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AN EPIZOOTIC OF EMERGING NOVEL AVIAN POX IN CARRION CROWS (*CORVUS CORONE*) AND LARGE-BILLED CROWS (*CORVUS MACRORHYNCHOS*) IN JAPAN

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ABSTRACT: In 2006–10, an epizootic of emerging avian pox occurred in Carrion Crows (*Corvus corone*) and Large-billed Crows (*Corvus macrorhynchos*), leading to mortality of juvenile crows in Hokkaido, the northernmost island of Japan. We diagnosed 27 crows with proliferative skin lesions (19 carcasses and eight biopsied cases [one in zoo captivity]) as avian pox clinically, histopathologically by detection of *Avipoxvirus*-specific 4b core protein (P4b) gene, and epidemiologically. The fatal cases demonstrated intensively severe infection and aggressive lesions with secondary bacterial infection. Since the first identification of avian pox in Sapporo, Japan, in 2006, the frequency of mortality events has increased, peaking in 2007–08. Mortalities have subsequently occurred in other areas, suggesting disease expansion. In Sapporo, prevalence of avian pox evaluated by field censuses during 2007–12 was 17.6% (6.6–27.2%), peaked during 2007–08 and 2008–09, and then decreased. All diseased crows were juveniles, except for one adult. The number of crows assembling in the winter roosts had been stable for >10 yr; however, it declined in 2007–08, decreased by about 50% in 2008–09, and recovered to the previous level in 2009–10, correlated with the avian pox outbreak. Thus, avian pox probably contributed to the unusual crow population decline. All P4b sequences detected in six specimens in Sapporo were identical and different from any previously reported sequences. The sequence detected in the zoo-kept crow was distinct from any reported clades, and interspecies transmission was suspected. This report demonstrates an emerging novel avian pox in the Japanese avifauna and in global populations of Carrion Crows and Large-billed Crows. Longitudinal monitoring is needed to evaluate its impact on the crow population.

Key words: 4b Core protein (P4b) gene, avian pox, *Avipoxvirus*, Carrion Crows, epidemiology, Large-billed Crows.

INTRODUCTION

Avian pox is an infectious disease caused by double-stranded DNA viruses of the genus *Avipoxvirus* (Skinner et al. 2012). Avipoxviruses (APVs) have been isolated from 278 bird species worldwide (van Riper and Forrester 2007). There is the possibility of interspecies transmission, as evidenced by phylogenetic analysis based on the APV-specific 4b core protein (P4b) gene (Weli and Tryland 2011; Gyuranecz et al. 2013). The named number of APV species is much smaller than the number of infected bird species, suggesting that further molecular characterization is needed. In Japan, there are a few case reports of avian pox in wild birds (Saito et al. 2009; Watanabe et al. 2009) and captive birds (Yu et al. 2007;

Terasaki et al. 2010), but the epidemiology among the Japanese avifauna is poorly studied.

In most birds, APV infections have been reported as mild, self-limiting, and rarely fatal; however, when lesions occur on eyelids with secondary infection or in diphtheritic form, they can be fatal (Tripathy et al. 2000; van Riper and Forrester 2007). Endemic bird populations on islands (e.g., the Hawaiian, Canary, and Galapagos islands) were more negatively affected by an introduced APV infection than those on continents where the hosts and viruses have had longer coevolutionary histories (Warner 1968; van Riper et al. 2002; Smits et al. 2005; Parker et al. 2011). Exotic APVs may have had a role in the population decline of Hawaiian Crows (*Corvus hawaiiensis*; Jenkins et al. 1989). In other corvids, there are reports of APV infection (Bolte et al. 1999; Miller et al. 2010; Wheeler et al. 2014), including a single case report in a Carrion Crow (*Corvus corone*; Poulding 1960) and a Large-billed Crow (*Corvus macrorhynchos*; Joshi et al. 2012). In Italy, an APV was isolated from a Hooded Crow (*Corvus corone cornix*), with phylogenetic analysis (Manarolla et al. 2010) based on P4b gene and H3L gene loci (Jarmin et al. 2006). In most parts of Japan, Carrion Crows and Large-billed Crows are common wild birds inhabiting urban and rural areas in close association with humans, but little is known of avian pox virus infection in these two species.

We report an outbreak and the subsequent epizootic of avian pox that emerged among the crow populations in Japan in 2006–10. We document the clinical and pathologic features, the phylogenetic classification of the detected APV P4b genes, and the results of an assessment of the effect on the local crow populations.

MATERIALS AND METHODS

Mortality reports, study areas, and specimens

Between November 2006 and December 2010, more than 29 mortality incidents of crows with proliferative skin lesions were reported by private citizens on Hokkaido, the northernmost island of Japan. We obtained 19 carcasses to evaluate the cause of death, mainly from Sapporo, Japan (43°03′43″N, 141°21′16″E; Table 1). Of these, 13 crows collected in 2006–09 were subjected to necropsy, histopathologic examination, and APV gene detection (cases 1–13, Table 2). The remaining six carcasses (cases 14–19), collected in 2010, were subjected to visual inspection of the lesions.

