Development of an Immunoglobulin M (IgM) Capture Enzyme-Linked Immunosorbent Assay for Detection of Equine and Swine IgM Antibodies to Vesicular Stomatitis Virus

EN-MIN ZHOU,* JOSE RIVA, AND ALFONSO CLAVIJO†
National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada

Received 19 October 2000/Returned for modification 11 December 2000/Accepted 16 January 2001

Vesicular stomatitis (VS) is a contagious viral disease that primarily affects cattle, horses, swine (7, 19), and some wild ungulates (9) in enzootic and epizootic forms in the tropical and subtropical areas of the Americas. VS, an important disease in the United States and many South American countries, spreads rapidly and has serious socio-economic and public health consequences. It is identified as a List A disease by the Office International des Epizooties (13) and is important in the health consequences. It is identified as a List A disease by the Office International des Epizooties (13) and is important in the international trade of animals and animal products. Vesicular stomatitis virus (VSV) is a Rhabdovirus of the Vesiculovirus genus with potential for arthropod transmission (7, 8, 17). Two VSV serotypes, VSV New Jersey (VSV-NJ) and VSV Indiana (VSV-IN), are serologically distinct and are of major etiological concern because both cause infections in cattle, horses, and swine. These viruses are morphologically similar, have some common antigens, and produce overt infection with similar lesions in susceptible animals. Early diagnosis of VS is necessary for the differential diagnosis of other vesicular diseases. Serodiagnostic tests available for distinguishing VSV-NJ and VSV-IN are neutralization tests (5, 15, 18), indirect enzyme-linked immunosorbent assay (I-ELISA) (1), and competitive ELISA (C-ELISA) (2). These tests are reliable but have some limitations. They can detect early and long-lasting antibody responses, but because of the nature of these assays, they are not able to differentiate a primary from a secondary VSV infection.

In a primary viral infection, immunoglobulin M (IgM) class antibody is the first to appear in the blood circulation, and it disappears shortly after the IgG antibodies develop (6). Because of the ontogeny of the antibody response, detection of specific IgM antibodies provides a differential serodiagnosis of virus infection. Thus, Vernon and Webb developed an IgM capture ELISA (MC-ELISA) that detected the recent infection of horse and cattle with VSV-NJ (16). In their assay, the ELISA plates were directly coated with rabbit anti-equine and anti-bovine IgM antibodies, and IgM antibodies to VSV-NJ were detected as early as 6 days postinfection (DPI). The objective of our study was to develop an MC-ELISA using an avidin-biotin system for detection of IgM anti-VSV-NJ and anti-VSV-IN antibodies from horses and pigs. The MC-ELISA utilized the unique nature of a biotin derivative, EZ-Link Sulfo-NHS-LC-Biotin, which has an extended space arm to reduce both steric hindrance and interference with the biological activity of the coupled IgG (3). The MC-ELISA was used for detection of both anti-VSV-NJ and anti-VSV-IN antibodies from horses and pigs in comparison with the C-ELISA and serum neutralization test.

**MATERIALS AND METHODS**

**VSV antigens.** VSV antigens were produced from Vero cells infected with the Ogden strain of VSV-NJ and San Juan strain of VSV-IN serotypes using a method described by Afshar et al. (1). These antigens were used in the MC-ELISA and the C-ELISA for detection of antibodies to VSV-NJ and VSV-IN, respectively, as described below.

**Purification and titration of mouse antibodies to VSV-NJ and VSV-IN.** Each 5 ml of mouse ascitic fluids produced and provided by Afshar et al. (2) against either VSV-NJ or VSV-IN serotypes was precipitated with 50% (vol/vol) saturated ammonium sulfate. Each globulin preparation was resuspended in 5 ml of phosphate-buffered saline (PBS) and was passed through a Sephacryl S-300 high-resolution gel filtration column (Pharmacia Biotech, Inc., Baire d’Urfe, Quebec, Canada). The fractions representing peak IgG were collected and concentrated using a microconcentrator with a molecular weight (MW) cutoff of

---

*M Corresponding author. Present address: Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, 2630 Veterinary Medicine Building, Iowa State University, Ames, IA 50011. Phone: (515) 294-4699. Fax: (515) 294-3564. E-mail: ezhou@iastate.edu.

