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Isolation and Epidemiology of Falcon Adenovirus

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An adenovirus was detected by electron microscopy in tissues from falcons that died during an outbreak of inclusion body hepatitis and enteritis that affected neonatal Northern aplomado (*Falco femoralis septentrionalis*) and peregrine (*Falco peregrinus anatum*) falcons. Molecular characterization has identified the falcon virus as a new member of the aviadenovirus group (M. Schrenzel, J. L. Oaks, D. Rotstein, G. Maalouf, E. Snook, C. Sandfort, and B. Rideout, J. Clin. Microbiol. 43:3402–3413, 2005). In this study, the virus was successfully isolated and propagated in peregrine falcon embryo fibroblasts, in which it caused visible and reproducible cytopathology. Testing for serum neutralizing antibodies found that infection with this virus was limited almost exclusively to falcons. Serology also found that wild and captive peregrine falcons had high seropositivity rates of 80% and 100%, respectively, although clinical disease was rarely reported in this species. These data implicate peregrine falcons as the natural host and primary reservoir for the virus. Other species of North American falcons, including aplomado falcons, had lower seropositivity rates of 43 to 57%. Falcon species of tropical and/or island origin were uniformly seronegative, although deaths among adults of these species have been described, suggesting they are highly susceptible. Chickens and quail were uniformly seronegative and not susceptible to infection, indicating that fowl were not the source of infection. Based on the information from this study, the primary control of falcon adenovirus infections should be based on segregation of carrier and susceptible falcon species.

An adenovirus was detected by electron microscopy in tissues from falcons that died during an outbreak of inclusion body hepatitis and enteritis that affected neonatal Northern aplomado falcons (*Falco femoralis septentrionalis*) (12). The outbreak occurred in 1996 at a raptor propagation facility that, in addition to neonatal falcons, housed 72 adult aplomado falcons, 94 American peregrine falcons (*Falco peregrinus anatum*), three Vanuatu peregrine falcons (*Falco peregrinus nesiotes*), three gyrfalcons (*Falco rusticolus*), 11 harpy eagles (*Harpia harpyja*), 20 California condors (*Gymnogyps californianus*), and two Andean condors (*Vultur gryphus*). The affected neonatal aplomado falcons were all housed together in a single brooder room. The outbreak had an overall attack rate of 72/110 (65%) and a case fatality rate of 62/72 (86%). Strikingly, 102 American peregrine falcon neonates that were being raised simultaneously in the same brooder room had an attack rate of only 6/102 (6%). None of the adult falcons, eagles, or condors had any illness or death compatible with viral infection during the disease outbreak. The exclusive food source for the neonatal aplomado and peregrine falcons was Japanese quail (*Coturnix japonica*) raised at the facility. The quail were raised in a building along with domestic chickens (*Gallus gallus domesticus*) that were used to feed the other raptors. In addition, large numbers of pigeons (*Columba livia*) and chukar (*Alectoris chukar*) were free ranging on the grounds of the facility. No unusual disease was noted in any of these other bird species during the outbreak.

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Epidemiologic investigation (12) indicated that the outbreak began with infection of a single neonatal aplomado falcon, with subsequent horizontal spread among the rest of the aplomado falcon chicks in the brooder room. However, the source of the initial infection was not known. The eggs from which these falcons hatched were naturally incubated for about 1 week by either peregrine or aplomado falcons, followed by artificial incubation. After hatching, the neonates were all hand raised and thus had no direct contact with any adult falcons. In the few previous reports of adenovirus infections of falcons (4, 10, 14), the source of infection was not determined. Forbes et al. (4) hypothesized that the agent was a poultry virus and that exposure of falcons occurred through food sources. This was based on isolation of an adenovirus from the chickens and turkeys used to feed the falcons. However, the ubiquitous nature of adenoviruses and the lack of any direct comparison of the falcon and fowl isolates made the association circumstantial (4).

Molecular characterization has identified the falcon virus as a new member of the aviadenovirus group (12); this new member is most closely related to the group I avian adenoviruses. The original working hypothesis concerning the outbreak was that the virus was introduced to the brooder room via the food quail. However, evidence that this adenovirus was distinct from the known poultry adenoviruses challenged the hypothesis that it originated from the food source. The purpose of this study was to isolate and propagate the falcon adenovirus and to use the virus for challenge studies and as an antigen in serologic assays to define the host range and natural history of the falcon adenovirus. This information would also be useful in determining the source of the virus in the original outbreak and in designing strategies to prevent future outbreaks.

