Pathogenic caliciviruses of the genus *Vesivirus* circulate in oceanic ecosystems and spread to and among terrestrial mammals. Isolation of *Vesivirus* from natural and laboratory infections in humans led to this investigation of *Vesivirus* seroprevalence and viremia. Sera from four groups were tested for antibodies to *Vesivirus* as follows: blood donors whose units were cleared for donation, blood donors whose units were not accepted for donation solely because of elevated blood liver alanine aminotransferase (ALT) concentrations, patients with clinical hepatitis of unknown but suspected infectious cause, and patients with clinical hepatitis of unknown cause but associated with blood transfusion or dialysis. Additionally, sera were tested for *Vesivirus* genome by three methods: dot-blot and two reverse transcription-polymerase chain reaction (RT-PCR) methods. The calculated seroprevalence against *Vesivirus* virions within these groups (N = 765) was 12%, 21%, 29%, and 47%, respectively (P < 0.001 for group differences). Additionally, 11 (9.8%) of 112 sera tested yielded RT-PCR amplicons that by nucleotide sequence were distinct from each other and related to known *Vesivirus*. These data indicate that some blood donors in the population tested have serologic evidence of previous *Vesivirus* infection and some also have *Vesivirus* viremia. These results justify further investigation of an association between *Vesivirus* infection and illness in humans. *J. Med. Virol.* **78:**693–701, 2006.

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**KEY WORDS:** calicivirus; *Vesivirus*; human; seroprevalence; viremia

**INTRODUCTION**

Recent spread of newly recognized human pathogens from animal reservoirs, for example, Nipah virus, coronavirus associated with severe acute respiratory syndrome (SARS), and avian influenza strains, has focused renewed study of viral ecology [Enserink, 2000, 2003; Smith, 2000; Trampuz et al., 2004]. A relatively under-explored potential reservoir of newly emergent human pathogens is the marine environment [Smith et al., 1998b; Culley et al., 2003]. We previously described laboratory and natural marine *Vesivirus* calicivirus infections in humans [Smith et al., 1998a].

*Vesivirus* is one of four genera in the *Caliciviridae*, the others being *Norovirus* and *Sapovirus*, common causes of gastroenteritis in humans, and *Lagovirus*, widespread in Eurasia in rabbits and hares [Green et al., 2000]. Within the *Vesivirus* genus, serotypes of marine origin form a polyphyletic grouping distinct from those designated feline caliciviruses (FCVs) [Berke et al., 1997]. *Vesivirus* cycles naturally in oceanic reservoirs and a single *Vesivirus* serotype has infected species as diverse as fish, seals, shellfish, swine, cattle, and primates, including humans, and caused disease, sometimes severe, in marine and domesticated food-producing animals and humans [Smith et al., 1998a,b]. Classical virologic investigations established that epidemics of vesicular exanthema of swine virus (VESV) in North America from 1932 through 1959 were caused by serotypes of marine origin [Smith et al., 1978, 1980b; Smith, 2000]. The VESV epidemic serotypes (N = 13) are closely related to serotypes (N > 25) of San Miguel sea lion viruses (SMVs) also of ocean origin and the few other *Vesivirus* serotypes (N = 5) isolated from terrestrial animals since 1970 [Smith et al., 1978, 1980b; Smith and Boyt, 1990; Neil et al., 1995, 1998; Matson et al., 1996; Reid et al., 1999; Smith, 2000]. Viruses in the marine environment other than caliciviruses also are...
Vesivirus infection can have a multiplicity of clinical outcomes, depending on the host and strain, which include encephalitis, hepatitis, myocarditis, pneumonia, spontaneous abortion, and dermatitis (reviewed in [Smith et al., 1998b, 2002a,b; Smith, 2000]). The human cases of marine Vesivirus disease which were described previously occurred in association with the North Pacific Basin, one in a laboratory scientist working with a marine Vesivirus who experienced a disseminated vesicular exanthem and the second in a field biologist working with marine mammals who had a vesicular exanthem on the face [Smith et al., 1998a]. One strain recovered from these human cases was by genome sequence related most closely to SMSV-5, the serotype known to infect more than 20 animal species and cause different clinical manifestations in them [Smith et al., 1997, 1980a, 1998b; Smith, 2000]. The other strain from a human case was of a new Vesivirus serotype. To assess whether Vesivirus infections are more widespread among humans, the seroprevalence of these two sets of samples, three additional sets of sera from cases of clinical hepatitis were acquired from an industry source. Two of these sets represented hepatitis cases of unknown but suspected infectious cause. Both were negative for known hepatitis viruses, but differed from each other in that some cases were associated with transfusion or dialysis. Other samples came from hepatitis cases caused by hepatitis B or C virus. The study was approved by the Institutional Review Boards of the authors’ institutions.

