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Case–Control Study of a Multistate Equine Herpesvirus Myeloencephalopathy Outbreak

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Background: A large multistate outbreak of equine herpesvirus myeloencephalopathy (EHM) occurred in May 2011 among horses that participated in a competitive event.

Objective: To identify EHM risk factors among horses with a common exposure venue.

Animals: A total of 123 horses: 19 horses with EHM, 14 equine herpesvirus-1 cases with no reported neurologic signs, and 90 control horses.

Methods: EHM case survey data were compared with data from EHV-1 cases with no neurologic signs and healthy controls using univariable and multivariable methods.

Results: Significant factors associated with higher risk for EHM compared with EHV-1 cases with no neurologic signs were (1) greater number of biosecurity risks at the event, (2) female sex, (3) increasing number of classes competed in at the event, and (4) an interaction between sex and number of classes competed in. In the EHM versus controls comparison, in addition to sex and biosecurity risks, factors associated with higher EHM risk included EHV-1 vaccination in the 5 weeks before the event and increasing number of events attended in April 2011; zinc dietary supplementation was associated with decreased risk. An interaction between sex and the number of events attended in April 2011 also was significant.

Conclusions and Clinical Importance: Findings from this study suggest that dietary zinc supplementation may be associated with decreased risk of EHM. Several factors were associated with increased risk of EHM. Additional investigations of factors associated with risk of EHM are warranted to evaluate the importance of these factors in this complex disease of horses.

Key words: EHM risk; Neurologic disease.

Equine herpesvirus-1 (EHV-1) infection occurs in horses of all ages throughout the world and typically is manifested as (1) sporadic mild respiratory disease associated with fever, primarily in horses <2 years of age, (2) abortion or delivery of an infected neonatal foal, and/or (3) neurologic disease causing morbidity, extensive movement restrictions, and loss of life.[1] Infection with EHV-1 typically occurs in the first weeks to months of a foal's life, and vaccination of pregnant mares currently does not prevent transmission of the virus to foals.[1] Once infected, latency and reactivation are important aspects of the epidemiology of EHV-1 infection.[1] Nasal shedding of virus also is an important epidemiologic feature of EHV-1 among horses attending events such as sales and shows.[2]

Several recent EHV-1 outbreaks resulting in EHM support the observation that morbidity and mortality during outbreaks are higher than in the past.[3] Animal health professionals involved in responding to these recent EHM outbreaks have underscored the need to improve our knowledge of this disease by systematic collection of epidemiologic data, specifically from EHV-1 outbreaks that prominently feature EHM cases.[4] The American College of Veterinary Internal Medicine (ACVIM) recently published a consensus statement on EHV-1, stating that the risks for disease attributable to EHV-1 are multifactorial in nature, in as much as they involve viral, host, and environmental factors.[1] In addition, the ACVIM consensus statement indicated that, although our understanding of EHV-1 and EHM is

Abbreviations:

ACVIM	American College of Veterinary Internal Medicine
CI	confidence interval
CTL	cytotoxic T-lymphocytes
EHM	equine herpesvirus myeloencephalopathy
EHV	equine herpesvirus
NCHA	National Cutting Horse Association
OMB	Office of Management and Budget
OR	odds ratio
PEH	primary exposed horses
POC	point of contact
USDA–APHIS	U.S. Department of Agriculture–Animal and Plant Health Inspection Service
VS	Veterinary Services

From the Colorado State University College of Veterinary Medicine and Biomedical Sciences, Animal Population Health Institute (APHI), Fort Collins, CO (Traub-Dargatz, Wiedenheft); the USDA–APHIS–VS Centers for Epidemiology and Animal Health, Fort Collins, CO (Traub-Dargatz, Kasari, Bush, Bjork); and the USDA–APHIS–VS Western Regional Office, Fort Collins, CO (Pelze-McCluskey, Creekmore, Geiser-Novotny). *Data collection for this study was performed in states with primary exposed horses. Study design, coordination, data analysis, and manuscript preparation were performed at USDA–APHIS–VS in Fort Collins, CO. This work was conducted as part of the regular duties of State Animal Health Officials and USDA–APHIS–VS personnel. No external funding supported the work. At the time of submission, this work has not been presented at a previous meeting.*

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increasing, future progress will be dependent on research into viral pathogenesis and epidemiologic determinants of this complex and economically important disease of horses.[1]

