Natural Infection with Attaching and Effacing *Escherichia coli* and Adenovirus in the Intestine of a Pigeon with Diarrhea

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**ABSTRACT.** Attaching and effacing *Escherichia coli* (AEEC) and adenovirus infections in a 6-month old pigeon were found by retrospective histologic examinations. Histologically, numerous gram-negative bacilli covered the surface of the enterocytes of the ileum. Brush borders were not sharply defined on the apical enterocytes at the sites of bacterial attachment. Scanning electron microscope showed that much of the mucosal surface was heavily colonized by bacilli and that the microvilli were lost. Many intranuclear inclusions were seen in enterocytes from the duodenum to the cecum. On transmission electron microscopy, adenovirus-like particles were observed in the nuclei of enterocytes.—**KEY WORDS:** adenovirus, *Escherichia coli*, pigeon


Enteropathogenic *Escherichia coli* (EPEC) generally colonizes the intestine and adheres closely to the enterocytes resulting in a characteristic effacement and cupping of the microvilli [5]. The term attaching and effacing *E. coli* (AEEC) has been used to designate *E. coli* that causes such lesions [6]. AEEC infection has been described in human beings, calves, rabbits, pigs, lambs, goats, dogs and cats [2, 4, 6, 7], and we already reported AEEC infection of a cow and a piglet in Japan [9, 10]. But there has been no previous report about AEEC infection of pigeons. On the other hand, adenovirus infection in pigeons is known as inclusion body hepatitis (IBH) [1, 3]. AEEC and adenovirus infections in a pigeon were found by retrospective histologic examinations. In this paper, we report the pathological findings observed in a pigeon naturally infected with AEEC and adenovirus.

A colony of about 100 pigeons was being reared for pleasure in Hokkaido, Japan. Out of these, 30 pigeons came back from pigeon racing, 14 days later, and showed depression and green loose diarrhea in September 1987.

A 6-month-old pigeon was humanely killed for necropsy. Discoloration of the liver was noted. There was no hemorrhagic lesion in alimentary tract. Tissue samples were collected and fixed in 20% neutral buffered formalin. The samples were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (HE). Sections of the intestine were stained by Gram stain.

Detection of *E. coli* antigen on paraffin sections of the ileum was examined by avidin-biotin complex immunoperoxidase technique (ABCIT: Wako Pure Chemical Industries, Osaka, Japan) with 73 *E. coli* 0 antisera as the primary antibody. Of these antisera, 30 were produced from rabbits immunized with bacterial cultures of serotypes 02, 03, 05, 07, 09, 11, 016, 017, 021, 023, 033, 056, 045, 056, 064, 073, 074, 077, 080, 084, 088, 098, 0103, 0116, 0117, 0120, 0132, 0133, 0139 and 0141 heated at 100°C for 2 hr. Forty-three rabbit antisera (01, 06, 08, 015, 018, 020, 025, 026, 027, 028*st,* 029, 044, 055, 063, 078, 086, 0111, 0112*st,* 0114, 0115, 0119, 0124, 0125, 0126, 0127*st,* 0128, 0129, 0136, 0142, 0143, 0144, 0146, 0148, 0151, 0152, 0153, 0157, 0158, 0159, 0164, 0166, 0167, 0168 and 0169) were commercial products (Denka Seiken, Tokyo, Japan). The antisera were used at a dilution from 1:100 to 1:400.

For transmission electron microscopy, sections of paraffin-embedded intestinal tissue were removed from the paraffin block and deparaffinized and processed in graded alcohol. After a brief washing in PBS, the tissues were fixed in 1% osmium tetroxide, embedded in epoxy resin, sectioned, stained with uranyl acetate and lead citrate. For scanning electron microscopy, sample of osmium-fixed intestine as mentioned above were dehydrated through graded alcohol solution and dried by t-butyl alcohol freeze-drying method. Specimens were mounted on stubs, coated with gold.

Histologically, many intranuclear inclusions with occasional halo were seen in enterocytes from the duodenum to the cecum. Most intranuclear inclusions were basophilic, but a few of them were eosinophilic. Edema and infiltration of heterophils were seen in the lamina propria. Numerous gram-negative bacilli covered the surface of the enterocyte of the ileum. The superficial epithelial cells were disrupted and irregular in outline. Brush borders were not sharply defined on the apical enterocytes at the site of bacterial attachment (Fig. 1). The intestinal crypts were dilated, and ileal lumen was filled with necrotic enterocytes, mucus and numerous bacilli. Vascular degeneration of hepatocytes and infiltration of lymphocytes were seen in the liver, but no intranuclear inclusion was seen in the hepatocytes. No lesions were observed in other organs. Bacilli at the surface of the enterocytes were not typed with 73 antisera against 0 antigens by ABCIT.

Scanning electron microscope showed that much of the mucosal surface was heavily colonized by bacilli and the microvilli were lost (Fig. 2).

On transmission electron microscopy, many virus particles were present in the nuclei of enterocytes. The virus particles were round or hexagonal and most of them consisted of a highly electron-dense core approximately 55 nm in diameter and a moderately electron dense capsid structure surrounding the core. The entire particle measured 80 to 100 nm in diameter, and frequently arranged in paracrystalline arrays (Figs. 3 and 4). From the morphology, the virus particles observed in the intestine of the pigeon was considered to be adenovirus [1, 3].
Bacterial culture from the intestines yielded non-hemolytic *E. coli*. The isolated *E. coli* was negative for fimbrial antigen (K88, K99, 987P) tests. *Salmonella* was not isolated from the intestines.

The lesions characterized by bacilli attaching closely to epithelial cells with effacing microvilli agreed with the lesion of AECC [6]. AECC strain in this case gave no reactions for 73 antisera against 0 antigens by an immunoperoxidase technique, suggesting that the possibility of this strain belongs to other 0-groups of *E. coli*. Since little has been reported on serotype of *E. coli* isolated from pigeons with diarrhea, further studies are needed to clarify this point.

Although Sueyoshi and Nakazawa reported recently an experimental infection of chick with AECC [8], there was no report on natural infection of AECC in birds. So we considered that this is the first report of natural infection of AECC in the bird including pigeon. It is also interesting that the pigeon enterocytes responded to AECC in the same manner as mammalian enterocytes as reported by Moon et al. [6].

On the other hand, IBH due to adenovirus in pigeons was found in 1986 in Japan [3], and this case reported here occurred in 1987. It is speculated that adenovirus infection of pigeons might be prevalent those years in Japan. The distribution of inclusion body in IBH were observed in the liver, kidney, and intestine. In this case inclusion body was districeted only to the intestine. The diarrhea in the pigeon...
was considered to be provoked by mixed infection of AEEC and adenovirus in the intestine.

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REFERENCES