### Materials and Methods

#### Serum Samples

Sequential, de-identified sera from blood donors were collected over a 4-year period (1996–1999), based upon availability, from the Regional Red Cross Blood Testing Laboratory in Portland, Oregon, which serves eight Western States and was processing samples from about 3,000 units of blood per day. One sample set included blood cleared for donation after clinical and laboratory screening, which included negative test results for hepatitis B surface and core antigens, HIV-1 and 2, HIV P24 antigen, human T-cell lymphotropic virus type 2, hepatitis C antigen, and serum alanine aminotransferase (ALT) levels below 120 International Units (IU). A second, concurrently acquired set of sequential blood specimens was collected from donors whose pre-donation laboratory values differed from the first set only in that the ALT level was ≥120 IU. Based upon testing of these two sets of samples, three additional sets of sera from cases of clinical hepatitis were acquired from an industry source. Two of these sets represented hepatitis cases of unknown but suspected infectious cause. Both were negative for known hepatitis viruses, but differed from each other in that some cases were associated with transfusion or dialysis. Other samples came from hepatitis cases caused by hepatitis B or C virus. The study was approved by the Institutional Review Boards of the authors’ institutions.

### Enzyme Immunoassay (EIA) for Antibody Detection

The EIA utilizing the SMSV pool antigen was described previously [Smith et al., 1998a]. SMSV pool antigen was applied directly to the plate and then incubated with a 1:100 dilution of human serum. Bound immunoglobulin was detected by an alkaline phosphatase-conjugated, anti-human immunoglobulin second antibody (1:40,000 dilution; Sigma, St. Louis, MO). Optical densities (ODs) of the color substrate Blue Phos (KPL, Gaithersburg, MD) were read at 650 nm on a Titertek Multiskan Plus EIA Plate Reader (Titertek, Huntsville, AL). For comparison, sera were tested against wells with no viral antigen. EIA data were
analyzed as P-N values, in which the OD of the antigen
negative well (N) was subtracted from the OD of the well
containing viral antigen (P). The OD values of antigen
negative (N) wells were ≤0.005 and most were 0.000.
The cut-point for positivity was a P-N ≥ 0.043. Previous
testing [Smith et al., 1998a] demonstrated antibody in
EIA-positive samples by Western blot and by preabsorp-
tion with SMSV-5 antigen, and that paired sera from
cases of Norwalk virus gastroenteritis showed no
seroresponse to the SMSV pool. Vesivirus typing anti-
sera show broad cross-reactivity of the SMSV-5 capsid
antigen with that of other Vesivirus serotypes [Kurth
et al., 2006].

The FCV antigen also was utilized in the same EIA, in
which all other reagents and steps were the same. The
cut-point for the FCV antigen also was a P-N value
≥ 0.043, which was derived by utilizing antigen of assay
potency comparable to that of the SMSV pool and a
graphical method for estimating cut-point [Matson,
1999].

**Vesivirus Nucleic Acid Detection and Characterization**

To test the possibility that sera may contain Vesivirus
genomes, as suggested by the clinical evidence of
viremia in cases of Vesivirus illness, including humans
[Smith et al., 1998a], three complementary methods
targeting three Vesivirus genomic regions were utilized
to detect Vesivirus RNA in serum (Fig. 1): dot blot for
the ORF1 3C protease region, reverse transcription-poly-
erase chain reaction (RT-PCR) for the 3′ terminal
region of ORF1 encoding a portion of the viral RNA
polymerase or for a portion of the viral capsid protein,
and nucleotide sequencing of RT-PCR amplicons.

