We report that a calicivirus of oceanic origin, San Miguel sea lion virus serotype 5 (SMSV-5), is a human pathogen. This biotype was isolated originally from blisters on the flippers of northern fur seals (Callorhinus ursinus) and replicates readily in primate and human cell lines. It infects a phylogenetically diverse array of hosts (poikilotherms to primates) and induces type-specific neutralizing antibodies in exposed humans. Group antibody against a pooled antigen of SMSV-5 and two other serotypes was also observed in 18% of 300 blood donors from a population in the northwestern United States. The human calicivirus isolate designated SMSV-5 Homosapien-1 (SMSV-5 Hom-1) was recovered from a laboratory worker with systemic illness, including vesicular lesions on all four extremities. We believe this newly described human disease represents a paradigmatic shift in calicivirus disease recognition.

Serological evidence can suggest that animal viruses are also human pathogens [1, 2], and occasionally this is confirmed by a single case, as we report herein and as was reported with regard to Cache Valley virus [3]. At other times, with no prior warning, a single case signals the arrival of a new zoonotic disease, as occurred with the death of a horse trainer in Australia infected with a previously unknown morbillivirus [4]. Such events can occur when shifts in ecologic relationships cause intermixing of species and result in increased ‘‘viral traffic’’ [5]. In turn, ‘‘viral traffic’’ encourages adaptive shifts in viral genomes and host-parasite relationships, which can result in emerging new diseases, especially zoonoses [5]. ‘‘Viral traffic’’ for one calicivirus was deliberately increased in Australia where a recently recognized (1984) hemorrhagic virus of rabbits [6–8] has been spread as a biological control agent, resulting in a continent-wide calicivirus challenge of naive and diverse populations.

The early detection of and appropriate responses to emerging viral diseases that may result from this or similar events that heighten ‘‘viral traffic’’ will require preventive programs involving research, early diagnosis, public health awareness, and reporting. In this context, the caliciviral traffic between oceanic hosts and humans that we report on herein, with regard to a laboratory worker’s illness caused by a San Miguel sea lion calicivirus (SMSV), provides an example of a new disease manifestation and observation. To provide further evidence of probable human exposure and infection with this group of pathogenic caliciviruses, we also report the serum reactivity to group SMSV antigens in blood donors living along the Pacific coast of North America.

Case Report

A 32-year-old male researcher developed a flu-like illness that was followed in 2 days by an initial blister on the right index finger. Within 12 hours, deep, painful blisters (not unlike those in hand, foot, and mouth disease caused by some enteroviruses) [11] appeared on the palms and fingers of both hands (figure 1), and by 24 hours they appeared on the soles and toes of both feet. In all, several dozen lesions occurred, ranging from small, red, raised areas to fluid-filled blisters 1 cm in diameter. Healing commenced within 1 week and was essentially complete in 2 weeks. The prototype San Miguel sea lion virus serotype 5 (SMSV-5) calicivirus was isolated from similar lesions on the flippers of northern fur seals (figure 2).

Virus particles having the morphological characteristics typical of the Caliciviridae were visualized by negative-contrast electron microscopy in the aspirated vesicular fluid [12] (figure...
3), and a calicivirus was isolated in Vero cells. Comparisons of reverse transcriptase PCR (RT-PCR) amplicons of SMSV-5 strain 205 [1] and the virus isolated from the hand lesions revealed that these were of the same genotype (figure 4) [11]. The human isolate, designated strain SMSV-5 Homosapien-1 (SMSV-5 Hom-1), was neutralized by typing antiserum to SMSV-5 but was not neutralized by typing antisera to 39 other calicivirus serotypes.

