Agents of Equine Viral Encephalomyelitis: Correlation of Serum and Cerebrospinal Fluid Antibodies

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ABSTRACT

A survey was conducted by testing 115 paired equine serum and cerebrospinal fluid samples by hemagglutination-inhibition for antibodies to Powassan and snowshoe hare viruses, and by virus neutralization for antibodies to equine herpesvirus type 1. Twenty-five samples were from horses with spontaneous neurological disease and the remainder from horses euthanized because of various nonneurological disorders. All sera and cerebrospinal fluids were negative for antibodies to Powassan virus. Fifty-one sera (44.3%) and 15 cerebrospinal fluids (13.0%) had antibodies to snowshoe hare virus. Ninety-eight sera (85.2%) and four cerebrospinal fluids (3.5%) were positive for antibodies to equine herpesvirus type 1.

Powassan virus was inoculated intracerebrally into one, and intravenously into four ponies. Neurological signs associated with a nonsuppurative encephalomyelitis occurred in three ponies. Antibodies to Powassan virus were detected in sera of all animals but in cerebrospinal fluids of only two. Powassan virus was isolated from brain and spinal cord of only the intracerebrally inoculated animal.

RÉSUMÉ

Cette étude portait sur 115 échantillons appariés de sérum et de liquide céphalo-rachidien de chevaux; elle consistait à y rechercher des anticorps contre le virus Powassan et celui du lièvre d'Amérique, par l'épreuve de l'inhibition de l'hémagglutination, de même que contre l'herpèsvirus équin du type #1, par la neutralisation du virus. Vingt-cinq échantillons provenaient de chevaux atteints d'une maladie neurologique spontanée et les autres, de chevaux sacrifiés à cause de diverses maladies d'origine autre que nerveuse. Aucun des 115 échantillons précités ne contenait d'anticorps contre le virus Powassan. Toutefois, 51 des échantillons de sérum, i.e. 44,3%, et 15 de ceux de liquide céphalo-rachidien, i.e. 13%, possédaient des anticorps contre le virus du lièvre d'Amérique, tandis que 98 échantillons de sérum, i.e. 85,2%, et quatre de ceux de liquide céphalo-rachidien, i.e. 3,5%, en possédaient contre l'herpèsvirus équin du type #1.

L'inoculation intracérébrale du virus Powassan à un poney et son injection intraveineuse à quatre autres se soldèrent par l'apparition de signes nerveux qui s'accompagnaient d'une encéphalo-myélite non suppurante, chez trois de ces poneys. Des anticorps contre ce virus se retrouvèrent dans le sérum des cinq poneys, mais dans le liquide céphalo-rachidien de seulement deux. On réussit à isoler le virus du cerveau et de la moelle épinière de seulement le poney qui l'avait reçu par la voie intracérébrale.

INTRODUCTION

Diagnosis of equine viral encephalomyelitis is optimally achieved by isolation of the etiological agent or demonstration of its antigens. Because brain tissue is the best source of antigen, postmortem examination is required to establish a diagnosis. However, although virus isolation can be accomplished relatively easily and reliably with rabies virus (1), it has been difficult to achieve with many other agents, such as equine herpesvirus type 1 (EHV-1) (2,3), eastern and western equine encephalomyelitis viruses (EEE, WEE) (4-6) and Powassan virus (POW) (7). More often a presumptive diagnosis is made on the basis of epidemiological and clinical history, serology and histopathology having ruled out other nonviral causes of central nervous system (CNS) disease.

The purpose of this study was to determine whether cerebrospinal fluid (CSF) antibodies may be of value in the antemortem diagnosis of equine viral encephalomyelitis. The three viruses chosen for this study were EHV-1, a cause of sporadic neurological disease in horses (8.9), POW, a tick-borne virus of the genus Flavivirus, shown experimentally to produce nonsuppurative encephalomyelitis in horses (7), and snowshoe hare virus (SSH), a mosquito-transmitted virus of the genus Bunyavirus, which has been implicated as an etiological agent of equine neurological disease (10). Antibody levels to these three viruses were determined in paired sera and CSF from 90 neurologically normal horses and from 25 horses with various spontaneous neurological disorders. In order to follow the

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development of CSF antibodies in acute encephalomyelitis due to a known agent, five ponies were inoculated with POW, paired sera and CSF were collected at various intervals postinoculation (PI) and POW antibody levels were determined by hemagglutination-inhibition (HI).

