Detection of antibodies against *Sarcocystis neurona* in cerebrospinal fluid from clinically normal neonatal foals

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Objective—To determine whether antibodies against *Sarcocystis neurona* could be detected in CSF from clinically normal neonatal (2 to 7 days old) and young (2 to 3 months old) foals.

Design—Prospective study.

Animals—15 clinically normal neonatal Thoroughbred foals.

Procedure—Serum and CSF samples were obtained from foals at 2 to 7 days of age and tested for antibodies against *S neurona* by means of western blotting. Serum samples from the mares were also tested for antibodies against *S neurona*. Additional CSF and blood samples were obtained from 5 foals between 13 and 41 days after birth and between 62 and 90 days after birth.

Results—Antibodies against *S neurona* were detected in serum from 13 mares and their foals; antibodies against *S neurona* were detected in CSF from 12 of these 13 foals. Degree of immunoreactivity in serum and CSF decreased over time, and antibodies against *S neurona* were no longer detected in CSF from 2 foals 83 and 84 days after birth. However, antibodies could still be detected in CSF from the other 3 foals between 62 and 90 days after birth.

Conclusions and Clinical Relevance—Results indicate that antibodies against *S neurona* can be detected in CSF from clinically normal neonatal (2 to 7 days old) foals born to seropositive mares. This suggests that western blotting of CSF cannot be reliably used to diagnose equine protozoal myeloencephalitis in foals < 3 months of age born to seropositive mares. (*J Am Vet Med Assoc* 2002;220:208–211)

Equine protozoal myeloencephalitis (EPM) caused by the protozoal organism *Sarcocystis neurona* is the most commonly diagnosed neurologic disorder of horses in the United States.[1] Retrospective surveys[2-4] indicate that horses < 4 years old are more susceptible to EPM than are older horses, and EPM has been diagnosed as early as 2 months of age.[3] Equine protozoal myeloencephalitis is currently diagnosed when results of **western blotting (WB)** performed on CSF from a horse with characteristic neurologic abnormalities are positive. Therefore, as part of a complete diagnostic workup, foals with neurologic abnormalities are often screened for EPM by WB of CSF.

It has been suggested that neonatal foals have a permeable blood-CSF barrier that allows passage of proteins into the CSF,[5-7] and previous studies[6,7] have documented that CSF protein and IgG concentrations in foals are higher than those in adult horses. In addition, authors of a previous study[8] found that maternal antibodies against *S neurona* were passively transferred from mares to their foals. In that study, 33 foals from seropositive mares were seronegative before ingestion of colostrum and seropositive afterwards. However, it is not known whether these maternal antibodies against *S neurona* can permeate the blood-CSF barrier in clinically normal foals.

The purpose of the study reported here, therefore, was to determine whether antibodies against *S neurona* can be detected in the CSF of clinically normal neonatal (2 to 7 days old) foals. In addition, because authors of a previous study[8] found that maternal antibodies in seropositive foals were metabolized over a period of a few months (mean time for seropositive foals to become seronegative was 4.2 months), a secondary objective of the present study was to determine whether *S neurona* antibodies could still be detected in the CSF of clinically normal foals up to 2 to 3 months of age.

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Materials and Methods

Foals—Fifteen healthy Thoroughbred foals were used in the study. All foals were from the Virginia Tech broodmare and foal herd maintained in Middleburg, Va, and had been delivered naturally. All births had been observed. Physical and neurologic examinations were performed on all foals prior to inclusion in the study, and foals that had had an abnormal delivery, had abnormal physical or neurologic examination findings, had a serum IgG concentration < 800 mg/dl, or had been given a colostrum supplement were excluded from the study. The study protocol was approved by the Virginia Tech Animal Care and Use Committee.

Sample collection—When foals were between 2 and 7 days old, they were anesthetized with xylazine hydrochloride (0.46 mg/kg [0.21 mg/lb] of body weight, IV), butorphanol (0.02 mg/kg [0.009 mg/lb], IV), and propofol (2.0 mg/kg [0.9 mg/lb], IV). Foals were placed in lateral recumbency, and

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anesthesia was maintained by administration of additional propofol as needed. Cerebrospinal fluid was collected aseptically from the atlanto-occipital space as described[9]; cephalic venous blood samples were obtained from the foals at the same time. Jugular venous blood samples were collected from the mares when CSF samples were collected from the foals.

Sample analysis—A WB assay was used to detect antibodies against *S neurona* in CSF from the foals and in serum from the foals and mares. The assay was performed by a commercial laboratory,[a] as described.[10] Results of WB were reported as negative, weak positive, low positive, or positive. Control samples representing various degrees of immunoreactivity were included on each blot, and results for the test samples were compared with results for these control samples. A weak positive result was interpreted as equivocal or borderline immunoreactivity, a low positive result was interpreted as an intermediate degree of immunoreactivity, and a positive result was interpreted as a higher degree of immunoreactivity.

Radial immunodiffusion, performed as described,[11,a] was used to measure concentrations of IgG in CSF and serum. Serum and CSF albumin concentrations were determined spectrophotometrically.[a] Cytologic examination and biochemical testing of CSF samples were performed within 2 hours of collection.