In 2011, a large outbreak of EHV-1 involving at least 242 exposed horse premises in 19 states occurred as a consequence of horses attending the National Cutting Horse Association (NCHA) Western National Championship in Ogden, UT, April 29 through May 8, 2011. Because of the multistate nature of the outbreak, the American Association of Equine Practitioners, the American Horse Council, and the National Assembly of State Animal Health Officials requested assistance from U.S. Department of Agriculture-Animal and Plant Health Inspection Service-Veterinary Services (USDA-APHIS-VS) to coordinate and communicate the outbreak situation. Ultimately, 90 laboratory-confirmed and 72 suspect cases of EHV-1 or EHM were identified among the primary and secondary exposed horses from this multistate outbreak.[5] As a result of the extensive involvement of the USDA-APHIS-VS from the onset of the outbreak, the agency initiated a case–control study to investigate risk factors associated with EHM that subsequently developed in many of these EHV-1 exposed animals. The findings of this case–control study are presented here. The descriptive epidemiologic features of the cases included in this study are described elsewhere.[6]

Materials and Methods

Questionnaire Development and Field Implementation of the Study

State Animal Health Officials (SAHO) from each state with primary exposed horses (PEH) that became cases categorized them based on reporting criteria defined for the outbreak.[5]

A cover letter from USDA–APHIS–VS was provided to the State Point of Contact (POC) for use when contacting participants to inform them of the study objectives and to encourage their participation. The NCHA notified members of the study via the association's newsletter, emailed to over 143,000 members, with a goal of informing them of the upcoming epidemiologic study and encouraging them to participate.[7] The study was granted regulatory compliance approval as an emergency epidemiologic investigation by the Federal Office of Management and Budget (OMB). Participation in the study was voluntary.

Data were collected from premises with ≥ 1 PEH, confirmed or suspect EHV-1 case with no reported neurologic signs or EHM case, and from selected controls using a horse-level questionnaire.

Horse-level questions were related to signalment, use, competition level, housing, EHV-1 vaccination history, areas housed and visited at the NCHA event, clinical signs with onset date, outcome of horse (disease consistent with EHV-1 infection or no disease), travel history, feed supplements, treatment history, and any diagnostic test results. Questionnaires were reviewed by several subject matter experts before finalization.

Sample Population

PEHs were those that attended the event. A total of 421 PEHs in the United States distributed across 242 premises in 19 states met the criteria for inclusion. Of the eligible horses, 13 of the 19 states produced 100 suspect or confirmed primary cases of EHV-1 or EHM.[8]

Selection of Cases

The 100 PEHs eligible for inclusion in the study were classified into 1 of 4 case categories by the SAHO: confirmed EHM, suspect EHM, confirmed EHV-1 case with no reported neurologic signs, or suspect EHV-1 case with no reported neurologic signs (see Supporting Information for detailed USDA–APHIS–VS case definitions).[5] All premises with ≥ 1 of the case-category horses were selected.

Selection of Controls

Only PEHs that attended the event May 3–8, 2011 and that were not classified as cases were eligible to serve as controls. This date range was used for inclusion of controls because no case horses left the event before May 3, 2011.

Selection of 165 control PEHs occurred at the state level. Simple random sampling was used to select one-half of these horses from a numbered list of noncase PEHs prepared by the VS study coordinator and respective state POC (see Supporting Information Table 1).

Questionnaire Review and Data Management

Each state POC collected questionnaire data either by face-to-face interview, by telephone interview, or by mailing the questionnaire before telephone follow-up. Responses were reviewed by the POCs and forwarded to the VS Study Coordinator for review and any needed follow-up. The respondents' and horses' identifying information was removed from questionnaires by the VS Study Coordinator and from any existing laboratory or medical records to maintain confidentiality of respondents and the identity of the horse. A premises-level code was assigned to the questionnaire.

The responses on the surveys were entered into an electronic database[a] and validated by data checks. Data collection began the week of July 18, 2011 and was concluded on October 8, 2011.

Statistical Analysis

Risk factors and covariates considered in this study were based primarily on previous findings summarized in a recently published consensus statement on EHV-1.[1] The questionnaire requested any and all commercial supplements to be listed by brand name, amount fed per day, and duration of supplementation. Labels for these supplements were reviewed for their zinc content and their respective feeding instructions to estimate daily supplemented zinc consumption (mg). The number of miles that each horse traveled from its home premises in the month preceding the event was estimated from reported travel locations. The number of biosecurity-related activities reported at the Ogden event was the sum of 6 reported activities dichotomized into 2 levels, 0–1 activities or ≥ 2 activities.

