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Short Communication

Surveillance of Marek's disease virus in migratory and sedentary birds in Hokkaido, Japan

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Abstract

Marek's disease virus serotype 1 (MDV-1) strains cause malignant lymphoma in chickens. MDV-1 has been previously reported to be widespread in white-fronted geese (*Anser albifrons*); however, the prevalence of MDV-1 in other wild birds has not been determined. In this study, we investigated the prevalence of MDV-1 in various wild birds in Hokkaido, Japan. The MDV-1 genome was widespread in geese and ducks, but was not detected in other birds. MDV-1 was detected in both sedentary and migratory species. These results suggest that, in Japan, MDV-1 is widespread in wild goose and duck populations, and that resident ducks may be significant carriers and reservoirs of MDV-1.

Keywords: Marek's disease virus serotype 1, Prevalence, Migratory birds, Sedentary ducks, Geese, *meq* gene

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by a cell-associated alphaherpesvirus, Marek's disease virus (MDV; family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Mardivirus*, species *Gallid Herpesvirus* 2 (GaHV-2)) (Calnek and Witter, 1997). Of the previously described serotypes of MDV, now classified as GaHV-2 or MDV serotype 1 (MDV-1), GaHV-3 or MDV-2, and *Meleagrid herpesvirus* 1 or MDV-3 (herpesvirus of turkeys); MD is caused by MDV-1 strains (exclusive of attenuated vaccine strains) (Calnek and Witter, 1997). Currently, MD is well controlled by the administration of live vaccines (Witter, 1997). However, MDV-1 tends to increase in virulence, and several MD cases in vaccinated chickens have been reported, suggesting that highly virulent MDV-1 could potentially induce future outbreaks (Witter, 1997).

Virological or serological evidence of MDV-1 infection has been reported in several genera of the order Galliformes; of which the most important natural host is the domestic chicken (Calnek and Witter, 1997). However, in 2001, an MD case was reported in a white-fronted goose (*Anser albifrons*) that had migrated from Russia to Hokkaido, Japan (Murata et al., 2007b). Subsequently, we investigated the prevalence of MDV-1 in wild waterfowl and ascertained that it was widespread in white-fronted geese (Murata et al., 2007b). Wild waterfowl usually flock together and migrate between breeding habitats and warm areas; thus they could play a significant role as reservoirs and carriers of MDV-1 to domestic poultry. Although MDV-1 is widespread in geese, clinical MD cases, except for that detected in 2001, have not been reported. This suggests that wild geese may be resistant

to MD. This may be the same situation in other wild birds, i.e. MDV-1 may be prevalent but the infected birds may be asymptomatic.

We investigated the prevalence of MDV-1 in wild birds by using nested PCR to screen for the *meq* gene in DNA samples extracted from feather tips. The *meq* gene is only detected in MDV-1 strains, and the length of the gene is 1 020 bp (Jones et al., 1992); however, in some low-virulent strains a 180-bp sequence is inserted in the *meq* open reading frame, termed L-*meq* (Chang et al., 2002; Shamblin et al., 2004). The *meq* gene, but not the L-*meq* gene, could be detected by nested PCR using feather-tip samples, even in chickens co-infected with high- and low-virulent strains (Murata et al., 2007a); therefore, strains carrying the L-*meq* gene could be distinguished from strains carrying the *meq* gene by this method. In this study, feather tip samples were collected from 309 wild geese and ducks, captured, in Hokkaido, by using ring nets and flat net traps with permission from the Ministry of Environment, Japan and from 40 other wild birds found dead in the Hokkaido region. These samples were analyzed as described previously (Murata et al., 2007a). We captured the wild geese and ducks harmlessly. The wild birds were birds. In DNA samples extracted from feather tips, the *meq* gene was detected in a high percentage of geese and ducks, whereas all other birds tested negative (Tables 1, 2). Because of the limited number of non-waterfowl species investigated, in these species DNA samples extracted from internal organs were also examined. The *meq* gene was not detected in these samples either (Table 2). These results suggest that MDV-1 is widespread in wild waterfowl but not in other birds. MDV-1 was

isolated from both migratory and sedentary waterfowl suggesting that transmission of MDV-1 from waterfowl to domestic poultry can occur throughout the year.

