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Development of a Neutralization Assay for Nipah Virus Using Pseudotype Particles

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Abstract

Nipah virus (NiV) and Hendra virus (HeV) are zoonotic paramyxoviruses capable of causing severe disease in humans and animals. These viruses require biosafety level 4 (BSL-4) containment. Like other paramyxoviruses, the plaque reduction neutralization test (PRNT) can be used to detect antibodies to the surface glycoproteins, fusion (F) and attachment (G), and PRNT titers give an indication of protective immunity. Unfortunately, for NiV and HeV, the PRNT must be performed in BSL-4 containment and takes 5–7 days to complete. Thus, we have developed a neutralization assay using VSV pseudotype particles expressing the F and G proteins of NiV (pVSV-NiV-F/G) as target antigens. This rapid assay, which can be performed at BSL-2, was evaluated using serum samples from outbreak investigations and more than 300 serum samples from an experimental NiV vaccination study in swine. The results of the neutralization assays with pVSV-NiV-F/G as antigen showed a good correlation with those of standard PRNT. Therefore, this new method has the potential to be a rapid and cost-effective diagnostic method, especially in locations that lack high containment facilities, and will provide a valuable tool for basic research and vaccine development.

1. Introduction

Nipah virus (NiV) and Hendra virus (HeV) are recently emergent, related paramyxoviruses that can cause severe diseases in humans and animals. These viruses are the only two members of a newly named genus, *Henipavirus*, within the family *Paramyxoviridae* (Bellini et al., 2005; Wang et al., 2001). HeV emerged in Australia in 1994 and caused severe respiratory disease in humans and horses (Murray et al., 1995). There have been sporadic cases of HeV

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through 2006 (Anonymous, 2008; Field et al., 2007; Westbury, 2000a; Westbury, 2000b), but the virus has not been detected outside of Australia. The first outbreak of NiV occurred in 1998–1999 in Malaysia and Singapore, and was manifested as a respiratory disease in swine and fatal, febrile encephalitis in humans (Chua et al., 2000). In 2001, NiV outbreaks occurred in Siliguri, India (Chadha et al., 2006; Harit et al., 2006). From 2001–2004, four outbreaks of NiV took place in Bangladesh involving 93 patients with a 73% case fatality rate (Hossain et al., 2008). In the Malaysian outbreak, pigs served as the amplifying host and transmitted the virus to humans (Chew et al., 2000; Chua et al., 2000; Lam and Chua, 2002; Paton et al., 1999). Unlike Malaysia (Mounts et al., 2001), person-to-person transmission and food-borne infection of NiV was reported in Bangladesh (Gurley et al., 2007a; Gurley et al., 2007b; Luby et al., 2006). Nucleotide sequencing studies showed that NiV strains in India were more closely related to those in Bangladesh than Malaysia (AbuBakar et al., 2004; Harcourt et al., 2005). Fruit bats were found to be the main reservoir of both HeV and NiV (Chua et al., 2002; Halpin et al., 2000). Five species of bats in peninsular Malaysia had antibodies to NiV (Yob et al., 2001). More recently, antibodies to Nipah-like viruses were detected among bats in Bangladesh (Hsu et al., 2004), Cambodia (Olson et al., 2002; Reynes et al., 2005), Thailand (Wacharapluesadee et al., 2005), Madagascar (Lehle et al., 2007), India (Epstein et al., 2008), China (Li et al., 2008) and Ghana (Hayman et al., 2008). The widespread distribution of fruit bats suggests that spillover of NiV from this reservoir into the human population could occur in many countries within Southeast Asia and sub-Saharan Africa with serious consequences for both human health and agriculture (Epstein et al., 2006; Hayman et al., 2008).

Enzyme-linked immunoassay (ELISA) and RT-PCR were used as a first-line diagnostic test in suspected NiV outbreaks (Daniels et al., 2001). However, since there is an approximately 2 percent false positive rate in the ELISA, confirmatory testing was performed by using a serum neutralizing antibody test (Daniels et al., 2001). The neutralization test requires live NiV and must be performed at biosafety level 4 (BSL-4) creating a significant challenge for countries lacking high containment facilities. Therefore, there is a need to develop alternative diagnostic methods for detecting antibodies to NiV that can be performed at BSL-2 conditions.

Like other paramyxoviruses, neutralizing antibodies to NiV are targeted to the fusion (F) and the attachment (G) glycoproteins (Bossart et al., 2002; Tamin et al., 2002). This manuscript reports on the development of a novel assay to detect neutralizing antibodies to NiV. The target antigen is a vesicular stomatitis virus (VSV) pseudotype particle (pVSV) displaying NiV F and G (pVSV-NiV-F/G) and expressing a luciferase reporter gene. The inhibition of entry of the pseudotype particle by antibodies to the surface glycoproteins of NiV can be detected by measuring the level of luciferase activity following infection of Vero cells. Because no infectious NiV is used, this assay can be performed at BSL-2 and completed within 48 hours and the results compared favorably to those of a standard plaque reduction neutralization test (PRNT).

2. Materials and Methods

2.1 Cells and viruses

Vero cells were maintained in Dulbecco's Modified Essential Medium (DMEM) containing 10% fetal calf serum (FCS), L-glutamine, penicillin, and streptomycin (GIBCO BRL). The production of pVSV-NiV-F/G was described previously (Aguilar et al., 2007; Negrete et al., 2005; Negrete et al., 2006). The experimental NiV vaccine based on recombinant canarypox (CP) expressing either the F (CP-F), or G (CP-G) proteins of NiV was described previously (Weingartl et al., 2006).

2.2 Experimental vaccination of pigs with CP-F and CP-G

The details of experimental design, vaccination and sample collections schedule were described previously (Weingartl et al., 2006). Briefly, pigs were vaccinated intramuscularly with 10^8 plaque forming units (pfu) (high dose) or 10^7 pfu (low dose) of CP-F, CP-G, or a mixture of both (four animals per group) under BSL-3 conditions. The pigs were boosted 21 days post vaccination (dpv) and serum samples were collected from each animal before vaccination (day 0) and at 7, 14, 21, 28, 35 and 48 dpv, and the samples were heat-treated at 56°C for 45 minutes prior to the neutralization test.