EHV-1 vaccination data were collected for the 12 months preceding the event (beginning May 1, 2010), categorized as number of EHV-1 vaccinations, type and administration of vaccine within certain intervals before the event (≤ 5 weeks, >5 weeks to ≤ 10 weeks, >10 weeks to ≤ 14 weeks), and whether vaccinated in the previous 12 months.

Univariable and multivariable analyses were conducted with generalized estimating equations applying a logit link[9,10] in standard statistical software,[a,b] to produce model parameter estimates, odds ratios, and confidence intervals. A premises identifier was entered into models to adjust for correlation of management practices among horses from the same premises. Univariable analyses were performed on individual risk factors (see Supporting Information). Variables with $P \leq .15$ in the univariable analysis were entered into corresponding multivariable models. Final multivariable models were selected based on statistical significance of explanatory variables, fit statistics (quasi-likelihood information criteria), and biological plausibility.[9]

Because of the small number of cases or controls experiencing some risk (or protective) factors, some candidate multivariable models became unstable. Factors that produced unstable but highly significant estimates because of small numbers were reported only univariably.

Results

A total of 123 PEHs met the inclusion criteria for the case–control study: 19 EHM cases, 14 EHV-1 cases with no reported neurologic signs, and 90 controls (Table 1).

Comparison of EHM with EHV-1 Cases with No Reported Neurologic Signs

Univariable analyses identified 2 individual risk factors that were statistically significant ($P \leq .05$) for association with EHM (Table 2). The odds of developing EHM compared with developing EHV-1 with no neurologic signs were greater among horses that were exposed to more biosecurity-related risks (2+) than those that experienced fewer risks (0 or 1). Horses that had been vaccinated in the 5 weeks before the Ogden event were 11 times more likely to develop EHM than EHV-1 without neurologic signs. In addition, mares were 4.3 times more likely than male horses to develop EHM ($P = .055$).

Table 1. Selected zoographic, health, and management characteristics of study population, by comparison group.*

Characteristic	All (n = 123)	Controls (n = 90)	EHV-1 Cases with No Reported Neurologic Signs (n = 14)	EHM (n = 19)
Female sex	50 (40.6%)	34 (37.8%)	4 (28.6%)	12 (63.2%)
Age				
<5 years	6 (5.0%)	2 (2.3%)	1 (7.7%)	3 (16.7%)
5–9 years	70 (58.8%)	53 (60.2%)	7 (53.8%)	10 (55.6%)
≥10 years	43 (36.1%)	33 (37.5%)	5 (38.5%)	5 (27.8%)
Number of events in April 2011: Mean (SD)	1.7 (1.3)	1.6 (1.2)	1.6 (1.5)	2.1 (1.5)
Horse use at Ogden event: Competing	108 (87.8%)	81 (90%)	12 (85.7%)	15 (79%)
Number of classes competed in at Ogden event: Mean (SD)	2.4 (2.2)	2.2 (1.8)	1.6 (1.9)	3.6 (3.2)
Number of classes competed in at Ogden event, May 3 and later: Mean (SD)	1.5 (1.7)	1.4 (1.6)	1.0 (1.5)	2.3 (2.3)
Number of biosecurity-related risks: Mean (SD)	1.6 (1.1)	1.4 (1.0)	1.5 (1.0)	2.6 (1.0)
Biosecurity-related risk: Responded "Yes"				
Tied in a barn	62 (50.4%)	39 (43.3%)	8 (57.1%)	15 (79%)
Used shared water	18 (14.6%)	11 (12.2%)	2 (14.3%)	5 (26.3%)
Grazed on grounds	22 (17.9%)	12 (13.3%)	0	10 (52.6%)
Used wash rack	79 (64.2%)	54 (60%)	9 (64.3%)	16 (84.2%)
Had a veterinary examination or treatment	9 (7.3%)	4 (4.4%)	1 (7.1%)	4 (21%)
Worked on by farrier	4 (3.2%)	3 (3.3%)	1 (7.1%)	0
Hours of travel before resting: Mean (SD)	6.4 (3.5)	6.6 (3.7)	7.6 (3.2)	4.9 (2.6)
Dietary supplementation: Responded "Yes"				
Receives any dietary supplement	71 (59.2%)	54 (61.4%)	7 (53.8%)	10 (52.6%)
Dietary supplement contains zinc	38 (30.9%)	33 (36.7%)	4 (28.6%)	1 (5.3%)
EHV-1 vaccination				
May 1, 2010–April 28, 2011	85 (73.3%)	61 (70.9%)	9 (75%)	15 (83.3%)
since April 29, 2011	36 (31.6%)	28 (32.9%)	5 (45.4%)	3 (16.7%)
in 35 days preceding April 29, 2011	29 (23.6%)	19 (21.1%)	1 (7.1%)	9 (47.4%)
36–70 days preceding April 29, 2011	22 (17.9%)	15 (16.7%)	4 (28.6%)	3 (15.8%)
71–100 days preceding April 29, 2011	8 (6.5%)	6 (6.7%)	1 (7.1%)	1 (5.3%)