† Present address: Pan American Foot-and-Mouth Disease Center Rio de Janeiro, Brazil.
50,000 (Millipore Canada Ltd., Nepean, Ontario, Canada). Protein concentration was calculated based on the extinction coefficient of 13.5 for a 1% preparation at an optical density at 280 nm (OD280).

An I-ELISA was used to titrate the purified mouse anti-VSV antibodies. The optimal dilution of puriﬁed IgM antibodies and the equine or swine serum samples in PBS-T. The purified IgM antibodies and the equine or swine serum samples in PBS-T were added into the wells (100 μl/well) and incubated for 1 h at 37°C. After removing unbound materials by washing ﬁve times with PBS-T, wells were ﬁlled with 100 μl of a 1:2,000 dilution of horseradish peroxidase-labeled goat anti-mouse IgG (Bethyl Laboratories, Montgomery, Tex.) in PBS-T and incubated at 37°C for 1 h. The substrate used was 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma Chemical Company, St. Louis, Mo.) in 0.05 M citrate buffer (pH 4.5) and 0.3% H2O2. The OD405 of each well was measured on an automatic ELISA plate reader (TiterTek plus; ICN Flow; Biomedical, Inc., Costa Mesa, Calif.).

Equine and swine serum samples. Sequential blood samples were collected from one horse (H-1) and two pigs (P-35-94 and P-36-94) experimentally infected with VSV-NJ and VSV-IN serotypes, respectively. Each 5 ml of equine serum was passed through a Sephacryl S-300 high-resolution gel ﬁltration column (Pharmacia Biotech, Inc.). Fractions containing IgM molecules were collected and concentrated to their original 5-ml volume using a microconcentrator with an MW cutoff of 100,000 (Millipore Canada Ltd.). A concentration of total IgM molecules of 3 mg/ml was determined using a method previously described (11). Their speciﬁc binding to VSV was experimentally infected with 105 TCID50 of VSV-NJ and one horse (H-107) infected with the same dose of VSV-IN, as described previously (10).

Puriﬁcation of IgM antibodies from equine serum samples. IgM molecules were puriﬁed from two serum samples collected at 6 DPI from two horses (H-1 and H-2) experimentally infected with VSV-NJ and VSV-IN serotypes, respectively. Each 5 ml of equine serum was passed through a Sephacryl S-300 high-resolution gel ﬁltration column (Pharmacia Biotech, Inc.). Fractions containing IgM molecules were collected and concentrated to their original 5-ml volume using a microconcentrator with an MW cutoff of 100,000 (Millipore Canada Ltd.). A concentration of total IgM molecules of 3 mg/ml was determined using the methods described by Lowery et al. (11). Their speciﬁc binding to VSV antigen was detected and titrated by the MC-ELISA, as described below.

