Antibodies Against Equine Herpesvirus 1 in the Cerebrospinal Fluid in the Horse

LINDA L. BLYTHE, DONALD E. MATTSON, E. DUANE LASSEN AND A. MORRIE CRAIG

College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97311-4802

ABSTRACT

Neutralizing antibodies against equine herpesvirus 1 were measured in serum and cerebrospinal fluid of 16 horses and ponies from a closed herd both before and after vaccination with modified live equine herpesvirus 1. These titers were also measured in 22 neurologically normal and 15 neurologically abnormal horses at a teaching hospital. Animals from the closed herd had prevaccination serum titers up to 1:8 and postvaccination serum titers up to 1:128. Horses from the teaching hospital had serum titers up to 1:64. Cerebrospinal fluid titers were not detected in the vaccinated horses or the neurologically normal horses but a low titer (1:8) was noted in one neurologically abnormal horse. This titer probably resulted from hemorrhage into the cerebrospinal fluid following trauma.

Key words: Herpesvirus 1, equine, antibodies, cerebrospinal fluid, serum.

RÉSUMÉ

Anticorps contre l'herpèsvirus 1 équin, dans le liquide céphalo-rachidien du cheval

Cette expérience consistait à déterminer le titre d'anticorps neutralisants contre l'herpèsvirus 1 équin, dans le sérum et le liquide céphalo-rachidien de huit chevaux et d'autant de poneys d'un troupeau fermé, avant et après l'administration d'un vaccin atténué contre la rhino-pneumonite équine. Elle visait aussi à effectuer la même détermination, chez 22 chevaux sains et chez 15 autres référés à un hôpital vétérinaire d'enseignement, à cause de troubles neurologiques. Avant leur vaccination, les sujets du troupeau fermé possédaient un titre d'anticorps sériques qui atteignait jusqu'à 1:8, tandis qu'après, ce titre s'éleva jusqu'à 1:128. Celui des chevaux de l'hôpital vétérinaire d'enseignement atteignait jusqu'à 1:64. Le liquide céphalo-rachidien des chevaux vaccinés et des témoins ne contenait pas d'anticorps contre le virus précité, tandis que celui d'un des chevaux atteints de troubles neurologiques affichait un titre de 1:8, probablement consécutif à une hémorragie qui s'y serait produite, à la suite d'un trauma.

Mots clés: herpèsvirus 1 équin, chevaux, anticorps, liquide céphalo-rachidien, sérum.

INTRODUCTION

Paresis and/or paralysis associated with equine herpesvirus 1 (EHVI) infection is a well-known syndrome (1-9). The pathogenesis of this disease is unknown. Both direct virus invasion of the central nervous system (CNS) and pathological activation of immune complexes at the level of the CNS vascular endothelium after reinfection or recurring infection have been implicated (10-12).

The presence of the following criteria have been used in diagnosing EHV1 induced neurological disease: 1) a history of and/or concurrent upper respiratory disease, 2) EHV1 abortion in the same animal or other horses in the herd, 3) increased protein and xanthochromia in cerebral spinal fluid (CSF) without concurrent pleocytosis (11), 4) a fourfold increase in serum-virus neutralizing antibody (SN) titer and 5) histological evidence of vasculitis with degeneration of adjacent nervous tissue (11,12). The latter two are the most definitive. The diagnosis is more difficult in a single animal, because the clinical signs are the same or are similar to those of a horse with acute wobbler syndrome, which has a multitude of causes (13,14). A correct diagnosis is desirable since treatment of the wobbler syndrome varies according to the etiology of disease. For example, steroid therapy for the neurological form of EHV1 would be contraindicated and would exacerbate a protozoal myelopathy (13).

Rising serum titers of SN antibodies are usually the most reliable antemortem indicator of viral disease. In a rapidly progressing case, the animal may die before paired samples can be obtained. In one investigation dealing with experimentally induced EHV1 paresis, high SN antibody titers and CSF antibody titers were present at or within two days of onset of signs (11). In human herpes simplex virus encephalitis, a low ratio (≤ 20) of serum to CSF antibody is diagnostic for that agent (15). Before EHV1 antibody titers in CSF can be used to aid in the differential diagnosis of paresis in a horse, baseline date on the presence of these antibodies in CSF must be obtained.

