

Occurrence of Reticuloendotheliosis in Chinese Partridge

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ABSTRACT. Two commercial flocks of Chinese partridge experienced increased mortality associated with a wasting disease at 120-day old in June 2006. Postmortem examination of dead chickens consistently showed visceral tissues mainly proventriculus, liver and spleen were diffuse enlargement. Microscopic examination revealed masses of immature lymphocytes with frequent mitotic figures were seen in various tissues including proventriculus, liver, spleen, kidney, heart, lung, thymus and intestine. Reticuloendotheliosis virus (REV) was isolated from each of four blood samples. Viral antigens were observed in cultured CEF (SPF embryos came from the Ji-nan poultry institute) inoculated blood samples via an indirect immunofluorescent assay. Three hundred bp fragments of LTR of REV obtained from liver samples of six chickens by PCR. This disease has not previously been reported in Chinese partridge. Chinese partridge may represent a potential reservoir of infection for other Chinese local chickens.

KEY WORDS: Chinese partridge, lesion, PCR, reticuloendotheliosis.

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Reticuloendotheliosis (RE) is a neoplastic disease of a number of avian species, including domestic chickens, ducks, quails, pheasants, and domestic turkeys [2, 7]. The disease is characterized by chronic lymphomas and a runt-ing-stunting syndrome. RE caused by a group of retroviruses that are unrelated antigenically, morphologically or structurally to avian leukosis viruses [7]. Two forms of REV are known, the defective (oncogenic REV-T) and non-defective (helper-virus REV-A). REV has a world-wide distribution and serologic surveys conducted in several countries have detected antibodies against this virus in 3.3%–25% of chicken flocks [2, 3]. China has much higher seropositive rate in recent decade, about 30–40% [6, 8]. RE has been reported in Hungarian partridge [5].

The Chinese partridge is also known as Chinese Ma Chicken, which distribute over south China, such as Fujian province and Guizhou province. This bird has been widely and successfully introduced from the South to the Northern region and prairie regions of the North Asia continent. Partridge from the commercial broiler enterprise described in this report were raised to provide meat and egg for human. RE has not previously been found to cause morbidity and mortality in Chinese partridge.

In June 2006, ten Chinese partridges from two flocks were submitted to College of Animal Husbandry and Veterinary Medicine of Shandong Agricultural University to identify the cause of debilitating visceral tumors. Upon arrival at the laboratory most chickens were inactive, reluctant to stand and had ruffled feathers (Fig. 1). Some had swollen abdomens and some had moderate to marked dyspnea. All partridges were 120-day old. The two sister flocks

consisted of 1,500 chickens respectively from the same production company, housed in two geographically distinct areas were affected with similar lesions and mortality. The problem was persistent mortality within the breeding flock and affected partridges tended to lose weight and waste away prior to death. The cumulative mortality of two flocks was 6% and 15%, respectively. All flocks had been vaccinated with a full dose of bivalent vaccine containing MDV serotype 3 (HVT) and MDV serotype 2 (SB-1). Other vaccines typical for broiler breeders were also administered.

Postmortem examinations were performed on the ten Chinese partridges. Livers and spleens showed diffuse enlargement (Fig. 2-A, B). Nodular lesions, resembling tumors were seen in proventriculus (Fig. 2-B), thymus, heart, ovary (Fig. 2-C), kidney (Fig. 2-D), lung and intestine. No gross lesions were seen in nerves, brains, eyes, or bursas of Fabricius.

The tissues including tumors, heart, proventriculus, kidney, liver, lung, spleen, bursa, thymus, peripheral nerve,



Fig. 1. Sick 120-day old Chinese partridge. It was inactive, reluctant to stand and had ruffled feathers.