TABLE 1. Summary of demographics of 19 carcasses of crows (Carrion Crows [*Corvus corone*] and Large-billed Crows [*Corvus macrorhynchos*]) with avian pox–suspected skin lesions from Hokkaido, Japan, 2006–11. Values are numbers of crows collected; numbers in parentheses are those collected in Sapporo.

Demographic	No.
Type of examination	
Necropsy (cases 1–13 in 2006–09)	13 (11)
Visual inspection (cases 14–19 in 2010)	6 (1)
Species	
Carrion Crows	10 (5)
Large-billed Crows	9 (7)
Location	
Sapporo	12
Hiroo	2
Asahikawa	5
Collected by year	
2006–07	1 (1)
2007–08	6 (6)
2008–09	3 (2)
2009–10	3 (2)
2010–11	6 (1)
Collected by month, 2006–11	
September	4 (3)
October	7 (7)
November	3 (2)
December	4 (0)
April	1 (0)

We also examined eight surviving crows with nodular skin lesions biologically and clinically and obtained biopsy specimens of the lesions for histopathologic examination and APV gene detection. The surviving crows included a clinical case in a Large-billed Crow kept for exhibition at a zoo in Asahikawa, Japan, after being trapped by the local government in 2008 (case 20), and seven crows captured in 2008–09 at banding surveys in Sapporo, Japan (cases 21–27).

Biological examination: age class, body condition, and body mass

We classified crows into two age groups: juveniles (<1 yr old) and adults (>1 yr old) (Goodwin 1977; Jenni and Winkler 1994). We assigned body condition as emaciated (scored as

TABLE 2. Characteristics of carcasses of 13 necropsied crows diagnosed with avian pox from Hokkaido, Japan.[a]

Case	Date carcass found	Species[b]	Location[c]	Sex[d]	Body condition[e]	Body mass (g)	Comorbidities and other histopathologic findings[f]	APV[g]
1	November 2006	*Cc*	S	M	3	570	SBI, hyperplasia and ballooning degeneration of epithelial cells in tracheal, bronchial, and air sac; congestion in lung	+
2	September 2007	*Cm*	S	F	2	457	SBI, HNE (*Clostridium perfringens* infection), airsacculitis, hepatic necrosis	+
3	October 2007	*Cc*	S	U	1	317	SBI, hepatic necrosis	−
4	October 2007	*Cc*	S	M	1	325	SBI, airsacculitis, hepatic necrosis	+
5	October 2007	*Cm*	S	M	1	482	SBI, airsacculitis, HNE (*C. perfringens* infection suspected)	+
6	October 2007	*Cm*	S	F	2	470	SBI, FI in air sac (*Aspergillus* infection suspected), hepatic necrosis	+
7	November 2007	*Cm*	S	M	1	428	SBI, FI in liver (*Aspergillus* infection suspected)	+
8	September 2008	*Cm*	S	M	4	850	Aspiration pneumonia, systemic congestion, hepatic necrosis	+
9	October 2008	*Cm*	S	F	2	464	SBI, septicemia, FI in gizzard (*Candida* infection suspected)	+
10	April 2009	*Cc*	H	M	1	430	SBI, airsacculitis, septicemia	−
11	September 2009	*Cc*	S	U	3	478	SBI, hemorrhage in lung	+
12	September 2009	*Cc*	H	M	1	416	SBI, aspiration pneumonia	−
13	October 2009	*Cm*	S	F	2	540	SBI, HNE (*C. perfringens* infection suspected), necrotic hepatitis	−

[a] Avipoxvirus (APV) infection was confirmed based on characteristic histopathologic findings (Bollinger body). One case (case 8) that had inactive avian pox lesions after the masses fell off (skin defects and necrosis in the adjacent bone) was diagnosed as avian pox by detection of APV-specific 4b core protein (P4b) gene.

[b] *Cc* = Carrion Crow (*Corvus corone*); *Cm* = Large-billed Crow (*C. macrorhynchos*).

[c] S = Sapporo; H = Hiroo.

[d] M = male; F = female; U = unknown.

[e] 1 = emaciated; 2 = poor; 3 = average; 4 = good.

[f] SBI = secondary bacterial infection in the lesions; HNE = hemorrhagic necrotic enteritis; FI = fungal infection.

[g] + = positive for APV P4b gene; − = negative for APV P4b gene.

1), poor (2), average (3), or good (4) based on visual inspection of pectoral muscle mass and fat deposits between the clavicles. We compared body masses between avian pox cases and healthy juveniles captured between October and February during 2008–12 (136 Carrion Crows and 74 Large-billed Crows) and statistically tested differences by Mann-Whitney *U*-test.

Avian pox diagnosis and evaluation of skin lesions

We confirmed avian pox histopathologically (van Riper and Forrester 2007) or by APV gene detection (Lee and Lee 1997). Additionally, we made diagnoses based on clinical findings of the characteristic, proliferative skin lesions (van Riper and Forrester 2007; Lawson et al. 2012) and epidemiologically.

We recorded the number and distribution of skin lesions on various parts of the body and scored the severity of infections as mild (1 lesion), moderate (2 lesions), or severe (3 or more lesions or one lesion on the head; van Riper et al. 2002).