*See Supporting Information Table 2 for all measured zoographic, health, management, and event-related characteristics.

Table 2. Selected univariable odds ratios (OR) and 95% confidence intervals (CI) for EHM risk factors and covariates comparing EHM horses (n = 19) with EHV-1 cases with no reported neurologic signs (n = 14).*

Variable	Category	OR	95% CI	*P*-Value
Sex	Male	Reference		
	Female	4.29	0.97–18.97	.055
Number of days per week exercised		0.33	0.09–1.21	.09
Number of classes competed in at event		1.47	0.91–2.37	.11
Number of classes competed in at event, May 3 and later		1.52	0.92–2.52	.10
Number of biosecurity-related activities (sum)	0–1	Reference		
	2+	1.51	1.16–1.96	.0024
Hours of travel before resting		0.71	0.50–1.01	.056
Distance traveled, April 1–29, 2011, before event, sum of miles		1.004	1.000–1.009	.08
Dietary supplementation	None	Reference		
	Supplement without Zn	2.00	0.38–10.58	.37
	Supplement with Zn	0.17	0.01–1.88	.17
Zinc-containing dietary supplement	No	Reference		
	Yes	0.14	0.01–1.42	.10
Horse vaccinated against EHV-1 since April 29, 2011	No	Reference		
	Yes	0.24	0.04–1.36	.10
Horse vaccinated in the 35 days previous to April 29, 2011	No	Reference		
	Yes	11.70	1.26–108.2	.03

*See Supporting Information Table 3 for all univariable ORs and 95% CIs.

Table 3. Multivariable horse-level parameter estimates ($\hat{\beta}$) and standard errors (SE [$\hat{\beta}$]) for model comparing EHM cases (n = 19) with EHV-1 cases with no reported neurologic signs (n = 14).

	Model Parameters		
Explanatory Variable	Estimate ($\hat{\beta}$)	SE ($\hat{\beta}$)	*P*-Value
Biosecurity-related activities at event (Greater, 2+ = 1)	2.400	0.974	.014
Sex: Female	4.325	1.354	.001
Number of classes competed in May 3 and later	1.885	0.698	.007
Interaction: Sex × Number of classes May 3 and later	−1.687	0.735	.022

Several predictor variables met the criteria for entry into the multivariable model: sex, average number of days per week exercised, dietary zinc supplementation, number of classes competed in at event, number of classes competed in May 3 and later, greater number of biosecurity risks, average hours traveled before resting, EHV-1 vaccination after April 29, 2011, and EHV-1 vaccination in the 5 weeks preceding the event.

Three main effects and 1 interaction term remained significant in the multivariable model: greater number of biosecurity-related risks (OR: 11.02; 95% CI: 1.63, 74.29), female sex, increasing number of classes competed in from May 3 and later, and the interaction between sex and number of classes competed in May 3 and later (Table 3). These multivariable horse-level parameter estimates are the basis for the following model equation and logit plot (Fig 1a): $G(x) = -5.046 + 2.400$ (Biosecurity-related activities (Greater, 2+ = 1)) + 4.325 (Sex, (Female = 1)) + 1.885 (#ClassesMay3+) – 1.687 (Sex*#ClassesMay3+).

The combined main and interaction terms indicate the risk of either sex of horse becoming an EHM case increased as the number of events competed in May 3 and later increased. For the lower number of classes, the likelihood of mares becoming cases was significantly greater than that for male horses. As the number of classes increased, the likelihood of a male horse becoming an EHM case increased more rapidly.