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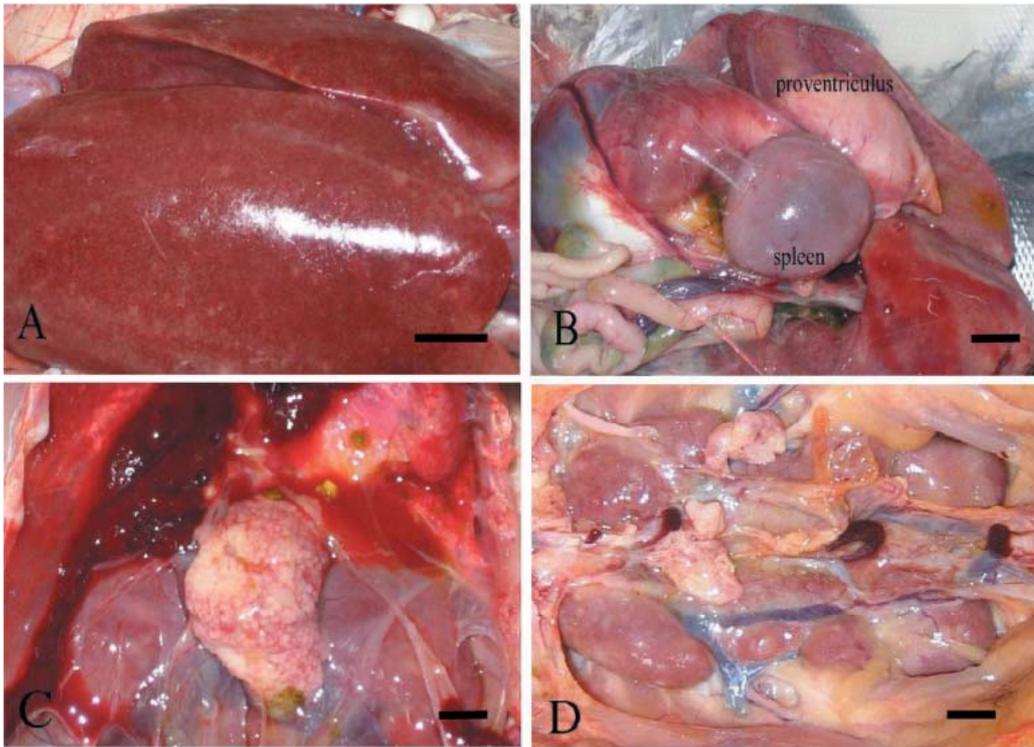


Fig. 2. A: liver, B: spleen and proventriculus, C: ovary, D: kidney. All showed diffuse enlargement, and white nodular lesions were seen in these tissues. Bar=2 cm.

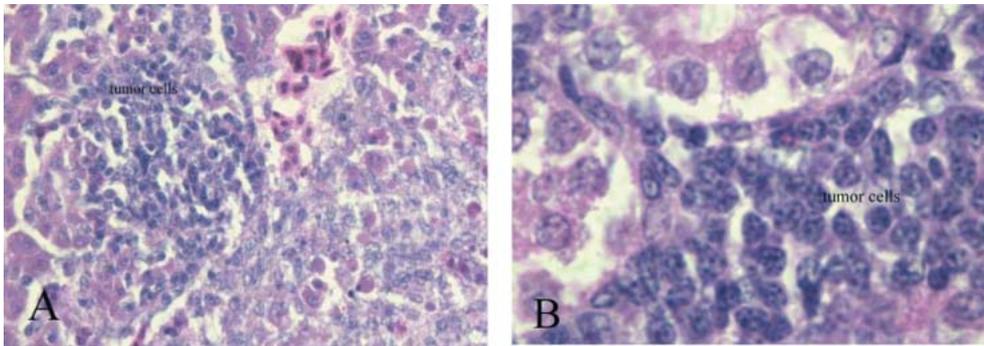


Fig. 3. A: many focal area of immature lymphocytes was seen in liver (HE, 400 ×); B: immature lymphocytes with large round to oval vesicular nuclei were seen in kidney (HE, 1,000 ×).

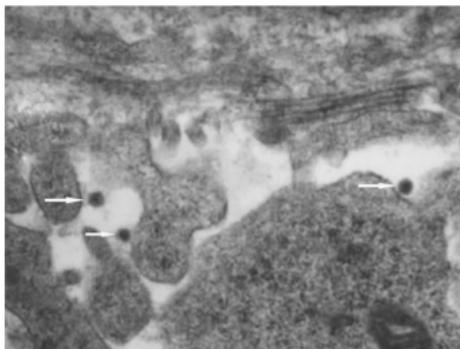


Fig. 4. Electron micrograph of mature reticuloendotheliosis virions (20K ×).

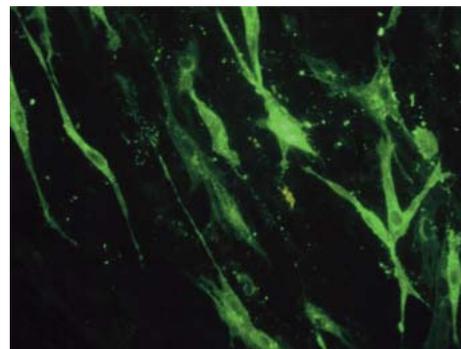


Fig. 5. REV antigen positive cultured CEF detected by indirect immunofluorescent (400 ×).

brain, pancreas, duodenum, intestine, skeletal muscle and bone marrow were collected and fixed in 10% neutral buffered formalin. The tissues were sectioned and mounted on glass slide, dewaxed and stained with hematoxylin and eosin (HE) for histological examination. The livers and tumor tissues were fixed with 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide, dehydrated in alcohol, and embedded in epoxy resin. Ultra thin sections were stained with saturated alcoholic uranyl acetate and Reynold's lead citrate and examined with a transmission electron microscope.