Currently, pathogenic MDV-1 strains have been classified as mild (mMDV-1), virulent (vMDV-1), very virulent (vvMDV-1), and very virulent+ (vv+MDV-1), based on their ability to breach vaccine-induced immunity (Witter, 1997). Several factors that could contribute to increased virulence of MDV-1 strains have been identified (Shamblin et al., 2004). Of these, the most important finding was the distinct diversity and point mutations in the *meq* gene product, Meq. Meq, which is a putative oncoprotein of MDV-1 (Jones et al., 1992), is a 339-amino-acid protein with an N-terminal basic region-leucine zipper (bZIP) domain and a C-terminal transactivation domain (Liu and Kung, 2000). The bZIP domain consists of 2 stretches of basic residues (BR) and a leucine zipper, which resembles the Jun/Fos family of oncoproteins (Liu and Kung, 2000). The transactivation domain is characterized by two and a half proline-rich repeats (PRR), which display the transrepression effect (Liu and Kung, 2000). Meq contributes to oncogenicity by altering the expression of various cellular genes (Levy et al., 2005). Therefore, changes in the structure of the Meq protein, such as amino acid substitutions in the BR and PRR, may alter transactivation activity, indicating that diversity in Meq may alter its function and subsequently contribute to oncogenicity (unpublished data). Consequently, we established the amino acid sequences in the Meq proteins of the detected *meq* genes.

As summarized in Table 3, distinct diversity was found in the BR of the waterfowl samples. Amino acid substitution at 77 in the BR has been identified in most of the virulent MDV-1 strains (vvMDV-1 and vv+MDV-1: lysine, vMDV-1 and mMDV-1: alanine/glutamic acid) (Shamblin et al., 2004). In samples of mallards, pintails, and spot-billed ducks, the amino acid residue at 77 was lysine, suggesting that highly virulent MDV-1 strains may be prevalent in wild ducks in Japan. However, no diversity was found in the PRR and the retinoblastoma binding domain (LXCXE). As other factors besides Meq are associated with MDV-1 pathogenicity (Osterrieder et al., 2006), experimental infection studies are required to establish whether the MDV-1 strains we isolated were virulent.

In conclusion, we investigated MDV-1 prevalence in wild birds and demonstrated that MDV-1 is widespread in both migratory and sedentary waterfowl. To control field outbreaks of MD in the future, periodic monitoring of MDV-1 in wild waterfowl is advisable.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Table 1. Occurrence of the *meq* gene in feather tips of wild geese and ducks collected in Hokkaido

Taxonomic order	Taxonomic family	Species	Common name	Lifestyle	Year	No. of positives/No. tested (%)
Anseriformes	Anatidae	*Anas platyrhynchos*	Mallard	Migratory (Partially sedentary)	2003–2005	64/92 (69.6)
		A. poecilorhyncha	Spot-billed duck	Sedentary	2003–2005	18/29 (62.1)
		A. penelope	European wigeon	Migratory	2004, 2005	13/30 (43.3)
		A. acuta	Pintail	Migratory	2003–2005	44/55 (80.0)
		A. crecca	Common teal	Migratory (Partially sedentary)	2004, 2005	14/16 (87.5)
		Anser albifrons	White-fronted goose	Migratory	2005	39/87 (44.8)

