Risk of postnatal exposure to *Sarcocystis neurona* and *Neospora hughesi* in horses

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Objective—To estimate risk of exposure and age at first exposure to *Sarcocystis neurona* and *Neospora hughesi* and time to maternal antibody decay in foals.

Animals—484 Thoroughbred and Warmblood foals from 4 farms in California.

Procedure—Serum was collected before and after colostrum ingestion and at 3-month intervals thereafter. Samples were tested by use of the indirect fluorescent antibody test; cutoff titers were ≥ 40 and ≥ 160 for *S neurona* and *N hughesi*, respectively.

Results—Risk of exposure to *S neurona* and *N hughesi* during the study were 8.2% and 3.1%, respectively. Annual rate of exposure was 3.1% for *S neurona* and 1.7% for *N hughesi*. There was a significant difference in the risk of exposure to *S neurona* among farms but not in the risk of exposure to *N hughesi*. Median age at first exposure was 1.2 years for *S neurona* and 0.8 years for *N hughesi*. Highest prevalence of antibodies against *S neurona* and *N hughesi* was 6% and 2.1%, respectively, at a mean age of 1.7 and 1.4 years, respectively. Median time to maternal antibody decay was 96 days for *S neurona* and 91 days for *N hughesi*. There were no clinical cases of equine protozoal myeloenchaphlitis (EPM).

Conclusions and Clinical Relevance—Exposure to *S neurona* and *N hughesi* was low in foals between birth and 2.5 years of age. Maternally acquired antibodies may cause false-positive results for 3 or 4 months after birth, and EPM was a rare clinical disease in horses ≤ 2.5 years of age. (*Am J Vet Res* 2004;65:1047–1052)

Sarcocystis neurona[1] and *Neospora* spp,[2-4] particularly *N hughesi*,[5-7] are the causal agents of **equine protozoal myeloencephalitis (EPM)**. Although the annual incidence of clinical EPM is reportedly[8] low (0.14%) among the general horse population, healthy horses in the United States are commonly exposed to *S neurona* and *Neospora* spp. Seroprevalence estimates range from 30% to 60% for *S neurona*[9-15] and 12% to 37% for *Neospora* spp.[7,14,16,17] Seropositivity to *S neurona* reportedly[9-13] increases with age and decreases with increasing numbers of days in which the ambient temperature is 0°C or colder, and it may possibly decrease with a decrease in humidity. In addition, greater likelihood of EPM was reported in race or show horses that were between 1 and 4 years of age, that had a history of a prior illness or injury, and that were from premises with a prior history of horses with EPM and evidence of opossums.[18]

To our knowledge, there have been no prospective studies to assess the risk of exposure and age at exposure to *S neurona* or *N hughesi* for field conditions. Furthermore, there has been limited assessment of decay of maternally derived antibodies to either parasite.[19] The objective of the study reported here was to estimate the risk of exposure and age at first exposure to *S neurona* and *N hughesi* and the patterns of decay of maternally derived antibodies in a cohort of foals from farms in California that had a history of horses with EPM.

Materials and Methods

Animals—Foals on 4 farms (3 Thoroughbred [farms 1 to 3] and 1 Warmblood [farm 4]) were selected for use in this study. Farms were selected on the basis of a history of horses with EPM on the farm, a willingness to cooperate with the investigators, and the ability to comply with the required sample collection scheme. Farms were located in San Diego, Fresno, Santa Barbara, and Ventura counties of California. Farms ranged from 37 to 226 hectares and had between 70 and 500 horses. Foaling season extended approximately from January through June of each year.

A total of 484 foals were enrolled in the study at the time of their birth during the 2000, 2001, and 2002 foaling seasons. Foals were from a sample of resident mares at each farm. Mares and foals were selected on the basis of the expectation that they would remain on the premises for at least the next 2 years. Farms 1 to 4 provided approximately 38%, 32%, 23%, and 7% of the foals, respectively. Approximately, 37%, 36%, and 27% of the foals were enrolled in the study during the 2000, 2001, and 2002 foaling seasons, respectively. Percentages of male and female foals were similar.

