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Clinical and Clinicopathological Factors Associated with Survival in 44 Horses with Equine Neorickettsiosis (Potomac Horse Fever)

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Background: The epidemiology of equine neorickettsiosis (EN) has been extensively studied but limited clinical and clinicopathological data are available concerning naturally infected horses.

Hypothesis: Factors predictive of survival will be identified in horses diagnosed with EN.

Animals: Convenience sample of 44 horses with EN admitted to 2 referral institutions.

Methods: A retrospective study was performed. A diagnosis of EN was based on the presence of positive blood or fecal PCR.

Results: The most common clinical signs included diarrhea (66%), fever (50%), anorexia (45%), depression (39%), colic (39%), and lameness (18%). The median duration of hospitalization was 6 days and 73% of horses survived to discharge. Laminitis was present in 36% of horses, 88% of which were affected in all 4 feet. Serum creatinine and urea nitrogen concentrations, as well as RBC count, blood hemoglobin concentration, hematocrit, band neutrophils, serum AST activity, serum CK activity, and anion gap, were significantly ($P < .05$) higher in nonsurvivors. Serum chloride and sodium, concentrations as well as duration of hospitalization were significantly lower in nonsurvivors. The results of forward stepwise logistic regression indicated that blood hemoglobin concentration on admission and antimicrobial treatment with oxytetracycline were independent factors associated with survival.

Conclusions and Clinical Importance: Severity of colitis as reflected by electrolyte loss, hemoconcentration, and prerenal azotemia were predictors of survival in horses diagnosed with EN. Treatment with oxytetracycline was associated with increased survival.

Key words: Colitis; Ehrlichiosis; Equine; Oxytetracycline.

Equine neorickettsiosis (EN) is caused by the bacterium *Neorickettsia risticii* (formerly *Erlichia risticii*) and is a common cause of equine colitis in endemic areas. Given the global distribution of the disease, the current designation of "Potomac horse fever" is suboptimal. Therefore, the term "equine neorickettsiosis" or "EN" will be used in this article. Horses appear to be accidental hosts in the complex lifecycle of *N. risticii*, and are infected upon ingestion of *N. risticii*-infected trematode metacercariae within aquatic insects, or free-living trematode cercariae.[1,2] Once in the gastrointestinal tract of the horse, *N. risticii* is released from the trematode and infects and replicates in colonic epithelial cells, as well as tissue macrophages, mast cells, and blood monocytes after translocation.[3]

Clinical signs of EN include decreased appetite progressing to anorexia, fever, diarrhea, abortion, and predisposition to laminitis.[4,5] Currently, there is no reference standard for diagnosis of EN, as no antemortem test is 100% sensitive or specific, and postmortem changes are often mild and nonspecific.[6,7] Available diagnostic tests include serum indirect fluorescent antibody (IFA) testing, enzyme-linked immunosorbent assay (ELISA), isolation of *N. risticii* from whole blood or feces, and polymerase chain reaction (PCR) for nucleic acid identification of conserved genes such as 16S rRNA from whole blood or feces.[8–12] Serum antibody titers have been shown to correlate poorly with infection and are associated with a high rate of false positive results; therefore, identification of antigen is considered the current standard for diagnosis.[11,13]

Four EN vaccines[a–d] containing a liquid suspension of inactivated *N. risticii* have been licensed for use in the United States, with 1 vaccine currently available. It has been demonstrated, however, that administering killed *N. risticii* bacterin fails to mitigate clinical signs and reduce treatment costs.[14] Vaccine failure is due, at least in part, to various strains of *N. risticii* expressing different major antigens such as 51 kD and 55 kD.[15,16] Vaccine failure has been reported to be as high as 89% and is associated with relatively low levels of vaccine-induced antibodies.[16]

Although the epidemiology of EN has been extensively investigated,[17–22] and the clinical and clinicopathological changes have been documented in 55 horses that were experimentally infected,[23] there are

Abbreviations:

DNA	deoxyribonucleic acid
EN	equine neorickettsiosis
IFA	indirect fluorescent antibody
PCR	polymerase chain reaction
OR	odds ratio

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limited data regarding clinical and clinicopathological aspects of EN in naturally occurring cases. Therefore, the objectives of this retrospective study were to describe the signalment, history (including vaccination status), clinical signs, clinicopathologic values, antimicrobial use, and survival status in horses exhibiting clinical signs of EN, and to identify variables associated with survival.