MATERIALS AND METHODS

Virus isolation. Primary and permanent cell cultures were used to attempt in vitro propagation of the adenovirus. Primary fibroblast cell cultures were prepared from 11- to 13-day-old domestic chicken embryos, 12-day-old Japanese quail embryos, 12- to 21-day-old domestic duck embryos, and 17- to 25-day-old peregrine falcon embryos by methods described by Docherty and Slota (3). Primary duck and quail embryo fibroblast-like cultures were also prepared from the livers of 20- to 25-day-old duck embryos and 12-day-old quail embryos. The procedure for preparing liver fibroblasts was adapted from the method of Docherty and Slota (3) to obtain fibroblasts from connective tissues. Fibroblasts from the liver were selected for, and hepatocytes were removed by, the level of protease digestion (0.05% trypsin and 0.02% EDTA at 37°C for 15 min), and cells were subcultured to select for dividing cells. Although these cells have not been characterized, they have the typical spindle-shaped morphology of fibroblasts. The chicken, quail, and duck cells were maintained in Eagle's minimal essential medium (MEM), and the falcon cells were maintained in M199 medium. All media were supplemented with 10% (vol/vol) fetal bovine serum, 2 mM L-glutamine, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin, and 2.5 μg ml^{-1} amphotericin B. Permanent cell lines of avian origin included a chicken liver cell line (LMH) and a Japanese quail fibroblast cell line (QT35) maintained as previously described (11).

Tissue pools of approximately 1 g of liver and 0.5 g of spleen from affected aplomado falcons were first homogenized with Dounce tissue grinders in 10 ml of MEM supplemented with 200 U ml^{-1} penicillin and 0.2 mg ml^{-1} streptomycin and then briefly centrifuged at 750 × *g* to remove tissue and cellular debris; then 500 μl of the sample was inoculated onto confluent monolayers of cells in 25-cm^2 flasks. The sample was incubated with the cells for 1 h at 37°C, after which time an additional 5 ml of supplemented medium was added to the flasks. The cell cultures were incubated under 5% CO_2 at 37°C for 7 to 14 days and observed for cytopathology. Cell cultures were passaged by freezing and thawing the flasks, collecting the cells and supernatants, and adding 1 ml of this lysate to new cells in 25-cm^2 flasks.

Electron microscopy. Portions (1.5) ml of cell supernatants were collected from cell cultures with visible cytopathology, centrifuged at 1,000 × *g* for 10 min to pellet large cellular debris, and centrifuged at 8,000 × *g* for 10 min to further remove debris; then 1 ml of the clarified supernatant was centrifuged at 13,000 × *g* for 30 min to pellet virions. The virus pellet was resuspended in 50 μl of distilled water, negatively stained with 1% phosphotungstic acid for 30 s, mounted onto 200-mesh Formvar-coated copper grids, and viewed by transmission electron microscopy.

PCR assay and sequencing. Falcon adenovirus was detected in cell cultures or tissues by using a PCR assay specific for the falcon adenovirus hexon gene as described by Schrenzel et al. (12). DNA was extracted and purified from infected cell monolayers or tissue samples by standard phenol-chloroform and ethanol precipitation methods, and 1 μg of total DNA was used in each PCR. The identity of the amplified DNA as falcon adenovirus was confirmed by cloning the amplicon into the pCR2.1 sequencing vector (TOPO TA cloning kit; Invitrogen, Carlsbad, CA), and sequencing by automated dideoxy DNA methods.

Serology. The serum neutralization (SN) assay was modified from a previously published microneutralization procedure (5). Serum or plasma was collected from various species of raptors and other birds, stored at −20°C, and heat inactivated at 56°C for 30 min prior to use. Stocks of the falcon adenovirus were prepared by propagating the virus in peregrine embryo fibroblasts in 25-cm^2 flasks until maximal cytopathology was evident. At this time, the cell monolayers were harvested by mechanically scraping free the cell monolayers, releasing intracellular virions by two freeze-thaw cycles, centrifuging to pellet cellular debris, and making 1-ml aliquots of the virus, which were stored at −80°C until use. The titer of the virus stock was determined by performing eight replicate 10-fold serial dilutions on peregrine embryo fibroblasts and scoring the well as positive or negative for cytopathology. The titer as the median tissue culture infective dose ($TCID_{50}$) was then calculated by the method of Reed and Muench (9). Stocks of fowl adenovirus type 1 (chicken embryo lethal orphan [CELO] strain) were similarly prepared and titered.