2.3 Enzyme immunoassay with pVSV-NIVF/G

pVSV-NiV-F/G or pVSV were diluted in PBS and added to duplicate wells of a flat-bottom 96 well tissue culture plate and incubated at 37°C for one hour. The plate was washed 5 times with PBS-Tween buffer (0.1M PBS with 0.05% Tween 20), and primary antibodies from humans or mice diluted 1:200 in blocking buffer (5% skim milk, 0.1M PBS, 0.05% Tween-20) were added to each well, and incubated at 37°C for one hour. The plates were washed 5 times with PBS-Tween buffer, and the corresponding secondary antibodies were added (goat anti-human-HRP, goat anti-mouse-HRP) and incubated at 37°C for one hour. The plates were washed 5 times with PBS-Tween buffer, 75 μl per well of TMB substrate was added and incubated at room temperature for 15 minutes. The reaction was stopped by adding 75 μl per well of 2M phosphoric acid. The absorbance was then read in an EIA reader at 450 nm (TECAN, Inc.).

2.4 Neutralization test using pVSV-NiV-F/G

The dilution of pseudotype virus used was calculated to produce 1500–2000 relative light units (RLUs) in control wells. Four fold serial dilutions of the serum samples were prepared in a 96 well plate beginning with an initial dilution of 1:20 in Minimal Essential Medium (MEM). An equal volume (55 μl) of pVSV-NiV-F/G was added to each well and the plates were incubated at 37°C for 45 minutes. One hundred microliters of the pseudotype particle-serum mixtures were transferred onto 96 well plates containing a monolayer of Vero cells. Plates were incubated at 37°C and 5% CO_2 for 2 hours for adsorption after which the innocula were replaced with 150 μl of DMEM with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS), and the plates were incubated at 37°C and 5% CO_2 for 17 hours. After incubation, the medium was removed by aspiration, and the plates were washed twice with 200 μl phosphate-buffered saline (0.01M PBS) per well. Cell lysis and detection of *Renilla* luciferase were performed according to the protocol recommended by the manufacturer (Promega, Inc). Briefly, *Renilla* Luciferase Assay Lysis Buffer was thawed to room temperature; diluted 1:5 with ddH20, and 30 μl was added to each well. Plates were placed on an orbital shaker for 1–2 hrs with periodic mechanical manipulation, or by freeze-thawing 3X to ensure complete lysis of the cells. Following lysis, 25 μl of the cell lysates was transferred to a Fluoroskan Ascent FL Fluorometer (Thermo Corporation, Finland) which was pre-programmed to read for 10 sec with a 2 sec delay after 100 μl of *Renilla* Luciferase Assay Reagent was added to the well. Percent inhibition was calculated by dividing the average RLU number in triplicate wells with the test antiserum samples by the average RLU of triplicate wells infected but without antiserum (positive control). A signal decrease of 90% or greater was considered to be inhibition. The titer of the pVSV-NiV-F/G neutralization assay was the reciprocal of the highest dilution of serum showing 90% or higher inhibition. Serum samples that failed to show at least 90% neutralization at the 1:40 initial dilution were considered negative.

2.5 Neutralizing assay by plaque reduction neutralization test (PRNT)

The method for PRNT using live NiV was described previously (Weingartl et al., 2006), and all procedures with live virus were performed under BSL-4 conditions. Briefly, PRNT titers were determined based on a 90% plaque reduction cut-off starting with a 1:20 dilution of serum.

2.6 Radioimmunoprecipitation (RIP) assays

RIP assays were carried out as described previously (Tamin et al., 1994) with the single exception that the ^{35}S-methionine-labeled vv/NiV-F and vv/NiV-G infected cells were resuspended in RIPA buffer (1% sodium deoxycholate, 1% Triton X-100, 0.2% SDS, 150 mM NaCl, and 50 mM Tris-HCl at pH 7.5). The human serum samples used were from the recent outbreaks of NiV in Bangladesh (Hossain et al., 2008). Normal human serum and NiV specific hyperimmune mouse ascites fluid (HMAF) were used as negative and positive controls, respectively. The ratio of antibody to ^{35}S-methionine labeled lysate was 1:40.

3. Results

3.1 Antigenic properties of pVSV-NiV-F/G

Infection of Vero cells with pVSV-NiV-F/G was inhibited by monospecific antiserum to NiV-F and NiV-G, HMAF prepared against NiV, or a human serum sample from a patient infected with NiV, all of which also neutralized NiV by standard PRNT (Table 1). Negative control serum samples included normal human and normal mouse serum and monospecific antiserum to NiV-N protein and measles virus F protein (Table 1). The results confirmed that pVSV-NiV-F/G particles had incorporated both the NiV F and G proteins and the infectivity of the particles was neutralized by serum samples that neutralized NiV in PRNT.

The pseudotype particles were able to serve as NiV-specific glycoprotein antigens in a standard enzyme immunoassay (Figure 1). HMAF prepared against NiV (Figure 1A), serum from mice vaccinated with vaccinia recombinants expressing NiV F and G (Figure 1B), and a human serum sample from a patient infected with NiV (Figure 1B) specifically recognized pVSV-NiV-F/G bound to the plastic plate. The second antibody-conjugate that was used to detect the mouse antibody gave lower OD readings than the second antibody that was used to detect the human antiserum. There was no background reactivity when pVSV particles were used as antigen (Figure 1A). Serum samples recognizing the surface glycoproteins of measles virus and normal human serum did not give a signal in the enzyme immunoassay format (Figure 1B). These results indicated that pVSV-NiV-F/G particles were antigenic and can serve as antigens in neutralization assays and enzyme immunoassays.

3.2 Antibody responses to NiV infection or experimental vaccination

Representative serum samples from pigs vaccinated with CP-F, CP-G or CP-F and CP-G recognized NiV-F and NiV G in a manner that was consistent with the vaccination scheme (Figure 2). However, the NiV proteins were not detected in the RIP assays until 1 week after the booster vaccination on day 21 with the exception of the day 21 serum samples from pigs vaccinated with CP-F and CP-G (Figures 2 and 4). Serum samples from humans infected with NiV recognized both NiV-G and NiV-F (Figure 3A). These results confirmed that the serum samples to be evaluated in the neutralization assay included antibodies specific to the surface glycoproteins of NiV.

3.3 Comparison of the pVSV-NiV-F/G assay and PRNT

The human serum samples containing antibodies to NiV F and G completely inhibited the luciferase production in Vero cells infected with pVSV-NiV-F/G at dilutions of up to1:640 (Figure 3B). Control serum samples did not inhibit luciferase production at the starting dilution

of 1:40. These data showed that the human serum samples were able to neutralize the infectivity of the pseudotype particles.

