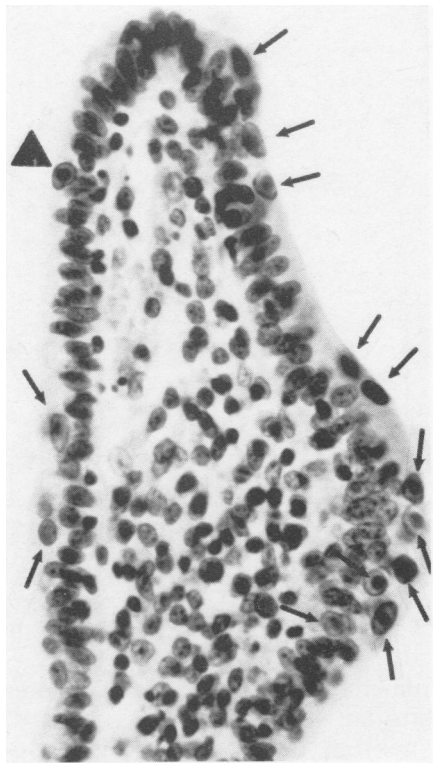

Fig. 3. Adenovirus infected enterocytes (arrows) are along the sides of this villus in the ileum. The lamina propria has a prominent mononuclear leukocyte infiltrate. H & E. X630.

intermixed with varying numbers of viral particles (Fig. 5). Virions were about 75 nm in diameter with cubic symmetry and an electron dense or an electron lucent core (Fig. 6). Dense concentrations of viral particles tended to form small crystalline arrays. Nuclear chromatin was aggregated in a narrow rim on the inside of the nuclear membrane and was often separated from the central granular electron dense material by an electron lucent zone. The affected epithelial cells had an altered brush border characterized by shortened, disorganized microvilli and sometimes loss of microvilli. Changes in the cytoplasm were usually mild and limited to swelling of endoplasmic reticulum. Intercellular junctions usually remained intact and adjacent enterocytes had minimal change. Viral particles were also seen occasionally in the nuclei of cells which were too degenerate to identify cell type. These degenerate cells were between epithelial cells and were probably of epithelial origin or were migrating cells of lymphocytic or histiocytic cell type.

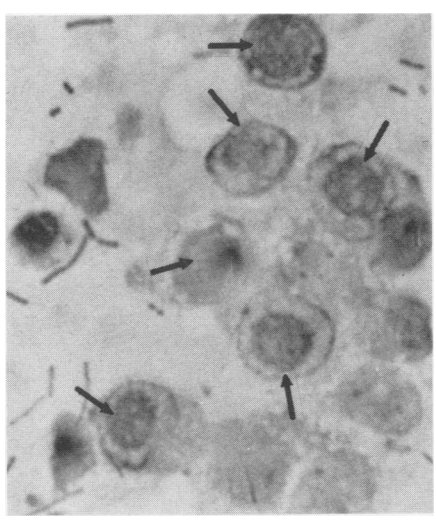

Fig. 4. Adenoviral inclusion bodies (arrows) in enterocytes in a cytology smear from the ileum. Modified Wright's stain. X500.

DISEASES DIAGNOSED

The diseases diagnosed and their incidence in the 43 pigs with adenovirus infection are given in Table I.

Clinical diarrhea or enteric abnormalities with diarrhea evident at necropsy occurred in 500 of the 979 pigs (51%) in this study and in 23 of the 43 pigs (53%) with enteric adenovirus infection. Agents other than adenovirus, recognized as causes of enteric disease, were identified in all but four of these 23 pigs. Viral particles were not detected by direct electron microscopy of the intestinal content of any of the 35 pigs sampled.

