Differences in Ibaraki Virus RNA Segment 3 Sequences from Three Epidemics

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(Received 6 December 2002/Accepted 29 July 2003)

ABSTRACT. Phylogenetic tree and partial nucleotide sequence analysis of RNA segment 3 were conducted to compare the Ibaraki virus (IBAV) strains from three epidemics in Japan, and serotype 2 epizootic hemorrhagic disease virus strains isolated in Australia, Taiwan, and Canada. Each strain was classified relative to the Ibaraki disease (IBAD) epidemics, which occurred in 1959–1960, 1987, or 1997–1998. In particular, major variation of the gene was identified in the strains isolated after 1997 when a new type of IBAD with the abnormal birth was confirmed. Ibaraki viruses isolated in Japan were more closely related to Taiwanese and Australian strains based on genetics, while the Canadian strain was more distantly related.

KEY WORDS: genetic analysis, Ibaraki virus, variant.

J. Vet. Med. Sci. 65(11): 1257–1263, 2003

Ibaraki virus (IBAV) was first isolated from defibrinated blood of affected cattle in Ibaraki Prefecture, Japan in 1959 [18]. IBAV is classified as belonging to the genus *Orbivirus* in the family *Reoviridae* and is now known as serotype 2 of epizootic hemorrhagic disease virus (EHDV) [2, 21]. It causes bluetongue-like disease clinical symptoms and pathological findings [17] and has been isolated from naturally infected cattle and sheep [8]. Ibaraki disease (IBAD) caused by IBAV typically manifests the clinical symptoms of a deglutitory disorder in cattle and has broken out in 1950–1952, 1959–1960, 1982, and 1987 in Japan [7, 8, 15, 17, 18]. After ten years, an epidemic of IBAD was observed in 1997–1998 in southwest Japan [15]. However, in the 1997 epidemic of IBAD, cows infected with IBAV frequently had abnormal pregnancies, characterized mainly by abortions and stillbirths. It is extremely likely that these new isolates of the virus were the causal agent of the abnormal pregnancies based on seroepidemiological and virological analyses [15].

IBAV has an icosahedral configuration; the virion measures about 55 nm in diameter, and the capsid consists of 32 capsomeres. One or more virions are enclosed in a pseudo-envelope of the cell membrane origin [9]. EHDV is composed of seven structure proteins (VP1–VP7) and three non-structural proteins (NS1–NS3) organized in a double-layered capsid containing the 10 double-stranded RNA segments of the viral genome [5, 6, 14]. RNA segment 3 (RNA3) encodes the major inner capsid protein VP3, which contains group-specific antigenic determinants [5, 11]. Researchers demonstrated that reverse transcription-polymerase chain reaction (RT-PCR) with primers based on the sequence of RNA3 was a useful tool for the detection and differentiation of the EHDV serogroup [4, 13, 15].

In this study, we performed comparative sequence analysis of RNA3 genes of IBAV isolated in Japan. A phylogenetic tree was also constructed to investigate the relationship

among IBAV strains from three epidemics.

IBAV isolates used in this study from Japan, Taiwan [12], Australia [19], and Canada [1, 2] are listed in Table 1. Seventeen isolates from Japan were classified into three epidemic groups: prototypes isolated during the first recognized epidemic of IBAD in Japan in 1959 (strain No. 2) and 1960 (strain Imaizumi), the 1987 epidemic strains (OA, OI-6, FC25-1, FC34-3, Iwagawa, OK75, OKC-13, OCH, OYE, and MS55P), and the 1997–1998 epidemic strains (Hyogo-6, KA84, 97MD70V2, 97MD60, and 98MD57). These isolates were chosen to allow comparison across generations. In addition, the following EHDV serotype 2 (EHDV-2) strains were added for geographic comparisons: CSIRO439 (Australia), Taiwan-41 (Taiwan), and Alberta (Canada).

The viruses were propagated in the HmLu-1 cell line. Total RNA was extracted from infected cells using TRIzol LS reagent (GIBCO BRL, U.S.A.), according to the manufacturer's instructions. A 660-base pair fragment of RNA3 of IBAV was amplified by RT-PCR by the method of McColl *et al.* [13]. The primers, IBA1 (5'-CCT AGA TGT TCA ATA GCA AAC CTA ATT-3') and IBA2 (5'-TAA CAT TTC GTT ATA ACA ATA ATA ATT-3'), were developed based on the nucleotide sequence of RNA3 of IBAV. RT-PCR products were cloned and sequenced.