MATERIALS AND METHODS

SEROLOGICAL SURVEY SAMPLES

One hundred and fifteen paired sera and CSF were collected from horses, one month to 24 years of age, between August 1984 and January 1986. Thirty-six animals were Standardbred, 27 Thoroughbred and the remainder included Quarter horse, Arab, Hunter, Belgian, Hannoverian, Clydesdale, pony and crossbreeds. The majority of animals were resident in southern Ontario with a few from northern and eastern Ontario. Twenty-five CSF samples were collected from horses with neurological disease, either aseptically from the cisterna magna in the anesthetized animal, or from the lumbosacral region in the conscious animal (11). The remainder of CSF samples were collected from the cisterna magna of horses immediately upon euthanasia, prior to postmortem examination.

HEMAGGLUTINATION-INHIBITION TESTS

Hemagglutination-inhibition tests were performed by the method of Clarke and Casals (12) as modified to a microtiter technique by Sever (13). All sera and CSF were prepared by acetone extraction (12), and 50 μL of horse serum, negative for antibodies to POW and SSH, were added to CSF samples to provide sufficient bulk of precipitate to assure quantitative recovery of antibody (12). Powassan virus antigen was prepared by sucrose-acetone extraction of infected suckling mouse brain (12), and SSH antigen by polyethylene glycol precipitation of virus propagated in Vero cells as described elsewhere for the preparation of Jamestown Canyon antigen (14). Four hemagglutination (HA) units were used for SSH, and eight HA units for POW antigen. Initial dilutions of sera and CSF were 1:10 and 1:2 respectively. Antibody titer was expressed as the reciprocal of the highest dilution of serum or CSF that exhibited inhibition of hemagglutination.

VIRUS NEUTRALIZATION TEST

Virus neutralization (VN) tests were conducted by the method of Blythe *et al* (15) with the modifications that Army strain 183 of EHV-1 (Dr. J.T. Bryans, Kentucky) and Madin-Darby bovine kidney cells were used. Each sample was tested in duplicate, initial serum and CSF dilutions were 1:2, and antibody titer was expressed as the reciprocal of the highest dilution of serum or CSF that neutralized the virus.

PREPARATION OF POWASSAN VIRUS SUSPENSION

Powassan virus, strain LB prototype M794, at passage level two, was used for horse inoculations. Stock virus, with a titer of $10^{7.75}$ median mouse intracerebral lethal doses ($MICLD_{50}$)/mL, was prepared in suckling mouse brain and stored in 1 mL aliquots at –70° C. A 1:5 dilution of stock virus was used for all horse inoculations: 2 mL for intracerebral (IC) and 5 mL for intravenous (IV) inoculation.

INOCULATION OF PONIES WITH POWASSAN VIRUS

Six ponies, varying in age from 1.5 to 7 years and in weight from 109 to 230 kg, were purchased from a sale yard in June 1985. The ponies were all treated with mebendazole (Equiverm, Davis and Lawrence, Toronto, Canada) and complete blood count (CBC) and biochemical profiles were obtained for each animal prior to the commencement of the experiment. The ponies were housed in isolation.

