Isolation of a Papovavirus-like Agent from Young Budgerigars with Feather Abnormalities

Katsuya HIRAI, Hiroko NONAKA, Hideto FUKUSHI, Seigo SHIMAKURA, Toshiaki MASEGI\(^1\), and Toru MIZOGUCHI\(^2\)

Department of Veterinary Microbiology and \(^1\)Veterinary Pathology, Faculty of Agriculture, Gifu University, Yanagido, Gifu, Gifu 501-11, and \(^2\)Shizuoka Prefectural Institute of Animal Health, Fujieda, Shizuoka 426, Japan

(Received 13 March 1984/Accepted 23 April 1984)

ABSTRACT. The outbreak of a feather abnormality known as "French molt" in young budgerigars was observed in a commercial breeding aviary. The disease of young birds (3 to 4 week of age) was characterized by the loss of flight and tail feathers. A papovavirus-like agent was isolated from the skin tissues, and liver and spleen specimens of 20 affected birds. Viral antigen was present in all sections of skin and kidney from affected birds.—Key words: French molt, papovavirus-like agent.


Outbreaks of a feather abnormality known as "French molt" have been reported among young breeding budgerigars kept in aviaries and by fanciers. The disease is characterized by the loss of fully developed flight and tail feathers. Although not fatal, the bird is extremely disfigured [10]. The etiology of the disease has not been established, but is suspected to be infectious [1–3, 10]. During 1981 Davis _et al._ in the United States [3] and Bernier _et al._ in Canada [1] observed a papovavirus-like agent in the tissues of fledgling budgerigars having high rates of mortality. Consequently, a papovavirus-like agent was isolated from budgerigars in the United States [2]. While investigating a feather abnormality in young budgerigars from a commercial breeding aviary in Shizuoka Prefecture, the authors isolated a papovavirus-like agent from 20 diseased birds. This is the first report on the isolation of a papovavirus-like agent from budgerigars in Japan.

Twenty diseased birds were sent to our laboratory for examination. All were held in isolation units before necropsy. After taking samples for bacterial examination, liver, spleen and skin tissues from each bird were homogenized separately in phosphate-buffered saline containing antibiotics. The supernatant fluid of each sample was inoculated onto budgerigar embryo fibroblast (BEF) cells. Primary monolayer cell cultures of BEF were prepared from embryos 12- to 16-day-old. The embryos were obtained from two aviaries having healthy birds. Methods for preparation of cells, culture media and incubation have been described [8]. Procedures for virus isolation, cytopathology, physicochemical examination, hemagglutination, serology, immunofluorescence (IF) and electron microscopy have also been described elsewhere [5–8].

The clinical history indicated that the submitted budgerigars were reared in several rooms (4 m\(^2\)) on a concrete floor within 3 houses containing 3,000 to 4,000 birds. The owner reported that almost all of the young budgerigars at the age of 3 to 4 weeks were found to lack flight and tail feathers during a 3-month period from March to May, 1981, and that no deaths occurred among very young
budgerigars less than 2 weeks old. Clinical signs of feather abnormality were not observed in adult breeding birds. The present cases could be divided into mild, moderate and severe according to the degree of macroscopic feather changes (Fig. 1). In the severe cases, most of the birds were affected bilaterally with the feather disease. Despite these disfigurations, no gross internal changes were evident in the birds when examined by necropsy. However, histologic examination of the kidneys revealed markedly enlarged nuclei with opaque inclusions most prominent in the epithelial cells of the renal tubules (Fig. 2). Similar nuclear inclusion bodies were also present in feather sheath cells of the feather follicles associated with infiltration of polymorphonuclear leukocytes and vasodilation (Fig. 3).