The amplified cDNA fragments were cloned into a pCR 2.1 vector using a TA cloning kit (Invitrogen Corporation, CA, U.S.A.). Competent *E. coli* cells were transformed, cultured, and plasmid DNA was isolated using the Plasmid Mini Kit (QIAGEN, Cologne, Germany). The cloned DNA was sequenced using an ALFII automated sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions. The partial RNA3 nucleotide sequences were analyzed by GENETYX Ver. 10 (Software Development, Tokyo, Japan) and a phylogenetic tree was constructed using the CLUSTAL W program in the

Fig. 1. Phylogenetic tree of RNA segment 3 amplified from cDNA of Ibaraki virus and serotype 2 of epizootic hemorrhagic disease virus strains from Japan, Australia, Taiwan, and Canada. Numbers indicate branch lengths.

No.2	1959	1	CCTAGATGTT	CAATAGCAAA	GCTAATTATG	AATATAGCGA	CGTGCTTACC	AACAGGAGAA	60
Imaizumi	1960	1							60
OA	1987	1							60
OI-6	1987	1				A			60
FC25-1	1987	1				A			60
FC34-3	1987	1				A			60
Iwagawa	1987	1				A			60
OK75	1987	1				A			60
OKC-13	1987	1				A			60
OCH	1987	1				A			60
OYE	1987	1							60
MS55P	1987	1				A			60
Hyogo-6	1997	1							60
KAB4	1997	1							60
97MD70V2	1997	1							60
97MD60	1997	1							60
98MD57	1998	1							60
CSIRO439	1980	1							60
Taiwan-41	1990	1				A			60
Alberta	1962	1	..G..G..	...T...	TT.G..A..		G.....		60

No.2	1959	61	TATGTATGTC	CAATCGCG	AATTGCATCA	ATAACGCTTA	GCCAGAGAAT	AACAACGACT	120
Imaizumi	1960	61							120
OA	1987	61							120
OI-6	1987	61	..C.....						120
FC25-1	1987	61	..C.....						120
FC34-3	1987	61	..C.....						120
Iwagawa	1987	61	..C.....						120
OK75	1987	61	..C.....						120
OKC-13	1987	61	..C.....			C			120
OCH	1987	61	..C.....						120
OYE	1987	61							120
MS55P	1987	61	..C.....						120
Hyogo-6	1997	61	C..		G.....		..G.....C		120
KAB4	1997	61	C..		G.....		..G.....C		120
97MD70V2	1997	61	C..		G.....		..G.....C		120
97MD60	1997	61	C..		G.....		..G.....C		120
98MD57	1998	61	C..C..		G.....		..G.....C		120
CSIRO439	1980	61							120
Taiwan-41	1990	61	..C.....						120
Alberta	1962	61	T..A..	..C.....T..	T..A..T..	..C..AT..G..	A..A.....	T..T..A..	120

No.2	1959	121	GGCCCTTTTG	CTATCTTAA	TGGATCAACT	GCAACTGCTC	AACAATTAGA	TGATGTGAGG	180
Imaizumi	1960	121							180
OA	1987	121	T						180
OI-6	1987	121		C.....	G.....		A		180
FC25-1	1987	121		C.....	G.....		A		180
FC34-3	1987	121		C.....	G.....		A		180
Iwagawa	1987	121		C.....	G.....		A		180
OK75	1987	121		C.....	G.....	C..	A		180
OKC-13	1987	121		C.....	G.....	C..	A		180
OCH	1987	121		C.....	G.....		A		180
OYE	1987	121							180
MS55P	1987	121		C.....	G.....		A		180
Hyogo-6	1997	121		T.....	G.....		C.....		180
KAB4	1997	121		T.....	G.....		C.....		180
97MD70V2	1997	121		T.....	G.....		C.....		180
97MD60	1997	121		T.....	G.....		C.....		180
98MD57	1998	121		T.....	G.....		C.....		180
CSIRO439	1980	121		T.....					180
Taiwan-41	1990	121		C.....	G.....		A		180
Alberta	1962	121	..A..A.....	A.....	G.....G.....	G..A..	C..A..A		180

