

Acknowledgments

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Human Herpesvirus 8, Southern Siberia

To the Editor: Human herpesvirus 8 (HHV-8) is the etiologic agent of Kaposi sarcoma. Sequence analysis of the highly variable open reading frame (ORF)–K1 of HHV-8 has enabled the identification of 5 main molecular subtypes, A–E (*1*). A and C subtypes are prevalent in persons in Europe, Mediterranean countries, northwestern China, and the United States; subtype B, in persons in sub-Saharan Africa; subtype D, in persons in the Pacific Islands and Japan (*2–6*); and subtype E, in Native Americans in the United States.

Considering that K1 gene polymorphisms of HHV-8–infected persons reflect the divergence accumulated during the early migrations of modern humans out of Africa (*1*), it is tempting to put the polymorphisms observed in the different subtypes into an evolutionary perspective with their geographic distribution. It is thought that Native Americans infected by subtype E and Pacific Islanders, including those infected by subtype D in the Japanese archipelago, originated from a common ancestral genetic stock in continental Asia. Because Siberia constitutes the geographic link between mainland Asia, North America, and the Pacific (online Technical Appendix, www.cdc.gov/eid/content/16/3/585-Techapp.pdf), it is likely that the Siberian region has served as a source or a corridor of human dispersals to these regions. Thus, we conducted a molecular epidemiology HHV-8 survey of the Buryat population, a major indigenous group in southern Siberia, to gain new insights into the origins, possibly common, of HHV-8 subtypes D and E.

After consent of local authorities and participants, we collected 745 human blood samples in 1995 in 17 medicosocial structures (homes for elderly persons, veterans of the Russian army,

hospitalized persons, blood donors) located near Lake Baikal and originating from Ulan Ude (344), Ust Orda (216), and Chita (185), Siberia, Russia (additional data can be obtained directly from the authors). The median age of those included was 52 years (range 25–98 years); 489 (66%) were women. Antibodies against HHV-8 latency-associated nuclear antigen were identified by immunofluorescent antibody assay by using the BC3 cell line (3). Punctuate nuclear staining of BC3 cells at a 1:160 dilution was observed for 187 (25.1%) patients with no difference according to investigated regions ($p = 0.32$ by χ^2 test) or between men (25.8%) and women (24.7%) ($p = 0.76$ by χ^2 test; online Technical Appendix). However, HHV-8 seroprevalence increased with patient age, rising from 12.9% (25–43 years) to 46.4% (≥ 61 years) ($p = 1.8 \times 10^{-13}$ by χ^2 test for trend) (Figure; online Technical Appendix). No significant difference was

observed in antibody titers according to age ($p = 0.45$ by Fisher exact test). These results demonstrate that HHV-8 infection is highly prevalent in the Siberian adult population tested.

HHV-8 infection was determined by nested PCR that amplified a 737-bp fragment of the ORFK1 in peripheral blood buffy coats of 85 HHV-8-seropositive and 10 HHV-8-seronegative persons (3). Amplification was positive in 19/85 (22.4%) samples; sequences were obtained for 18 of these samples (online Technical Appendix). These sequences showed 0%–7.31% nucleotide divergence and 0%–3.55% amino acid divergence. Nevertheless, 17 strains were found to be closely related with $<1.75\%$ nucleotide differences for 684 nt, and only 1 sequence (1445 strain) displayed higher nucleotide divergence.

A comparative sequence analysis, including 66 representatives of K1 gene sequences of the HHV-8 A/C

subtypes/subgroups, and sequences obtained from persons originating from Russia, was performed (7–9). Seventeen of the 18 HHV-8 strains from Siberia belonged to the A subtype; 15 clustered in a newly identified specific subclade (online Technical Appendix). Notably, the 1445-Siberian strain, which exhibits the typical 5 aa deletion at positions 201–205, belongs to subtype C and clustered with the 7848 strain previously described by Lacoste et al. (9). Furthermore, both strains originate from Chita.