Comparison of EHM Cases with Controls

Univariable analyses identified numerous individual risk factors significantly ($P \leq .05$) associated with EHM when compared with controls (Table 4). Similar to the above results, experiencing ≥ 2 biosecurity-related risk factors increased the likelihood of becoming an EHM case (OR: 1.24). There was a 3.3 times greater odds of EHM among horses reported to be vaccinated against EHV-1 in the 5 weeks preceding the event and a 1.9 greater odds with each

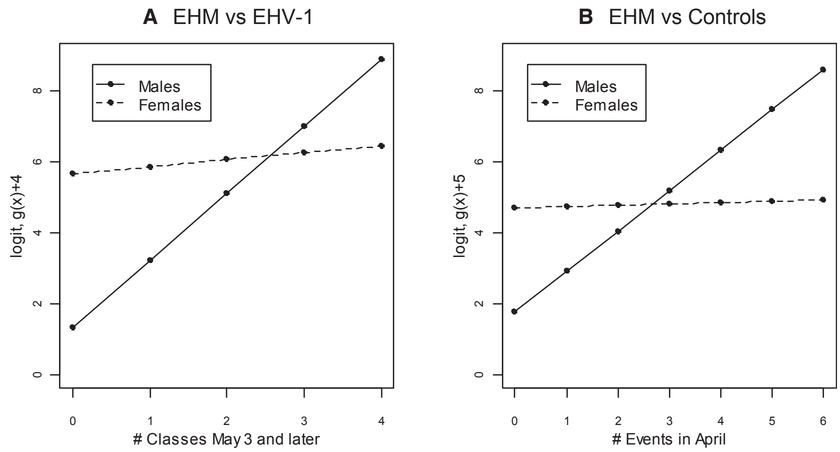

Fig 1. Logit plots for significant interactions in multivariable models: (a) comparison of EHM cases with EHV-1 cases, interaction of sex with number of classes competed in at event May 3 and later; (b) comparison of EHM cases with control horses, interaction of sex with number of events attended in April 2011.

Table 4. Selected univariable odds ratios (OR) and 95% confidence intervals (CI) for EHM risk factors and covariates comparing EHM horses (n = 19) with nonclinical horses (controls, n = 90).*

Variable	Category	OR	95% CI	*P*-Value
Sex	Male	Reference		
	Female	2.82	1.01–7.87	.047
Age	<5 years	Reference		
	5–9 years	0.13	0.02–0.85	.03
	≥ 10 years	0.10	0.01–0.76	.03
Number of events participated in April 1–28, 2011		1.33	0.90–1.95	.15
Hours to next event, categorized	0 – ≤ 6 hours	Reference		
	6 – ≤ 12 hours	0.63	0.18–2.24	.48
	>12 hours	0.35	0.09–1.41	.14
Number of classes competed in at event		1.29	1.06–1.58	.01
Number of classes competed in at event, post-May 3		1.29	0.998–1.67	.052
Number of biosecurity-related	0–1	Reference		
activities (sum)	2+	1.24	1.07–1.44	.0034
Hours of travel before resting		0.84	0.70–1.01	.066
Dietary supplement	None	Reference		
	Supplement without Zn	1.62	0.55–4.73	.37
	Supplement with Zn	0.11	0.01–0.95	.045
Zinc-containing dietary supplement	No	Reference		
	Yes	0.11	0.013–0.83	.032
Horse vaccinated against EHV-1	No	Reference		
in the 35 days previous to April 29, 2011	Yes	3.36	1.20–9.45	.02
Number of multivalent vaccinations, May 1, 2010–April 28, 2011 (0–3)		1.85	0.98–3.48	.058
Number of vaccinations, May 1, 2010–April 28, 2011 (0–4)		1.90	1.07–3.35	.028

*See Supporting Information Table 4 for all univariable ORs and 95% CIs.

increase in number of EHV-1 vaccinations in the year before the event. Mares were 2.8 times more likely than male horses to be EHM cases. The risk of being an EHM case increased as the number of classes increased at the event, but, feeding a nutritional supplement containing zinc was associated with decreased risk of being an EHM case. Horses >5 years of age had a lower risk of being an EHM case, but the number of horses in the youngest age category was small, and most cases were 5–9 years of age.

Several predictor variables met the criteria for entry into the multivariable model: sex, age, biosecurity-related risk factors, number of events participated in

Table 5. Multivariable horse-level parameter estimates ($\hat{\beta}$) and standard errors (SE [$\hat{\beta}$]) for model comparing EHM cases (n = 19) with control horses (n = 90).

