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Expression Cloning and Humoral Immune Response to the Nucleocapsid and Membrane Proteins of Equine Arteritis Virus

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To provide a convenient and sensitive method for the detection of equine arteritis virus (EAV)-specific serum antibodies, we developed an immunoblot assay employing the EAV nucleocapsid (N) and membrane (M) proteins expressed in a procaryotic expression vector (pMAL-c2) for the production of recombinant maltose-binding (MBP) fusion proteins (MBP-N and MBP-M). The antigenic reactivity of the recombinant fusion proteins and their Xa factor cleavage EAV products was confirmed by immunoblot using horse antisera to EAV. Some horse sera, however, showed immune reactivity to the MBP fusion partner protein. Based on a total of 32 horse sera analyzed for the presence of EAV antibodies by immunoblot, using the MBP-N or -M fusion proteins and the Xa factor cleavage EAV products, and in the serum neutralization test, there was 100% concordance between the assays. Sera from horses experimentally infected with EAV were reactive in the immunoblot test with both the MBP-N and the MBP-M fusion proteins by day 14 after EAV exposure. The reactivity continued to the end of the experiment at day 145 after infection. This immune reactivity correlated with the detection of neutralizing antibodies in the serum samples. Based on these findings, the recombinant N and M proteins might be useful for serodetection of EAV-infected animals.

Equine arteritis virus (EAV), the causative agent of equine viral arteritis, is an enveloped positive-stranded RNA virus of 70 nm in diameter that was first isolated in Bucyrus, Ohio, in 1953 (16). The virus, which is restricted to members of the family *Equidae*, is present in horse populations in many countries (32). The clinical outcome following EAV exposure varies widely from subclinical infection to systemic equine viral arteritis with the risk of abortion in pregnant mares (32). The respiratory route appears to be a major means of EAV transmission from acutely infected to susceptible in-contact horses. However, a carrier state exists in 10 to 60% of seropositive stallions which are persistent shedders of EAV in their semen (31). Such stallions can infect seronegative mares at the time of breeding, and these, in turn, are capable of transmitting the virus during the acute viremic phase to susceptible in-contact horses.

EAV is the prototype virus of the arterivirus group, which also includes lactate dehydrogenase-elevating virus, porcine reproductive and respiratory syndrome (PRRS) virus, and simian hemorrhagic fever virus (5). The EAV genome is a polyadenylated positive single-stranded RNA of 12.7 kb in length (12). Eight open reading frames (ORFs) have been identified in EAV (12). ORFs 1a and 1b encode the viral polymerase (28), and ORF3- and 4-encoded products are believed to be glycosylated nonstructural proteins (12, 14). One minor and three major structural EAV proteins have been identified and assigned to ORFs 2 and 5 to 7, respectively. ORFs 6 and 7 encode an unglycosylated membrane (M) protein of 16 kDa and a 14-kDa nucleocapsid (N) protein, respectively (12, 14). ORF 5 encodes the heterogeneously glycosylated 30- to 42-kDa large (G_L) membrane protein (14), which expresses the major EAV neutralizing determinants (2, 8, 13, 18). ORF 2 encodes a glycosylated 25-kDa small (G_s) membrane protein which represents only 1 to 2% of the total virion protein (14, 15).

Although the reverse transcription-PCR technology has been applied in detection of EAV in biological samples (4, 6, 26, 29), currently detection of EAV is still based on virus isolation in cell cultures (19, 31). Serodiagnosis of EAV is also of importance and is presently based on antibody determination by the sensitive but time-consuming complement fixation or virus neutralization tests (17, 27). Because the ORF 5-encoded G_L protein expresses the neutralizing epitopes of EAV (2, 8, 13, 18), a G_L recombinant protein might be a likely candidate for a subunit vaccine against EAV. A serological test based on the detection of serum antibodies specific to other EAV gene products would be useful for the diagnosis of EAV infection and for differentiating animals naturally infected with EAV from EAV-vaccinated animals.

