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RISK OF TRANSPLACENTAL TRANSMISSION OF *SARCOCYSTIS NEURONA* AND *NEOSPORA HUGHESI* IN CALIFORNIA HORSES

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ABSTRACT: The study objective was to assess the risk of transplacental transmission of *Sarcocystis neurona* and *Neospora hughesi* in foals from 4 California farms during 3 foaling seasons. Serum of presuckle foals and serum and colostrum of periparturient mares were tested using indirect fluorescent antibody tests for *S. neurona* and *N. hughesi*. Serum antibody titers were ≤10 in 366 presuckle foals tested. There was no serologic or histologic evidence of either parasite in aborted fetuses or placentas examined. Positivity for *S. neurona* and *N. hughesi* in mares increased with age. Mares ≤9 yr that originated from Kentucky were 3.8 and 1.4 times more likely to be positive for *S. neurona* and *N. hughesi*, respectively, than mares from California. The strength of association between positivity to either parasite and state of birth decreased as age increased. Mares positive for *S. neurona* and *N. hughesi* were 2.2 and 1.7 times more likely, respectively, to have a previous abortion than negative mares, adjusted for age and state of birth. The annual mortality rate for mares was 4%. The annual incidence rate of equine protozoal myeloencephalitis was 0.2%. In conclusion, there was no detectable risk of transplacental transmission of *S. neurona* and *N. hughesi*. Prevalence of antibodies against both parasites in mares increased with age.

Infections with Apicomplexa protozoa belonging to species of *Sarcocystis*, *Neospora*, and *Toxoplasma* are acquired horizontally through ingestion of contaminated food or water or vertically (transplacentally) from dam to fetus during pregnancy (Dubey, 1994; Tenter, 1995; Dubey, 2003).

Transplacental transmission in species of all 3 genera has been reported in naturally infected (Dubey, 1981; Dubey and Bergeron, 1982; Dubey et al., 1990, 1996; Barr et al., 1991, 1992; Duncanson et al., 2001; Pitel et al., 2003) and, in some cases, experimentally infected (Dubey, 1988; Dubey et al., 1988; Conrad et al., 1993; Owen et al., 1998) animals of different species. In horses, *Sarcocystis* spp., *Neospora* spp., and *Toxoplasma gondii* have been detected in fetuses, newborn foals, and placentas, indicating that vertical transmission of the parasites may occur (Cunningham, 1973; Roperto et al., 1983; Dubey and Porterfield, 1990; Turner and Savva, 1990, 1992; Lindsay et al., 1996; Pronost et al., 1999; Pitel et al., 2003).

Sarcocystis neurona (Dubey et al., 1991) and, less frequently, *Neospora* spp (Daft et al., 1996; Marsh, Barr, Madigan, Lakritz, Nordhausen et al., 1996; Hamir et al., 1998), most notably *Neospora hughesi* (Marsh et al., 1998; Cheadle et al., 1999; Dubey et al., 2001), are the causal agents of equine protozoal myeloencephalitis (EPM). Knowledge of the modes of transmission of these parasites has important implications for selection of effective preventive measures for EPM. Thus far, investigation of the risk of transplacental transmission of *S. neurona* and *N. hughesi* in horses has been limited. In a recent study, lack of antibodies to *S. neurona* in a selected group of 33 presuckled foals born to Western blot–seropositive mares suggested a low risk of vertical transmission (Cook et al., 2001). In other studies, an association between abortion and seropositivity for *Neospora caninum* in mares was suggested (McDole and Gay, 2002; Pitel et al., 2003). To our knowledge, large prospective epidemiologic studies designed to assess the risk of vertical transmission of both EPM parasites have not been conducted. The objective of this study was to assess the risk of transplacental transmission of *S. neurona* and *N. hughesi* in a cohort of mares and foals on 4 California farms with a history of EPM.

This study was conducted between January 2000 and July 2002 as part of a prospective cohort study designed to assess epidemiologic aspects of EPM in California.