Necropsy and histopathologic examination

We tested all submitted carcasses for avian influenza virus and West Nile virus (WNV) using commercial rapid diagnostic test kits (ESPLINE® Influenza A and B–N Fujirebio, Inc., Tokyo, Japan;

and VecTest®, Medical Analysis Systems, Camarillo, California, USA, respectively). We performed complete necropsies with subsequent microbiologic and histopathologic examinations. Skin lesions and tissue samples from all major organs in the carcasses and biopsy skin specimens in the surviving crows were fixed in 20% neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined.

Detection of APV P4b genes and phylogenetic analysis

We performed P4b gene detection by PCR on the specimens (Lee and Lee 1997; Saito et al. 2009). We extracted DNA from homogenates of the specimens using SepaGene® (Sanko Junyaku Co., Ltd., Tokyo, Japan). The purified DNA was applied for the PCR with two *Fowlpox virus*–specific primers (P1X [5′-TCAGCAGGTGC TAAACAACA-3′] and P2 [5′-CGGTAGCT TAACGCCGAATA-3′]) (P1XP2 PCR). For P4b gene detection of APVs closely related to *Canarypox virus*, we used another primer pair (P1canF [5′-TGCAAGATTTAATCAGCGTG-3′] and P2canR [5′-GCTTCATCTGATCTCCTATTC-3′]) (canFR PCR). The expected sizes of the amplified DNA fragments were 579 and 183 base pairs, respectively. We performed PCR amplifications of the DNA at 94 C for 1 min, 59 C (55 C for canFR PCR) for 1 min, and 72 C for 1 min for 35 cycles after initial denaturation for 5 min at 94 C. We purified the PCR products and determined the DNA sequences using Applied Biosystems® 3130 Genetic Analyzer (Life Technologies Corp., Carlsbad, California, USA). The APV P4b gene sequences, detected and obtained from the DNA Databank of Japan (DDBJ; Japanese National Institute of Genetics 2015), were aligned using the CLUSTALW program in MEGA (Molecular Evolutionary Genetics Analysis) 6.06 (Tamura et al. 2013). We constructed a phylogenetic tree using the neighbor-joining method (Saitou and Nei 1987).

Epidemiologic field surveys and population monitoring

To provide an index of avian pox prevalence within the crow populations in Sapporo, we conducted fixed-spot censuses and banding surveys. Fixed-spot censuses were conducted between July (when almost all juveniles had left their nests) and the following February (the last month of the winter roosting season before dispersal for breeding) during 2007–08 and 2011–12 (341 censuses: 10–59 times in each 1-yr period) (Reynolds et al. 1980). The censuses focused on spotting diseased individuals and evaluating adults that are rarely trapped at banding surveys. We recorded the number of crows observed, their behavior, age, and the presence of skin lesions within a radius of 50 m for 5–10 min between 0800 and 1900 hours. We accumulated the number of crows observed at each census (one to 56) and calculated proportions of crows with lesions for each year.

Banding surveys were conducted in the suburbs between October and the following February, the period in which crows assemble in winter roosting sites, during 2008–09 and 2011–12 (54 surveys: nine to 17 times each year). We banded 262 crows (170 Carrion Crows and 92 Large-billed Crows) captured in traps, and recorded age, body mass, general physical condition, and the presence of skin lesions, before release. When skin lesions were found, they were sampled. We accumulated the number of trapped crows at each session (one to 17) and calculated proportions of crows with lesions for each year.

For assessments of the crow populations, we counted the number of incoming crows in the winter roosts from 1 h to 2 h before sunset until 0.5–1.5 h after sunset in 2006–07 and 2011–12.

RESULTS

Mortality incidents and distribution

Most of the carcasses (12/19, 63%) were found in Sapporo and between September and December (18/19, 95%; Table 1). In Sapporo, we confirmed the first avian pox case (case 1) in 2006. Subsequently, mortalities from avian pox increased, peaking in 2007, and then occurring sporadically until 2010. The remaining seven carcasses were collected at two areas in Hokkaido: Hiroo, Japan (42°17′10″N, 143°18′42″E, approximately 270 km SE of Sapporo; n=2), in 2008–09 (case 10) and in 2009 (case 12), and Asahikawa, Japan (43°46′15″N, 142°21′54″E, approximately 130 km NE of Sapporo; n=5) in 2010. In Hiroo, the two carcasses were collected for subsequent examination, although 12 carcasses with similar skin lesions were found between February 2008 and September 2009.

Biological findings: species, age class, body condition, and body mass

All 27 examined crows (13 Carrion Crows and 14 Large-billed Crows) were juveniles. Of the 13 necropsied carcasses, 77% (10/13) were in emaciated or poor body condition (Table

2). There were significant differences in body mass between avian pox cases and healthy Carrion Crows (422.7±95.5 g versus 646.1±73.6 g) and Large-billed Crows (527.3±146.3 g versus 773.9±77.9 g) ($P<0.01$ for both comparisons).

