

Falcon adenovirus infection in breeding Taita falcons (*Falco fasciinucha*)

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Abstract. Four female and 3 male Taita falcons (*Falco fasciinucha*) out of a breeding colony of 14 Taita falcons (7 pairs) died during the breeding season after showing lethargy and anorexia for 1 to 2 days. All animals were submitted for necropsy. Gross lesions in the female falcons were characterized by anemia secondary to marked hemorrhage into the ovary and oviduct, serofibrinous effusion into the cardioabdominal cavity and serosal petechiae. In addition, marked necrotizing splenitis and pulmonary hemorrhage were present. Histologically, the female falcons had mild necrotizing hepatitis with numerous intranuclear inclusion bodies and necrotizing splenitis with rare inclusion bodies. There were no gross lesions in the male falcons, and the histological lesions were characterized by urate deposition and rare intranuclear inclusion bodies in the renal tubular epithelial cells. Adenoviral particles were found by electron microscopy in the cloacal contents of the female Taita falcons but not in the male falcons. DNA in situ hybridization revealed widespread aviadenoviral nucleic acid within the nuclei of hepatocytes, renal tubular epithelial cells, and adrenal cells in the female falcons but no aviadenoviral nucleic acid in 1 male falcon and only a low quantity of adenoviral nucleic acid in the liver and kidney of another male Taita falcon. PCR amplified aviadenoviral DNA in the liver and intestine of all Taita falcons. The amplicons were sequenced, and the virus was identified as falcon adenovirus. The deaths of the female and male birds were attributed to the aviadenovirus infection.

Key words: *Falco fasciinucha*; falcon adenovirus; in situ hybridization; pathology; PCR; Taita falcon.

Clinical aviadenovirus infection is rare in raptors. It has been reported in few species, including a free-ranging goshawk (*Accipiter gentilis*), a group of 9 captive juvenile American kestrels (*Falco sparverius*), a free-ranging merlin (*Falco columbarius*), a captive Bengalese eagle owl (*Bubo bengalensis*), and a captive white-bellied sea eagle (*Haliaeetus leucogaster*).^{9,10,13} In addition, an outbreak of adenovirus infection occurred in a Mauritius kestrel (*Falco punctatus*) breeding colony.¹ A falcon adenovirus, distantly related to serotypes 1 and 4 of the group I avian adenoviruses, was identified for the first time in a recent outbreak in an aplomado falcon (*Falco femoralis*) breeding colony.^{8,11} Peregrine falcons (*Falco peregrinus*) were identified as a possible reservoir species for falcon adenovirus.⁸

This report describes an outbreak of falcon adenovirus infection killing 7 of 14 captive adult Taita falcons (*Falco fasciinucha*) during the breeding season of 2005. In 2002, 3 Taita falcon nestlings, approximately 25 to 30 days old, from the same breeding colony had died of falcon

adenovirus infection after they had been shipped to a different facility.^{11,16} In these cases, the shipping stress was thought to be a predisposing factor for the fatal infection. As a consequence of the 2002 outbreak, eleven Taita falcons from the breeding colony, including 6 of the 7 dead falcons reported on herein and 5 of the surviving Taita falcons, were tested serologically in the fall of 2002 by one of the authors (L. Oaks) according to described methods.⁸ The sera of all animals were negative for antibodies against aviadenoviruses at that time.

Seven pairs of Taita falcons and 1 pair of peregrine falcons were housed in 1 building at the breeding facility. The peregrine falcons and most of the Taita falcons had been housed together since 2002, but 1 male Taita falcon was introduced into the group in February of 2005. The pairs were individually housed in 8 large flight chambers that were located in an approximately 15 m by 30 m building. The chambers were connected by a common corridor. Two other Taita falcons that were hatched in 2004 were kept in another, smaller building that was located at a distance of approximately 100 m from the main building. The Taita falcon diet consisted of quail (*Coturnix japonica*) and day-old chickens, fed on alternating days. The peregrine falcons received quail alternating with 5-week-old chickens. The breeder had successfully reared Taita falcons since 1994, with 6, 9, and 5 Taita falcons being reared in 2002, 2003, and 2004, respectively. The breeding season of 2005 started with mating in the beginning of March. The first animal, a 17-year-old male bird, died on March 16. The females started laying eggs on March 21, 2005. Two females died 1 to 2 days after laying an egg, within a time span of 2.5 weeks. Two more females and 2 more males also died during that time period. The animals had not shown any signs of illness before death, or

