Case Report

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Retrospective Analysis of Postmortem Findings in Oriental Stork (*Ciconia boyciana*) from Korea (2019-2023)

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Abstract The Oriental stork (*Ciconia boyciana*), an endangered species, faces significant threats despite extensive conservation efforts aimed at their reintroduction and long-term survival in South Korea. Understanding the primary causes of mortality and identifying predominant pathological lesions are crucial for enhancing these efforts. This study aimed to investigate the causes of unexplained mortality in Oriental storks between 2019 and 2023 through comprehensive postmortem examinations. Twelve storks, including both wild and captive birds, classified as "unknown" deaths, were examined. The results revealed that parasitic infections, particularly in wild storks, were frequently observed (41.7%). Additionally, gastrointestinal obstruction due to the ingestion of foreign materials was identified in a wild stork, highlighting the impact of environmental pollution. Despite the prevalence of inflammatory lesions in liver (58.3%), no cases of hepatitis were confirmed, except for one case of liver rupture in a wild stork. Notably, three nestling death cases from a single litter of captive storks suggested potential parental infanticide or lack of parental care. The overall results highlight the importance of addressing parasitic infections and environmental risks to improve the survival of wild Oriental storks, and emphasize the need for effective management in breeding facilities. Continuous monitoring and pathological evaluations for mortality cases are essential for understanding disease trends and developing adaptive conservation strategies to ensure the long-term preservation of this endangered species.

Key words mortality, oriental stork, pathologic diagnosis, retrospective, South Korea.

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Introduction

The Oriental stork (*Ciconia boyciana*), belonging to the family Ciconiidae and order Ciconiiformes, is listed as endangered (EN) in the International Union for Conservation of Nature Red List of Threatened Species (1). This large migratory wading waterbird is distributed throughout Eastern Asia, including Russia, Taiwan, Mongolia, China, Hong Kong, Japan, and South Korea (1). In South Korea, this species is protected as a Natural Monument, managed in breeding facilities for captive propagation, and released into government-designated reintroduction sites. The release of captive Oriental storks began in September 2015, following significant preparatory work since the late 1990s, including the establishment of the Eco-Institute for Oriental Stork by Korea National University of Education in 1996.

The reintroduced storks have been monitored to ensure their survival and reproduction in the wild, with significant efforts made to create suitable habitats and minimize human-wildlife conflicts. Despite these efforts, the population faces numerous threats including habitat destruction, environmental pollution, and various human activities. A recent Japanese study analyzing the causes of injury and death among reintroduced storks between 2005 and 2021 reported that nearly half of the deaths were attributable to human activities and 22.9% of cases were "unknown" causes (10).

Postmortem examination of wildlife can elucidate the causes of unexplained (unknown) deaths. Retrospective analyses of these findings provide essential insights into the health challenges faced by wildlife species, including Oriental storks. This valuable information enables the implementation of targeted interventions to mitigate theses health problems in wild and captive storks, and helps control potential diseases outbreak in their populations (2,4,9). Furthermore, these data are crucial for developing effective conservation strategies and improving the success of reintroduction program (3,10). However, few studies have focused on the cause of death in Oriental storks in Korea, and most available data limited to individual case reports rather than retrospective analyses (2,4,7).

This study aimed to analyze the causes of unexplained mortality (unknown death) in Oriental storks in Korea from 2019 to 2023 through whole postmortem examinations. Additionally, we compared major pathological lesions in wild and captive Oriental storks, which could provide valuable insights into disease trends and major mortality factors in this endangered species.

Case Report

Of 42 Oriental stork mortality cases from February 2019 to December 2023, causes of death were categorized according to a previous study (10). Fourteen cases (33.3%) were classified as "unknown," prompting postmortem examinations on 12 storks to confirm the cause of death. These cases included storks found dead in the wild (wild Oriental storks, n = 6) and in the breeding facility at Yesan Oriental stork Park (captive Oriental storks, n = 6). The dead storks were immediately delivered to Animal Diagnostic center, Jeonbuk National University, or stored at 4°C during 1-2 days before necropsy. Cases with a clear cause of death (e.g., predation by other species or collision with electric power-lines) were not subjected to histopathological examinations.