MATERIALS AND METHODS

Farms and horses

Three California Thoroughbred farms (farms 1, 2, and 3) and 1 Warmblood (farm 4) farm located in San Diego, Fresno, Santa Barbara, and Ventura counties, respectively, participated in the study. Farm size ranged from 37 to 226 ha, and the total number of resident mares varied between 23 and 350. All farms were selected based on a previous history of EPM occurrence, willingness to cooperate, and ability to comply with the required sampling scheme. Management at these farms was considered typical of equine breeding operations in the state. Breeding and foaling seasons extended from approximately February through July and from January through June, respectively. Horses were fed commercial feed and alfalfa hay and had variable access to pasture. Deworming was performed approximately every 2 mo. Vaccination protocols varied slightly among farms and included immunization against tetanus, influenza, herpes virus, rabies, eastern and western equine encephalitis, strangles, and rotavirus. None of the farms used *S. neurona* vaccine during the study period.

A sample of resident mares on each farm was selected based on the expectation that mares and their foals would remain on the premises for at least the next 2 yr. A total of 189 mares were enrolled in the study at the beginning of the 2000 foaling season. An additional 148 mares were added to the study for the 2001 foaling season. Out of these 148, 72 participated in the study only during the 2001 season. Approximately 34% of the mares were on farm 1, 42% on farm 2, 17% on farm 3, and 7% on farm 4. All foals delivered by the selected mares were enrolled in the study on birth.

Sample collection and testing

Serum samples were collected from mares at the start of the study, as close as possible to parturition (on average 3 days after parturition), and at approximately 3-mo intervals from June 2000 to June 2002, for mares participating in the study for more than 1 foaling season. Colostrum samples were collected as close as possible to the parturition day.

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Serum samples from newborn foals were collected before and after (≤4 days) colostrum ingestion. Blood samples were collected by jugular venipuncture from mares and by the same procedure or umbilical cord drainage from foals. Most blood and all colostrum samples were collected, processed, and stored in a −20 C freezer by farm personnel. Samples stored at the farms were shipped to the laboratory of 1 of the investigators (P.A.C.) on approximately a quarterly basis by overnight mail. Upon arrival, samples were aliquoted in triplicate and stored at −80 C until tested.

Farms were instructed to submit aborted fetuses, placentas, and serum samples from aborting mares to the California Animal Health and Food Safety Laboratory (CAHFSL). Complete examination of the submitted specimens was performed, and whenever possible, fetal peritoneal fluid was collected for antibody analysis. A search of the CAHFSL records was also performed to identify cases of abortion occurring in the study farms between 1999 and 2002.

All serum, colostrum, and fetal peritoneal fluid samples were tested in 1 of the author's (P.A.C.) laboratory. Samples were tested for antibodies against *S. neurona* and *N. hughesi* using the indirect fluorescent antibody test (IFAT), as described previously (Packham et al., 2002; Duarte et al., 2003). Merozoites of the UCD-1 *S. neurona* (Marsh, Barr, Madigan, Lakritz, and Conrad, 1996) and tachyzoites of the EN-1 *N. hughesi* (Marsh et al., 1998) isolates were used as test antigens. Serum and colostrum dilutions started at 1:10 and the reciprocal end-point titer was the last dilution with evidence of distinct, whole-parasite fluorescence (Conrad et al., 1993). If no fluorescence was evident at a 1:10 dilution, horses were classified as having a titer of <10. Reciprocal titers of 40 and 160 for *S. neurona* and *N. hughesi*, respectively, were used as cutoff values for test interpretation when dichotomization of test results was necessary (Packham et al., 2002; Duarte et al., 2004). To verify the possibility of colostrum ingestion, serum samples from foals having an IFAT titer ≥10 and reported to be collected before colostrum ingestion or those that had time of collection unknown were tested for total immunoglobulin G (IgG) concentration, using a commercial single radial immunodiffusion kit (Veterinary Medical Research Development, Pullman, Washington). Samples having a total IgG concentration ≥200 mg/dl were considered to be from postsuckled foals (Leblanc, 1990).

Data analysis

Prevalence estimates were calculated as the total number of horses with an IFAT titer ≥40 or ≥160 for *S. neurona* and *N. hughesi*, respectively, divided by the total number of horses tested at a given time. Associations between prevalence and age, state of birth, and previous abortions (lifetime occurrence before the study period) were determined using test results from serum samples collected before parturition and serum or colostrum samples collected in the periparturient period during at least 1 foaling season. Mares with a positive serum IFAT result before parturition or a positive periparturient serum or colostrum result in at least 1 foaling season were considered positive for comparison purposes. The odds ratio and its 95% confidence interval (CI) were used as the measure of strength of association between variables. The chi-square test was used to compare and identify a statistical trend in proportions. A 5% level of significance was used for all comparisons.