Microscopic examination of formalin fixed tissues, except nerves, brains, eyes, and bursas of Fabricius from partridges revealed nodular infiltration of immature lymphocytes exist in the rest of tissues. Hepatic tissue contained wide bands of neoplastic cells morphologically indistinguishable from those in the preventriculus. These cells infiltrated the connective tissue stroma surrounding central veins and portal triads (Fig. 3-A). Mucosal stratified squamous epithelium was focally ulcerated, and ulcers were covered by necrotic cell debris enmeshed in fibrin. Immature lymphocytes diffusely distributed in the lamina propria were enmeshed within and surrounded by stromal connective tissue stromal fibers and consisted entirely of neoplastic immature lymphocytes. These cells had large round to oval vesicular nuclei with prominent chromatin clumps adjacent to the inner surface of the nuclear envelope. Each cell had moderate to abundant pale basophilic cytoplasm. Mitotic figures were numerous and an occasional cell was undergoing lysis (Fig. 3-B).

Virus particles were observed within tumor cells. These particles were round, approximately 110–120 nm in diameter, and had a uniform space between the outer edge of the electron-dense core and the inner edge of the viral envelope. Some cores had a polygonal shape and, in some sections, regularly spaced small electron-dense structures suggestive of peplomers were observed in association with the viral envelope (Fig. 4).

Attempts were made to isolate REV from heparinized blood samples taken from four female tumor-bearing Chinese partridges (other chickens could not be taken blood because they were almost dying). The embryos prepared for Chick embryo fibroblasts (CEF) were from breeders maintained at the Ji-nan poultry institute that tested negative for exogenous avian retroviruses and other common pathogens. CEF was used for REV isolation. Monoclonal antibody 11B118+11B154 of REV used for an indirect immunofluorescent assay was kindly provided by Professor Zhizhong Cui. A second passage of the CEF was stained for REV antigens. All four blood samples were REV antibody positive (Fig. 5).

A Polymerase chain reaction (PCR) was conducted with primers at the region of the 5' long terminal repeat (LTR), about 300-bp length [1]. All products generated by PCR amplification were analyzed in agarose gels. Six liver samples were positive by PCR for REV LTR. Four samples from Chinese partridges livers were PCR-negative (Fig. 6).

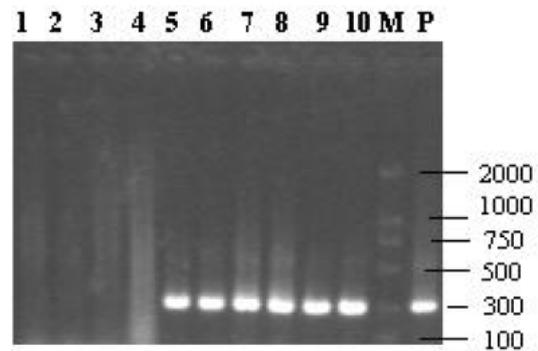


Fig. 6. PCR results: Six liver samples were positive and 4 samples were negative of 10 sick chickens. M=Marker, P=Positive sample.

Histopathological differentiation among RE, AL and MD tumor is important. The character of MDV tumor cells are pleomorphic types of cellular infiltration composed of a mixed population of small to large lymphocytes. In cases of infection with REV and ALV the tumors are infiltrated with monomorphic cells, composed of homogenous lymphoblasts in the case of ALV-infection, T or B lymphocytes in the case of REV-infection and myeloid cells in the case of ALV-J-infection. While the cytological picture is typical in the case of MDV and ALV-J, there is no possibility to distinguish histologically between ALV and REV-infections.

A consistent feature of reticuloendotheliosis in Chinese partridges in this outbreak was the presence of neoplastic immature lymphocytes in most of tissues, except bursas of Fabricius, nerves, brains and eyes.

The disease may have been present but not detected due to the timing of sampling. Further data are needed to evaluate the likelihood of these possibilities. In addition, it could be useful to investigate whether individual partridge have antibodies to the RE viruses. The detection of antibodies may help to identify birds that have been exposed to the virus but carry proviral copies at numbers below threshold sensitivity of the PCR test, and may provide information about past exposure to the disease.

The possible presence of RE in the Chinese partridge populations should be carefully considered in any future relocation and reintroduction efforts because of the potentially serious consequences of RE described in Drew *et al.* [4].

Chinese partridge may represent a potential reservoir of infection for other upland birds. The susceptibility of Chinese partridge and the character of this virus were needed to be further studied.

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