High prevalence of TT virus in a rural community of South Africa

To the Editor: The development of new molecular techniques in recent years has resulted in the discovery of a number of novel viruses. Hepatitis C virus (HCV) was the first to be discovered in 1989 using immunoscreening methods[1] and since 1995 a further five new viruses have been identified using the same or modified technologies: GB virus-C / hepatitis G virus (GBV-C / HGV),[2,3] human herpesvirus 8 (HHV8),[4] TT virus (TTV),[5] SEN virus (SEN-V)[6] and human metapneumovirus.[7] In each case the prevalence of the virus in different communities, its association with disease and therefore its relevance in the hospital and blood transfusion settings needed to be established.

These new techniques have the potential to find viruses that have no association with disease. The determination of a disease association or lack thereof has major implications for the health care sector, and particularly the blood transfusion services. For example, confirmation that the majority of non-A non-B post-transfusion hepatitis cases were associated with HCV infection has resulted in screening of blood donors and a subsequent dramatic drop in the number of transfusion-association hepatitis cases.[8] On the other hand, GBV-C / HGV was initially thought to play a role in liver disease but this has been disproved and to date the virus has not been consistently associated with any other disease.[9] Thus no new diagnostic interventions were required in hospitals or in the blood transfusion services.

The following are other examples of recently discovered viruses. HHV8 has been causally associated with Kaposi's sarcoma and other neoplasms in immunodeficient patients and although transmission via organ transplantation has been demonstrated, it is not transmitted by blood transfusion.[10] Recent studies[6] have suggested that certain genotypes of SEN virus may play a role in non A-E hepatitis, but this has not been confirmed. Clarification of this association is critical as multiply transfused patients are at greater risk of acquiring the virus. Human metapneumovirus, the most recently discovered virus, causes a range of upper respiratory tract diseases varying from severe bronchiolitis to pneumonia in both children and adults.[7]

When TTV, a small non-enveloped DNAvirus isolated from a patient with non A-G post-transfusion hepatitis, was originally described it was thought to play a role in the development of fulminant hepatitis and acute and chronic liver disease of unknown aetiology.[11] However, evidence of the high prevalence of TTV infection in healthy blood donors and the lack of an evident epidemiological link to disease now suggests that the role it may play in liver disease in uncertain. The prevalence in blood donor groups varies from 1.9% to 83%[12,13] depending on the geographical area studied and the region of the viral genome amplified by the polymerase chain reaction (PCR). The early TTV PCR studies underestimated the true prevalence of infection in different population groups because little was known about the genetic variation of the virus. However, by targeting a more conserved region of the TTV genome detection has improved, with more sensitive and reliable results.[14,15]

Sequence analysis of full-length and partial sequences of TTV from around the world indicates that the virus can be classified into six different genotypes (G1 - G6) with a sequence divergence of > 30%.[16] The most commonly recognised genotypes worldwide are G1, G2 and G3, although the latter has yet to be found in Africa.

This study was undertaken to determine the true prevalence of TTV infection in a rural community in South Africa using three different PCR primer sets and to identify the genotypes circulating within this population.

Blood samples were obtained from 75 consenting adults living in the Eastern Cape, as previously described.[17] All participants lived in traditional mud houses with no access to an indoor water supply or waterborne sewerage.

DNAwas extracted from serum and amplified by PCR with three independent primer sets targeting different regions of the TTV genome to assess which would detect virus most sensitively.[13-15] The PCR products amplified from the N22 region of open reading frame 1 (ORF1) were cloned, sequenced and aligned with known sequences obtained from the GenBank database. A phylogenetic tree was constructed to demonstrate the genetic relatedness, using the neighbour-joining method.

The TTV prevalence varied from 17% to 84% depending on the primer set used for genome amplification. The most sensitive PCR assays were those using primers designed to amplify the conserved 5' non-coding region (5'NCR) of the genome. Of the 75 samples tested, 15 (20%) were positive in all three systems and 10 (13%) negative.