All of the serum samples from the pigs vaccinated with CP-F, CP-G or CP-F and CP-G were tested in both the standard PRNT and the assay using pVSV-NiV-F/G as an antigen (Figure 4). Both assays consistently detected neutralizing antibodies to NiV in the serum samples obtained after the booster vaccination on day 21, and the assays based on the pVSV-NiV-F/G demonstrated high sensitivity and specificity compared to standard PRNT (Figure 5). However, the sensitivity of the pVSV-NiV-F/G assay was decreased to approximately 50% with serum samples collected 7, 14 and 21 dpv. PRNT detected low levels of antibodies more efficiently than the pseudotype assay or RIP. Overall, the specificity of the pseudotype assay was extremely high (94–100%) and none of the pre-vaccination serum samples were positive in either assay (Figures 4–5). When samples were scored as positive or negative, there was a 96% correlation between the results of the pseudotype assay and PRNT with only 16 discordant results for 363 serum samples, 15 of which occurred for samples taken 7–21 dpv (Figure 5). There was nearly 100% correlation for all of the pre-bleed samples and for samples taken 28–49 dpv. Comparing the titers for the PRNT and pseudotype assay gave a coefficient of correlation of 0.51 for all samples collected on days 28–49. Serum samples from the pigs receiving the high dose of the vaccines had an overall correlation coefficient of 0.62 and 0.68 for the samples from days 28 and 35 post vaccination, respectively.

4. Discussion

Several strategies have been employed to develop new diagnostic techniques to detect infections with the henipaviruses with an emphasis on assays that do not require BSL-4 containment. Expression systems were used to produce antigens for enzyme immunoassays including the NiV N protein (Chen et al., 2006; Yu et al., 2006), a truncated phosphoprotein antigen (Chen et al., 2007), or the glycoproteins of NiV (Eshaghi et al., 2005a; Eshaghi et al., 2004; Eshaghi et al., 2005b). Other assays described the use of monoclonal antibodies for immunohistochemical based diagnosis (Tanimura et al., 2004; Xiao et al., 2008), or a modified plaque assay in which the plates are prepared in BSL-4 and inactivated by gamma irradiation before staining at BSL-2 (Crameri et al., 2002). Soluble G proteins and/or ephrin B2 coupled with a bioplex protein array have been used to measure antibody responses to the glycoproteins of henipaviruses (Bossart et al., 2007).

The pVSV-NiV-F/G was initially developed to support studies to identify the cellular receptors for the henipaviruses and has recently been used as antigen for a neutralization test (Wang et al., 2006). A similar approach using pseudotype particles or reporter virus particles (RVP) was used in a neutralization assay for SARS (Temperton et al., 2005) and West Nile virus (Pierson et al., 2006). This report describes the first comparison between the neutralization assay using pVSV-NiV-F/G and PRNT. The VSV-NiV-F/G neutralization assay offers several significant advantages over PRNT. The assay can be performed entirely at BSL-2, completed in less than 48 hours, and relies on automated read-outs in a 96-well format instead of manual counting of plaques. Overall, the results obtained by the assay using pVSV-NiV-F/G were consistent and correlated well with the results from PRNT. Both methods (Figure 4) confirmed previous reports of the ability of NiV G to induce higher neutralizing titers than NiV-F (Bossart et al., 2005; Guillaume et al., 2006; Mungall et al., 2006; Tamin et al., 2002). Also, the pseudotype particles were immunogenic when used as an antigen in an indirect enzyme immunoassay format suggesting that this may be a promising technique for developing glycoprotein specific enzyme immunoassays for NiV and other paramyxoviruses.

There is considerable variation in all diagnostic methods, including PRNT, in detecting low levels of antibodies. In this study, the results of the PRNT were regarded as the gold standard.

Fifteen of the 16 discordant results between PRNT and the pseudotype neutralization assay were recorded from serum samples collected 7, 14 and 21 dpv when antibody levels were very low. With only one exception, the PRNT titers did not exceed 40 for any of the samples that appeared to give false negative results in the pseudotype assay. Some of these low titers could have been due to toxic effects of the serum in the PRNT assay. All of the five apparent false positive results in the pseudotype assay were from the serum samples collected on day 21 and these had the titers of 40.

Henipaviruses are zoonotic viruses, and their natural reservoir is fruit bats of the genus *Pteropus* (Halpin et al., 2000; Chua et al., 2002). The geographical distribution of *Pteropus* fruit bats includes parts of West Africa, South Asia and Southeast Asia, the Pacific Islands, and the eastern coast of Australia. This vast area includes some of the most heavily populated areas in the world including many developing countries (Epstein et al., 2006). Unlike other paramyxoviruses, these viruses have the ability to infect a wide range of host species (Bellini et al., 2005). Therefore, there is always the potential for spillover of henipaviruses from the natural reservoir into humans or farm animals. Good laboratory tests, including both serologic and virus detection methods are needed to rapidly detect outbreaks of henipaviruses.

The standard first-line serologic test for henipaviruses has been enzyme immunoassay along with neutralization tests used for confirmation. The pseudotype neutralization assay described here could be used to replace PRNT as the confirmatory serologic test in areas lacking high containment facilities. Pseudotype particles could easily be prepared in advance and stored frozen until required. The local laboratory would need only cell culture capacity and a luminometer. This new novel, high-throughput assay could also be a valuable tool for performing serologic surveillance of various bat species and domestic animals. Finally, this assay would provide a convenient and safe way to measure protective antibodies induced by experimental vaccines against henipaviruses.

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References

AbuBakar S, Chang LY, Ali AR, Sharifah SH, Yusoff K, Zamrod Z. Isolation and molecular identification of Nipah virus from pigs. Emerg Infect Dis 2004;10:2228–2230. [PubMed: 15663869]

Aguilar HC, Matreyek KA, Choi DY, Filone CM, Young S, Lee B. Polybasic KKR motif in the cytoplasmic tail of Nipah virus fusion protein modulates membrane fusion by inside-out signaling. J Virol 2007;81:4520–4532. [PubMed: 17301148]

Australian vet dies from Hendra virus infection. Veterinary Record 2008;163:257–259.

