

Table 6

## Antigenic analysis of B virus isolated in 1999/2000 season in Japan.

Virus antigens Reference strains	Reference ferret anti-sera								Date of isolation	
	1	2	3	4	5	6	7	8		
1. CH B/Beijing/243/97	160	160	<10	<10	<10	<10	<10	<10	<10	
2. CH B/Shangdong/07/97	160	320	<10	<10	<10	<10	<10	<10	<10	
3. CH B/Beijing/184/93	<10	20	320	160	80	160	80	160	160	
4. CH B/Harbin/07/94	20	40	320	320	160	160	80	80	80	
5. JA B/Yamanashi/166/98	<10	20	320	320	640	160	80	80	80	
6. JA B/Kouchi/193/99	<10	10	40	40	20	160	80	80	160	Feb. 2, 99
7. JA B/Fukushima/220/99	<10	<10	20	20	10	160	160	160	160	Mar. 1, 99
8. CH B/Shenzhen/654/99	<10	<10	<10	<10	<10	40	20	20	640	
<u>1999/2000 isolate</u>										
9. JA B/Fukuoka/c-103/99	<10	<10	80	160	80	160	160	160	NT	Dec.27, 1999
10. JA B/Hiroshima/17/2000	<10	<10	160	40	320	80	20	40	40	Jan. 27, 2000
11. JA B/SendaiH/N153/2000	<10	<10	10	40	40	160	160	160	160	Jan. 31, 2000
12. JA B/Sendai/355/2000	<10	<10	20	40	80	160	160	160	NT	Jan. 31, 2000
13. JA B/Osaka/c1/2000	<10	<10	<10	20	20	160	80	80	NT	Feb.24, 2000

Table 1. Accession numbers of influenza B virus sequence data determined in this study and their abbreviations

Strain	Abbreviation	Accession number of nucleotide sequence of the following genes:			
		PB2	PB1	PA	
B/Lee/40	LEE40	AFI01982	M14880*	AFI02017	
B/Ann Arbor/1/66	AA66	M20168†	M20479#	M20172†	
B/Singapore/222/79	SIN79	AFI01987	AFI02004	M16711§	
B/Norway/1/84	NOR84	AFI01984	AFI02001	AFI02019	
B/Ibaraki/2/85	IBA85	AFI01981	AFI01999	AFI02016	
Yamagata/16/88-like strains					
B/Yamagata/16/88	YAM88	AFI01989	AFI02006	AFI02023	
B/Panama/45/90	PAN90	AF005737	AF005736	AF005738	
B/Beijing/184/93	BEI93	AFI01974	AFI01992	AFI02009	
B/Mie/1/93	MIE93	AFI01983	AFI02000	AFI02018	
B/Harbin/07/94	HAR94	AFI01979	AFI01997	AFI02014	
B/Shiga/T30/98	SHI3098	AFI01986	AFI02003	AFI02021	
B/Yamanishi/166/98	YAN98	AFI01990	AFI02007	AFI02024	
Victoria/2/87-like strains					
♯/Victoria/2/87	VIC87	AFI01988	AFI02005	AFI02022	
B/Aichi/5/88	AIC88	AFI01973	AFI01991	AFI02008	
B/Guangdong/08/93	GUA93	AFI01977	AFI01996	AFI02012	
B/Guangdong/05/94	GUA94	AFI01978	AFI01995	AFI02013	
B/Beijing/243/97	BEI97	AFI01975	AFI01993	AFI02010	
B/Henan/22/97	HEN97	AFI01980	AFI01998	AFI02015	
B/Chiba/447/98	CHI98	AFI01976	AFI01994	AFI02011	
B/Shiga/51/98	SHI5198	AFI01985	AFI02002	AFI02020	

\* Kemdirim *et al.* (1986).

† DeBorde *et al.* (1988).

# DeBorde *et al.* (1987).

§ Akoto-Amanfu *et al.* (1987).

|| Jambrija *et al.* (1997).



Table 9

Table 3. Evolutionary rates of human influenza virus PB2, PB1 and PA genes

Subtype	Lineage	Nucleotide evolutionary rate (ns/site/year $\times 10^{-3}$ )			Protein evolutionary rate (aas/site/year $\times 10^{-3}$ )		
		PB2 gene	PB1 gene	PA gene	PB2 protein	PB1 protein	PA protein
Influenza B Virus	I	0.76	1.20	1.24	ND	0.57	0.26
	II	0.76	0.83	1.16	0.14	0.01	0.37
Human H3N2 influenza virus		1.27	1.41	1.46	0.40	0.12	0.49

\* Nucleotide evolutionary rates were based on the entire gene segment of each gene.  
ND, Not detectable.

Table 10

Table 4. Evolutionary profiles of influenza B virus genes

Y and V indicate the respective phylogenetic lineage of each gene based on the Yamagata-like and Victoria-like lineage evolutionary profile of the HA gene, respectively.

