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Parvoviruses PARV4/5 in Hepatitis C Virus- infected Persons

To the Editor: Parvoviruses are small, nonenveloped DNA viruses that infect both vertebrate and invertebrate hosts. Until recently, parvovirus B19 and adeno-associated viruses, which belong to the genera *Erythrovirus* and *Dependovirus*, respectively, were the only known members of the family *Parvoviridae* that infected humans (1). However, 2 recent publications have identified 2 distinct, novel parvoviruses in humans by using the DNase sequence-independent single-primer amplification technique and a related method (2,3). The first of these viruses, termed PARV4, was observed in a patient with symptoms of acute viral infection syndrome after high-risk behavior for infection with HIV-1, although the patient was subsequently confirmed as negative for HIV-1 (2). The second parvovirus was identified in respiratory samples from children

with lower respiratory tract infections and termed human bocavirus (3).

Parvovirus B19 is a frequent contaminant of plasma pools that are used in the manufacture of blood products, which results in high viral loads in pools and viral transmission in recipients of clotting factors (4). We identified PARV4 in such pools (5), albeit at a lower frequency and titer than parvovirus B19, when parvovirus B19 was not excluded by screening with nucleic acid amplification techniques. Sequence analysis identified a second genotype of PARV4, which we have termed PARV5, that shares 92% nucleotide identity with PARV4 (5).

PARV4 was originally identified in a plasma sample from a homeless, injection drug user with fatigue, night sweats, pharyngitis, neck stiffness, vomiting, diarrhea, arthralgia, and confusion (2). This person was coinfecting with hepatitis B virus. In this study, we looked retrospectively for PARV4 and PARV5 in blood samples from a similar cohort of persons, many of whom were known to be infected with hepatitis C virus (HCV) (as determined by the presence of both HCV RNA and antibodies to HCV), and some of whom were intravenous drug users (IVDUs) (6).

Blood samples were collected from 26 cadavers in London and the surrounding area as part of a study to investigate the inhibition of nucleic acid amplification techniques for bloodborne viruses in tissue samples (6). The cohort was composed of 10 HCV RNA-positive IVDUs, 8 HCV RNA-positive non-IVDUs, 4 HCV RNA-negative IVDUs, and 4 HCV RNA-negative non-IVDUs (Table). Nucleic acid was extracted as previously described (4) by using the

MagNA Pure LC instrument (Roche, Basel, Switzerland). PCR was performed with primers specific for the second open reading frame (ORF2) in the PARV4 genome (2), which is homologous to the VP1 capsid of parvovirus B19. Primers PVORF2F (5'-AGGAGCAGCAAACAACTCAGAC-3') and PVORF2R (5'-TCCTTCATCGCGGCTGTCACTAA-3') amplify a 268-bp region of ORF2 (nucleotides 2710–2977, GenBank accession no. AY622943). The PCRs were performed and analyzed as previously described (5). The assay is highly specific (no cross-reactivity with parvovirus B19) and sensitive (detects 5–10 copies of PARV4 virus DNA per reaction).

PCR products were cloned, sequenced, and compared with the prototype PARV4. Two blood samples were positive for PARV4, and a third sample was positive for PARV5, with 99%–100% nucleotide identity. These positive samples were from HCV RNA-positive IVDUs (Table). The titer of PARV4 and PARV5 DNA in the positive samples was low and did not exceed >700 copies/mL of plasma, as determined by using a consensus TaqMan assay (J. Fryer, unpub. data). None of the other blood samples tested was positive for PARV4 and PARV5, including those for persons who were HCV RNA negative and not IVDUs.

In our previous study (5) of >130 fractionation pools (composed of thousands of units from screened healthy donors) for PARV4, the only positive pools were from North America and no European pools were positive for PARV4 or PARV5. These viruses may be present in such pools but diluted to undetectable levels. In

Table. Analysis of 26 cadavers for parvoviruses PARV4 and PARV5*

Group	No. positive/no. tested	
	PARV4 and PARV5 in HCV RNA-positive cadavers	PARV4 and PARV5 in HCV RNA-negative cadavers
IVDUs	3/10	0/4
Non-IVDUs	0/8	0/4

*HCV, hepatitis C virus; IVDUs, intravenous drug users.

the present study, PARV4 and PARV5 have been identified in blood samples obtained from persons from the United Kingdom. For parvovirus B19, there is evidence of persistent virus infection, at low levels, in bone marrow of previously exposed persons (7) and in plasma of immunocompromised and immunocompetent persons (8,9). There is also evidence for the lifelong persistence of parvovirus B19 (genotypes 1 and 2) in tissues such as skin and synovia (10). PARV4 and PARV5 virus genomes share only limited homology with parvovirus B19 (<30% amino acid similarity). Although they have been detected in blood and plasma, nothing is known about the role of these viruses in human disease or their ability to persist in infected persons, healthy or otherwise. Further studies will be required to determine the prevalence of PARV4 and PARV5 in healthy persons compared with its prevalence in those with chronic infections and at high risk, such as IVDUs, and to investigate the nature of persistence of these novel viruses.

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Saint Louis Encephalitis Virus, Brazil

To the Editor: Saint Louis encephalitis virus (SLEV), a member of the *Flaviviridae* family, is widely dispersed in the Americas (1,2). In Brazil, SLEV was first isolated in the 1960s from a pool of mosquitoes at the

Amazon Basin. Subsequently, the virus was repeatedly isolated from animals and arthropods in the Amazon region and São Paulo state (3). Nonetheless, isolation of SLEV from humans is rare; only 2 isolates from humans were described before 2005. Each isolate was from a patient who had jaundice and febrile illness without any neurologic symptoms (1,3). Recently in São Paulo, SLEV was isolated from a patient who had an incorrect diagnosis of dengue fever (2,4).

Despite the rare isolation of SLEV from humans, antibodies to this virus have been found in ≈5% of studied populations in the north and southeast regions of Brazil. However, because of antibody cross-reactivity among different flaviviruses and the fact that this population is vaccinated against yellow fever and exposed to dengue virus (DENV), such results should be interpreted carefully. Nevertheless, in these areas, SLEV may circulate and infect humans, although most infections are undiagnosed (1,3,5).

In contrast to previous instances in which the disease was detected in only 1 patient, we describe the first community outbreak of SLEV in Brazil. The outbreak was detected in São José do Rio Preto (population 400,000), in northwest São Paulo state. This outbreak was concurrent with a large outbreak of DENV serotype 3 (DENV-3), which occurred during the first half of 2006, with >15,000 possible cases reported to public health authorities. During this time, we were involved in an epidemiologic study to monitor the disease. We tested ≈250 samples for DENV, and 65% were positive. We tested for SLEV only those patients who were in our hospital or those who were referred to us for SLEV testing after an initial diagnosis of SLEV or DENV. The protocol approved by our ethical committee allowed us to test only samples from these patients (process no. 300/2004).