On day 0, serum was collected from each animal and frozen for later POW antibody determination. Each pony was then anesthetized using a mixture of xylazine (1.1 mg/kg) (Rompun, Haver-Lockhart, Bayvet Division, Miles Laboratories Ltd., Rexdale, Ontario) and ketamine (1.0 mg/kg) (Vetalar, Parke-Davis Canada Inc., Scarborough, Ontario) and CSF was collected aseptically from the cisterna magna (11). Protein determination (15) and total and differential cell counts were performed on a portion of the CSF and the remainder was frozen for later POW antibody determination. Powassan virus was inoculated IC into pony #798 while it was still under general anesthesia, using the technique described by Little *et al* (7). Powassan virus was inoculated IV into four ponies (#s 808, 943, 939, 944) after they had recovered from anesthesia and the sixth pony (#950) was given 5 mL of phosphate buffered saline (PBS) IV.

Rectal temperatures were monitored twice daily for two weeks and daily thereafter. The ponies were observed daily for evidence of clinical disease, and whole blood, serum and CSF samples were collected at various intervals PI. The animals were euthanized with an overdose of pentobarbitol (Euthanyl, MTC Pharmaceuticals, Mississauga, Ontario) IV. A postmortem examination was performed and the following tissues preserved in 10% buffered formalin for histological examination: thyroid, heart, lung, kidney, spleen, liver, thymus, mesenteric lymph node, adrenal, skeletal muscle, pancreas, esophagus, stomach, duodenum, jejunum, ileum, pituitary, trigeminal ganglia, spinal cord and brain. Frontal cortex, spleen, liver and lymph node were cultured for bacteria, and frontal cortex, cerebellum, trigeminal ganglia and spinal cord were frozen at –70° C for viral isolation.

VIRUS ISOLATION

Virus isolation was attempted from frontal cortex, cerebellum, trigeminal ganglia and spinal cord of all inoculated animals as described by Shope (17) with the modification that a 20% uncentrifuged suspension of brain tissue was prepared and inoculated into three to four-day-old suckling mice. Suspected positive suckling mouse brains were inactivated by treatment overnight at 4° C with 0.3% beta-propiolactone (Sigma, St. Louis, Missouri) (18) and virus identification made by the complement-fixation test (19).

RESULTS

SEROLOGICAL STUDIES OF SURVEY SAMPLES

All sera and CSF were negative for antibodies to POW.

TABLE I. Horses With High Serum Antibody Titers (≥ 320) and/or Positive Cerebrospinal Fluid (CSF) Antibody Titers to Snowshoe Hare Virus (SSH)

ID No.	Age	Breed[a]	Sex	Test Month	Serum SSH Titer[b]	CSF SSH Titer[b]	Ratio Serum: CSF Titer	Clinical Diagnosis
531043	14 yr	Qtr	M	May 85	1280	16	80	Fractured radius
530034	8 yr	Qtr	F	Oct 84	1280	4	320	Colonic torsion
528294	18 mo	Thb	F	Oct 84	1280	4	320	Osteomyelitis
530320	2 yr	Std	M	Jan 85	640	4	160	Cervical stenotic myelopathy
30242	3.5 yr	Clydes	F	Dec 84	640	2	320	Colic
530912	12 yr	Std	F	May 85	640	2	320	Colic
527034	4 mo	Thb	M	May 85	640	Neg[c]	—	Fractured femur
530490	13 yr	Qtr	F	Feb 85	320	4	80	Respiratory disease
529902	3 yr	Qtr	F	Sept 84	320	4	80	Encephalitis
530636	4 yr	Std	G	Mar 85	160	2	80	Arthritis
531206	12 yr	Hunter	G	June 85	160	2	80	Lameness
530500	5 yr	Qtr	G	Feb 85	80	4	20	No history available
530998	2 mo	Std	M	May 85	80	2	40	Physitis
530898	7 yr	Std	F	May 85	80	2	40	Nephrosis
530693	4 yr	Thb	G	Apr 85	80	2	40	Colic
530612	13 yr	Thb	F	Mar 85	20	2	10	Colic

[a]Qtr — Quarter horse; Thb — Thoroughbred; Std — Standardbred; Clydes — Clydesdale
[b]Antibody titer expressed as the reciprocal of the highest dilution of CSF or serum that exhibited hemagglutination-inhibition
[c]CSF SSH titer interpreted as < 2

Fifty-one of 115 sera (44.3%) and 15 CSF (13.0%) had antibodies to SSH antigen. Serum titers ranged from 10 to 1280 and CSF titers from 2 to 16. Of the nine serum samples with titers of 320 or greater, eight had measurable antibodies in the CSF (Table I). There was an increasing prevalence of serum SSH antibodies with age, 8/33 horses (24.2%) < 2 years, 10/32 horses (31.3%) two to six years and 34/50 horses (68.0%) older than six years having serum antibodies to SSH antigen.