Initially, the dead storks were grossly inspected by designated veterinary pathologists regarding with their history. The gross lesions were recorded, and lesioned organ tissues were fixed in 10% neutral-buffered formalin, and washed for >8 hours with tap water. Tissue samples were subjected to routine processing using a Myr Spin Tissue Processor-STP 120 (Tarragona, Spain). The processed tissue samples were embedded in paraffin wax (Leica Biosystems, Newcastle, UK) using an embedding station (Sakura, CA, USA). Three-micrometer-thick tissue sections were obtained using a craftex CR-603 microtome (Leica Biosystems) and mounted on glass slides (Muto Pure Chemicals, Tokyo, Japan). Hematoxylin and eosin (H&E) staining was performed automatically using a Myr Eva-slide stainer SS-30 (Tarragona). Digital imaging was conducted using an Olympus microscope (model BX53F; Olympus, Tokyo, Japan) in conjunction with Olympus digital imaging software.

The pathological diagnostic profiles of 12 necropsied Oriental storks [wild (n = 6) and captive (n = 6)] are shown in Table 1. Two storks (16.7%) showed gross traumatic lesions, including an open fracture in the leg (Fig. 1A) and hemorrhage in the pectoralis muscles (Fig. 1B). In addition, two cases (16.7%) had a low body condition score (BCS). Half of the cases (n = 6, 50.0%) had macroscopic lesions in the gastrointestinal tract (Fig. 1C), and most cases (n = 5) presented with numerous small (1-2 cm) globular nodules with thick walls in the serosal layers of small intestine, and in proventriculus as well as gizzard. Ulcerated lesions were observed in the ventriculus (Fig. 1D), and small intestine (Fig. 1E) in two cases (16.67%). In the heart, one case (B06) showed cardiovascular system dysfunction (hypertrophy of the bicuspid valve) (Fig. 1F). The multifocal white foci (Fig. 1G), and ruptures (Fig. 1H) was observed in the liver in two cases. In addition, multifocal white foci were observed in the kidneys (n = 2, 16.67%) and

Table 1. Pathological diagnostic profiles of the necropsied Oriental storks

Group	Case ID	Sex	Developmental stage (year of birth)	Death date (MM/YY)	Major gross lesions	Major histopathological lesions	Pathological diagnosis
Wild Oriental stork	E42	M	Juvenile (2020)	01/2021	Numerous nodules in gastrointestinal tracts	Parasitic infection in gastrointestinal tract, acute enteritis	Acute enteritis
	E46	F	Adult (2001)	01/2021	Crust formation in esophagus, multifocal white foci in liver, starvation	Ulceration in gastrointestinal tract, proventriculitis, perivascular inflammatory cell infiltration in liver, anthracosis in parabronchus	Occlusion of the passage in proventriculus
	B10	F	Adult (2006)	05/2021	Numerous nodules in gastrointestinal tracts	Parasitic infection in gastrointestinal tract	Unknown
	E63	F	Fledgling (2021)	06/2021	Numerous nodules in gastrointestinal tracts, liver rupture, hemorrhage in pectoralis muscle	Parasitic infection in gastrointestinal tract, enteritis, severe perivascular inflammatory cell infiltration in liver	Physical trauma (liver rupture), enteritis
	A01	M	Adult (2013)	04/2022	-	Ulceration in gastrointestinal tract, bronchitis	Unknown
	C60	M	Adult (2019)	04/2023	Numerous nodules in gastrointestinal tracts, open fracture in leg	Parasitic infection in gastrointestinal tract, perivascular inflammatory cell infiltration in liver, bronchitis, anthracosis	Physical trauma
Captive Oriental stork	22-0220(1)	M	Nestling (2022)	03/2022	-	Enteritis, perivascular inflammatory cell infiltration in liver, interstitial nephritis	Severe interstitial nephritis
	22-0220(2)	M	Nestling (2022)	03/2022	Multifocal white foci in kidney	Perivascular inflammatory cell infiltration in liver, interstitial nephritis	Severe interstitial nephritis
	22-0220(3)	M	Nestling (2022)	03/2022	Multifocal white foci in kidney	Perivascular inflammatory cell infiltration in liver, interstitial nephritis	Severe interstitial nephritis
	C03	M	Adult (2009)	05/2022	-	Perivascular inflammatory cell infiltration in liver	Unknown
	B06	F	Adult (2017)	05/2023	Numerous nodules in gastrointestinal tracts, ulceration in gastrointestinal tract, multifocal white foci in spleen, hypertrophy of bicuspid valve, starvation	Parasitic infection in gastrointestinal tract, enteritis, ulceration in gastrointestinal tract, multifocal foci in liver, multifocal necrosis in spleen	Necrotic enteritis, cardiovascular disorder
	C25	F	Adult (2012)	06/2023	-	Calcification in trabecular artery of spleen	Unknown