Biotechnique of sheep antibodies against equine and swine IgM molecules. The EZ-Link Sulfo-NHS-LC-Biotin purchased from Pierce (Rockford, Ill.) was used for biotinylating sheep IgG antibodies against equine and swine IgM according to the manufacturer’s instructions. Briefly, 2 mg of sheep IgG antibodies against equine or swine IgM molecules (Cedarlane Laboratories Ltd., Canada, Hornby, Ont., Canada) in 1 ml of 50 mM sodium bicarbonate buffer, pH 8.5, was mixed with 74 μl of EZ-Link Sulfo-NHS-LC-Biotin (1 mg/ml in water). The reaction between sheep IgG and biotin was allowed to proceed for 2 h on ice, and free biotin was removed using a microconcentrator (MW cutoff of 5,000). The biotinylated sheep antibody preparations were mixed with equal volume of glycerol and stored at −20°C. The optimal dilution of the biotinylated sheep antibody preparations was determined using a checkerboard titration method. Various dilutions of the biotinylated sheep antibody preparations were bound to the streptavidin-coated plate for 1 h at 37°C followed by three washing steps with PBS-T. Equine and swine IgM antibodies at various concentrations were added to the plate and incubated for 1 h at 37°C, and this was followed by washing steps. The conjugate was sheep antibody against equine or swine IgM coupled to horseradish peroxidase at a dilution of 1:3,000 in dilution buffer (100 μl/well). The substrate was ABTS, as recommended by the manufacturer (Cedarlane Laboratories Ltd., Canada). The substrate was ABTS, as recommended by the manufacturer (Cedarlane Laboratories Ltd., Canada). The substrate was ABTS, as recommended by the manufacturer (Cedarlane Laboratories Ltd., Canada). The substrate was ABTS, as recommended by the manufacturer (Cedarlane Laboratories Ltd., Canada).

RESULTS

Puriﬁcation and titration of mouse antibodies. Mouse polyclonal IgG antibodies against VSV-NJ and VSV-IN were puriﬁed individually and titrated against the homologous and heterologous antigens. The results shown in Fig. 1 demonstrated that the endpoint titers of 0.78 and 1.2 μg/ml were generated for the anti-VSV-NJ antibody (F38–51) and the anti-VSV-IN antibody (F39–52), respectively. The endpoint titer was determined by the dilution of antibody preparation giving an OD410 value of 1.0 after 10 min of development time in the I-ELISA. The 0.78- and 1.2-μg/ml concentrations of antibody preparations against VSV-NJ and VSV-IN, respectively, were used in the MC-ELISA and C-ELISA.

Sensitivity of MC-ELISA. The puriﬁed IgM antibodies and nonpurified sera from two horses (H-1 and H-2) were used to determine the sensitivity of the MC-ELISA. As demonstrated in Fig. 2, the endpoint titer for both the serum sample and puriﬁed IgM antibodies against VSV-NJ was 1:50,000. Similarly, the antibodies against VSV-IN were detected with the endpoint titer of 1:8,000 for the serum sample and 1:50,000 for the puriﬁed IgM antibodies. No cross-reaction between homologous and heterologous anti-VSV antibodies was observed, and no IgM antibodies in prebleed serum samples were detected.

Detection of antibodies from equine serial bleed samples. The MC-ELISA was compared with the C-ELISA and MTSN test for detection of equine antibodies to VSV (Table 1). For VSV-NJ IgM antibody detection, sequential serum samples were collected from three horses infected with VSV-NJ. In the MC-ELISA, using a cutoff value of an OD of 0.3, IgM antibodies from animal H-1 were detected in the 5-DPI sample. Earlier samples (0, 1, and 3 DPI) were negative for IgM antibodies. Similarly, IgM anti-VSV-NJ antibodies were detected at 6 DPI in sera collected from two other animals (H-106 and
H-109). The samples collected from these two animals between 0 and 4 DPI were not available in this study, but Katz et al. (10) previously reported them to be negative for VSV antibodies by the complement fixation test, IgM capture ELISA, serum neutralization test, and C-ELISA (10). The IgM antibodies from these animals were detected for up to 35 DPI. The peak IgM titer was observed between 8 and 11 DPI, with an average OD of 1.04.

For VSV-IN IgM antibody detection, two horses (H-2 and H-107) were infected with VSV-IN. IgM antibodies against VSV-IN were detected in these samples by the MC-ELISA at 5 and 6 DPI. Antibodies remained at high levels up to 12 DPI (OD > 1.2) and diminished after 28 DPI. No IgM antibodies were detected before 5 DPI in samples from animal H-2 (Table 1) or animal H-107 by another IgM capture ELISA (10).