Characteristics of skin lesions and clinical findings

In the 21 crows we assessed (13 carcasses and eight biopsied surviving cases), the severity of infection was scored as *severe*, except for one clinical case assessed as *moderate* (Table 3). In that case (case 20), the lesions on the toes were evaluated as having healed with scars 1.5 mo after biopsy.

Most lesions occurred on toes (90%; 19/21) followed by eyelids (57%; 12/21) (Fig. 1). Most fatal cases had lesions on the eyelids and three or more digits of the toes (62%; 8/13). Only fatal cases had lesions elsewhere (e.g., bill, mouth commissure). In the epidemiologic field surveys, two diseased crows (cases 2 and 6) were found still alive and unable to fly, leading to their capture. They became lethargic and died the next day (Fig. 1).

Pathologic findings, APV P4b genes, and diagnoses

Grossly, 26/27 cases had multiple proliferative wart-like skin lesions. All lesions of the eight biopsied cases were solitary, distinct nodules. In contrast, all lesions of 18/19 fatal cases were extensively proliferative and aggressive masses, subsequently involving injury, ulceration, and necrosis with a putrid odor. The largest coalescent mass lesions measured up to 2.4 cm in diameter (Fig. 1).

Histopathologically, 18 of 21 crows, had epithelial cell hypertrophy and hyperplasia with ballooning degeneration, containing intracytoplasmic Bollinger bodies, and were confirmed as avian pox cases. In 19/21 cases, infiltrating bacterial colonies were visible in the ulceration of the keratinized superficial epidermis. All fatal cases had comorbidities (Table 2). We detected APV P4b genes in 15/21 cases, including three histopathologically avian pox-negative cases. We also assumed the remaining six carcasses were avian pox cases based on the severe skin lesions. We diagnosed all examined 27 crows as avian pox cases.

TABLE 3. Skin lesions in 21 crows (Carrion Crows [*Corvus corone*] and Large-billed Crows [*Corvus macrorhynchos*]) from Hokkaido, Japan, diagnosed with avian pox.

	Carcasses (n=13)[a]	Biopsied cases (n=8)[b]	Total (n=21)
Severity of infections			
Moderate	0	1	1
Severe	13	7	20
Distribution			
Toe	12	7	19
Eyelid	9	3	12
Bill, cere, and mouth commissure	5	0	5
Foot	4	0	4
Bend of wing	1	0	1
Other head region[c]	4	0	4
Gross findings: proliferative skin lesion[d]	12 (masses)	8 (nodules)	20
Histopathologic findings: Bollinger body[e]	12	6	18
APV P4b gene: P1XP2 PCR; canFR PCR	7; 9	3; 6	10; 15

[a] Cases 1–13.

[b] Cases 20–27.

[c] Other head region included jaw, crown, and neck.

[d] One case had inactive lesions (case 8).

[e] Three cases had no Bollinger bodies. One case (case 8) was diagnosed having necrosis in the adjacent bone. Two biopsied cases (cases 24 and 26) involved suppurative dermatocellulitis.

Phylogenetic analysis of detected APV P4b genes

We submitted the nucleotide sequences of the APV P4b gene from seven specimens (cases 1, 2, 9, 11, 23, 26, and 20) to the DDBJ database under accessions LC055558–64). In six Sapporo specimens (Carrion Crows in 2006 [n=1] and 2009 [n=2]; Large-billed Crows in 2007 [n=1], 2008 [n=1], and 2009 [n=1]), the nucleotide sequences of the P1XP2 PCR products were identical (Sapporo strain). The P4b nucleotide sequence of the

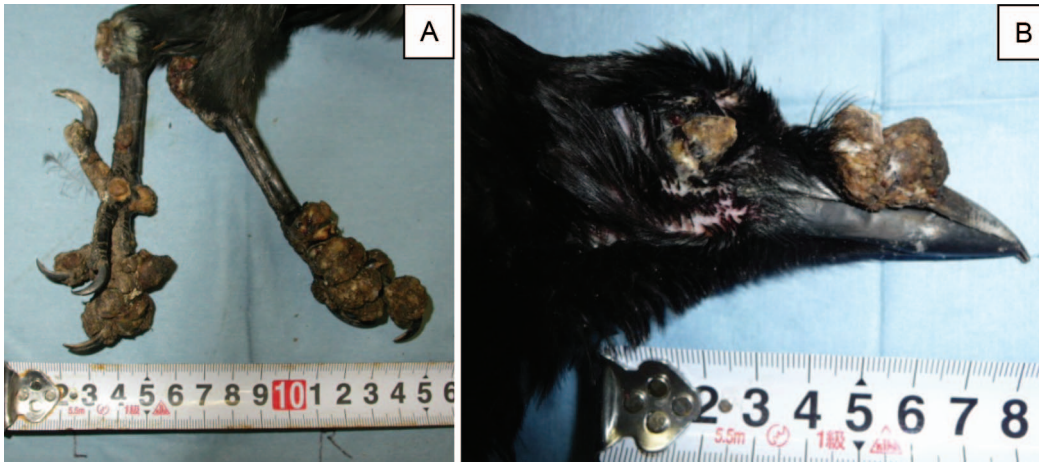


FIGURE 1. Gross findings of skin lesions in crows with *Avipoxvirus* infection. Nodular and proliferative coalescent masses grow: (A) on both feet and toes, leading to inability to walk, in a Large-billed Crow (*Corvus macrorhynchos*) carcass collected in Sapporo, Japan, in October 2007 (case 6); (B) on both right upper and lower eyelids (leading to impaired vision) and cere (infiltrating into the nasal cavity), in a Carrion Crow (*Corvus corone*) carcass collected in Hiroo, Japan, in September 2009 (case 12).

