Use of a Surrogate Chimeric Virus To Detect West Nile Virus-Neutralizing Antibodies in Avian and Equine Sera

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Received 11 June 2008/Returned for modification 7 July 2008/Accepted 3 November 2008

A chimeric yellow fever virus/West Nile virus (WNV) was compared to WNV alone as a biosafety level 2 reagent in the plaque reduction neutralization test for determining WNV infection histories. Concordance was 96.3% among 188 avian and equine serum samples. Neutralizing antibody titers were frequently more than twofold lower against the wild-type virus. Of these, 47 were positive for YF/WN Nt Abs, indicating a diagnostic test sensitivity of 0.89. Specificity was 0.99, with an overall concordance (κ) of the binary result (positive versus negative) of 0.96. Limiting the evaluation to the 100 samples derived from horses yielded a sensitivity of 0.90, a specificity of 1.00, and a κ of 0.96. Among the 88 samples derived from birds (representing a variety of wild and domestic species), the sensitivity was 0.91, the specificity was 0.98, and κ was 0.97. Sensitivity was reduced for low-titer samples (titer of 40 or lower). Among samples with titers of 80 or greater, sensitivity was 100% for both avian and equine sera.

We evaluated whether both tests produced equivalent titers (i.e., twofold or less difference). Agreement of titers differed significantly between avian and equine subsets (Fisher’s exact test; P = 0.004). Agreement was 0.86 among 22 avian samples but only 0.45 among 31 equine samples (Fig. 1). In all cases of disagreement, titers were greater (by fourfold or more) when the wild-type WNV was used. Agreement in titer increased when the neutralization threshold was reduced to 70%.

The biosafety rating of WNV has prevented widespread use of the PRNT as a diagnostic tool, in spite of its broad acceptability as the gold standard serologic technique for detecting Nt Abs specific to arboviruses. The availability of an attenuated virus with similar immunologic reactivity and attenuated neurovirulence in the form of the chimeric YF/WN recombinant virus permits a broader use of the PRNT for surveillance and diagnostic purposes (8). We show that the sensitivity and specificity of the PRNT using the chimeric virus in place of wild-type WNV are adequate for diagnostic testing of avian and equine serum samples. A limitation of the chimera compared with the wild type is the length of the PRNT assay. The chimera requires 5 days for plaque formation, whereas the wild type requires 3 days.

Titers of Nt Abs in bird sera determined by the two PRNT tests were similar. However, agreement of titers was much lower for equine sera, with titers derived using the chimera consistently lower than those derived using the wild type. One possible reason for this difference is that a conformational change(s) in the chimeric virus envelope may lower the avidity or block the recognition of certain equine but not avian immunoglobulins. Interspecies variation in humoral immune responses to certain WNV antigenic determinants between mice and humans (7) and between mice and horses (9) has been noted. However, in spite of the lower titers observed for horses

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‡ Published ahead of print on 12 November 2008.

Diagnosic assays for West Nile virus (WNV) (Flavivirus; Flaviviridae) infection in vertebrate blood samples often rely on the plaque reduction neutralization test (PRNT), considered the most sensitive and specific antibody detection test for arboviruses (2). However, WNV is characterized as a biosafety level 3 (BSL-3) virus and thus is restricted in its use to specialized bioscure laboratories. The premembrane and envelope protein genes of WNV (strain New York 1999, flamingo isolate 383-99) were inserted, without modifications in the gene sequences, into the attenuated yellow fever vaccine virus (17D strain) genome, resulting in an infectious, chimeric virus (1). The chimeric virus is significantly more attenuated than either the wild-type WNV New York 1999 strain or 17D strain, currently licensed as an equine vaccine (Prevenil; Intervet, Millsboro, DE) and classified as a BSL-2 agent (6). We evaluated whether this recombinant attenuated virus could be used in place of wild-type WNV as the challenge virus in the PRNT.

All avian and equine serum samples selected for this evaluation were obtained from WNV outbreak investigations in New York City in 1999 and 2000 or experimental infection studies (3–5, 10). Samples were chosen by availability and quantity of archived sera and assayed using a blind testing procedure. Serum samples were tested for WNV-specific neutralizing antibodies (Nt Abs) by the PRNT as previously described (2). All samples were tested for Nt Abs against WNV strain New York 1999 (wild type; crow brain isolate NY99-4132) and the yellow fever vaccine virus/WNV chimera (hereafter referred to as YF/WN), and end point 90% neutralization titers (PRNT90) were determined using twofold serial dilutions beginning at 1:10. A PRNT90 of 10 or greater was considered positive.