In the heterologous MC-ELISA, no cross-reactions were observed in samples from animals H-1 and H-2 (Table 1), and only a low level of cross-reaction was seen in samples from three other animals (H-106, H-109, and H-107). The cross-reactions in the VSV-IN MC-ELISA were detected for animals H-106 and H-109 (infected with VSV-NJ) at 10, 11, and 14 DPI and at 10 and 11 DPI, respectively. Cross-reactive samples produced ODs of 0.46 or less. A similar cross-reaction was observed in samples from one VSV-IN-infected animal (H-107) in the VSV-NJ MC-ELISA at 11 and 14 DPI, with an OD of ≤0.38.

The C-ELISA, with a 50% inhibition cutoff value, detected...
equine anti-VSV-NJ antibodies as early as 6, 10, and 11 DPI from animals H-1, H-109, and H-106, respectively (Table 1). Similarly, anti-VSV-IN antibodies were also detected at 11 and 12 DPI from animals H-107 and H-2, respectively. No cross-reactivity in the heterologous C-ELISAs was observed in any of the five horses.

The MTSN test detected equine antibodies to VSV-NJ as early as at 5, 6, and 5 DPI for animals H-1, H-106, and H-109, respectively (Table 1). Similarly, anti-VSV-IN antibodies were also detected at 11 and 12 DPI from animals H-107 and H-2, respectively. No cross-reactivity in the heterologous C-ELISAs was observed in any of the five horses.

The MTSN test detected equine antibodies to VSV-NJ as early as at 5, 6, and 5 DPI for animals H-1, H-106, and H-109, respectively (Table 1). Similarly, anti-VSV-IN antibodies were also detected at 11 and 12 DPI from animals H-107 and H-2, respectively. No cross-reactivity in the heterologous C-ELISAs was observed in any of the five horses.

The MTSN test detected equine antibodies to VSV-NJ as early as at 5, 6, and 5 DPI for animals H-1, H-106, and H-109, respectively (Table 1). Similarly, anti-VSV-IN antibodies were also detected at 11 and 12 DPI from animals H-107 and H-2, respectively. No cross-reactivity in the heterologous C-ELISAs was observed in any of the five horses.

The MTSN test detected equine antibodies to VSV-NJ as early as at 5, 6, and 5 DPI for animals H-1, H-106, and H-109, respectively (Table 1). Similarly, anti-VSV-IN antibodies were also detected at 11 and 12 DPI from animals H-107 and H-2, respectively. No cross-reactivity in the heterologous C-ELISAs was observed in any of the five horses.

FIG. 2. Sensitivities of sera and IgM antibodies to VSV-NJ (A) and VSV-IN (B) measured in the VSV-NJ MC-ELISA (A) and VSV-IN MC-ELISA (B). Serum samples and purified IgM antibodies were collected from horses experimentally infected with VSV-NJ (H-1) and VSV-IN (H-2) at 0 DPI (0d) and 6 DPI (6d). An OD<sub>410</sub> of 0.3 (horizontal lines) was selected as the cutoff.

Detection of antibodies from swine serum samples. The MC-ELISA was compared to the C-ELISA and MTSN assay by testing sequential blood samples from pigs experimentally infected with VSV-NJ or VSV-IN. As shown in Table 2, IgM antibodies were detected in the MC-ELISA as early as 4 DPI and up to 28 DPI from pigs infected with both VSV serotypes. Cross-reactions were observed in samples from these animals in the heterologous MC-ELISAs, with ODs of <0.45. The
cross-reactions were from samples collected at 6, 7, and 14 DPI from two pigs infected with VSV-NJ and at 6 and 7 DPI from two other pigs infected with VSV-IN.