No.2	1959	181	AAGATATATT	TAGCGTTAAT	GTTCCCAGGT	CAAATAATCT	TAGATTTAAA	AATAGATCCG	240
Imaizumi	1960	181							240
OA	1987	181							240
OI-6	1987	181							240
FC25-1	1987	181							240
FC34-3	1987	181							240
Iwagawa	1987	181							240
OK75	1987	181							240
OKC-13	1987	181							240
OCH	1987	181							240
OYE	1987	181							240
MS55P	1987	181							240
Hyogo-6	1997	181		A.....	T.....				240
KAB4	1997	181		A.....	T.....				240
97MD70V2	1997	181		A.....	T.....				240
97MD60	1997	181		A.....	T.....				240
98MD57	1998	181		A.....	T.....				240
CSIRO439	1980	181							240
Taiwan-41	1990	181							240
Alberta	1962	181	C..	G..	T.....A	T..	A.....		240

No.2	1959	241	GGTGAGAGGA	TGGACCCAGC	GGTACGTATG	GTGGCGGGGG	TTGTTGGTCA	TTTAATGTTT	300
Imaizumi	1960	241							300
OA	1987	241							300
OI-6	1987	241					C.....		300
FC25-1	1987	241					C.....		300
FC34-3	1987	241					C.....		300
Iwagawa	1987	241					C.....		300
OK75	1987	241					C.....		300
OKC-13	1987	241					C.....		300
OOH	1987	241					C.....		300
OYE	1987	241							300
MS55P	1987	241					C.....		300
Hyogo-6	1987	241	A..A..	T..		A..		C.....	300
KA84	1987	241	A..A..	T..		A..		C.....	300
97MD70V2	1987	241	A..A..	T..		A..		C.....	300
97MD60	1987	241	A..A..	T..		A..		C.....	300
98MD57	1988	241	A..A..	T..		G..A..		C.....	300
CSIRO439	1980	241							300
Taiwan-41	1990	241					C.....		300
Alberta	1962	241	A..A..	T..	G..	A..A..	G..		300

No.2	1959	301	ACAGCGGGAC	CTAGGTTTAC	GAACATAACC	CAAAATATGG	CACGACAGCT	TGATATTGCC	360
Imaizumi	1960	301							360
OA	1987	301							360
OI-6	1987	301							360
FC25-1	1987	301							360
FC34-3	1987	301							360
Iwagawa	1987	301							360
OK75	1987	301							360
OKC-13	1987	301							360
OOH	1987	301							360
OYE	1987	301							360
MS55P	1987	301							360
Hyogo-6	1987	301			T..		G.....		360
KA84	1987	301			T..		G.....		360
97MD70V2	1987	301	A..		T..		G.....		360
97MD60	1987	301			T..		G.....		360
98MD57	1988	301	A..	A..T..			G.....T..		360
CSIRO439	1980	301							360
Taiwan-41	1990	301							360
Alberta	1962	301	..G..A..C..	..AC..A..	T..		..A..G..	A.....C..A	360

No.2	1959	361	TTAGCTGATT	TTCTTCTCTA	CATGTACAAC	ACGAGGATCC	AGGTTCAATA	TGGTCCAACG	420
Imaizumi	1960	361							420
OA	1987	361							420
OI-6	1987	361			T..				420
FC25-1	1987	361			T..				420
FC34-3	1987	361			T..				420
Iwagawa	1987	361			T..				420
OK75	1987	361			T..				420
OKC-13	1987	361			T..				420
OOH	1987	361			T..				420
OYE	1987	361			T..				420
MS55P	1987	361			T..				420
Hyogo-6	1987	361		G..		A..	A..G..		420
KA84	1987	361		G..		A..	A..G..		420
97MD70V2	1987	361		G..		A..	A..G..		420
97MD60	1987	361		G..	A..	A..	A..C..G..		420
98MD57	1988	361		G..		AG..	A..G..	C..	420
CSIRO439	1980	361							420
Taiwan-41	1990	361			T..				420
Alberta	1962	361	C..G..C..	..T..AT..A..	T.....T..T	..C..A..	..A.....G..	..A..G..T	420

