

Case Report—

Fungal Airsacculitis Associated with Multiple Helminth Infestations in a Black-Eared Kite (*Milvus migrans*)

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SUMMARY. A young, female black-eared kite was rescued from a small reservoir adjacent to a rice paddy in Nagano Prefecture, Japan. The bird was given a fluid diet through the esophagus and started to eat by herself from the fifth day. Her fecal samples were examined for parasites on the seventh day and fluke eggs were detected. *Capillaria* and Ascarididae eggs were also detected from day 19 and day 32, respectively. The bird started to show loss of appetite from day 22 and finally showed no appetite on day 35. On day 38, the bird was treated with Profender Spot (Bayer Health Care, Tokyo, Japan) but died on day 41. A necropsy revealed a thickened air sac associated with considerable fungal growth. Histopathologic examination showed that the mucous membrane of the saccobronchus was thickened with hyphal proliferation, and the fungus was identified as *Aspergillus fumigatus*. A number of trematodes, thin nematodes, and four roundworms were obtained from the alimentary tract. Parasitologically, they were identified as *Neodiplotomum pseudattenuatum*, a *Capillaria* sp., and *Porrocaecum phalacrocoracis*, respectively. In conclusion, the bird was diagnosed as having fungal airsacculitis associated with multiple helminth infestations.

RESUMEN. *Reporte de Caso*—Aerosaculitis fúngica asociada con una infestación múltiple por helmintos en un milano negro (*Milvus migrans*).

Se rescató un milano negro joven de sexo femenino de un pequeño reservorio adyacente a un campo de arroz en la Prefectura de Nagano, Japón. El ave recibió una dieta de líquidos a través del esófago y empezó a comer por sí misma a partir del quinto día. Sus muestras fecales fueron examinadas para detectar parásitos en el séptimo día y se detectaron huevos de tremátodos. También se detectaron huevos de *Capillaria* y de Ascarididae a partir de los 19 y 32 días, respectivamente. El ave empezó a mostrar pérdida del apetito a partir del día 22 y finalmente, dejó de comer a los 35 días. En el día 38, el ave fue tratada con Profender Spot (Bayer Health Care, Tokyo, Japan), pero murió al día 41. Durante la necropsia se observó un saco aéreo engrosado asociado con un considerable crecimiento de hongos. El examen histopatológico demostró que la membrana mucosa de los bronquios recurrentes (saccobronchus) estaba engrosada con proliferación de hifas, el hongo fue identificado como *Aspergillus fumigatus*. Se obtuvieron del tracto digestivo varios trematodos y nemátodos pequeños y cuatro nematodos redondos. Estos parásitos fueron identificados como *Neodiplotomum pseudattenuatum*, una *Capillaria* sp., y *Porrocaecum phalacrocoracis*, respectivamente. En conclusión, se diagnóstico que el ave sufría una aerosaculitis fúngica asociada con una infestación por múltiples helmintos.

Key words: *Aspergillus fumigatus*, black-eared kite, *Capillaria*, fungal airsacculitis, helminthiosis, *Porrocaecum phalacrocoracis*, *Neodiplotomum pseudattenuatum*

Abbreviations: EPG = eggs per gram; H&E = hematoxylin and eosin stain; Grocott = Grocott-Gomori methenamine-silver stain; PAS = periodic acid-Schiff stain

There has been much public concern about infectious diseases in wild birds since 2007, when a highly pathogenic avian influenza virus was isolated from diseased, wild swans in the northern part of Japan. The authors have already reported some infectious diseases in birds, such as avian tuberculosis in a hooded merganser, *Salmonella* infection in cage birds and zoo birds, *Pseudomonas* infection in a Japanese ptarmigan, and candidiasis in cage birds (8,9,10,11,12,13). Recently, the authors encountered a case of fungal disease associated with multiple helminthiosis in a rescued young black-eared kite (*Milvus migrans*). As far as we know, there have been only a few reports of fungal disease and helminthiosis in black-eared kites (7). Occurrences of *Aspergillus fumigatus* infection in a black-eared kite, and identification of helminthes detected in the alimentary tract of the bird, are described in this report.