To perform the SN assay, 100 $TCID_{50}$s of virus stock were mixed with test sera/plasma and cell culture media to give final serum dilutions of 1:5, 1:10, or 1:40 in a total volume of 100 μl. For selected cases, twofold serial dilutions of the sera/plasma were made (range, 1:10 to 1:5,120) to detect the end point titers. The serum-virus mixture was incubated at 37°C for 1 h, and then the entire 100 μl was added to newly confluent peregrine embryo fibroblasts in 96-well plates. The plates were then incubated at 37°C under a 5% CO_2 atmosphere for 10 to 14 days, after which time the wells were scored as positive or negative for cytopathology. The titer was reported as the reciprocal of the highest dilution that completely inhibited cytopathology.

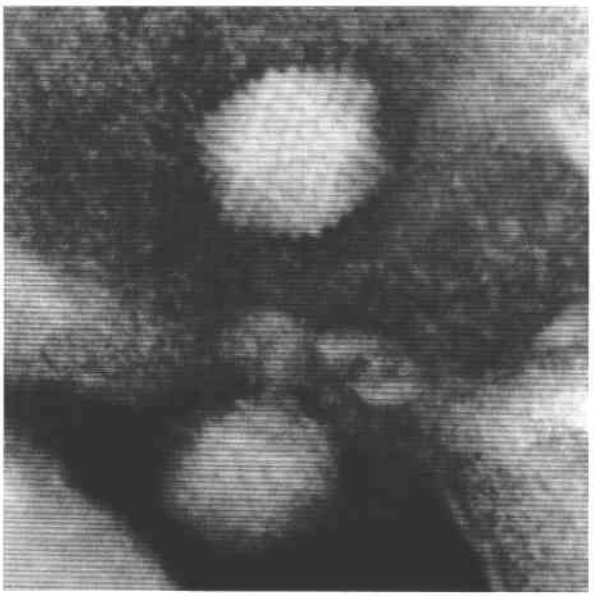

FIG. 1. Electron micrograph of virus particle identified in quail embryo fibroblast cell culture of aplomado falcon RP5958. Virions have the characteristic icosahedral shape of adenoviruses. Magnification, ×60,000.

Experimental infections of live quail. All experiments using live birds were approved by the Washington State University Institutional Animal Care and Use Committee. To attempt to reproduce disease and determine the host range for the virus, Japanese quail were infected with tissue pools from infected aplomado falcons. These tissue pools were shown to have infectious virus by isolation in cell culture. The source flock for the quail was negative for adenovirus titers by agar gel precipitation assays against group I and group II aviadenoviruses (performed commercially by the Poultry Diagnostic and Research Center at the University of Georgia). Two groups of three 2-day-old quail each were inoculated either orally with 10 μl or by intramuscular injection with 10 μl of liver/spleen tissue homogenates from aplomado falcons RP5958 and 96JT1,6. One quail in each group was also left uninoculated as a contact control. At 19 days postinoculation, all of the quail in both groups, including the contact controls, were reinoculated by intramuscular injection with 100 μl of tissue homogenates from the respective falcons. Another two groups of two 8-week-old quail each were inoculated using 100 μl for the initial oral and intramuscular administrations and 200 μl for the subsequent intramuscular reinoculation. Groups of four age-matched control quail were housed in the same room but in separate cages. The birds were observed daily for any clinical evidence of disease. At day 29 postinoculation, all the birds were euthanized for collection of serum and tissues. SN assays for antibodies to fowl adenovirus type 1 in experimentally infected quail were performed commercially by the Poultry Diagnostic and Research Center, University of Georgia.

RESULTS

Virus isolation. Tissue pools were prepared from three aplomado falcons (RP5958, RP5963, and 96JT1,6) that were shown to be infected by histopathology and PCR (12). These three tissue pools were initially inoculated onto quail embryo fibroblasts and quail liver fibroblasts, because Japanese quail were hypothesized to be the primary host for the virus. Very slight cytopathology characterized by rounded, refractile cells was noted by day 3 postinfection in both cell types for all three cases. Electron microscopy on the supernatants from cases RP5958 and RP5963 collected at day 14 postinfection identified an adenovirus in the cultures from these two cases (Fig. 1).