A mare was considered censored when it died or left the study before the end of the study period. The cumulative censoring by year of enrollment was calculated as the total number of mares enrolled in the study in a given foaling season that died or left during the whole study period, divided by the total number of mares enrolled in the study in that season. Overall mortality and EPM incidence rates were calculated as the total number of deaths or EPM cases divided by the sum of days each mare was in the study and expressed as annual percentages. Ninety-five percent CIs for rates were calculated from previously published tables (Haenszel et al., 1962). A suspected clinical case of EPM was defined as a horse presenting neurologic signs with a serum IFAT titer ≥40 for *S. neurona* or ≥160 for *N. hughesi* or a cerebrospinal fluid (CSF) titer ≥5 to either parasite (or both) or a Western blot–positive test for *S. neurona* in serum or CSF. The data analysis was performed using SPSS 10.0 (SPSS, Chicago, Illinois) statistical software.

RESULTS

Foals

Presuckle serum samples for 366 (66%) of 556 foals born during the 3 consecutive foaling seasons were collected and tested for antibodies against *S. neurona* and *N. hughesi*. Approximately, 22% of the samples were collected in 2000, 53% in 2001, and 25% in 2002. Ninety-five percent of the foals tested were Thoroughbreds from farms 1 (34%), 2 (45%), and 3 (16%), and 5% were Warmbloods from farm 4. The percentage of foals from mares that had *S. neurona* or *N. hughesi* positive serum or colostrum was approximately 11 and 7%, respectively. One foal (foal 1) had a presuckle IFAT titer of 10 for *S. neurona*, and 5 other foals (foals 2–6) had a presuckle titer of 10 for *N. hughesi*. All 6 foals had a total IgG <200 mg/dl. The remaining 360 foals had titers of <10 to both parasites. Foal 1 maintained the same IFAT titer after ingestion of colostrum, and its dam had a *S. neurona* colostrum titer of 160. Foals 2–6 had *N. hughesi* titers of <10 after colostrum ingestion, and their dams had periparturient serum or colostrum titers of <10 for this parasite. The dams of foals 4, 5, and 6 had serum tested at least once during the 6-mo period before parturition, and all titers were <10 for *N. hughesi*. Foals 1 and 6 were from farm 1, and foals 2–5 were from farm 2. Four of these 6 foals were born during the 2001 foaling season and 2 during the 2002 foaling season.

Abortions

A total of 22 aborted fetuses (including 1 stillbirth) and 12 placentas from mares enrolled (n = 10) or not (n = 12) in the study and belonging to 2 of the farms (68% farm 2, 32% farm 1) were examined at the CAHFSL between September 1999 and December 2002. Approximately 77% of the abortions occurred at 6 mo of gestation or later and most (55%) were not definitively diagnosed. Equine herpes virus and bacterial infections accounted for approximately 27% of the abortions. Hydrocephalus, umbilical cord torsion, twins, and a partially mummified fetus accounted for 18% of the abortions. The abortion attributable to twinning was from a mare that developed clinical signs compatible with EPM. The abortion occurred at 6 mo of gestation, approximately 2 mo after the onset of clinical signs and 1 mo after the completion of a 4-wk treatment with ponazuril (Marquis™, Bayer Corporation, Shawnee Mission, Kansas). No serologic or histologic evidence of *S. neurona* and *N. hughesi* infection was found in the fetuses. The mare's serum IFAT titer for *S. neurona* was <10 at the time of abortion but varied between 10 and 40 before and during the course of the disease. Serum IFAT titers for *N. hughesi* were <10 at all sampling times including on the date of abortion.

Mare serum and fetal fluid were available for another 7 mare–fetus pairs and for a single fetus. All fetal fluid titers were <10 for both parasites. Minimal perivascular macrophage infiltrate was observed in the central nervous system (CNS) of 1 of these fetuses. No other CNS lesion, except for the hydrocephalus, was observed in any of the fetal brains evaluated (n = 21). Serum titers of 160, 80, and 10 for *S. neurona* and <10 or 10 for *N. hughesi* were found in 3 of the 7 mares. There was no evidence of either parasite in any of the placentas examined.

Mares

Baseline information on the study mares is presented in Table I. The percentage of parturition events, abortion, and barren mares in each farm for each foaling season is presented in Table II. The prevalence of antibodies against *S. neurona* and *N.*

TABLE I. Age and reproductive indices before the beginning of the study for mares on 4 California farms.