It was previously reported that rabbit and equine antisera recognize EAV N, M, G_L, and G_s native or recombinant fusion proteins in immunoprecipitation and immunoblot assays (14, 33). In this paper, we report on the expression of the N and M genes of the virus in the procaryotic expression vector pMAL to produce maltose-binding (MBP) fusion proteins. Horse sera were compared for their reactivity with these proteins in Western blots and in the classical serum neutralization (SN) test. In a kinetic study using serum samples from horses experimentally infected with EAV, we also showed that both the N and the M recombinant-protein-specific serum antibodies were detected as early as 14 days post-virus exposure (PVE) and lasted for at least 145 days.

MATERIALS AND METHODS

Sources of sera. Field horse sera and serum samples from horses that were convalescent from vaccination against EAV with the Arvac vaccine strain (32) or experimental EAV infection with the reference Bucyrus strain (32) or field isolate 84KY-A1 (21, 30), IL93, or IL94 (IL93 and -94 were isolated during EAV outbreaks in the state of Illinois in 1993 and 1994, respectively [23a]) were

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provided by W. H. McCollum, Gluck Equine Research Center, University of Kentucky, Lexington. Field sera from horses naturally infected with equine herpesvirus types 1 to 3, equine rhinovirus types 1 and 2, equine influenza virus types 1 and 2, and equine infectious anemia virus were provided by W. H. McCollum and Susy Carman (Veterinary Laboratory Services, Ontario Ministry of Agriculture Food and Rural Affairs, Guelph, Ontario, Canada). Serum samples used in the kinetic study of the antibody response to EAV were generously provided by Claude Dubuc (Virology Section, Animal Diseases Research Institute, Canadian Food Inspection Agency, Nepean, Ontario). The porcine serum specific to the PRRS virus (5) was kindly provided by Ronald Magar (Health of Animals and Food Laboratory, Canadian Food Inspection Agency, St-Hyacinthe, Québec).

Virus and cell cultures. The reference Bucyrus strain of EAV was propagated in confluent monolayers of rabbit kidney (RK-13) cells (ATCC no. CCL37) as previously described (29). When 80 to 100% of the cells exhibited cytopathic effect, the tissue culture supernatant was clarified by centrifugation at 5,000 × *g* for 30 min. Virus was then pelleted by centrifugation (100,000 × *g* for 3 h) at 5°C over a 25% sucrose cushion. The virus pellet was resuspended in TNE buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA [pH 7.4]) to provide the working virus stock.

Oligonucleotide primers. The oligonucleotide primer sequences for reverse transcription-PCR amplification of EAV ORFs 6 and 7 were designed according to the published sequence of the EAV Bucyrus strain genome (12) and were synthesized with a Gene Assembler Plus DNA synthesizer (Pharmacia, Uppsala, Sweden). The specific sense and antisense primers of ORF 6 were 5′-CGGATCCATGGGAGCCATAGATTCA-3′ (nucleotides 11884 to 11901) and 5′-CCTGCAGGCGCAGTAGGTCATTGTA-3′ (nucleotides 12382 to 12365), respectively. The specific sense and antisense primers of ORF 7 were 5′-CTCTAGAATGGCGTCAAGACGATC-3′ (nucleotides 12296 to 12312) and 5′-CAAGCTTTATCCACGTCTTACGGC-3′ (nucleotides 12638 to 12622), respectively. The underlined nucleotides are short 5′ extensions which contain the restriction enzyme site *Bam*HI (GGATCC), *Pst*I (CTGCAG), *Hin*dIII (AAGCTT) or *Xba*I (TCTAGA) for subcloning the cDNA amplified fragment encoding EAV ORFs 6 and 7 in the expression vector.

Reverse transcription, PCR amplification, cloning, and sequencing. Genomic RNA from EAV virions was isolated according to the method of Chomzynski and Sacchi (11). The EAV RNA was reverse transcribed and amplified as previously described (29). The expected amplified cDNA fragments encoding EAV ORFs 6 and 7 were subsequently cloned into the pCR II TA cloning vector (Invitrogen, Palo Alto, Calif.) in accordance with the supplier's instructions and sequenced by the chain termination method of Sanger (25) to confirm the EAV-specific nature of the amplified product.