Materials and Methods

Data Collection

Medical records from horses presented to Institution 1 and Institution 2 over a 15-year period (January 1996–December 2011) were reviewed. Inclusion criteria included complete medical records and a diagnosis of EN by positive blood or fecal PCR. Animals with incomplete medical records were excluded. Data collected included signalment, date of presentation (day, month and year), vaccination status, historical complaint, treatment before presentation, physical examination upon presentation, routine blood work upon presentation, method of diagnosis, in-hospital treatment, duration of hospitalization, and outcome. Presentation with or development of laminitis was also included. Clinical laminitis was defined as an Obel grade of 1, 2, 3, or 4 with subjectively increased digital pulse pressure, whereas confirmed laminitis was defined as clinical laminitis with radiographic or histologic changes.[24]

Data Analysis

Horses were grouped by outcome (discharged alive or not) and compared with $P < .05$ considered significant. Normally distributed data were reported as mean ± SD and compared using unpaired *t*-tests. Non-normally distributed data were reported as median and range and compared using the Mann–Whitney *U*-test. Categorical data were compared using a Chi-square test; Fisher's exact test was used when the expected count in >20% of the cells was <5. The association of clinical signs, clinicopathologic factors, and treatments with outcome was investigated using forward stepwise logistic regression and $P < .05$ to enter and $P < .05$ to remain in the model. The final logistic regression model fit was evaluated using the Hosmer–Lemeshow Goodness-of-Fit test. Statistical analysis was performed using commercially available statistical software.[e]

Results

Signalment/History

Forty-four horses met the criteria for entry into the study: 21 from Institution 1 and 23 from Institution 2. Horses ranged from 4 months to 29 years of age with a median age of 7 years. Twenty-five horses were female and 19 were male (6 stallions and 13 geldings). Breeds included Thoroughbreds (n = 25), Quarter Horses (n = 6), Tennessee Walking Horses (n = 2), mixed breeds (n = 2), and one each of Standardbred, Paint, American Saddlebred, Paso Fina, Haflinger, Arabian, Pony of the Americas, Appaloosa, and Morgan. Although no breed or sex predilection was identified in this study, it is interesting that 6 horses (14%) were less than 1 year of age. The month of presentation ranged from June to December with a peak in August (34% of cases). Eleven horses had available immunization records, 9 (82%) of which were vaccinated against EN. The date of vaccination ranged from 1 to 5 months before presentation with a median of 3.5 months before presentation. The vaccine manufacturer was not recorded for any of the vaccinated horses. Two animals were known to be not vaccinated and 33 had an unknown vaccination history. Common recent complaints included diarrhea (52% of horses), anorexia (39%), fever (34%), colic (27%), lethargy (27%), and lameness (11%).

Clinical Signs

The most common clinical signs at presentation included diarrhea (66% of horses), fever (50%), anorexia (45%), depression (39%), colic (39%), and lameness (18%). The median duration of clinical signs before presentation was 5 days (range: 12 hours to 15 days). Eight horses (18%) presented with clinical laminitis and an additional 8 horses (18%) developed clinical laminitis in the hospital. Among the 16 horses that developed laminitis, 14 were lame on all 4 limbs and 2 were lame on the front limbs only. Ten of the 14 horses that were lame on all 4 limbs had distal extremity radiographs performed, 9 of which had all 4 feet radiographed and 1 had only front feet radiographed. Four had evidence of rotation on all 4 feet, 2 had evidence of rotation on the front feet only and 4 had no evidence of rotation. Distal extremity radiographs were not taken in the 2 horses with front limb lameness only. Overall, clinical laminitis was found in 16 horses (36%) and confirmed by radiographic demonstration of P3 rotation in 60% of horses with radiographs. Among horses with clinical laminitis, all demonstrated laminitis on both front feet and 14 horses (88%) demonstrated clinical laminitis on all 4 feet. Among all cases, the median time for development of laminitis was 24 hours after hospitalization (range: 0–4 days).