Strain	Evolutionary lineages of influenza B virus genes									
	HA*	PB2	PB1	PA	NP*	M/BM2*	NS1/NS2*			
SIN79	Y	Y	V	Y	Y	Y	Y	Y	Y	Y
NOR84	Y	V	V	V	V	V	V	V	V	V
YAM88	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
PAN90	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
BEI93	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
MIE93	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
HAR94	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
SHI3098	Y	Y	Y	Y	Y	Y	Y	Y	V	V
YAN98	Y	Y	Y	Y	Y	Y	Y	Y	Y	V
IBA85	V	V	V	V	V	V	V	V	V	Y
VIC87	V	V	V	V	V	V	V	V	V	Y
AIC88	V	V	V	V	V	V	V	V	V	Y
GUA93	V	V	V	V	Y	V	V	V	V	V
GUA94	V	V	V	V	Y	V	V	V	V	V
BEI97	V	V	V	Y	Y	Y	Y	Y	Y	V
HEN97	V	V	V	Y	Y	Y	Y	Y	Y	V
CHI98	V	V	V	Y	Y	Y	Y	Y	Y	V
SHI5198	V	V	V	Y	Y	Y	Y	Y	Y	V

\* Evolutionary profiles of the HA, NP, M and NS genes were determined previously (Lindstrom *et al.*, 1999).

Table 11

Table 1. GenBank accession numbers of nucleotide sequences of H5N1 influenza A viruses determined in the present study

Strain	PB2	PB1	PA	NP	M	NS
HK156	AF036363	AF036362	AF084267	AF036359	AF036358	AF036360
HK481	AF115290	AF115292	AF115294	AF115284	AF115286	AF115288
HK482	AF084261	AF084264	AF084268	AF084276	AF084282	AF084285
HK483	AF084262	AF084265	AF084269	AF084277	AF084283	AF084286
HK485	AF084263	AF084266	AF084270	AF084278	AF084284	AF084287
HK486	AF115291	AF115293	AF115295	AF115285	AF115287	AF115289
ckHK220*	AF046086	AF046085	AF046087	AF046084	AF046082	AF046083
ckHK258*	NA	NA	NA	AF057293	NA	NA

\* These strains were not sequenced in the present study (see Methods).  
NA, Sequence not available.

Table 12

**Table 2. Nucleotide and amino acid sequence differences among the internal genes of human H5N1 influenza viruses**

For each gene comparison, the values in the upper right and lower left represent the numbers of nucleotide and amino acid differences, respectively.

Strain	Number of differences					
	HK156	HK482	HK486	HK483	HK485	HK481
<b>PB2</b>						
HK156	—	16	15	18	19	30
HK482	4	—	1	25	26	36
HK486	4	0	—	24	25	35
HK483	6	8	8	—	13	27
HK485	7	9	9	3	—	28
HK481	8	10	10	6	7	—
<b>PB1</b>						
HK156	—	11	12	29	15	11
HK482	4	—	1	30	16	12
HK486	5	1	—	30	17	13
HK483	5	4	5	—	28	28
HK485	4	4	5	5	—	16
HK481	2	2	3	3	2	—
<b>PA</b>						
HK156	—	4	6	25	23	9
HK482	0	—	2	27	25	11
HK486	0	0	—	27	25	13
HK483	9	9	9	—	18	28
HK485	5	5	5	4	—	26
HK481	0	0	0	9	5	—
<b>NP</b>						
HK156	—	6	6	18	14	25
HK482	2	—	0	14	12	21
HK486	2	0	—	14	12	21
HK483	5	3	3	—	12	21
HK485	6	4	4	3	—	17
HK481	8	6	6	6	7	—
<b>M1</b>						
HK156	—	8	8	5	4	6
HK482	1	—	0	5	6	8
HK486	1	0	—	5	6	8
HK483	1	2	2	—	3	5
HK485	1	1	1	2	—	4
HK481	0	1	1	1	1	—
<b>M2</b>						
HK156	—	3	3	0	0	0
HK482	2	—	0	3	3	3
HK486	2	0	—	3	3	0
HK483	0	2	2	—	0	0
HK485	0	2	2	0	—	0
HK481	0	2	2	0	0	—
<b>NS1</b>						
HK156	—	1	1	9	10	10
HK482	0	—	1	10	11	11
HK486	1	1	—	11	12	12
HK483	5	5	6	—	3	19
HK485	5	5	6	2	—	20
HK481	7	7	8	12	12	—

Table 13

Table 3. Predicted amino acid differences observed among human and chicken H5N1 viruses isolated in Hong Kong

Only those residues that differ from that shown for HK156 are given. NA, Sequence not available.

