Frequent Development of Inflammatory Lesions and Lymphoid Foci in the Kidneys of Japanese Wild Crows (Corvus macrorhynchos and Corvus corone) as a Result of the Entry of Causal Agents via the Renal Portal Blood

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ABSTRACT. Although the increase in the number of wild crows is causing social problems in urban areas, crows play an increasingly important role in monitoring serious infectious diseases, such as highly pathogenic avian influenza and West Nile fever. To gain a better understanding of normal conditions and common disorders in crows, we conducted a retrospective study of wild crows captured in central Japan in the 1990s and examined the necropsy findings from 166 jungle crows (Corvus macrorhynchos) and 74 carrion crows (Corvus corone). We found frequent development of lymphoid foci and inflammatory lesions in the kidneys of both species of crows. These findings were unrelated to place or date of capture, indicating the universality of renal lesion developments in the Corvus species. In the kidneys, suppurative granulomas were concentrated in the renal cortex and the vein wall, indicating the haematoegenous spread of causal agents. However, the glomeruli remained intact, unlike the spreading of causal agents via arterial blood, which strongly suggested the renal portal blood as a possible entry route of causal agents. The renal lymphoid foci showed the same distribution as the granulomas, supporting the possibility of external agents entering through renal portal blood. We also identified types of parasites in Japanese wild crows by means of histopathological analysis. We hope that our data will contribute to the appropriate evaluation and a better understanding of pathological conditions in Japanese wild crows.

KEY WORDS: crow, granuloma, kidney, lymphoid focus.

Crows have increased in number in urban areas around the world, including Japan. Although the increase in the number of crows has caused social problems, recent research indicates that crows also provide useful information in terms of public health and animal hygiene. Because they share their habitat with humans, they have attracted attention as biological indicators for monitoring environmental pollution in urban areas [6, 13]. More importantly, they are gaining significance as potential sentinel animals for monitoring epidemics of infectious diseases, including highly pathogenic avian influenza and West Nile fever. Actually, during the recent outbreaks of highly pathogenic H5N1 avian influenza in Japan, the viruses were isolated from dead or prostrate jungle crows (Corvus macrorhynchos) in the infected area, although other wild birds tested negative [12]. Recent remarkable incidents concerning crows have included cases of West Nile fever in the United States. Crowd mortality has proven valuable as a sentinel surveillance system for West Nile virus in the United States [2] and could play an important role also in other regions where the virus has not been detected. Jungle crows are widely distributed in East Asia and have been shown to be highly susceptible to experimental West Nile infection [11].

Though monitoring of wild crows is gaining importance, fundamental data is not enough to evaluate their conditions appropriately in a pathological examination. In this study, in order to better understand normal conditions and common disorders in crows, we conducted a retrospective study on Japanese wild crows using necropsy findings from jungle crows and carrion crows (Corvus corone) captured around Gifu City in central Japan in the 1990s. This report describes the characteristic concentrations of lymphoid foci and inflammatory lesions in the kidneys of Japanese wild crows and discusses the possible entry route of causal agents into the kidneys.

MATERIALS AND METHODS

Necropsy findings from 166 jungle crows (Corvus macrorhynchos) and 74 carrion crows (Corvus corone) were examined. The crows were captured around Gifu City as part of an animal control program in the 1990s, mostly between 1991 and 1992, and sent to Gifu University, Department of Veterinary Pathology. In addition, we examined seven more jungle crows sent from Hyogo Prefecture in 2005 to compare our samples with flocks located in a distant habitat. Crows were routinely necropsied and tissue samples from the heart, lung, esophagus, proventriculus, gizzard, intestine, liver, pancreas, spleen, bursa of Fabricius, kidney, genital gland and brain were fixed in 10% buffered formalin. The kidneys were trimmed across both sides of the cranial lobes together with the genital glands. The tis-
sues were routinely processed and stained with hematoxylin and eosin for histological examination. Some kidney sections, in which multiple granulomas were observed, were also stained with Ziehl-Neelsen stain to examine for the presence of acid-fast organisms.