Ninety-eight of 115 sera (85.2%) were positive for EHV-1-neutralizing antibody at a titer of 2 or greater, and there was an increasing prevalence with age, 22/33 horses (66.7%) < 2 years, 27/32 horses (84.4%) two to six years and 49/50 horses (98.0%) older than six years having antibodies to EHV-1 antigen. Four CSF samples (3.5%) were positive with titers of 2 or 3 (Table II), and three of these had serum to CSF titer ratios of 96 or greater.

Of the 25 horses withi spontaneous neurological disease, only three had encephalitis of possible viral etiology. One (531027) had serum and CSF antibody to EHV-1 (Table II) and a postmortem diagnosis of possible viral encephalomyelitis was confirmed. The second (529902) had serum and CSF antibody to SSH (Table I) but convalescent serum and CSF samples revealed no change in antibody titers. The third animal did not have viral antibody in serum or CSF.

INOCULATION OF PONIES WITH POWASSAN VIRUS

Clinical Signs — Three ponies (# 939, 943, 950) remained normal throughout the experimental period. Animal #798 was clinically normal until day 6 PI at which time it was hyperesthetic. The following day it made constant chewing movements. On the eighth and ninth days PI, the pony was depressed, anorexic, had muscle tremors over its entire body, constantly shifted from one hind leg to the other and continued to make chewing movements. Clinical improvement was apparent on day 10 PI but the pony remained hyperesthetic especially to sudden sound or movement and still had muscle tremors. These signs remained until two days later when the animal was euthanized. Pony #808 was clinically normal until day 12 PI (the day it was euthanized) at which time it was irritable, hyperesthetic and had trembling of its lower lip. On day 8 PI pony #944 was depressed, anorexic and had trembling of its lower lip. The following day it began to eat again but remained hyperesthetic and its lower lip continued to tremble. Muscle tremors

TABLE II. Horses With Neutralizing Antibodies to Equine Herpesvirus Type 1 (EHV-1) in Cerebrospinal Fluid (CSF)

ID No.	Age	Breed[a]	Sex	Test Month	Serum EHV-1 Titer[b]	CSF EHV-1 Titer[b]	Ratio Serum: CSF Titer	Clinical Diagnosis
527034	4 mo	Thb	M	May 85	384	3	128	Fractured femur
531268	yearling	Thb	M	July 85	256	2	128	Brain abscess
531503	mature	Std	G	Sept 85	192	2	96	Laminitis
531027	5 yr	Hunter	F	May 85	64	2	32	Encephalomyelitis

[a]Thb — Thoroughbred; Std — Standardbred
[b]Titers expressed as the reciprocal of the highest dilution of CSF or serum that neutralized the virus

were apparent over its entire body but were more pronounced over the head and neck. There was gradual improvement over the following two weeks.

Rectal Temperatures — Four animals developed fever (temperature ⩾ 39.0°C) during the experimental period and this coincided with the onset of clinical signs in two ponies (Table III). The third animal which developed clinical signs (#944) also had a moderate increase in temperature (38.7°C) on day 8 PI.

Hematology — Complete blood count determinations made preinoculation and at intervals PI were either normal or indicative of a physiological leukocytosis, i.e. mild to moderate leukocytosis characterized by mild neutrophilia without a left shift and either a normal lymphocyte number or a lymphocytosis.

CSF Analysis — The CSF protein values were variable and only two ponies had greater than 1.0 g/L in any sample examined (Table III).