Residue	HK156	HK482	HK486	HK481	HK483	HK485	ckHK220
PB2							
71	N	—	—	—	—	K	—
72	E	—	—	D	—	—	—
199	S	—	—	A	A	A	A
278	A	—	—	—	—	V	—
318	R	—	—	K	K	K	K
336	F	—	—	S	—	—	—
339	K	—	—	R	—	—	—
355	K	Q	Q	—	—	—	—
382	I	—	—	—	V	—	—
390	D	—	—	N	—	—	—
508	Q	—	—	R	R	R	R
627	E	—	—	—	K	K	—
675	L	I	I	—	—	—	NA
683	T	A	A	—	—	—	NA
727	R	G	G	G	G	G	NA
PB1							
119	M	V	V	V	V	V	V
171	M	V	V	—	—	—	—
198	K	R	R	—	N	—	—
317	I	—	M	—	—	—	—
387	K	—	—	—	—	T	—
397	V	—	—	—	I	—	I
430	R	—	—	—	—	K	—
610	G	C	C	C	C	C	C
653	K	—	—	—	R	—	R
PA							
127	V	—	—	—	I	I	I
181	E	—	—	—	D	—	—
241	C	—	—	—	Y	—	—
336	L	—	—	—	M	M	M
409	N	—	—	—	S	S	S
497	R	—	—	—	K	K	K
505	V	—	—	—	I	I	I
560	P	—	—	—	A	—	—
580	E	—	—	—	D	—	—
NP							
41	I	—	—	V	—	—	—
63	I	—	—	—	—	V	—
194	I	—	—	V	—	—	—
210	E	—	—	D	—	—	—
236	K	—	—	R	—	—	—
283	P	L	L	L	L	L	L
284	A	—	—	—	T	—	—
319	K	—	—	N	N	N	N
329	V	—	—	—	—	I	—
377	T	S	S	S	S	S	S
423	V	—	—	S	A	A	A
M1							
15	I	V	V	—	—	T	—
23	E	—	—	—	Q	—	—
M2							
64	S	P	P	—	—	—	—
69	P	L	L	—	—	—	—



Table 14

**Table 4. Comparison of amino acids found in the internal proteins of H5N1 Hong Kong viruses with those of avian and human viruses**

Amino acids are shown that were found in the predicted amino acid sequences of H5N1 Hong Kong viruses in comparison with the consensus sequences for avian and human viruses.

Virus	PB2						PA	NP	M2	
	199	627	661	667	409	136			16	28
Avian	A	E	A	V	S	L	E	I	L	L
Human	S	K	T*	I†	N	M	G	V	M	F
HK156	S	E	T	I	N	M	G	V	M	F
HK481	A	E	T	I	N	M	G	V	M	F
HK482	S	E	T	I	N	M	G	V	M	F
HK483	A	K	T	I	S	M	G	V	M	F
HK485	A	K	T	I	S	M	G	V	M	F
HK486	S	E	T	I	N	M	G	V	M	F
Chicken H5N1	S/A§	E‡	T	I‡	S/N§	M§	G§	V§	M§	F§

\* Residue found in 21 of 22 sequences published previously.

† Residue found in 20 of 22 sequences published previously.

‡ From the sequence of A/chicken/Hong Kong/220/97 (ckHK220).

§ As reported by Zhou *et al.* (1999).

Table 15

**Table 5. Phylogenetic differentiation of RNA segments of H5N1 viruses isolated from humans**

The evolutionary locations of the various segments in the dendrograms shown in Fig. 1 are given as i (HK156-like branch cluster) or ii (HK483-like cluster, with the exception of the M gene, which was represented by HK482). The divergent PB2, NP and NS genes of HK481 did not belong to either minor lineage.

Strain	HA	NA	PB2	PB1	PA	NP	M	NS
HK156	i	i	i	i	i	i	i	i
HK481	i	i	HK481	i	i	HK481	i	HK481
HK482	i	i	i	i	i	i	ii	i
HK486	i	i	i	i	i	i	ii	i
HK483	ii	ii	ii	ii	ii	ii	i	ii
HK485	ii	ii	ii	i	ii	ii	i	ii

