Characterization of Immunodominant Linear B-Cell Epitopes on the Carboxy Terminus of the Rinderpest Virus Nucleocapsid Protein

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The nucleocapsid (N) protein of rinderpest virus (RPV) is one of the most abundant and immunogenic viral proteins expressed during natural or experimental infection. To identify immunogenic epitopes on the N protein, different forms of RPV N protein, including the full-length protein (N1-525), an amino-terminal construct (N1-179), and a carboxy-terminal construct (N414-496), were expressed in Escherichia coli as glutathione S-transferase (GST) fusion proteins. The antigenicity of each recombinant protein was evaluated by Western immunoblotting. All recombinants were recognized by hyperimmune RPV bovine antisera, indicating that immunoreactive epitopes may be present at both ends of the N protein. However, GST-N414-496 was much more antigenic than GST-N1-179 when tested with sera from vaccinated cattle, suggesting that an immunodominant or highly immunogenic epitope(s) may be located at the carboxy terminus of the N protein. Epitope mapping with overlapping peptides representing different regions of the carboxy terminus (amino acids 415 to 524) revealed three nonoverlapping antigenic sites in regions containing the residues 440VPQVRKETRASSR452 (site 1), 479PEADTDPL486 (site 2), and 520DKDL524 (site 3). Among these, antigenic site 2 showed the strongest reactivity with hyperimmune anti-RPV bovine sera in a peptide enzyme-linked immunosorbent assay but did not react with hyperimmune caprine sera raised against peste-des-petits-ruminants virus, which is antigenically closely related to RPV. Identification of an immunodominant linear antigenic site at the carboxy terminus of the N protein may provide an antigen basis for designing diagnostics specific for RPV.

Rinderpest is an acute and highly contagious viral disease causing high morbidity and mortality in cattle and wild bovine species. The disease has accounted for significant economic losses to the livestock industry in Africa, Europe, the Middle East, the Near East, and South Asia (27, 30). Rinderpest is now largely eradicated, but it is still endemic in some areas in East Africa and Asia (20, 27, 29).

Rinderpest virus (RPV), the causative agent of rinderpest, is an enveloped RNA virus belonging to the Morbillivirus genus in the family Paramyxoviridae. The other members of the genus include peste-des-petits-ruminants virus (PPRV), measles virus (MV), canine distemper virus, phocine distemper virus, and dolphin morbillivirus (1, 11). RPV, despite having a single genus, is antigenically closely related to RPV. Identification of an immunodominant linear antigenic site at the carboxy terminus of the N protein may provide an antigen basis for designing diagnostics specific for RPV.

Although the N protein is known to play an important role in humoral immunity against morbillivirus, many questions related to the locations and antigenicities of antigenic sites, especially immunodominant epitopes, that would be very useful for diagnostic applications remain unclear. Buckland et al. (4) have identified two antigenic sites (amino acids [aa] 457 to 476 and 519 to 523) on the carboxy terminus and one antigenic site (aa 122 to 150) on the amino terminus of the MV N protein by using N deletion mutants and monoclonal antibodies, but they did not investigate the degrees of immunogenicity of these epitopes. The carboxy-terminal region of the morbillivirus N protein protrudes from nucleocapsid core (16, 25, 26), so it has the potential to contain immunodominant antigenic sites, as found in N protein of Sendai virus, one of the morbilliviruses (13).

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In a previous study conducted by our laboratory (8), antigenic sites in the amino terminus of the N protein of RPV were mapped and identified by using anti-N monoclonal antibodies and N deletion mutants. However, results obtained by use of hyperimmune RPV antisera indicated that antigenic sites might exist in the carboxy terminus and that they might be more continuous (i.e., be linear epitopes) than ones in the amino terminus. In this study, linear antigenic sites in the carboxy terminus of the N protein of RPV were identified by using synthetic peptides and hyperimmune RPV antisera. The relative degree of antigenicity of each site was evaluated by using sera from RPV-infected cattle.