The albumin quotient and IgG index were calculated with the following formulas:

$$\text{Albumin quotient} = \frac{\text{CSF albumin concentration}}{\text{Serum albumin concentration}} \times 100$$

$$\text{IgG index} = \frac{\text{CSF IgG concentration}}{\text{Serum IgG concentration}} \times \frac{\text{Serum albumin concentration}}{\text{CSF albumin concentration}}$$

Follow-up CSF samples—At least 7 days after initial CSF and serum samples were obtained, follow-up CSF and serum samples were obtained from 5 foals for which results of WB of initial CSF samples were positive. Age at the time follow-up samples were collected ranged from 13 to 41 days. A third set of CSF and serum samples was obtained from these 5 foals 49 days later, at which time foals ranged from 62 to 90 days old.

Statistical analyses—A Pearson χ^2 test was used to test for an association between results of WB for serum and CSF samples from the neonatal foals. Analyses were performed with computer software.[12,b] Values of $P < 0.05$ were considered significant.

Results

Cerebrospinal fluid samples were obtained from the atlanto-occipital space without difficulty. Eleven of 15 samples contained < 6 RBC/µl, with the remaining samples containing 11, 16, 20, and 40 RBC/µl. Serum IgG concentration, CSF IgG concentration, albumin quotient, and IgG index were higher than previously reported (**Table 1**).

Thirteen of the 15 mares had serum antibodies against *S neurona*. The 13 foals from these mares all also had serum antibodies against *S neurona* when tested at 2 to 7 days of age. The remaining 2 mares and the 2 foals from these mares were all seronegative. Twelve of the 13 seropositive foals also had positive CSF WB results, and degree of CSF immunoreactivity was significantly ($P < 0.001$) associated with degree of serum immunoreactivity (**Table 2**). The 1 seropositive foal that had negative CSF WB results had only weakly positive serum immunoreactivity. The 2 seronegative foals had negative CSF WB results.

Results of WB of follow-up serum and CSF samples obtained at 13 to 41 days of age from 5 foals were still positive. The degree of CSF immunoreactivity had decreased in 4 foals, the degree of serum immunoreactivity had decreased in 2 foals, and the CSF IgG concentration had decreased in 4 foals (**Table 3**). For the third set of serum and CSF samples obtained at 62 to 90 days of age from these foals, results of WB of CSF were negative for 2, results of WB of serum were negative for 1, and CSF IgG concentration had decreased in all 5.

Table 1—Results of analysis of CSF from 15 clinically normal neonatal (2 to 7 days old) foals

Variable	Mean ± SD	Median	Range	Reference range[6]
RBC count (cells/µl)	8 ± 11	3	0–40	208 ± 471
WBC count (cells/µl)	0.47 ± 0.81	0	0–3	1.3 ± 1.2
CSF albumin (mg/dl)	51.5 ± 15	51.5	21.2–78.4	52.0 ± 8.6
Albumin quotient	2.7 ± 1.1	2.6	1.0–5.1	1.86 ± 0.29
CSF IgG (mg/dl)	25.6 ± 10.8	22.4	9.5–43.1	10.2 ± 5.5
IgG index	0.35 ± 0.14	0.33	0.19–0.67	0.519 ± 0.284

Table 2—Cross-correlation of results of western blotting (WB) of CSF and serum from 15 neonatal foals for antibodies against *Sarcocystis neurona*

	CSF WB results			
Serum WB results	Negative	Weak positive	Low positive	Positive
Negative	XX			
Weak positive	X			
Low positive		XXX	XX	
Positive			XXXX	XXX

Results for CSF and serum were significantly ($P < 0.001$) associated. Each X represents a single foal.

Table 3—Results of WB for antibodies against *S neurona* and IgG concentration in CSF samples collected at 3 times from 5 foals

	First sample			Second sample			Third sample		
Foal No.	Age (d)	WB result	IgG (mg/dl)	Age (d)	WB result	IgG (mg/dl)	Age (d)	WB result	IgG (mg/dl)
1	6	Low positive	23.6	13	Weak positive	17.6	62	Weak positive	3.4
2	6	Low positive	17.5	13	Weak positive	18.4	62	Weak positive	1
3	4	Positive	22.4	41	Low positive	6.5	90	Weak positive	< 1
4	4	Weak positive	35.6	34	Weak positive	12.1	83	Negative	8.4
5	5	Low positive	15	35	Weak positive	2.4	84	Negative	< 1

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Discussion

Results of the present study suggest that antibodies against *S neurona* can be detected in CSF from clinically normal neonatal (2 to 7 days old) foals born to seropositive mares. In addition, antibodies could be detected in CSF from some foals for as long as 2 to 3 months after birth. These findings are clinically important, because they suggest that WB of CSF cannot be reliably used to diagnose EPM in foals < 3 months of age born to seropositive mares. On the other hand, results of WB of CSF were negative for the 2 foals in the present study born to seronegative mares. Thus, positive CSF WB results for a neonatal foal born to a seronegative mare can be considered supportive of a diagnosis of EPM.

