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Avian Poxvirus Epizootic in a Breeding Population of Lesser Flamingos (*Phoenicopterus minor*) at Kamfers Dam, Kimberley, South Africa

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ABSTRACT: Avian pox has a worldwide distribution, but prior to this investigation has not been reported in free-ranging flamingo populations. During observations of the first successful breeding of Lesser Flamingos on a purpose-built island, at Kamfers Dam near Kimberley, South Africa, multiple small, raised, crusted plaques on the legs and facial area were noticed on 30% of the fledgling flamingos. A diagnosis of avipoxvirus infection was made on the basis of the macroscopic, histologic, and electron microscopic features, and was further confirmed by DNA sequence analysis. The avipoxvirus detected was very similar to that previously detected in albatross and falcons.

Key words: Avipoxvirus, epizootic, Lesser Flamingos, *Phoenicopterus minor*, South Africa.

Avian pox is caused by infection with members of the genus *Avipoxvirus* of the family *Poxviridae*. The virus has a worldwide distribution with more than 232 affected species described in 23 orders of birds (Bolte et al., 1999). Prior to this investigation, avipoxviruses had only been reported in captive flamingos (Arai et al., 1991; Mondal et al., 2008).

Two forms of avian pox have been described: 1) a cutaneous form with proliferative, wart-like lesions on the unfeathered parts of the body (the legs, feet, face at the base of the beak, and eyelids) and 2) a diphtheritic form with the development of caseous necrotic lesions in the mucous membrane of the upper respiratory tract, mouth, and pharynx (Hansen, 2001).

Kamfers Dam (28°40′S, 24°45′E), a large (400 ha), perennial wetland near Kimberley, South Africa, supports the largest permanent population of Lesser Flamingos (*Phoenicopterus minor*) in southern Africa. Between late 2007 and early 2008, the Lesser Flamingos bred on a purpose-built island on the dam, producing an estimated 9,000 chicks (Anderson, 2008). This was the first successful breeding of Lesser Flamingos in South Africa, with Kamfers Dam now one of only four breeding localities in Africa; other breeding sites include Sua Pan in Botswana, Etosha Pan in Namibia, and Lake Natron in Tanzania (Childress et al., 2008).

From January to June 2008 regular observations were made of the Kamfers Dam flamingo breeding event. This involved monthly surveys to count chicks and weekly ground visits to check on progress of the breeding event. After fledging, juvenile flamingos left the island and there were occasionally small groups of up to 100 birds on the mainland. During observations of these fledged flamingos we noticed multiple small dermal lesions on the birds' legs and faces. By May 2008, toward the end of the breeding season, an estimated 30% of the juvenile flamingos had developed lesions.

To conduct a more detailed examination of the lesions, four juvenile Lesser Flamingos with gross lesions were euthanized and examined. Their estimated ages ranged from 3 to 4.5 mo. All four birds had lesions on their limbs. One had a small lesion on its lower bill. Most limb

lesions were located on or near the tibiotarsal joint, just distal to the junction of the feathered and scaled area of the limb and on the web of the foot. The lesions were raised, crusted plaques, some with a necrotic center, varying in size from a few millimeters to 1 cm in diameter. No other gross abnormalities were observed.

Tissue samples were taken from the skin and various organs and fixed in 10% buffered formalin. Histopathologic examination of the skin lesions showed multifocal, subacute, heterophilic, hyperplastic, and necrotizing dermatitis characterized by focal areas of marked epidermal hyperplasia and hypertrophy with many cells containing large, eosinophilic, granular, cytoplasmic inclusions (Bollinger bodies) and edema. In some cases, marked heterophilic or lymphoplasmacytic superficial dermatitis was present.

Formalin-fixed skin biopsies were processed for transmission electron microscopy with the use of standard techniques. Ultrathin resin sections were examined in a Philips CM10 transmission electron microscope (Philips Electronics, Eindhoven, Netherlands) operated at 80 kV. Many large cytoplasmic inclusion bodies containing numerous poxvirus particles were detected in the epidermal cells. The brick-shaped virions measured 360×280×170 nm, and, depending on the sectioning plane, exhibited a biconcave core and an envelope.