For the dot-blot method, a 280-bp biotin-labeled
riboprobe (5R.3) specific for the Vesivirus 3C protease
genomic region (designed from SMSV-5) and corre-
sponding to nts 3,727–4,007 of primate Vesivirus prototype
Pan-1, GenBank number AF091736) was synthesized by
in vitro transcription from recombinant plasmid
pCN5R.3 (not shown) and probed against 50 ng of total
RNA extracted from blood donor serum using Trizol
(Invitrogen, Inc., Carlsbad, CA) blotted onto a nylon
membrane. Comparison controls also blotted included
20 ng of RNA extracted from CsCl-banded SMSV-5 and
50 ng of total RNA from mouse embryo (Clontech, Inc.,
Palo Alto, CA). The membrane was probed at 50°C in
Northern Max Hyb Buffer (Ambion, Inc., Austin, TX)
and washed at high stringency (2X SSC, 1% SDS, 60°C).

For one RT-PCR (RT-PCR 1), total RNA was extracted
from 100 μl of serum using Trizol. A positive control
(tissue culture of primate calicivirus) and a negative
control (water) were included in each round of extraction
in order to control for the quality of the extraction.
Extracted RNA was diluted in water containing 0.4 U/μl
of RNAsin (Promega, Madison, WI) and used directly
in RT-PCR assays. The RNA was amplified with primers
D3A 10F (5′-CCAAAC GCCAA CACAT TTGCA TGCA
TG-3′, designed from reverse transcriptase (Sigma) according to manufacturer’s instructions and
Platinun Pfx DNA polymerase (Invitrogen) also follow-
ing the manufacturer’s recommended protocol. For the
second RT-PCR (RT-PCR 2), total RNA from serum was amplified in a similar manner using primers 289rc, 5′-
TATGG TGATG ACCTAG GTCTA CA-3′ (designed from
nucleotides 5,123–5,144 of Pan-1), and primer “pre-
capsid,” 5′-CACCT CACCA GTCA CGTG CTCG CCC-3′ (designed
from nucleotides 5,650–5,633 of Pan-1), and yielding a product of approximately 510 base pairs. RT-PCR
amplicons were resolved in a 1% agarose gel and
visualized by ethidium bromide under UV light.

For nucleotide sequencing of RT-PCR amplicons, RT-
PCR products were sequenced directly or after cloning.
Cloning used the pGEM-T vector system I (Promega)
following the manufacturer’s protocol. Recombinants
obtained from cloning were screened by PCR using
forward and reverse primers targeting the SP6 and T7
polymerase promoters bracketing the multiple cloning
cassette. Plasmid DNA from the positive clones was
purified by QIA prep Spin Miniprep Kit (Qiagen,
Valencia, CA) and sequenced with Sequi Therm EXCEL
II Long-read DNA Sequencing Kit (Epiconentre Technolo-
gies, Madison, WI) on an automated sequencer
(ALF Express DNA Sequencer, Amersham Pharmacia,
Piscataway, NJ).

**Sequence Comparisons**

Raw DNA sequences were edited in OMIGA 2.0
(Accelrys, San Diego, CA). Sequences were compared
and aligned in CLUSTAL W with prototype sequences
retrieved from the NCBI public databases using BLAST
searches. Comparison Vesivirus sequences included
human Hom-1 (GenBank number U623227), primate
Pan-1 (AF091736), SMSV-2, SMSV-5, SMSV-6, SMSV-
7, SMSV-13, and SMSV-14 (U18730, U18731, U18732,
U18733, U18734, and U18739), VESV strains A48, C52,
E54, I55, and 1934b (U76874, U18738, U18739, U18740,
and U18736), bovine (U18741), feline strain F9
(M86379), rabbit (AJ866991), skunk strains 4-1L, 4-2S,
and 7-2 (U14668, U14670, and U14672), and

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walrus (AF321298), Two sequences of SMSV-1 (U15301 and U14676), called here SMSV-1A and SMSV-1B, and of SMSV-4 (U15302 and U14674), called here SMSV-4A and SMSV-4B, were included.

GenBank numbers of sequences utilized or determined in this study include: for the 3C region riboprobe, DQ300285; for the capsid region amplicon of RT-PCR 1: sample 4, DQ300286; for the polymerase region amplicons of RT-PCR 2: sample N102, DQ300287; sample N104, DQ300288; sample 214, DQ300289; sample 298, DQ300290; sample 310, DQ300291; and sample N330, DQ300292.