The blood-CSF barrier is a functional and anatomic separation of the CSF from the vascular compartment of the body.[13] If the blood-CSF barrier is intact, then antibodies in the CSF can be assumed to originate from antigenic stimulation in the CNS. Theoretically, therefore, antibodies against *S neurona* detected in the CSF can be considered an indication that the organism is in the CNS. However, it has been speculated that an enhanced intracellular tubular transport system in neonatal foals and in neonates of other species allows for enhanced transport of proteins and protein fractions across the blood-CSF barrier.[14] A recent study[6] found that CSF IgG concentrations in clinically normal neonatal foals were substantially higher than values reported for adults horses, even though serum IgG concentrations in these foals were similar to reported values for adult horses. In clinically normal neonatal foals, as in neonates of other species, the CSF protein concentration is significantly higher at birth and for the first several weeks after birth than it is in adults.[5,7,15]

In a previous study[8] of 33 foals, maternal antibodies against *S neurona* were passively transferred from seropositive mares to their foals. Foals in that study were seronegative for antibodies against *S neurona* at birth and became seropositive within 24 hours after ingestion of colostrum. Results of the present study suggest that antibodies against *S neurona* can also be found in the CSF of clinically normal foals born to seropositive mares, likely as a result of the enhanced potential for transport of serum immunoglobulins into the CSF in neonatal foals. Analysis of follow-up samples from 5 foals in the present study indicated that these antibodies against *S neurona* can persist in the CSF for at least several months. Therefore, a positive WB result on CSF from a young foal may simply be a result of ingestion of antibody-rich colostrum, rather than an indication of EPM.

To our knowledge, in utero exposure of foals to *S neurona* has not been reported, and foals tested prior to ingestion of colostrum in a previous study[8] were seronegative. Unfortunately, in the present study, we did not collect CSF from the foals before they had ingested colostrum, and maternal antibodies were not labeled to allow specific detection in the foals. However, given that all foals born to seropositive mares were themselves seropositive and all foals born to seronegative mares were seronegative, it is most likely that antibodies detected in the foals were of maternal origin.

Analysis of multiple samples from 5 foals in the present study indicated that antibodies against *S neurona* persisted in the CSF for 2 to 3 months after birth. Although it is possible that foals were exposed to *S neurona* during the time of the study, the degree of immunoreactivity in the CSF and serum decreased over time. If foals had been exposed to *S neurona* during the study period, we would have expected an increase in degree of serum and CSF immunoreactivity.[8]

High CSF IgG concentrations can result from damage to the blood-CSF barrier, an increase in local production of IgG secondary to inflammatory neurologic disease, or contamination of the CSF sample with blood during collection. As little as 0.001 µl of blood, which could correspond to as few as 8 RBC/µl of CSF, could result in a false-positive WB result if the blood were strongly immunoreactive for *S neurona*.[16] As indicated, blood concentrations in CSF samples in the present study ranged from 0 to 40 RBC/µl, with 11 of 15 having < 6 RBC/µl.

It has been suggested that calculation of the albumin quotient and IgG index can help in differentiating the source of IgG in CSF samples.[6] However, values for foals in the present study were appreciably different from those published in a previous study[6] of healthy foals of the same age range. This disparity between studies may, in part, be attributable to differences in the degree of blood contamination in CSF samples. Mean ± SD number of RBC in CSF samples in the present study (8 ± 11 RBC/µl) was substantially lower than mean number of RBC in CSF samples in the previous study (208 ± 471 RBC/µl). In addition, although mean CSF albumin concentration in the present study (51.5 ± 15 mg/dl) was similar to mean CSF albumin concentration in the previous study (52 ± 8.6 mg/dl), serum albumin concentration was lower in the present study (2,007 ± 608 mg/dl) than in the previous study (2,900 ± 240 mg/dl), which would have affected calculated albumin quotients and IgG indices. The discrepancy in serum albumin concentrations between studies is probably attributable to methodologic differences. In the present study, CSF and serum albumin concentrations were determined spectrophotometrically, whereas in the previous study, CSF albumin concentrations were determined by means of electrophoresis, and serum albumin concentrations were determined by use of chemical analysis.

Rossdale et al[15] suggested that CSF protein concentrations decrease to adult concentrations within 2 weeks after birth in foals. In the present study, however, the decrease in CSF IgG concentration was slower than this, and low CSF IgG concentrations were not detected in some foals until 2 to 3 months after birth.

[a]Equine Biodiagnostics Inc, Lexington, Ky.
[b]SAS system, version 8.1, SAS Institute Inc, Cary, NC.

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Correction: Role of dietary carbohydrate and fat in horses with equine polysaccharide storage myopathy

In "Role of dietary carbohydrate and fat in horses with equine polysaccharide storage myopathy" (*J Am Vet Med Assoc* 2001;219:1537–1544), the second sentence from the end of the first paragraph on the top of the left column on page 1541 is incorrect. The correct statement appears below:

Instead, addition of fat may actually increase concentrations of serum α-tocopherol.

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