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Authors: PROCTOR, S. J., MATTHEWS, P. J., and KLUGE, J. P.

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DUCK PLAGUE IN GNOTOBIOTIC DUCKS

S. J. PROCTOR and P. J. MATTHEWS, Agricultural Research Service,
U.S. Department of Agriculture, National Animal Disease Center, Ames, Iowa 50010, USA

J. P. KLUGE, Department of Pathology, Iowa State University, Ames, Iowa 50010, USA

Abstract: Four-week-old gnotobiotic and conventional ducks were inoculated orally with duck plague virus. Both groups of ducks died on the third and fourth day after inoculation. Gross and microscopic lesions of duck plague were similar in gnotobiotic and conventional ducks, indicating the synergistic action of species of *Salmonella* and *Pasteurella* was not essential for development of lesions.

INTRODUCTION

Duck plague (duck virus enteritis) is an acute, contagious herpesvirus of Anatidae.[9,12,13,17] Secondary bacterial infections are common occurrences in viral infections, and *Salmonella* and *Pasteurella spp.* have been isolated from parenchymatous organs of half the ducks that died in some epornitics.[2] Lesions were reported to be more diffuse in those ducks from which duck plague virus (DPV) and bacteria were isolated, and species of *Pasteurella* and *Salmonella* were thought to act synergistically with DPV.[3]

Some organisms, such as *Entamoeba histolytica*[16] and *Histomonas meleagridis*,[1] do not cause diseases in germfree animals. Other organisms such as canine distemper virus,[6] feline infectious enteritis virus,[20] and *Eimeria tenella*[10] are less pathogenic in germfree animals than in conventional counterparts. In contrast, feline rhinotracheitis virus[8] and canine hepatitis virus[7] cause similar diseases in germfree and conventional animals.

The purpose of this study was to determine if DPV was pathogenic for gnotobiotic ducks and to describe the lesions of duck plague in gnotobiotic ducks.

MATERIALS AND METHODS

Ducks

White Pekin duck eggs were obtained from a *Salmonella pullorum* and DPV-free flock.[1] After 26 days of incubation, eggs containing viable embryos were entered into a flexible film isolator and hatched; seven ducks were maintained under germfree conditions.[14,21] A commercial chick-starter[2] sterilized with ethylene oxide gas and sterile water were available at all times in the isolator. Thirteen conventional ducks were raised in a separate room and fed the same sterilized starter and water.

Gnotobiotic ducks were monitored weekly and prior to viral inoculation for anaerobic and aerobic bacteria, but ducks were not monitored for other microorganisms such as viruses, *Mycoplasma* or *Chlamydia*. Cloacal swabs were taken from each duck and incubated in liquid thioglycollate broth and trypticase soy broth and isolates were identified.[19]

Virus

The DPV used to inoculate ducks was isolated from a mallard duck that died in the 1973 epornitic at Lake Andes, South Dakota. After 3 passages in primary

[1] Truslow Farms, Inc.

[2] Specially-formulated chick starter for NADC, supplied by United Supplier, Inc., Eldora, Ia.

duck embryo fibroblast, a suspension containing 10^4 duck embryo median lethal doses/ml was prepared.[4]

Duck inoculation

A sealed vaccine bottle containing 10 ml of the DPV suspension was entered into the isolator, using germfree techniques, when the ducks were 4 weeks old.[11] One gnotobiotic duck was removed from the isolator, killed, and necropsied immediately. Six ducks in the isolator were inoculated orally with 1 ml of DPV. Twelve conventional 4-week-old ducks were inoculated orally with 1 ml of the same virus from another bottle. One uninoculated conventional duck was killed and necropsied.

Necropsy

The 4-week-old gnotobiotic duck removed from the isolator prior to inoculation and a 4-week-old conventional duck were necropsied. Sections of spleen, bursa of Fabricius, thymus, liver, kidney, lung, trachea, adrenal, esophageal-proventricular junction, ventriculus, duodenum, jejunum, Meckel's diverticulum, ileum, cecum, large intestine, cloaca and the entire brain were fixed in 10% neutral buffered formalin. Necropsies also were conducted on conventional and gnotobiotic ducks which died, and sections of the same tissues were fixed in formalin. Formalin-fixed specimens were processed by routine paraffin-embedding techniques, and sections were stained with hematoxylin and eosin. The cloaca, liver, lung and kidney of each gnotobiotic duck were cultured for anaerobic and aerobic bacteria using liquid thioglycollate broth and trypticase soy broth.

RESULTS

Escherichia coli and *Streptococcus fecalis* were isolated from the cloacal swabs of gnotobiotic ducks prior to inoculation and at necropsy. No bacteria were isolated from the liver, lung and kidney of gnotobiotic or conventional ducks at necropsy.