CASE REPORT

Treatment of the bird. A young, female black-eared kite was rescued from a small reservoir adjacent to a rice paddy along the Chikuma River in Nagano Prefecture, Japan on 20 July 2007. At the time when the bird was rescued, the reservoir was 180 cm in length, 98 cm in width, and 98 cm in depth and was filled with water to a depth of about 40 cm. Because a pair of kites, probably her parents, was flying above the paddy field and crying for 2–3 days, it was thought that the young bird had fallen into the reservoir soon after leaving the nest.

The day after it was rescued, the young kite was brought to the author's clinic because the bird was extremely weakened. The young kite was emaciated and weighed 900 g at initial presentation. The feathers and down around the back and abdomen had hardened from being in water for several days and the bird could not fly. The bird was immediately placed in an admission room in a stainless steel cage 60 cm in length, 55 cm in width, and 50 cm in height. The kite was given 5% glucose solution and a pet fluid diet (a/d, Hill's Pet

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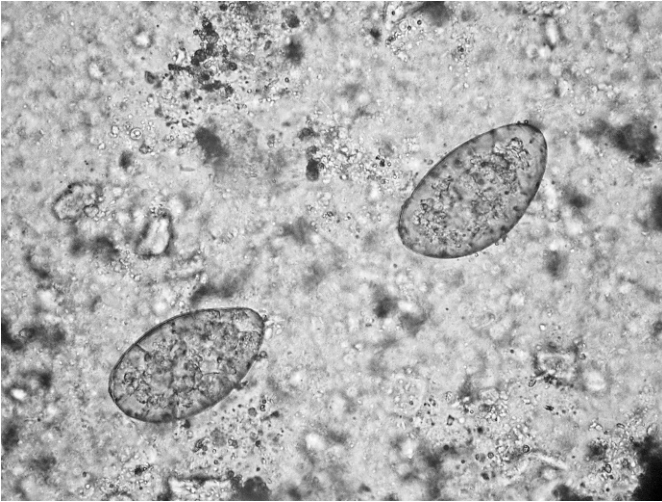


Fig. 1. Fluke eggs detected in a fecal sample from the kite.

Nutrition, Tokyo, Japan) through the esophagus using a catheter. The kite started to eat by herself from day 5 and was given canned food for cats (FPW, Schering-Plough Animal Health, Tokyo, Japan). The kite's weight gradually increased to 920 g on day 7 and to 940 g by day 20.

Although there were no apparent clinical symptoms, such as diarrhea, fecal samples were examined for parasites on day 7 and numerous fluke eggs, roughly 15,000 eggs per gram (EPG), were found (Fig. 1). Fecal examination was carried out every day from the seventh day. A certain number of *Capillaria* eggs, and moderate numbers of Ascarididae eggs (roughly 3000 EPG), were detected from day 19 and from day 32, respectively (Figs. 2, 3). Average lengths and widths were $97.0 \times 59.1 \mu\text{m}$ ($n = 30$) for the fluke eggs, $95.7 \times 68.4 \mu\text{m}$ ($n = 20$) for the Ascarididae eggs, and $57.1 \times 27.0 \mu\text{m}$ ($n = 5$) for the *Capillaria* eggs.

The bird remained in good health until the 21st day, although the down of the abdomen remained matted and the bird could not fly. However, the bird started to show loss of appetite from day 22. When canned food was repeatedly given to the bird through the mouth, using forceps, the bird ate the food and regained appetite until day 26. The kite showed anorexia again on day 34 and finally showed no appetite at all by day 35. Though canned food was given through the esophagus, using forceps, the kite vomited it



Fig. 2. *Capillaria* sp. egg detected in a fecal sample from the kite.