Of 188 nonhuman vertebrate serum samples, 53 were positive for WNV Nt Abs against the wild-type virus. Of these, 47 were positive for YF/WN Nt Abs, indicating a diagnostic test sensitivity of 0.89. Specificity was 0.99, with an overall concordance (κ) of the binary result (positive versus negative) of 0.96. Limiting the evaluation to the 100 samples derived from horses yielded a sensitivity of 0.90, a specificity of 1.00, and a κ of 0.96. Among the 88 samples derived from birds (representing a variety of wild and domestic species), the sensitivity was 0.91, the specificity was 0.98, and κ was 0.97. Sensitivity was reduced for low-titer samples (titer of 40 or lower). Among samples with titers of 80 or greater, sensitivity was 100% for both avian and equine sera.

We evaluated whether both tests produced equivalent titers (i.e., twofold or less difference). Agreement of titers differed significantly between avian and equine subsets (Fisher’s exact test; P = 0.004). Agreement was 0.86 among 22 avian samples but only 0.45 among 31 equine samples (Fig. 1). In all cases of disagreement, titers were greater (by fourfold or more) when the wild-type WNV was used. Agreement in titer increased when the neutralization threshold was reduced to 70%.

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tested with YF/WN, the overall result—positive or negative—was the same for both the chimera and the wild-type virus.

We recommend use of the YF/WN chimera as a surrogate diagnostic reagent in place of wild-type WNV for PRNT assays applied to nonhuman vertebrate sera, with the caveat that use of the chimera may result in reduced sensitivity for detecting low levels of Nt Abs. Similar assays have already been applied to human sera (8). These assays should be permissible in BSL-2 biocontainment laboratories.

REFERENCES

1. Arroyo, J., C. A. Miller, J. Catalan, and T. P. Monath. 2001. Yellow fever vector live-virus vaccines: West Nile virus vaccine development. Trends Mol. Med. 7:350–354.

2. Beaty, B. J., C. H. Calisher, and R. E. Shope. 1995. Arboviruses, p. 189–212. In E. H. Lennette, D. A. Lennette, and E. T. Lennette (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 7th ed. Public Health Association, Washington, DC.

3. Komar, N., J. Burns, C. Dean, N. A. Panella, S. Dusza, and B. Cherry. 2001. Serological evidence for West Nile virus infection in birds in Staten Island, New York after an outbreak in 2000. Vector Borne Zoonot. Dis. 1:191–196.

4. Komar, N., N. A. Panella, J. E. Burns, S. W. Dusza, T. M. Macearenaus, and T. O. Talbot. 2001. Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. Emerg. Infect. Dis. 7:621–625.

5. Komar, N., S. Langevin, S. Hinten, N. Nemeth, E. Edwards, D. Hettle, B. Davis, R. Bowen, and M. Bunning. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg. Infect. Dis. 9:311–322.

6. Long, M. T., E. P. Gibbs, M. W. Mellenkamp, R. A. Bowen, K. K. Seino, S. Zhang, S. E. Beachboard, and P. P. Humphrey. 2007. Efficacy, duration, and onset of immunogenicity of a West Nile virus vaccine, live flavivirus chimera, in horses with a clinical disease challenge model. Equine Vet. J. 39:492–497.

7. Oliphant, T., G. E. Nybakken, S. K. Austin, Q. Xu, J. Bramson, M. Loeb, M. Throsby, D. H. Fremont, T. C. Pierson, and M. S. Diamond. 2007. Induction of epitope-specific neutralizing antibodies against West Nile virus. J. Virol. 81:11828–11839.

8. Pugachev, K. V., F. Guirakhoo, F. Mitchell, S. W. Ocran, M. Parsons, B. W. Johnson, O. L. Kosoy, R. S. Lanciotti, J. T. Roehrig, D. W. Trent, and T. P. Monath. 2004. Construction of yellow fever/St. Louis encephalitis chimeric virus and the use of chimeras as a diagnostic tool. Am. J. Trop. Med. Hyg. 71:639–645.

9. Sánchez, M. D., T. C. Pierson, M. M. Degrace, L. M. Mattei, S. L. Hanna, T. Del Rio, and R. W. Doms. 2007. The neutralizing antibody response against West Nile virus in naturally infected horses. Virology 359:336–348.

10. Trock, S. C., B. J. Meade, A. L. Glasser, E. N. Ostlund, R. S. Lanciotti, R. C. Cropp, V. Kulasekera, L. D. Kramer, and N. Komar. 2001. West Nile virus outbreak among horses in New York State, 1999 and 2000. Emerg. Infect. Dis. 7:745–747.

FIG. 1. Scatterplots depict WNV titers versus YF/WN titers derived from plaque reduction neutralization tests on avian (A) and equine (B) sera exposed to natural or experimental WNV infection. End point titers were not measured beyond 320 or below 10. Titers of <10 were considered as 5 in this chart. Samples with points falling within the dashed lines had equivalent titers (less-than-fourfold difference) by both PRNT methods. Points falling outside the dashed lines indicate disagreement in titer.