Table 2. Occurrence of the *meq* gene in wild birds

Taxonomic order	Taxonomic family	Species	Common name	Year	No. of positives/No. tested (%)			
					Feather	Spleen	Liver	Kidney
Passeriformes	Turdidae	*Turdus naumanni*	Dusky thrush	2003	0/3 (0)	0/1 (0)	N.D.	0/3 (0)
	Fringillidae	*Coccothraustes coccothraustes*	Hawfinch	2003	0/1 (0)	N.D.	N.D.	0/1 (0)
	Motacillidae	*Motacilla alba*	White wagtail	2003	0/1 (0)	N.D.	N.D.	0/1 (0)
	Emberizidae	*Emberiza spodocephala*	Black-faced bunting	2003	0/1 (0)	N.D.	N.D.	0/1 (0)
	Sturnidae	*Sturnus cineraceus*	Grey starling	2003	N.D.	N.D.	0/1 (0)	0/1 (0)
	Corvidae	*Corvus macrorhynchos*	Jungle crow	2009	0/10 (0)	0/2 (0)	0/2 (0)	N.D.
Charadriiformes	Laridae	*Larus schistisagus*	Slaty-backed gull	2010	0/1 (0)	0/1 (0)	0/1 (0)	N.D.
	Scolopacidae	*Calidris subminuta*	Long-toed stint	2003	0/1 (0)	N.D.	N.D.	0/1 (0)
	Alcidae	*Cerorhinca monocerata*	Hornbilled puffin	2010	0/1 (0)	N.D.	0/1 (0)	N.D.
Anseriformes	Anatidae	*Cygnus cygnus*	Whooper swan	2003, 2009	0/2 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Falconiformes	Accipitridae	*Milvus migrans*	Black kite	2009	0/1 (0)	N.D.	N.D.	N.D.
Gruiformes	Gruidae	*Grus japonensis*	Manchurian crane	2009	0/16 (0)	0/14 (0)	N.D.	N.D.
Procellariiformes	Procellariidae	*Oceanodroma furcata*	Fork-tailed storm petrel	2010	0/1 (0)	N.D.	N.D.	0/1 (0)

N.D.: not determined

Table 3. Amino acid substitutions in the Meq proteins of MDV strains from wild geese and ducks

Strains	Birds	Basic region				Transactivation domain							Virulence	Accession No.
		71	77	80	115	119[a] LXCXE	153[b] PPPP	176[b] PPPP	180	217/276[b, c] PPPP	283/342[c]	320/379[c]	326/385[c]	
Tokachi-m1	Mallard	A	K	D	V	C	P	P	T	P	A	I	T		AB638841
Tokachi-m2	Mallard	A	E	Y	A	C	P	P	T	P	A	I	T		AB638842
Tokachi-p1	Pintail	A	K	D	V	C	P	P	T	P	A	I	T		AB638843
Tokachi-s1	Spot-billed duck	A	K	D	V	C	P	P	T	P	A	I	T		AB638844
Tokachi-s2	Spot-billed duck	S	E	D	V	C	P	P	T	P	A	I	T		AB638845
Tokachi-w1	White-fronted goose	S	E	D	V	C	P	P	T	P	A	I	T		AB638846
N/595	Chicken	A	K	D	V	R	Q	A	A	A	A	I	T	vv+/vv	AY362718/AY362715
New	Chicken	A	K	D	V	R	Q	A	T	A	V	T	T	vv+	AY362719
643P	Chicken	A	K	D	V	R	Q	A	A	A	A	I	T	vv	AY362716
W/Md-5	Chicken	A	K	D	V	C	P	P	T	A	V	T	T	vv+/vv	AY362723/AF243438
RB1B/GA	Chicken	A	K	D	V	C	P	P	T	P	A	I	T	vv/v	AY243332/M89471
567	Chicken	A	E	Y	V	R	P	P	T	P	A	I	T	v	AY362709
Jm[c]	Chicken	S	A	D	A	C	P	P	T	P	A	I	T	v	AY243331
CVI988[c]	Chicken	S	E	D	V	C	P	P	T	P	A	I	T	v	AY243333
CU-2[c]	Chicken	S	E	D	V	C	P	P	T	P	A	I	T	m	AY362708

a) Putative retinoblastoma binding domain “LXCXE”

b) The second position of direct repeats of 4 prolines in PRR

c) Strains containing 59 amino acid insertion in PRR