Definitions

Healthy blood donors (Normal) are adults volunteering to donate blood and whose donation cleared screening tests and was accepted for donation. Most are repeat donors. Persons with elevated ALT (high ALT) are other blood donors, like the healthy donors, except their blood donation was rejected solely because of the detection of elevated serum ALT values in the blood at the time of donation. Samples from persons with infectious hepatitis (B and C) and hepatitis of unknown etiology (non-A-G hepatitis) came from an industry repository established to discover new causes of human hepatitis.

Statistical Analysis

Optical density (P-N) values were compared between groups by ANOVA and, for correlation, by regression. Difference in estimated prevalence of anti-Vesivirus antibodies among groups were assessed by the \( \chi^2 \) test. The trend of estimated prevalence among groups with increasingly greater evidence of hepatitis was assessed by the Mantel \( \chi^2 \) for the trend. \( P \)-values < 0.05 were considered significant.

RESULTS

Prevalence of Vesivirus Antibody in Different Groups

A total of 374 sera from Normal and 350 sera from high ALT blood donors were tested against both Vesivirus antigens. Results from this testing led to obtaining a set of sera from patients with hepatitis (see Materials and Methods) and retesting against the SMSV pool antigen because the estimated prevalence of antibody to both antigens in the high ALT donors was about twice that observed among the Normal blood donors (\( P < 0.05 \), Yates’ corrected \( \chi^2 \) for each comparison) and mean P-N values were significantly higher among the high ALT donors than among the Normal donors when tested against the SMSV pool antigen but not when tested against the F9 antigen (\( P < 0.05 \), Student’s \( t \)-test). The sera from all groups were recoded and tested together; the results of this combined testing are presented in Table I.

In the retesting, high ALT donors had a higher prevalence of antibody to both test antigens (21% for the SMSV pool and 14% for F9) than the Normal donors (\( P < 0.001 \) and \( P = 0.026 \), respectively). The retesting also yielded a higher mean P-N value among positive samples in the high ALT donors for antibody to the SMSV pool (\( P < 0.001 \)), but not for antibody to F9. Sera from 51 cases of clinical hepatitis had a yet higher estimated prevalence (25%) of anti-SMSV pool antibody.

### TABLE I. Anti-Vesivirus Serum Antibody Among Different Study Groups

| Groupa | SMSV antigen | No. positive/no. testedb | Mean (SD) P-N of Pos. samplesb | Feline CV antigen | No. positive/No. tested# | Mean (SD) P-N of Pos. samples |
|--------|--------------|--------------------------|-----------------------------|-------------------|--------------------------|-----------------------------|
| Normal | 44/374 (12)c,d,e,f,1 | 145 (243)h,i,j | 31/374 (8.2)k | 214 (320) |
| High ALT | 73/350 (21)c,e,1 | 241 (303)h | 48/350 (14)k | 192 (250) |
| Hepatitis cases | 13/51 (25)h | 267 (408)i | ND | ND |
| Non-A-G hepatitis | 12/41 (29)e | 278 (425)h | ND | ND |
| Associated with transfusion or dialysis | 7/15 (47)d,e,1 | 102 (65)h | ND | ND |
| Exposures source unknown | 5/26 (19) | 524 (560)h | ND | ND |
| HBV or HCV hepatitis | 1/10 (10) | 139 (—) | ND | ND |

**a**Normal** = blood donors cleared for donation by all screening procedures. **Elevated ALT** = blood donors whose units were rejected only because of elevated liver ALT; **Non-A-G hepatitis** = cases of clinical hepatitis suspected to be of infectious etiology, but not linked to any known etiologic agent of hepatitis.