This study confirms the lack of sensitivity of the PCR system which targets the N22 region of ORF1 as originally described by Nishizawa *et al.*,[5] and is certainly due to the high genetic variability in this region of the genome between different viral genotypes. Enhanced sensitivity can been achieved by amplifying the conserved 5'NCR and in this study, depending on the primer set used, 55% or 84% of rural adults showed evidence of TTV infection compared with 17% using primers to the N22 region.

The high prevalence of TTV infection (84%) in this South African rural community is comparable with figures from other

healthy rural populations in Africa (Tanzania (74%), Gambia (83%), Ghana (88%) and Egypt (85%)) and in developed countries around the world (34 - 90%).[12] This suggests that routes other than the parenteral route must play a role in TTV transmission. Evidence for the presence of TTV in faeces[18] and saliva[19] indicates that horizontal transmission via the faecal-oral route and aerosols may be important. In addition, domestic animals have also been shown to harbour the TT virus[14] and this could be another source for human infections, although at present there are no data available on TTV infections in domestic animals from Africa.

Phylogenetic analysis showed that of the 15 sequences analysed, 14 grouped with G1 and only one with G2 (Fig. 1), thereby confirming the worldwide distribution of these two genotypes. This study also confirmed previous reports that there is no evidence for the presence of genotype 3 in Africa, but this may be due to the low number of isolates reported from this continent.

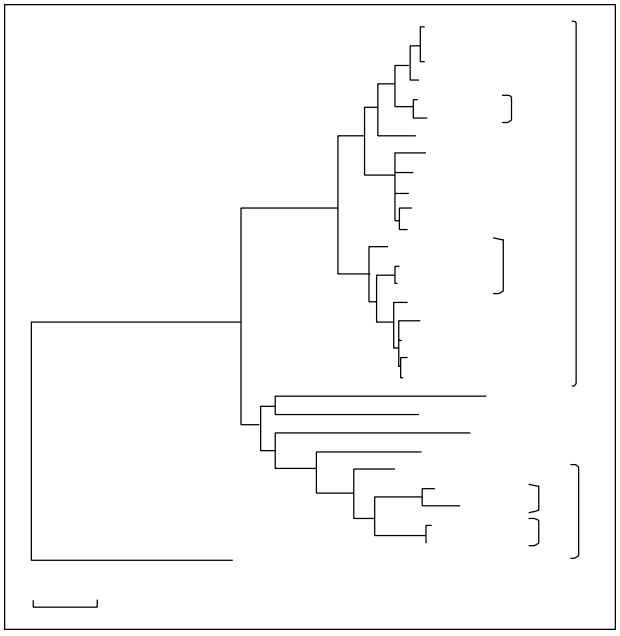

Fig. 1. Phylogenetic tree generated by neighbour-joining analysis of the N22 region of ORF 1. Bootstrap values of greater than 75% are indicated on the nodes of the tree. The branch lengths are proportional to the evolutionary distances as shown on the scale. Sequences from this study are represented in bold type. Representative sequences of the different genotypes (indicated by accession numbers) including a divergent TTV isolate (PMV), used as the outgroup, were obtained from GenBank.

This is a new paradigm, where discovered viruses have no obvious disease association, and are only found because of the use of molecular techniques. As a result of these findings, we need to be cautious in interpreting them and be meticulous in the epidemiological links that are made.However, these novel techniques also open the window for many potential microbes to be discovered which do cause diseases for which no aetiological agent is evident.

Studies like this need to be undertaken around the world in both healthy populations and in patients suffering from a variety of diseases to establish global prevalence rates and to ascertain any possible disease association. This can then be used to assess the relevance in the blood transfusion setting. The high prevalence of TTV in healthy populations in this and other studies currently suggests that TTV is non-pathogenic and probably does not cause disease and is not important to blood transfusion.

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