**MATERIALS AND METHODS**

**Virus.** The LATC strain of RPV (6), the seed virus for the production of rinderpest vaccine for emergency use in Korea, was used for the generation of recombinant N proteins. The LATC strain belongs to Asian lineage. The virus was propagated in Vero cells prepared in roller bottle cultures, concentrated, and semipurified as previously described (8).

**Preparation of full-length N and truncated mutants.** The pGEX recombinants carrying the full-length open reading frame and truncations of the RPV N protein gene were constructed by previously described procedures (8), using the following three sets of PCR primers with XhoI and NotI sites (underlined): NP-F1 (5′-CTGAGTTTACGTAATCGGTTCT-3′) and NP-R1 (5′-GGCCGCGCATTCTATTGCTGGG-3′) for pGEX-N1,252, NP-F1 and NP-R179 (5′-GGCCGCGCACAAGACCGAGATGCTGA-3′) for pGEX-N1,179, and NP-F141 (5′-CTGAGACGCCAGGCGGAGCTTG-3′) and NP-R496 (5′-GGCGGGCTTCTGCTGACTTCGCTCT-3′) for pGEX-N4,144-496. Three pGEX constructs containing full-length protein (pGEX-N1,252), the amino-terminal construct (pGEX-N1,179), or the carboxy-terminal construct (pGEX-N4,144-496) were transformed into Escherichia coli strain BL21 (Amersham Biosciences) and were expressed by using isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 0.2 mM. The resulting fusion proteins glutathione-S-transferase (GST)-N protein were constructed by previously described (8).

**Antisera.** Hyperimmune antisera raised against viruses strains representing three lineages of RPV (Asian lineage and African lineages I and II) and four lineages of PPRV (lineages I, II, III, and IV) were used in this study. Two bovine antisera against RPV African lineages I (aRPV-I) and II (aRPV-II) were kindly supplied by E. Couacy-Hymann (LANADA/LCPA, Bingerville, Ivory Coast). One bovine antisera against RPV Asian lineage (aRPV-Asian), stored in our laboratory, was used. One bovine antisera against RPV RBOK (vaccine) was supplied by G. Libeau (CIRAD-EMVT, Montpellier, France). Four goat antisera against PPRV lineages I (aPPRV-I), II (aPPRV-II), III (aPPRV-III), and IV (aPPRV-IV) were kindly supplied by E. Coaucy-Hymann (LANADA/LCPA, Bingerville, Ivory Coast). All hyperimmune sera had neutralizing antibody titers of ≥1:256 against the respective virus. In addition to hyperimmune goat sera, anti-PPRV bovine sera were obtained by the courtesy of E. Couacy-Hymann in Africa. These sera were already confirmed positive by cross neutralization tests for PRV and PPRV (data not shown).

Anti-RPV sera were also obtained from 10 Holstein cows vaccinated with an attenuated RPV vaccine (LATC strain) at a rate of approximately 10^7.5 50% tissue culture infective doses per dose and used for the study. Sera designated 1 to 10 were collected from all vaccinated cattle 3 weeks after vaccination. All animals were kept in a biosafety level 3 containment animal facility during the entire study period, in accordance with regulations of the Korean government.