Table 16

Comparative Analysis of HK156 In Mouse Organs  
after I.n. Infection<sup>a</sup>

Days p.i.	Virus	Virus Infectivity (log <sub>10</sub> pfu/ml)			
		Lung	Brain	Liver	Kidney
2	HK156-CK	6.7/- <sup>b</sup>	-/-	-/-	-/-
	B1-1-1	6.0/6.6	-/-	-/-	-/-
	B1-1-2	6.9/7.2	-/-	-/-	-/-
	B3-1-1	5.9/6.5	2.1/1.5	-/-	-/-
	HK156-E3	-/-	-/-	-/-	-/-
	L7-4-1	0.7/4.7	-/-	-/-	-/-
	D5-7-1	5.0/5.1	-/-	-/-	-/-
4	HK156-CK	5.8/6.2	-/-	-/-	-/-
	B1-1-1	6.3/6.5	1.3/1.7	-/-	-/-
	B1-1-2	6.0/6.8	1.4/1.6	-/-	-/-
	B3-1-1	6.6/6.8	2.7/2.7	-/-	-/-
	HK156-E3	5.3/5.4	-/-	2.5/-	1.0/-
	L7-4-1	5.4/5.4	-/-	-/-	-/-
	D5-7-1	4.4/4.9	-/-	-/-	-/-
6	HK156-CK	4.5/4.9	3.3/3.1	-/-	-/1.2
	B1-1-1	ND <sup>c</sup>	ND	ND	ND
	B1-1-2	ND	ND	ND	ND
	B3-1-1	ND	ND	ND	ND
	HK156-E3	3.5/4.0	-/-	-/-	-/-
	L7-4-1	4.8/5.0	-/-	-/-	-/-
	D5-7-1	4.2/4.3	-/-	-/-	-/-

<sup>a</sup> Mice were infected with I.n.  $2 \times 10^5$  pfu of each virus. To determine virus titers, two mice were sacrificed on days 2, 4, and 6 p.i. Virus titers were determined by plaque assay in MDCK cells in the presence of trypsin.

<sup>b</sup> -, virus titer less than 10 pfu/ml.

<sup>c</sup> ND, not determined.

Table 17

**TABLE 2**  
**Infectivity of HK156 Viruses Used In the Study In Mouse Brain**  
**after I.c. Inoculation<sup>a</sup>**

Virus	Virus titer ( $\log_{10}$ pfu/ml) In mouse brain on days p.i.		
	2	4	6
HK156-CK	ND <sup>b</sup>	4.2/4.3	ND
B1-1-1	4.3/4.3	4.7/5.0	ND
B1-1-2	3.5/4.7	5.2/5.3	ND
B3-1-1	4.2/4.7	4.9/5.0	ND
HK156-E3	ND	2.5/2.6	ND
L7-4-1	2.7/3.2	3.5/4.5	1.2/2.7
D5-7-1	3.2/3.2	3.5/3.7	- <sup>c</sup> /3.1
Aichi68 <sup>d</sup>	ND	-/-	ND

<sup>a</sup> Two mice were infected i.c. with  $1 \times 10^4$  pfu of each virus. After 2, 4, and 6 days p.i., mouse brain were collected and virus titers were determined by plaque assay in MDCK cells in the presence of trypsin.

<sup>b</sup> ND, not determined.

<sup>c</sup> -, virus titer less than 10 pfu/ml.

<sup>d</sup> Aichi68 was used as a negative control as a non-neurovirulent virus.

Table 18

TABLE 3  
Comparison of Growth Characteristics and Virulence of HK156 Virus and Its Cloned Viruses Used in the Study

Virus	Passage history <sup>a</sup>	Plaque size in MDCK cells <sup>b</sup>	log <sub>10</sub> pfu/ml in MDCK cells	EID <sub>50</sub> (log <sub>10</sub> /ml)	ELD <sub>50</sub> (pfu)	MLD <sub>50</sub> (pfu)
Pathogenic in mice HK156-CK	MX/M2	Pinpoint (30%) Medium (70%)	7.3	7.5	0.6	8.0 × 10 <sup>2</sup>
Mouse brain clone B1-1-1	MX/Ms1M4	Pinpoint (74%) Medium (26%)	7.2	7.5	4.0 × 10 <sup>2</sup>	3.2
B1-1-2	MX/Ms1M4	Pinpoint	7.7	7.5	1.0 × 10 <sup>4</sup>	3.2
B3-1-1	MX/Ms1M4	Medium	7.8	8.0	6.0 × 10 <sup>3</sup>	3.2
Less pathogenic in mice HK156-E3	MX/M1E3	Medium	7.3	8.0	0.2	1.3 × 10 <sup>4</sup>
HK156-E3 clone L7-4-1	MX/M1E3C3E1	Medium	7.6	8.2	0.2	>2 × 10 <sup>6</sup>
D5-7-1	MX/M1E3C3E1	Medium	7.8	8.0	0.2	>2 × 10 <sup>6</sup>

<sup>a</sup> Passage history in Hong Kong/our laboratory. MX, MDCK cells (passage number is unknown); M, MDCK cells; Ms, mouse; C, CE cells; E, eggs. The number indicates the number of passages. Method for virus cloning was described in Materials and Methods.

<sup>b</sup> Pinpoint plaque formed <1 mm in diameter; medium plaque formed 1-2 mm in diameter.