zoo-kept Large-billed Crow specimen (case 20) differed from those of the Sapporo strain, with 71% similarity (Asahikawa strain). We found no identical P4b nucleotide sequences among strains in the DDBJ by BLAST (Basic Local Alignment Search Tool) search. In the phylogenetic tree (Fig. 2), the Sapporo strain clustered in subclade B1 with a *Fowlpox virus* isolated from a Hooded Crow (GQ180211) (Jarmin et al. 2006) with 99% nucleotide and amino acid similarity. The evolutionary distance was 0.008. In the Asahikawa strain, the closest nucleotide similarity was with an APV isolate from a Macaw (*Ara* spp.; AM050382) in clade C (75% identity with the other three accessions). The genetic distance was 0.303. The Asahikawa strain formed a single outlier branch with a bootstrap value of 82%.

Avian pox prevalence and number of roosting crows

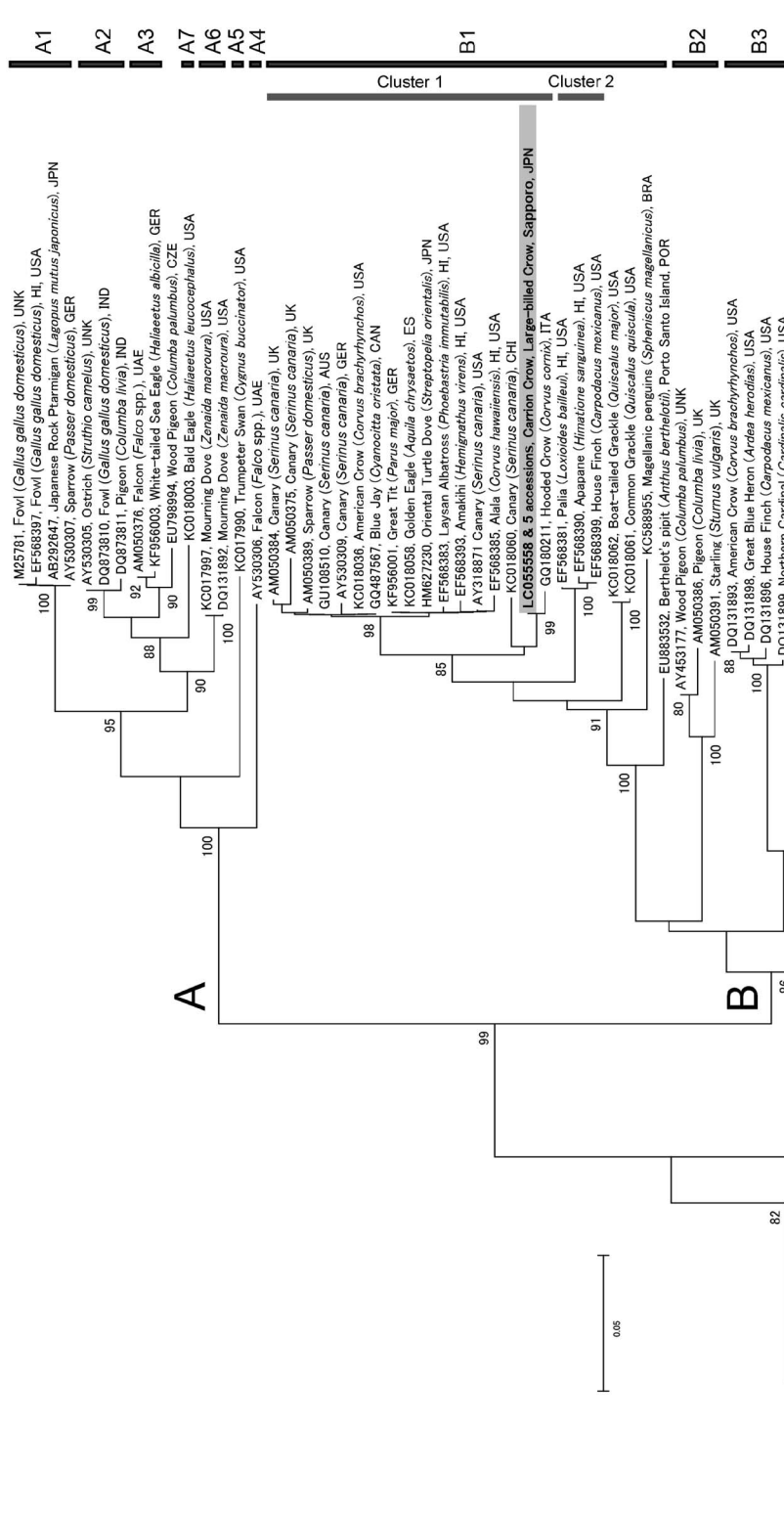
In the fixed spot censuses, 43.8% (892/2,036) of observed crows were juveniles. All diseased crows were juveniles, except for one adult. The total 5-yr avian pox rate was 17.6% (358/2,036) in all crows and 40.0% (357/892) in juveniles. Avian pox prevalence in all crows was highest at 22.7% in 2007–08 and 27.2% in 2008–09 (Fig. 3), and decreased to 6.6% in 2011–12. In juveniles, avian pox prevalence was highest at 57.3% in 2007–08 and 52.1% in 2008–09, and decreased to 17.0% in 2011–12.

