CATARRHAL PROVENTRICULITIS ASSOCIATED WITH A FILAMENTOUS ORGANISM IN PET BIRDS

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ABSTRACT

Catarrhal proventriculitis due to infection by an unidentified organism was diagnosed in 79 of 534 pet birds examined histologically. It was more prevalent in domestic birds (70 cases) than in imported ones (9 cases). A high incidence of the disease was encountered in budgerigars (Melopsittacus undulatus) and it was occasionally found in finches (Poephila gouldiae gouldiae), parakeets (Psittacula krameri manillensis), Amazona parrots (Amazona aestiva aestiva) and cockatiels (Nymphicus hollandicus). The agent was a large filamentous rod, and was stained positively with Gram, GMS and PAS methods. Histologically, it induced a mild to moderate exudative or proliferative inflammation in the proventriculus. All the cases had an erosion in the gizzard. Ultrastructurally, the organism had a eukaryotic nucleus and three cell-wall layers.

Concurrent infections were very common, including adenoviruses (37 cases), giardiasis (31 cases), candidiasis (13 cases), papovaviruses (11 cases) and knemidocoptic mites (11 cases).

Key words: proventriculitis, filamentous organism, pet bird, histopathology.

INTRODUCTION

Pathologic lesions of avian proventriculus attributable to infectious agents have rarely been encountered¹⁰. A recent report indicated that a large, rod-shaped, Gram-positive, non-spore forming bacterium caused proventriculitis in canaries, usually with a clear mucus on the mucosal surface¹². This paper describes a similar disease, although it might be caused by a different etiological agent, in imported and domestic pet birds.

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MATERIALS AND METHODS

We histologically examined 293 budgerigars (*Melopsittacus undulatus*) collected from pet bird stores in Japan and 241 birds imported from several other countries. The imported birds consisted of 67 parakeets (*Psittacula krameri manillensis*), 52 cockatiels (*Nymphicus hollandicus*), 29 parrots (*Amazona aestiva aestiva* and *Psittacus erithacus erithacus*), 47 budgerigars (*Melopsittacus undulatus*), 18 lories (*Agapornis roseicollis*), 2 rosellas (*Platycercus emimius*) and 18 finches (*Poephila gouldiae gouldiae*) which died within 2 weeks of quarantine in a bird shop.

For histopathological examination, tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Grocott’s methenamine silver-nitrate (GMS) and Gram methods.

For electron microscopic examination, formalin-fixed tissues were post-fixed in 1% osmium tetroxide and embedded in Quetol 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a JEOL electron microscope, model JEM–100SX.

RESULTS

On histological examination, catarrhal proventriculitis due to infection by a filamentous organism occurred in 79 of 534 birds examined. The incidence was higher in the domestic birds than in the imported ones (70: 9). The affected birds consisted of 72 budgerigars (91.1%), 3 finches (3.8%), 2 parakeets (2.5%), 1 Amazona parrot (1.3%) and 1 cockatiel (1.3%). The caudal portion of the proventriculus and the proventricular-gizzard junction were the target sites for this organism.

The organism was often localized in the lumina of superficial glands of the proventriculus, while it did not invade either the mucosal layer or the common duct (Figs. 1, 2). The tissue reaction to the organism was not so severe, although some proliferative and exudative changes were present (Table 1).

Most of the cases showed mild to moderate epithelial hyperplasia in the superficial glands, and collecting ducts of the deep glands (Fig. 1). This alteration was often associated with an inflammatory cell reaction in the lamina propria consisting mainly of lymphocytes, plasma cells and fibroblasts. Occasionally, foreign-body giant cells around cystic dilations were seen in the deep glands. An excess of mucus accumulated in the proventricular lumen or the sulcus, often forming a mixture with the desquamated epithelial cells of the superficial glands (Fig. 2). Infiltration of heterophils and edema occurred in the lamina propria, although it was mild in degree.

The gizzards of all the cases had erosions without cell reaction in the superficial portion of the koilin layer.