The infected individual developed type-specific neutralizing antibodies measured with use of 100 tissue-culture-infective doses (TCID$_{50}$) of prototype SMSV-5 of test virus as follows. The preexposure serum was negative at a 1:4 serum dilution. On day 4 postinfection (PI), the neutralization titer was 1:10, and it was 1:80 on days 28 and 180 PI. Convalescent serum (day 28 PI) immunoprecipitated the single capsid protein (60 kD) of the prototype SMSV-5 and SMSV-5 Hom-1 but not proteins in mock-infected Vero cells. SMSV-5 typing serum also immunoprecipitated the capsid protein of both virus stocks (figure not shown). In addition, we previously reported findings of neutralizing antibody in humans [1, 2].

**Methods**

*Electron microscopy.* A blister on the patient’s hand was cleansed with alcohol, and vesicular fluid (~25 μL) was aspirated and placed into 1 mL of sterile distilled water. The sample was vortexed and clarified for 10 minutes at 850g. The resulting supernatant was transferred to a new tube and centrifuged 20 minutes at 15,000g. The pellet was resuspended in 10 μL of distilled water and transferred to parafilm. A carbon-coated, 300-mesh copper grid was floated on the drop for 2 minutes, blotted dry with filter paper, stained with phosphotungstic acid, and examined at an accelerating voltage of 80 kilovolt with use of a Phillips 300 transmission electron microscope. Subsequent to electron microscopic examination, the remainder of the sample was processed for virus isolation.

*Virus propagation.* Isolation was carried out in Vero cell monolayers inoculated with 0.2 mL of a 1:10 sterile distilled water dilution of vesicular fluid aspirate and then incubated and examined for cytopathic effect; isolates were plaque-passaged three times, as previously described [1]. For comparisons, the clinical human isolates and prototype SMSV-5 strain 205 originally isolated from an ill pinniped [1] were used for agar gel electrophoresis (SDS-PAGE) capsid protein comparisons. Vero cells were inoculated with a multiplicity of infection (MOI)
>10 of the respective viruses, incubated for 1 hour at 37°C, and then rinsed and fed with minimum essential media (MEM) plus 1% fetal bovine sera (FBS) and incubated at 37°C.

At 3.5 hours postinoculation, the medium was removed and the cells were rinsed once with MEM and then fed for 30 minutes with methionine-free MEM containing actinomycin D (2 μg/mL). At 4 hours postinoculation, fresh methionine-free medium with 35S-methionine (25 μCi/mL) was added, and uptake of labeled methionine was allowed to continue for 30 minutes. The cells then were rinsed once with 0.5 M of ice-

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**Figure 4.** Comparisons of the nucleotide and predicted amino acid sequences of prototype San Miguel sea lion virus serotype 5 (SMSV-5), pri
dicate calicivirus (PCV Pan-1), and SMSV-5 Homosapien-1 (SMSV-5 Hom-1) in the RNA polymerase gene region. SMSV-5 Hom-1 has 97% nucleotide and 96% amino acid identity with the prototype SMSV-5 and 85% nucleotide and 92% amino acid sequence identity with PCV Pan-1 in this most highly conserved region of calicivirus genomes. These differences are consistent with the designation of SMSV-5 Hom-1 as a strain of SMSV-5 [11]. The following GenBank accession numbers have been assigned to these sequences: SMSV-5 [U52093], SMSV-5 Hom-1 [U623227], and PCV Pan-1 [U52086].
cold Tris-HCl (pH, 7.4) and samples were collected with lysis buffer (0.05 M of Tris-HCl and 2% SDS [pH, 7.4]). Samples were processed for immunoprecipitation and were electrophoresed under reducing conditions.

Serum neutralization. Neutralization for typing and to determine antibody titers in patient sera was completed with use of Vero cells in 96-well plates and replicates of 4 wells with twofold dilution of test serum against 100 TCID$_{50}$ of virus [1]. The human isolate was typed with use of 20 antibody units of heterologous typing serum from each of 40 calicivirus types, including SMSV-5 [1].