**b**Statistically significant comparisons between groups are indicated by lower-case letters, as follows.

- \( P = 0.001 \)
- \( P = 0.013 \)
- \( P = 0.004 \)
- \( P = 0.001 \)
- \( P = 0.041 \)
- \( P = 0.001 \)
- \( P = 0.022 \)
- \( P = 0.034 \)
- \( P = 0.026 \)
- \( P = 0.001 \)

Tests of proportions between groups by Yates’ corrected \( \chi^2 \) or Mantel \( \chi^2 \) for the trend. Tests of means of samples reacting positively by Student’s \( t \)-test when variances were normally distributed or by the Kruskal–Wallis H test when significant differences between variances occurred [Dean et al., 1994].
and mean P-N value among positive samples. When these hepatitis cases were analyzed by subgroup, the highest prevalence (47%) of antibody to the SMSV pool was in the cases of Non-A-E hepatitis associated with transfusion or dialysis. Cases of Non-A-E hepatitis with an unknown exposure source had an estimated prevalence (19%) similar to that (21%) of the high ALT donors and cases of HBV or HCV hepatitis had an estimated prevalence (10%) similar to that of healthy donors. The increase of anti-SMSV pool prevalence along the axis “Normal donor–high ALT donor–Non-A-E hepatitis case associated with transfusion or dialysis” was highly significant ($P < 0.001, \chi^2$ for the trend). The mean P-N value of the positive samples from the Non-A-E hepatitis group was lower than that of the other groups studied ($P > 0.05$ for these comparisons). The patterns of significant differences in estimated prevalence were similar and statistically significant for a range of antibody assay cut-points, up to 0.200, although estimated prevalence declined as the cut-point increased.

**Detection of Vesivirus RNA in Human Sera**

Total RNA was extracted from 30 donor sera and tested by RT-PCR 1. The laboratory performing this testing had no prior experience with Vesivirus genomes in the facility. RT-PCR 1 generated a Vesivirus amplicon in one of these samples that was confirmed by dot-blotting (Fig. 2) and sequencing.

To reduce the possibility that a positive result for serum Vesivirus genome detection was generated by laboratory contamination at the first facility, a further 82 donor samples were tested in a second laboratory routinely performing RT-PCR and that had prior experience with Vesivirus RNA, but using newly designed primers to amplify a genomic region not previously amplified (RT-PCR 2). Ten of these 82 samples yielded amplicons of the expected size (Fig. 3).

Ten (11%) amplicons came from 91 high ALT donor samples and 1 (4.8%) amplicon from 21 Normal donor samples ($P = 0.64$, Yates’ corrected $\chi^2$). Five (15%) of the amplicons were from 34 sera that scored EIA-positive and 6 (7.7%) from 78 sera that scored EIA-negative ($P = 0.42$, Yates’ corrected $\chi^2$).

**Sequence Comparisons of the RT-PCR Amplicons**

Six amplicons from the RT-PCR 2 (polymerase region) sample set and one from the RT-PCR 1 (capsid region) sample set were successfully sequenced. Amplicons not successfully sequenced yielded a band too faint for successful direct sequencing and were not successfully cloned. Five of the six polymerase region amplicons were distinct from each other but closely related (1–6% divergence). The sixth amplicon, study number N104, was distinct from the other five polymerase region amplicons (24–38% divergence from N104). When the five similar polymerase region amplicons were compared with GenBank entries spanning the amplification region of RT-PCR 2, the two best matches were with primate Vesivirus Pan-1 (88–96% identity) and SMSV-6 (88–94%) and lower match was with SMSV-5 (84–86%).
The sixth polymerase region amplicon (N104), was closest (68% identity) to SMSV-13 and 44% identical to F9. The capsid gene sequence from RT-PCR 1 best (97% identity) matched SMSV-5.

A smaller nucleotide (113 nt) region of the six polymerase region amplicons was shared with 22 SMSV-like Vesivirus entries in GenBank (Fig. 4). The five similar polymerase region amplicons were distinct from each of the GenBank entries and diverged from published Vesivirus sequences for the most part at sites where other Vesivirus strains also differed from each other (Part A). The amplicon from donor N104 also had a Vesivirus sequence, but was distinct from other Vesivirus strains, including F9, differing from published Vesivirus most at the 5′ end of the sequence, in a manner akin to how FCV differed from SMSV-like Vesivirus strains. Strain Hom-1 derived from a skin vesicle of a previously reported case of human Vesivirus infection [Smith et al., 1998a] had a short (47–64 nt) region of overlap with the serum-derived polymerase region amplicons. In this region of overlap, nucleotide sequence identity with the polymerase region amplicons ranged from 66% to 83%.