Clinicopathologic Values

A complete blood count (CBC) and serum biochemical profile were available for 34 (77%) and 32 (73%) horses, respectively. The most common CBC abnormalities included neutropenia (47%) and increased hematocrit (38%). The most common serum biochemical abnormalities included hypocalcemia (76%), hyponatremia (64%), hyperglycemia (59%), hypochloremia (53%), azotemia (50%), hyperbilirubinemia (50%), and hypoalbuminemia (34%). Nineteen percent of horses had increased serum creatine kinase (CK) activity, whereas 6% had increased serum AST activity Blood lactate concentration at admission was available for 8 horses, 5 of which (63%) demonstrated hyperlactatemia. Serum creatinine and urea nitrogen concentrations, as well as RBC count, blood hemoglobin concentration, hematocrit, band neutrophils, serum CK activity, serum AST activity, and anion gap were higher in nonsurvivors. Serum chloride and sodium

Table 1. Complete blood count (n = 34) and serum biochemical profile (n = 32) data from horses with clinical signs of EN. Horses were categorized as survivors (discharged alive) or nonsurvivors (euthanized or died). Data are expressed as mean ± SD or median and range (in parentheses). Only clinicopathologic variables that were significantly different ($P < .05$) between survivors and nonsurvivors are included. [a]Serum creatine kinase activity values were available for 26 horses.

Factor	Survivors	Nonsurvivors	*P*-Value
Serum chloride (mEq/L)	94.2 ± 8.3	81.6 ± 11.9	.0016
Hemoglobin (g/dL)	15.7 (10.8–21.2)	20.8 (14.2–24.6)	.0034
Hematocrit (volume%)	45.3 ± 7.7	55.2 ± 10.0	.0035
Serum urea nitrogen (mg/dL)	28 (5–104)	71 (26–145)	.0047
Serum creatinine (mg/dL)	1.7 (1.3–5.0)	3.2 (1.1–11.0)	.0072
Serum creatine kinase (IU/L)[a]	195 (108–710)	356 (124–10,435)	.0097
Serum AST (IU/L)	265 (150–449)	343 (205–1158)	.0119
Serum sodium (mEq/L)	129.3 ± 6.9	122.3 ± 8.3	.0177
Band neutrophils (cells/L)	0.25 (0–4.0)	0.70 (0.24–5.9)	.0327

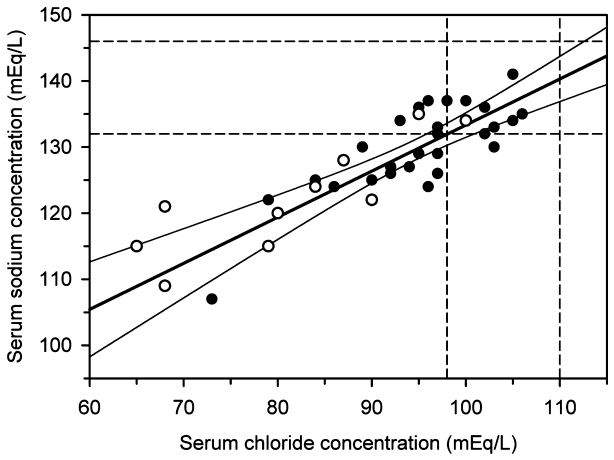

Fig 1. Scatterplot of the relationship between serum sodium and chloride concentrations of 32 horses with clinical signs of EN. Horses were categorized as survivors (discharged alive, n = 22; closed circles) or nonsurvivors (euthanized or died, n = 10; open circles). The dashed lines indicate the upper and lower limits of the reference range for serum sodium and chloride concentrations.

concentrations were lower in nonsurvivors (Table 1). All horses with increased anion gap were azotemic, and the majority of horses with available blood lactate concentrations at admission demonstrated hyperlactatemia. Serum sodium and chloride concentrations were linearly related ($R^2 = 0.74$) (Fig 1). Forward stepwise logistic regression indicated that the blood hemoglobin concentration on admission was the only independent clinicopathologic factor associated with survival (Table 2).

Table 2. Results of forward stepwise logistic regression analysis of clinical and clinicopathologic findings and treatment as predictors of survival in 32 horses with clinical signs of EN. Horses were categorized as survivors (discharged alive, n = 22) or nonsurvivors (euthanized or died, n = 10). The Hosmer–Lemeshow Goodness-of-Fit test indicated a good fit ($P = .32$) to the logistic regression model. N/A = not applicable.

