Veterinary Microbiology 183 (2016) 30–36

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Ferret hepatitis E virus infection induces acute hepatitis and persistent infection in ferrets

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ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 4 November 2015
Accepted 14 November 2015

Keywords:
Ferret HEV
ALT
AST
Persistent infection
Animal model

ABSTRACT

Ferret hepatitis E virus (HEV), a novel hepatitis E virus, has been identified in ferrets. However, the pathogenicity of ferret HEV remains unclear. In the present study, we compared the HEV RNA-positivity rates and alanine aminotransferase (ALT) levels of 63 ferrets between before and after import from the US to Japan. We found that the ferret HEV-RNA positivity rates were increased from 12.7% (8/63) to 60.3% (38/63), and ALT elevation was observed in 65.8% (25/38) of the ferret HEV RNA-positive ferrets, indicating that ferret HEV infection is responsible for liver damage. From long term-monitoring of ferret HEV infection we determined that this infection in ferrets exhibits three patterns: sub-clinical infection, acute hepatitis, and persistent infection. The ALT elevation was also observed in ferret HEV-infected ferrets in a primary infection experiment. These results indicate that the ferret HEV infection induced acute hepatitis and persistent infection in ferrets, suggesting that the ferrets are a candidate animal model for immunological as well as pathological studies of hepatitis E.

1. Introduction

Hepatitis E virus (HEV) is a single-stranded positive-sense RNA virus that belongs to the genus *Hepevirus* in the family *Hepeviridae*, which is primarily transmitted by the fecal-oral route, and causes hepatitis E (Emerson and Purcell, 2003; Meng et al., 2012). Molecular characterization of various HEV strains circulating among humans has led us to recognize four major genotypes (G1–G4), in which G1 and G2HEV are restricted to humans and often transmitted via contaminated water in developing countries (Balayan et al., 1983; Huang et al., 1992; Reyes et al., 1990; Schlauder et al., 1998; Wang et al., 1999). G1HEV circulates mainly in Asian and African countries, and G2HEV was first isolated in Mexico (Huang et al., 1992). G3 and G4HEV are detected not only in humans but also in monkeys, swine, wild boar, deer, and mongooses (Li et al., 2005; Meng et al., 1997; Nakamura et al., 2006; Sato et al., 2011; Tei et al., 2003; Yamamoto et al., 2012). Because the transmission of HEV from deer, swine and wild boar to humans is well known, hepatitis E is recognized as a zoonosis, mainly in association with G3 and G4HEV infection (Meng, 2010).

In addition to G1-G4HEVs, many novel HEV or HEV-like viruses have been identified in wild boars, rabbits, rats, minks, moose, ferrets, red foxes, camels, chickens, bats and cutthroat trout (Batts et al., 2011; Bodewes et al., 2013; Drexler et al., 2012; Haqshenas et al., 2001; Jay et al., 2014; Johne et al., 2010a; Krog et al., 2013; Raj et al., 2012; Takahashi et al., 2014; Woo et al., 2014; Zhao et al., 2009). A recent study proposed that the family *Hepeviridae* be divided into two genera, *Orthohepevirus* and *Piscihepevirus* (Smith et al., 2014), and this division was recently approved by the ICTV (http://ictvonline.org/virusTaxonomy.asp). The *Orthohepevirus* includes four species: *Orthohepevirus A,* which includes isolates from humans, pigs, wild boar, deer, mongooses, rabbits and camels; *Orthohepevirus B,* which includes isolates from chickens; *Orthohepevirus C,* which includes isolates from rats, greater bandicoots, Asian musk shrews, ferrets and mink; and *Orthohepevirus D*, which includes isolates from bats. Cutthroat trout virus belongs to the genus *Piscihepevirus*. It is not clear yet whether

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http://dx.doi.org/10.1016/j.vetmic.2015.11.014
0378-1135/

animal HEVs other than G3 and G4HEV are transmitted to humans. Although it has been confirmed that avian HEV infection caused big liver and spleen syndrome in chickens, the pathogenicity of other animal HEVs remains undefined (Yugo et al., 2014).

Ferret HEV was first detected in ferrets (*Mustela putorius*) in The Netherlands (Raj et al., 2012). Since then, many ferret HEV strains have been detected in laboratory and pet ferrets in the US and Japan, and four full genome sequences have demonstrated that the genome contains 6820 or 6854 nucleotides (nt) not including the 3′ poly-A tail (Li et al., 2015a,b; Raj et al., 2012; Yang et al., 2013). The genome structure is similar to that of other HEVs, and contains three open reading frames (ORFs 1–3). ORF1 encodes a nonstructural protein of 1589 or 1596 amino acids (aa), ORF2 encodes a capsid protein of 654 aa, and ORF3 encodes a functionally unknown phosphoprotein of 108 aa. In addition, a putative ORF4 encoding 183 aa was observed in the ferret HEV genome, although its function is unknown (Li et al., 2014). The nucleotide sequence analyses indicated that the ferret HEV genome shares the highest nucleotide sequence identity (72.3%) with rat HEV, and the identity with G1–4HEV, rabbit HEV and avian HEV ranges from 54.5% to 60.5%.