Virus was recovered from the tissues of 20
affected birds. All of the skin tissues, and 20 of liver and spleen specimens, respectively, produced CPE within 7 days after inoculation. CPE on BEF cultures consisted on foci of rounded cells characterized by swollen nuclei (Fig. 4). Physicochemical properties of a representative isolate, GFM-1 strain, are shown in Table 1. The addition of IUdR to the medium of BEF cultures at the time of infection prevented the development of CPE. The result indicated that the nucleic acid type of the isolate is DNA. The isolate was thermostable, acidstable, and resistant to lipolytic solvent. The virus passed through a 50 nm filters, but not through a 25 nm filter. In thin-section preparations of GFM-1 infected BEF cells, apparent virus particles usually filled entire nuclei often enlarging their diameters up to two-fold (Fig. 5). Virions were spherical in shape and measured about 40 to
Fig. 5. Electron micrograph of virus particles. (a) Virus particles were seen in the nucleus of BEF cell infected with GFM-1 isolate. $\times22,500$. (b) Higher magnification of virus particles in the nucleus of infected-BEF cell. $\times75,000$. (c) Single virus particle of the isolate GFM-1 negatively stained. Spherical form is evident. $\times247,500$. 
48 nm in diameter. In the partially purified preparation of the isolate, the particles were also more or less spherical having an average diameter of 44 nm (Fig. 5). The isolate did not agglutinate erythrocytes of budgerigars, chickens, guinea pigs, rabbits, sheep, goats, cattle, pigs, horses, dogs, cats and human type 0. Antiserum against the GFM-1 isolate neutralized the GFM-1 strain and all of the other isolates recovered from the diseased birds. IF examinations indicated that viral antigen was present in all sections of skin and kidney from affected birds, especially within the epithelial cells of feather follicles and renal tubules (Fig. 6). No such fluorescence was observed in tissue sections from healthy birds used as negative controls. No evidence of apparent infection was achieved by intraperitoneal or subcutaneous inoculation of the GFM-1 strain into 20 4-week-old budgerigars, 10 newborn hamsters and 20 newborn nude mice.

The fatal disease in very young budgerigars described by Davis et al. [3] and Bernier et al. [1] was characterized by abdominal distention, lack of down feathers on the back and abdomen, lack of filoplumes on the head and neck, and retarded growth of the tail and contour feathers in birds that either survived or died later. They have suggested that "French molt" is a nonfatal form of the above infection caused by a papovavirus-like agent in which diseased birds have some of the same clinical signs such as retardation of growth of flight and tail feathers. Our results regarding the clinical disease in young budgerigars support this hypothesis including histopathology, detection of viral antigen in infected birds and isolation of a viral agent with biological, physicochemical and morphological characteristics of Papovaviridae [2, 4, 9]. Consequently, "French molt" does occur in Japan causing nonfatal feather abnormalities in young budgerigars. The lack of mortality as sequel of this disease may depend upon the age of an animal at the time of infection. The fatal papovavirus infection under natural conditions has been observed in very young budgerigars less than 2 weeks of age [1–3]. However, experimental exposure of young budgerigars at 4 weeks of age produced histologic lesions of variable severity but none of the inoculated birds died [1]. Bozeman et al. [2] reported recovery of papovavirus from the tissues of apparently normal young budgerigars kept in the infected aviaries. These
works suggested the possibility that many budgerigars after recovery from the clinical infection remained carriers capable of transferring the virus to the healthy bird. Further work is necessary to determine if there is a relationship between latent papovavirus infection in adult budgerigars and neonatal mortality. Additional characterization of our virus isolate is also necessary regarding serological comparisons with papovaviruses of mammalian isolates, determination of DNA molecular weight and identification of virus structural proteins.

ACKNOWLEDGMENTS. We wish to thank Dr. T. R. Meyers, University of Alaska at Juneau, and Dr. S. B. Hitchner of Professor emeritus of Cornell University, for critical reading and suggestion of this manuscript. We thank Dr. M. Inoue, of the Department of Veterinary Pathology, Faculty of Agriculture, Gifu University, for his encouragement. This work was supported by a Grant-in Aid for Scientific Research Nos. 574800079 and 58480082 from Ministry of Education, Science and Culture of Japan.

REFERENCES

セキセイインコの翼羽疾患から分離されたバポバウイルス様ウイルスについて（短報）：平井克哉・野中博子・福士秀人・鳥倉省吾・樋口広・関本和 （岐阜大学農学部家畜共生物学教室，1）家畜病理学教室，2）静岡県家畜衛生研究所）——1981 年 3 月～5 月の間に浜松市のセキセイインコ繁殖場で，孵化後 3 ～ 4 週齢の幼若鳥に，飛翔翼羽および腹羽が欠損し，致死的ではないが外観を損う，疾病が発生した。20羽の翼羽，胸部および肝臓の乳剤を BEF 細胞に接種して，全例からバポバウイルス様ウイルスを分離した。病鳥の全例で羽根部および尿細管上皮細胞にウイルス抗原が認められ，分離ウイルスが本病の病因と考えられた。