Factor	Estimate	SE	Odds Ratio	95% CI	*P*-Value
Intercept	7.01	3.2	N/A	N/A	.031
Hemoglobin (g/dL)	−0.42	0.180	0.6	0.464–0.9	.020
Oxytetracycline administration	2.20	1.04	9.0	1.2–69.7	.035

Table 3. Results of diagnostic tests of 44 horses with clinical signs of EN. The 2 diagnostic tests were not run on all horses.

	Positive	Negative	Percent Positive
PCR whole blood (n = 40)	33	7	83
PCR feces (n = 16)	12	4	75

Diagnostic Tests

Forty horses were tested for EN by PCR of whole blood, 33 of which were positive (83%). Sixteen horses were tested for EN by PCR of feces, 12 of which were positive (75%). These results are presented in Table 3. Serum IFA titers were performed on admission in 13 horses, only one of which had a second, convalescent titer performed. There was a wide variation in acute titers, with a range of 40–10,240 and a median of 640. Among the 13 horses with an acute IFA titer performed, 5 had been vaccinated within manufacturer recommendations for protection against EN. Despite this, there was a wide variation among titers in vaccinated horses, which ranged from 40–10,240.

Treatment

Four horses (9%) received oxytetracycline before presentation to the referral institution. Oxytetracycline was the most commonly used antibiotic (30 horses, 68%) in-hospital at a median dose of 6.5 mg/kg (range: 5–15 mg/kg), with most dosing regimens of twice daily treatment for 5 consecutive days. Fifteen horses (34%) received a different antimicrobial than oxytetracycline before admission and 23 horses (52%) received a different antimicrobial at the referral

hospital. Ceftiofur sodium was the most common antimicrobial used before admission (n = 7, 16%), whereas metronidazole was most commonly used in-hospital (n = 15, 34%). Eleven horses (25%) received oxytetracycline in combination with metronidazole in-hospital. There was a significant ($P = .0043$) difference in survival between horses treated with oxytetracycline before the admission or in-hospital (survival 87%, 26/30) and horses not treated with oxytetracycline (survival = 43%, 6/14). There was no significant ($P = .40$) difference in survival between horses treated with oxytetracycline alone and those treated with oxytetracycline and metronidazole.

Intravenous isotonic crystalloid fluids were administered to 42 horses (95%) at an initial median rate of 5 mL/kg/h (range: 2.5–10 mL/kg/h). Flunixin meglumine was administered to 33 horses (75%) within 12 hours of admission, with 26 horses (79%) receiving 1.1 mg/kg twice daily.

Outcome

Thirty-two horses (73%) survived to discharge and the median duration of hospitalization was 6 days (range: 1–15 days). Hospitalization duration was significantly shorter for nonsurvivors ($P = .018$) as 4 horses (33% of nonsurvivors) were euthanized on the day of presentation. No other statistically significant difference in epidemiologic data between survivors and nonsurvivors was detected and the only signalment variable associated with survival was age (survivors, 6.0 years [0.3–23.0 years]; nonsurvivors, 12.0 years [2.0–23.0 years], $P = .045$). Seven of 9 horses (78%) vaccinated against EN survived, which is not significantly different from the overall survival rate of 73%.

Logistic regression was confined to variables identified as being significantly associated with survival in Table 1, except that CK activity was not evaluated because data were available for a smaller number of horses. Forward stepwise logistic regression using data from 32 horses that had complete blood counts and serum biochemical analyses (serum electrolyte and creatinine concentrations) performed indicated that the blood hemoglobin concentration on admission was independently associated with survival (Table 2). Oxytetracycline administration was the only independent treatment factor associated with survival (Table 2).

Discussion

The main findings of the study reported here were that the blood hemoglobin concentration on admission and antimicrobial treatment with oxytetracycline were independent factors associated with survival of horses with EN. Oxytetracycline administration appears indicated as part of the treatment of all horses that live in an endemic area and that develop clinical signs consistent with EN. This recommendation is based on the documented effectiveness of oxytetracycline against *N. risticii* in vitro[25] and in treating horses with naturally acquired infection (this study), coupled with the lack of clear evidence that oxytetracycline at a dose rate of 6.6 mg/kg IV q12–24 h for 5 days induces or exacerbates colitis.[26]

Blood hemoglobin concentration as an independent predictor of survival was likely a reflection of the degree of hypovolemia. Although other hematologic indices such as RBC count and hematocrit were not independent factors associated with survival, they were significantly elevated in nonsurvivors. It is not surprising that the degree of hypovolemia was associated with severity of disease and prognosis, and is consistent with previous reports.[27,28] The predictive ability of blood L-lactate concentration could not be evaluated because it was measured in only 8 (18%) horses.