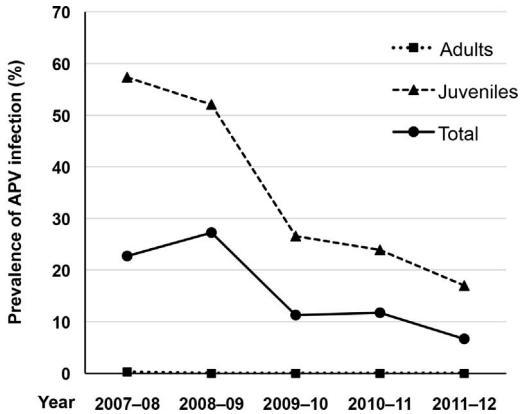
Most (93.9%, 246/262) trapped crows were juveniles (Carrion Crows: 155/170, Large-billed Crows: 91/92), and all diseased crows were juveniles. The total 4-yr avian pox prevalence was 14.5% (38/262): 12.4% (21/170) in Carrion Crows and 18% (17/92) in Large-billed Crows. Avian pox prevalence increased from 7% in 2008–09 to a peak of 30% in 2009–10 (Fig. 4) and decreased to 13% in 2011–12. No difference in changes of avian pox prevalence was observed between the two species.

The number of roosting crows was stable at around 7,000 to 8,500 between 2002–03 and 2006–2007 when we detected the first avian pox case (Fig. 5). The crow numbers decreased to 6,133 in 2007–08 and then dropped to 4,680 in 2008–09 as the avian pox prevalence increased, and recovered to around 7,500 in 2009–10.

DISCUSSION

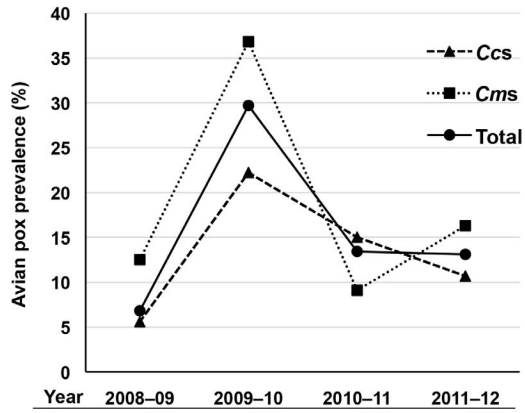
Avipoxvirus infection has been frequently documented in a variety of hosts worldwide (van Riper and Forrester 2007); however, in





Year	2007–08	2008–09	2009–10	2010–11	2011–12
Adults	1/421	0/175	0/236	0/165	0/147
Juveniles	157/274	100/192	46/173	38/159	16/94
Total	158/695	100/367	46/409	38/324	16/241

FIGURE 3. The proportions of crows (Carrion Crows [*Corvus corone*] and Large-billed Crows [*Corvus macrorhynchos*]) with avian pox–suspected skin lesions are shown as avian pox prevalence. Crows were counted by fixed spot censuses in Sapporo, Japan, between July and February in 2007–12. Values below the year indicate number of diseased individuals/counted individuals in adult, juvenile, and total crows. APV=Avipoxvirus.



Year	2008–09	2009–10	2010–11	2011–12
Ccs	2/36	4/18	9/60	6/56
Cms	1/8	7/19	2/22	7/43
Total	3/44	11/37	11/82	13/99

FIGURE 4. The percentages of crows with avian pox–suspected skin lesions are shown as avian pox prevalence for Carrion Crows (*Corvus corone*; *Cc*) and Large-billed Crows (*Corvus macrorhynchos*; *Cm*), captured at banding surveys in the suburbs of Sapporo, Japan, between October and February 2008–12. Most (93.9%, 246/262) trapped crows were juveniles. All diseased crows were juveniles. Values below the year indicate number of diseased/banded crows.

Japan, APV infection has not been reported in corvids. Herein, we report an epizootic in 2006–10 of avian pox in crows emerged in Hokkaido, thereby documenting an emerging avian pox outbreak in the Japanese avifauna and in global populations of Carrion Crows and Large-billed Crows.