Foals were fed commercial feed and hay and allowed to graze on pasture. They were typically weaned between 4 and 6

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months of age and dewormed regularly. Vaccinations included immunization against tetanus, influenza virus, herpesvirus, rabies, eastern and western equine encephalomyelitis, *Streptococcus equi*, and rotavirus. Vaccination against *S neurona* was not performed on any of the farms.

Collection and analysis of samples—Serum samples were obtained from newborn foals ≤ 4 days after initial colostrum ingestion (postcolostrum). Subsequent samples were obtained at intervals of approximately 3 months from June 2000 to June 2002. Blood samples were collected from a jugular vein by farm personnel or the investigators. Samples collected by farm personnel were centrifuged at the farm, and the serum was harvested and stored at –20°C before shipment to the laboratory. Samples collected by the investigators were transported to the laboratory on the day of collection or the following day, and serum was harvested after centrifugation on that same day. All processed samples were divided into triplicate aliquots and stored frozen at –80°C until tested.

Samples were tested at the laboratory of 1 of the investigators (PAC). Antibodies against *S neurona* and *N hughesi* were each detected by use of an **indirect fluorescent antibody test (IFAT)**, as described elsewhere.[20,21] Serum dilutions started at 1:10, and the reciprocal end point titer was the last dilution with distinct whole-parasite fluorescence.[22] Samples without fluorescence at a dilution of 1:10 were classified as a titer of < 10. On the basis of prior validation studies,[20,23] reciprocal titers of ≥ 40 and ≥ 160 were considered indicative of exposure to *S neurona* and *N hughesi*, respectively.

Data analysis—Kaplan-Meier survival analysis[24] was used to estimate and compare the risk of exposure (first exposure) to the parasites during the study period and the median time to decay of maternally derived antibodies. First exposure was defined as the first titer ≥ 40 (*S neurona*) or ≥ 160 (*N hughesi*) for foals that had a postcolostrum titer < 10 to either parasite or foals that did not have a postcolostrum titer measurement but that were at least 3 months old at the time of exposure. Foals with a postcolostrum titer ≥ 10 for either parasite were considered exposed when the subsequent titer was at least 2 dilutions higher than the preceding value and equal to or higher than the defined cutoff values for exposure to either parasite. Foals that had 1 or more missing titer measurements between 2 titers below the defined cutoff values were assumed as not exposed to either parasite during the time of the missing measurements.

Risk of exposure was defined as the complement of the cumulative probability of a titer < 40 (*S neurona*) or < 160 (*N hughesi*), which was obtained by use of the Kaplan-Meier survival analysis. Age at first exposure was calculated as the interval between birth and exposure to either parasite. Time to decay of maternally derived antibodies was defined as the interval between an IFAT titer ≥ 40 for *S neurona* or ≥ 160 for *N hughesi* (in postcolostrum samples) and a titer of < 10 for either parasite. Prevalence estimates were calculated as the total number of foals with an IFAT titer ≥ 40 for *S neurona* or ≥ 160 for *N hughesi* divided by the total number of foals tested at a given time.

Censoring was performed when a foal died or left a farm (ie, exited the study) before the end of the study period. The overall proportion of censoring (cumulative censoring) was calculated as the total number of foals that exited the study (died or left) divided by the total number of foals enrolled in the study. Cumulative censoring for each year was calculated as the total number of foals that exited the study in a given calendar year divided by the total number of foals at risk (ie, population at risk) of exiting the study during that calendar year. The population at risk was the total number of foals born in a specified year plus the total number of foals born in preceding years of the study that were alive on January of the specified year, except for the first year of the study in which the population at risk was the total number of foals born in 2000.