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were anorexic and lethargic for a period from a few hours to about 1 day. After the first 2 birds died, the remaining animals were prophylactically treated for 6 days with oral administration of 5 mg enrofloxacin per bird per day against possible bacterial infections and intramuscular injections of 7.5 mg mefloquine per bird per day against a suspected *Plasmodium* sp. infection.^{a,b} None of the surviving birds showed any clinical signs of illness during the outbreak according to the owner, who monitored the animals very closely after the second animal had died. The 2 remaining paired females each went on to lay a clutch of eggs, beginning 1 and 6 days after the last death occurred. One clutch was infertile, but 2 eggs from the second clutch hatched in the laboratory after an approximately 30-day full-term incubation by the pair. The offspring was successfully hand-reared. These 2 animals were reared in a separate side building until they were fledging in late June, just before the blood sampling. At that time they were put into a chamber in the same building as the birds raised in 2004.

The carcasses of the 7 falcons (4 females and 3 males) were sent to the Veterinary Diagnostic Laboratory of the University of Minnesota (bird numbers 1 to 5) and the Texas Veterinary Medical Diagnostic Laboratory (bird numbers 6 and 7) for necropsy. The Taita falcons were in a good to fair postmortem preservation state. Tissue samples including lung, heart, liver, spleen (not collected in 1 male falcon), kidney, esophagus, proventriculus, ventriculus, pancreas, intestine, thyroid, brain, and skeletal muscle were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Sections were cut at 4 μ m, mounted on glass slides, and stained with hematoxylin-eosin. Fresh samples of heart, liver, kidney, lung, and intestine of bird numbers 1 and 2 and heart and liver of bird numbers 3, 4, and 5 were cultured for bacteria by routine laboratory procedures using sheep blood agar, MacConkey agar, Columbia CNA agar, and brilliant green (BG) agar. In addition, pooled tissues were incubated in a Hajna tetrathionate broth at 42°C for 24 hours and plated to BG agar and xylose lysine desoxycholate agar to detect a possible *Salmonella* sp. infection.

Feces of falcon numbers 1 and 2 were submitted for detection of parasites by ZnSO₄ centrifugal flotation method.¹⁴ Cloacal content of 5 falcons (bird numbers 1 to 5) were investigated for viral particles using negative staining electron microscopy as previously described.³ Furthermore, homogenated liver tissue of 5 falcons (bird numbers 1 to 5) was inoculated into the allantoic fluid of chicken eggs with 9- to 11-day-old embryos using a standard protocol.¹² A total of 5 passages, incubated for 5 days at 37°C, were examined. The allantoic fluid was analyzed by hemagglutination test using turkey erythrocytes and by electron microscopy. In addition, homogenated liver was inoculated onto chicken hepatic cell culture and incubated for 3 weeks. Ultrastructural studies were performed on formalin-fixed sections of liver of 1 falcon (bird 2). Sections of liver were embedded in Epon and thin-sectioned for transmission electron microscopy.

DNA in situ hybridization to detect aviadenoviral infection was performed on replicate sections of formalin-