Expression and purification of fusion proteins. The cDNA fragments encoding EAV ORFs 6 and 7 were excised from the pCR II TA cloning vector with the appropriate restriction enzymes and purified by using a low-melting-temperature agarose gel (29). The cDNA bands were then recovered and ligated into a similarly digested pMAL-c2 expression vector (New England Biolabs, Portland, Maine). This enabled the amplified cDNA products encoding EAV ORFs 6 and 7 to be cloned in frame with the *malE* gene of *Escherichia coli*, which encodes MBP, resulting in the expression of an MBP fusion protein. The cDNA clones were sequenced as previously described to confirm the MBP-EAV DNA junction in the correct reading frame.

For protein expression, overnight stationary-phase cultures of *E. coli* (DH5 α) containing the pMAL-c2 recombinant plasmids of EAV ORFs 6 or 7 were diluted 100-fold in Luria-Bertani–ampicillin (200 μg/ml) medium and incubated at 37°C to an A_{600} of 0.5 before induction with isopropyl-β-D-thiogalactopyranoside (IPTG) (final concentration, 0.3 mM). The bacterial cell cultures were further incubated for 3 h at 37°C, and the bacterial cells were harvested by centrifugation at 4,000 × *g* for 10 min. Cell pellets were lysed by sonication with shortwave pulses of 15 s in buffer (10 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA) which contained aprotinin (1 μg/ml) and polymethylsulfonylfluoride (1 μg/ml) as proteinase inhibitors. The crude sonicates were centrifuged at 9,000 × *g* at 4°C for 20 min to remove insoluble material. The supernatants (crude extracts) were then analyzed on a sodium dodecyl sulfate (SDS)–12% polyacrylamide gel (1).

Fusion proteins were purified either by using an MBP-resin affinity column (New England Biolabs) or by electroelution (Microeluter, Bio-Rad, Palo Alto, Calif.) of the protein that was previously cut out of the SDS-polyacrylamide gel. Where appropriate, fusion proteins were treated with the Xa factor (New England Biolabs) to cleave off the virus protein from the MBP. The cleavage products were then resolved by SDS-polyacrylamide gel electrophoresis as described above.

Immunoblotting. Following electrophoretic transfer of proteins from SDS–12% polyacrylamide gels onto a nitrocellulose membrane, immunoblots were performed as previously described (1) by using, as the blocking reagent solution, 4% nonfat dried milk solids, 1% Triton X-100, 0.05% Tween 20, 50 mM Tris hydrochloride (pH 7.5), and 10 mM EDTA. The horse serum samples were used at a final dilution of 1:50.

SN test. The complement-dependent virus neutralization (SN) test was performed in accordance with the method described by Senne et al. (27). The antibody titer of each sample was expressed as the reciprocal of the highest serum dilution giving 75% or higher neutralization of the test virus (100 PFU of EAV).

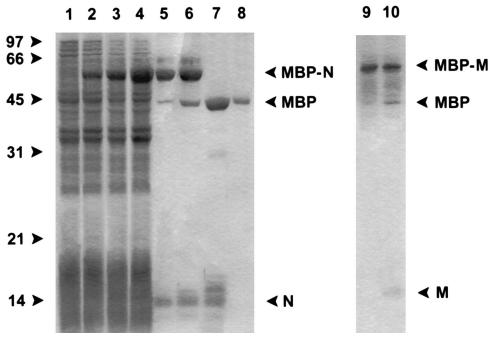

FIG. 1. Expression of EAV N (MBP-N) and M (MBP-M) fusion proteins in *E. coli*. Proteins synthesized in *E. coli* were analyzed by SDS-polyacrylamide gel electrophoresis and visualized by staining with Coomassie brilliant blue. Lane 1, extracts of control noninduced cultures incubated for 3 h; lanes 2 to 4, extracts of cultures induced with IPTG and incubated for 1, 2, and 3 h, respectively; lane 5, sample of MBP-N fusion protein purified by the small-scale amylose resin method; lane 6, sample of MBP-N fusion protein purified by an affinity chromatography amylose column and maltose; lane 7, Xa factor cleavage products from a sample of purified MBP-N fusion protein; lane 8, MBP control protein; lane 9, sample of MBP-M fusion protein purified by an affinity chromatography amylose column and maltose; lane 10, Xa factor cleavage products from a sample of purified MBP-M fusion protein. Molecular weight standards are indicated on the left (in thousands).

