Prevalence of Recovirus-Neutralizing Antibodies in Human Serum Samples

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To investigate recovirus infections and their association with zoonosis, the prevalence of the virus-neutralizing antibody against three recovirus serotypes was tested in the general population and in zookeepers. Neutralizing antibodies were detected in a significantly higher number of zookeepers than in the general population but with significantly lower titers than in macaques.

Norovirus, Sapovirus, Lagovirus, Vesivirus, and Nebovirus are the 5 established genera within the family Caliciviridae. “Recovirus,” “Valovirus,” and “Chicken Calicivirus” are three new proposed genera (1). The Tulane virus is the prototype recovirus (ReCV) strain (2).

Caliciviruses (CVs) cause enteric, respiratory, vesicular, and hemorrhagic diseases in animals, while human CVs, including noroviruses (NoVs) and sapoviruses, are one of the leading causes of acute gastroenteritis in both developed and developing countries (1).

One of the major obstacles in human CV research is the inability to grow human CVs in cell culture. ReCVs not only can be grown in cell culture, but evolutionarily and biologically are closely related to human noroviruses (NoV) and are a promising surrogate for human NoV research (3–5).

ReCVs are endemic in captive rhesus macaque colonies, and based on serological surveys, they also infect other primate species, including humans (3, 6). Human ReCV infections were first suggested by the detection of Tulane virus-neutralizing (VN) antibodies in archived serum samples collected from animal caretakers at the Tulane National Primate Research Center (3). Recently, the molecular detection of a novel ReCV strain in stool samples of Bangladeshi patients exhibiting clinical symptoms of acute gastroenteritis was reported (7). Since the human ReCV strain genetically is significantly different from any of the known rhesus isolates, the zoonotic origin of these human cases is not clear.

Recently, the characterization of 10 cell culture-adapted genogroup 1 ReCV isolates, including the Tulane virus, revealed the existence of at least four serotypes (4). This presented the opportunity for further investigation of ReCV infections in humans and their possible zoonotic origin by evaluating seroprevalence against different serotypes and in different human populations. To represent the general population without exposure to nonhuman primate (NHP) contact, 100 randomly selected serum samples collected in 2013 were obtained from the Biobank of Cincinnati Children’s Hospital Medical Center (CCHMC). These samples had been previously collected by Antibody Systems, Inc. (Fort Worth, TX), for research purposes. No unique identifiers or information on the age, race, and sex of these individuals were available. The work performed in this study with human serum samples did not meet the definition of human subject research because all samples were deidentified. The institutional review board (IRB) deemed the study exempt from IRB review.

One hundred serum samples, collected between September 2009 and February 2010 during a previous study from animals between 1.5 and 26.5 years old (average, 5.9), both males (n = 45) and females (n = 69) housed at the Tulane National Primate Research Center (TNPRC), and provided by Karol Sestak, were used to establish seroprevalence in rhesus macaques (Macaca mulatta) (6).

Heat-inactivated (56°C, 30 min) serum samples were tested in a cytopathic effect (CPE)-based virus neutralization assay to inactivate 100 tissue culture infective doses (TCID50) of ReCVs as described in our previous studies (3, 6). Briefly, the virus-serum mixture was incubated for 1 h at 37°C and transferred into duplicate wells of 96-well tissue culture plates seeded with 104 LLC-MK2 cells/well. Virus- and mock-infected control wells were included. Plates were stained with crystal violet when all cells in the virus control wells were rounded and detached (~72 h post-inoculation). All sera were tested at a 1:10 dilution. Positive samples were end titrated. Neutralization was established when the cell monolayer was ≥50% intact in both of the wells for a given dilution. Statistical significance of antibody prevalence between the sample populations and between the mean titers of positive samples was evaluated by Fisher’s exact test and one-way analysis of variance (ANOVA), respectively.

The prevalence of neutralizing antibodies against the three ReCV serotypes tested in this study ranged between 96 and 99% (≥1:10 dilution) in the rhesus macaques. Interestingly the human samples collected from zookeepers exhibited a significantly higher...
prevalence of virus-neutralizing antibodies ($P < 0.0001$) against all serotypes (28 to 100%) than samples collected from the normal population (3 to 18%) (Fig. 1). Among the Cincinnati samples, where demographic information was available, the presence or level of VN antibodies was independent of age, sex, or race. The marked difference in the prevalence of ReCV-neutralizing antibodies between the two human populations is consistent with a zoonotic transmission of ReCVs. However, this was not supported by the antibody titers of the positive samples. The mean titers of virus-neutralizing antibodies in both human populations were significantly lower ($P < 0.05$) than those in the macaques. No statistically significant difference was detected between the mean titers of the two human populations (Fig. 2).

The possibility that ReCVs infect humans had been previously indicated by the detection of Tulane virus-neutralizing antibodies in serum samples collected from animal caretakers and more importantly by the molecular detection of a novel ReCV strain in stool samples from Bangladeshi patients (3, 7). These findings also raised the question about the zoonotic potential of ReCVs, which could be supported by the close genetic relationship of the nonhuman primate host and humans and by the evolutionary relatedness and shared biological features of ReCVs and human NoVs, including the role of histo-blood group antigen (HBGA) binding in susceptibility.