Besides antisera raised in the natural host of morbillivirus, polyclonal antibodies were generated by immunizing rabbits (aRPV-Asian, aRPV-I, aRPV-II, aRPV-III, aRPV-I, aPPRV-I, aPPRV-II, aPPRV-III, and aPPRV-IV), vaccine (no. 1 to 10), and N protein-specific (aN1,179) antisera. GST was used as a control protein. A 50-μl volume of one of the purified fusion proteins or GST control protein was added alternately to a row of wells in each of the ELISA plates (MaxiSorp; Nunc, Roskilde, Denmark) at a concentration of 1.0 μg/ml in 0.01 M PBS containing 3% skim milk, 0.5% rabbit serum, 1% bacterial lysates, and 0.05% Tween 20 and 50 μl of each dilution was added to a pair of two wells (one with one of the recombinant fusion protein and the other with GST control protein). After 60 min of incubation at 37°C, the plates were washed three times with 0.002 M PBS containing 0.05% Tween 20 (PBST). After incubation for 60 min at 37°C, the plates were washed with PBST. Antigen-antibody reactions were visualized by adding 50 μl of optimally diluted anti-species IgG labeled with peroxidase (Kirkegaard-Perry Laboratories, Inc.) to each well, incubating for 1 h at 37°C, and then adding ortho-phenylenediamine substrate. After 10 min, the colorimetric reaction was stopped by adding 1.25 M sulfuric acid. The optical density (OD) of each well was measured at a wavelength of 492 nm. The net absorbance of each serum sample was corrected by subtracting the OD for the GST control from that for the recombinant fusion protein. Sera with net OD values of ≥0.2 were considered positive. The test was repeated three times.

**Preparation of synthetic peptides.** Twelve overlapping synthetic peptides covering the carboxy terminus (aa 414 to 524) were used in this study. Their amino acid sequences were based on those of the N protein of the RPV LATC strain (Table 1). Nonoverlapping peptide P1 was used as an unrelated control peptide. All synthetic peptides were produced by a commercial manufacturer (Peptron Co., Daejeon, Korea) by the 9-fluorenlymethoxycarbonyl solid-phase method on amide resin. Peptides synthesized on the resin were cleaved from the resin with reagent K (950 μl of trифluoroacetic acid, 25 μl of tri-isopropyl silane, and 25 μl of water), precipitated with a mixture of ether and hexanes at a ratio of 2:1, washed with distilled water, and dried by lyophilization. For each well, one of the purified peptide products were adjusted to a concentration of 5 mg/ml in distilled water. The amino acid position in the native N protein corresponding to the first amino acid of the peptide is indicated in parentheses.

**TABLE 1. Synthetic peptides used for identifying antigenic sites**

| Peptide* | Amino acid sequence (5′ to 3′) |
|----------|-------------------------------|
| R415     | (415).................................TOYSLRTDGQGEY |
| R418     | (418).................................CTOYSLRTDGQGEY |
| R440     | (440).................................VPOYRKTRESSRSDRYK |
| R453     | (453).................................SDRYKEDTNDN |
| R457     | (457).................................KEDTDNESVPSVP |
| R467     | (467).................................PSVKTILDVDT |
| R470     | (470).................................KTLIDVDTTPEADTDPL |
| R475     | (475).................................LITDVTADPTDGLKSK |
| R487     | (487).................................GSKKSEAI |
| R488     | (488).................................SKKSEAEALLKLQAM |
| R501     | (501).................................MASILEGLSTGNLRTVY |
| R508     | (508).................................STLGNDLRTYNDKDL |
| P1       | (control)............................CPVNTEGKVMSIEAY |

* The amino acid position in the native N protein corresponding to the first amino acid of the peptide is indicated in parentheses.

 purchased from a commercial supplier (Amersham Biosciences) and used to identify fusion protein-reactive antibodies.

**SDS-PAGE and Western immunoblotting.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein materials (i.e., purified whole virus or recombinant GST fusion proteins) was performed with 12% vertical slab gels under denaturing conditions (21). Polypeptides separated on a polyacrylamide gel were subsequently electrotransferred to nitrocellulose membranes (36). Immunoblotting was performed by standard techniques with 1:100-diluted antiserum, species-specific anti-immunoglobulin G (anti-IgG) conjugated with alkaline phosphatase (1:1,000), and a BCIP (5-bromo-4-chloro-3-indolylphosphate)-nitroblue tetrazolium solution (Kirkegaard-Perry Laboratories, Inc.) as the substrate.

