Outbreak of Listeria Monocytogenes in Pheasants

Yufang Gu, Xiongyan Liang, Zhuan Huang, and Yuying Yang

College of Animal Science, Yangtze University, Jingzhou 434025, P.R. China

ABSTRACT Listeria monocytogenes is capable of infecting almost all animals. However, outbreaks of listeriosis are infrequent in birds. This report describes an outbreak of listeriosis in a small pheasant (Phasianus colchicus) breeder farm with more than 2,000 pheasants from Hubei province of the People’s Republic of China. The affected flock consisted of adult and young birds. Approximately 300 young birds and a few adult birds were found dead within a few days of the onset of clinical signs. Twenty-five dead birds were collected for further examination. Histopathological lesions in the visceral organs were characterized by monocyte infiltration and proliferation. Localized encephalitis and meningitis were detected in the brains of dead birds. Gram-positive organisms were observed in heart blood smear, liver, and brain impression smears. The organisms were isolated from fresh liver and were identified as L. monocytogenes serotype 4b based on multiplex polymerase chain reaction (PCR) and hlyA gene sequence analysis. This is the first report describing outbreak of listeriosis in pheasant flock.

Key words: Listeria monocytogenes, pheasant, pathology, isolation, multiplex PCR

INTRODUCTION

Listeria monocytogenes is a bacterial pathogen capable of causing listeriosis with a high mortality rate in a wide range of animal hosts and in humans (Cossart and Lebreton, 2014). Infected animals were manifested as invasive diseases, including encephalitis and meningitis, septicemia, perinatal infections, and abortion. L. monocytogenes is widely distributed among avian species including chickens, turkeys, waterfowl, game birds, pigeons, parrots, wood grouse, snowy owls, eagles, and canaries (Berrang et al., 2010; Chinivasagam et al., 2010; Dhama et al., 2013a; Siriken et al., 2014; Zhang et al., 2013). Chickens are thought to be the carriers of Listeria and also the prime reservoirs for the infection in the litter and environment of the poultry production units. It has been reported that an outbreak in adult chicken flock was associated with L. monocytogenes infection in Washington State in the United States (Crespo et al., 2013). However, listeriosis in pheasants is rare, and there has been no report regarding the pathological changes in pheasants due to listeriosis. This study investigated an outbreak of listeriosis in a pheasant (Phasianus colchicus) breeder farm of Jingzhou, Hubei Province, P.R. China.

MATERIAL AND METHODS

Flock Background

More than 2,000 free-ranch pheasants were bred in a phasianus colchicus breeder farm of Jingzhou, Hubei Province, P. R. China. The flock consisted of adult and young pheasants. In July 2014, some birds showed depression, feather drooping, and a loss of appetite. The sick pheasants also showed significant neurological symptoms such as being not able to stand with their heads backwards, and a swimming pose of two legs. Some pheasants had diarrhea with yellow-white feces. Adult female birds stopped producing eggs. Approximately 300 young birds at 22 days of age and a few 9 months old adult birds died within two days from the onset of clinical signs. Twenty dead young birds and 5 dead adult birds were taken to the laboratory for post-mortem and histopathological examinations and pathogen detection.

Histopathological Examination

The twenty-five dead birds were dissected and the different organs were examined histologically. Organs including the heart, liver, spleen, lungs, kidneys, brain, cloacal bursa, pancreas, and duodenum were examined. Tissues were fixed in 10% buffered neutral formalin, routinely processed, embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin, and examined by light microscopy.
Pathogen Detection

Bird heart blood smear, liver and brain impression smears were taken and examined using Wright’s staining for bacterial observation under the microscope. Heart, brain, and liver samples from the dead pheasants were also inoculated onto tryptic soy agar plates, Listeria identification agar (PALCAM), and MacConkey agar plates, and incubated for 24 hours at 37°C. A single suspect colony was picked for the preparation of smear followed by Gram’s stain and examination of staining and morphological characteristics of the isolate. The suspect colonies were further examined using a commercial trace biochemical identification tube for Listeria (Micro-Biochemical Identification Tube for Bacteria, Qingdao Hopebio-Technology Co. Ltd., China).

Division-specific Multiplex Polymerase Chain Reaction and hlyA Gene Sequencing

The genomic DNA was extracted from the isolated bacteria using the Bacteria Genomic DNA Purification Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Division-specific multiplex polymerase chain reaction (PCR) assay was performed based on the procedures described previously using following sets of primers that amplify marker genes of L. monocytogenes genome: lmo0737, lmo1118, ORF2819, ORF2110, prs (Doumith et al., 2004). Each PCR product was designed for amplifying distinct fragment sizes between 370 and 906 bp. These primer pairs can accurately classify L. monocytogenes into 4 major serovars (1/2a, 1/2b, 1/2c, and 4b). Further, PCR was also performed to amplify complete hlyA gene of L. monocytogenes using primer pairs of GAG GAT CCA GAG TGA AAC CCA TG and TGG AGC TCA TTC GAT TGG ATT AT giving an amplicon of 1,616 bp (Soni and Dubey, 2014). The hlyA gene obtained was cloned into the pMD19-T vector (Takara Biotechnology Dalian Co., Ltd., China), sequenced and aligned against the known sequences in the GenBank database using the BLAST program of the National Centre for Biotechnology Information.