It is well established that chimpanzees and perhaps other nonhuman primate species can experimentally be infected with human NoVs (8). However, whether human NoV transmission to nonhuman primates occurs under natural circumstances is unknown. The interspecies transmission of several other viral agents between different primate species is well documented, including Cercopithecine herpesvirus I, herpes simplex viruses I and 2, measles virus, hepatitis A and C viruses, and the Ebola viruses. The zoonotic transmission of several animal CVs, including NoVs that are genetically closely related to human NoVs (e.g., genogroup 2 [G2] swine and G3 bovine NoVs) has been suggested previously (9), but even with decades of worldwide surveillance of human NoV infections, the detection of swine or bovine NoVs in human samples has not yet been reported. On the other hand, human NoVs can replicate in gnotobiotic pigs, which indicates the possibility of the emergence of swine-human recombinant NoVs or that swine could serve as reservoir for human NoVs. Recently, the detection of human G2.3, G2.4, and G2.13 NoVs that were associated with human outbreaks in the same year was reported in swine in Japan (10). However, the low copy numbers of these viruses compared to swine NoV strains raises questions about whether the human NoVs replicated in the pigs or were the result of environmental contamination.

The interspecies transmission or zoonotic potential of several CVs, including human and animal NoVs, was also suggested previously based on serological studies. Since a cell culture system is not available for most of these viruses, seroprevalence studies mostly relied on virus-like particle (VLP)-based immunoassays. For example, the interspecies transmission of human NoVs was suggested based on the detection of enzyme-linked immunosorbent assay (ELISA) antibodies against human NoVs in pigs (11). Widdowson and colleagues reported higher prevalence of ELISA antibodies against bovine NoVs in veterinarians (28%) than in the general population (20%), indicating the possible zoonotic transmission of bovine NoVs (12). However, the existence of cross-reactive epitopes between human and swine or bovine NoVs has also been reported (11, 13). This makes the detection of anti-human NoV ELISA antibodies in different animal species or vice versa difficult to interpret.

In our study, not ELISA but neutralizing antibodies against three ReCV serotypes were evaluated in individuals with known exposure to nonhuman primates and in the general population. The prevalence of ReCV-neutralizing antibodies was significantly

![FIG 1 Prevalence of ReCV-neutralizing antibodies. The percentage of samples with a VN titer of ≥10 is shown. **, statistically significant difference between the Cincinnati samples and samples obtained from zookeepers ($P < 0.0001$).](http://jcm.asm.org/)

![FIG 2 Virus neutralization antibody titers. Means and standard errors of the means (SEM) of positive samples are shown. *, statistically significant difference between macaque and human samples ($P < 0.05$). No statistically significant difference was detected between the two human populations.](http://jcm.asm.org/)
significantly lower levels of neutralizing antibodies in the human population with documented exposure to nonhuman primates points to the zoonotic potential of ReCVs. However, the significantly lower levels of neutralizing antibodies in the human samples compared to that in the macaques (Fig. 2) raises several questions that need to be further investigated. The low level of neutralizing antibody titers in the human samples could be explained by restricted infection and replication of the nonhuman primate ReCVs in the human host, resulting in weaker humoral immune responses. The existence of cross-neutralizing antibodies between nonhuman primate and human host-specific ReCVs, human NoVs, or other primate CVs that are able to infect humans cannot be excluded. However, cross-neutralization with human host-specific CVs, including NoVs and sapoviruses, would not explain the difference between the prevalence rates among the two human populations in this study. Furthermore, in our previous study, multivalent anti-NoV hyperimmune sera did not neutralize the Tulane virus, although the number of NoVs included was limited. While the zoonotic nature of most animal CVs is not clearly established, vesicular disease of a laboratory worker caused by San Miguel sea lion virus 5 (SMSV-5) has been described (14). Vesivirus types have been isolated from a wide variety of domestic and wild animals, including nonhuman primates. Cross-reactive neutralizing antibodies generated by a vesivirus vesivirus with lower affinity to ReCVs could explain the higher seroprevalence in zookeepers and the lower antibody titers in the human samples. Due to the unavailability of vesivirus serotyping reagents and the large number of vesivirus serotypes, exclusion of cross-neutralization between ReCVs and vesivirus will be challenging. However, since clear serotypic differences were observed even between closely related ReCVs (4), cross-neutralization between genetically distant CVs is less likely.

Nonspecific neutralization is more likely to occur at lower serum dilutions. The highest VN antibody titer detected in the human samples was 1:320, while the highest in macaques was 1:1,280. The prevalences of individuals with VN antibody levels of ≥1:80 against any of the three ReCVs were 84% in the macaques, 30% in the zookeepers, and 9% in the Cincinnati population, still indicating an association with nonhuman primate exposure. To clearly establish the zoonotic nature of ReCVs, detection of ReCV strains endemic in nonhuman primates is needed in human populations. This could be achieved in a targeted cohort study of animal caretakers working at nonhuman primate centers.

Close to 60% of modern emerging infectious diseases are considered to have a zoonotic origin. Furthermore, over half of the shared pathogens listed as emerging in humans are viruses, and a large number of them have been isolated from wild NHPs (15). Due to the high biological relatedness of ReCVs and human NoVs and their hosts, the risk of cross-species adaptation by genetic mutations or the emergence of recombinant viruses with unknown pathogenic potential should be considered high. Our findings together with the recent detection of ReCVs in human stool samples (7) call for detailed investigations of ReCV infections in both nonhuman primate and human populations.

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