Our results indicate that HHV-8 infection is highly prevalent in the population tested in southern Siberia and extend current knowledge on the worldwide distribution of HHV-8 genotypes. The presence of a Siberian strain monophyletic subclade suggests the existence of HHV-8 strains preferentially spreading among this population in southern Siberia.

To ascertain the maternal ancestry of these persons, we sequenced the hypervariable region I (HVS-I) of the maternally-inherited mitochondrial DNA (mtDNA) and assigned haplogroups on the basis of the HVS-I motifs. Our analyses showed that 17/18 persons analyzed showed a mtDNA motif of clear continental east Asian origin (e.g., A, D correspond to different mtDNA haplogroups). One person (1474-strain) had a lineage (i.e., HV1) that is thought to have a western Eurasian origin. Overall, these mtDNA analyses indicate that the maternal ancestry of the persons examined here can be unambiguously attributed to East Asia, and not to Western Eurasia. K1 subtype A sequences recently found in the Xinjiang Uygur region in China (10) do not correspond to the specific Siberian clade described in our study. Thus, we must now consider that the widely distributed HHV-8 A/C subtype, so far mainly observed in Europe and Mediterranean countries, is also largely predominant in continental Asia.

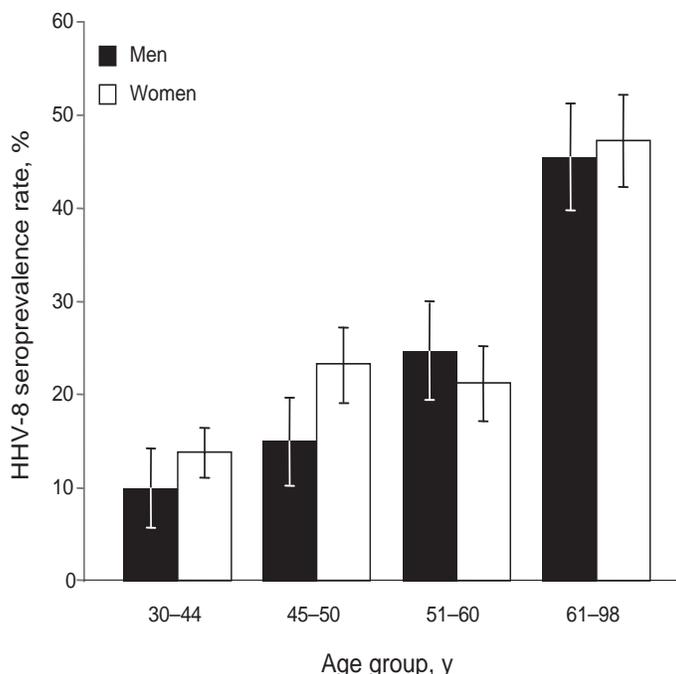


Figure. Age-dependent human herpesvirus 8 (HHV-8) seroprevalence rates for 745 persons in southern Siberia 25–98 years of age who lived in the Ust Orda, Ulan Ude, or Chita districts during 1995. Seropositivity was based on strict criteria; only samples showing punctuate nuclear staining clearly reactive at a dilution $\geq 1:160$ were considered HHV-8 positive. All 187 HHV-8-seropositive samples were tested for antibodies directed against HIV-1/2 by using Genscreen HIV-1/2 Antibody Assay (Bio-Rad Laboratories, Marnes-la-Coquette, France); only 2 were seropositive. Error bars indicate 95% confidence intervals.