Bellini WJ, Harcourt BH, Bowden N, Rota PA. Nipah virus: an emergent paramyxovirus causing severe encephalitis in humans. J Neurovirol 2005;11:481–487. [PubMed: 16287690]

Bossart KN, Crameri G, Dimitrov AS, Mungall BA, Feng YR, Patch JR, Choudhary A, Wang LF, Eaton BT, Broder CC. Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. J Virol 2005;79:6690–6702. [PubMed: 15890907]

Bossart KN, McEachern JA, Hickey AC, Choudhry V, Dimitrov DS, Eaton BT, Wang LF. Neutralization assays for differential henipavirus serology using Bio-Plex protein array systems. J Virol Methods 2007;142:29–40. [PubMed: 17292974]

Bossart KN, Wang LF, Flora MN, Chua KB, Lam SK, Eaton BT, Broder CC. Membrane fusion tropism and heterotypic functional activities of the Nipah virus and Hendra virus envelope glycoproteins. J Virol 2002;76:11186–11198. [PubMed: 12388678]

Chadha MS, Comer JA, Lowe L, Rota PA, Rollin PE, Bellini WJ, Ksiazek TG, Mishra A. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006;12:235–240. [PubMed: 16494748]

Chen JM, Yaiw KC, Yu M, Wang LF, Wang QH, Crameri G, Wang ZL. Expression of truncated phosphoproteins of Nipah virus and Hendra virus in Escherichia coli for the differentiation of henipavirus infections. Biotechnol Lett 2007;29:871–875. [PubMed: 17322967]

Chen JM, Yu M, Morrissy C, Zhao YG, Meehan G, Sun YX, Wang QH, Zhang W, Wang LF, Wang ZL. A comparative indirect ELISA for the detection of henipavirus antibodies based on a recombinant nucleocapsid protein expressed in Escherichia coli. J Virol Methods 2006;136:273–276. [PubMed: 16769130]

Chew MH, Arguin PM, Shay DK, Goh KT, Rollin PE, Shieh WJ, Zaki SR, Rota PA, Ling AE, Ksiazek TG, Chew SK, Anderson LJ. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000;181:1760–1763. [PubMed: 10823780]

Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ, Mahy BW. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000;288:1432–1435. [PubMed: 10827955]

Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, Chan YP, Lim ME, Lam SK. Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes Infect 2002;4:145–151. [PubMed: 11880045]

Crameri G, Wang LF, Morrissy C, White J, Eaton BT. A rapid immune plaque assay for the detection of Hendra and Nipah viruses and anti-virus antibodies. J Virol Methods 2002;99:41–51. [PubMed: 11684302]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001;3:289–295. [PubMed: 11334746]

Epstein JH, Field HE, Luby S, Pulliam JR, Daszak P. Nipah virus: impact, origins, and causes of emergence. Curr Infect Dis Rep 2006;8:59–65. [PubMed: 16448602]

Epstein JH, Prakash V, Smith CS, Daszak P, McLaughlin AB, Meehan G, Field HE, Cunningham AA. Henipavirus infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008;14:1309–1311. [PubMed: 18680665]

Eshaghi M, Tan WS, Chin WK, Yusoff K. Purification of the extra-cellular domain of Nipah virus glycoprotein produced in Escherichia coli and possible application in diagnosis. J Biotechnol 2005a; 116:221–226. [PubMed: 15707682]

Eshaghi M, Tan WS, Mohidin TB, Yusoff K. Nipah virus glycoprotein: production in baculovirus and application in diagnosis. Virus Res 2004;106:71–76. [PubMed: 15522449]

Eshaghi M, Tan WS, Ong ST, Yusoff K. Purification and characterization of Nipah virus nucleocapsid protein produced in insect cells. J Clin Microbiol 2005b;43:3172–3177. [PubMed: 16000431]

Field HE, Breed AC, Shield J, Hedlefs RM, Pittard K, Pott B, Summers PM. Epidemiological perspectives on Hendra virus infection in horses and flying foxes. Aust Vet J 2007;85:268–270. [PubMed: 17615038]

Guillaume V, Contamin H, Loth P, Grosjean I, Courbot MC, Deubel V, Buckland R, Wild TF. Antibody prophylaxis and therapy against Nipah virus infection in hamsters. J Virol 2006;80:1972–1978. [PubMed: 16439553]

Gurley ES, Montgomery JM, Hossain MJ, Bell M, Azad AK, Islam MR, Molla MA, Carroll DS, Ksiazek TG, Rota PA, Lowe L, Comer JA, Rollin P, Czub M, Grolla A, Feldmann H, Luby SP, Woodward JL, Breiman RF. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infect Dis 2007a;13:1031–1037. [PubMed: 18214175]

Gurley ES, Montgomery JM, Hossain MJ, Islam MR, Molla MA, Shamsuzzaman SM, Akram K, Zaman K, Asgari N, Comer JA, Azad AK, Rollin PE, Ksiazek TG, Breiman RF. Risk of nosocomial transmission of Nipah virus in a Bangladesh hospital. Infect Control Hosp Epidemiol 2007b;28:740–742. [PubMed: 17520553]

Halpin K, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. J Gen Virol 2000;81:1927–32. [PubMed: 10900029]

Harcourt BH, Lowe L, Tamin A, Liu X, Bankamp B, Bowden N, Rollin PE, Comer JA, Ksiazek TG, Hossain MJ, Gurley ES, Breiman RF, Bellini WJ, Rota PA. Genetic characterization of Nipah virus, Bangladesh, 2004. Emerg Infect Dis 2005;11:1594–1597. [PubMed: 16318702]

Harcourt BH, Tamin A, Ksiazek TG, Rollin PE, Anderson LJ, Bellini WJ, Rota PA. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. Virology 2000;271:334–349. [PubMed: 10860887]

Harit AK, Ichhpujani RL, Gupta S, Gill KS, Lal S, Ganguly NK, Agarwal SP. Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. Indian J Med Res 2006;123:553–560. [PubMed: 16783047]

Hayman DT, Suu-Ire R, Breed AC, McEachern JA, Wang L, Wood JL, Cunningham AA. Evidence of henipavirus infection in West African fruit bats. PLoS ONE 2008;3:e2739. [PubMed: 18648649]

Hossain MJ, Gurley ES, Montgomery JM, Bell M, Carroll DS, Hsu VP, Formenty P, Croisier A, Bertherat E, Faiz MA, Azad AK, Islam R, Molla MA, Ksiazek TG, Rota PA, Comer JA, Rollin PE, Luby SP, Breiman RF. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008;46:977–984. [PubMed: 18444812]

Hsu VP, Hossain MJ, Parashar UD, Ali MM, Ksiazek TG, Kuzmin I, Niezgoda M, Rupprecht C, Bresee J, Breiman RF. Nipah virus encephalitis reemergence, Bangladesh. Emerg Infect Dis 2004;10:2082–7. [PubMed: 15663842]

Lam SK, Chua KB. Nipah virus encephalitis outbreak in Malaysia. Clin Infect Dis 2002;34(Suppl 2):S48–51. [PubMed: 11938496]

Lehle C, Razafitrimo G, Razainirina J, Andriaholinirina N, Goodman SM, Faure C, Georges-Courbot MC, Rousset D, Reynes JM. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007;13:159–161. [PubMed: 17370536]