**DISCUSSION**

In this report, the detection of antibodies to Vesivirus and of SMSV-like Vesivirus viremia in blood donors is described at a U.S. regional blood bank serving eight Northwestern states. A higher prevalence of anti-Vesivirus antibody was observed, in comparison with normal blood donors, in donors with elevated serum ALT values, and the highest prevalence, among the groups tested, in cases of clinical hepatitis of unknown etiology associated with transfusion or dialysis. In addition, Vesivirus RNA was detected in healthy and high ALT blood donors, occurring numerically but not statistically significantly more frequently in the high ALT blood donors than among the healthy donors tested. The findings of this study and the attributes of Vesivirus calicivirus strains in mammals extend the potential for Vesivirus disease in humans from a few well-described cases to a broader population.

The complementary evidence for viremia included positive results from separate laboratories testing separate sample sets, utilization of RT-PCR assays that amplified non-overlapping genomic regions, direct detection of genomic RNA by hybridization, and ampli- con sequencing that revealed non-identity of sequence compared with known Vesivirus, including strains characterized in one of the laboratories previously [Rinehart-Kim et al., 1999] and among the set of characterized amplicons themselves. These results together indicate that the positive laboratory results were not because of contamination in sample collection and handling.

The antigens utilized for detection of anti-Vesivirus antibody were purified by cesium chloride banding, which yields a homogenous population of viable particles. A pool of three SMSV strains was utilized, with each strain representing a different potential mechanism for exposure of humans to Vesivirus: SMSV-5 detected previously in a human case, SMSV-13 known to cause disease in two livestock species, and SMSV-17 recovered from edible shellfish. The separate phyloge- netic cluster within the Vesivirus genus defined by FCV was represented by a live vaccine strain widely administered to cats. The noted differences among serum groups in patterns of estimated prevalence remained across a broad range of potential assay cut-points. It would be unexpected for humans to have Vesivirus viremia, but not exhibit serologic evidence of infection.

Viremia was expected to be part of the natural history of Vesivirus infections in humans because one of two cases described had a disseminated vesicular exanthem of the hands and feet from which Vesivirus was cultivated [Smith et al., 1998a]. The Vesivirus strains causing Vesivirus viremia in this study were closest by genome sequence comparison to SMSV, marine Vesivirus. SMSV Vesivirus are widely distributed in marine and some terrestrial animals and may routinely “traffic” among these hosts, cycling from their large marine reservoirs onto land and perhaps back again. An ocean presence has been established by the isolation of virus, the presence of specific neutralizing antibodies, or by genome amplification and sequencing for 43 of the 46 known serotypes of the genus Vesivirus, including serotypes isolated initially from terrestrial hosts and named feline (FCV), primate (PCV Pan-1), bovine (BCV Bos-1), reptilian (RCV Cro-1), swine (VESV-A46-K56), mink (MCV), and human (SMSSV-5 Hom-1 and HuCV Hom-1) caliciviruses [Evermann et al., 1983; Smith et al., 1983, 1998a,b, 2002b; Seal et al., 1995; Reid et al., 1999]. Another marine Vesivirus isolated from walrus (WCV) causes hepatitis in domestic animals [Smith, 2000; Ganova-Raeva et al., 2004]. Vesivirus calici- viruses are resistant to environmental degradation; stable in aquatic substrates; multiply to high titer, with an estimated 10^13 virions released into the ocean daily by a single California gray whale (Eschrichtius gibbosus) [Smith et al., 1998b, 2004] and, in the case of FCV, have a cosmopolitan distribution [Studdert, 1978; Smith et al., 1999b]. Such attributes indicate the potential for frequent contact between a diversity of Vesivirus biotypes and hosts.