RESULTS AND DISCUSSION

Necropsy showed that all affected pheasants examined were emaciated. Liver was celadon and enlarged with a small amount of visible ecchymosis under capsule. Spleen was swollen and kermesimus with a small amount of petechia under capsule. Kidney was enlarged and sallow, while, some kidney was red. Portions of lungs became hardened with a small amount of visible ecchymosis. Cardiac hypertrophy and the brain were gray in color. Other organs did not generally show abnormal changes.

Figure 1. L. monocytogenes-induced encephalitis. Histologic section of the brain showing mononuclear and heterophilic granulocyte infiltration around necrotic foci (HE stain; 100 ×).

Figure 2. Hepatic inflammation. Histologic section of the liver showing different degrees of hepatocyte degeneration with infiltration of various numbers of monocytes and heterophilic granulocytes (HE stain; 200 ×).

The main histopathological changes of the sick birds were encephalitis and meningitis with extensive infiltration of monocyte-macrophage. The meninges of cerebrum and cerebellum were thickened at some locations with monocyte and heterophilic granulocytes hyperplasia. Perivascular infiltration of mononuclear cells, degeneration of some neurons and several scattered necrosis areas with the infiltration of a large number of monocytes and heterophilic granulocytes around the necrotic foci (Figure 1) were observed in the brain. Various degrees of hepatocyte degeneration and lysis were observed in the liver. There were varying amounts of monocytes and heterophilic granulocytes between hepatocytes (Figure 2). In lung sections, alveolar wall capillaries and interlobular vascular were congested and mononuclear cells and heterophilic granulocytes proliferated around the vein. In renal sections, glomerular volume was increased with significant proliferation of intracapsular capillary endothelial cells and
mesangial cells. There were numerous monocytes in the mesenchymal of the kidney. The number of lymphocytes in lymph nodules of splenic white pulp decreased significantly. Splenic red pulp was hyperemia and hemorrhage, containing scattered mononuclear cells and heterophilic granulocytes. Pancreas showed degeneration of pancreatic epithelial cells and focal proliferation of mononuclear cells. The numbers of lymphocytes was decreased in the lymphoid follicle medulla of the cloacal bursa. The cardiac muscle showed congestion, interstitial edema, and degeneration of partial muscle fiber.

There were many rounded ends and darker colored poles brevibacterium in the bird liver smears and heart blood smears. Some of the bacteria were grouped into a V-shape, and the others were scattered individually. Suspect colonies on the tryptic soy agar plates were identical smooth rounded and like dew, 1 to 1.5 mm in diameter, and a light blue fluorescent color under a tilted light. The grayish green colonies appeared on the PALCAM Listeria agar plates. However, there was no growth on the MacConkey agar plates. The isolated bacillus was Gram-positive rod, deep colored with rounded ends. Biochemically, the isolates were positive for catalase, MR-VP, and esculin, and negative for nitrate reduction test, fermented glucose, maltose, and rhamnose, xylose and mannitol, which are typical biochemical reactions of \textit{L. monocytogenes}.

Five suspect isolates of \textit{L. monocytogenes} were obtained from 5 birds using buffered Listeria enrichment broth followed by plating onto Listeria identification agar. These pure isolates were confirmed to be \textit{L. monocytogenes} by division-specific multiplex PCR, namely positive using ORF2819, ORF2110 and \textit{prs} primer sets, but negative for \textit{lmo0737} and \textit{lmo1118} primer pairs tested. These results indicated that the isolates belonged to \textit{L. monocytogenes} serotype 4b (Figure 3). The amplicon of \textit{L. monocytogenes} \textit{hlyA} gene amplified by PCR showed an expected size of 1,616 bp. \textit{HlyA} gene sequence from the isolated strains showed 99% similarity with several \textit{L. monocytogenes} strains in the GenBank database.

According to the characteristics of the clinical symptoms, necropsy findings, the results of bacterial identification, and pathology of the sick pheasants, it is concluded that the outbreak in these birds was due to the infection of \textit{L. monocytogenes} serogroup 4b. This is the first report describing an outbreak of listeriosis in a pheasant flock. \textit{L. monocytogenes} is recognized as an opportunistic, food-borne pathogen of human, cattle, and wild animals. Most human infections are caused by 3 major serotypes (1/2a, 1/2b, and 4b). The feces and secretions excreted by the chickens containing \textit{L. monocytogenes} could be potential sources of \textit{L. monocytogenes} infection. Therefore, the dead chickens with listeriosis and the relevant farm environment including pheasantry, feed, and straw used for the infected chickens had to be disinfected to prevent further infection. A public awareness of zoonotic potential of \textit{L. monocytogenes} infection is necessary since this organism is a common foodborne pathogen and has the ability to survive and grow in harsh conditions (Aury et al., 2010; Voidarou et al., 2011; Dhama et al., 2013b; Shen et al., 2013).

**ACKNOWLEDGMENTS**

This study was supported by grants from the National Natural Science Foundation (No. 31060342). Authors thank Dr. Hongsheng Huang of Canadian Food Inspection Agency, Ottawa Laboratory (Fallowfield), Ottawa, Ontario, Canada, for scientific comments and revising the manuscript.

**REFERENCES**