spleen (n = 1, 8.33%) (Fig. 1I, J, respectively).

Histopathological examination revealed a parasitic invasion (n = 5, 41.7%) (Fig. 2A, B, C) and proventriculus/enteritis lesions (n = 5, 41.7%) (Fig. 2D, E), which were most frequently observed in H&E stained gastrointestinal tract sections, followed by ulcerations (n = 3, 25.0%) (Fig. 2F). Eight cases of liver tissue sections (66.7%) showed multifocal inflammatory foci (Fig. 3A) or perivascular inflammatory cell infiltration (Fig. 3B). In the spleen, one case (n = 1, 8.3%) showed multiple necrotic lesions (Fig. 3C), and another case (n = 1, 8.3%) presented with calcification in the trabecular artery of the spleen (Fig. 3D). Severe nephritic lesions, primarily consisting of heterophiles, were observed in the kidney tissue sections of three birds (25.0%) (Fig. 3E, F). Among the heart tissue sections, one case (n = 1, 8.3%) showed fibrosis of the bicuspid valve (Fig. 4A). Two necropsied Oriental storks (16.7%) had bronchial lesions (Fig. 4B) in the lungs, and anthracosis was observed in two cases (16.7%) (Fig. 4C).

Despite detailed gross and histological observations, the cause of death could not be determined in 33.3% (n = 4) of cases. Except for these unknown cases, severe interstitial nephritis was the most common cause of death (n = 3). However, these three storks were all young birds, under 10 days of age, from the same litter. Although inflammatory lesions in the liver (n = 7, 58.3%) and helminth infections (n = 5, 41.7%) were observed in many cases, they were seldom determined to be the final cause of death. Three storks with severe parasitic infection lesions were finally diagnosed with