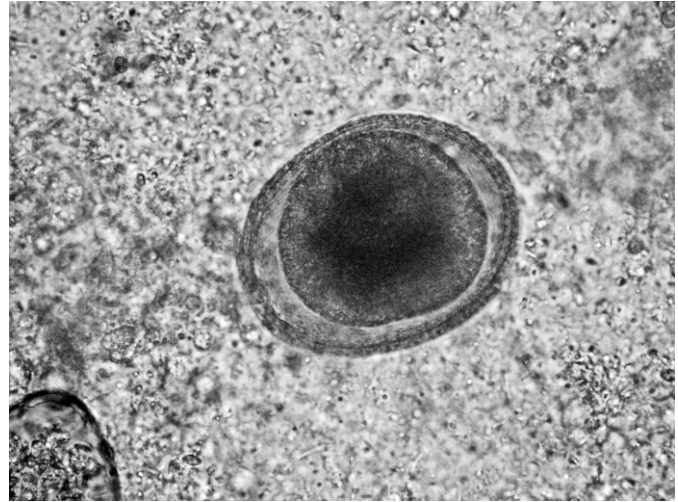


Fig. 3. Ascarididae roundworm egg detected in a fecal sample from the kite.

immediately. The bird was treated on the 38th day with an anthelmintic, Profender Spot (0.35 ml pipet, Bayer Health Care, Tokyo, Japan), containing 30 mg of praziquantel and 7.5 mg of emodepside. On day 40, the bird regurgitated a male roundworm and then died in the morning on day 41.

Pathologic examination. Necropsy was performed on the morning of 30 August 2007 (the day the kite died). The bird weighed only 680 g and the air sac was conspicuously thickened and yellowish-brown.

Considerable fungal growth was observed all over the air sac, and the fungal colonies were bluish-green in the center and silky-white in the periphery (Fig. 4). No specific lesions were recognized in other organs.

For histopathologic examination, the heart, lungs, liver, kidneys, spleen, and ovary were fixed in 15% buffered formalin, and paraffin sections were prepared and stained with hematoxylin and eosin stain (H&E), periodic acid-Schiff stain (PAS), and Grocott-Gomori methenamine-silver stain (Grocott). The mucous membrane of the saccobronchus was thickened with hyphal proliferation (Fig. 5). The hyphae were septate and the wall was thin and positive for PAS and Grocott. Numerous conidial heads, with hyphal proliferation, were

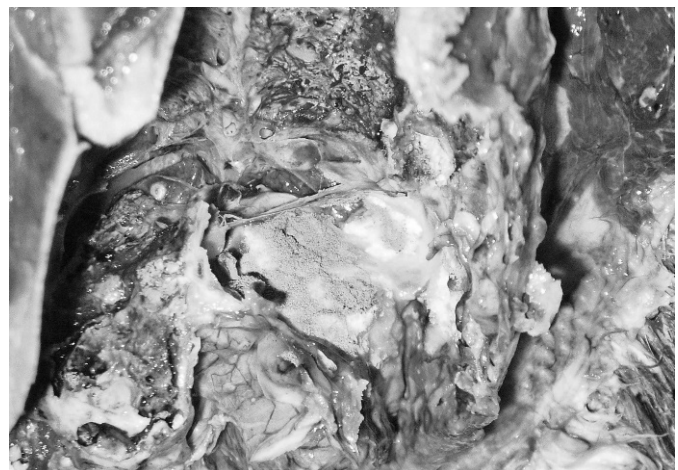


Fig. 4. The air sac was conspicuously thickened and considerable fungal growth was observed. The fungal colonies were bluish-green in the center and silky-white in the periphery.

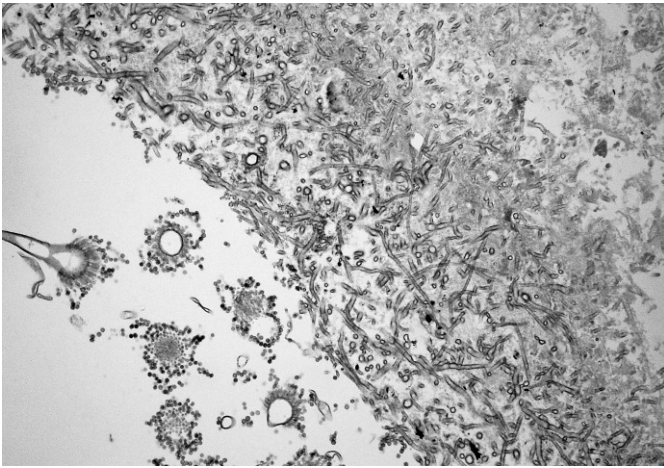


Fig. 5. The mucous membrane of the saccobronchus was thickened with hyphal proliferation. Numerous conidial heads were observed in the lumen of the saccobronchus (H&E).