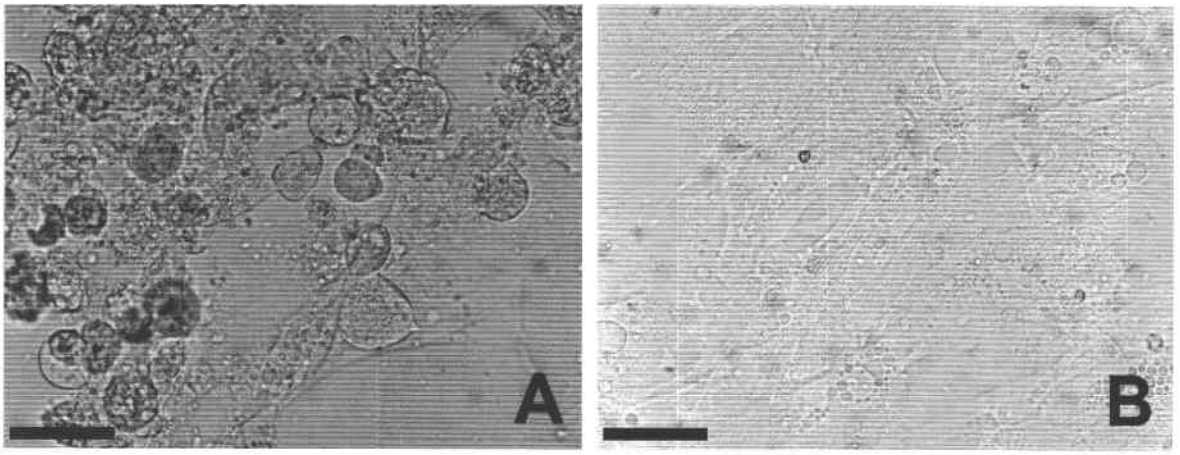

FIG. 2. (A) Cytopathic effect of the falcon adenovirus in peregrine falcon embryo fibroblasts. (B) Uninfected control cells. Bars, 20 μm.

However, the cytopathology did not progress after day 3 postinfection and was lost upon subsequent passage. LMH and QT35 cells inoculated with samples from cases RP5958, RP5963, and 96JT1,6 did not have detectable cytopathology after three passages.

Duck embryo fibroblasts and duck liver fibroblasts were also inoculated with the tissue pool from 96JT1,6. Both cell types showed moderate cytopathology characterized by round, refractile cells up to the third passage. In the embryo fibroblasts, visible cytopathology was lost at the fourth passage and not detected with seven additional passages. However, in the liver fibroblasts, weak and sporadic cytopathology was detected between passages 4 and 11. Attempts to rescue cytopathology by passaging material from infected duck liver fibroblasts back to primary duck or chicken fibroblasts were unsuccessful. Subsequent electron microscopy of the third-passage duck liver fibroblasts detected adenovirus particles, and PCR testing revealed that all of the passages of both the duck embryo fibroblasts and duck liver fibroblasts were infected with the falcon adenovirus, although visible cytopathology was not apparent.

The cell culture supernatant from the third passage of duck liver fibroblasts for case 96JT1,6, which was positive by PCR and electron microscopy, was used to inoculate peregrine falcon embryo fibroblasts. In the peregrine cells, cytopathology characterized by rounded, refractile cells was detectable by day 4 to 5 postinfection and then progressed over the next 5 days to extensive cytopathology with many pyknotic cells (Fig. 2). Five subsequent passages onto fresh peregrine falcon fibroblasts resulted in extensive and reproducible cytopathology in all passages by about day 10 postinfection and titers of approximately 2×10^3 to 3×10^3 $TCID_{50}s$ ml^{-1}. These passages were also all PCR positive. Comparison of the sequences of the PCR amplicons from falcon tissues and cell cultures confirmed that the cultured adenovirus was the falcon adenovirus.