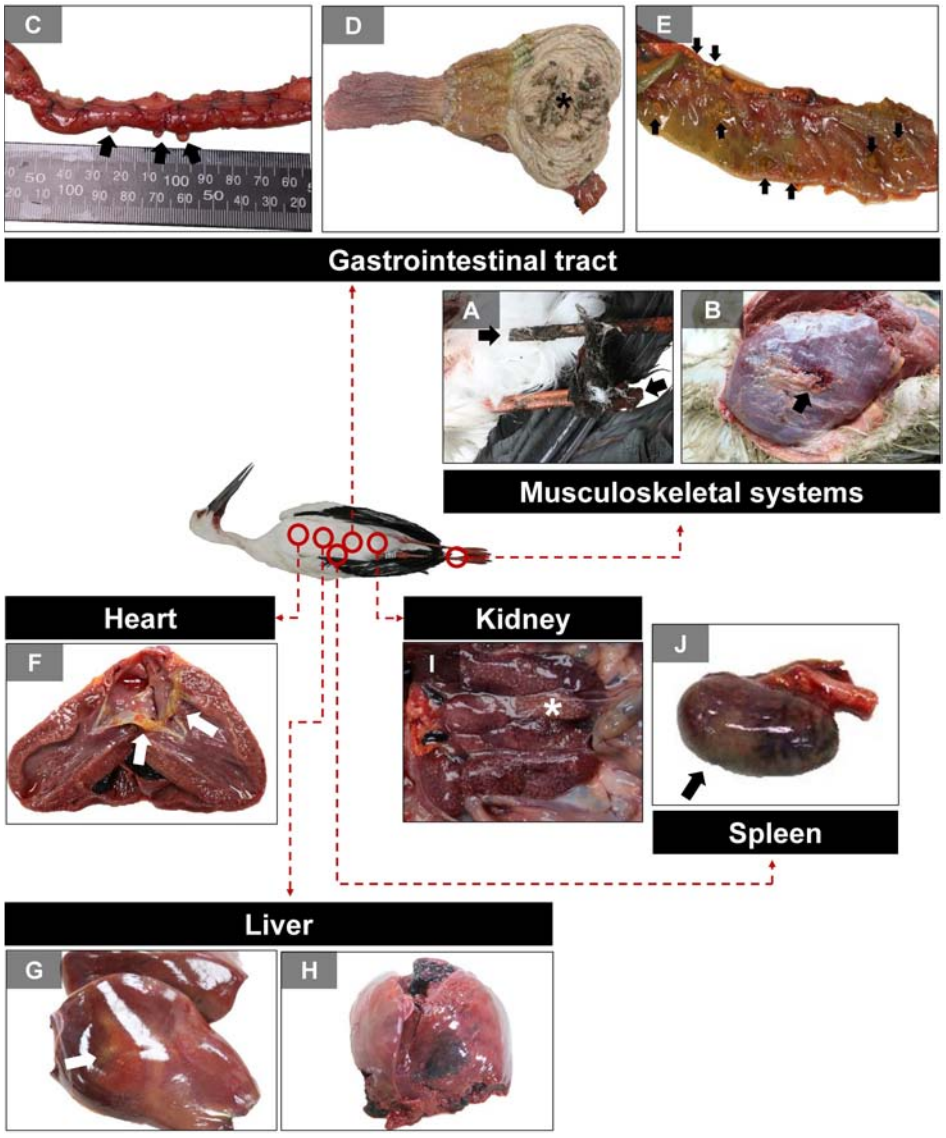

Fig. 1. Representative gross lesions in necropsied Oriental storks (n = 12). (A) C60 case. Open fractures (arrows) in the right leg. (B) E63 case. Hemorrhage (arrows) in the pectoralis muscle. (C) E42 case. Numerous nodules (arrows) in the small intestine. (D) B06 case. Severe multifocal ulceration lesions (asterisk) in the gizzard. (E) B06 case. Severe multifocal ulcers (arrows) in the jejunum. (F) B06 case. Hypertrophy of the bicuspid valve (white arrows) in the heart. (G) E46 case. Multifocal white foci (white arrow) in the liver. (H) E63 case. Liver rupture. (I) 22-0220(3) case. Multifocal white foci (white asterisk) in kidneys. (J) B06 case. Multifocal white foci (arrow) in the spleen.

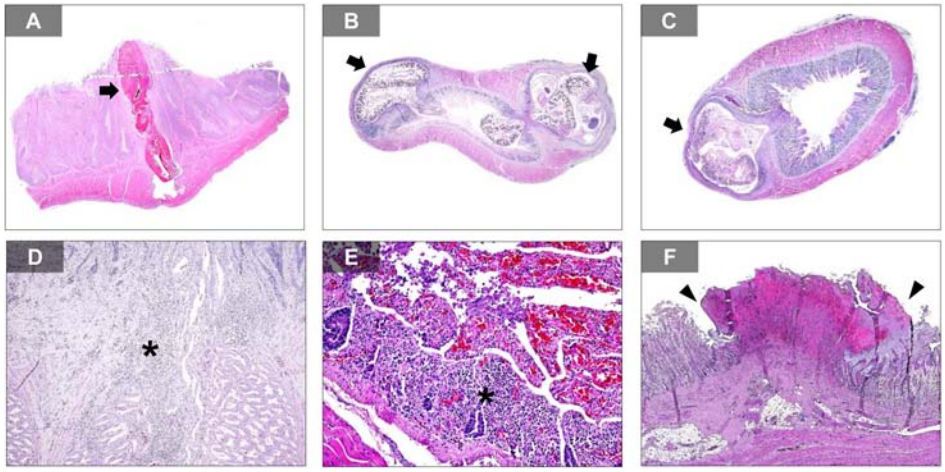

Fig. 2. Representative histopathological lesions in the gastrointestinal tract of necropsied Oriental storks (n = 12). (A) C60 case. Parasite infection (arrow) in the proventriculus. (B) E63 case. Parasite infection (arrows) in the small intestine. (C) B10 case. Parasite infection (arrows) in small intestine. (D) E63 case. Severe inflammatory cell infiltration (asterisk) in submucosal layer of proventriculus. (E) E42 case. Inflammatory cell infiltration (asterisk) in the mucosal layer of the jejunum. (F) B06 case. Severe ulceration (arrowheads) in gizzard. H&E.