RESULTS

Expression of EAV ORFs 6 and 7 in *E. coli*. Segments of the EAV genome containing the predicted ORFs of the M and N gene polypeptides were successfully inserted into the pMAL-c2 expression vector, where the β-galactosidase promoter could be adequately controlled by IPTG. When the bacterial cells were transformed with the EAV ORF 7 construct and induced with IPTG, a band migrating at a position corresponding to the MBP-N fusion protein with a molecular size of approximately 56 kDa was readily observed on a Coomassie brilliant blue-stained gel 1 h after IPTG induction (Fig. 1, lane 2). More intense protein bands were obtained when the bacterial cell cultures were incubated for 2 or 3 h after IPTG induction (Fig. 1, lanes 3 and 4). In contrast, such a band was not detected when cells not induced with IPTG were used (Fig. 1, lane 1). The soluble fraction was greatly enriched after purification using either the small-scale method in a microcentrifuge tube with the amylose resin or an affinity chromatography procedure with the amylose resin column and maltose according to the protocols of the manufacturer (New England Biolabs) (Fig. 1, lanes 5 and 6, respectively). However, after these purification procedures, extra protein bands of 42 and 14 kDa were observed, which corresponded to the MBP and N proteins, respectively (Fig. 1, lanes 5 and 6). These protein bands were derived from the spontaneous cleavage of a fraction of the MBP-N fusion protein. Nevertheless, when the purified fusion protein preparation was treated with the Xa factor, the MBP-N fusion protein disappeared and the N protein band increased in intensity (Fig. 1, lane 7).

We were also able to express the M protein as an MBP-M fusion protein with a molecular size of approximately 58 kDa, as observed on the Coomassie brilliant blue-stained gel 3 h after bacterial-cell IPTG induction (Fig. 1, lane 9). When the purified MBP-M fusion protein preparation was treated with the Xa factor, we could readily observe a protein band of 16 kDa, which corresponds to the molecular weight of the predicted M protein (Fig. 1, lane 10). However, the MBP-M

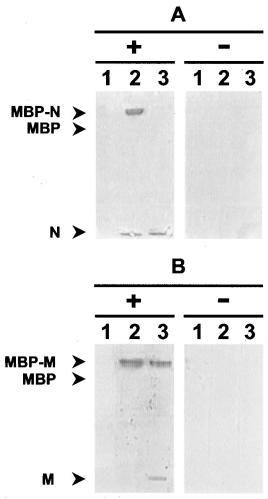

FIG. 2. Immunoreactivity of bacterially expressed EAV MBP-N (A) and MBP-M (B) fusion proteins. A total of 2 μg of MBP (lanes 1), MBP-N or MBP-M fusion protein (lanes 2), or Xa factor cleavage product (MBP and N or M) of MBP-N or MBP-M fusion protein (lanes 3) was electrophoresed, transblotted to nitrocellulose, and exposed to a 1:50 dilution of horse serum (no. 85) positive (+) (i.e., 40 days after exposure to the EAV reference strain Bucyrus) or negative (−) (i.e., before EAV exposure) for EAV antibodies. No immune reactivity to the MBP protein was observed with either horse serum.

protein was only partially cleaved, as demonstrated by the presence of a residual fraction of the MBP-M protein observed after the cleavage reaction (Fig. 1, lane 10).