Serology. Identification of peregrine embryo fibroblasts as an in vitro cell culture system with a reproducible detection method (visible cytopathology) was an important prerequisite for developing the SN assay. The initial focus was to determine SN titers in birds at the breeding facility. Serum samples were collected in September 1996, 2 months after the end of the outbreak, from surviving neonatal aplomado falcons ($n = 10$), adult aplomado falcons ($n = 6$), and adult peregrine falcons ($n = 6$). All 22 falcons tested were seropositive, with SN titers of 5 to ≥40. End point titers were determined for five of the adult peregrines; they ranged from 640 to 1,280. Attempts to determine the end point titers for five of the surviving neonatal aplomado falcons showed that all birds had very high titers of ≥5,120. These results are summarized in Table 1.

Archived plasma samples from 1995 and January 1996 provided an opportunity to test for SN titers in the six adult aplomado falcons and six adult peregrine falcons prior to the outbreak. All of these birds were positive, with titers of 5 to ≥40, indicating that the virus was present at the facility at least 1.5 years prior to the outbreak. Seven adult aplomado falcons and five adult peregrine falcons were tested in 1999, 3 years after the outbreak. Four of the aplomado falcons were positive with titers of 10 to ≥40, and three were seronegative at a dilution of 1:10. All five of the peregrine falcons were strongly seropositive, with titers of ≥40. These data indicated either that the titers were persistent or that exposure was ongoing, especially in the peregrine falcons. These results are summarized in Table 1.

Subsequently, other raptors and species of birds at the breeding facility were also tested for SN antibody titers (Table 1). This included seven adult gyrfalcons tested in 2002, of which three (43%) had titers of 10 to ≥40 and four were seronegative at a dilution of 1:10. All other birds tested were seronegative at titers of 1:10, including 5 harpy eagles in 1999, 4 pigeons in 2001, 2 chukar partridges in 2001, 4 of the food quail in 2001, and 10 of the food chickens in 2002. These initial results indicated that infection with the falcon adenovirus was limited to birds in the genus *Falco*, and the high and persistent titers in the adult peregrine falcons suggested that infection was endemic in this species. An additional five chickens from mixed flocks not associated with the breeding facility were also tested and found seronegative at dilutions of 1:10 (Table 2).

Testing for SN titers was also performed on raptors not associated with the breeding facility (Table 2). All species of hawks and eagles tested were seronegative at a dilution of 1:10, including six red-tailed hawks (*Buteo jamaicensis*), two rough-legged hawks (*Buteo lagopus*), two bald eagles (*Haliaeetus leu-*

TABLE 1. Reciprocal serum neutralization titers against the falcon adenovirus in raptors and other birds at the breeding facility, 1995 to 2002[a]