RESULTS

Findings relating to the kidneys: Development of lymphoid tissues and inflammatory lesions was found at varying frequencies in the kidneys of both the jungle crows and carrion crows. The incidences of renal lesions are summarized in Table 1 and representative histologies are illustrated in Fig. 1. Each lesion is described in detail below.

Frequent occurrence of other particular lesions was not observed in other organs, except for the parasitic diseases described below.

Lymphoid cell infiltrations in the kidneys: Lymphoid cell infiltrations comprised the most common histological finding in the kidneys of both species of crows. These lesions were characterized by mild to moderate infiltrations of mature small lymphocytes around the renal tubes in the renal cortex without definite demarcation and were found multifocally in most cases (Fig. 1A). Though the incidences reached nearly 100% in both species, we found considerable individual variability in the number and size of the lesions.

Lymphoid foci in the kidneys: In addition to lymphoid cell infiltrations, formations of lymphoid foci were frequently observed in the renal cortical regions of both species. Round- to oval-shaped lymphoid foci were demarcated by narrow connective tissues and consisted mainly of immature medium and large lymphocytes (Fig. 1B). The lymphoid foci differed distinctly from the lymphoid cell infiltrations in the presence of the connective tissue capsule and the difference in maturity of the lymphoid cells comprising it. Multiple lymphoid foci were randomly distributed in the renal cortex, with infrequent aggregation of several foci.

Lymphoid foci were also encountered in association with the veins of the kidneys, which developed adjacent to the parenchymatous small veins, including the intralobular vein and interlobular veins, and within the vessel walls of the larger veins, including the renal veins, renal portal veins and their branches. The foci sometimes developed just beneath endothelial cells within the vessel wall and protruded into the lumen of the large veins as shown in Fig. 1C. Multiple lymphoid foci were often observed around a single large vein. The avian kidney possesses not only efferent veins but also an afferent portal vein. Thus, we additionally classified the large veins into afferent veins and efferent veins, depending on whether they ran close to the artery (efferent vein) or not (afferent vein) [3], and found a predominance of efferent veins (Table 1).

Granulomatous inflammation in the kidneys: Both species of crows demonstrated a high incidence of suppurative granulomatous inflammation in the kidneys, characterized by solitary to multifocal formations of granulomas in the cortical regions and/or in the veins (Fig. 1D and E). Granulomatous lesions were composed of central areas of degenerated heterophils surrounded by multinucleated giant cells, macrophages and variable numbers of lymphocytes. In the veins, granulomas protruded into the vascular lumen from the vessel walls and occupied a large portion of the lumen with variable amounts of fibrin accumulation in some cases. Venous granulomas were concentrated in the efferent renal veins and their branches, as with the lymphoid foci. The causal agents were not observed within the granulomas. We further examined some kidney sections with multiple granulomas using Ziehl-Neelsen stain, but acid-fast organisms were not demonstrated in the affected or unaffected areas of the kidneys at any point. Renal lesions are occasionally found as a part of systemic infectious disease, but heterophilic granulomas have been preferentially found in the kid-
Fig. 1. Histologies of the renal lesions in the jungle crow. H&E staining. (A) Lymphoid cell infiltrations. The mature small lymphocytes had infiltrated the area around the renal tubules in the renal cortex. Scale bar=50 μm. (B) Lymphoid foci in the renal cortex. The two lymphoid foci developed adjacently in the renal cortex. Note that they were demarcated by narrow connective tissues and consisted mainly of immature medium and large lymphocytes. Scale bar=50 μm. (C) Lymphoid foci in the wall of the efferent renal vein which runs close to the arteries. The lymphoid focus protruded into the lumen of the large veins from the vessel walls. Scale bar=100 μm. (D) Heterophilic granuloma that developed in the renal cortex. Scale bar=100 μm. (E) Suppurative granulomatous inflammation of the efferent renal vein. A large granuloma had protruded into the vascular lumen and occupied a large portion of the lumen. The adjacent artery is shown at the lower right in the photograph. Scale bar=100 μm.
neys, without other organs of involvement, including the liver.