Table 19

TABLE 4  
Comparison of Amino Acid Sequences of HK156 Viruses Used in the Study<sup>a</sup>

Virus	Amino acid at position (nucleotide at position)										
	HA	NS1	PB1	PA	NP	PB2	NA				
Pathogenic for mice HK156-CK	155 (464)	211 (631)	101 (301)	17 (50)	456 (1366)	712 (2134)	631 (1891)	127 (378)	701 (2101)	64 (161)	83 (248)
	Ser (G) <sup>b</sup> or Asn (A)	Pro (C) <sup>b</sup> or Thr (A)	Asp (G)	Ala (C) <sup>b</sup> or Asp (A)	His (C)	Ser (T)	Gly (G)	Glu (G)	Asn (A)	Asn (A)	Lys (A)
B1-1-1	Ser (G)	Pro (C)	•	Ala (C)	Tyr (T)	Pro (C)	Ser (A)	Lys (A)	Asp (G)	•	•
B1-1-2	Ser (G)	Pro (C)	•	Ala (C)	Tyr (T)	Pro (C)	Ser (A)	Lys (A)	Asp (G)	•	•
B3-1-1	Ser (G)	Pro (C)	•	Ala (C)	Tyr (T)	Pro (C)	Ser (A)	Lys (A)	Asp (G)	•	•
Mice brain homogenate <sup>c</sup>	Ser (G)	Pro (C)	•	Ala (C) <sup>d</sup>	Tyr (T) <sup>d</sup>	Pro (C) <sup>d</sup>	Ser (A) <sup>d</sup>	Lys (A) <sup>d</sup>	Asp (G) <sup>d</sup>	-- <sup>d</sup>	--
Less pathogenic for mice											
HK156-E3	Ser (G)	Thr (A)	Asn (A)	Ala (C)	•	•	•	•	Asp (G)	•	•
L7-4-1	Ser (G)	Thr (A)	Asn (A)	Ala (C)	•	•	•	•	Asp (G)	Ile (T)	•
D5-7-1	Ser (G)	Thr (A)	Asn (A)	Ala (C)	•	•	•	•	Asp (G)	•	Arg (G)

<sup>a</sup> No amino acid substitutions were found in M1, M2, or NS2 proteins. <sup>b</sup> Identical amino acids.

<sup>c</sup> Mixture of nucleotides was observed in sequence gel image.

<sup>d</sup> Sequences were determined directly from mouse brain homogenate infected i.n. with HK156-CK on 6 days p.i.

<sup>e</sup> Partial sequence was determined in this region.

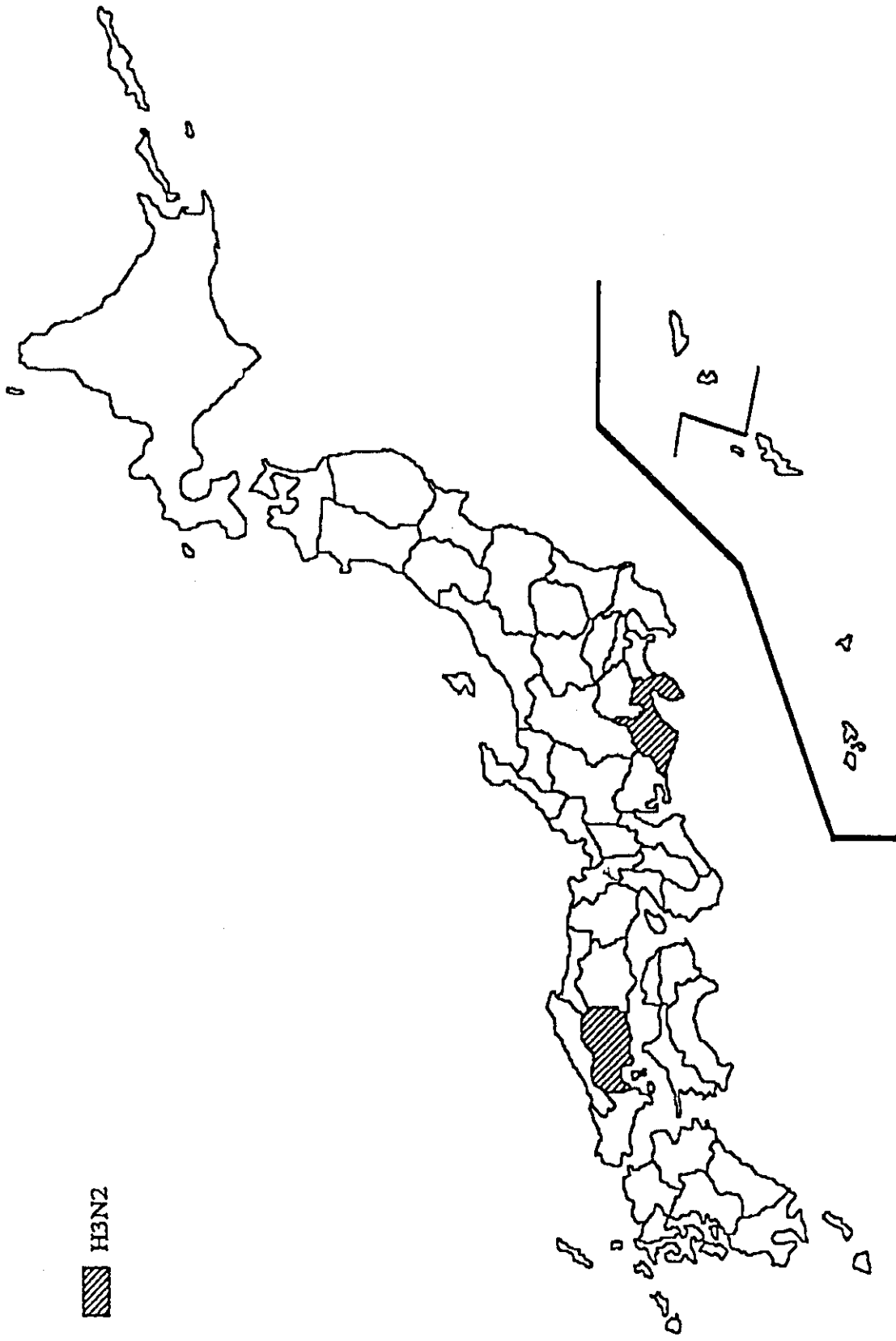
• —, not determined.