Exposure rate for each parasite was calculated as the total number of exposed foals divided by the sum of the number of days each foal was in the study. Overall mortality rate was calculated as the number of foals that died or were euthanized divided by the sum of the number of days each foal was in the study. Mortality rate attributable to neurologic disease was calculated as the number of foals that died or were euthanized because of neurologic conditions divided by the total number of days each foal was in the study. The EPM incidence rate was calculated as the number of foals suspected of having EPM divided by the sum of the number of days each foal was in the study. Rates were expressed as percentages for each year. A suspected clinical case of EPM was defined as a horse with neurologic signs of disease that had a titer ≥ 40 for *S neurona* or ≥ 160 for *N hughesi* (as determined by use of the IFAT) in a serum sample; a titer ≥ 5 to either parasite in a sample of CSF; or a positive result for *S neurona* in a serum or CSF sample tested by use of western blot analysis. Exact 95% **confidence intervals (CIs)** were determined for proportions. The 95% CIs for rates were calculated on the basis of tables published elsewhere.[25] A χ^2 test was used to assess the association between exposure to *S neurona* and exposure to *N hughesi*. For all comparisons, a value of $P \leq 0.05$ was considered significant.

Results

Exposure to *S neurona* and *N hughesi*—Risk of exposure to *S neurona* and *N hughesi* during the study period (2.5 years) and the annual rate of exposure to each parasite for each farm were determined **(Table 1)**. Median age at first exposure was 1.2 years (95% CI, 0.9 to 1.4 years) for *S neurona* and 0.8 years (95% CI, 0.5 to 1.1 years) for *N hughesi*. There was a significant ($P = 0.005$) difference in the risk of exposure to *S neurona* among farms. There was no significant ($P = 0.83$) difference in the risk of exposure to *N hughesi* among

Table 1—Risk of exposure and annual rate of exposure to *Sarcocystis neurona* and *Neospora hughesi* in foals from 4 farms in California.

		S neurona		*N hughesi*	
Farm	**No. of foals**	**Risk (%)***	**Rate (%/y)**	**Risk (%)***	**Rate (%/y)**
1	182	1.9 (0–4.4)	1 (0.1–3.7)	1.3 (0–3.1)	1 (0.1–3.7)
2	156	12.3 (3.8–20.9)	4.4 (1.9–8.6)	3.3 (0–7)	1.6 (0.3–4.7)
3	113	4.6 (0–13.3)	0.8 (0.02–4.5)	3.9 (0–8.1)	2.4 (0.5–7.1)
4	33	24.1 (0.4–47.7)	10.4 (2.8–26.6)	7.7 (0–22.2)	2.4 (0.1–13.2)
Total	**484**	**8.2 (3.7–12.6)**	**2.8 (1.5–4.6)**	**3.1 (1–5.1)**	**1.7 (0.8–3.1)**

Values in parentheses are 95% confidence intervals.
*Represents the cumulative risk of exposure to each parasite during the 2.5-year study period.

farms. Risk of exposure to both parasites did not differ on the basis of the sex of foals ($P = 0.53$). Farm of origin, sex of foal, serum titers, and age at first exposure for the foals exposed to *S neurona* and *N hughesi* were determined (**Tables 2 and 3**). Of the 15 foals exposed to *S neurona* and the 9 foals exposed to *N hughesi*, 14 (93%) and 7 (78%), respectively, were born in 2000. The rates of exposure to *S neurona* and *N hughesi* during the first 1.5 years after birth were 2.8% (95% CI, 1.1% to 5.9%) and 2.0% (95% CI, 0.7% to 4.7%), respectively, for foals born in 2000 and 0.5% (95% CI, 0.01% to 2.8%) and 1.0% (95% CI, 0.1% to 3.6%), respectively, for foals born in 2001. Two of the foals exposed to *S neurona* were from the same mare (1 born in 2000 and the other born in 2001). There was no significant ($P = 0.75$) association between exposure to *S neurona* and *N hughesi*.