This study was undertaken to evaluate titers of EHV1 neutralizing antibody in serum and CSF of horses before and after vaccination with a modified live virus vaccine and in horses that were presented to the Veterinary Teaching Hospital.

MATERIALS AND METHODS

Vaccinated Horses

A closed herd of eight horses and eight ponies of various ages (2-17 years), breeds and sexes was used.

Supported by Institutional Research Funds.

Animals were isolated six months prior to and for the duration of the study. Atlanto-occipital CSF samples were aseptically obtained under general anesthesia induced by intravenous xylazine (Rompun, Haver-Lockhart) and ketamine (Vetalar, Parke-Davis). A prevaccination blood sample was taken.

Following recovery from the initial sampling, all animals were vaccinated with a commercial modified live virus vaccine (Rhinomune, Norden Laboratories, Inc.). Repeat CSF and serum samplings were undertaken at one, two, four, six and ten weeks after vaccination using the same procedures. Final serum samples were taken at four months.

Hospitalized Horses

Twenty-two horses that were normal by clinical neurological examination and 15 horses with primary neurological disease had serum and CSF neutralizing antibody titers measured in a manner similar to the experimental, vaccinated horses. Signalment and disease diagnosis of the neurologically abnormal horses are presented in Table I.

EHV1 Neutralization Tests

Virus neutralization (VN) tests were conducted using 96-well microtiter plates. Serum or CSF (0.05 mL) was serially diluted in twofold steps. Each sample was tested in triplicate. An equal volume of EHV1 which contained 100 $TCID_{50}$ was added to each well. Initial serum or CSF dilution was 1:2 in experimentally vaccinated horses and 1:4 in horses presented from clinical cases. The serum- or CSF-virus mixture was incubated for one hour at 25°C. After incubation, 0.05 mL bovine turbinate cells (1×10^5 cells per mL) were added to each well. Bovine turbinate cells were suspended in Eagle's minimal essential medium with 10% bovine serum. Cultures were incubated at 37°C in an atmosphere of four percent CO_2. Each well was examined for presence of cytopathic effect after five days of incubation. The neutralization end-point was defined as the last serum or CSF dilution which prevented the expression of cytopathic effect in at least two of the three wells. Antibody titer was expressed as the reciprocal of the highest dilution of CSF or serum that neutralized the virus.

Reference EHV1 and bovine turbinate cells were obtained from the Veterinary Service Laboratory, NADC, Ames, Iowa. The EHV1 virus was isolated from an aborted fetus and not specified as to a strain designation.

Pathological Studies

Brain and spinal cord of all clinical horses were examined both grossly and microscopically.

RESULTS

All animals had measurable SN titers to EHV1 prior to vaccination. Initial titers to EHV1 were generally low, the highest titer being 1:8 in four animals. Peak elevations occurred at varying times between one and six weeks postvaccination. The SN titers ranged from 8 to 128. Serum neutralizing titers began declining in the majority of animals by ten weeks. Equine herpesvirus 1 antibodies were not found in the CSF at any test date. Throughout the study, all animals remained free from clinical signs of upper respiratory disease and/or abortion. The one pregnant mare foaled normally three months postvaccination.

Hospitalized horses that were free from neurological signs had SN titers that varied from negative to 64. None of these horses had VN antibodies in their CSF. Cerebrospinal fluid protein in all cases was within normal range.

In the horses with known neurological disease, SN titers ranged from negative to 128. One (Horse CN6) had detectable EHV1 antibodies in the CSF (Table 1). This horse had an acute fracture of the petrous temporal bone extending into the cranial vault. The cisternal CSF obtained from this animal was read and CSF protein was elevated at 251 mg/100 mL. Evidence of hemorrhage into the CSF was also seen in horse CN11 which had recent trauma to the skull and in CN13 as a