Table 20. ウイルスが分離された患者数を基礎にしたインフルエンザウイルス間の重症度の比較

症 状	A・香港型(H3N2)	A・ソ連型(H1N1)	B型
発 熱 (℃) ～38.5	14.8	15.5	20.1
38.5～39.0	29.9	32.3	35.3
39.1～40.0	34.4	34.0	27.2
40.1 以上	4.3	3.7	1.8
不明	0.6	3.0	4.3
上気道炎	59.3	54.9	61.5
気管支炎及び細気管支炎症	8.4	5.1	5.6
肺炎	2.4	0.9	1.5
胃腸炎	6.6	6.0	8.2
筋肉痛・関節痛	8.7	10.2	7.4
脳炎・脳症	1.4	0.4	0.4
結膜炎	0.4	0.8	0.6
頭痛	13.0	16.0	17.3
咳	22.3	21.0	26.9
痰	1.2	0.6	1.8
下痢	16.2	3.4	3.1

全項目調査数：A・香港型 11,615人 A・ソ連型 5,972人 B型 8,888人

Fig. 1



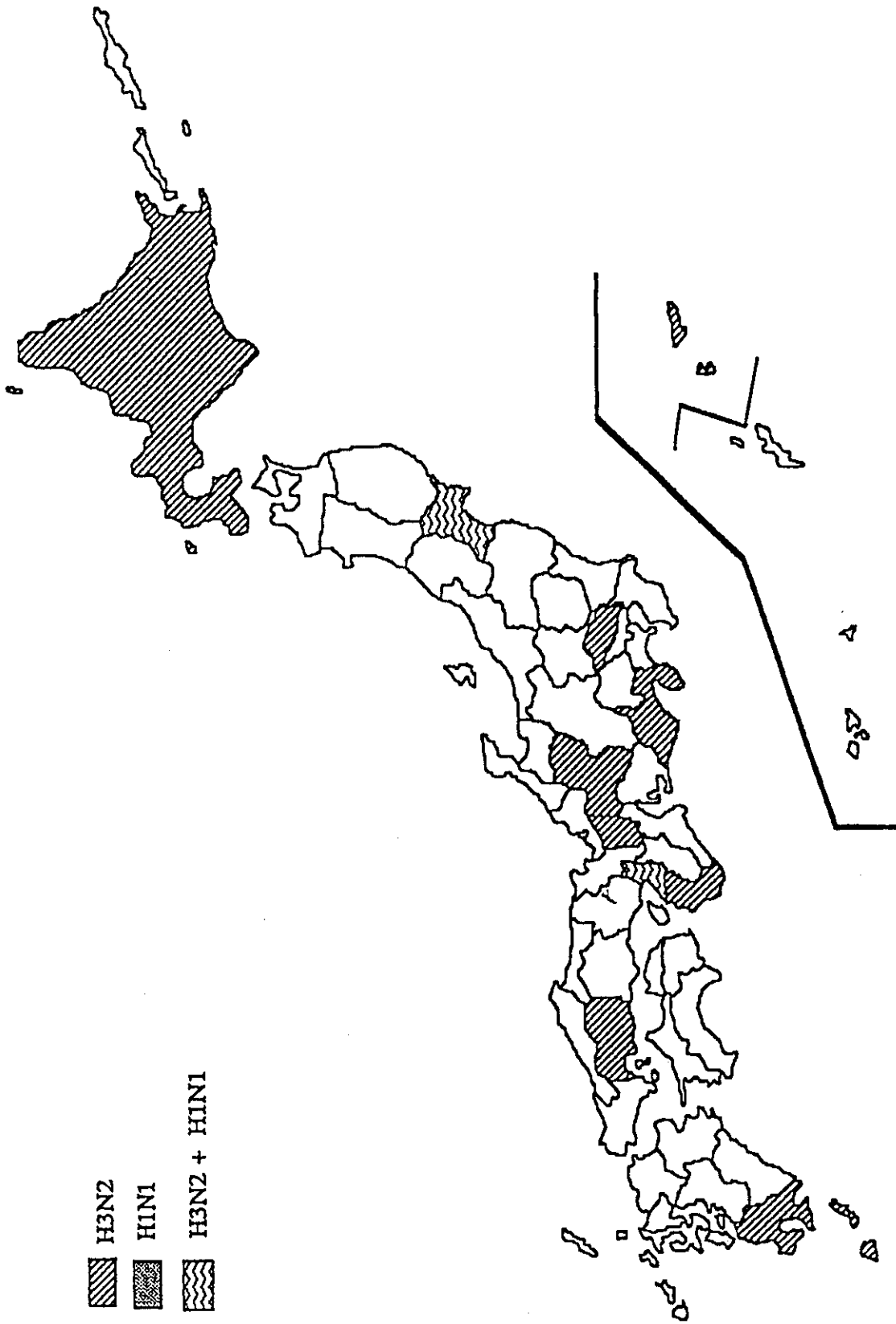
Geographical distribution of influenza virus isolates between October 1 and October 31, 1999.