The highest prevalence of serum antibodies to *S neurona* and *N hughesi* was 6% (95% CI, 2.4% to 11.9%) and 2.1% (95% CI, 0.4% to 6.1%), respectively, for foals born in 2000; highest prevalence of serum antibodies to *S neurona* and *N hughesi* was detected at a mean age of 1.7 and 1.4 years, respectively. Prevalence of antibodies to either parasite for the entire cohort during the study period ranged between 0% and 2.9% for *S neurona* and 0% and 1% for *N hughesi*. Prevalence estimates for postcolostrum foals and foals at the first 3-month sample collection were excluded because of possible interference with maternally acquired antibodies.

Decay of maternally derived antibodies—Median time for decay of maternally derived antibodies for *S neurona* (21 foals) and *N hughesi* (13 foals) was 96 days (95% CI, 92 to 100 days) and 91 days (95% CI, 72 to 110 days), respectively. Complete decay for all foals was evident by 230 days for *S neurona* and 145 days for *N hughesi*. The interval between birth and the initial 3-month sample collection ranged between 0 and 124 days. The difference in median time to decay between foals with an initial titer ≥ 160 and foals with an initial titer of 40 or 80 for *S neurona* was 2 days; these values did not differ significantly ($P = 0.55$). The difference in median time to decay between foals with an initial titer ≥ 40 and foals with an initial titer of 10 or 20 for *S neurona* was 10 days, but these values did not differ significantly ($P = 0.16$).

Mortality and censoring—Overall annual mortality rate during the study period was 5.7% (95% CI, 3.9% to 8.1%). Annual farm-specific mortality rate was 10.6%, 1.6%, 5.6%, and 2.3% for farms 1 to 4, respectively. The main causes of fatalities (death or euthanasia) were musculoskeletal problems (25%), wobbler syndrome (25%), and colic (9%). Approximately 16% of the foals that died or were euthanatized had no known cause of death. The mortality rate attributable to neurologic disease was approximately 1.6%/y (95% CI, 0.6%/y to 2.8%/y). On the basis of antibody titers, none of the 9 foals that died or were euthanatized because of neurologic disease were exposed to either parasite during the study. Seven of these foals were from farm 1, and the other 2 were from farm 3. There were no study foals with clinical evidence of EPM during the 2.5 years of the study.

The overall percentage of censoring during the study period was 31.2%. Cumulative censoring was 7.8% for 2000, 17.8% for 2001, and 18.8% for the first 6 months of 2002. Approximately, 39.1%, 22.5%, 33.8%, and 4.6% of the censored foals were from farms 1 to 4, respectively. A total of 56% of the censored foals were males, whereas 44% were females. Overall, approximately 21% of the censoring was attributable to foals that died or were euthanatized and the remaining 79% to other reasons. Among foals censored because of reasons other than death or euthanasia, 34% were sold and 26% left the farm of origin for various purposes. Training was the most common cause for leaving a farm (61%).

Table 2—Age at first exposure and titers* to *S neurona* in 15 foals on 4 farms during the 2.5-year study.

Farm	Sex	Date of birth	Age (y)‡	Date of sample collection†								
				Jun-00	Sep-00	Dec-00	Mar-01	Jun-01	Sep-01	Dec-01	Mar-02	Jun-02
1	M	Mar-01	0.7	—	—	—	—	ND	0	160	0	10
	M	Feb-00	1.0	ND	ND	ND	320	160	10	80	10	ND
2	M	Apr-00	0.7	0	0	40	0	0	0	0	0	0
	M	Feb-00	0.9	0	0	80	0	0	0	0	ND	0
	M	Apr-00	1.1	0	0	0	0	1,280	0	80	160	0
	F	Apr-00	1.2	0	0	0	0	320	160	ND	10	0
	M	Apr-00	1.6	0	10	0	0	0	0	40	0	0
	F	Mar-00	1.7	0	0	0	0	10	0	40	0	ND
	F	Mar-00	1.7	0	0	0	0	0	0	40	0	0
	F	Jan-00	1.8	0	0	0	0	0	0	40	ND	ND
3	F	Jan-00	1.6	0	0	0	0	0	320	0	ND	ND
4	M	May-00	0.3	0	40	0	0	0	0	ND	ND	0
	M	Apr-00	0.4	0	40	0	0	0	0	0	0	0
	M	Jun-00	1.2	10	0	0	0	0	80	0	0	0
	F	Mar-00	1.8	0	0	ND	0	0	0	40	0	ND

*A titer of ≥ 40 was considered evidence of exposure to *S neurona*. †Results for samples collected at 3-month intervals. ‡Age at first exposure was the interval from birth until foal had a serum titer of ≥ 40 for *S neurona*.
M = Male. F = Female. — = Foal was not yet born. ND = Not determined.