All animals except #950 and #943 had a marked increase in the number of white blood cells in the CSF (12-20/μL) at some time PI (Table III). The majority of cells seen were mononuclear with low numbers of neutrophils present in some samples.

Bacteriology — Bacterial pathogens were not cultured from tissues of any of the animals.

Serology — All preinoculation samples were negative for antibodies to POW. With the exception of pony #950, all animals seroconverted to POW (Table IV). Titers ranged from 40 (animal euthanized at day 12 PI) to 640.

Antibodies to POW were present in the CSF of only two animals — #798 and #944. By day 9 PI, pony #798 had a titer of 8 in the CSF and this had increased by day 12 PI. Pony #944 had a CSF titer of 4 by day 13 PI and this reached a maximum of 32 in the fourth week PI, and thereafter declined. Cerebrospinal fluid antibodies were of lower magnitude and were detected later than serum antibodies in the IV inoculated animal.

Virus Isolation — Powassan virus was isolated from cerebellum, cerebrum, spinal cord and trigeminal ganglia of only one pony, #798.

Histopathology — Histological lesions were not detected in ponies #950 or #943. In the remaining animals lesions were confined to the nervous system and were typical of a nonsuppurative meningoencephalomyelitis. Focal and diffuse gliosis, mononuclear cell perivascular cuffing, neuronal degeneration and meningitis were evident (Figs. 1 and 2). The most severe lesions were in brain, spinal cord and trigeminal ganglia of pony #798. Ponies #808 and #944 had mild to moderate lesions which were more severe in spinal cord than in brain and in gray matter than in white matter. Pony #939 had only mild meningitis with occasional perivascular cuffs and gliosis in the spinal cord.

DISCUSSION

Serological data obtained in this study indicates that horses have a much higher exposure to SSH than to POW in Ontario. This is probably because SSH is much more ubiquitous than POW in Ontario (20) and because horses are more likely to be bitten by mosquitoes, the SSH vector, than by ticks, the POW vector. Antibodies to POW were not detected in serum or CSF of any animal in contrast to the report by Little *et al* (7) in which 13% of 101 Ontario horses had serum antibodies to POW, albeit at very low levels. Some of this difference may be due to differing geographical selection of horses since POW virus is known to be more prevalent in more northerly areas of Ontario. Exposure of horses to SSH antigen is, on the other hand, very high with 44.3% of 115 horses in the present study having antibodies ranging in titer from 10 to 1280. These results are comparable to previous surveys conducted in southern Ontario in 1976 and 1977 in which 21.1% of 228 and 41.4% of 112 sera respectively were positive for antibodies to SSH antigen (21,22). Serum antibody titers in the latter surveys were much lower than in the present survey, possibly because

TABLE III. Inoculation Regime and Occurrence (Day postinoculation PI) of Clinical Disease, Pyrexia, Increased White Blood Cells (WBC) in Cerebrospinal Fluid (CSF), Euthanasia, and Histology of Brain and Spinal Cord of Ponies Experimentally Inoculated with Powassan Virus (POW)

Pony #	Inoculation Regime	Days PI When Clinical Signs Were Apparent	Days PI When Temp ⩾ 39.0°C	Days PI When Inc. Protein in CSF	Days PI When Inc. WBC in CSF[a]	Day PI of Euthanasia	Histological Evidence of Encephalomyelitis
798	IC POW	6-12	6-8	12[b]	9,12	12	Present Mod-Severe[c]
808	IV POW	12	11,12	—	12	12	Present Mild-Mod
943	IV POW	—	7	—	—	19	Absent
939	IV POW	—	8	—	0,21	26	Present Mild
944	IV POW	8-24	—	23[d]	13,19,23	33	Present Mild-Mod
950	IV PBS	—	—	—	—	16	Absent

[a] > 5 WBC/μL in CSF
[b] 1.20 g/L protein in CSF
[c] Powassan virus isolated from brain and spinal cord
[d] 1.03 g/L protein in CSF