Species	Age	Sex	ID	Titer (end point titer) at the following time:					
				1995	Jan. 1996	Sept. 1996	1999	2001	2002
Aplomado	Juv	M	8G			≥40 (≥5,120)			
Aplomado	Juv	F	11G			≥40 (≥5,120)			
Aplomado	Juv	M	12G			≥40 (≥5,120)			
Aplomado	Juv	M	13G			≥40 (≥5,120)			
Aplomado	Juv	F	15G			≥40 (≥5,120)			
Aplomado	Juv	F	24B			≥40			
Aplomado	Juv	M	53B			≥40			
Aplomado	Juv	F	53Y			≥40			
Aplomado	Juv	F	53W			≥40			
Aplomado	Juv	M	53R			≥40			
Aplomado	Ad	F	MEMI		10	≥40	≥40		
Aplomado	Ad	F	TWA		≥40	≥40	≥40		
Aplomado	Ad	F	LO		5	5	Neg @ 10		
Aplomado	Ad	M	LO		5	10	Neg @ 10		
Aplomado	Ad	M	MOE		10	10			
Aplomado	Ad	F	MOE				Neg @ 10		
Aplomado	Ad	M	AP30		5	≥40	≥40		
Aplomado	Ad	F	AP30				10		
Peregrine	Ad	F	TJ2	Neg @ 5		≥40 (1,280)	≥40		
Peregrine	Ad	M	TJ2	≥40		≥40 (1,280)	≥40		
Peregrine	Ad	F	CW3	≥40		≥40 (1,280)			
Peregrine	Ad	F	CW5	≥40		≥40 (1,280)			
Peregrine	Ad	M	CW5	≥40		≥40 (640)			
Peregrine	Ad	M	CZ2	10		10			
Peregrine	Ad	F	CW7				≥40		
Peregrine	Ad	M	CHP				≥40		
Peregrine	Ad	F	YO				≥40		
Gyrfalcon	Ad	M	Konkel						Neg @ 10
Gyrfalcon	Ad	M	Shane						≥40
Gyrfalcon	Ad	M	Vic						Neg @ 10
Gyrfalcon	Ad	F	Berry						Neg @ 10
Gyrfalcon	Ad	M	Berry						Neg @ 10
Gyrfalcon	Ad	F	WG						≥40
Gyrfalcon	Ad	M	WG						10
Harpy eagle	Ad	F	Olafa				Neg @ 10		
Harpy eagle	Ad	F	Olivia				Neg @ 10		
Harpy eagle	Ad	M	Panama				Neg @ 10		
Harpy eagle	Ad	M	SD 98				Neg @ 10		
Harpy eagle	Ad	F	LA 1				Neg @ 10		
Pigeon	Ad	Unk	A					Neg @ 10	
Pigeon	Ad	Unk	B					Neg @ 10	
Pigeon	Ad	Unk	C					Neg @ 10	
Pigeon	Ad	Unk	D					Neg @ 10	
Chukar	Ad	Unk	A					Neg @ 10	
Chukar	Ad	Unk	B					Neg @ 10	
Quail	Ad	Unk	A					Neg @ 10	
Quail	Ad	Unk	B					Neg @ 10	
Quail	Ad	Unk	C					Neg @ 10	
Quail	Ad	Unk	D					Neg @ 10	
Chicken	Ad	Unk	1						Neg @ 10
Chicken	Ad	Unk	2						Neg @ 10
Chicken	Ad	Unk	3						Neg @ 10
Chicken	Ad	Unk	4						Neg @ 10
Chicken	Ad	Unk	5						Neg @ 10
Chicken	Ad	Unk	6						Neg @ 10
Chicken	Ad	Unk	7						Neg @ 10
Chicken	Ad	Unk	8						Neg @ 10
Chicken	Ad	Unk	9						Neg @ 10
Chicken	Ad	Unk	10						Neg @ 10

[a] Abbreviations used: ID, identification; Juv, juvenile; Ad, adult; M, male; F, female; Unk, unknown; Neg, negative; Neg @ 10, negative at a dilution of 1:10.

cocephalus), and two golden eagles (*Aquila chrysaetos*). Among owls, three great-horned owls (*Bubo virginianus*) were seronegative at a dilution of 1:10, while the single barred owl (*Strix varia*) tested had a titer of 10. Among falcons, two American kestrels (*Falcon sparverius*) were seronegative at a dilution of 1:10 while two others each had a titer of 10. Eleven captive teita falcons (*Falco fasciinucha*) and seven orange-breasted falcons (*Falco deiroleucus*) tested were all seronegative at dilutions of 1:10. In contrast, among 30 wild-caught tundra peregrine falcons (*Falco peregrinus tundrius*), 16 (53%) had titers

TABLE 2. Reciprocal serum neutralization titers against the falcon adenovirus in raptors and chickens not from the breeding facility[a]