**GST capture ELISA.** The immunoactivity of whole or partial recombinant GST fusion N proteins was assessed by using hyperimmune (aRPV-Asian, aRPV-I, aRPV-II, aRPV-III, aRPV-I, aPPRV-I, aPPRV-II, aPPRV-III, and aPPRV-IV), vaccination (no. 1 to 10), and N protein-specific (aN1,179) antisera. GST was used as a control protein. A 50-μl volume of one of the purified fusion proteins or GST control protein was added alternately to a row of wells in each of the ELISA plates (MaxiSorp; Nunc, Roskilde, Denmark) at a concentration of 1.0 μg/ml in 0.01 M PBS containing 3% skim milk, 0.5% rabbit serum, 1% bacterial lysates, and 0.05% Tween 20 and 50 μl of each dilution was added to a pair of two wells (one with one of the recombinant fusion protein and the other with GST control protein). After 60 min of incubation at 37°C, the plates were washed three times with 0.002 M PBS containing 0.05% Tween 20 (PBST). After incubation for 60 min at 37°C, the plates were washed with PBST. Antigen-antibody reactions were visualized by adding 50 μl of optimally diluted anti-species IgG labeled with peroxidase (Kirkegaard-Perry Laboratories, Inc.) to each well, incubating for 1 h at 37°C, and then adding ortho-phenylenediamine substrate. After 10 min, the colorimetric reaction was stopped by adding 1.25 M sulfuric acid. The optical density (OD) of each well was measured at a wavelength of 492 nm. The net absorbance of each serum sample was corrected by subtracting the OD for the GST control from that for the recombinant fusion protein. Sera with net OD values of ≥0.2 were considered positive. The test was repeated three times.
Expression and characterization of the GST fusion proteins. GST-N₁₋₅₂₅, GST-N₁₋₁₇₉ and GST-N₄₁₄₋₄₉₆ and GST polypeptides were expressed with apparent molecular masses of 87, 48, 37, and 28 kDa, respectively, which correlated with the predicted molecular mass of each product (Fig. 1A). The presence of recombinant GST fusion protein and GST itself was confirmed by Western immunoblotting with anti-GST antibody (Fig. 1B) and anti-N polypeptide antibody (Fig. 1C). Their expression was optimized by inducing the cells with 0.2 mM IPTG for 6 h at room temperature. After purification on a glutathione-Sepharose 4B affinity column, the recombinant GST fusion N proteins were recovered at concentrations of approximately 4, 3.8, and 4 mg per liter of culture, respectively. GST itself was recovered at a concentration of 4 mg per liter of culture after affinity column chromatography. GST and two truncated forms of the N protein (GST-N₁₋₁₇₉ and GST-N₄₁₄₋₄₉₆) were successfully expressed in soluble form, while the full-length N protein (GST-N₁₋₅₂₅) was insoluble under nondenaturing conditions after mechanical lysis of the bacteria and incubation with 5% Triton X-100 (data not shown). Insoluble protein GST-N₁₋₅₂₅ was successfully extracted from bacterial cells after treatment with lysozyme (0.2 mg/ml) and sodium dodecyl sulfate. Insoluble protein GST-N₁₋₅₂₅ was insoluble under nondenaturing conditions and the full-length N protein (GST-N₁₋₅₂₅) proteins were assessed by Western immunoblot analysis and ELISA with three hyperimmune RPV antisera (αRPV-Asian, αRPV-Ⅰ, and αRPV-Ⅱ). All three forms of recombinant N protein reacted with the RPV-specific bovine hyperimmune antisera in Western immunoblotting (Fig. 2), suggesting that both the carboxy-terminal and the amino-terminal regions of the protein contained an antigenic determinant(s) recognized by antibodies raised against RPV in cattle. The reactivity pattern was similar to that with anti-N polypeptide guinea pig antisera (Fig. 1C). However, based on band intensity, there was a difference between the amino-terminal form (GST-N₁₋₁₇₉) and the carboxy-terminal form (GST-N₄₁₄₋₄₉₆) in the degree of antigenicity of GST-N fusion proteins, their reactivities with sera from cattle vaccinated against RPV, and the reactivities with sera from cattle vaccinated against RPV, in which relatively low levels of RPV neutralizing antibody (1:8 to 1:16) were detected, were evaluated by Western immunoblotting. As shown in Fig. 3, eight sera reacted with both the native N (whole virus) and the full-length N (GST-N₁₋₅₂₅) proteins.