The C-ELISA detected antibodies against VSV-NJ from one animal (P-36–94) at 6 DPI and at 28 DPI from the second animal (P-35–94) (Table 2). For VSV-IN, antibodies were detected from both animals (P-39–94 and P-40–94) at 14 DPI. Antibodies against both VSV serotypes remained positive at 42 DPI and over 160 DPI (data not shown). No cross-reactions were identified for these animals in the heterologous C-ELISAs (Table 2).

The MTSN test detected neutralizing antibodies to both VSV serotypes as early as 4 or 5 DPI (Table 2) that persisted more than 160 DPI (data not shown). No cross-reactions were observed for two pigs infected with the VSV-NJ and tested in the VSV-IN MTSN. However, some cross-reaction occurred with samples from two other animals infected with VSV-IN and tested in the VSV-NJ MTSN (Table 2). In this case,
cross-reactions were observed in samples collected at 5 and 6 DPI with antibody titers of \( \leq 1:64 \).

**DISCUSSION**

Currently, the tests most commonly used for the detection of antibodies against VSV are the serum neutralization test and the C-ELISA described in the *Manual of Standards for Diagnostic Tests and Vaccines* (13). These assays are not able to distinguish between IgM and IgG antibodies and therefore are not able to differentiate a primary from a secondary infection. In this study, we report the development of an MC-ELISA for the detection of primary infection of VSV-NJ and VSV-IN serotypes in horses and pigs based on a streptavidin-biotin system.

The MC-ELISA had an analytical sensitivity of 60 ng of total purified IgM antibodies per ml (or 6 ng/well) against both VSV serotypes (Fig. 2), i.e., a concentration of 3 mg of IgM preparation diluted 50,000 times. The purified IgM antibodies had higher binding values than the whole serum samples (end point titers of 1:50,000 for anti-VSV-NJ and of 1:80,000 for anti-VSV-IN), which may be due to the homology of the purified IgM antibody preparation that has no other molecules to cause potential nonspecific blocking of the antigen-antibody interaction. This level of analytical sensitivity is similar to that of the IgM capture ELISA developed by Vernon and Webb (16) in which ELISA plates were coated with rabbit antibodies against bovine and equine IgM molecules and a high-salt buffer was used as a dilution buffer. In our laboratory, the same format using rabbit and sheep antibodies against equine and swine IgM diluted in the same high-salt buffer resulted in high background levels of binding (data not shown). This led us to the

| Infection and animal | DPI | Result of test for indicated VSV serotype |
|---------------------|-----|----------------------------------------|
|                     |     | MC-ELISA | C-ELISA | MTSN |
|                     |     | NJ | IN | NJ | IN | NJ | IN |
| VSV-NJ              |     |   |   |   |   |   |   |
| P-35-94             | 0   | - | - | - | - | - | - |
| P-36-94             | 0   | - | - | - | - | - | - |
| P-35-94             | 4   | + | - | - | - | - | - |
| P-36-94             | 4   | + | - | - | - | - | - |
| P-35-94             | 5   | + | - | - | - | - | - |
| P-36-94             | 5   | + | - | - | - | - | - |
| P-35-94             | 6   | + | - | - | - | - | - |
| P-36-94             | 6   | + | - | - | - | - | - |
| P-35-94             | 7   | + | + | - | - | - | + |
| P-36-94             | 7   | + | + | - | - | - | + |
| P-35-94             | 14  | + | - | - | - | - | - |
| P-36-94             | 14  | + | + | + | - | - | - |
| P-35-94             | 21  | + | - | - | - | - | - |
| P-36-94             | 21  | + | - | - | - | - | - |
| P-35-94             | 28  | + | - | - | - | - | - |
| P-36-94             | 28  | + | - | - | - | - | - |
| P-35-94             | 35  | - | - | - | - | - | - |
| P-36-94             | 35  | - | - | - | - | - | - |
| P-35-94             | 42  | - | - | - | - | - | - |
| P-36-94             | 42  | - | - | - | - | - | - |