Immunoblot reactivity of the bacterially expressed fusion proteins. In order to verify that the 56- and 58-kDa bacterially expressed proteins contained products derived from the EAV ORF 7 (N protein) and ORF 6 (M protein), the MBP-N and MBP-M fusion proteins and their cleavage products were subjected to immunoblotting and probed with a serum from a horse 40 days after the horse was experimentally exposed to the EAV reference Bucyrus strain (Fig. 2). This serum recognized the MBP-N and MBP-M fusion proteins as well as the cleaved EAV N (Fig. 2A) and M (Fig. 2B) proteins. As expected, the serum also reacted with the fraction of the MBP-M protein that was not cleaved by the Xa factor (Fig. 2B). However, the serum did not react with the MBP protein (Fig. 2). No immune reactivity was obtained when the MBP, the MBP-N, and MBP-M fusion proteins and the EAV N and M proteins were allowed to react with the horse serum prior to EAV exposure, (the serum was negative for EAV antibodies as determined by the virus neutralization test) (Fig. 2). No cross-reactions were detected when the EAV N and M proteins were allowed to react with horse antisera specific to equine herpesvirus types 1 to 3, equine influenza virus types 1 and 2, and equine rhinovirus types 1 and 2 and with a porcine serum specific to PRRS virus (data not shown).

Based on these results, the immunoblot that has been described appears to be suitable for the detection of antibodies to EAV. It offers the important advantages of a plentiful supply of test antigens and a more rapid procedure at lower cost compared to the SN test. It should be noted, however, that reactivity with MBP alone was obtained with certain horse sera, either positive or negative for antibodies to EAV. This is illustrated in Fig. 3: serum samples from a horse before and after experimental infection with EAV (84KY-A1 isolate) reacted with both the MBP-N fusion protein (lane 2) and the MBP protein (lanes 1 to 3). However, only the postinfection

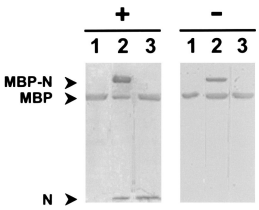

FIG. 3. Immunoreactivity of MBP and bacterially expressed EAV MBP-N fusion protein. A total of 2 μg of MBP (lanes 1), MBP-N fusion protein (lanes 2), or Xa factor cleavage product (MBP and N) of MBP-N fusion protein (lanes 3) was electrophoresed, transblotted to nitrocellulose, and exposed to a 1:50 dilution of horse (no. 403) serum positive (+) (i.e., 4 months after exposure to the EAV 84KY-A1 field isolate) or negative (−) (i.e., before EAV exposure) for EAV antibodies. Note the immune reactivity to the MBP protein for both EAV-positive and -negative antisera.

serum reacted with the N protein present in the partially spontaneously cleaved (lane 2) or Xa factor-cleaved (lane 3) preparations, indicating that this serum was indeed positive for EAV antibodies. The overall results suggest that the Xa factor cleavage products of either MBP-N or MBP-M fusion protein, or both, could be used as test antigens in our immunoblot procedure to detect sera positive for antibodies to EAV.

Kinetics of the antibody response to EAV N and M proteins. The kinetics of the appearance of antibodies directed to the N and M proteins of EAV was followed for up to 145 days in a horse experimentally exposed to the EAV reference strain Bucyrus. By using the fusion proteins as test antigens, specific antibodies to both the N and the M proteins were detected from 21 days PVE to the end of the experiment at day 145 PVE (Fig. 4). This correlated with the detection of serum neutralizing antibodies, the titers of which were in the range of 64 to 256 (data not shown). As evident from the immunoblot, the intensity of the signal for the N protein peaked at 28 and 40 days PVE and returned to the level of the signal for the 21-day serum sample at 96 or 145 days PVE. In contrast, the intensity of the signal obtained for the M protein appeared relatively stable throughout the 145-day experimental period. No immune reactivity was observed to the MBP protein alone in sera collected before exposure to EAV or throughout the 145 days of the experiment. By using another set of serum samples from another horse experimentally exposed to EAV and testing samples taken at days 7, 14, and 21 PVE, we were able to detect antibodies to both the N and the M proteins as early as 14 days PVE, which again correlated with the appearance of neutralizing antibodies in the same sera (data not shown). Together, these results suggest that either recombinant N pro-