	Farm 1 (n = 115)	Farm 2 (n = 141)	Farm 3 (n = 58)	Farm 4 (n = 23)
% of total*	58	40	73	100
Age†	9.6 (3–22)‡	10.6 (3–25)	9.4 (4–17)	7.7 (2–18)
Reproductive years§	5.3 (1–18)	5.9 (1–20)	4.9 (1–12)	3.6 (1–10)
Live foals	3.9 (0–15)	3.9 (0–14)	2.9 (0–10)	1.5 (0–8)
Abortions	0.19 (0–2)	0.16 (0–2)	0.22 (0–2)	0.09 (0–1)
Barren years	0.44 (0–7)	0.27 (0–3)	0.22 (0–2)	0.95 (0–8)

* Represents the proportion of sampled mares (n) in relation to the total number of reported resident mares.
† Approximate age of the mares in years in January 2000.
‡ All values are means with ranges in parentheses. Live foals, abortions, and barren years are mean numbers per mare.
§ Calculated as the age of the mare at the beginning of the study minus the reported age in the first year of reproduction.

hughesi in serum before parturition (≤6 mo) and in periparturient serum or colostrum samples (only paired results shown) among mares tested during each foaling season is presented in Table III. The age-specific prevalence for both parasites is presented in Figure 1. There was a positive, statistically significant age trend for *S. neurona* positivity before or during the periparturient period over all foaling seasons ($P = 0.024$). The age trend for *N. hughesi* positivity was marginally statistically significant ($P = 0.066$). Mares ≤9 yr of age (median) born in Kentucky (90% of the 88 non-California mares) were 3.8 (95% CI = 1.4–10.7) and 1.4 (95% CI = 0.4–4.7) times more likely to be positive for *S. neurona* and *N. hughesi*, respectively, than mares in the same age group born in California. The strength of association between *S. neurona* and *N. hughesi* positivity and place of birth (California and Kentucky) decreased as age increased ($P = 0.02$ and $P = 0.89$, respectively). Mares from Kentucky were 2.5 (95% CI = 1.1–5.5) times more likely to have a previous abortion or stillbirth than mares from California. Mares positive for *S. neurona* and *N. hughesi* were 2.2 (95% CI = 0.7–7.1) and 1.7 (95% CI = 0.5–5.6) times more likely, respectively, to have a previous abortion or stillbirth than negative mares, adjusted for age and state of birth. There was no association between occurrence of abortion during the study period and seropositivity for *S. neurona* and *N. hughesi*, across foaling seasons ($P \geq 0.42$).

The cumulative censoring for the mares permanently enrolled in the study in the 2000 and 2001 foaling seasons were 35 and 24%, respectively. The median time for censoring among censored mares was 1.7 and 0.7 yr for the 2000 and 2001 enrollment seasons, respectively. Overall, approximately 25% of the censoring was attributable to death and the remaining 75% to other causes including sale or shipment to other farms for breeding purposes (67%). The overall mortality rate was 4% (95% CI = 2.6–6.5%) per year. The annual mortality rate varied between 0 and 6% among farms. The main cause of death for both enrollment seasons was colic or colic-derived problems (62%) followed by parturition complications (19%). There was no reported death related to neurologic disease. The annual incidence rate of suspected clinical EPM among mares (1 mare) was 0.2% (95% CI = 0.01–1.1%) per year.

DISCUSSION

In this study, there was no serologic or histologic evidence of in utero infection with either *S. neurona* or *N. hughesi*, despite varying degrees of exposure of mares to both parasites on all 4 farms. Previous studies in horses and cattle have indicated that high antibody titers are expected in presuckle foals and calves infected with different agents during gestation (Morgan et al., 1975; Anderson et al., 1997; Sheoran et al., 2000). In this study, 6 foals had low IFAT titers (10) to either *S. neurona* or *N. hughesi*. These titers were at least 4-fold lower than the cutoffs considered indicative of infection (Packham et al., 2002; Duarte et al., 2004) and were probably attributable to nonspecific reactions because trace amounts of IgG might be present in sera of newborn foals before colostrum ingestion (Perryman et al., 1980). Findings of this study corroborate, in part, the results of 2 prior studies indicating that transplacental transmission of *S. neurona* and *Neospora* spp. in horses is not frequent (Cook et al., 2001; Pitel et al., 2003). In the first study (Cook et al., 2001), no serum antibody to *S. neurona* was found in a selected group of 33 presuckled foals born to Western blot–seropositive mares in 2 different locations. In the second study (Pitel et al., 2003), there was no evidence of DNA of *N. caninum* in fetal tissues from aborting mares with various agglutination serum titers for *N. caninum*. In the first study, there was no histologic examination of placentas and fetal tissues, and in neither of the studies the potential for vertical transmission of *N. hughesi* was assessed. All fetuses examined in this study were from farms 1 and 2. Approximately 78% of the fetal fluids tested were from fetuses ≥180 days of age and presumably capable of an immune response should infection have occurred during gestation (Perryman et al., 1980).