| VSV-IN              |     |   |   |   |   |   |   |
|                     |     |   |   |   |   |   |   |
| P-39-94             | 0   | - | - | - | - | - | - |
| P-40-94             | 0   | - | - | - | - | - | - |
| P-39-94             | 4   | - | + | - | - | - | - |
| P-40-94             | 4   | - | + | - | - | - | - |
| P-39-94             | 5   | - | + | - | - | - | - |
| P-40-94             | 5   | - | + | - | - | - | - |
| P-39-94             | 6   | + | + | - | - | - | - |
| P-40-94             | 6   | + | + | - | - | - | - |
| P-39-94             | 7   | + | + | - | - | - | - |
| P-40-94             | 7   | + | + | - | - | - | - |
| P-39-94             | 14  | - | + | - | - | - | - |
| P-40-94             | 14  | - | + | - | - | - | - |
| P-39-94             | 21  | - | + | - | - | - | - |
| P-40-94             | 21  | - | + | - | - | - | - |
| P-39-94             | 28  | - | - | - | - | - | - |
| P-40-94             | 28  | - | - | - | - | - | - |
| P-39-94             | 35  | - | - | - | - | - | - |
| P-40-94             | 35  | - | - | - | - | - | - |
| P-39-94             | 42  | - | - | - | - | - | - |
| P-40-94             | 42  | - | - | - | - | - | - |

*See footnotes to Table 1.*
development of an avidin-biotin system to capture sheep antibodies to equine and swine IgM. To test the performance of the MC-ELISA for anti-VSV IgM antibody detection in VSV-infected swine and horses, the C-ELISA and MTSN test were included in this study and compared with the MC-ELISA.

As demonstrated in Table 1, the MC-ELISA detected the specific IgM anti-VSV antibodies between 6 and 35 DPI. The IgM antibodies did diminish after 49 DPI, based on the study by Katz et al. (10). In comparison, the C-ELISA detected the equine anti-VSV antibodies at later DPI, remained positive at 35 DPI and more than 49 DPI. The antibodies detected by the MTSN test were developed as early as 6 DPI but lasted more than 60 DPI as reported by Katz et al. (10). However, because it is not an immunoglobulin isotype-specific assay, the MTSN test cannot provide information of whether the antibodies are produced by a primary or a secondary stimulation of the virus infection.

It was not surprising that some degree of cross-reaction was observed between the homologous and heterologous MC-ELISAs due to the polyclonal nature of the captured IgM and the heterologous nature of the antigens used in this assay. However, this cross-reaction was not observed in every animal serum sample. As shown in this study, the value of cross-reaction was much lower than that obtained from the homologous assay and only lasted a few days until the titer of homologous antibody reached its peak. Similar results were generated with swine antisera against VSV-NJ and VSV-IN.

From this study, we can conclude that the MC-ELISA provides relatively higher sensitivity for the detection of equine and swine IgM antibodies to VSV-NJ and VSV-IN. The specificity of the MC-ELISA needs to be further evaluated by testing large numbers of negative equine and swine serum samples. The MC-ELISA, like the MTSN test, was able to detect the IgM antibodies at early days of infection. However, due to the nature of these assays, only the MC-ELISA can differentiate a primary from a secondary infection of VSV.

Therefore, the C-ELISA can be used as a screening test for the serodiagnosis of VSV infection. Samples identified as positive by the C-ELISA should be confirmed by the MTSN test and subsequently tested by the MC-ELISA to provide further information on antibody isotypes. Results of the MC-ELISA would indicate if the infection is primary or secondary. This information would be valuable for routine disease surveillance and of particular use as an epidemiological tool during VS outbreaks.

ACKNOWLEDGMENTS

We thank Lisa Fernando and Deidre Ridd for their excellent technical assistance.