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Sus-Pense

R.L. Bernstein

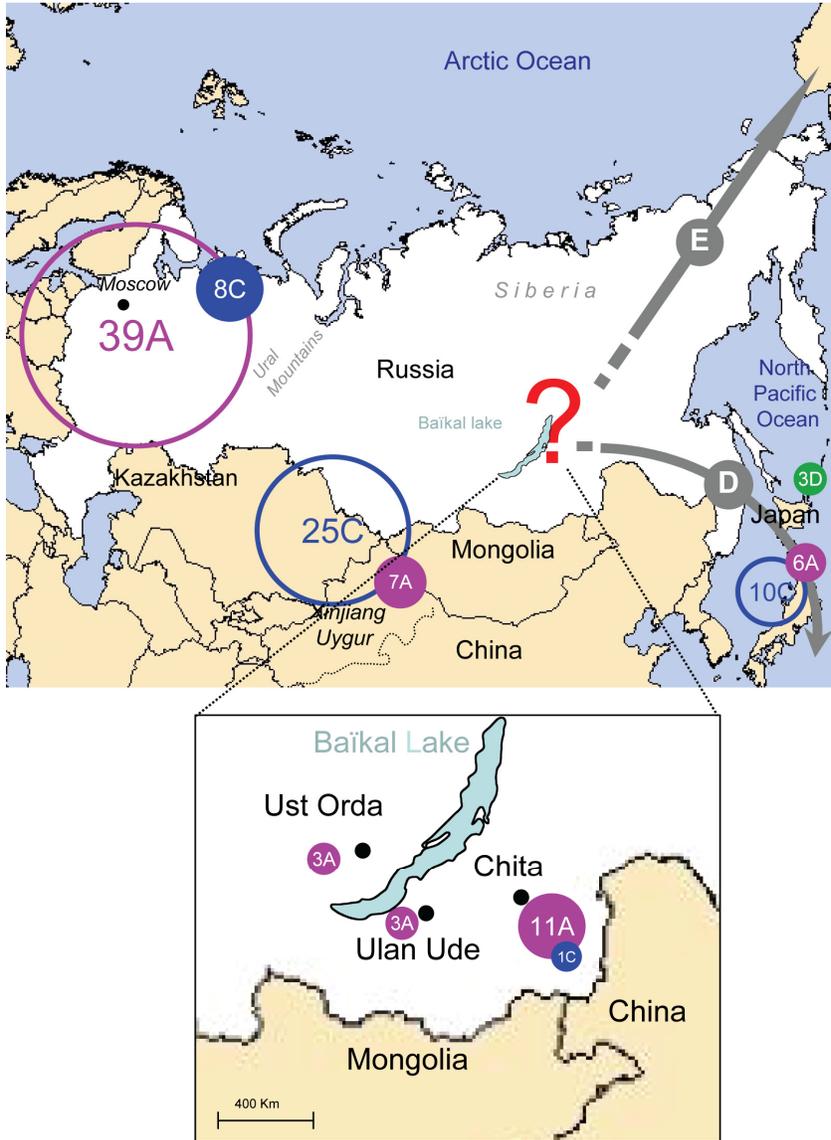
Don't call it swine flu:
if you dine on pork, you
can't catch the flu, as we know.
But the pork people say
some customers may
be confused by the name and just go.
Now look at the news:
around the world views
show people with a mask on their face.
Despite the name change,
folks who find H1N1 strange
still want to keep swine in their place.
For me it is clear:
I don't have the fear
of swallowing bacon-wrapped figs.
While others may hurry
to wear masks, I don't worry.
I really don't plan to kiss pigs.