observed in the lumen of the saccobronchus. The vesicle of the conidial head was flask-shaped and covered with monoseriate phialides having chains of conidia. According to the morphologic features, the fungus was identified as *Aspergillus fumigatus* (1,2). No histopathologic lesions were recognized in the heart, liver, kidneys, spleen, or ovary.

Parasitologic examination. The stomach and intestine that had been fixed in 15% buffered formalin were examined for helminthes. A number of trematodes, thin nematodes, and four roundworms were obtained from the intestine.

Ten flukes stained with hematoxylin and carmine solution were 1.6–1.87 mm in length and were divided into anterior (0.83–0.98 × 0.41–0.60 mm) and posterior (0.83–0.98 × 0.41–0.60 mm) parts of the body (Fig. 6). The testes were tandem and showed the shape of a butterfly, and the anterior and posterior testes measured 0.16–0.20 × 0.24–0.34 mm and 0.20–0.22 × 0.27–0.34 mm, respectively. A round ovary was in front of the anterior testis and was 0.20–0.24 × 0.13–0.17 mm in size. From those traits, the

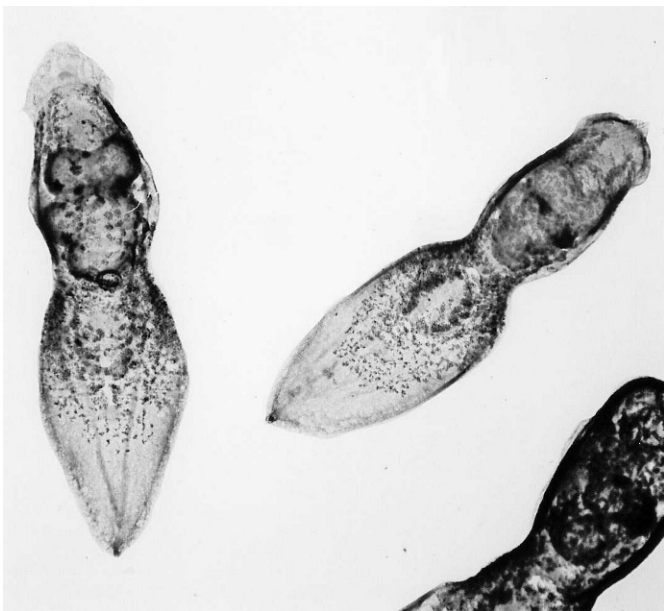


Fig. 6. *Neodiplotomum pseudattenuatum* stained with hematoxylin and carmine solution.

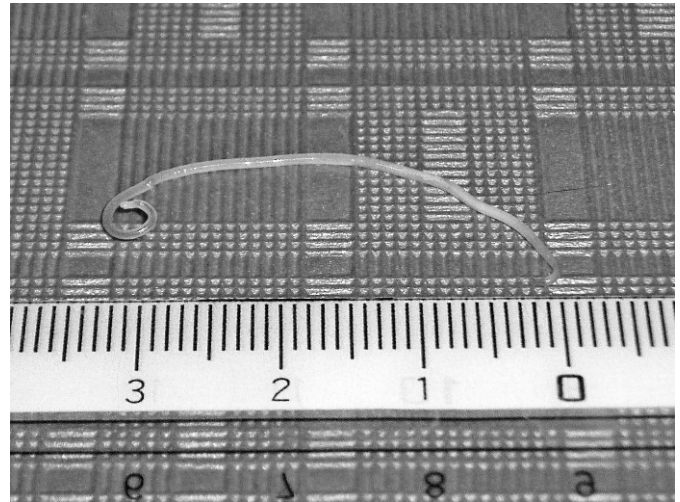


Fig. 7. A roundworm regurgitated by the kite on the 40th day. Scale = cm.

flukes were identified as *Neodiplotomum pseudattenuatum* (Diplostomatidae).