REFERENCES

1. Afshar, A., G. C. Dulac, P. F. Wright, and D. Martin. 1993. Application of indirect ELISA for detection of bovine antibodies against vesicular stomatitis viruses. J. Vet. Diagn. Invest. 5:26–32.
2. Afshar, A., H. Shakarchi, and G. C. Dulac. 1993. Development of a competitive enzyme-linked immunosorbent assay for detection of bovine, ovine, porcine, and equine antibodies to vesicular stomatitis virus. J. Clin. Microbiol. 31:1860–1865.
3. Alton, J. G., and E. B. Pagler. 1995. A one-step procedure for biotinylation and chemical cross linking of lymphocyte surface and intracellular membrane-associated molecules. Anal. Biochem. 224:382–389.
4. Boulanger, P. 1995. Complement-fixation tests of swine serum. I. In the diagnosis of vesicular stomatitis. Can. J. Comp. Med. 59:37–47.
5. Brown, F., D. H. L. Bishop, and J. Crick. 1979. Rhabdoviridae. Interviroli. 12:1–17.
6. Carayannopoulos, L., and I. D. Capra. 1993. Immunoglobulins: structure and function, p. 283–314. In W. E. Paul (ed.), Fundamental immunology, 3rd ed. Raven Press, New York, N.Y.
7. Hanson, R. P. 1952. The natural history of vesicular stomatitis. Bacteriol. Rev. 16:179–204.
8. Hanson, R. P. 1981. Vesicular stomatitis, p. 517–539. In E. P. J. Gibbs (ed.), Viruses of food animals, vol. 2. Academic Press, New York, N.Y.
9. Karstad, L. H., E. V. Adams, R. P. Hanson, and D. H. Ferris. 1956. Evidence for the role of wildlife in epizootics of vesicular stomatitis. J. Am. Vet. Med. Assoc. 129:95–96.
10. Katz, J. B., K. A. Eernisse, J. G. Landgraf, and B. J. Schmitt. 1997. Comparative performance of four serodiagnostic procedures for detecting bovine and equine vesicular stomatitis virus antibodies. J. Vet. Diagn. Investig. 9:329–333.
11. Lawery, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
12. Nielsen, K. H., P. F. Wright, and E. B. Nielsen. 1984. Enzyme immunoassay: application to the detection of bovine antibody to Brucella abortus. Agriculture Canada Monograph.
13. Office International des Epizooties, 1996. Manual of standards for diagnostic tests and vaccines, p. 57. Office International Des Epizooties, World Organization for Animal Health, Paris, France.
14. Snyder, M. L., E. W. Jenney, G. A. Erickson, and E. A. Carbrey. 1982. The 1981 resurgence of vesicular stomatitis in the United States; a summary of laboratory diagnostic findings. Proc. Annu. Meet. Am. Assoc. Vet. Lab. Diagn. 19:221–228.
15. Tesh, R. B., Da Rosa A. P. A., Travassos, and D. R. J. S. Travassos. 1983. Antigenic relationship among arboviruses infecting terrestrial vertebrates. J. Gen. Virol. 64:169–176.
16. Vernon, S. D., and P. A. Webb. 1985. Recent vesicular stomatitis virus infection detected by immunoglobulin M antibody capture enzyme-linked immunosorbent assay. J. Clin. Microbiol. 22:582–586.
17. Webb, P. A., and F. R. Holbrook. 1989. Vesicular stomatitis, p. 1–29. In T. P. Monath (ed.), The arboviruses: epidemiology and ecology, vol. 5. CRC Press, Inc., Boca Raton, Fla.
18. Wilks, C. R., and J. A. House. 1985. The glycoproteins of seven vesiculoviruses are antigenically distinct. Arch. Virol. 86:335–340.
19. Wuill, T. M. 1981. Vesicular stomatitis, p. 125–142. In G. W. Beran (ed.), CRC handbook series in zoonoses, sectio B. Viral zoonoses, vol. 1. CRC Press, Inc., Boca Raton, Fla.