The organism was a large, filamentous, non-spore forming, and non-branching rod.
Catarrhal Proventriculitis Associated with Filamentous Organism in Pet Birds

Table 1. Proventricular tissue changes in proventriculitis due to a filamentous organism infection

<table>
<thead>
<tr>
<th>Tissue change</th>
<th>Severity of lesion in each case</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Exudative change in lamina propria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucus</td>
<td>46</td>
<td>20</td>
</tr>
<tr>
<td>Edema</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>Heterophil</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Proliferative change in lamina propria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>47</td>
<td>25</td>
</tr>
<tr>
<td>Macrophage</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Plasma cell</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Foreign body giant cell</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Glandular epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>44</td>
<td>13</td>
</tr>
<tr>
<td>Cystic dilation</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

*a + : mild, ++ : moderate, and +++ : severe.
*b No. of case.

Fig. 1. Many long, unbranched, Gram-positive filamentous organisms (arrow-heads) inhabit the mucosal surface of the proventriculus and sulci of the superficial glands, inducing epithelial necrosis and desquamation. Gram stain. ×350.
Fig. 2. Many filamentous organisms (arrowhead) invade the sulci of the superficial glands, causing catarrhal proventriculitis. HE stain. ×230.

It was stained positively with Gram, GMS and PAS methods. Ultrastructurally, the longitudinal section of the organism had a distinct nucleus enclosed by a nuclear membrane (Fig. 3). The organism was surrounded by three layers; the inner layer was thick and had mild indentations into the cytoplasm, the middle one was very thin.

Fig. 3. Electron micrograph of an organism in the proventricular lumen. It has a distinct nucleus (N) enclosed by a nuclear membrane and is surrounded by a three-layered wall. ×31,000.
and more electron-dense, and the outer layer was very thick and more electron-opaque.

Based on the histological examination, most of the cases (67/79) in this study had concurrent infections. The frequency of the occurrence was as follows; adenovirus infection (37 cases), giardiasis (31 cases), candidiasis (13 cases), papovavirus infection (11 cases), knemidocoptic mite (11 cases), cryptosporidiosis (1 case), and sarcosporidiosis (1 case).

**DISCUSSION**

Proventriculitis is usually associated with a generalized condition rather than constituting a specific problem by itself\(^{11}\). Proventricular dilatation had been reported in macaws, cockatoos and psittacines with unknown causes\(^7\). Candidiasis might be the most common agent involving the proventriculus of pet birds\(^8\). Recently, high morbidity (30%) of bacterial proventriculitis has been reported in canaries from which the agent has not been isolated\(^{12}\). Electron microscopically, the agent lacked a definite nuclear structure and cytoplasmic granules.

Based on the lesion distribution, and the organism morphology and stainability examined by light microscope, the disease of our cases was identical to the bacterial proventriculitis\(^{12}\). However, the organism observed here had a eukaryotic nucleus, suggested that it was not a bacterium. Morphologically, blastospores and pseudohyphae typical for Candida species\(^8\) were not demonstrated in our cases. The present study indicated that the agent had a wide host range, including canaries, budgerigars, finches, parakeets, Amazona parrots and cockatiels.

Gizzard erosion has been attributed to water deprivation\(^4\) or a bile acid deficiency\(^1\). It has also been associated with the use of quaternary ammonium compounds as disinfectants in drinking water\(^9\), deficiencies of sulfur-containing amino acids\(^{10}\), and B-6 avitaminosis\(^5\), as well as feeding moldy corn\(^2\). All the cases in the present study had an erosive lesion in the gizzard. However, the pathogenesis seemed to be different from those mentioned above.

The koilin layer of the gizzard is composed of a scaffolding of interconnecting vertical rods embedded in a horizontal matrix, which contains a carbohydrate-protein complex\(^6\). The vertical rods are secreted by the gland of the lamina propria and harden within the lumen as filaments. The horizontal matrix is a secretion of the crypt cells and surface epithelium, which is hardened by a fall in its pH as a result of diffusion, through the membrane, of hydrochloric acid from the proventriculus. Bacterial proventriculitis causes an increase in the pH of the proventriculus, from 0.7 – 2.4 in the uninfected stomach to 7.0 – 7.3 in the infected stomach. This change in pH causes poor precipitation of the protein-carbohydrate in the koilin layer of the gizzard, resulting in formation of erosions. This might be the reason why gizzard erosion has been found in all the cases of this study. The identification and pathogenicity of the
organism need to be studied further.

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