	Model Parameters		
Explanatory Variable	Estimate ($\hat{\beta}$)	SE ($\hat{\beta}$)	*P*-Value
Biosecurity-related activities at event (Greater, 2+ = 1)	2.905	0.975	.0029
Sex: Female	2.945	0.870	.007
Number of events in April 2011	1.139	0.340	<.001
EHV-1 vaccination in preceding 5 weeks (Yes = 1)	1.963	0.800	.014
Dietary zinc supplementation (Yes = 1)	−3.661	1.134	.001
Interaction: Sex × Number of events in April 2011	−1.104	0.485	.023

during the month before the event, zinc supplementation, number of hours traveled immediately after the event, average hours traveled before resting while in transit, EHV-1 vaccination in the 5 weeks before the event, and number of EHV-1 vaccinations of any type in the year before the event.

In the multivariable model, 5 main effects and 1 interaction term remained significant (Table 5). As in the previous multivariable model, greater biosecurity risk (OR: 18.27; 95% CI: 2.67, 123.61) and female sex remained significant. Other main effects associated with increased EHM risk were EHV-1 vaccination in the 5 weeks preceding the event (OR: 7.12; 95% CI: 1.48, 34.17) and increasing number of events attended in April 2011. One main effect significantly associated with decreased risk of becoming an EHM case was zinc dietary supplementation (dichotomous in the model; OR: 0.03; 95% CI: 0.003, 0.24). A significant interaction occurred between sex and the number of events attended in April 2011.

The resulting multivariable model was $G(x) = -6.114 + 2.905$ (Biosecurity-related activities (Greater, 2+ = 1)) + 1.963 (EHV-1 vaccination in 5 weeks (Yes = 1)) – 3.661 (Zinc Supplement (Yes = 1)) + 2.945 (Sex (Female = 1)) + 1.139 (#Events in April) – 1.104 (Gender*#Events in April).

The logit plot (Fig 1b) suggests that the likelihood of mares becoming EHM cases increased slightly, and the likelihood of males becoming EHM cases increased more rapidly, with the increasing number of events in April 2011 before the Ogden event.

Discussion

This study was undertaken to investigate plausible risk factors for EHM based primarily on those previously reported in the literature. By analysis of epidemiologic data collected via questionnaires completed by owners or trainers of horses that attended the NCHA event in Ogden, UT, in May of 2011, multiple factors were identified to be associated with development of EHM. Factors contributing to development of EHM continue to be investigated by many researchers. EHV-1 cell-associated viremia is a necessary element of EHM pathogenesis. In addition, host and environmental factors play a role.[1] Future epidemiologic investigations can be focused on risk factors identified in this and previous EHM outbreak reports. Clinical research may be focused on risk factors identified in this study, perhaps as potential intervention strategies to decrease EHM risk. Although this study represents one of the largest collections of data on EHM cases from a single outbreak, statistical inferences were limited by the relatively small number of EHM and EHV-1 cases with no reported neurologic signs for which questionnaire data were available. Any statistically significant association identified in this epidemiologic field study does not prove that a factor is truly causative (or protective).

In light of the important role that zinc may play in the control of herpesvirus infection in humans, our surveys included questions regarding nutritional supplementation of this trace mineral. We found a significant association between reported dietary zinc supplementation and a decreased risk of EHM when EHM cases were compared with healthy exposed control horses. However, this association must be viewed cautiously as only 1 reported EHM case was receiving zinc supplementation. No zinc measurements were made from tissues or sera taken from these horses and zinc variables were calculated based on assumptions that zinc was included in supplements according to manufacturers' packaging and that horses consumed and efficiently utilized the estimated amount of daily supplemental zinc.

A potential explanation of the role of zinc is that it is second only to iron as the most abundant trace metal found in eukaryotic organisms.[11] It is a cofactor for more than 300 enzymes associated with DNA replication, signal transduction, and cell proliferation, particularly that taking place in immune cells (T and B cells) that produce immunoglobulins and cytokines.[11,12] Regarding EHV-1-induced myeloencephalopathy, cytotoxic T-lymphocytes (CTL) are recognized as important for controlling the magnitude of postinfection leukocyte-associated viremia and subsequent development of neurologic disease.[1] One study has implicated low pre-exposure concentrations of CTL precursor cells in horses as a predisposing factor of horses to the neuropathogenic form of EHV-1.[13] Bodily zinc status also influences the quantity of circulating CTLs as evidenced by decreases in the total number of lymphocytes in concert with zinc deficiency.[14]