The percentage of abortions varied by farm and foaling season and was, in some cases, higher than that initially reported by the farms (approximately 2–5%). However, abortions occurring during the study period included fetal loss between detection of pregnancy and parturition (stillbirths included), possibly increasing the estimates. In this study, there was a weak association between positivity for *S. neurona* and *N. hughesi* and occurrence of previous abortion. In addition, the association between abortion during the study period and positivity for either parasite was established based on IFAT results available for mares at any time during the study. Therefore, IFAT results did not represent the serologic status of the mare at the time of abortion. Recent reports showed a potential association between seroprevalence for *N. caninum* and fetal loss (McDole and Gay, 2002; Pitel et al., 2003). However, whether *S. neurona* or *N. hughesi* are causes of reproductive failure remains equivocal.

TABLE II. Percentage of parturition events, abortions, barren mares, and mares with unknown reproductive status (NA) by farm and foaling season.

	2000					2001					2002				
Farm*	n	Parturitions (%)	Abort (%)	Barren (%)	NA (%)	n	Parturitions (%)	Abort (%)	Barren (%)	NA (%)	n	Parturitions (%)	Abort (%)	Barren (%)	NA (%)
1	57	91.2	3.5	1.8	3.5	97	74.2	4.1	18.6	3.1	77	64.9	2.6	26	6.5
2	53	96.2	1.9	0	1.9	67	71.6	1.5	25.4	1.5	60	80	6.7	11.7	1.6
3	58	75.9	13.8	10.3	0	56	71.4	5.4	23.2	0	54	63	9.2	25.9	1.9
4	21	71.4	9.6	19	0	22	50	0	50	0	12	41.7	0	58.3	0
Overall	189	85.7	6.9	5.8	1.6	242	70.7	3.2	24.4	1.7	203	67.6	5.4	23.6	3.4

* All percentages were calculated as the total number of parturition events, abortions (including stillbirths), barren mares (bred but not pregnant or not bred), or mares with unknown reproductive status divided by the total number of mares alive in the study in January of each foaling season.

The increase in percentage of barren mares after the 2000 foaling season most likely reflects a tendency to include pregnant mares in the first foaling season (2000) rather than an increase in reproductive failure in subsequent years. The most common reason for a mare being barren was not having been bred (≥54%).

The reason for an apparent decrease in seroprevalence during foaling season is not obvious. Possible reasons include an association between censoring and seropositivity, a dilution effect caused by addition of seronegative mares in the 2001 foaling season, variation in test performance, or a true reduction in exposure to the parasites during the study period. Mares tested consecutively for 2 or more seasons showed the same decreasing pattern of seroprevalence across foaling season. This finding indicates no association between censoring and seropositivity or a dilution effect. Variation in the IFAT performance seems unlikely because serum IgG appears to be stable after multiple freeze–thaw cycles (Pinsky et al., 2003), the test was shown to be reliable, and all samples in the study were tested and read by the same person (Duarte et al., 2004). At least 1 of the farms reported a change to a higher-quality hay that might suggest lower parasite exposure levels over time. However, a formal association between changes in management and a decrease in prevalence could not be established. In addition, the implementation of the study might have increased awareness to factors associated with exposure, potentially leading to changes in management and reduction in exposure.