No.2	1959	421	GGAGAACCGC	TAGATTTCGG	AATAGGACGT	GGGCAGTATG	ATTGTAACGC	TTTTCGCACG	480
Imaizumi	1960	421							480
OA	1987	421							480
OI-6	1987	421			G..				480
FC25-1	1987	421			G..				480
FC34-3	1987	421			G..				480
Iwagawa	1987	421			G..				480
OK75	1987	421			G..				480
OKC-13	1987	421			G..				480
OOH	1987	421			G..				480
OYE	1987	421							480
MS55P	1987	421			G..				480
Hyogo-6	1987	421	A..				C.....		480
KA84	1987	421	A..				C.....		480
97MD70V2	1987	421	A..				C.....		480
97MD60	1987	421	A..				C.....		480
98MD57	1988	421	A..				C.....		480
CSIRO439	1980	421							480
Taiwan-41	1990	421			G..				480
Alberta	1962	421	CT	TA..	C..T..	..A..G..	T	A.....G..	480

No.2	1959	481	AATTTCCAAA	CTGGTGCGGG	ATATAATGGA	TGGGGATTAG	TAGATGTTGA	AAATAGAGAA	540
Imaizumi	1960	481							540
OA	1987	481							540
OI-6	1987	481							540
FC25-1	1987	481							540
FC34-3	1987	481							540
Iwagawa	1987	481							540
OK75	1987	481							540
OKC-13	1987	481							540
OCH	1987	481							540
OYE	1987	481							540
MS55P	1987	481							540
Hyogo-6	1997	481	G	A	G			G	540
KA84	1997	481	G	A	C			G	540
97MD70V2	1997	481	G	A	C			G	540
97MD60	1997	481	G	A	C			G	540
98MD57	1998	481	G	A	C			G	540
CSIRO439	1980	481							540
Taiwan-41	1990	481			C				540
Alberta	1962	481	C.....T	G.....A	T	T	T	C.....G	540

No.2	1959	541	CCAGCACCAT	ATGATCATGT	ACAAGCGCTTT	ATACGATATT	GTAACATTGA	TTCCAGAGAA	600
Imaizumi	1960	541						C	600
OA	1987	541							600
OI-6	1987	541						C.....T	600
FC25-1	1987	541						C.....T	600
FC34-3	1987	541						C.....T	600
Iwagawa	1987	541						C.....T	600
OK75	1987	541						C.....T	600
OKC-13	1987	541						C.....T	600
OCH	1987	541						C.....T	600
OYE	1987	541						C.....T	600
MS55P	1987	541						C.....T	600
Hyogo-6	1997	541		C					600
KA84	1997	541		C					600
97MD70V2	1997	541		C					600
97MD60	1997	541		C					600
98MD57	1998	541		C					600
CSIRO439	1980	541							600
Taiwan-41	1990	541						C.....T	600
Alberta	1962	541	G.....T	C	G.....A		C.....T.....A	C.....AC	600

No.2	1959	601	CTGATACACC	CAGCGAGGTT	CGGTATTGGT	ATGAATTATT	ATTGTCATAA	CGAAATGTTA	660
Imaizumi	1960	601		A					660
OA	1987	601							660
OI-6	1987	601		A					660
FC25-1	1987	601		A					660
FC34-3	1987	601		A					660
Iwagawa	1987	601		A					660
OK75	1987	601		A					660
OKC-13	1987	601		A					660
OCH	1987	601		A					660
OYE	1987	601							660
MS55P	1987	601		A					660
Hyogo-6	1997	601	T	G					660
KA84	1997	601	T	G					660
97MD70V2	1997	601	T	G		T			660
97MD60	1997	601	T	G					660
98MD57	1998	601	T	G		T			660
CSIRO439	1980	601							660
Taiwan-41	1990	601		A					660
Alberta	1962	601	A.....T	C.....A	T	CC	T	T.....G	660

Fig.2. Alignment of the nucleotide sequences of RNA segment 3 of Ibaraki virus and serotype 2 of epizootic hemorrhagic disease virus strains isolated in Japan, Australia, Taiwan, and Canada. Dots indicate identity with IBAV sequence, No. 2.