TABLE IV. Serum (Ser) and Cerebrospinal Fluid (CSF) Antibody Titers to Powassan Virus (POW) in Inoculated Ponies, at Various Times Postinoculation (PI)

	Pony Number											
	798		808		943		939		944		950	
Day PI	Ser	CSF	Ser	CSF	Ser	CSF	Ser	CSF	Ser	CSF	Ser	CSF
-6	Neg[a]	ND	Neg	ND	Neg	ND	Neg	ND	Neg	ND	Neg	ND
0	Neg	Neg[b]	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
6			Neg	Neg								
7									80	Neg		
8	80	ND					Neg	Neg			Neg	Neg
9	80	8			80	Neg			160	ND		
12	80	> 8	40	Neg								
13					640	ND	320	ND	320	4		
14					320	Neg						
16							640	Neg			Neg	Neg
19					160	Neg			160	8		
21							160	Neg				
23									80	32		
26							160	Neg				
28									80	16		
33									160	4		

[a]Negative sample interpreted as < 10
[b]Negative sample interpreted as < 2
ND = Not tested

samples were collected in the late fall and winter, some time after peak mosquito activity. Prevalence of SSH antibodies increased with age, perhaps reflecting increased probability of exposure to the agent. The high serum titers present in several horses may represent anamnestic responses in previously sensitized animals.

Antibody prevalence indicates high endemicity of EHV-1 infection in horses in Ontario and increasing age prevalence. Similar results have been reported by other authors (23,24).

Thirteen percent of equine CSF samples had antibodies to SSH and 3.5% to EHV-1 albeit at low levels. The difficulty regarding these low CSF titers arises in determining whether or not they are significant and indicative of CNS infection. There are several possible explanations for the origin of the immunoglobulins detected in the CSF. Firstly the immunoglobulins may represent transudation of serum antibodies across the blood-brain (BBB) or blood-CSF barriers (25,26). Sherwin *et al* (27) showed that antibodies could be detected in the CSF of rabbits after either active or passive immunization with bovine serum albumin or ovalbumin and that the ratio of serum antibody to that of the CSF antibody titer varied between 100 and 1600. Immunoglobulins may also be produced locally within the CNS (28). Reid *et al* (29) inoculated sheep with egg albumin intramuscularly to provide a circulating antibody that could be used as a serum marker and three weeks later inoculated the majority of the same animals with louping-ill virus subcutaneously (SC). They found the ratio of antibodies to egg albumin in serum to that in CSF to be 80 to 1280 whereas the ratio of serum to CSF antibodies to louping-ill virus was 5 to 40. This indicates that there was some transudation of serum antibodies into the CSF but that there was also local production of antibodies to louping-ill virus within the CNS. Greater than expected serum to CSF antibody ratios may also indicate either increased permeability of the BBB or blood-CSF barrier due to inflammation or mechanical damage, or contamination of CSF by blood during collection (28).