Li Y, Wang J, Hickey AC, Zhang Y, Wu Y, Zhang H, Yuan J, Han Z, McEachern J, Broder CC, Wang LF, Shi Z. Antibodies to Nipah or Nipah-like viruses in bats, China. Emerg Infect Dis 2008;14:1974–1976. [PubMed: 19046545]

Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, Khan R, Ahmed BN, Rahman S, Nahar N, Kenah E, Comer JA, Ksiazek TG. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006;12:1888–1894. [PubMed: 17326940]

Mounts AW, Kaur H, Parashar UD, Ksiazek TG, Cannon D, Arokiasamy JT, Anderson LJ, Lye MS. A cohort study of health care workers to assess nosocomial transmissibility of Nipah virus, Malaysia, 1999. J Infect Dis 2001;183:810–813. [PubMed: 11181159]

Mungall BA, Middleton D, Crameri G, Bingham J, Halpin K, Russell G, Green D, McEachern J, Pritchard LI, Eaton BT, Wang LF, Bossart KN, Broder CC. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. J Virol 2006;80:12293–12302. [PubMed: 17005664]

Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B. A morbillivirus that caused fatal disease in horses and humans. Science 1995;268:94–97. [PubMed: 7701348]

Negrete OA, Levroney EL, Aguilar HC, Bertolotti-Ciarlet A, Nazarian R, Tajyar S, Lee B. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. Nature 2005;436:401–405. [PubMed: 16007075]

Negrete OA, Wolf MC, Aguilar HC, Enterlein S, Wang W, Muhlberger E, Su SV, Bertolotti-Ciarlet A, Flick R, Lee B. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. PLoS Pathog 2006;2:e7. [PubMed: 16477309]

Olson JG, Rupprecht C, Rollin PE, An US, Niezgoda M, Clemins T, Walston J, Ksiazek TG. Antibodies to Nipah-like virus in bats (Pteropus lylei), Cambodia. Emerg Infect Dis 2002;8:987–988. [PubMed: 12194780]

Paton NI, Leo YS, Zaki SR, Auchus AP, Lee KE, Ling AE, Chew SK, Ang B, Rollin PE, Umapathi T, Sng I, Lee CC, Lim E, Ksiazek TG. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999;354:1253–6. [PubMed: 10520634]

Pierson TC, Sanchez MD, Puffer BA, Ahmed AA, Geiss BJ, Valentine LE, Altamura LA, Diamond MS, Doms RW. A rapid and quantitative assay for measuring antibody-mediated neutralization of West Nile virus infection. Virology 2006;346:53–65. [PubMed: 16325883]

Reynes JM, Counor D, Ong S, Faure C, Seng V, Molia S, Walston J, Georges-Courbot MC, Deubel V, Sarthou JL. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005;11:1042–1047. [PubMed: 16022778]

Tamin A, Harcourt BH, Ksiazek TG, Rollin PE, Bellini WJ, Rota PA. Functional properties of the fusion and attachment glycoproteins of Nipah virus. Virology 2002;296:190–200. [PubMed: 12036330]

Tamin A, Rota PA, Wang ZD, Heath JL, Anderson LJ, Bellini WJ. Antigenic analysis of current wild type and vaccine strains of measles virus. J Infect Dis 1994;170:795–801. [PubMed: 7930720]

Tanimura N, Imada T, Kashiwazaki Y, Shahirudin S, Sharifah SH, Aziz AJ. Monoclonal antibody-based immunohistochemical diagnosis of Malaysian Nipah virus infection in pigs. J Comp Pathol 2004;131:199–206. [PubMed: 15276859]

Temperton NJ, Chan PK, Simmons G, Zambon MC, Tedder RS, Takeuchi Y, Weiss RA. Longitudinally profiling neutralizing antibody response to SARS coronavirus with pseudotypes. Emerg Infect Dis 2005;11:411–416. [PubMed: 15757556]

Wacharapluesadee S, Lumlertdacha B, Boongird K, Wanghongsa S, Chanhome L, Rollin P, Stockton P, Rupprecht CE, Ksiazek TG, Hemachudha T. Bat Nipah virus, Thailand. Emerg Infect Dis 2005;11:1949–1951. [PubMed: 16485487]

Wang L, Harcourt BH, Yu M, Tamin A, Rota PA, Bellini WJ, Eaton BT. Molecular biology of Hendra and Nipah viruses. Microbes Infect 2001;3:279–87. [PubMed: 11334745]

Wang X, Ge J, Hu S, Wang Q, Wen Z, Chen H, Bu Z. Efficacy of DNA immunization with F and G protein genes of Nipah virus. Ann N Y Acad Sci 2006;1081:243–245. [PubMed: 17135518]

Weingartl HM, Berhane Y, Caswell JL, Loosmore S, Audonnet JC, Roth JA, Czub M. Recombinant nipah virus vaccines protect pigs against challenge. J Virol 2006;80:7929–7938. [PubMed: 16873250]

Westbury H. Hendra virus: a highly lethal zoonotic agent. Vet J 2000a;160:165–6. [PubMed: 11061952]

Westbury HA. Hendra virus disease in horses. Rev Sci Tech 2000b;19:151–159. [PubMed: 11189712]

Xiao C, Liu Y, Jiang Y, Magoffin DE, Guo H, Xuan H, Wang G, Wang LF, Tu C. Monoclonal antibodies against the nucleocapsid proteins of henipaviruses: production, epitope mapping and application in immunohistochemistry. Arch Virol 2008;153:273–281. [PubMed: 17978885]

Yob JM, Field H, Rashdi AM, Morrissy C, van der Heide B, Rota P, bin Adzhar A, White J, Daniels P, Jamaluddin A, Ksiazek T. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–441. [PubMed: 11384522]

Yu F, Khairullah NS, Inoue S, Balasubramaniam V, Berendam SJ, Teh LK, Ibrahim NS, Abdul Rahman S, Hassan SS, Hasebe F, Sinniah M, Morita K. Serodiagnosis using recombinant nipah virus nucleocapsid protein expressed in Escherichia coli. J Clin Microbiol 2006;44:3134–3138. [PubMed: 16954238]