Info-SINCA system (Fujitsu Kyushu System Engineering, Fukuoka, Japan), employing the unweighted pair-group method using arithmetic mean and neighbor-joining methods [20]. The distances between sequences were estimated using three parameter methods. The reliability of the phylogenetic tree was evaluated by the bootstrap test (n=500).

In a phylogenetic tree, the 20 EHDV-2 strains segregated into five clusters (Fig. 1). The sequences formed two main clusters: one including the Alberta strain isolated in Canada in 1962 and the other consisting of the rest of the isolates. Within the second cluster, the strains were further divided

into two groups according to the isolation date: 1959–1987 and 1997–1998. Isolates OA and OYE in 1987 and CSIRO439 isolated in Australia in 1980 clustered with the prototype strains. The other major strains of the 1987 isolates clustered with Taiwan-41 isolated in Taiwan in 1990. The 1997 isolates formed one cluster, while the 1998 isolate placed in a separate neighboring cluster. Consequently, greater variation of the gene was observed among the strains isolated after 1997 than among the strains isolated earlier.

The alignment of the RNA3 nucleotide sequences of IBAV and EHDV-2 strains is shown in Fig. 2. With the

Table 1. Ibaraki virus and serotype 2 of epizootic hemorrhagic disease virus strains used in this experiment

Isolates	Year and Place of isolation		Source	DDBJ Accession No.
No. 2	1959	Ibaraki	affected cattle blood	AB106900
Imaizumi	1960	Aichi	affected cattle blood	AB106901
OA	1987	Oita	affected cattle blood	AB106902
OI-6	1987	Oita	affected cattle blood	AB106903
FC25-1	1987	Kagoshima	<i>Culicoides</i> species	AB106904
FC34-3	1987	Kagoshima	<i>Culicoides</i> species	AB106905
Iwagawa	1987	Kagoshima	<i>Culicoides maculatus</i>	AB106906
OK75	1987	Okinawa	affected cattle blood	AB106907
OKC-13	1987	Okinawa	<i>Culicoides</i> species	AB106908
OCH	1987	Okinawa	<i>Culicoides</i> species	AB106909
OYE	1987	Okinawa	affected cattle blood	AB107802
MS55P	1987	Miyazaki	affected cattle blood	AB107801
Hyogo-6	1997	Hyogo	affected cattle blood	AB107800
KA84	1997	Kagoshima	RNA from blood ^{a)}	AB107799
97MD70V2	1997	Fukuoka	aborted fetus brain	AB107798
97MD60	1997	Fukuoka	affected cattle blood	AB107797
98MD57	1998	Fukuoka	affected cattle blood	AB107803
CSIRO439	1980	Australia	affected cattle blood	AB107796
Taiwan-41	1990	Taiwan	affected cattle blood	AB107795
Alberta	1962	Canada	affected deer blood	AB107794

a) Viral RNA was obtained by RT-PCR from the blood of cattle, but the virus was not directly isolated.

Table 2. Pairwise comparisons of nucleotide sequence homology of RNA segment 3 Ibaraki virus and serotype 2 of epizootic hemorrhagic disease virus strains