Zinc ions themselves also may have a direct antiviral effect on herpesviruses. For example, zinc chloride added to herpes simplex virus-infected tissue culture cells completely blocked formation of herpes-induced giant cells.[15] The mechanism of action may relate to zinc's ability to cross-link the double helix of DNA typical for herpesviruses, thus increasing the structural stability of the molecule and inhibiting the scission necessary for viral replication. In a recent report, serum concentrations of zinc along with other micronutrients were measured in horses with EHV-1 infection.[16] Results showed concentrations of zinc and copper to be lower in the 9 naturally EHV-1-infected horses when compared with 9 healthy horses. Unfortunately, given the observational nature of our study, measurements of pre- and postinfection zinc concentrations in EHV-1-infected horses were not available. An argument can be made that other ingredients contained in the various dietary supplements fed to the horses in our study may have influenced our findings. However, whereas the risk of EHM was not influenced by feeding a dietary supplement as compared with feeding no supplement, feeding supplements that contained zinc was associated with decreased risk of EHM. These findings suggest that further evaluation of dietary zinc supplementation should be included in subsequent epidemiologic studies of EHM. Appropriately designed randomized clinical trials also are worthy of consideration to investigate more directly the role of zinc in control of EHV-1 infection.

Increased age has been shown to influence expression of EHM.[13] In previous studies, horses >20 years typically exhibited high viremias after EHV-1 challenge, and some of the challenged horses developed EHM.[13] In our univariable analysis of age, younger age was significantly associated with higher EHM risk. The number of horses in the youngest age group was small, however, and age did not remain significant in the multivariable model suggesting other factors played a stronger role.

Mares were more likely to develop EHM when compared with controls, similar to previous reports from other outbreaks.[17,18] In our 2 comparisons, the sex effect appeared to be modified by the level of competition the horse experienced (ie, the number of classes competed in at Ogden or the number of events competed before the Ogden event). Mares may respond differently than males to competition stress or some mares may have been managed in a way (eg, receiving progesterone supplementation) that altered their response to stress.

Current USDA-licensed vaccine products containing EHV-1 have been approved based on efficacy studies that supported claims of aiding in control of respiratory disease due to EHV-1, prevention of abortion associated with EHV-1, or reduction in viral shedding. None of these products carries a claim to protect against EHM. The majority of EHM cases in our study were reportedly vaccinated against EHV-1 in the 12 months before the Ogden event. Furthermore, EHV-1 vaccination in the 5 weeks before the event was associated with an increased risk of EHM. Henninger et al suggested an association between horses having received 3–4 EHV-1 vaccinations in the 12 months before an EHV outbreak and the development of EHM.[19] These authors recommended caution in interpretation of their findings, because horses that were more frequently vaccinated in the previous 12 months also were older, and older horses were found in their outbreak and challenge studies to be more likely to develop EHM.[13] Henninger et al suggested that previous exposure to EHV-1 and individual responses to vaccination may have influenced the immune response and susceptibility to EHM.[19] Collection of detailed EHV-1 vaccination history, including the type of vaccine, in future EHM outbreaks could assist in further defining the role of vaccination in development of EHM. Collecting serum samples for specific immunoglobulin subtype testing may be of value, because there have been reports that inactivated EHV-1 vaccines produce a different IgG subtype than does modified live vaccine.[20]

In both comparisons reported here, the risk of EHM increased with increasing biosecurity risks while at the Ogden event. This may be a result of the level of exposure to EHV-1, and potentially could be dose-related based on multiple exposures.

In conclusion, further investigations in both natural outbreaks and experimental settings are warranted to evaluate the potential protective effect of dietary zinc supplementation and the contributions of sex, stress of competition, and recent EHV-1 vaccination on the development of EHM. More attention to implementing good biosecurity practices while at equine events is also indicated to decrease the risk of exposure to infectious disease agents. Epidemiologic investigations of EHV-1 outbreaks are a valuable tool for discovery of potential factors that contribute to development of EHM.