Known examples of such interaction include mussels and oysters, aquatic filter feeders that concentrate particulates, including viruses, from the water column, preserve viral viability for 60 days or more and thereby can deliver large doses of viable Vesivirus to species ingesting contaminated shellfish [Smith, 2000; Burkhart et al., 2002]. Another example is from the mid-Pacific (French Frigate Shoals), where Vesivirus-infected fingerlings of two fish genera (Aterinomorous spp. and Encrasicoelina spp.) were eaten by white tern (Gygis alba) hatchlings that developed a Vesivirus-associated blistering disease of the feet [Poet et al., 1996]. As mentioned above, whales (California gray) that can shed large numbers of Vesivirus particles per
Fig. 4. Nucleotide (A) and amino acid (B) alignment of shared sequence among Vesivirus polymerase region amplicons from blood of study subjects and from Vesivirus strains represented in GenBank. Study strain sequences shared with these GenBank entries are aligned and placed next to the strains with which they had the highest sequence identity. GenBank sequences are grouped according to similarity to each other. Five of the study strains were similar to each other and closest to chimpanzee Pan-1 and San Miguel sea lion serotype 6 strains. The other polymerase study strain also was closest to known Vesivirus, but in the pattern of sequence homology similar to feline calicivirus.
gram of feces per day also migrate thousands of miles annually between the Sea of Cortez and the Arctic Ocean [Akers et al., 1974; Smith et al., 2004]. *Vesivirus* has been recovered at titers of $10^7$ infectious virions per gram of spleen in naturally and experimentally infected opal-eye fish (*Girella nigricans*). These fish are resident along the Southern California coast [Smith et al., 1980a,b, 1981] and are a sports and commercial fish and a common food source for seals, some of which also have extensive migration cycle. Seals reproduce on land or ice, where *Vesivirus*-induced reproductive failure and death occur, where foraging scavengers can further redistribute the viruses into terrestrial ecosystems, and where exposure to *Vesivirus* likely occurred for one human case [Smith et al., 1998a].

The present findings indicate a broader potential for *Vesivirus* infection and, perhaps, illness in humans than previously recognized. The strains causing viremia and to which antibody was detected in this study are similar to the *Vesivirus* with an ocean reservoir. A finding of subclinical viremia and the detected highest seroprevalence in cases of clinical hepatitis associated with transfusion or dialysis suggest that blood exposure that may have led to hepatitis also could lead to higher exposure to *Vesivirus*. If *Vesivirus* causes hepatitis in humans, then an estimated rate for such causation can be derived from the study findings, as follows: the rate of 10 of 91 donors amplicon-positive in the serum who also had elevated serum liver transaminases (ALT) values, together with the rate of high ALT values occurring in about 1 in 1,000 blood donors, would correspond to a rate of $\sim 1$ in 10,000 blood donors who might have active, subclinical *Vesivirus* hepatitis. The association of higher anti-*Vesivirus* antibody prevalence with clinical hepatitis of unknown etiology would require further study. The finding of *Vesivirus* viremia in otherwise normal blood donors indicates that blood exposure to caliciviruses of the genus *Vesivirus* could occur by multiple routes of exposure. The diversity of host species, mechanisms of exposure and tissue tropisms for *Vesivirus* with the findings of this study suggest additional *Vesivirus* disease manifestations might be found in humans with further investigation.

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*B*  

| *Vesivirus* Strain | Amino Acid Sequence |
|-------------------|---------------------|
| Pan-1             | PLISSVPKV FTNLKQGKLK PTRTDKDAE ITPIPAD |
| Study N330        |                     |
| Study N102        |                     |
| Study N102        |                     |
| Study N102        |                     |
| Study 310         | R-                  |
| Study 214         | A-                  |
| Study 298         | A-                  |
| SMSV-6            |                     |
| VESV E54          |                     |
| VESV A48          |                     |
| SMSV-4A           |                     |
| skunk 4-2S        |                     |
| SMSV-4B           |                     |
| VESV 1934b        |                     |
| skunk 4-1L        |                     |
| skunk 7-2 & 3L    |                     |
| SMSV-1A           |                     |
| SMSV-2            |                     |
| SMSV-1B           |                     |
| SMSV-5            |                     |
| walrus            |                     |
| SMSV-7            |                     |
| rabbit            |                     |
| VESV C52          |                     |
| bovine            |                     |
| VESV B5           |                     |
| SMSV-14           |                     |
| SMSV-13           |                     |
| Study N104        | -EAHCSRA-- K-G-E-- M-- |
| feline F9         | IMYA-ISDQI G-SSY--- SVGA R--DP- |

Fig. 4. (Continued)
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