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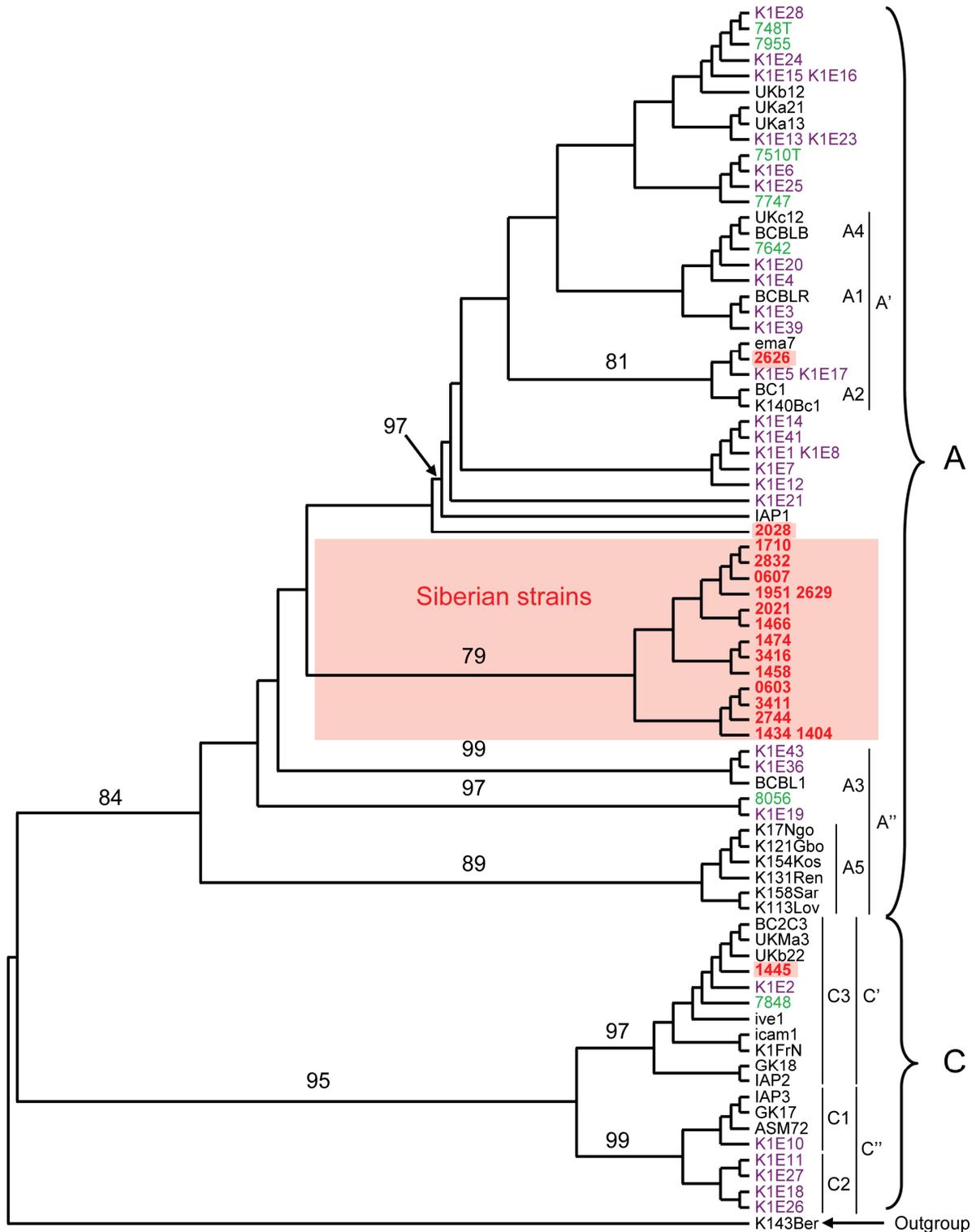
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Human Herpesvirus 8, Southern Siberia



Technical Appendix Figure 1. Map of Siberia (top) showing geographic distribution of human herpesvirus 8 (HHV-8) subtypes according to previous phylogenetic studies based on the complete or partial K1 gene/protein analysis. Russian A/C subtype strains were obtained from patients with Kaposi sarcoma living in the Moscow area (1–3). Chinese A/C strains were obtained in persons originating from Xinjiang Uygur region and reviewed in (4). D subtype strains were obtained from inhabitants of Japan, Pacific Islands, and Australia and subtype E strains were found among Native American populations of the Brazilian, Ecuadorian and French Guyanan regions. Gray

arrows correspond to the migration routes of human derived from genetic, archaeological and anthropologic studies. The inset box shows the location of the 3 districts where samples were obtained during this study, Ust-Orda, Ulan-Ude, and Chita, as well as distribution of the molecular subtypes of HHV-8 strains characterized.