In Sapporo, since the first identification of an avian pox case in a Carrion Crow in 2006, mortality incidents from avian pox increased, with a peak in 2007. The avian pox prevalence (6.6–27.2%, 17.6% for 5 yr) was much higher than those reported for avian pox in wild birds in endemic regions (0.5–1.5%) (van Riper and Forrester 2007). Moreover, the prevalence was similar to those of novel APV introduced in naïve populations on islands (>10%) (van Riper et al. 2002; Smits et al. 2005). In general, avian pox has been reported as mild and rarely resulted in death in most birds (van Riper and Forrester 2007); however, a newly introduced APV can cause severe infection (Warner 1968; Jenkins et al. 1989; van Riper and Forrester 2007). In Hawaiian Crows, it was reported that avian pox could cause direct

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FIGURE 2. Neighbor-joining phylogenetic tree based on DNA sequences of *Avipoxvirus* (APV)-specific 4b core protein genes detected in crows in Japan and other isolates downloaded from the DNA Databank of Japan database. Sequences of *Molluscum contagiosum virus* (MOCV: MCU60315) P4b gene were used as an outgroup. The APV clades A, B, and C, with subclades A1–7 and B1–3, are labeled according to previous reports (Jarmin et al. 2006; Gyuranecz et al. 2013). Bootstrap values for 1,000 replicates (posterior probability values of >80) are shown at the respective nodes. The bar indicates one substitution per 100 nucleotides. Isolate accession numbers, hosts, and origins are indicated with the following abbreviations: AUT=Austria; BRA=Brazil; CAN=Canada; CZE=Czech Republic; CHI=Chile; GER=Germany; HI=Hawaiian Islands; IND=India; ITA=Italy; JPN=Japan; MAR=Morocco; POR=Portugal; ES=Spain; UAE=United Arab Emirates; UK=United Kingdom; USA=United States of America; UNK=unknown. The APVs detected in Carrion Crows (*Corvus corone*) and Large-billed Crows (*Corvus macrorhynchos*) in the present study are highlighted in gray.

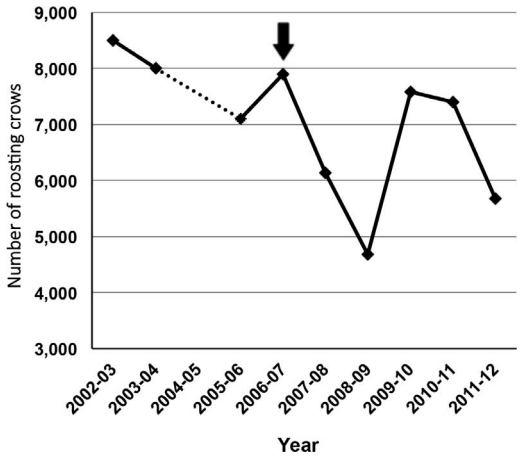

FIGURE 5. Changes in the numbers of roosting crows, including Carrion Crows (*Corvus corone*) and Large-billed Crows (*Corvus macrorhynchos*), counted in the winter roosts in Sapporo, Japan, between 2006–07 and 2011–12 (on 11 February, except for one survey on 19 January, in 2007–08). Our previous data between 2002–03 and 2005–06 are also indicated for comparison (not obtained in 2004–05). The period when the first carcass was diagnosed with avian pox is shown by an arrow.

mortality and skin lesions around the eye and bill, resulting in limited survival (Jenkins et al. 1989; Tripathy et al. 2000). In this study, the APV infection was fatal in juveniles of both species, and most cases were scored as *severe* infection. In the fatal cases, the skin lesions grew distinctively, more as large invasive masses than in previous reports (Poulding 1960; Joshi et al. 2012), and the lesions were likely to cause deaths because of disabilities (i.e., impaired vision or inability to feed). Additionally, secondary bacterial infections in the lesions and debilitation caused by the avian pox were considered to lead to immunosuppression and subsequent complications (e.g., fungal infection). These results indicate that a new APV incursion into the endemic avifauna in Sapporo and an epizootic of emerging novel APV infection occurred within the crow population. The reason why almost only juveniles were affected might be related to their weak immune capacity or the lack of acquired immunity, as reported by Buenestado et al. (2004).

The number of roosting crows in Sapporo was approximately 8,000 in 1996 and 1999 (M.T. unpubl. data), and it had been stable for 10 yr; however, an unusual decline in the numbers during 2007–08 and 2008–09 (nearly half of that in 2006–07) was concurrent with the avian pox outbreak. In 2009–10, the number of crows recovered to the previous level, which was correlated with decreasing avian pox prevalence. In Japan, crow mortality incidents from *Clostridium perfringens*–induced enteritis (Asaoka et al. 2004) and pesticide poisoning (Hosono et al. 2006) were previously reported; however, such unusual crow die-off incidents were not seen in Hokkaido during our investigation period. In Hawaii, an exotic, emerging APV had a negative impact on the population of Hawaiian Crows (Jenkins et al. 1989). Similarly, the outbreak of emerging novel avian pox might have contributed to the unusual crow population decline. The number of crows tended to decrease again in 2011–12, but the reason for this is unclear. Further monitoring is important to evaluate the subsequent potential impact on the crow populations because the long-term influence of juvenile mortality on population growth is unpredictable, and the APV infection prevalence might be changed by crow influx and dispersal movements.