ELISA for antibody to SMSVs. Assays were completed with use of a 96-well microtiter plate (#76-371-04; ICN Biomedicals, Horsham, PA). The test antigen consisted of a 100-µL pool of three CsCl-banded caliciviruses (SMSV-5, 13, and 17) in 0.1 M of carbonate buffer (pH, 7.6). The test antigen was adsorbed to the plate for 2 hours at 37°C and then washed twice with Tris-buffered saline containing Tween 20 (TBST) and blocked overnight at 4°C with TBST containing 0.25% BSA. After two TBST washes, each serum (diluted 1:100 in blocking buffer) was added for 2 hours at 37°C and then washed six times with TBST.

Next, human antibody IgG–alkaline phosphatase (Sigma Chemical, St. Louis), diluted 1:40,000 in blocking buffer, was added and incubated for 2 hours at 37°C and then the wells were washed six times with TBST and twice with TBS without Tween. The chromogenic substrate pnpp (Sigma) was added (200 µL per well at 1 mg/mL) and incubated overnight at 37°C. Optical densities (ODs) were read at 405 nm on an ELISA plate reader (TiterTek Multiskan, ICN Biomedicals, Costa Mesa, CA). The OD value used was the test OD value minus the OD of a serum control well. The OD values of control wells were ≤0.005, and most were 0.000. Values above an OD of 0.2 were called positive.

RT-PCR amplicon generation. Amplicons were generated by RT-PCR using primers Pr35 and Pr36 based upon the primate calicivirus (PCV Pan-1) sequence [9, 10]. RT-PCR products were cloned into pGem-T (Promega, Madison, WI) and sequenced with use of “forward” and “reverse” primers and dideoxy chain termination.

Results and Discussion

To determine whether an unselected adult population had been infected with SMSVs in the past, we measured serum reactivity by ELISA with use of a calicivirus group antigen test. Eighteen percent of 300 sera collected from adults donating blood in Portland, Oregon, during a 6-month period in 1996 had reactivity (OD value >0.20, or 8% at OD >0.50) in an ELISA using as test antigen three CsCl-banded marine calicivirus serotypes, including SMSV-5 (figure 5). Two of two ELISA-positive samples (OD values, 0.884 and 1.690) were confirmed to have antibody to the 60-kD capsid protein of a marine calicivirus (strain McAll) by western blot analysis, while one ELISA-negative sample (OD, 0.011) was also negative by western blotting.

Fourteen serum samples (paired samples, acute and convalescent) from seven individuals with confirmed diagnoses of Norwalk gastroenteritis did not test positive against the SMSV group antigen, and the OD readings of convalescent vs. acute sera showed no detectable rise (data not shown). For these reasons we believe the reactivity to SMSV antigen cannot be discounted, as cross-reactivity to infection from other human caliciviruses of the Norwalk group and ELISA reactivity to our calicivirus antigen could be eliminated by preadsorbing the sera with calicivirus antigen.

SVM-5 was recovered originally in 1973 from a vesicle on the flipper of a severely affected northern fur seal (Callorhinus ursinus) necropsied on St. Paul Island, Alaska [1] (figure 2). Altogether, more than 21 other calicivirus serotypes have been isolated from marine mammals, and some of these also have been isolated from domestic livestock [13]. Further evidence of “viral traffic” between marine and terrestrial species is shown in terrestrial animals by the presence of type-specific serum antibodies to a variety of marine calicivirus serotypes [13].

In retrospect, caliciviruses circulating in the 1930s, 1940s, and 1950s in southern California’s marine populations are believed to have been the origins of the historic nationwide epizootic of vesicular exanthema of swine (VES) [13, 14]. The feeding of raw or poorly cooked fish products is the recognized mechanism whereby these “ocean” caliciviruses were introduced into terrestrial mammals [13]. Once VES was established in swine, then pork scraps from these infected pigs were discarded into garbage and fed to others, thereby initiating a devastating pig-to-pig disease cycle [13, 14]. These historical events
involving caliciviruses of oceanic origin are not unlike the current bovine spongiform encephalopathy (BSE) cycle in which cattle are thought to have been originally infected by some other species (scrapie in sheep) and then bovine tissues containing the BSE agent were fed to cows, resulting in an epidemic of BSE among cattle [15].