RESULTS

Expression and characterization of the GST fusion proteins. GST-N₁₋₅₂₅, GST-N₁₋₁₇₉ and GST-N₄₁₄₋₄₉₆ and GST polypeptides were expressed with apparent molecular masses of 87, 48, 37, and 28 kDa, respectively, which correlated with the predicted molecular mass of each product (Fig. 1A). The presence of recombinant GST fusion protein and GST itself was confirmed by Western immunoblotting with anti-GST antibody (Fig. 1B) and anti-N polypeptide antibody (Fig. 1C). Their expression was optimized by inducing the cells with 0.2 mM IPTG for 6 h at room temperature. After purification on a glutathione-Sepharose 4B affinity column, the recombinant GST fusion N proteins were recovered at concentrations of approximately 4, 3.8, and 4 mg per liter of culture, respectively. GST itself was recovered at a concentration of 4 mg per liter of culture after affinity column chromatography. GST and two truncated forms of the N protein (GST-N₁₋₁₇₉ and GST-N₄₁₄₋₄₉₆) were successfully expressed in soluble form, while the full-length N protein (GST-N₁₋₅₂₅) was insoluble under nondenaturing conditions after mechanical lysis of the bacteria and incubation with 5% Triton X-100 (data not shown). Insoluble protein GST-N₁₋₅₂₅ was successfully extracted from bacterial cells after treatment with lysozyme (0.2 mg/ml) and sodium dodecyl sulfate (0.5%).

Antigenicity of GST-N fusion proteins. The antigenicities of three different forms of recombinant GST-N fusion proteins were assessed by Western immunoblot analysis and ELISA with three hyperimmune RPV antisera (αRPV-Asian, αRPV-Ⅰ, and αRPV-Ⅱ). All three forms of recombinant N protein reacted with the RPV-specific bovine hyperimmune antisera in Western immunoblotting (Fig. 2), suggesting that both the carboxy-terminal and the amino-terminal regions of the protein contained an antigenic determinant(s) recognized by antibodies raised against RPV in cattle. The reactivity pattern was similar to that with anti-N polypeptide guinea pig antisera (Fig. 1C). However, based on band intensity, there was a difference between the amino-terminal form (GST-N₁₋₁₇₉) and the carboxy-terminal form (GST-N₄₁₄₋₄₉₆) in the degree of antigenicity of GST-recombinant N fusion proteins, their reactivities with sera from cattle vaccinated against RPV, and the reactivities with sera from cattle vaccinated against RPV, in which relatively low levels of RPV neutralizing antibody (1:8 to 1:16) were detected, were evaluated by Western immunoblotting. As shown in Fig. 3, eight sera reacted with both the native N (whole virus) and the full-length N (GST-N₁₋₅₂₅) proteins.
With the same set of antisera, the GST-N\textsubscript{414-496} reacted with seven of the sera, whereas the GST-N\textsubscript{1-179} reacted only with serum 1, suggesting that highly immunogenic epitopes were present within the carboxy terminus.

**Identification of immunodominant epitopes on the carboxy terminus.** Immunodominant epitopes at the carboxy terminus of N protein were investigated by use of overlapping peptides covering the carboxy terminus (aa 415 to 524) of the N protein. Guinea pig polyclonal antibodies (\(\alpha\text{H9251 N1-525}\)) recognized 4 peptides (R440, R470, R473, and R508), as illustrated in Fig. 4. Comparison of amino acid sequences of immunoreactive peptides revealed that the carboxy terminus of the N protein possessed at least three nonoverlapping antigenic sites. The first antigenic site (site 1) was considered to be present in residues 440VPQVRKETRASSR452 of immunoreactive peptide R440, since peptide R440 shared amino acid residues 453SDRYK457 with nonimmunoreactive peptide R453. The second antigenic site (site 2) appeared to be in residues 479PEADTDPL486, since these residues were included in immunoreactive peptides (R470 and R473) but not in nonimmunoreactive peptide R487. The last antigenic site (site 3) was considered to be in residues 520DKDLL524 of immunoreactive peptide R508, since R508 shared residues 508PTLGNDSLRTYN519 with nonimmunoreactive peptide R501.