In general, prevalence estimates for antibodies against *S. neurona* and *N. hughesi* found in this study were lower than previous estimates in the western United States (Blythe et al., 1997; Vardeleon et al., 2001; Daft et al., 2002). However, comparisons between different studies, particularly those using different diagnostic tests, should be made cautiously because estimates vary with the population sampled and test accuracy. Younger mares born in California were less likely to be positive for *S. neurona* and, to a lesser extent, *N. hughesi* than mares born in Kentucky. In addition, the overall prevalence for *S. neurona* and *N. hughesi* increased with age as reported previously (Bentz et al., 1997; Blythe et al., 1997; Saville et al., 1997; Tillotson et al., 1999), although the trend varied between parasites and state of birth (California and Kentucky). Prevalence of *S. neurona* antibodies in mares born in California consistently increased with age, whereas it appeared to initially decrease in mares born in Kentucky. This could indicate a relative reduction in exposure across geographic locations and reemphasize the importance of the oral route of exposure to the parasites. This pattern was not obvious for *N. hughesi*, although older mares (≥14 yr) tended to have higher prevalence estimates for this parasite. Seroconversion among mares tested consecutively during the study period was infrequent (data not presented).

The sensitivity and specificity of the IFAT for the serodiagnosis of *S. neurona* infection were estimated to be approximately 83 and 97%, respectively, at a cutoff titer of 80, using horses with and without CNS infection as the gold standard (Duarte et al., 2004). In addition, the IFAT was shown to be more accurate than the Western blot test for diagnosis of EPM caused by *S. neurona* (Duarte et al., 2003). In horses, the sensitivity and specificity of the IFAT for the serologic diagnosis of *N. hughesi* were estimated to be 100% at a cutoff titer of

TABLE III. Prevalence of antibodies against *Sarcocystis neurona* (SN) and *Neospora hughesi* (NH) in serum before parturition and in serum or colostrum during the periparturient period among mares on 4 California farms during the 2000, 2001, and 2002 foaling seasons.

	2000					2001					2002				
		SN		NH			SN		NH			SN		NH	
Farm	n	Prior (%)	Partur.* (%)	Prior (%)	Partur. (%)	n	Prior (%)	Partur. (%)	Prior (%)	Partur. (%)	n	Prior (%)	Partur. (%)	Prior (%)	Partur. (%)
1	41	2.4	39	2.4	36.6	38	0	2.6	0	0	39	0	2.6	0	0
2	43	27.9	9.3	20.9	7	34	0	5.9	0	2.9	28	7.1	7.1	0	0
3	14	0	0	7.1	0	36	0	22.2	2.8	8.3	28	0	0	0	0
4	11	27.3	27.3	27.3	9.1	7	0	0	0	14.3	5	0	0	0	0
Overall	109	14.7	21.4	12.8	17.4	115	0	9.6	0.9	4.3	100	2	3	0	0

* Periparturient period.

FIGURE 1. Age-specific prevalence of *Sarcocystis neurona* and *Neospora hughesi* antibodies in serum before parturition or in serum or colostrum during the periparturient period among mares on 4 California farms during all foaling seasons.

640 in a limited number of experimentally infected and control horses (Packham et al., 2002). In this study, lower cutoff values were used to enhance test sensitivity for screening for exposure to the parasites. Cross-reactivity between *S. neurona*, *Neospora* spp., and other related apicomplexan parasites appears to be low but might occur at low IFAT cutoff values and cannot be discounted (Packham et al., 2002; Duarte et al., 2004). Therefore, lower cutoff values might decrease specificity and increase seroprevalence estimates. The accuracy of the IFAT in colostrum or milk of mares has not been determined. It has been shown that concentration of IgG antibodies in colostrum decreases markedly within the first 12 hr after birth; therefore, sensitivity might be reduced in colostrum samples collected after foaling (Pearson et al., 1984). In this study, 93% of the 331 colostrum samples tested, which had an exact date of collection available, were obtained between 0 and 5 days before parturition. Nonspecific factors in milk were reported to cross-react with antibodies and also to reduce specificity of certain milk-based assays (Neuteboom et al., 1992; Cullor et al., 1994). However, the effect of such factors on the IFAT specificity in the colostrum of mares is unknown.

Limited data on mare mortality and EPM incidence have been published in the United States. A previous report estimated the annual equid mortality rate in the western United States and the national EPM incidence to be 1.3 and 0.14%, respectively (NAHMS, 1998, 2001). In this study we found a higher overall mortality rate and a similar incidence rate of EPM among mares. However, the sampled populations and case definition differed between studies, and comparisons of estimates should be made cautiously.

In conclusion, there was no detectable risk of transplacental transmission of either *S. neurona* or *N. hughesi* in the farms participating in this study. Prevalence of antibodies against both parasites among mares was lower than previous reports, decreased during the study period, and increased with age. The incidence of suspected clinical EPM was low among broodmares.

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