Isolate	No. 2	Imaizumi	OA	OI-6	FC25-1	FC34-3	Iwagawa	OK75	OKC-13	OCH	OYE	MS55P	Hyogo-6	KA84	97MD70V2	97MD60	98MD57	CSIRO439	Taiwan-41	Alberta
No. 2	1959	—																		
Imaizumi	1960	99.7	—																	
OA	1987	99.8	99.5	—																
OI-6	1987	98.3	98.3	98.2	—															
FC25-1	1987	98.3	98.3	98.2	100	—														
FC34-3	1987	98.3	98.3	98.2	100	100	—													
Iwagawa	1987	98.3	98.3	98.2	100	100	100	—												
OK75	1987	98.2	98.2	98.0	99.8	99.8	99.8	99.8	—											
OKC-13	1987	98.0	98.0	97.9	99.7	99.7	99.7	99.7	99.8	—										
OCH	1987	98.3	98.3	98.2	100	100	100	100	99.8	99.7	—									
OYE	1987	100	99.7	99.8	98.3	98.3	98.3	98.3	98.2	98.0	98.3	—								
MS55P	1987	98.3	98.3	98.2	100	100	100	99.8	99.7	100	98.3	—								
Hyogo-6	1997	95.6	95.3	95.5	93.9	93.9	93.9	93.8	93.6	93.9	95.6	93.9	—							
KA84	1997	95.6	95.3	95.5	93.9	93.9	93.9	93.8	93.6	93.9	95.6	93.9	100	—						
97MD70V2	1997	95.3	95.0	95.2	93.6	93.6	93.6	93.6	93.5	93.3	93.6	95.3	93.6	99.7	99.7	—				
97MD60	1997	95.3	95.0	95.2	93.6	93.6	93.6	93.6	93.5	93.3	93.6	95.3	93.6	99.7	99.7	99.4	—			
98MD57	1998	94.1	93.8	93.9	92.4	92.4	92.4	92.3	92.1	92.4	94.1	92.4	98.5	98.5	98.8	98.2	—			
CSIRO439	1980	99.8	99.5	100	98.2	98.2	98.2	98.2	98.0	97.9	98.2	99.8	98.2	95.5	95.5	95.2	95.2	93.9	—	
Taiwan-41	1990	98.2	98.2	98.0	99.8	99.8	99.8	99.8	99.7	99.5	99.8	98.2	99.8	94.1	94.1	93.8	93.8	92.6	98.0	—
Alberta	1962	82.4	82.4	82.2	82.2	82.2	82.2	82.2	82.1	82.2	82.4	82.2	80.9	80.9	80.9	80.6	80.0	82.2	82.1	—

exception of isolates OA and OYE, the sequences obtained from the isolates in the 1987 epidemic differed from strain No. 2 at 11 positions: 49, 63, 136, 144, 180, 282, 387, 447, 591, 594, and 618. Strains OA and OYE were isolated from cows infected with IBAD. Neither of the affected cows had been inoculated with the IBAD vaccine, so these strains were not of the same origin as the vaccine. Therefore, it is

possible to conclude that the isolates of the 1987 epidemic consist of two varieties of IBAV strains closely related to the prototype and strains with gene structures differing from the prototype. There are 29 nucleotide substitutions similarly in the isolates in 1997 and 1998 as compared with strain No. 2. However, they were not found in the isolates in 1987, and the 11 nucleotide changes that observed simi-

larly in the 1987 isolates were not detected in the 1997–1998 isolates.

Pairwise nucleotide sequence homology analyses of RNA3 of the IBAB and EHDV-2 strains are shown in Table 2. Strains OA and OYE showed very high identities of 99.8% and 100%, respectively, with strain No. 2, while other 1987 isolates had identities varying from 98.0 to 98.3%. The 1997–1998 isolates showed lower identity with strain No. 2; nucleotide homologies varied from 94.1 (98MD57) to 95.6% (Hyogo-6 and KA84). Sequences homologies within the group of 1987 isolates were 97.9 to 100%, and 98.2 to 100% for the group of 1997–1998 isolates, suggesting that the strains are closely related within each group.

Sequence homology between the 1987 and 1997–1998 isolate groups ranged from 92.1 to 95.6% showing lower identities than between 1987 group and strain No. 2. Isolate 97MD60 from the blood of IBAD affected cattle and isolate 97MD70V2 from the brain of an aborted fetus, isolated in the same year and place, showed a high identity of 99.4%. This suggests that the 1997 isolates had the same origin, but spread widely causing abnormal births in addition to the previously observed manifestations of the disease. In addition, the nucleotide sequence homology also recognized the 1998 isolates as being slightly different from the 1997 isolates, with the alignment of RNA3 showing that the 1997 isolates and 1998 isolates consistently varied at 29 positions. Consequently, it was suggested that the 1998 isolate is a variant of the 1997 isolates.