**Antigenicity and specificity of each epitope on the carboxy terminus.** Although all antigenic sites were recognized equally by the anti-N polypeptide antibody (\(\alpha\text{N}_{1-525}\)) in the peptide ELISA, peptides representing antigenic site 2 showed much stronger reactivity with the hyperimmune PRV-specific bovine antisera than with peptides representing the other two antigenic sites (Fig. 4). Similar observations were also made with sera from RPV-infected cattle (Table 2). Peptide R470 (antigenic site 2) was recognized by six of seven sera that reacted with GST-N\textsubscript{414-496}, whereas none and one of seven sera reacted with peptides R440 (antigenic site 1) and R508 (antigenic site 3), respectively. These results indicated that site 2 may be more antigenic or immunodominant than the other two sites when cattle are infected with RPV. Antigenic sites 2 and 3 reacted with all hyperimmune RPV sera, whereas site 1 reacted with \(\alpha\text{RPV-Asian}\) only (Table 2). Antigenic sites 1 and 2 did not show reactivity with sera from goats and cattle infected with PPRV, while antigenic site 3 showed reactivity to some PPRV hyperimmune caprine sera.

**DISCUSSION**

In this study, Western immunoblot analyses of denatured GST-recombinant N fusion proteins with hyperimmune anti-RPV bovine sera (\(\alpha\text{RPV-Asian}, \alpha\text{RPV-I}, \alpha\text{RPV-II}\)) and anti-N polypeptide antibody (\(\alpha\text{N}_{1-525}\)) demonstrated that both ends of the N protein were antigenic. This observation is consistent with reports on MV N protein (4). Furthermore, both full-length N recombinant protein (GST-N\textsubscript{1-525}) and the GST fusion protein representing the carboxy terminus of the N protein (GST-N\textsubscript{414-496}) showed stronger reactivity with antisera in Western immunoblotting (Fig. 2) than did the fusion protein (GST-N\textsubscript{1-179}) containing the amino terminus of the N
protein. This suggests the presence of highly immunogenic epitopes on the carboxy terminus of RPV N protein that are probably independent of conformation and/or are natively unfolded (26). This would not be a surprise, since the carboxy terminus is outside a normally folded protein (16, 25, 26). GST-N1-525 also detected anti-N antibodies in all of the vaccinated cattle by indirect ELISA (Table 2), suggesting that the MV N protein. Our results from epitope mapping with overlapping peptides and polyclonal antibodies to both native and recombinant N proteins were used to map linear epitopes and assess the comparative antigenicities of identifiable epitopes. To our knowledge, this approach was the first attempt at defining epitopes on the N proteins of the morbilliviruses. Since amino-terminal epitopes of RPV N protein were identified in previous studies (7, 8), we attempted to determine the genomic locations of epitopes on the carboxy terminus of RPV N protein and obtained results similar to those for MV N protein (4). At least three antigenic sites (sites 1 to 3) were identified on the carboxy-terminal part of the N protein by using polyclonal anti-N polypeptide antiserum (αN1-525), as illustrated in Table 2 and Fig. 4. Both antigenic sites 1 and 3 were strongly recognized by αN1-525 but showed weak or no reactivity with hyperimmune anti-RPV bovine sera, which is probably due to weaker immunogenicity. In contrast, antigenic site 2 reacted strongly with both αN1-525 and these hyperimmune sera. The greater immunogenicity of antigenic site 2 was also demonstrated with sera from RPV-infected cattle (Table 2). Several factors might account for the difference in immunogenicity between recombinant N protein and RPV (i.e., native N protein). First, perhaps the antigenicities of sites 1 and 2. Several factors might account for the difference in immunogenicity between recombinant N protein and RPV (i.e., native N protein). First, perhaps the antigenicities of sites 1 and 2 were somewhat different from those with anti-N polypeptide guinea pig serum (αN1-525), as illustrated in Table 2 and Fig. 4. Both antigenic sites 1 and 3 were strongly recognized by αN1-525 but showed weak or no reactivity with hyperimmune anti-RPV bovine sera, which is probably due to weaker immunogenicity. In contrast, antigenic site 2 reacted strongly with both αN1-525 and these hyperimmune sera. The greater immunogenicity of antigenic site 2 was also demonstrated with sera from RPV-infected cattle (Table 2). Several factors might account for the difference in immunogenicity between recombinant N protein and RPV (i.e., native N protein). First, perhaps the antigenicities of sites 1 and 3 were reduced by conformational changes through formation of N–P complexes in infected cattle, since the carboxy terminus is intrinsically disordered and folds upon binding to the carboxy-terminal moiety of the phosphoprotein (3, 25, 26, 33). Second, the specificity of both species and the route of inoculation may also contribute to some degree of immunodominance. An alternative explanation, aside from actual differences in antigenicity, is that other protein regions may contribute to the antibody response. Antigenic site 2 may interact with other immune components, such as a helper T-cell