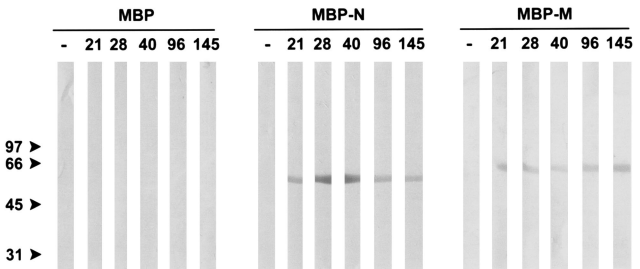

FIG. 4. Kinetics of the appearance of specific antibodies to EAV N (MBP-N) and EAV M (MBP-M) fusion proteins. MBP was used as a control antigen. The horse was bled before (−) and at 21, 28, 40, 96, and 145 days after exposure to the EAV reference Bucyrus strain. Molecular weight standards are indicated on the left (in thousands).

TABLE 1. Comparison of the immunoblot with the SN test for the detection of serum antibodies to EAV

Test	No. of serum samples				
	MBP		EAV		With the same result by immunoblot (% concordance)
	+	−	+	−	
Immunoblot	13[a]	19	18[b]	14[c]	
SN			18[d]	14	32 (100)

[a] Six of the sera were positive for EAV N and M antibodies.
[b] These sera were reactive against the MBP-N and MBP-M fusion proteins as well as the Xa factor-cleaved EAV N and M proteins. These sera were convalescent-phase sera from horses experimentally infected with the reference Bucyrus strain ($n = 5$) or field isolate 84KY-A1 ($n = 4$), IL93 ($n = 3$), or IL94 ($n = 4$) and from horses vaccinated with the Arvac vaccine strain ($n = 2$).
[c] These sera were from field horses ($n = 7$) and from horses prior to EAV experimental infection ($n = 7$).
[d] The SN titers of the serum samples positive for EAV by immunoblot varied from 32 to 256.

tein or recombinant M protein could be used in an immunoblot procedure for the detection of antibodies to EAV.

Comparison of the EAV immunoblot with the SN test. A total of 32 horse sera were tested for antibodies to EAV by immunoblot and the SN test. For the immunoblot, each serum sample was tested against the MBP, the MBP-N, and the MBP-M proteins and the M and N proteins derived from the Xa factor-cleaved products of the fusion proteins. The results are summarized in Table 1. When the immunoblot was used, we were able to detect 18 positive serum samples from horses either experimentally infected with the reference Bucyrus strain ($n = 5$) or the field isolates 84KY-A1 ($n = 4$), IL93 ($n = 3$), and IL94 ($n = 4$) or vaccinated with the Arvac vaccine strain ($n = 2$). In all cases, the immunoblot detected both the N and the M proteins. A total of 13 serum samples (40.6% of all sera tested) from horses experimentally exposed to either EAV strains or EAV isolates reacted with the MBP protein. Of these MBP-positive sera, six were found to be positive for EAV antibodies and seven were negative, as determined by their immune reactivity against the M and N proteins derived from the Xa factor cleavage products of the fusion proteins. This suggests that the Xa factor cleavage products of the fusion proteins are suitable for use in an immunoblot assay. When the immunoblot results were compared with the SN test results, a concordance of 100% was observed. The SN titers of the horse sera positive for EAV by immunoblot ranged from 32 to 256.

DISCUSSION

The EAV system offers important advantages for studying the mechanisms of arterivirus pathogenesis and persistence. Disease can be elicited by certain strains of EAV in a relatively short time after infection, and in pregnant mares this may result in abortion (32). The occurrence of EAV strains of varying virulence has been reported (22, 23), and many cases of EAV infection are clinically inapparent (24, 32). Moreover, a significant proportion of EAV-infected stallions are asymptomatic shedders of the virus in their semen (31, 32) and, consequently, constitute a major reservoir of EAV which is transmitted through breeding.