fixed liver, kidney, and spleen of 2 male and 2 female Taita falcons (bird numbers 1, 2, 4, and 5) following published guidelines using a cocktail of oligonucleotide probes FN-23 and FN-96.⁵ These probes were designed to detect target sequences of the penton gene that codes for capsid structural protein. Hepatic and intestinal samples of 3 female and 2 male Taita falcons (bird numbers 1 to 5) were examined by polymerase chain reaction (PCR) assay specific for adenovirus, and the amplicons were cloned and sequenced following a previously published protocol.¹¹ Briefly, DNA was extracted from frozen tissues using the Qiagen Tissue Kit.^{c,11} PCR for the falcon adenovirus hexon region was performed as previously described,¹¹ with the sense primer (AAGAAACACCACAACAGGG) and the antisense primer (GTAAGTAACCAGATCGAAGGTG). Cycling conditions were used at 95°C for 6 minutes, followed by 35 cycles of 95°C for 45 seconds, 54°C for 1 minute, 72°C for 1 minute, and then 72°C for 8 minutes to generate a 385-bp product; PCR for *Herpesviridae* was done with nested degenerate primers that target consensus regions of the DNA polymerase gene of all herpesviruses, as previously described.¹⁵ PCR for *Circoviridae* was done with nested degenerate primers that target consensus regions of the *rep* protein for all circoviruses, as previously described.⁶ PCR for *Parvovirinae* was done with primers that target consensus areas of the capsid protein for parvoviruses, including adeno-associated virus (AAV) or dependovirus, as previously described.² Two weeks after the outbreak, droppings of the peregrine falcons and of 2 surviving Taita falcons were collected over a period of 10 days, frozen, and investigated for presence of viral particles by electron microscopy and for the presence of adenoviral nucleic acid by PCR, as previously described.¹² Sera of all surviving Taita falcons, including the approximately 8-week-old chicks and both peregrine falcons, were collected approximately 14 weeks after the beginning of the outbreak and assayed for titers of adenovirus-specific antibody, as previously described.⁸

At gross necropsy, the 4 female Taita falcons were anemic and had marked hemorrhage into the reproductive tract, including ovaries and oviduct. The females had mild accumulation of a serosanguineous fluid in the cardioabdominal cavity. The livers of the females were mildly enlarged and diffusely beige. The spleens of the females were mildly to moderately enlarged and had multifocal beige well-demarcated foci, up to 2 to 3 mm in diameter (Fig. 1). The females had pulmonary hemorrhage. In contrast, the male birds did not have any gross lesions.

Histologically, the female Taita falcons had moderate to marked hepatocellular necrosis, lymphoplasmacytic portal hepatitis and basophilic intranuclear inclusion bodies surrounded by a clear halo in numerous hepatocytes (Fig. 2). The females had acute necrotizing splenitis with occasional intrahistiocytic intranuclear inclusion bodies. A mild lymphoplasmacytic nephritis was present in the females, with occasional inclusion bodies in tubular epithelial cells. Histological lesions in the males consisted of urate deposition in the kidney. One of the male birds had a focally extensive heterophilic nephritis. Very few and rare inclusion bodies were present in renal tubular epithelial

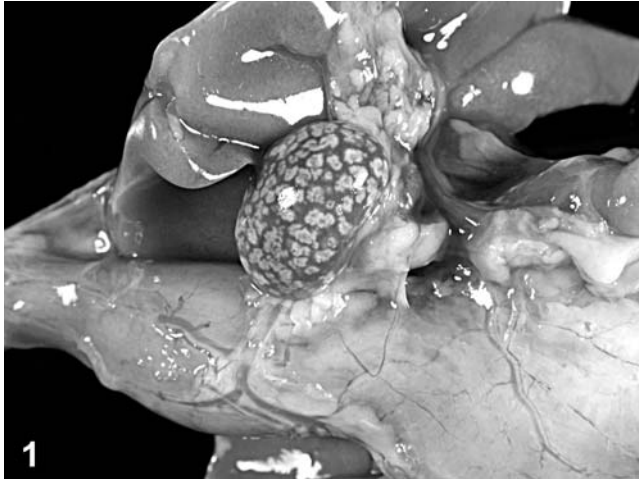


Figure 1. In situ depiction of the enlarged spleen with multifocal areas of necrosis in a female Taita falcon (Taita falcon number 2).