TABLE I

SERUM AND CSF NEUTRALIZATION TITERS[a] COMPARED TO CSF PARAMETERS IN HORSES WITH KNOWN NEUROLOGICAL DISEASE

	Breed[b]	Age	Sex[c]	Problem	Titers[d] Serum	CSF	Protein mg/100 mL	CSF Parameters WBC/cmm	RBC/cmm
CN1	Morgan	5	G	Cauda equina neuritis	64	N	107	12	5
CN2	Arab	4	F	Nonsuppurative encephalomyelitis	16	N	60	0	0
CN3	Draft	0.5	F	Cerebellar abiotrophy	N	N	70	1	0
CN4	TW	12	F	Nonsuppurative encephalomyelitis	8	N	34	0	0
CN5	TB	2	F	Cervical stenotic myelopathy	16	N	55	1	1
CN6	QH	18	S	Fractured temporal bone	128	8	251	11,700	60,400
CN7	QH	2	S	Cervical stenotic myelopathy	16	N	65	1	1
CN8	TW	1	F	Eq. degenerative myelopathy	N	N	76	1	2
CN9	App	2	S	Cervical stenotic myelopathy	4	N	92	0	13
CN10	QH	0.2	F	Eq. degenerative myelopathy	N	N	61	0	11
CN11	Pony	0.8	G	Head trauma	4	N	135	2,500	1,780,000
CN12	TB	0.3	S	Cervical stenotic myelopathy	8	N	94	0	1
CN13	QH	2	S	Cervical stenotic myelopathy	32	N	97	21	135,000
CN14	App	1.5	S	Eq. degenerative myelopathy	N	N	43	0	0
CN15	App	4	S	Eq. degenerative myelopathy	16	N	62	2	787

[a]Data expressed as the reciprocal of the serum or CSF neutralization titers
[b]Arab = Arabian; App = Appaloosa; TW = Tennessee Walker; TB = Thoroughbred; QH = Quarterhorse
[c]S = Stallion; F = Female; G = Gelding
[d]N = Negative

result of a traumatic tap. In these cases, no cerebrospinal fluid EHV1 antibodies were present in spite of elevated CSF protein.

The acute neural form of rhinopneumonitis was not diagnosed in any of the horses with neurological disease. The majority of horses with primary neurological disease in this study had degenerative, compressive, or traumatic types of disorders. Two horses had a nonsuppurative encephalomyelitis, but the onset of the nonprogressive ataxia had been noted months previously in both cases. The causative agent remains undefined.

Pathology Results

No lesions were evident on gross and microscopic examination of brain and spinal cords of neurologically normal hospitalized horses. Diagnoses for neurologically abnormal hospitalized horses are listed in Table 1.

DISCUSSION

The very low SN titers present in the experimental animals before vaccination were expected since the horses had been isolated for six months prior to the study. The SN antibody response observed in the vaccinated horses was comparable to that reported by some (16,17), but lower than in another study (18). Vaccination elicited an anamnestic response in serum only. In the normal horse, the ratio of serum protein to CSF protein ranges from approximately 100:1 to 250:1 (19). Normally, there is a barrier to diffusion of antibody from blood to the CSF so that much lower levels of antibody, usually $<$ 100-fold are maintained in the CSF (20). The postvaccination serum titers which were observed in this study would not be high enough to result in a measurable amount of antibody in CSF from passive diffusion across the normal blood-CSF barrier.

The presence of low SN antibody titers present in the majority of the hospitalized horses (18/21) is comparable to findings by other investigators (21-22), and reflects both the widespread occurrence and the poor antigenic nature of the herpesvirus. The presence of a positive low antibody titer in the CSF of horse CN6 was considered a result of serum protein leakage concurrent with hemorrhage into the CSF. While this animal had the highest SN titer (128), no histological evidence of the vasculitis that is the hallmark of EHV1 central nervous system infection was seen (4,10,11). Similar evidence for hemorrhage was seen in horses CN11 and CN13. It is suggested that the SN titers were too low to produce detectable CSF titers in these horses.

With the absence of EHV1 antibody titers in CSF of postvaccination horses, of neurologically normal horses, and of all but one of the neurological cases where an altered blood-CSF barrier might have existed, one can conclude that the presence of cerebrospinal fluid EHV1 antibodies in the CSF would indicate either marked blood-CSF breakdown with concurrent high serum titers or localized production on antibodies in the CNS. Further studies are needed to determine the usefulness of EHV1 titers in horses with known neurological form of EHV1 disease.

ACKNOWLEDGMENTS

The authors thank Marilyn Slizeski and Rocky Baker for their technical assistance.

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