In the present study serum to CSF ratios for SSH antibodies varied from

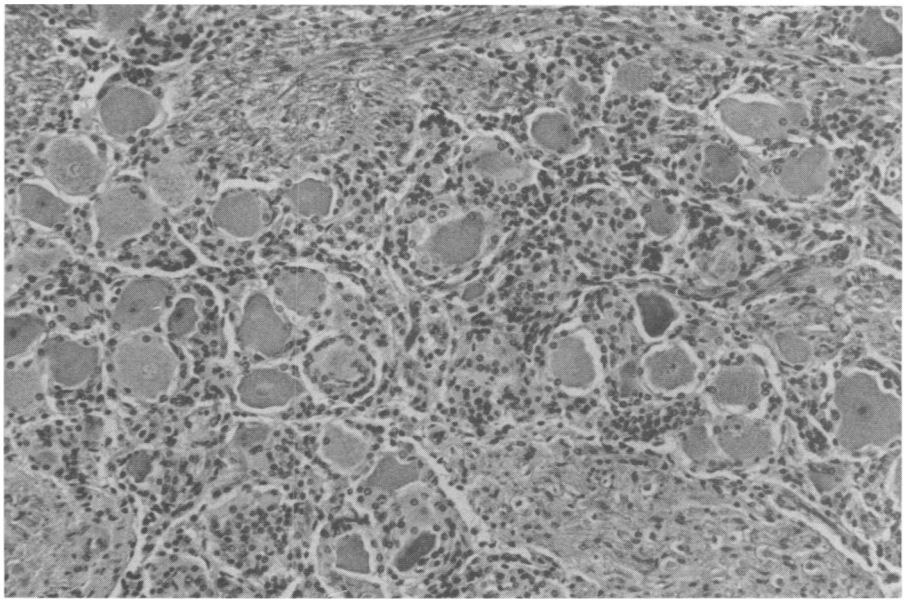

Fig. 1. Trigeminal ganglia pony #798 inoculated intracerebrally with Powassan virus. Note ganglionitis and neuronophagia. H & E. X10.

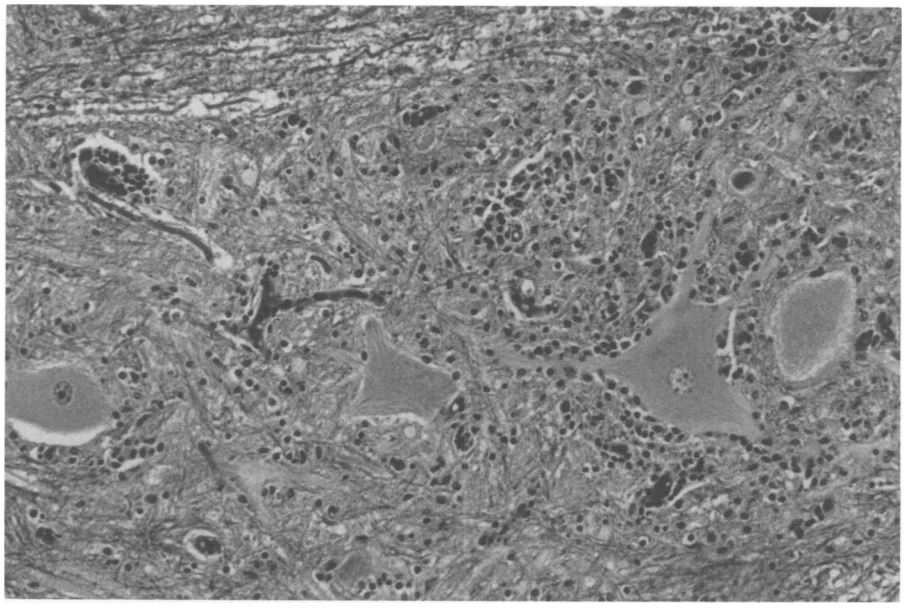

Fig. 2. Diffuse gliosis grey matter spinal cord pony #808 inoculated intravenously with Powassan virus. H & E. X10.

10 to 320, with 10 of the 15 horses having ratios of 80 or greater. In these latter animals, the CSF antibodies probably represent transudation of immunoglobulin across the BBB or blood-CSF barrier. One of these animals (529902) had serum and CSF antibody titers of 320 and 4 respectively. This animal was diagnosed clinically as having encephalitis but convalescent serum and CSF samples revealed no change in either the serum or CSF antibody titers. Hence the CSF antibodies may have resulted from transudation secondary to increased permeability of the BBB due to inflammation caused by an agent other than SSH. None of the remaining animals had a history of neurological disease, three of the CSF samples were grossly contaminated with blood and as the serum and CSF titers were very low in all cases, these CSF titers were not considered to be significant.

Only four animals had CSF antibodies to EHV-1. In three of these the serum to CSF ratio was 96 or greater, which probably indicates transudation of antibodies across the BBB. In addition, one of these animals (531268) had serum and CSF titers of 64 and 2 respectively. This horse had a ten day history of neurological disease and on postmortem examination histological lesions were suggestive of encephalomyelitis due to EHV-1. In this case the CSF antibodies were probably significant.