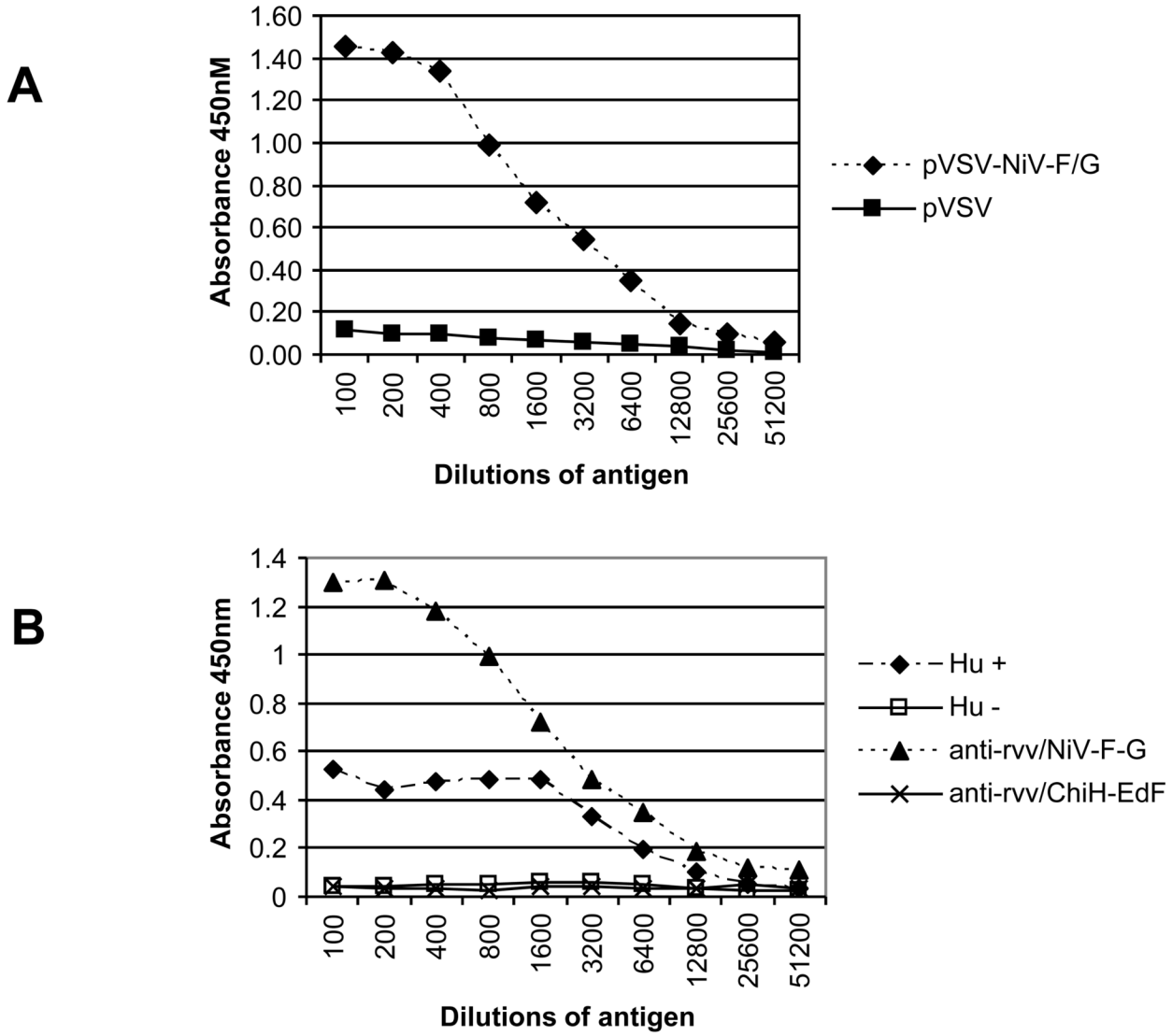

Figure 1.

Pseudotype particles are can serve as antigens in an enzyme immunoassay. Panel A shows reactivity of HMAF prepared against NiV with pVSV-NiV-F/G and pVSV. Plates were coated with dilutions of the pseudotype particles as indicated before being tested by enzyme immunoassay as described in Materials and Methods. Panel B shows the reactivity of pseudotype pVSV-NiV-F/G with positive (Hu+) and negative (Hu−) human serum samples and samples from mice vaccinated with recombinant vaccinia viruses expressing either NiV F and G (anti-rvv/NiV-FG) or measles H and F (anti-rvv/ChiH-EdF).

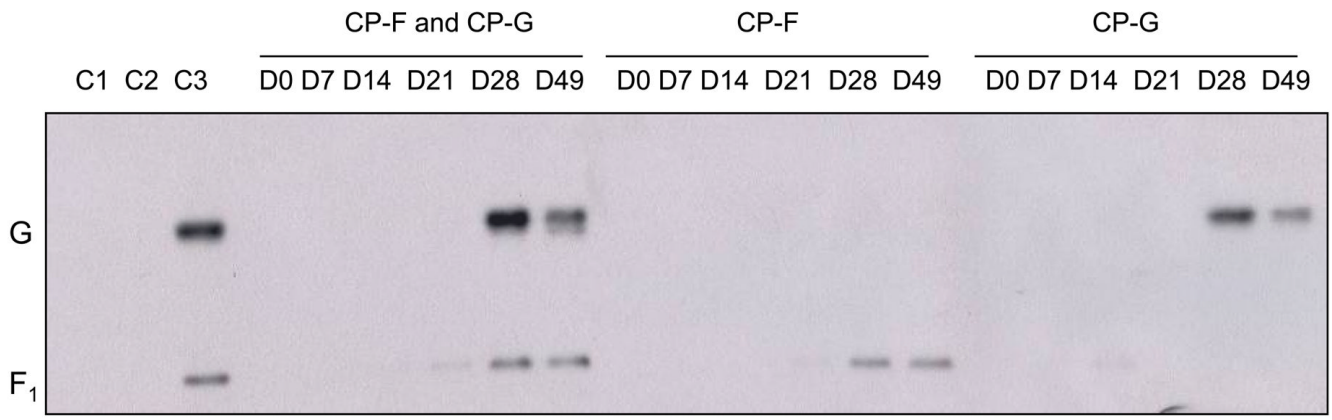

Figure 2.

Antibody responses following experimental vaccination for NiV. Figure shows results of the RIP assay. Antigens were prepared by infecting Vero cells with vaccinia viruses expressing NiV F and NiV-G and labeling with ^{35}S-methionine as described in Materials and Methods. Representative serum samples from pigs that had been vaccinated with recombinant canarypoxvirus vaccines expressing NiV glycoproteins, CP-F, CP-G, or CP-F and CP-G at 0 (D0), 7 (D7), 14 (D14), 21 (D21), 28 (D28), and 49 (D49) dpv were used. Lanes C1 and C2 show reactions with mock infected and labeled Vero cells and C3 shows reaction with HMAF prepared to NiV. RIP assays were performed as described in the Materials and Methods.