DISCUSSION

The virus particles observed in the intestinal epithelium of our

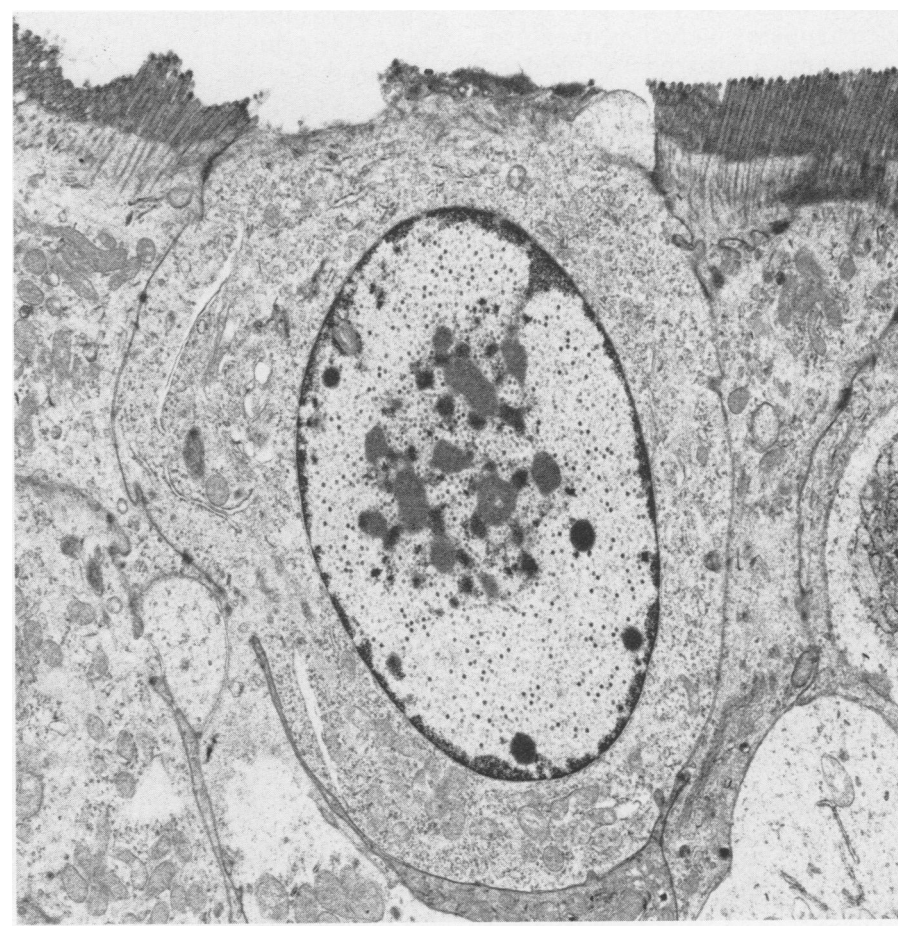

Fig. 5. Adenovirus infected enterocyte in the ileum. The nucleus is swollen, chromatin is marginated and adenovirus particles are present throughout the karyoplasm. Microvilli are distorted and reduced in number. Cell junctions are intact. X12,000.

pigs were considered to belong to the adenovirus group based on size, morphology and the intranuclear location (8). Inclusion bodies similar to those in our pigs have been described in intestinal epithelial cells of other pigs reported to have spontaneous adenovirus disease (5, 6) and in pigs experimentally infected with adenovirus (6, 9). Enterocytes were the only cell type identified which contained virus particles in the pigs in our study. Although we did not identify them in our pigs, lymphocytes as well as enterocytes were observed to contain adenovirus particles in the ileum of one pig used as an experimental control in a study of transmissible gastroenteritis (10).

Enteric adenovirus infections and disease have been seen in other animal species (11, 12, 13). In calves the disease is characterized by hemorrhagic and necrotizing gastroenteritis associated with viral damage to endothelial cells (11, 12). In contrast, enteric adenovirus infection in mice is characterized by intranuclear inclusion bodies in enterocytes with little associated pathology (13). The infection in mice is asymptomatic and appears to be relatively innocuous (13), similar to the adenoviral infection in our pigs. In both adenovirus infected mice and in the pigs we examined, the lesion was essentially limited to individual enterocytes which contained inclusion bodies. It was not possible in our study to make any definite association between viral infection and disease in affected animals. Almost half of the adenovirus infected pigs in our study did not have any clinical enteric disease or gross intestinal changes. Similarly, about 50% of the pigs submitted alive to our laboratory have diarrhea. Also, of the adenovirus infected pigs with enteric disease, 19 of 23 had other known enteropathogenic agents which by themselves may have caused the diarrhea. In only four pigs was no agent other than the adenovirus identified. In these four pigs, it is possible that some agent other than the adenovirus was present but was not identified. Adenovirus cannot be excluded as a possible cause of disease in these animals since, experimentally, adenovirus is capable of producing diarrhea in neonatal pigs (6).