O : Date of first isolation at the period (Oct.1-Oct.31)

(2/8/2000)



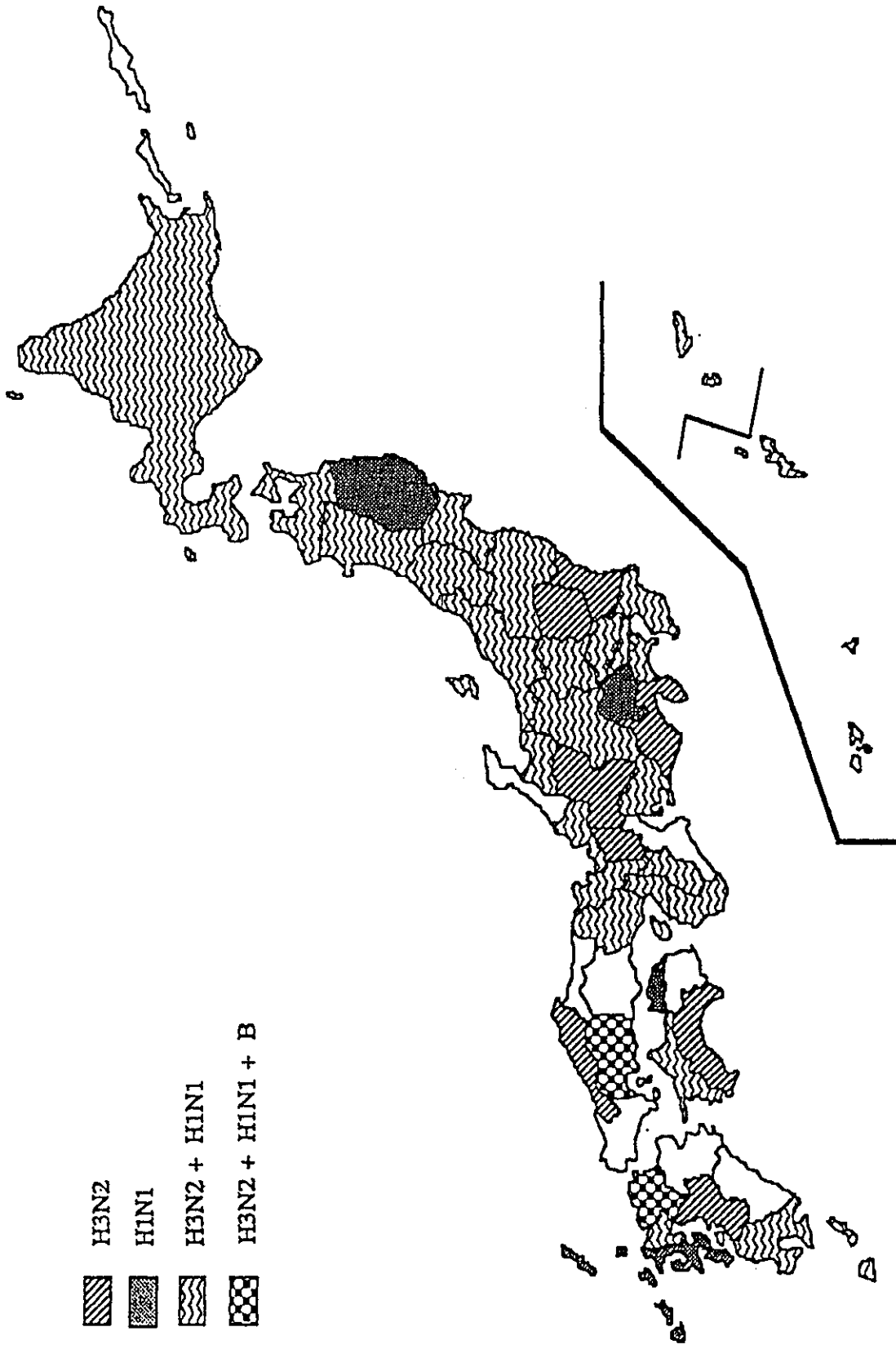
Fig. 2



Geographical distribution of influenza virus isolates between November 1 and November 30, 1999.  
○ : Date of first isolation at the period (Nov.1- Nov.30)