The spleen in the uninoculated gnotobiotic control duck was one-third smaller than the spleen of the uninoculated conventional duck. Fewer lymphocytes were found around splenic arterioles, and germinal centers were reduced in number in the spleen of the gnotobiotic duck. The decreased number of splenic lymphocytes caused the periarteriolar reticular sheaths of the gnotobiotic duck to be histologically prominent when compared with spleen from the conventional duck.

Intestinal annular bands could not be identified grossly in the uninoculated conventional or gnotobiotic ducks. Lymphoid nodules were scattered throughout the intestinal submucosa of the conventional ducks, but only an occasional nodule was found in the submucosa of the gnotobiotic ducks. Since annular bands could not be identified in histologic examination, Meckel's diverticulum and the esophageal-proventricular junction were examined to evaluate intestinal lymphoid development. Submucosal lymphoid nodules were found in both locations in the conventional duck. Only irregular shaped accumulations of lymphocytes were found at the esophageal-proventricular junction and in Meckel's diverticulum of the gnotobiotic duck.

Three inoculated gnotobiotic ducks died 3 days post-infection (DPI), and 3 died 4 DPI. Four of the inoculated conventional ducks died 3 DPI, and 8 died 4 DPI. Gross lesions of duck plague were similar in gnotobiotic and conventional ducks. Submucosal petechiae at the esophageal-proventricular junction were covered by diphtheritic plaques. Annular bands in the small intestine were hemorrhagic and visible on the serosal surface. On the mucosal surface they were covered by diphtheritic membranes (Fig. 1). Scattered submucosal petechiae covered by diphtheritic plaques were present between the annular bands; these submucosal hemorrhages were less numerous in gnotobiotic ducks. Meckel's diverticulum was hemorrhagic in gnotobiotic and conventional ducks, and a fibrinous plug was present in the lumen. Submucosal petechiae were present in the ceca, large intestine, cloaca, and bursa of Fabricius (Fig. 2); and tags of fibrin adhered to the mucosal surface of the intestine. Livers were swollen, and the edges

bulged on cut surface. Numerous petechiae were present in all livers and pinpoint white foci of necrosis were present in the livers of two gnotobiotic and five conventional ducks.

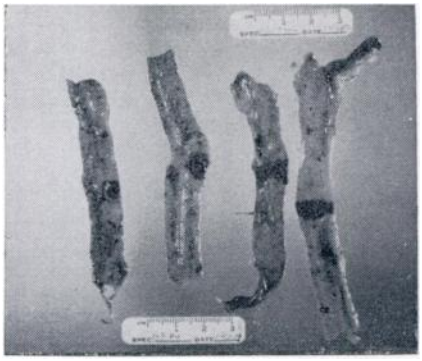

FIGURE 1. The 2 sections of intestine on the left are from a gnotobiotic duck, and the 2 on the right are from a conventional duck. Annular bands in both ducks are hemorrhagic.

FIGURE 2. Terminal ileum, ceca, large intestione, cloaca, and bursa of Fabricius from a gnotobiotic duck inoculated with DPV. The posterior ileal annular band is hemorrhagic; and scattered submucosal petechiae are present in the ceca, large intestine, cloaca, and bursa of Fabricius.

Histologically, hydropic degeneration and intranuclear inclusion bodies were present in mucosal epithelial cells of the esophagus and cloaca. Diffuse cytolysis of lymphocytes and hemorrhage were present in the submucosa at the esophageal-proventricular junction. Fibrocytes, macrophages, and submucosal glands in the esophageal mucosa were degenerate and contained intranuclear inclusion bodies. Diffuse necrosis of lymphocytes and hemorrhage also were observed in Meckel's diverticulum and in intestinal annular bands. The overlying mucosa was degenerate, and fibrin was present on its surface. Necrotic fibrocytes and macrophages were present in the villous lamina propria. Crypt epithelium was degenerate and contained intranuclear inclusion bodies.

Multiple nonzonal foci of necrosis and hemorrhage were present in the liver (Fig. 3). Intranuclear inclusion bodies

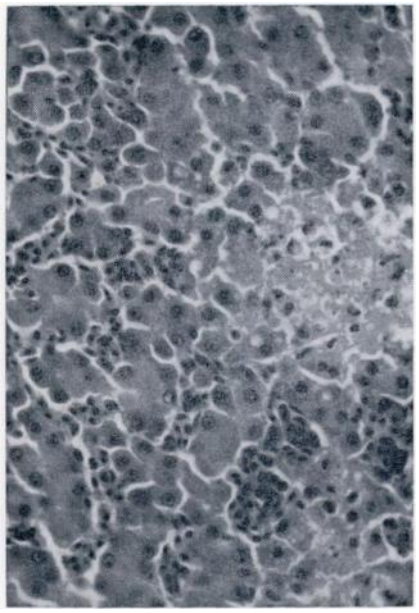

FIGURE 3. Liver from a gnotobiotic duck inoculated with DPV. Nonzonal focal hepatic necrosis is present. The surrounding hepatic sinusoids are congested. Pyknosis and marginated chromatin are present in some of the surrounding hepatocytes. H & E X900.

were present in surrounding hepatocytes. Bile duct epithelium was also degenerate, and inclusion bodies were seen in their nuclei. Scattered foci of necrosis also were present in pancreatic acinar, islet, and ductal cells.