Coussement *et al* described blunting of individual adenovirus infected villi in the terminal jejunum and ileum with associated infiltration by histiocytes, plasma cells and lymphocytes in the lamina propria of the pigs in their study (6). In pigs in our study, intestinal villi were usually not

TABLE I. Incidence of Diseases Diagnosed in the 43 Pigs with Adenovirus Infection

Enteric Diseases — 23 Cases	Number of Cases
Coccidiosis	7
Colibacillosis	6
Cryptosporidiosis	5
Swine Dysentery	1
Adenovirus Only	4
Nonenteric Diseases — 19 Cases	
Bacterial Pneumonia	8
Pasteurella multocida [4]	
Streptococcus suis type II [2]	
Haemophilus pleuropneumoniae [1]	
Mixed bacterial culture [1]	
Meningitis	7
Streptococcus suis type II [4]	
Escherichia coli [3]	
Polyarthritis	2
β-Hemolytic Streptococcus	
Septicemia	1
Salmonella cholerae-suis var. *Kunzendorf*	
Submandibular Lymph Node Abscessation	1
Corynebacterium pyogenes	
No Disease — 1 Case	

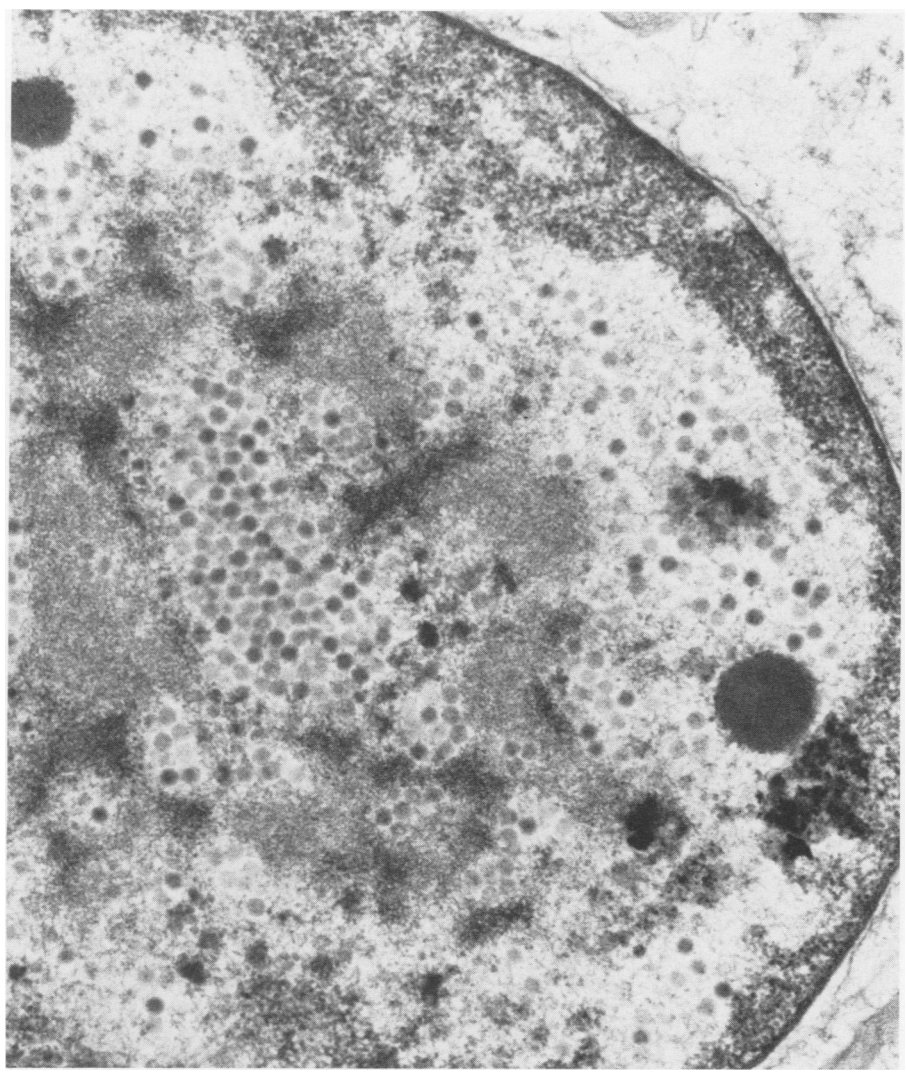

Fig. 6. Array of adenovirus particles in an enterocyte nucleus. X48,000.

noticeably atrophied, but the intranuclear inclusion bodies were often in enterocytes of so-called dome epithelium (14) which covers the short, blunted, or triangular villi located over hyperplastic lymphoid aggregates of Peyer's patches, especially in the lower ileum. In our experience conventional pigs without enteric disease often have lymphoplasmacytic and histiocytic aggregates in these villi and blunt villi over Peyer's patches are common in conventional pigs of all ages. This probably represents a normal morphological host response to numerous everyday environmental antigenic challenges (14, 15).

From our study, the limited histological changes and in most cases the lack of enteric disease suggest that enteric adenovirus infection in conventional pigs is an incidental and largely asymptomatic infection.

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