An animal calicivirus of oceanic origin, having ocean reservoirs involving cycles through marine invertebrates, poikilotherms, and mammals, now is shown to be a human pathogen. This is a new paradigm for disease occurrence, and the history of this previously unknown zoonotic virus cycle is as follows. Caliciviruses of oceanic origin were first isolated in primate and human cell lines [1], exposed laboratory workers developed type-specific neutralizing antibodies [2, 13], and the host range of single serotypes included species as phylogenetically diverse as fish, seals, swine, and primates, therefore suggesting host nonspecificity that could extend to humans [13].

Three serotypes (SMSV-4, SMSV-5, and VESV-C52) induced vesicular lesions at inoculation sites in monkeys [2]. PCV Pan-1 is in the marine calicivirus genogroup and was recovered from five species of nonhuman primates, including two species of great apes (pygmy chimpanzee Pan paniscus and lowland gorilla Gorilla gorilla) [10, 13, 16, 17]. In one species (pygmy chimpanzee), the virus was recovered from a herpesvirus-like lip lesion and was excreted in oropharyngeal secretions for at least 6 months [10]. Another PCV Pan-1 isolate was recovered from the brain of a baby douc langur (Pygathrix nemaeus) that died with encephalitis [16].

There have been occasional field reports of unusual blistering skin infections among wildlife biologists working with pinnipeds (personal communication, Robert L. DeLong, National Marine Mammal Laboratory, Sand Point, Seattle, 1994). One individual who handled diseased Steller’s sea lions (Eumetopias jubatus) in the Bering Sea required emergency medical treatment for deep, painful blistering of the hard palate, upper lip, and facial area. Although the attending physician made a clinical diagnosis of herpesvirus infection, throat washings sampled 30 days post-onset produced a calicivirus isolate (currently designated strain McAll). This virus was not neutralized by the 40 different type-specific antisera available at the Laboratory for Calicivirus Studies and will be designated a new calicivirus type. Serum samples were collected at 30, 45, and 60 days post-onset. Only the 30-day sample was positive by ELISA with use of the McAll virus as antigen. Preliminary sequence data indicate this isolate is in the same genogroup as the SMSVs of oceanic origin (unpublished data of Smith AW, Skilling DE, Matson DO, and Berke T), thereby providing evidence of a second, although not fully documented, case of zoonotic calicivirus disease.

SMSV-5 is one of 40 cultivatable calicivirus serotypes. Subtypes include additional marine and closely related strains isolated from terrestrial animals. All appear to share a common genomic organization [18]. Many serotypes replicate in feline, canine, pig, primate, and human cell lines [19], and their similar genomic organization, close sequence identity, and common replicative properties in host animals and cell cultures suggest that SMSV-5 is only one of many calicivirus serotypes in the marine genogroup that could be expected to infect humans if exposure occurs [2]. In fact, the untyped SMSV isolate recovered from the biologist and designated strain McAll, the high levels of neutralizing antibody to serotype SMSV-4 in humans, and the presence of antibodies to SMSVs in blood donors living in the Pacific Rim are three additional pieces of evidence that suggest that this has occurred [2].

This communication provides the first description of human disease produced by a calicivirus of oceanic origin. The apparent viremia, with dissemination of virus to multiple sites of replication on all four extremities, mimics the disease observed in pinnipeds and swine infected with the same and related serotypes [1, 13, 14, 20]. Pneumonitis, myocarditis, encephalitis, hemorrhagic disease or disseminated intravascular coagulation, gastroenteritis, hepatitis, blistering, and reproductive disorders with secondary and cross-species transmission, involving birds, reptiles, amphibians, primates, and other mammals, have been described in association with calicivirus infections in animals [2, 13, 19–22]. In humans, caliciviruses are known to cause gastroenteritis and hepatitis and now blistering [23–25]; however, improved diagnostic methods and an understanding of this newly recognized virus cycle should provide insight for the future diagnosis of human diseases for which an etiology is currently unknown.

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References