Footnotes

[a] SAS v. 9.3, SAS Institute, Cary, NC
[b] R v 2.13.1, www.r-project.org

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References

1. Lunn DP, Davis-Poynter N, Flaminio MJBF, et al. Equine herpesvirus-1 consensus statement. J Vet Intern Med 2009;23: 450–461.
2. Carlson JK, Traub-Dargatz JL, Lunn DP. Equine viral respiratory pathogen surveillance at horse shows and sales. J Eq Vet Sci 2012:1–9. In press.
3. USDA–APHIS–VS. Equine herpesvirus myeloencephalopathy: a potentially emerging disease. 2007. Available at: http://www.aphis.usda.gov/animal_health/emergingissues/downloads/ehv1final.pdf. Accessed January 22, 2013.
4. USDA–APHIS–VS. Equine herpesvirus myeloencephalopathy: mitigation experiences, lessons learned and future needs. 2008. Available at: http://www.aphis.usda.gov/vs/nahss/equine/ehv/equine_herpesvirus_nahms_2008report.pdf. Accessed January 22, 2013.
5. USDA–APHIS–VS. Recommendations for horses exposed to EHV-1 or EHM. 2011. Available at: http://www.aphis.usda.gov/vs/nahss/equine/ehv/ehv_ehm_recommendations_051611.pdf. Accessed January 22, 2013.
6. USDA-APHIS-VS. Technical report: Descriptive epidemiologic characteristics of cases from the 2011 multistate outbreak of EHV-1. Available at: http://www.aphis.usda.gov/animal_health/nahms/equine/index.shtml. Accessed January 22, 2013.
7. NCHA. News from the National Cutting Horse Association. 2011. Available at: http://www.nchacutting.com/ag/pubs/enews/2011/enews20110713.html. Accessed January 22, 2013.
8. USDA–APHIS–VS. Final situation report. 2011. Available at: http://www.aphis.usda.gov/vs/nahss/equine/ehv/ehv_2011_final_sitrep_062311.pdf. Accessed January 22, 2013.
9. Hardin JW, Hilbe JM. Generalized Estimating Equations. Chapman and Hall: CRC Press; 2003.
10. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986;73:13–22.
11. Coleman JE. Zinc proteins: Enzymes, storage proteins, transcription factors, and replication proteins. Ann Rev Biochem 1992;61:897–946.
12. Goswami TK, Bhar R, Jadhav SE, et al. Role of dietary zinc as a nutritional immunomodulator. Asian-Aust J Anim Sci 2005;18:439–452.
13. Allen GP. Risk factors for development of neurologic disease after experimental exposure to equine herpesvirus-1 in horses. Am J Vet Res 2008;69:1595–1600.
14. Bises G, Mengheri E, Gaetani S. T-lymphocyte subsets in zinc deficient and in protein malnourished rats. Nutr Rep Int 1987;1987:1371–1378.
15. Eby GA, Halcomb WW. Use of topical zinc to prevent recurrent herpes simplex infection: Review of literature and suggested protocols. Med Hypoth 1985;17:157–166.
16. Yörük I, Deger Y, Mert H, et al. Serum concentration of copper, zinc, iron and cobalt and the copper/zinc ratio in horses with equine herpesvirus-1. Biol Trace Elem Res 2007; 118:38–42.
17. Goehring LS, van Winden SC, van Maanen C, et al. Equine herpesvirus type 1-associated myeloencephalopathy in The Netherlands: A four-year retrospective study (1999–2003). J Vet Intern Med 2006;20:601–607.
18. Pronost S, Legrand L, Wegge B, et al. Outbreak of equine herpesvirus myeloencephalopathy in France: A clinical and molecular investigation. Transbound Emerg Dis 2012;59: 256–263.
19. Henninger RW, Reed SM, Saville WJ, et al. Outbreak of neurologic disease caused by equine herpesvirus-1 at a university equestrian center. J Vet Intern Med 2007;21:157–165.
20. Goodman LB, Wagner B, Flaminio JBF, et al. Comparison of efficacy of inactivated combination and modified-live virus vaccines against challenge infection with neuropathogenic equine herpesvirus type 1 (EHV-1). Vaccine 2006;24:3636–3645.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Case definitions used in USDA–APHIS–VS recommendations for exposed horses to EHV-1 or EHM.

Table S1. List of the number of primary exposed horses, number of PEH cases (EHV-1 or EHM suspect or confirmed cases eligible for inclusion in study), number of eligible controls, and number of selected controls by state.

Table S2. Characteristics of horses, housing, health management, and travel reported by owner/trainer survey respondents for the three comparison groups and for all horses.

Table S3. Univariable odds ratios (OR) and 95% confidence intervals (CI) for EHM risk factors comparing EHM cases (n = 19) with EHV-1 cases with no reported neurological signs (n = 14).

Table S4. Univariable odds ratios (OR) and 95% confidence intervals (CI) for EHM risk factors and covariates comparing EHM cases (n = 19) with controls (n = 90).

Data S2. Case and Control Horse questionnaire.