Diffuse cytolysis of lymphocytes and hemorrhage were observed in the spleen. Reticuloendothelial cells lining the sinusoids and in the periarteriolar reticular sheaths were degenerate, and inclusion bodies were present in the nuclei. Diffuse necrosis of medullary lymphocytes was seen in the thymus and bursa of Fabricius. Lymphocytic degeneration also was evident in the cortex of the thymus and bursa of Fabricius (Fig. 4). Epithelial cells in thymic Hassall's corpuscles and epithelial cells separating the cortex and medulla of the bursal follicles were degenerate and contained intranuclear inclusion bodies.

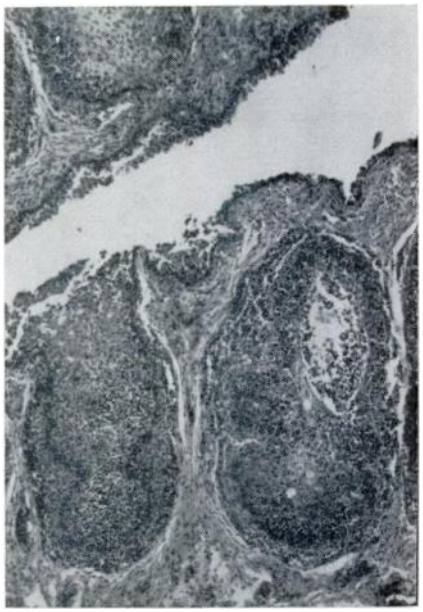

FIGURE 4. Bursa of Fabricius from a gnotobiotic duck inoculated with DPV. Follicles of the bursa are depleted of lymphocytes. A cystic space containing cellular detritus is present in one follicle. Mucosal epithelial cells lining the bursa of Fabricius are degenerate, and some are sloughed into the lumen. H & E X250.

Small foci of degeneration were found in the adrenal cortex, and intranuclear inclusion bodies were present in a few cells. Tracheal epithelium was degenerate, and lymphoid nodules in the peribronchial tissues were necrotic. Air capillary walls were thickened and intranuclear inclusion bodies were present in cells in the air capillary walls. Bone marrow cells, osteoblasts lining bony spicules, and osteoclasts were degenerate; and inclusion bodies could be found in a few of these cells. Microscopic lesions were not found in the brain, kidney, and eyes of 4-week-old gnotobiotic or conventional ducks.

DISCUSSION

DPV, like feline rhinotracheitis virus,[8] and canine hepatitis virus,[7] produced similar lesions in gnotobiotic and conventional hosts. In the absence of *Salmonella* or *Pasteurella*, or their endotoxins, DPV caused nonzonal hepatic necrosis in gnotobiotic ducks. These degenerate hepatocytes contained intranuclear inclusion bodies similar to those observed in conventional ducks.[5,18] Tracheal lesions in gnotobiotic ducks also were similar to those produced in germfree cats by rhinotracheitis virus,[8] emphasizing that virulent herpesviruses can produce disease without the synergistic action of bacteria.

Lesions of the digestive tract in gnotobiotic ducks were similar to those in conventional ducks. The slow lymphoid tissue development in uninoculated gnotobiotic ducks has been reported previously in germfree chickens.[22] Previous reports indicated intestinal lymphoid tissue in day-old, conventional ducks was developed sufficiently to initiate annular band lesions typical of duck plague.[4] Unlike feline infectious enteritis virus, which did not cause lesions in crypt epithelium in the germfree cat,[20] DPV caused lesions in the intestinal crypts of gnotobiotic ducks. It has been suggested that the sluggish proliferation of crypt epithelium in germfree cats was responsible for the crypt epithelial resistance to feline infectious enteritis virus.[11] The rate of crypt cell proliferation in conventional and gnotobiotic ducks has not been studied.

Since the death rates in conventional and gnotobiotic ducks were similar, and bacteria were not isolated from the liver, kidney, or lung of either group of ducks, bacteremia was not considered essential for DPV to cause lesions and kill ducks. In irradiated mice[11,15] bacteremia also was not a sequela to degeneration of intestinal crypt as observed in duck plague. Hyperplastic crypt cells reported in irradiated mice that died within 3-6 days were not observed in ducks.

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