### Table 2: Reactivities of hyperimmune, vaccination, and N protein-specific sera with various recombinant GST-N fusion proteins and synthetic peptides

| Serum tested | GST capture ELISA | Peptide ELISA |
|--------------|-------------------|---------------|
|              | GST-N1-525 | GST-N1-179 | GST-N414-496 | R440 (site 1) | R470 (site 2) | R508 (site 3) |
| Hyperimmune sera |            |            |            |            |            |            |
| αN1-525      | +        | +        | +        | +        | +        | +        |
| αRPV-Asian   | +        | +        | +        | +        | +        | +        |
| αRPV-I       | +        | +        | +        | +        | +        | +        |
| αRPV-II      | +        | +        | +        | +        | +        | +        |
| αRPV-RBOK    | +        | +        | +        | +        | +        | +        |
| αPPRV-I      | +        | −        | −        | −        | −        | +        |
| αPPRV-II     | +        | −        | −        | −        | −        | −        |
| αPPRV-III    | +        | −        | −        | −        | −        | −        |
| αPPRV-IV     | +        | −        | −        | −        | −        | −        |
| Bovine sera |            |            |            |            |            |            |
| RPV positive | 10/10 (8/10) | 1/10 (1/10) | 7/10 (7/10) | 0/10 | 6/10 | 1/10 |
| PPRV positive | NTa | NT | NT | 0/13 | 0/13 | 0/13 |

*For the GST capture ELISA, sera with net OD values of ≥0.2 were considered positive; for the peptide ELISA, sera with T/C values of ≥2.0 were considered positive. For bovine sera, results are shown as number of sera positive/number of sera tested, and data in parentheses represent the results of Western immunoblotting (see Fig. 3).

a NT, not tested.
epitope, influencing the repertoire specificity of antibody-producing cells (2, 31). In RPV, a hypervariable region (aa 452 to 501) of the N protein has been identified as a helper T-cell epitope (28). If this was the case in the antibody response to NP, differences in the response elicited by the site may affect properties of other sites, which remain to be further investigated.

Cross-reactivity between PRV and PPRV has been a diagnostic challenge, particularly for serological monitoring in areas of endemicity, which raises the need for a diagnostic antigen conserved among PRV strains but distinct from PPRV. In areas of endemicity, which raises the need for a diagnostic antigen conserved among PRV strains but distinct from PPRV. In endemic areas, which raises the need for a diagnostic antigen conserved among PRV strains but distinct from PPRV.

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