Commonly, APV transmission can be facilitated mainly by arthropods or direct contact with diseased birds (van Riper and Forrester 2007). In Sapporo, the mortality incidents from avian pox were concentrated between September and December, correlated with the avian pox high-prevalence period: we usually sighted diseased crows from the end of July to the following March (data not shown). In the Sapporo area, blood-sucking insects (e.g., *Culicidae* mosquitoes, *Culicoides* midges) emerge from May until October with peaks in late July and early August (Kanasugi and Sasaki 1994; Niizuma and Sasaki 1994). During field surveys, we observed these biting insects between June and August, correlating with high avian pox prevalence. Additionally, we observed that juvenile crows (including diseased individuals) flocked and foraged together from June with a peak in the

population density after they left their natal areas, consistent with a previous report (Tamada and Fujimaki 1993). Family members of the fatal cases tended to have similar skin lesions: in field observation of the 13 collected carcasses, four cases had diseased siblings (n=3) and one a diseased mother. Subsequently, the crows assembled at winter roosts from around August, followed by increased avian pox mortalities. Diseased crows were often observed being pecked on the skin lesions by other crows. Therefore, it was possible that the avian pox seasonal increase was facilitated and enhanced by arthropod vectors (Jenkins et al. 1989; van Riper et al. 2002) or by direct contact via sharing the same behavioral patterns (Lachish et al. 2012a), particularly among family members at their nests and congregation sites (e.g., foraging spots or winter roosts). The temporal lag between the peak mortality/prevalence and the blood-sucking insect emergence or the period of leaving their nests might be explained by a latent period between infection and disease for the APV or the disease progress (Lachish et al. 2012b).

Our P4b sequence analysis revealed that there are two APV strains affecting crows in Hokkaido. The Sapporo strain was genetically close to a *Fowlpox virus* from a Hooded Crow in Italy (Manarolla et al. 2010). In general, APVs tend to be specific to host family, and phylogenetically, each clade infects a similarly diverse range of bird hosts (Gyuranecz et al. 2013). Thus, the Sapporo strain may form an APV group that infects corvids as a natural host. The source and route of introduction of the Sapporo strain into the crow population were unclear. However, a new APV could have been introduced via infected, migrating birds or through vectors, such as mosquitoes (Jenkins et al. 1989; Lawson et al. 2012). The mean annual temperature in Japan has increased by about 1.0 C during the past century, indicating the possibility of a change in the distribution of migrating birds or arthropods (Case and Tidwell 2007). Indeed, recently, new records of wild birds, including corvids, and an expansion of their range have been reported in Hokkaido (Hokkaido-Block Council of Wild Bird Society of Japan 2007). Thus, it is possible that the virus was brought by 1) an overlooked endemic host, including corvids in Hokkaido or crows from the main island of Japan; 2) infected birds from overseas migrating into Sapporo, which is located on their East Asian–Australasian Flyway; or 3) infected arthropods.

The Asahikawa strain, detected only in a zoo-kept crow, seems to be independent from any of the three major clades and is considered a novel APV. The severity of infection was scored as *moderate* only in this case. It is unclear whether the Large-billed Crow is a natural host of this APV, and the source of introduction into the zoo is unknown. In zoos, which house a variety of bird species at much higher densities than in the wild, there is concern that the APV might have spilled over from the other species of captive birds (interspecies transmission) (Weli and Tryland 2011; Gyuranecz et al. 2013). For zoo biosecurity, we conducted health management practices to prevent disease spread to captive birds in nearby cages (e.g., isolation of the patient, screening of captive birds).

In conclusion, an outbreak and epizootic of emerging novel *Avipoxvirus* infection caused juvenile crow mortality and likely led to local population decline in Sapporo, Japan, during 2006–10. Emerging wildlife infectious diseases have been frequently driven by anthropogenic environmental changes (Daszak et al. 2001). In Galapagos finch species (e.g., Small Ground Finch [*Geospiza fuliginosa*]), human disturbance (i.e., changes in land use) could have affected immune function in the host species that contribute to APV emergence (Zylberberg et al. 2013). Avipoxviruses can spread more rapidly as host and vector abundance increase (van Riper et al. 2002). In our study, the environmental changes that could have resulted in exposure to stressors were unknown; however, the emerging APV prevalence in the crows could have been enhanced by human factors, such as crow congregation in urban areas (e.g., garbage depots; Becker et al. 2015), or expansion of the range of migrating birds and arthropods influenced by climate change (Fuller et al.

2012). Additionally, the emergence of novel APVs represents a possibility for the incursion of other viruses into Japan, including WNV, which is a significant zoonotic arthropod-borne virus. In the US, the American Crow (*Corvus brachyrhynchos*) is routinely used as a sentinel for WNV surveillance (Eidson et al. 2005). Similarly, APVs and WNV-sensitive Carrion Crows (Dridi et al. 2013) or Large-billed Crows (Shirafuji et al. 2008) can be useful as an epidemiologic model for critical diseases driven by wild birds and arthropods. Continued monitoring of the APVs in crows and further nationwide APV surveillance based on molecular epidemiology will be important to understand the APV epidemiology in Japanese avifauna, which should contribute to ecosystem health.

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