Table 3—Age at first exposure and titers* to *N hughesi* in 9 foals on 4 farms during the 2.5-year study.

Farm	Sex	Date of birth	Age (y)‡	Date of sample collection†								
				Jun-00	Sep-00	Dec-00	Mar-01	Jun-01	Sep-01	Dec-01	Mar-02	Jun-02
1	M	Apr-00	0.1	640	0	10	0	0	0	0	0	ND
	F	Feb-00	0.3	160	0	0	0	160	0	0	0	ND
2	M	Apr-00	0.2	320	0	0	160	ND	ND	ND	ND	ND
	F	Jan-00	1.2	0	0	80	160	0	160	0	ND	ND
	F	Jun-00	1.3	0	ND	0	10	0	320	0	0	0
3	M	Jan-00	0.7	0	160	0	0	0	0	ND	ND	ND
	F	May-01	0.8	—	—	—	—	0	0	0	320	0
	M	Apr-01	0.9	—	—	—	—	0	0	40	160	0
4	M	May-00	1.4	0	0	ND	80	40	160	0	0	0

*Titer of ≥ 160 was considered evidence of exposure to *N hughesi*.
See Table 2 for remainder of key.

Discussion

In the study reported here, the risk of exposure to either parasite between birth and 2.5 years of age was low. Exposure was characterized, in most cases, by samples with spikes in titers between samples with titers of < 10. This may indicate transient infection or, possibly, the inability to detect an increase and decrease in antibody titers because of the 3-month interval between samples. Other studies[20,23] in which the IFAT was used to monitor parasite-specific antibody response in horses experimentally infected with *S neurona* and *N hughesi* have indicated that serum titers may reach values above the cutoff values used in our study as soon as 1 to 2 weeks after inoculation, and that these titers may be mantained for at least 3 or 4 months. However, the duration of a detectable antibody response may vary with infective dose and pathogenicity of the parasites.[23] Hence, there is a possibility that horses exposed to the parasites immediately before or after a sample was collected or that had missing collections may not have been detected by use of a 3-month interval between samples.

Differences in the risks and rates of parasite exposure among farms may reflect variation in definitive host abundance and, hence, environmental contamination. Opossums are the only known definitive host for *S neurona*, and they shed sporocysts in their feces.[26] Studies[27-30] have indicated that oral exposure is a route of transmission for *S neurona* and that horses presumably become infected by ingestion of feed or water contaminated with sporocysts. The complete life cycle for *N hughesi* has not been determined. Dogs and potentially other canids have been identified as definitive hosts and shedders of a closely related parasite, *N caninum*.[31-33] For *N caninum*, transplacental transmission (primarily in cattle) appears to be the main mode of infection.[34] Farms in the study reported here were located in 4 counties in central and southern California with differing microclimates. Farm 4 was densely vegetated with oak trees (*Quercus* sp) and had the highest exposure risk for both parasites. Whether density and type of vegetation are associated with increased prevalence of definitive hosts and, consequently, environmental contamination is not known. However, wooded terrain around equine premises has been identified as a possible risk factor for EPM.[18]

In the study reported here, exposure rates for foals born in 2000 were higher than those of foals born in 2001 and monitored for an equivalent amount of time. Whether this was attributable to changes in management during the study period or a reduction in definitive host abundance and consequent environmental contamination was not determined. The older age of foals at first exposure to *S neurona*, compared with the age at first exposure to *N hughesi*, and the lack of association between titers for each parasite most likely reflected differences in parasite life cycles and risk factors associated with exposure.