Species	Age	Sex	ID	Date	Titer (end point titer)
Peregrine falcon	Ad	F	1807-34626	2001	≥40 (≥640)
Peregrine falcon	Juv	F	1807-52540	2001	≥40
Peregrine falcon	Juv	F	1807-52541	2001	Neg @ 10
Peregrine falcon	Juv	F	1807-52542	2001	10
Peregrine falcon	Juv	F	1807-52563	2001	10
Peregrine falcon	Juv	F	1807-52566	2001	≥40
Peregrine falcon	Juv	F	1807-52567	2001	≥40
Peregrine falcon	Ad	F	1807-52571	2001	≥40
Peregrine falcon	Juv	F	1807-52578	2001	Neg @ 10
Peregrine falcon	Juv	F	1807-52581	2001	10
Peregrine falcon	Juv	F	1807-52583	2001	Neg @ 10
Peregrine falcon	Juv	F	1807-52589	2001	≥40
Peregrine falcon	Juv	F	1807-52590	2001	10
Peregrine falcon	Ad	F	1807-52593	2001	≥40
Peregrine falcon	Ad	F	1807-52597	2001	≥40
Peregrine falcon	Juv	F	1807-52600	2001	≥40
Peregrine falcon	Juv	F	1807-52611	2001	10
Peregrine falcon	Juv	F	1807-52612	2001	≥40
Peregrine falcon	Juv	F	1807-52613	2001	≥40
Peregrine falcon	Juv	F	1807-52616	2001	10
Peregrine falcon	Ad	F	1807-82485	2001	Neg @ 10
Peregrine falcon	Juv	F	1807-82493	2001	Neg @ 10
Peregrine falcon	Juv	F	1807-82494	2001	10
Peregrine falcon	Juv	F	1807-86336	2001	≥40
Peregrine falcon	Juv	F	1807-86341	2001	Neg @ 10
Peregrine falcon	Juv	F	1807-86352	2001	≥40
Peregrine falcon	Juv	F	1807-86357	2001	10
Peregrine falcon	Ad	F	1807-86375	2001	≥40
Peregrine falcon	Juv	F	1807-86383	2001	≥40
Peregrine falcon	Juv	F	1807-86387	2001	≥40
Teita falcon	Ad	F	Chimani	2002	Neg @ 10
Teita falcon	Ad	M	Chimani	2002	Neg @ 10
Teita falcon	Ad	M	NPM	2002	Neg @ 10
Teita falcon	Ad	F	NPF	2002	Neg @ 10
Teita falcon	Ad	F	Group	2002	Neg @ 10
Teita falcon	Ad	M	T7M	2002	Neg @ 10
Teita falcon	Ad	F	OR	2002	Neg @ 10
Teita falcon	Ad	M	Green	2002	Neg @ 10
Teita falcon	Ad	M	0.78	2002	Neg @ 10
Teita falcon	Ad	M	HBM	2002	Neg @ 10
Teita falcon	Ad	M	SengM	2002	Neg @ 10
Orange-breasted falcon	Ad	M	A2	2003	Neg @ 10
Orange-breasted falcon	Ad	F	W3	2003	Neg @ 10
Orange-breasted falcon	Ad	M	K9	2003	Neg @ 10
Orange-breasted falcon	Juv	F	H6	2003	Neg @ 10
Orange-breasted falcon	Ad	M	A8	2003	Neg @ 10
Orange-breasted falcon	Ad	M	E9	2003	Neg @ 10
Orange-breasted falcon	Ad	M	V9	2003	Neg @ 10
American kestrel	Ad	Unk	WSU 82622	2001	10
American kestrel	Juv	F	WSU 87219	2002	Neg @ 10
American kestrel	Ad	M	WSU 87307	2002	Neg @ 10
American kestrel	Juv	F	WSU 87163	2002	10
Barred owl	Ad	Unk	WSU 18485	2001	10
Great-horned owl	Ad	Unk	WSU 88326	2002	Neg @ 10
Great-horned owl	Juv	Unk	WSU 87889	2002	Neg @ 10
Great-horned owl	Ad	Unk	WSU 85630	2002	Neg @ 10
Red-tailed hawk	Ad	Unk	WSU 82941	2001	Neg @ 10
Red-tailed hawk	Ad	Unk	WSU 80996	2001	Neg @ 10
Red-tailed hawk	Ad	Unk	WSU 81514	2001	Neg @ 10
Red-tailed hawk	Ad	Unk	WSU 83063	2001	Neg @ 10
Red-tailed hawk	Ad	Unk	WSU 85004	2002	Neg @ 10
Red-tailed hawk	Ad	Unk	WSU 85107	2002	Neg @ 10
Rough-legged hawk	Unk	Unk	WSU 85560	2002	Neg @ 10
Rough-legged hawk	Ad	Unk	WSU Feb 2002	2002	Neg @ 10
Bald eagle	Ad	Unk	WSU 84725	2002	Neg @ 10
Bald eagle	Ad	Unk	WSU 84483	2002	Neg @ 10
Golden eagle	Ad	Unk	WSU 84359	2002	Neg @ 10
Golden eagle	Ad	Unk	WSU 85586	2002	Neg @ 10
Chicken	Ad	F	1	2002	Neg @ 10
Chicken	Ad	F	2	2002	Neg @ 10
Chicken	Ad	F	3	2002	Neg @ 10
Chicken	Ad	F	4	2002	Neg @ 10
Chicken	Ad	F	5	2002	Neg @ 10

[a] Abbreviations used: ID, identification; Juv, juvenile; Ad, adult; M, male; F, female; Unk, unknown; Neg, negative; Neg @ 10, negative at a dilution of 1:10.