cells. Hepatocellular inclusion bodies were detected in 2 of the 3 birds (numbers 5 and 6), but only in very low numbers (less than 1 per 10 high power fields). Inclusion bodies were not detected in the 2 spleens that were evaluated. Adenoviral particles were found in the cloacal content of the female Taita falcons but not in the male falcons. The viral particles were approximately 70 nm in diameter and had an icosahedral symmetry characteristic of aviadenoviruses. Electron microscopy of liver tissue demonstrated adenoviral particles in the nucleus of hepatocytes (Fig. 3). DNA in situ hybridization revealed widespread aviadenoviral nucleic acid within the nuclei of hepatocytes, renal tubular epithelial cells, and adrenal cells in the female falcons, but no aviadenoviral nucleic acid in 1 male falcon

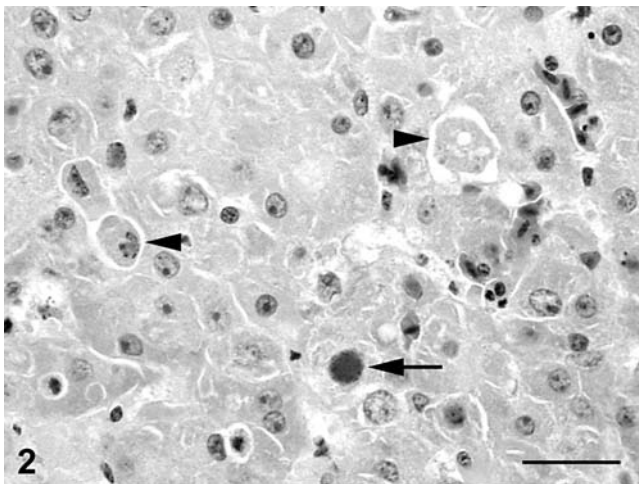


Figure 2. Histological section of the liver of a female Taita falcon with acute adenovirus-induced diffuse hepatic necrosis (Taita falcon number 1). Occasional hepatocytes are necrotic and numerous hepatocytes contain inclusion bodies (arrows). The affected nuclei have a hyperchromatic nuclear membrane and a halo. Hematoxylin and eosin stain; bar = 25 µm.

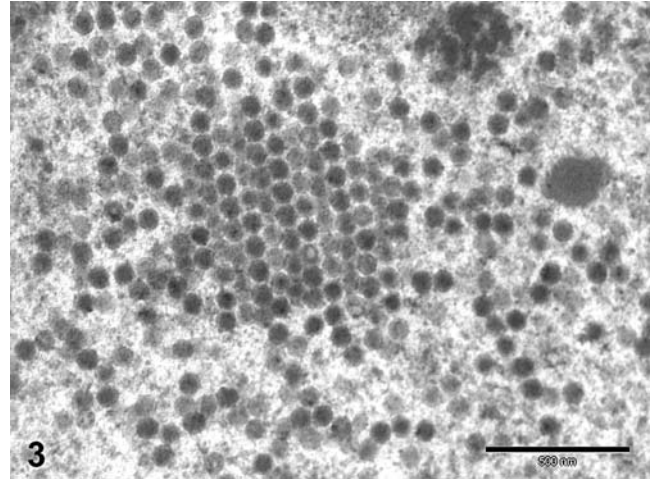


Figure 3. Transmission electron microscopic demonstration of a pseudocrystalline array of isometric 75-nm adenovirus particles in the nucleus of a hepatocyte of a female Taita falcon (Taita falcon number 2); bar = 500 nm.

(number 4) and only a low quantity of adenoviral nucleic acid in the liver and kidney of the second male Taita falcon (number 5). PCR amplified aviadenoviral nucleic acid in the liver and intestine of all tested Taita falcons (numbers 1 to 5) (Fig. 4). The DNA sequence of the hexon region was 100% identical to previously published taita falcon adenovirus (GenBank AY683554).¹¹ The liver samples were negative for herpesvirus, dependovirus (parvovirus), polyomavirus, and chlamydia by PCR.

Few nonhemolytic *Escherichia coli* were isolated from various organs of bird numbers 1 and 2. There was no significant aerobic bacterial growth from liver and heart of bird numbers 3, 4, and 5. Attempted virus isolation by egg inoculation or cell culture was unsuccessful in all animals.