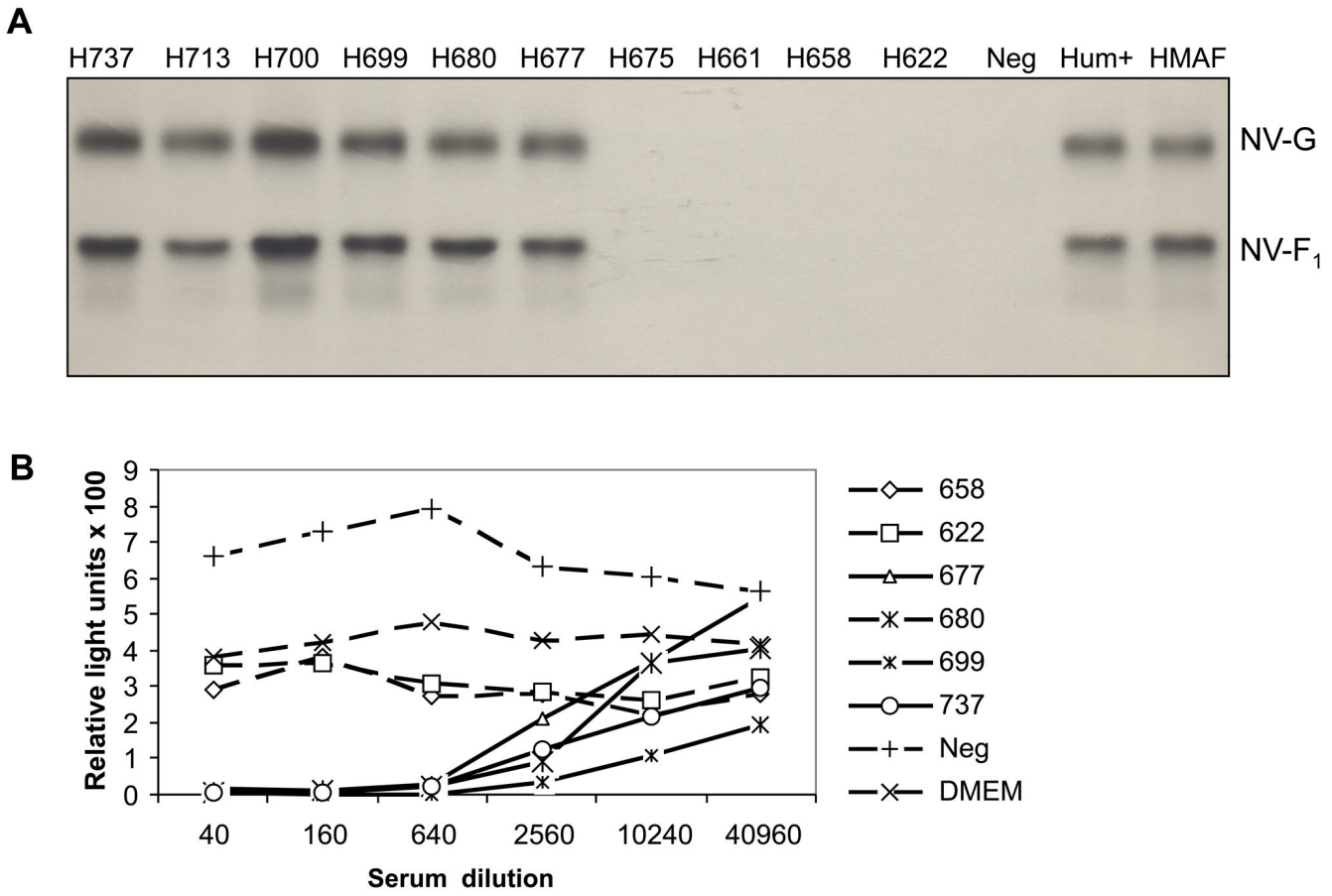

Figure 3.

Analysis of serum samples from NiV infected humans by RIP and pseudotype neutralization assay. Panel A shows immunoprecipitation of radio labeled NiV F and G (expressed from recombinant vaccinia virus) with human serum samples collected during outbreaks of NiV. Samples H737, H713, H700, H699 and H677 were from NiV-infected individuals; samples H675, H661, H658, H680 and H622 were from uninfected contacts. The controls include a serum sample from a volunteer in the laboratory (Neg), a positive control human serum sample from the initial outbreak of NiV in Malaysia (Hum+), and HMAF to NiV. Panel B shows the ability of the serum samples to inhibit luciferase activity in Vero cells infected with pVSV-NiV-F/G. Serum samples were tested at four fold dilutions beginning at 1:40 and the infections and luciferase assays were performed as described in the Materials and Methods. Negative controls samples are indicated by dashed lines and NiV positive samples have solid lines.