A diagnosis of avipoxvirus infection was made on the basis of the macroscopic, histologic and electron microscopic features, and was further confirmed by DNA sequence analysis. The P4b gene has been used in analyses of avian poxvirus phylogenetics (Jarmin et al., 2006; Carulei et al., 2009). This gene encodes the 75.2-kDA P4b protein, a precursor of the 4b virion core protein and is one of 49 genes conserved in all poxviruses (Binns et al., 1989; Upton et al., 2003). A partial P4b gene sequence from one of the infected flamingos was amplified by polymerase chain reaction with the use of primers 5′-TAAATGAGTTTGCG TATAAAAATC-GATAAG-3′ (corresponding to FWPVUS [AF198100] nt 159599–159628); 5′-CTT-CCGTATCCATAACTATCTTTGACTC-3′ (corresponding to FWPVUS [AF198100] nt 160307–160280). The analysis was carried out using a total volume of 50 µl, containing 1 µl of template (obtained by lysis of crude virus stock), 24 µl H_2O and 25 µl Biomix (Bioline, Tauton, Massachusetts, USA). The sample was subjected to 25 cycles (30 sec at 94 C, 30 sec at 45 C, 2 min at 72 C). A product of 708 base pairs (bp) was amplified and sequenced. The sequence was compared to the equivalent region in other avipoxviruses. All sequences were truncated to 428 bp to allow comparison (Fig. 1 and Table 1).

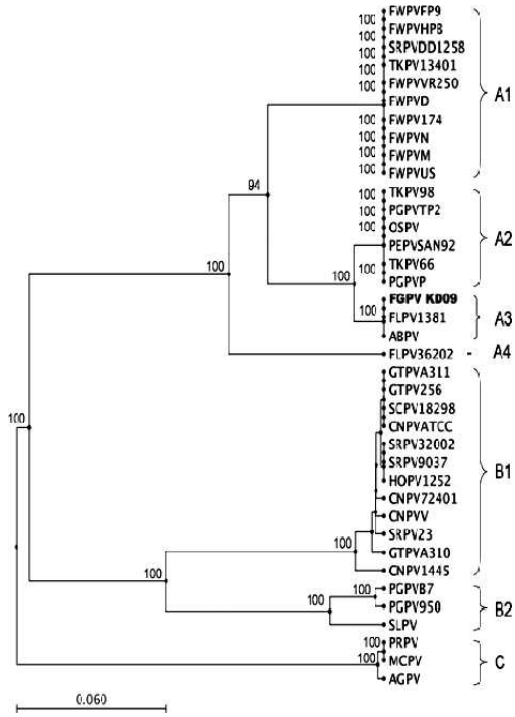

FIGURE 1. Neighbor-joining phylogenetic tree based on the MUSCLE alignment of partial P4b nucleotide sequences of 38 avian poxvirus isolates (Table 1). Flamingopox virus (FGPV KD09), shown in bold type, was isolated from a Lesser Flamingo at Kamfers Dam in 2009. Bootstrap values from 1,000 replicate samplings are shown as percentages.

TABLE 1. Virus acronyms, host species, and GenBank sequence accession numbers of all isolates or loci of avian poxviruses used in the study of avian poxviruses and Lesser Flamingos at Kamfers Dam, Kimberley, South Africa, 2008. Flamingopox virus (FGPV) isolated from a Lesser Flamingo is the subject of this report.