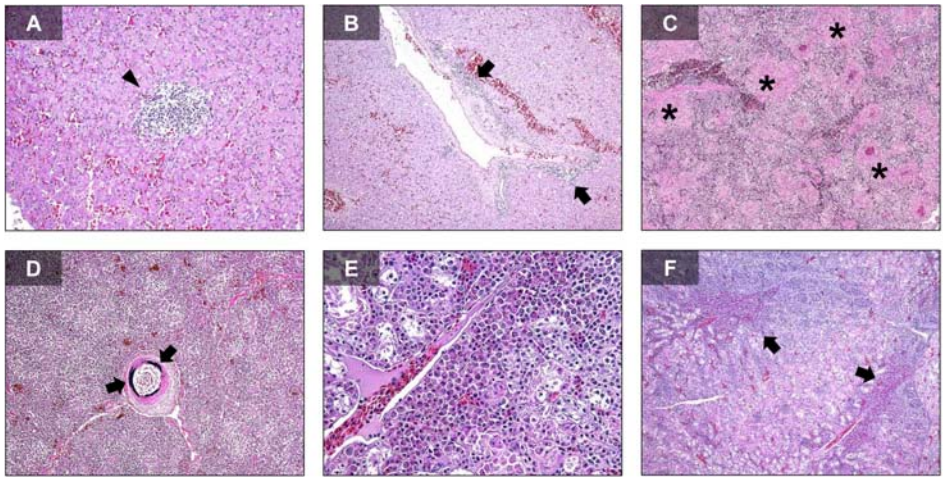

Fig. 3. Representative histopathological lesions in the liver, spleen, and kidneys of necropsied Oriental storks (n = 12). (A) E63 case. Inflammatory foci (arrowhead) in the liver. (B) E63 case. Perivascular inflammatory cell infiltration (arrows) in the liver. (C) B06 case. Multifocal necrosis (asterisk) in the spleen. (D) C25 case. Calcification (arrows) in the splenic trabecular artery. (E) 22-0220(2) case. Severe nephritis at low magnification. (F) 22-0220(2) case. Severe nephritis (arrows) primarily consists of heterophils observed at a high magnification. H&E.

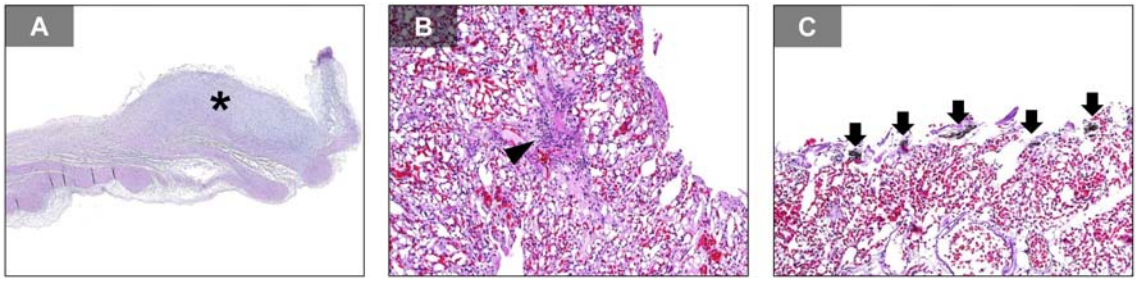

Fig. 4. Representative histopathological lesions in the heart and lungs of necropsied Oriental storks (n = 12). (A) B06 case. Fibrosis (asterisk) in the bicuspid valve. (B) C60 case. Inflammatory foci (arrowhead) in the lungs. (C) E46 case. Anthracosis (arrows) in lungs. H&E.

enteritis, including two wild and one captive Oriental stork.