(2/8/2000)

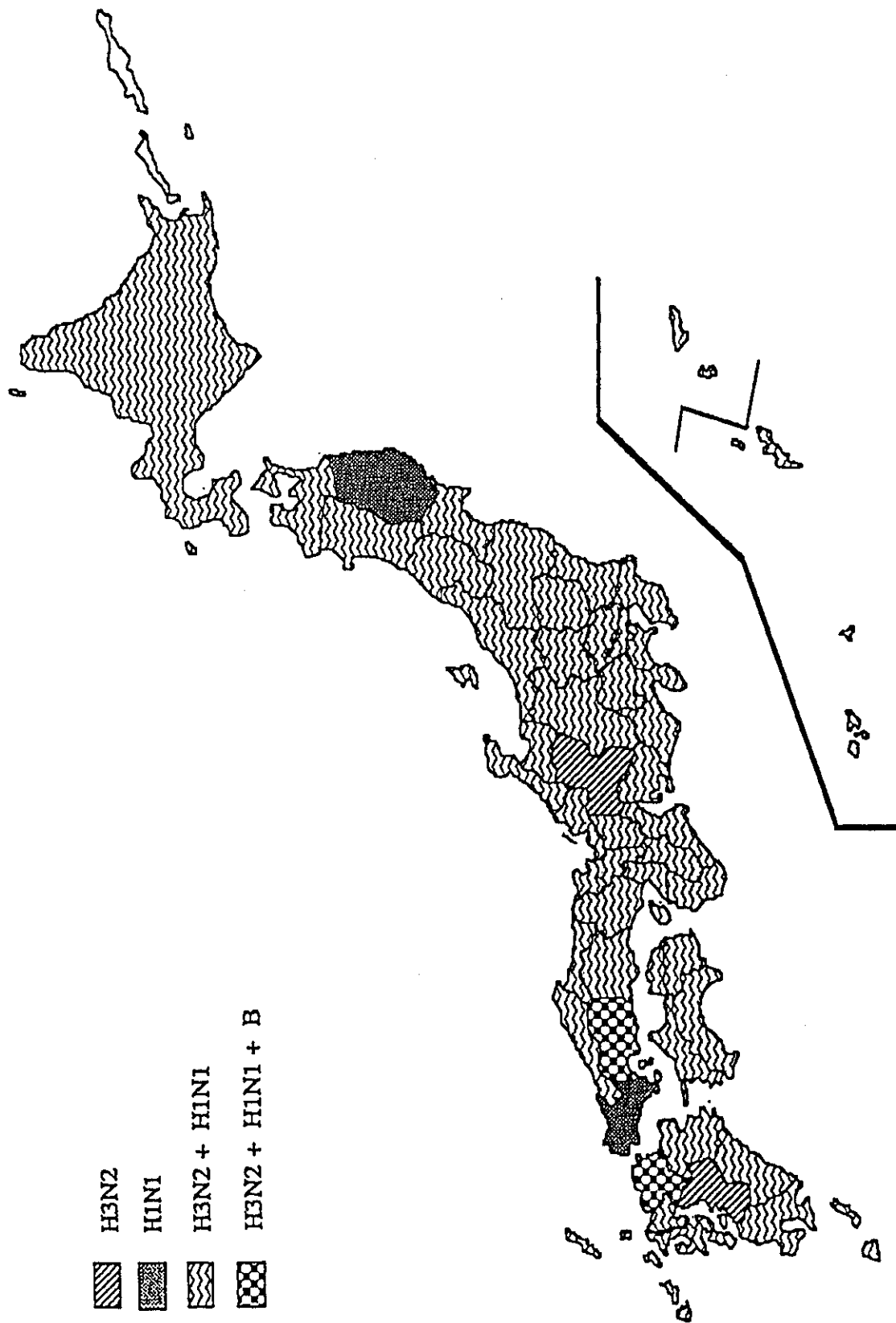
Fig. 3



Geographical distribution of influenza virus isolates between December 1 and December 31, 1999.  
○ : Date of first isolation at the period (Dec.1- Dec.31)

(2/8/2000)

Fig. 4



Geographical distribution of influenza virus isolates between January 1 and January 31, 2000.

○ : Date of first isolation at the period (Jan.1- Jan.31)

(2/8/2000)

Fig. 5