Avian poxvirus acronym	Host common name	Host species or family	Accession number
ABPV	Black-browed Albatross	*Thalassarche melanophrys*	AM050392
AGPV	Agapornis	*Agapornis* sp.	AY530311
CNPVATCC	Canary	Fringillidae	AY318871
CNPV72401	Canary	Fringillidae	AY530306
CNPV1445	Canary	Fringillidae	AM050375
CNPVV	Canary	Fringillidae	AM050384
FGPV	Lesser Flamingo	*Phoenicopterus minor*	GU204249
FLPV36202	Falcon	Falconidae	AY530306
FLPV1381	Falcon	Falconidae	AM050376
FWPVVR250	Chicken	*Gallus gallus*	AY453172
FWPVUS	Chicken	*Gallus gallus*	AF198100
FWPVHPB	Chicken	*Gallus gallus*	AY530302
FWPVFP9	Chicken	*Gallus gallus*	AJ581527
FWPV174	Chicken	*Gallus gallus*	AM050377
FWPVD	Chicken	*Gallus gallus*	AM050380
FWPVN	Chicken	*Gallus gallus*	AM050379
FWPVM	Chicken	*Gallus gallus*	AM050378
GTPV256	Great Tit	*Parus major*	AY453175
GTPVA310	Great Tit	*Parus major*	AY453173
GTPVA311	Great Tit	*Parus major*	AY453174
HOPV1252	Houbara bustard	*Chlamydotis undulata*	AM050381
MCPV	Macaw	Psittacidae	AM071513
OSPV	Ostrich	*Struthio camulus*	AY530305
PEPV	African Penguin	*Spheniscus demersus*	FJ948105
PRPV	Parrot	Columbidae	AM050383
PGPVB7	Pigeon	Columbidae	AY453177
PGPVTP2	Pigeon	Columbidae	AY530303
PGPVP	Pigeon	Columbidae	AM050385
PGPV950	Pigeon	Columbidae	AM050386
SCPV18298	Stone curlew	Scolopacidae	AY530310
SLPV	Starling	Sturnidae	AM050391
SRPVDD1258	Sparrow	Passeridae	AY530307
SRPV32002	Sparrow	Passeridae	AY530308
SRPV9037	Sparrow	Passeridae	AM050389
SRPV23	Sparrow	Passeridae	AM050390
TKPV13401	Turkey	*Meleagris gallopavo*	AY530304
TKPV66	Turkey	*Meleagris gallopavo*	AM050387
TKPV98	Turkey	*Meleagris gallopavo*	AM050388

FGPVKD09 (flamingopox virus Kamfers Dam 2009—GU 204249) was shown to group in clade A, subclade A3, showing greater similarity to fowlpox-like viruses (clade A) than canarypox-like viruses (clade B) or psittacine-like viruses (clade C). At the nucleotide level, FGPVKD09 was shown to be 100% identical to falcon isolates FLPV1381 (AM 050376) and Black-browed Albatross (*Thalassarche melanophrys*) ABPV (AM 050392), respectively. FGPVKD09 showed 100% identity to all other isolates in clade A at the amino acid level.

The similarity of the poxvirus detected in Lesser Flamingos to that previously detected in albatross and falcons suggests that the flamingos were infected with a virus that was already in circulation in wild birds. The locality of the lesions on the limbs is suggestive of a biting insect vector. The skin over the tibiotarsal joint

and at the junction of the feathered area of the limb is less protected by dermal scales and as a result more exposed than the rest of the limb, making these areas more prone to biting insects.

The effect of poxviruses varies considerably and in some populations, such as endemic Hawaiian forest birds (VanderWerf, 2001), it may be a significant mortality factor. Young and VanderWerf (2008) found that fledging success of Laysan Albatross chicks in years with high pox prevalence did not differ from fledging success in years with low prevalence and most of the chicks recovered from the infection, indicating a strong immunity to avian poxvirus. All the lesions seen in the Kamfers Dam flamingo population have been of the cutaneous form, which have regressed over time, indicating that they may also have a strong immunity to avian poxvirus.

The Lesser Flamingo is listed as near-threatened in South Africa and internationally, mainly because of a declining population, few breeding sites, and human-induced threats to breeding sites (Anderson, 2000). There is concern that this epizootic could cause additional stress on this declining population. The unsuitable habitat (larger pans with minimal vegetation) for mosquito vectors at other Lesser Flamingo breeding sites may, however, preclude the maintenance and spread of the disease at these sites. This, however, does not rule out the possibility of other biting arthropods, such as mites, acting as potential vectors for the virus.

In general, Lesser Flamingos are very gregarious and tend to congregate into small areas for breeding and foraging, making them especially vulnerable to infectious diseases. It will be important to monitor the prevalence of avian pox in the Kamfers Dam population annually, to get a better understanding of the epidemiologic factors of the disease and its long-term impact on the breeding population.

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