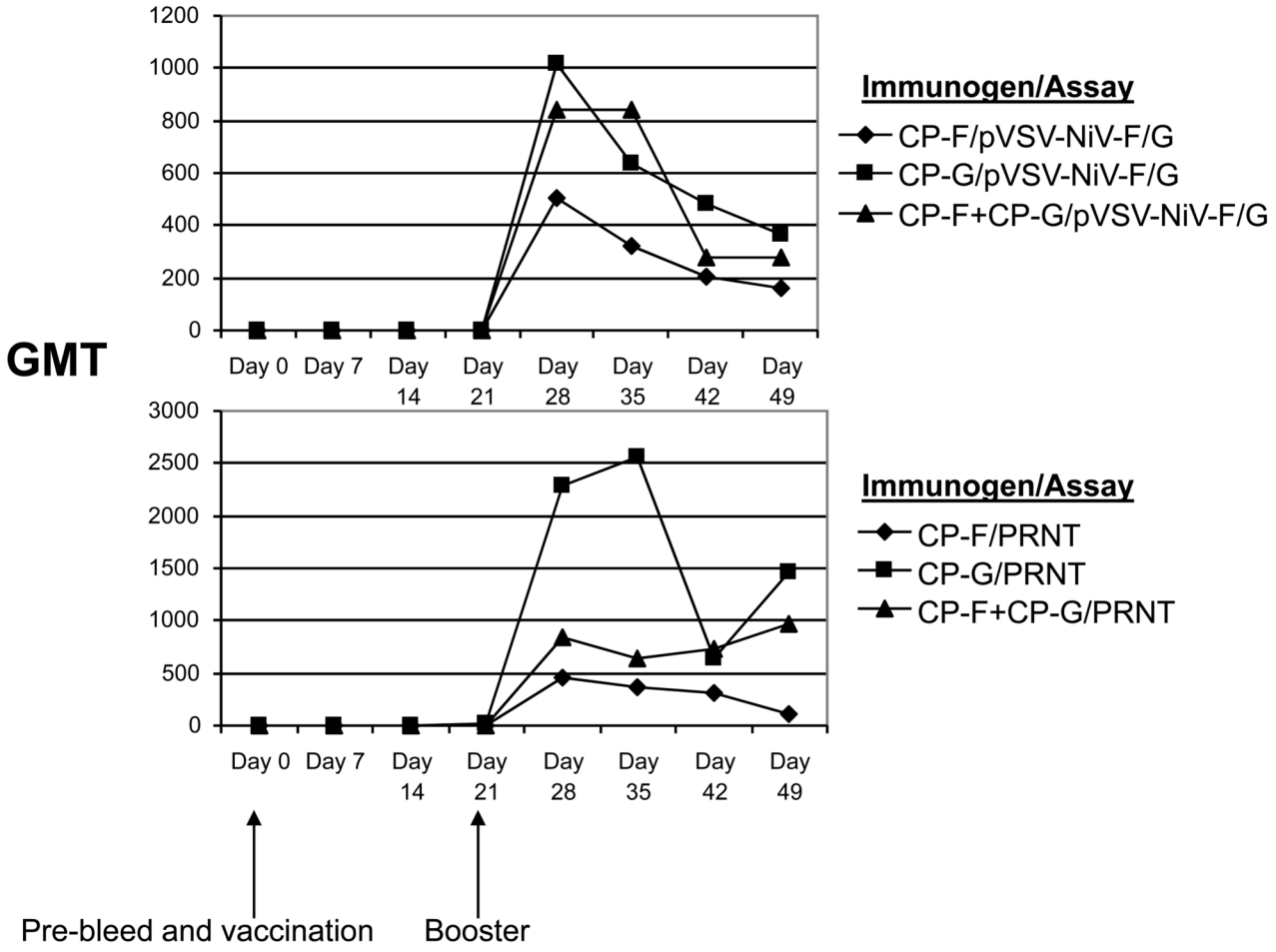

Figure 4.

Comparison of the results of PRNT with the neutralization assays using pVSV-NiV-F/G as antigen. Graphs show geometric mean titers (GMT) from both assays (pVSV-NiV-F/G in top graph; PRNT in lower graph) for serum collected 0, 7, 14, 21, 28, 35, 42 and 49 dpv (pigs receiving high dose vaccine only are shown).

Comparison of pVSV-NiV-F/G with PRNT:

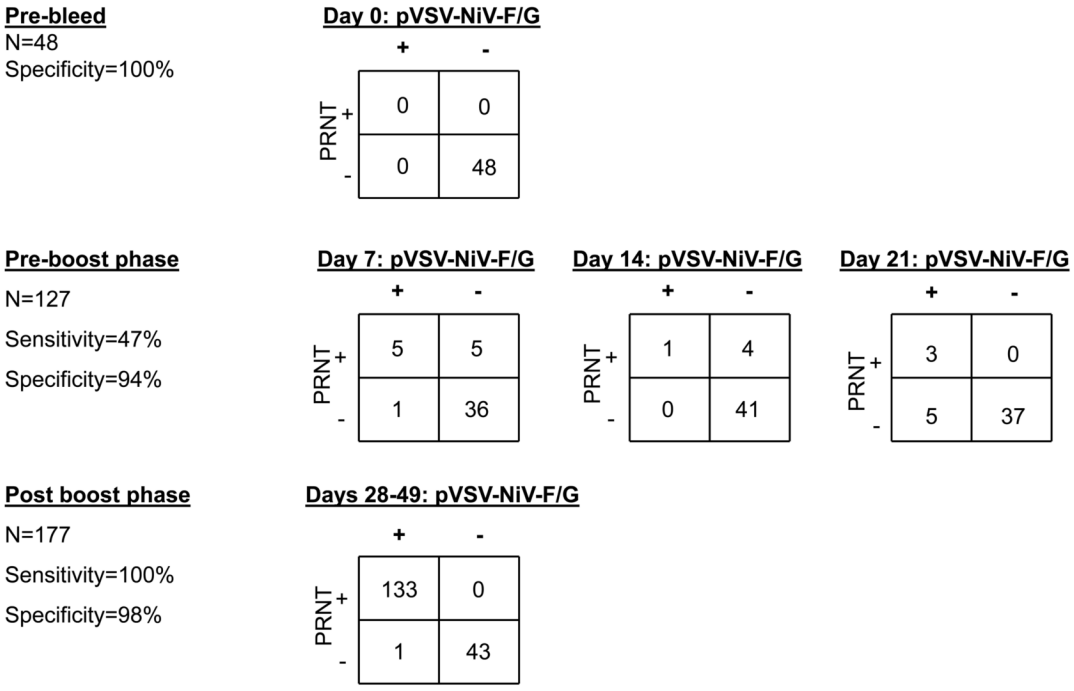

Figure 5.

Sensitivity and specificity of the pseudotype neutralization assays compared to PRNT. Figure shows comparison tables of the results from the neutralization assay using VSV-NiV-F/G and PRNT. Serum samples were collected at 0, 7, 14, 21, 28, 35, 42 and 49 dpv. All animals were boosted on day 21. Results from pigs receiving high dose and low dose vaccinations are included.

Table 1

Antigenic properties of VSV-NiV-FG pseudotype particles.

Antiserum[a]	PRNT[b]	pVSV-NiV-F/G[c]
vv/NiV-F	+	+
vv/NiV-G	+	+
vv/NiV-N	−	−
vv/MeV-F	−	−
NMS	−	−
pVSV-NiV F/G	ND[d]	+
Soluble NiV G	ND[d]	+
NHS	−	−
+ HS	+	+
CP	−	−
CP-NiV F, CP-NiV G	+	+

[a] Serum samples were from mice vaccinated with recombinant vaccinia viruses expressing NiV F (vv/NiV-F), NiV-G (vv/NiV-G), NiV-N (vv/NiV-N), measles virus F (vv/MeV-F) as described previously (Tamin et al., 2002; Tamin et al., 1994), normal mouse serum (NMS), rabbits vaccinated with pVSV-NiV-F/G or soluble NiV-G (Negrete et al., 2005; Negrete et al., 2006), normal human serum (NHS), serum from a human infected with NiV (+HS), and pigs vaccinated with the canarypox vector alone (CP) or the recombinant viruses expressing NiV-F and NiV-G (Weingartl et al., 2006).

[b] PRNT results based on previous publications (Harcourt et al., 2000; Weingartl et al., 2006).

[c] Results from the neutralization assay using pVSV-NiV-F/G as antigen described in this paper.

[d] ND=not done