The diagnosis of EAV infection is currently based on serum antibody determination by complement fixation or virus neutralization tests and/or virus isolation in cell cultures (17, 19, 27, 31). However, these laboratory procedures are laborious and time-consuming. We have produced recombinant N and M fusion proteins and investigated their ability to detect serum EAV antibodies using an immunoblot procedure. The recombinant fusion proteins of the virus were also used to study the kinetics of the appearance of N- or M-specific antibodies in horses experimentally infected with EAV. The N and M proteins, each of which constitutes approximately 30% of the virion protein (14), were targeted as potential test antigens because they have been shown previously to induce the production of specific serum antibodies to EAV (14, 33). Moreover, although genetic variation has been reported among strains of EAV (3, 7, 20, 21, 24, 30), the nucleic and deduced amino acid sequences of ORFs 6 and 7 have been found to remain well conserved among several different EAV isolates (7), thereby suggesting that these proteins are less likely to experience antigenic variation.

The results of this study confirm that both M and N fusion proteins and the Xa-cleaved products thereof could be successfully used in an immunoblot procedure to detect EAV antibodies in sera from horses infected with various strains or isolates of EAV. The results also show that, due to the reactivity of MBP with some negative sera, the Xa-cleaved products are the antigens of choice to be used in the immunoblot. In addition, both fusion proteins, when used to independently immunize rabbits, were found to be highly immunogenic (data not shown).

In previous studies (9, 10), recombinant proteins containing fusions to the G_L and N proteins and derived by using the pGEX glutathione *S*-transferase (GST) bacterial expression system were evaluated in Western blot and an enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to EAV in horse sera. Surprisingly, even though the fusion proteins were immunologically reactive, certain horse sera were found to react with the GST protein in both the ELISA and Western blot. In the present study, immune cross-reactivity with the MBP fusion partner was also observed in the case of a significant number of equine serum samples tested. This discouraged us from using the fusion proteins in an ELISA to differentiate sera negative for antibodies to EAV from positive sera. There was too high a background reactivity to the MBP fraction of the antigen preparation (data not shown). This prompted us to use the Xa factor-cleaved products of the fusion proteins for the specific detection of EAV M or N antibodies by immunoblot. No interference by way of immune cross-reactivity to MBP was observed by using these cleaved products. It should be noted that immune cross-reactivity to GST or MBP has also been observed with the sera of other animal species such as swine, cows, and rabbits (1a).

Comparison of the results of testing a group of horse sera by immunoblot (targeting either the N or M protein) and the SN test revealed that the two tests were 100% in agreement, regardless of the magnitude of the serum neutralizing antibody titers. Reactivity to both M and N proteins was detected in all sera positive for neutralizing antibodies to EAV. This finding was somewhat surprising in light of the fact that analysis of the M protein indicates that it is very hydrophobic. This would suggest that this protein is most likely submerged within the virus particle (14) and, thus, less accessible to the host's immune system. Nevertheless, the results presented by this study clearly indicate that the M molecules, which are present mostly as heterodimers with the G_L molecules in the virions (14), are immunogenic in the rabbit (inoculated with the fusion protein) and in the horse (during EAV infection). This is supported by the results of the kinetic study in which both N and M proteins were shown to be strongly reactive (although less for the M protein) with horse sera collected from day 14 to day 145 PVE.

In summary, the results of this study confirm that the recombinant M and N virus proteins can be used as test antigens

for serodetection of EAV infection by immunoblot. Although this immunoassay does not allow serum titration, it could be useful adjunct to existing tests for diagnosis of EAV, for epidemiologic surveillance, or to prescreen samples prior to a confirmatory SN test on immunoblot-positive sera. The test appears to be highly sensitive and has several advantages over conventionally used assays including a shorter test time. A much more in-depth evaluation of these antigens produced in the vector pMAL-c2 is needed in order to confirm their suitability as test reagents for detection of antibodies to EAV. Further research should investigate other expression vectors such as the baculovirus system for the production of non-bacterium-derived recombinant EAV antigens that might be more suitable, in terms of reduced background activity, for the development of an ELISA for this infection.

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