Variation of renal lesion development in the crows: These various renal findings were observed in the crows independently of place and date of capture, but most of the crows examined were captured in relatively limited regions in central Japan in 1991 and 1992. We therefore examined seven other jungle crows sent from Hyogo Prefecture in the western part of Japan in 2005 and frequently found the same renal lesions, including lymphoid cell infiltrations, lymphoid foci and granulomatous inflammations (data are not shown) as those in the crows captured around Gifu City, indicating the universality of renal lesion developments in corvids, even in the case of serious infections. Other parasites were more frequently observed in carrion crows than in jungle crows. Hepatic Trematode was the second most frequent parasite in carrion crows, followed by Nematode in the esophagus (Capillaria spp.), gizzard and small intestine. Adult trematodes containing a large number of eggs were observed in the dilated interlobular bile ducts but did not cause any inflammatory reaction in most cases.

Table 2. Major parasites in the jungle crows and carrion crows

<table>
<thead>
<tr>
<th>Host</th>
<th>Parasite</th>
<th>Stage</th>
<th>Infectious organs</th>
<th>Infectious tissues</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jungle crow</td>
<td>Coccidium</td>
<td>Gametogony</td>
<td>Small intestine</td>
<td>Villous epithelium</td>
<td>74.7% (124/166)</td>
</tr>
<tr>
<td>(Corvus macrorhynchos)</td>
<td>Trematode</td>
<td>Adult</td>
<td>Liver</td>
<td>Interlobular bile duct</td>
<td>5.4% (9/166)</td>
</tr>
<tr>
<td>Carrion crow</td>
<td>Coccidium</td>
<td>Gametogony</td>
<td>Small intestine</td>
<td>Villous epithelium</td>
<td>83.8% (62/74)</td>
</tr>
<tr>
<td>(Corvus corone)</td>
<td>Trematode</td>
<td>Adult</td>
<td>Liver</td>
<td>Interlobular bile duct</td>
<td>44.6% (33/74)</td>
</tr>
<tr>
<td></td>
<td>Nematode</td>
<td>Adult and/or Egg</td>
<td>Esophagus</td>
<td>Squamous epithelium</td>
<td>33.8% (25/74)</td>
</tr>
<tr>
<td></td>
<td>Nematode</td>
<td>Adult and/or Egg</td>
<td>Grizzard</td>
<td>Boundary of epithelial layer and keratinoid layer (Adult)</td>
<td>29.7% (22/76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Keratinoid layer (Egg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nematode</td>
<td>Adult</td>
<td>Small intestine</td>
<td>Lamina propria of villi</td>
<td>18.9% (14/74)</td>
</tr>
</tbody>
</table>

Fig. 2. Blood supply and distribution of lesions in the kidneys of crows. The avian kidneys receive a dual blood supply of arterial and renal portal blood. The distribution of the lesions strongly indicates the spread of the causal agents via renal portal blood.

Parasites

Parasites were frequently observed in various organs, with the prevalence and parasitic organs/tissues of major parasites being summarized in Table 2. Intestinal Coccidium was the most common parasite in both jungle crows and carrion crows. Gametocytes were observed in the villous epithelium of the small intestine, and there was no apparent inflammatory host tissue response to the presence of coccidia, even in the case of serious infections. Other parasites were more frequently observed in carrion crows than in jungle crows. Hepatic Trematode was the second most frequent parasite in carrion crows, followed by Nematode in the esophagus (Capillaria spp.), gizzard and small intestine. Adult trematodes containing a large number of eggs were observed in the dilated interlobular bile ducts but did not cause any inflammatory reaction in most cases.