Three EHD-2 strains isolated outside of Japan were also analyzed. CSIRO439 isolated in Australia showed high identities with prototype strains (99.5 to 99.8%) and the 1987 isolates (97.9 to 100%), but lower identities with the 1997–1998 isolates (93.9 to 95.5%). Taiwan-41 showed high identity with prototype strains (98.2%), 1987 isolates (98.0 to 99.8%), and CSIRO439 (98.0%). Isolate Alberta from Canada showed low identities with the prototype strains (82.4%), 1987 isolates (82.1 to 82.4%), 1997–1998 isolates (80.0 to 80.9%), CSIRO439 (82.2%), and Taiwan-41 (82.1%).

It was demonstrated that IBAB isolated in Japan genetically changed each year. In particular, large variation of the gene was recognized in the strains isolated in 1997–1998 when the new type of IBAD with the abnormal birth was confirmed. EHD-2 viruses isolated in Japan were recog-

nized to be genetically close to Asian and Australian strains, while the North America strain was distantly related, as described previously [3, 10, 16]. More sequence data of isolates from many countries are needed to further elucidate the origin of the IBAB isolated in 1997.

REFERENCES

1. Campbell, C. H., Barber, T. L. and Jochim, M. M. 1978. *Vet. Microbiol.* **3**: 15–22.
2. Campbell, C. H. and St. George, T. D. 1986. *Aust. Vet. J.* **63**: 233.
3. Cheney, I. W., Larson, M. D., Mecham, J. O. and Wilson, W. C. 1995. *Virus Res.* **36**: 279–286.
4. Harding, M. J., Prud'homme, I., Rola, J. and Dulac, G. C. 1996. *Can J. Vet. Res.* **60**: 59–64.
5. Huismans, H., Bremer, C. W. and Barber, T. L. 1979. *Onderstepoort J. Vet. Res.* **46**: 95–104.
6. Huismans, H. and Erasmus, B. J. 1981. *Onderstepoort J. Vet. Res.* **48**: 51–58.
7. Inaba, Y., Tanaka, Y., Ishii, S., Morimoto, T. and Sato, K. 1970. *Jpn. J. Microbiol.* **14**: 351–360.
8. Inaba, Y. 1975. *Aust. Vet. J.* **51**: 178–185.
9. Ito, Y., Tanaka, Y., Inaba, Y. and Omori, T. 1973. *Arch. Gesamte Virusforsch.* **40**: 29–46.
10. Iwata, H., Manabe, S., Yoshida, A., Pereira, E. M. and Inoue, T. 2001. *J. Vet. Med. Sci.* **63**: 73–78.
11. Le Blois, H., Fayard, B., Urakawa, T. and Roy, P. 1991. *J. Virol.* **65**: 4821–4831.
12. Liao, Y. K., Lu, Y. S., Huang, S. T. and Lee, S. H. 1996. *J. Chin. Soc. Vet. Sci.* **22**: 183–191.
13. McColl, K. A. and Gould, A. R. 1991. *Virus Res.* **21**: 19–34.
14. Mecham, J. O. and Dean, V. C. 1988. *J. Gen. Virol.* **69**: 1255–1262.
15. Ohashi, S., Yoshida, K., Watanabe, Y. and Tsuda, T. 1999. *J. Clin. Microbiol.* **37**: 3800–3803.
16. Ohashi, S., Yoshida, K., Yanase, T. and Tsuda, T. 2002. *J. Clin. Microbiol.* **40**: 3684–3688.
17. Omori, T., Inaba, Y., Morimoto, T., Tanaka, Y., Ishitani, R., Kurogi, H., Munakata, K., Matsuda, K. and Matsumoto, M. 1969. *Jpn. J. Microbiol.* **13**: 139–157.
18. Omori, T., Inaba, Y., Morimoto, T., Tanaka, Y., Kono, M., Kurogi, H. and Matsumoto, M. 1969. *Jpn. J. Microbiol.* **13**: 159–168.
19. Parsonson, I. M. and Snowdon, W. A. 1985. *Prog. Clin. Biol. Res.* **178**: 27–35.
20. Saitou, N. and Nei, N. 1987. *Mol. Biol. Evol.* **44**: 406–425.
21. Sugiyama, M., Hirayama, N., Sasaki, H., Sugimura, T., Minamoto, N. and Kinjo, T. 1989. *Res. Vet. Sci.* **46**: 283–285.