The feces of the peregrine falcons and 2 surviving Taita falcons that were collected after the outbreak were negative for viral particles by negative staining electron microscopy and negative for adenoviral nucleic acid by PCR. All Taita falcons within the main building and both Taita falcons that were hatched in 2004 had an adenovirus-specific antibody titer of > 1 : 40, while the 2 Taita falcons that were hatched in 2005 were seronegative.

An outbreak of aviadenovirus infection in a captive breeding colony of Taita falcons was presumptively diagnosed based on the microscopic finding of intranuclear inclusion bodies. The diagnosis was confirmed by the detection of adenoviral particles in the cloacal content, by detection of adenoviral particles in the nuclei of hepatocytes, by demonstration of aviadenoviral nucleic acid within infected hepatocytes and renal tubular epithelial cells via in situ hybridization, by detection of adenoviral nucleic acid in liver and intestine tissue via polymerase chain reaction, and by serology.

The origin of the falcon adenovirus in the current outbreak was not determined. It is speculated that the peregrine falcons may have been carriers and may have shed adenovirus during the breeding season. The adenovi-

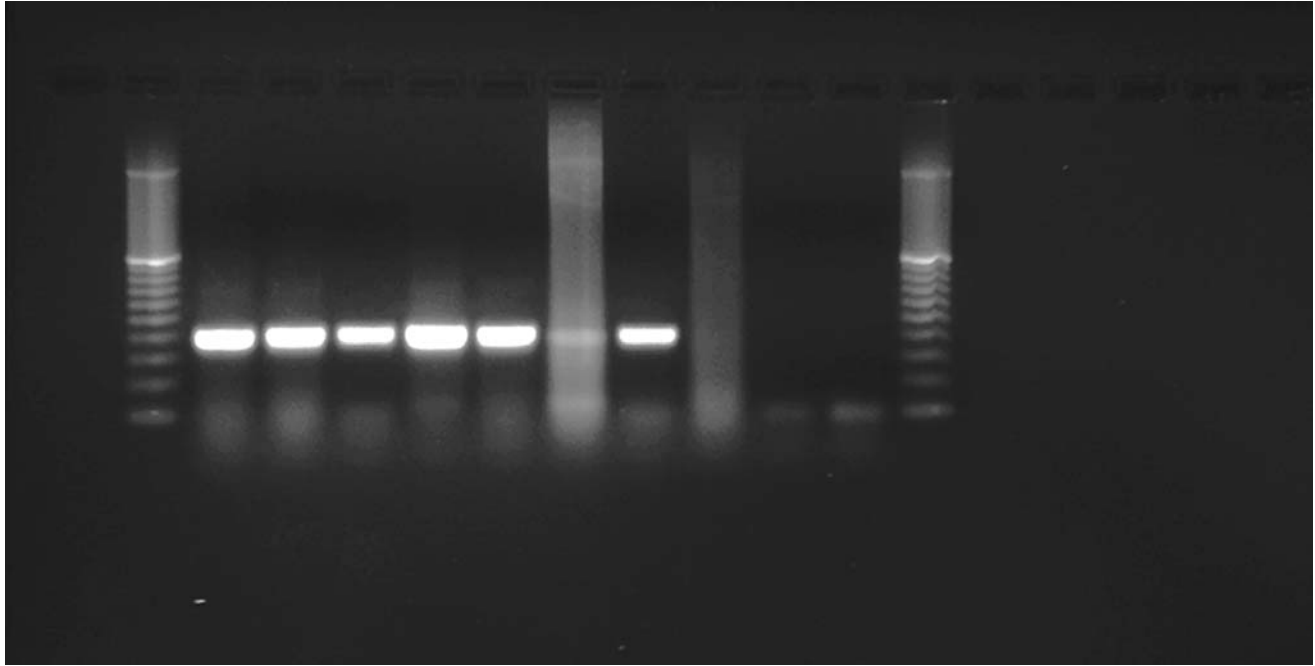


Figure 4: PCR reaction for the detection of falcon adenovirus in the livers and intestine of five Taita falcons (bird number 1 to 5: 1 = liver of female falcon number 2; 2 = liver of female falcon number 1; 3 = intestine of male falcon number 5, 4 = liver of female falcon number 3; 5 = intestine of female falcon number 3; 6 = liver of male falcon number 4; 7 = intestine of male falcon number 4; 8 liver of male falcon number 5; 9 = negative control irrelevant DNA and 10 = negative control no DNA; 100 bp ladder). PCR lanes 1-7 were positive.