Recombinant ferret HEV-like particles (VLPs) have been produced by the expression of a partial ferret HEV ORF2 gene using a baculovirus expression system, and an enzyme-linked immunosorbent assay (ELISA) for detection of anti-ferret HEV IgG and IgM antibodies has been established using VLPs as the antigen (Yang et al., 2013). A preliminary seroepidemiological study indicated that the positivity rates of the IgG and IgM were 23.3% and 24.4%, respectively, in a ferret farm in the US, and ferret HEV detected in the US is genetically different from that detected in The Netherlands. Furthermore, the antibody against VLPs does not neutralize G3HEV, suggesting that the serotypes of these two HEVs are different (Yang et al., 2013). However, the pathogenicity of ferret HEV remains unclear.

In our previous study, we examined stool specimens from 63 ferrets, and found that 63.5% (40/63) of these animals were positive for ferret HEV RNA (Li et al., 2014). In the present study, we monitored the kinetics of ferret HEV RNA and liver enzymes in these ferrets and observed three patterns of infection, sub-clinical, acute and persistent, suggesting that ferrets are a candidate animal model for hepatitis E.

2. Materials and methods

2.1. Ferrets and samples collection

Sixty-three ferrets born in a ferret farm in the US between January 14 and 28, 2013 were imported to the National Institute of Infectious Diseases (NIID), Japan on May 17, 2013. The serum samples were collected on April 30, 2013 before import. The stool and serum samples were collected on May 24, 2013 (7 days post-import) and May 27, 2013 (10 days post-import), respectively. Nine out of 63 ferrets were used for long-term observations. The monitoring period was 74 days long (from the day of import until July 30, 2013) except in the case of one ferret, ferret no. 4351, which was monitored for 153 days (from import to October 17, 2013). The blood samples were taken through the cranial vena cava weekly, and the fresh fecal samples were collected from each cage one or two times per week. The serum samples were used to detect liver enzymes, ferret HEV-specific IgG and IgM antibodies, and ferret HEV RNA. The stool samples were used for the detection of ferret HEV RNA. In addition, 41 serum samples were collected from 22 ferrets at 109–153 days post-import and used for the observation of persistent infection (Table 1).

Table 1
Detection of ferret HEV RNA and ALT in ferret serum.

		Days post-importation					
Group	Ferret No.	−17	10	109	117	130	153
A	4339	264	158				
	4341	220	121	71/−*			
	4343	181	160				
	4350	203	107				
	4352	352	100				
	4356	211	83				
	4357	203	135	193/−			
	4359	246	147	422/+**	257/+	213/−	115/−
	4361	166	132				
	4362	264	145				
	4364	185	153				
	4367	115	114				
	4368	112	110				
	4369	154	178				
	4377	172	129				
	4381	135	141				
	4386	194	194	178/−			
	4388	183	137	132/−			
	4390	115	150	102/−			
	4391	97	141	104/−			
B	4319	282	266				
	4320	224	102				
	4326	137	147				
	4340	220	164				
C	4321	189	182				
	4322	129	177	115/−			
	4323	170	161	80/−			
	4324	101	291				
	4325	184	302	127/−			
	4327	178	775				
	4328	188	528	161/−	208/−		
	4329	189	612				
	4330	185	415				
	4331	130	386	681/+	354/+		493/+
	4335	205	422				
	4342	235	351				
	4349	162	183				
	4351	203	209				
	4354	149	967				
	4358	165	350				
	4360	100	288	381/+	511/+	270/+	187/-
	4365	117	159				
	4366	116	266				
	4370	133	200				
	4371	125	385				
	4372	125	172	331/+	244/+	290/+	
	4373	152	473				
	4374	129	231	219/+		196/+	294/+
	4375	170	191	449/+	328/+	309/+	624/+
	4376	132	786				
	4378	112	290				
	4379	160	269				
	4380	148	245	505/+	464/+	407/+	547/+
	4382	62	450				
	4383	127	193	131/−			
	4384	82	273	176/−			
	4385	119	166	168/−			
	4387	66	182	152/−			
	4389	101	327	99/+			
D	4318	184	1693				
	4344	158	348				
	4345	172	2376				
	4363	150	629				
		ALT	ALT	ALT/RNA	ALT/RNA	ALT/RNA	ALT/RNA

*−, negative for ferret HEV RNA; **+, positive for ferret HEV RNA.

All of the ferret experiments were reviewed by the Ethics Committee of NIID, and carried out according to the Guides for Animal Experiments Performed at NIID under approval code 113089. Ferrets were individually housed in BSL-2 facilities.