Discussion

One-third of stroke deaths (33.3%) in Korea over the recent five-year period (2019-2023) could not be initially classified based on clinical history. Postmortem examinations were conducted for 12 "unknown" cases to confirm the pathological diagnosis.

The presence of numerous small globular nodules (n = 5, 41.7%) in the gastrointestinal tract, indicative of parasitic infection, was one of the most frequently observed gross

lesion. Notably, the majority of these lesions (n = 4) were observed in wild storks, suggesting that wild Oriental storks are more exposed to the risk of parasitic infections than captive storks. Although the specific parasite species could not be confirmed in this study, it was assumed to be *Chaunocephalus ferox* based on previous studies (2,5,6). *Chaunocephalus ferox* was reported to cause several health problems in other stork species within *Ciconia* genus, such as the white stork (*C. ciconia*) and black stork (*C. nigra*) in different regions (5,12,13). These results highlighted the need for further investigation into its potential impact on Oriental storks (*C. boyciana*) in Korea. Interestingly, more than half of parasite infection cases (3 of 5 cases, 60.0%) presented enteritis on histopathological examination with two of these cases involving young wild storks (<1 year old). These findings were consistent with previous studies suggesting that a large number of adult parasitic infections are required to cause cachexia and eventual death (5,12). However, parasitic infections in young wild birds could impair flight performance, predatory effectiveness, and predisposition to secondary trauma (12). Therefore, continuous monitoring of *Chaunocephalus ferox* infections in Oriental storks is crucial, especially in young storks, and this parasitic infection should be carefully considered during the rescue and treatment processes.

There have been many case reports in Oriental storks of gastrointestinal tract obstruction due to ingestion of foreign materials, including plastic or rubber (4,9). In this study, one wild stork (8.3%) was pathologically diagnosed as an obstruction of the passage in the proventriculus. Given the significant threat that environmental pollution poses to wildlife, habitat restoration with effective waste management and regular health monitoring in reintroduced Oriental storks should be prioritized to reduce mortality related with the ingestion of foreign materials.

Interestingly, despite the high prevalence of inflammatory cell infiltration in the liver (58.3%), none of the case was diagnosed with hepatitis. These results suggest that histopathological lesions are common in Oriental storks, but may not be the primary cause of mortality. However, our findings are limited in establishing a general conclusion because of the lack of viral investigations in liver tissues with lesions. In Europe, various viral infections (e.g., herpesvirus and hepadnavirus) have been reported in white storks with necrotic lesions in the liver (8,11). Further studies are needed to fully understand the causes of death in Oriental storks with liver lesions, and to determine the prevalence of viral infections threatening the stork population.

In this study, severe interstitial nephritis was diagnosed in three nestlings from the same litter of captive Oriental storks. Although the exact causes of renal lesions in these cases were unclear, it is presumed that parental infanticide or lack of parental care was responsible for the death. Previous studies have reported parental behaviors such as "throwing-out-of-the-nest," which could explain these findings (5,13). These results suggested that effective management and monitoring of newly hatched storks, particularly in breeding facilities, are needed to ensure their survival and to eventually preserve Oriental stork populations.

Since 2015, the health status of Oriental storks in South Korea has been monitored by tracking their movements using GPS and analyzing their physical condition as well as feeding behaviors. Continuous accumulation of these data should be need to identify major health-threatening factors in Oriental storks, which could facilitate the development of risk management strategies for successful conservation of the Oriental stork population in South Korea. In addition, future studies are essential for developing detailed treatment protocol manuals specifically for the management of diseased Oriental storks.

Conclusions

Our results provide a comprehensive pathological analysis of the multifactorial causes of mortality in Oriental storks from South Korea. These findings highlight the importance of addressing parasitic infections and environmental pollution for wild Oriental storks to improve their survival rate after reintroduction. In captive storks, effective management of newly hatched birds in breeding facilities is essential for the success of reintroduction program with preservation of this species. Furthermore, continuous monitoring and comprehensive pathological evaluations in cases of unexplained (unknown) mortality are critical for understanding disease dynamics in wild and captive storks, and developing adaptive conservation strategies to ensure the long-term preservation of the Oriental stork population.

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Conflicts of Interest

The authors have no conflicting interests.

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