rus implicated in an outbreak in a breeding colony of aplomado falcons as well as in the falcons of this report was a recently described member of the genus *Aviadenovirus*, named falcon adenovirus. This virus is closely related to fowl adenovirus types 1 and 4 but is genetically sufficiently different from these viruses to merit classification as a new species of adenovirus.^{8,11} Therefore, the fed chicken and quail were not considered to be the source of the infection. Peregrine falcons were suspected to be the reservoir for the virus, although aviadenovirus infections are usually considered to be species-specific.^{4,7,8} Alternatively, it may be speculated that the virus was introduced by the Taita falcon that was added to the breeding group approximately 4 weeks before the outbreak, since this animal survived the outbreak and was seropositive after the outbreak.

Interestingly, the adenovirus infection presented differently in the female versus the male falcons regarding the gross and microscopic lesions and regarding the shedding of the virus into cloacal content. In fact, there was no evidence of significant inflammatory or degenerative lesions in the male falcons, so that only auxiliary diagnostic tools including *in situ* hybridization and polymerase chain reaction were able to demonstrate viral infection. It seems possible that this occurred because of immunosuppression during the breeding season in the female birds due to an altered metabolism during oogenesis.

The serological results of the surviving animals indicate that some of the Taita falcons survived the adenovirus infection without showing obvious signs of illness. Five of the surviving animals had tested negative for adenovirus-

specific antibodies in 2002 but were positive in 2005. It is uncertain why these animals survived without showing any clinical signs, whereas other Taita falcons in the same environment died of the infection. The serological results suggest that the outbreak had an infection rate of 100% with a 50% morbidity and mortality rate. The significance of positive antibody titers in respect to reinfection with falcon adenovirus is uncertain. A previous study presented evidence that adenovirus-specific titers may not necessarily be protective.⁸ Interestingly, the 2 Taita falcons that hatched shortly after the outbreak did not have detectable adenovirus-specific antibody titers. It is possible that these animals had maternal antibodies that had waned in the time between hatching and blood collection. In chickens, it is well established that adenovirus can be transmitted vertically, but the authors of a previous study of falcon adenovirus speculated that vertical transmission did not occur in aplomado falcons.¹¹ The negative isolation results were not surprising, considering that falcon fibroblasts appear to be necessary to isolate the virus.⁸

Biosafety measures, including quarantine protocols and high hygiene standards resulting in prevention of cross-contamination from 1 nesting chamber to the neighboring chamber, appear to be critical for the prevention and control of infectious diseases, including aviadenovirus infection, in falcon breeding facilities. Aviadenoviruses are known to be fairly resistant to inactivation and may remain infectious for long periods.^{4,7} Recommended disinfection for aviadenoviruses in poultry consists of chlorine releasing agent, iodophors, and quaternary am-

monium compounds, while chlorhexidine may be fairly ineffective. Removal of any organic matter, rinsing of the soap before the application of the disinfectant, and a sufficiently long incubation time with the disinfectant at room temperature are known to be important for successful disinfection. Stress, such as that induced by the breeding activity, may predispose the animals to clinical and fatal adenovirus infection. Cohousing of domestic falcons, particularly peregrine falcons, and tropical falcon species, as well as introduction of new birds into the breeding colony close to the breeding season, are likely risk factors and should be avoided.

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Sources and manufacturers

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- b. Mefloquine (Lariam), Roche, Nutley, NJ.
- c. Qiagen, Valencia, CA.

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