Technical Appendix Figure 2. Unrooted phylogenetic tree of human herpesvirus 8 (HHV-8) strains generated by using the neighbor joining (NJ) method with a 586-bp fragment of the K1 gene. The phylogeny was derived by using the GTR model in PAUP* version 4.0b10 (Sinauer Associates, Inc.,

Sunderland, MA, USA). Reliability of the inferred tree was evaluated by bootstrap analysis on 1,000 replicates. Numbers on each node indicate the percentage of bootstrap samples (1,000) in which the cluster is supported. Only bootstrap values ≥ 75 are given. K143Ber strain was used as an outgroup. The 18 new ORFK1 HHV-8 sequences labeled in red (GenBank accession nos. GQ861475–GQ861492) were analyzed with 66 HHV-8 available sequences from the GenBank database. Previously Russian sequences generated by Lacoste et al. (3) and Kadyrova et al. (2) are labeled in green and purple, respectively. Bars on the right indicate subtypes, groups and subgroups. A and C correspond to 2 of the 5 main HHV-8 subtypes and A1–A5 and C1–C3 to subgroups within the subtypes described by Zong et al. (5). The A', A'', C' and C'' grouping reflects the Cook et al. (6) classification scheme. Phylogenetic analyses show that all the Siberian sequences, except two pairs (1951/2629 and 1434/1404), are different.

Technical Appendix Table 1. Demographic, geographic and serologic data of 19 HHV-8 seropositive persons from Siberia confirmed by molecular analysis*

Virus strain	Place of origin	Age, y	Sex	Maternal ancestry†	IFA titers (LANA)	PCR K1	HHV-8 molecular subtype	GenBank accession no.
603	Ulan Ude	47	F	East Asia	10 240	+	A	GQ861475
607	Ulan Ude	49	F	East Asia	640	+	A	GQ861476
1710	Ulan Ude	78	F	East Asia	10 240	+	A	GQ861479
1737	Ulan Ude	47	F	East Asia	640	±	NA	NA
1404	Chita	66	F	East Asia	2 560	+	A	GQ861487
1434	Chita	64	M	East Asia	320	+	A	GQ861477
1445	Chita	73	F	East Asia	320	+	C	GQ861486
1458	Chita	67	M	East Asia	2 560	+	A	GQ861485
1466	Chita	68	F	East Asia	10 240	+	A	GQ861484
1474	Chita	43	F	West Asia	640	+	A	GQ861478
2626	Chita	83	F	East Asia	10 240	+	A	GQ861482
2629	Chita	44	M	East Asia	640	+	A	GQ861489
2744	Chita	59	M	East Asia	640	+	A	GQ861483
2832	Chita	69	F	East Asia	1 280	+	A	GQ861490
3411	Chita	39	F	East Asia	160	+	A	GQ861491
3416	Chita	35	M	East Asia	160	+	A	GQ861492
1951	Ust Orda	31	F	East Asia	640	+	A	GQ861488
2021	Ust Orda	64	M	East Asia	5 120	+	A	GQ861480
2028	Ust Orda	50	F	East Asia	5 120	+	A	GQ861481

*HHV-8, human herpesvirus 8; IFA, immunofluorescence assay; LANA, HHV-8 specific antibody directed against latent nuclear antigen; PCR K1, amplification of a 737-bp fragment of the ORFK1 genomic region of HHV-8; NA, data not available.

†Genetic feature revealed by mtDNA analysis.

‡Weak PCR signal.

Technical Appendix Table 2. Age-dependent HHV-8 seroprevalence rates, by sex, for 745 persons in southern Siberia 25–98 years of age who lived in the Ust Orda, Ulan Ude, or Chita districts during 1995*

Age group, y	Men n/N (%)	Women n/N (%)	Total n/N (%)
25–43	5/50 (10.0)	23/167 (13.8)	28/217 (12.9)
44–50	9/60 (15.0)	26/112 (23.2)	35/172 (20.3)
51–60	17/69 (24.6)	22/104 (21.2)	39/173 (22.5)
61–98	35/77 (45.5)	50/106 (47.2)	85/183 (46.4)
Total	66/256 (25.8)	121/489 (24.7)	187/745 (25.1)

*HHV-8, human herpesvirus 8. Seropositivity was based on strict criteria; only samples showing punctuate nuclear staining clearly reactive at a dilution $\geq 1:160$ were considered HHV-8 positive.

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