Following experimental inoculation of ponies with POW, clinical signs were observed in only two of the four IV inoculated animals and in the IC inoculated pony. Cerebrospinal fluid antibodies were present in two animals and all POW-inoculated ponies developed serum antibodies. In the IC-inoculated animal, CSF antibodies appeared simultaneously with serum antibodies, a similar finding to that reported by Gerhard *et al* (28) who inoculated mice IC with parainfluenza type 1 virus. The IC-inoculated horse, however, was probably killed before maximal CSF titers had developed. In the IV-inoculated animal, CSF antibodies were detected later than serum antibodies and were of lower magnitude. The CSF antibodies, however, were increasing in the face of decreasing serum levels suggesting that they were being produced locally within the CNS. The decline in CSF antibodies by day 33 PI suggests that POW had been cleared from the CNS and may explain why viral isolation was negative.

Cerebrospinal fluid antibodies were not detected in pony #808 because this animal was euthanized the day clinical signs first appeared and hence CSF antibodies would not have had a chance to develop. Although pony #939 did not have clinical signs, there were very mild histological lesions of encephalomyelitis, and CSF antibodies in this animal may only have been present in very low or undetectable quantities. Hofmann *et al* (30) reported similar problems in detecting antibodies against tick-borne encephalitis (TBE) in CSF of patients by means of a HI test, but they developed an enzyme-linked immunosorbent assay (ELISA) that was senstitive and specific for the detection of TBE antibodies in serum and CSF. Perhaps a similar test is required for the detection of POW antibodies in the CSF.

Normal CSF protein values for ponies are reported to range from 0.20 to 1.05 g/L with a mean of 0.60 g/L (31). This makes interpretation of relatively high values difficult. The CSF protein in pony #798 was significantly increased at 1.20 g/L although there were large numbers of red blood cells (RBC) present — 2500/μL (Reference values 0-2000/μL (31)). Interpretation of protein values in the CSF of pony #944 are more difficult as the sample obtained at day 23 PI, in which there was 1.03 g/L protein also had 2710 RBC/μL. Four of the five inoculated ponies had an increase in white blood cells (WBC) ($>$ 12 WBC/μL) in the CSF at some time during the experimental period. Normal WBC counts in CSF are reported to be from 0 to 6/μL (31). The majority of cells present were mononuclear with low numbers of neutrophils in many samples. These findings are consistent with other reports of viral inflammation of the CNS in which neutrophils may be seen early in the infection but mononuclear cells predominate (32). All animals with abnormal CSF cell counts also had histological lesions of a nonsuppurative encephalomyelitis. Lesions tended to be more severe in spinal cord than in brain and more severe in gray matter than in white matter. This distribution of lesions is compatible with hematogenous spread of POW to the CNS.

Powassan virus was only isolated from brain and spinal cord tissue of the IC inoculated animal. Viral isolation from brain of horses with encephalomyelitis has been notoriously difficult for many agents. Little *et al* (7) were unable to isolate POW

from any of their experimental ponies, Platt *et al* (2) and Dinter and Klingeborn (3) were unable to isolate EHV-1 from horses with the neurological form of the disease and Kissling *et al* (4) and Monath *et al* (5) showed that viral isolation from the brain of horses with EEE was only successful if attempted within one or two days of the onset of disease.

It is difficult to draw any definitive conclusions on the antemortem diagnostic use of CSF antibodies in equine viral encephalomyelitis from the survey results as only 3 of the 115 animals had a history of encephalitis during our survey interval. However, data from the experimental inoculation of ponies with POW shows that CSF antibodies do develop in clinically affected animals given time and we feel that measurement of CSF and serum antibodies in clinically affected horses is a worthwhile diagnostic procedure, especially in those with subacute or nonfatal disease.

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