Veterinary Microbiology 166 (2013) 68–75

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Molecular characterization and phylogenetic analysis of the reticuloendotheliosis virus isolated from wild birds in Northeast China

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ARTICLE INFO

Article history:
Received 8 April 2013
Received in revised form 9 May 2013
Accepted 22 May 2013

Keywords:
Reticuloendotheliosis virus
Wild birds
Isolation
Characterization

ABSTRACT

To analyze the status of reticuloendotheliosis (RE) infection of wild birds in China, 585 samples from wild birds collected in Liaoning, Jilin and Heilongjiang provinces China were investigated and analyzed. The sampled birds represent 3 orders and more than 40 species. Virus isolation and PCR amplification showed that some of the wild birds were infected with REV, and 10 REV strains were isolated. The gp90 gene from each of the 10 REV strains was amplified, cloned, and sequenced. Sequence analysis indicated that the gp90 genes of the 10 REV strains isolated in this study were more similar at the nucleotide level with the northeast Chinese strains HLJR0901 and HLJR0801 and some REV strains found in the US and Taiwan than with the early Chinese REV isolate HA9901. Furthermore, phylogenetic analysis indicated that the gp90 genes of the 10 REV strains were more similar to the REV subtype III-representing strain (CSV) than to strains 170A (subtype I) or SNV (subtype II). This is the first study to investigate the status of wild birds infected with REV. The results of this paper will not only provide necessary information for further understanding the evolution of REV, but they also identify the potential role of wild birds in REV transmission and furthers our understanding of the ecology of REV in wild bird species.

1. Introduction

Reticuloendotheliosis (RE) is a neoplastic disease caused by a group of reticuloendotheliosis viruses (REVs) (Barbosa et al., 2007; Walker et al., 1983). This disease is characterized by immunosuppression, oncogenicity, chronic lymphomas, a runting–stunting syndrome and chronic neoplasm. REVs belong to the family Retroviridae, subfamily Orthoretrovirinae and genus gammaretrovirus (Barbacid et al., 1979). REVs comprise a variety of strains, including defective REV-T (Barth et al., 1990), non-defective REV-A (Baxter-Gabbard et al., 1971), spleen necrosis virus (SNV) (Kewalramani et al., 1992), duck infectious anemia virus (DIAV) (Ludford et al., 1972), and chick syncytial virus (CSV). REV has extensive avian hosts, including chickens, turkeys, ducks, geese, peafowl,

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http://dx.doi.org/10.1016/j.vetmic.2013.05.008

pheasants, Japanese quail, Hungarian Partridges, Chinese Partridges and Attwater's prairie chickens (Barbosa et al., 2007; Cheng et al., 2007; Miller et al., 1998; Schat et al., 1976; Trampel et al., 2002). REV has a worldwide distribution (Bagust et al., 1979; Zavala et al., 2006). Serologic surveys conducted in several countries have detected antibodies against this virus in 3.3–25% of chicken flocks. REV infection is also highly prevalent in China (Qin et al., 2010; Deng et al., 2011); the seropositive rate has recently reached 30–40% and has caused severe damages to the poultry industry (Wang et al., 2006). Thus, it is imperative to research the molecular epidemiology of REV to better understand the continuous evolution of this virus. Previous molecular epidemiology studies focused primarily on poultry and waterfowl, and reports of infection in wild birds were rare. In this study, 10 REV strains were isolated from wild birds in Northeast China. Our study represents the first report of REV infection in wild birds. To better understand the molecular characteristics of REV isolated from wild birds, we sequenced and analyzed the gp90 genes to begin to elucidate the epidemiology of REV in wild birds in China.

2. Materials and methods

2.1. Cells

DF1 cells were cultured in Dulbecco modified Eagle's medium (DMEM; Invitrogen, CA) supplemented with 10% fetal bovine serum (FBS) at 37 °C under 5% CO_2.

2.2. Clinical samples

A total of 585 clinical samples were collected from wild birds from 3 provinces in China (Heilongjiang, Jilin and Liaoning) between May 2010 and May 2012. Of these, 436 samples were from different species of wild duck (Anseriformes), 20 samples were from different types of

Table 1
Wild birds tested for REV.

Order	Family	Species		Number of birds	Number of isolates
		English name	Latin name		
Anseriformes	*Anatidae*	Baikal teal	*Anas formosa*	157	4
		Pintail	*Anas acuta*	7	1
		Mallard	*Anas platyrhynchos*	40	1
		Pochard	*Aythya ferina*	39	0
		Tufted duck	*Aythya fuligula*	12	1
		Baer's pochard	*Aythya baeri*	18	0
		Spot-billed duck	*Anas poecilorhyncha*	17	1
		Common shelduck	*Tadorna tadorna Linnaeus*	15	0
		Northern shoveller	*Anas clypeata*	35	0
		Falcated duck	*Anas falcata*	2	0
		Eurasian wigeon	*Anas Penelope*	1	0
		Gadwall	*Anas strepera*	8	0
		Green-winged Teal	*Anas crecca*	57	0
		Common goldeneye	*Bucephala clangula*	14	0
		Canvasback	*Aythya valisneria*	12	0
		Fulica atra	*Eurasian Coot*	2	0
Podicipediformes	*Podicedidae*	Crested grebe	*Podiceps cristatus*	3	0
		Black necked grebe	*Podiceps nigricollis*	9	0
		Little grebe	*Podiceps ruficollis*	8	0
Passeriformes	*Ardeidae*	Yellow bittern	*Ixobrychus sinensis Gmelin*	9	1
		Brambling	*Fringilla montifringilla*	7	0
		Long-tailed rosefinch	*Uragus sibiricus*	8	0
	Paridae	Great tit	*Parus major*	5	0
		Pallas's rosefinch	*Carpodacus roseus*	3	0
		Marsh tit	*Parus palustris*	7	0
	Laniidae	Brown shrike	*Lanius cristatus*	5	0
	Passeridae	Gray wagtail	*Motacilla cinerea*	3	0
		Tristram's bunting	*Emberiza tristrami*	5	0
	Emberizidae	Yellow-throated bunting	*Emberiza elegans*	4	0
		Black-faced bunting	*Emberiza spodocephala*	4	0
		Rustic bunting	*Emberiza rustica*	5	0
	Turdidae	Gray-backed thrush	*Turdus hortulorum*	4	0
		Red-breasted flycatcher	*Ficedula parva*	5	0
	Muscicapidae	Pallas's warbler	*Phylloscopus proregulus*	11	0
		Red-flanked bush robin	*Tarsiger cyanurus*	9	1
	Sittidae	Sitta europaea	*Sitta europaea*	5	0
		Yellowbrowed warbler	*Phylloscopus inornatus*	7	0
	Sylviidae	Eastern crowned warbler	*Phylloscopus coronatus*	5	0
		Dusky warbler	*Phylloscopus fuscatus*	5	0
	Corvidae	Azure-winged magpie	*Cyanopica cyana*	9	0
	Fringillidae	Black-billed magpie	*Pica pica*	4	0
3	13	41	41	585	10