

Parvovirus 4 in Blood Donors, France

To the Editor: In the past few years, several novel parvoviruses have been identified, including human parvovirus B19-related strains V9 and A6, and bocavirus. In 2005, parvovirus 4 (PARV4), a new putative member of the family *Parvoviridae*, was identified in the plasma of a patient in North America who had an acute virus infection (1). This virus had limited sequence homology with parvovirus B19 (<30% aa similarity) despite a conserved genomic organization showing 2 large nonoverlapping open reading frames (ORF). Phylogenetic studies performed with near-complete sequences have proposed that human PARV4 can be described by ≥ 3 genogroups (2).

Little information is available about the natural history of the virus, and its clinical role remains unknown. Initial studies showed that PARV4 was present in the blood of febrile patients, intravenous drug users, and persons positive for hepatitis C virus or HIV at prevalences of 6%–30% (3–5). Two recent studies also showed the virus in cohorts of kidney transplant patients (6,7). PARV4 has also been identified in persons without apparent pathologic changes, such as blood donors (1.0%–3.95%), and in blood products negative for parvovirus B19 DNA (4–6). Virus DNA also was found in bone marrow and various tissues or organs, suggesting possible dispersion of the virus in diverse biologic locations (8).

To provide new insights into the dispersion of PARV4 in healthy persons, we assessed the frequency of PARV4 viremia in a cohort of blood donors by using a dual real-time assay. A total of 304 volunteer blood donors (154 men; mean age of all volunteers 40 years) living in south-eastern France entered the study. The sex ratio (men:women: 1.03) and age

distribution (19–65 years) of the cohort were considered representative of the population of blood donors at that time. Blood samples were collected in vacuum tubes (Vacutainer, SST, Becton Dickinson, Meylan, France) and centrifuged, and 1-mL plasma aliquots were stored at -80°C until use. Nucleic acids were extracted as described previously (7) and tested for PARV4 genomic DNA immediately after extraction to avoid freezing/thawing cycles.

Five microliters of the eluted material served for PARV4 DNA detection using real-time PCR TaqMan methods (StepOne Plus, Applied Biosystems, Courtaboeuf, France) and primers (ORF2) compatible with the detection of the 3 virus genogroups described at this time (2,4,7). Two probes were tested in separate amplification assays: PARV4-O (5'-FAM-TGTTCAACTTTCTCAGGTCCTACCGCCC-TAMRA-3') (4,7) and PARV4-N (5'-FAM-TCCTACYGCCSCCTCCTCCTTCTT-TAMRA-3'). The second primer was designed after identification and sequencing of several in-house real-time PCR products (GenBank accession nos. FJ883557–61), and more particularly those detectable on agarose gels but showing

negative signal with PARV4-O TaqMan assay because of mismatches identified on the probe recognition site (Figure). Amplification reactions were performed as described previously (7). Both TaqMan assays were estimated to detect as few as 10 copies of PARV4 DNA per reaction using dilutions of a synthetic template (7).

Fifteen (4.9%) blood donors gave positive signal with probe PARV4-O; 62 (20.4%) were positive with probe PARV4-N; 6 (2.0%) samples were positive in both assays. Overall prevalence for PARV4 DNA was 24.0% in the blood donors tested.

PARV4 origin of randomly selected PCR products from PARV4-O and PARV4-N TaqMan assays (5 each) was confirmed after cloning and M13 sequencing. Point mutations located on the PARV4-O recognition site were retrieved in amplicons originating from samples positive with PARV4-N assay (Figure). However, subsequent molecular studies aiming to characterize virus strains were not feasible because of the low titer of PARV4 DNA (<500 copies/mL of plasma) in positive samples. No specific correlation was identified between sex or geographic origin and PARV4 viremia, whereas analysis of the distribution of PARV4-positive

A

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TGTTCAACTTTCTCAGGTCCTACCGCCC PARV4-O
FJ883557 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
FJ883558 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
FJ883559 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
FJ883560 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
FJ883561 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
PARV4 ref gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
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TCCTACYGCCSCCTCCTCCTTCTT PARV4-N

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B

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TGTTCAACTTTCTCAGGTCCTACCGCCC PARV4-O
DS33 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
DS36 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
DS43 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
DS72 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
DS120 gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
PARV4 ref gtgatattgctGATGTTCAACTTTCTCAGGTCCTACCGCCCCTCCTCCTTCTTCGGACCAACAACCGgcttattccgc
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TCCTACYGCCSCCTCCTCCTTCTT PARV4-N

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Figure. Alignment of parvovirus 4 (PARV4) sequences showing the location of the 2 probes used in the real-time experiments. A) Partial sequences used for the design of probe PARV4-N: PARV4 prototype isolate (AY622943) and in-house PCR products characterized initially (GenBank accession nos. FJ883557–61). B) Examples of point mutations located on the PARV4-O recognition site identified in amplicons originating from samples positive with PARV4-N assay. Mismatches identified in the alignments are underlined. Nucleotides shown in lowercase letters correspond to 5'/3' ends of the real-time primers. Mismatches identified in the alignments are underlined and in **boldface**.

samples in age groups highlighted a relative homogeneity throughout the corresponding cohort.

Our study shows that PARV4 infection is readily detectable in French blood donors. Prevalence results using probe PARV4-O were comparable to those obtained in previous studies involving healthy persons originating from various countries (4–6). Conversely, the high prevalence obtained by using probe PARV4-N was unexpected because only 1 study demonstrated a higher value (45.7%) after the investigation of PARV4 DNA in bone marrow aspirates of AIDS patients from Italy (9).

This finding suggests a larger dispersion of PARV4 than expected initially in the general population and highlights the need for improvement in detection systems directed toward PARV4 DNA, particularly by interlaboratory collaborations, in direct connection with studies investigating PARV4 genetic diversity. These considerations are consistent with the recent description of a new PARV4 genogroup in humans and characterization of highly divergent variants in bovine and porcine species (10). In addition, such data raise the question of the consequent persistence of PARV4 infection in healthy persons. Future studies need to explore both dispersion and potential clinical impact of PARV4 on infected hosts.

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Otomastoiditis Caused by *Mycobacterium abscessus*, the Netherlands

To the Editor: Nontuberculous mycobacteria (NTM) are increasingly recognized as human pathogens (1). Otomastoiditis is a rare extrapulmonary NTM disease type first described in 1976; *Mycobacterium chelonae*–*M. abscessus* group bacteria, which are rapidly growing NTM, are the most frequent causative agents and the disease mostly affects children (1–3). In the Netherlands, *M. chelonae*–*M. abscessus* group isolates have been reported from the otologic samples of an average of 2 patients annually since 2006, as compared to 6 patients in the preceding 10 years. This emergence is not a likely result of improved laboratory facilities or awareness in clinicians because liquid culture and molecular identification techniques predate the rise in notification and Dutch guidelines advise against performing cultures for chronic otorrhea.

We identified 10 patients from the national reference laboratory database with otologic samples yielding *M. chelonae*–*M. abscessus* group bacteria during January 1995–June 2007. We resubjected these isolates to molecular identification by *rpoB* gene sequencing (4) and performed a medical file review.