DISCUSSION

The present study revealed the characteristic distribution of lymphoid tissues and inflammatory lesions in Japanese wild crows, which were concentrated in their kidneys. Interestingly, there was little difference in the prevalence of renal lesions between jungle crows and carrion crows, and these findings were found independently of the place and date of capture, indicating the universality of renal lesion development in the Corvus species.

The high incidences of renal inflammatory lesions were considered to reflect the frequent entry of causal agents into the kidneys. We found heterophilic granulomas preferentially formed in the kidneys of the crows without involvement in other organs. In avian species, granulomas develop in response to a wide spectrum of external causal agents,
including various kinds of bacteria [7] which can enter the kidney either by hematogenous spread or via the ascending ureters [10]. Initial lesions would be present in the glomeruli in the former case and in the renal tubules in the latter case [10]. However, the granulomas were concentrated in the renal cortex and the veins, while the glomeruli and urinary tract remained intact in the kidneys of the crows, indicating the possibility of causal agents entering via other pathways. We subsequently considered the avian renal portal system as a candidate for the route of entry into the kidney. In addition to the arterial blood supply, the afferent venous blood enters the avian kidneys through the renal portal vein from the lower part of the body, including the hind limb, tail region and lower intestinal tract. Whereas arterial blood first passes the glomeruli before flowing into the peritubular capillary sinuses in the renal cortex, the portal venous blood flows directly into the capillary sinuses and then drains into the efferent vein with arterial blood [3, 5, 10]. Thus, causal agents could reach the renal cortex and the efferent veins via the renal portal blood without affecting the glomeruli, and the distribution of the renal lesions strongly indicates the spread of the causal agents via the hematogenous route (Fig. 2). Interestingly, Aruji et al. [1] isolated many kinds of enterobacteria from cloacal and cecal contents of jungle crows captured in Tokyo, and members of Enterobacteriaceae are known to affect the avian kidney [10].

The high frequency of lymphoid foci and lymphoid cell infiltrations and their distribution strongly support frequent entry of external agents through renal portal blood. The same distribution was seen in the kidneys through the granulomas, which had developed in the cortex and within or adjacent to the walls of veins. In histological analyses, we differentiated cortical lymphoid foci from vein-associated lymphoid foci, but the former also developed in association with the blood circulating in the peritubular capillary sinus of the renal cortex. Anatomically, birds lack the encapsulated lymph nodes that mammals have, and instead have organized lymphoid tissues in areas commonly stimulated by external antigens, such as the respiratory and digestive tracts [4]. Similarly, the hematogenous entry of the antigens via the renal portal vein lends itself to a high frequency of lymphoid foci and lymphoid cell infiltrations in the kidneys of crows.

In this study, we were unable to identify the causal agents involved in renal lesion development in Japanese crows. We demonstrated the absence of acid-fast organisms in the affected renal tissues by means of Ziehl-Neelsen stain. However, further analyses, including bacteriological and virological tests, are required to identify the causal agents because avian species form granulomas in response to a wide range of pathogens other than mycobacteria [7].

We also identified the types of parasites in the jungle crows and carrion crows based on the histological analyses. Though further studies are necessary to identify the species of parasites, our histopathological data will provide useful information for identifying the parasites and understanding their pathogenicities in Japanese wild crows. Intestinal Coccidium and hepatic Trematode are high on the list of parasites. The high prevalence and the lack of host tissue response to the parasites suggest that they are parasitic in nature. Though there have been no reports concerning coccidiosis in Japanese wild crows, five species of Isospora have previously been reported from Corvus hosts, including Corvus macrorhynchos and Corvus corone, living in other regions [8, 9].

The present study revealed the characteristic concentrations of the lymphoid tissues and inflammatory lesions in the kidneys of Japanese wild crows. Monitoring the conditions of wild crows is becoming increasingly important, especially in monitoring epidemics of infectious diseases, such as the highly pathogenic avian influenza and West Nile fever. We hope that our data will contribute to the appropriate evaluation and a better understanding of these pathological conditions.

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REFERENCES