Most of the thin nematodes were broken, and only one male worm and one female worm that had complete bodies were useful for identification. The male and female worms, cleaned in lactophenol, measured 17.9 × 0.06 mm and 20.9 × 0.09 mm, respectively. They had sticocytes in the esophagus, although the exact number of sticocytes was unclear, and the body width hardly differed between the anterior and posterior portions of the body. The spicules of the male were 1.1–1.2 mm in length. Those traits supported the identification of the nematodes as a *Capillaria* sp.

The roundworms included two males and two females. They were cleaned in lactophenol, revealing three lips (1.9–2.1 mm in size) in the anterior tip and the ventricular appendix. The mean measurements of the body were 47.4 × 0.94 mm for the males and 64.7 × 10.3 mm for the females, and the tail lengths were 0.29–0.30 mm (Fig. 7). There were 22–23 pairs of preanal and five pairs of postanal papillae and the spicules were 0.8 mm. Based on these traits, the roundworms were identified as *Porrocaecum phalacrocoracis*.

Diagnosis. According to the results of pathologic, histopathologic, and parasitologic examinations, the bird was diagnosed as having fungal airsacculitis associated with multiple helminthiasis.

DISCUSSION

In Nagano Prefecture, where the present case was found, the breeding season of the black-eared kite usually starts the end of February and the chicks generally hatch in the middle of April (3). Since the young female bird in this case was rescued on 20 July, her age was estimated to be about 3 months old at initial presentation, and it was strongly suspected that the bird had fallen into the reservoir soon after leaving the nest.

Helminthes, including *N. pseudattenuatum*, have been detected in black-eared kites in Japan (14,15). However, to our knowledge, there have been no reports on detection of *P. phalacrocoracis* and *Capillaria* sp. in black-eared kites. Thus, this is the first record of these helminthes in this host species. Although *Capillaria tenuissima* has been found in the small intestine of *M. migrans* in Spain (7), the present *Capillaria* nematodes could not be identified as *C. tenuissima* because we could not precisely calculate their sticocytes, which is one of the characteristics required for species identification.

Respiratory fungal disease commonly occurs in birds of prey such as the northern goshawk (*Accipiter gentilis*), gyrfalcon (*Falco rusticolus*), red-tailed hawk (*Buteo jamaicensis*), golden eagle (*Aquila chrysaetos*) and Blakiston's fish owl (*Ketupa blakistoni*), and *A. fumigatus*, as well as *Aspergillus flavus* and *Aspergillus niger*, have been isolated as their causative agents (6). As far as we know, the present case is the first report of severe airsacculitis due to *A. fumigatus* in a black-eared kite, one of the most common raptors in Japan.

Aspergillosis has been divided into acute and chronic types in poultry as well as in wild birds (2,6). Acute aspergillosis occurs when birds are exposed to a large amount of *Aspergillus* conidia and is usually characterized by high mortality in young birds. Chronic aspergillosis occurs when immunocompetence of birds is impaired (2,6). After removal from the reservoir, the present kite had little chance to be exposed to *Aspergillus* conidia as the kite was kept in a clean, stainless steel cage in an admission room. Factors such as recent capture, change of keepers, inadequate ventilation, and young or advanced age of birds have been reported to be triggers to induce clinical aspergillosis in birds of prey (6). Additionally, the present kite had been severely infected with parasites. Although parasitic infestation in raptors appears to cause little or no distress to healthy individuals, helminth infestation can lead to serious health problems when combined with other factors or at times of stress (4,5). The severe helminthiasis in the present case would have been one of the main factors predisposing the kite to aspergillosis. Clinical diagnosis of respiratory fungal disease in birds of prey is very difficult because the disease advances chronically, without showing any specific symptoms except for anorexia.

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