Other studies[9-12] have used the western blot test and estimated that *S neurona* seroprevalence for horses between 1 and 3 years of age is between 15% and 59%. In the study reported here, the highest estimate of *S neurona* prevalence (6%) was obtained for foals born in 2000 and tested between 1.5 and 2 years of age. This difference may have been the result of variation in test accuracy, differences in the amount of exposure among sampled populations, or a combination of both. The IFAT is more accurate than the western blot test for the diagnosis of EPM caused by *S neurona*.[21] In addition, farms enrolled in the study reported here were large, well-managed equine operations and may not have been representative of typical equine breeding operations in California or other types of facilities such as racetracks and training barns. A validation study of the western blot test that involved use of necropsied horses in California yielded a seroprevalence estimate of 52% for *S neurona*.[15] However, that study comprised horses of various ages, breeds, and origin from those of this study, and the results may not be directly comparable.

Limited information is available on seroprevalence patterns for *N hughesi*.[7,14,16,17] Other tests and cutoff values have been used in various studies,[7,14-17] making it difficult to compare results from those studies with results of the study reported here. In general, exposure and disease caused by *Neospora* spp appear to be less frequent than exposure and disease caused by *S neurona*.[7,14-17]

The IFAT used in this study has been validated for use in the diagnosis of infection with *S neurona*

and *N hughesi*.[20,23] In those reports,[20,23] the cutoff titers suggested for a positive test result were 80 and 640 for *S neurona* and *N hughesi*, respectively. In the study reported here, the IFAT was used for screening healthy foals to detect exposure to either parasite; hence, lower cutoff values were considered appropriate.

Knowledge about decay of maternally derived antibodies may guide the choice of the appropriate time for vaccination and help to determine the period of greatest likelihood of false-positive results should the test be used for screening or clinical diagnosis in young horses. The time to decay of maternally derived antibodies to *S neurona* reported here corroborates, in part, the results obtained by use of the western blot test that revealed a mean time to decay of 4.2 months and a maximal time to decay of 9 months.[19] Time to decay was longer in foals with titers ≥ 40, compared with that of foals with lower titers, but the magnitude of the difference was smaller when higher titers were compared with each other. In general, longer time to decay of maternally derived antibodies would be expected in foals with higher initial titer values.[35] The observed variation in the magnitude of the differences may have been the result of the method used to categorize the data. In our study, some of the times to decay for each parasite may have been overestimated because of the extended interval between collection of the postcolostrum sample and the subsequent 3-month sample.

The mortality rate among the cohort included horses that were euthanatized and was higher than that reported in another study.[8] However, the methods and population used for the calculations, as well as differences in age categorization, preclude direct comparisons between that study and the study reported here. Horses that died or were euthanatized because of neurologic disease did not have detectable IFAT titers to either parasite at various time points prior to death; therefore, the likelihood of EPM in these horses was considered minimal. However, necropsy was not performed, and a definitive diagnosis was not established. Most of the foals that had neurologic disease were at farm 1. On that farm, several male foals that had neurologic signs and were considered by a veterinarian to have a poor prognosis for subsequent racing were euthanatized.

There was no apparent reason for a higher rate of exposure among censored foals; hence, censoring was not believed to be an important source of bias. The farm distribution of the censored foals was approximately the same as that for the enrollment of foals, indicating no preferential censoring on the basis of farm. There was a higher proportion of censored males than females. However, this was probably because females may be more likely to be retained on the basis of their greater potential for use as brood mares.

Exposure to *S neurona* and *N hughesi* was low in foals between birth and 2.5 years of age. Maternally acquired antibodies may cause false-positive results in foals tested during the first 3 or 4 months after birth, and EPM was a rare clinical disease of horses ≤ 2.5 years of age. Exposure to the parasites that cause EPM in older horses and the role of environment and management factors associated with a change in activity (ie, training and racing) should be investigated.

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