Band neutrophilia was associated with survival, and suggested a more profound systemic inflammatory response in nonsurvivors. Higher band neutrophil counts have been observed in nonsurviving horses diagnosed with *Clostridium difficile* enterocolitis.[29] Increased anion gap in nonsurvivors was likely a reflection of presumed hyperlactatemia in hypovolemic animals as well as the presence of strong anions associated with uremia. The significance of serum CK and AST activities in the current study is unclear. There was no association between enzyme activity and the presence of colic. It is possible that decreased tissue perfusion may have led to acute muscle injury, as 80% of horses with an elevated serum CK concentration also had elevated hematocrit and blood hemoglobin concentrations. However, the magnitude of elevation was too low to draw definitive conclusions regarding etiology of muscle injury.

Serum sodium and chloride concentrations at admission were significantly lower in nonsurvivors. A well-established mechanism of diarrhea in horses is decreased chloride reabsorption in the colon.[30] *Neorickettsia risticii* microorganisms are consistently found in the wall of the large colon of equids that develop clinical signs of EN.[3] The organism multiplies in the cytoplasm of colonic glandular epithelial cells inducing exfoliation of the mucosa. It has been shown that functional colonic mucosa is required for physiologic sodium and chloride reabsorption.[29] Therefore, it is not surprising that following experimental *N. risticii* infection, sodium and chloride reabsorption is inhibited.[30] Clinically, this finding is important because serum sodium and chloride concentrations can be used to indirectly assess the severity of colitis in horses with EN. Although this may be confounded by the fact that hyponatremia and hypochloremia may be found in horses with colitis caused by *Clostridium* sp. or *Salmonella* sp.,[31,32] the agreement between our clinical observations and the precise mechanism of inhibition of sodium and chloride reabsorption in the colon by *N. risticii* makes this unlikely. Furthermore, clinical and clinicopathological description of infections by *Clostridum difficile* type A and *Salmonella* sp. failed to identify hyponatremia or hypochloremia in diseased horses.[29,33] Although serum calcium concentration was not associated with survival, hypocalcemia was the

most commonly observed serum biochemical abnormality. This was attributed primarily to decreased absorption secondary to colitis and has been observed in horses with *C. difficile* and *C. perfringens* enterocolitis.[29] Although hypoalbuminemia likely contributed to hypocalcemia in some cases, less than half of the horses with hypocalcemia demonstrated hypoalbuminemia.

Oxytetracycline had been previously shown to be effective in vitro at treating EN.[34] Administration of oxytetracycline was an independent factor associated with survival in the study reported here and should be promptly used in horses diagnosed with EN. Theoretical complications of using oxytetracycline before a definitive diagnosis of EN is made include the potential for prolonged shedding of *Salmonella enterica* in cases of salmonellosis and fatal colitis after administration of tetracyclines.[35] Our results, however, suggest that administration of oxytetracycline to horses with compatible clinical signs in endemic areas during summer months should be considered before a definitive diagnosis in order to increase the likelihood of survival.

Horses in the current study demonstrated clinical signs typical of EN.[23] The most common presenting complaints, as previously reported, were depression, anorexia, diarrhea, and lameness.[23] Laminitis was diagnosed in approximately 1/3 of horses with EN, with some horses presenting with laminitis before the onset of diarrhea. To the authors' knowledge, this is the first study to document prevalence of laminitis in horses with EN. The presence of laminitis in the current study was not associated with outcome. Although this finding may be confounded by the fact that all cases were presented to a referral hospital and therefore horses diagnosed with colitis-induced laminitis in the field may have been euthanized before presentation, the agreement between our calculated prevalence of laminitis and the estimated occurrence in horses with clinical EN makes this unlikely.[5,36]

Younger horses with EN were more likely to survive than older horses with EN. This is difficult to explain, given that the median "older" age was only 12 years, which is not typically associated with age-related disease. Interestingly, 14% of horses in this study were under 1 year of age, highlighting the importance of considering EN in foals with diarrhea. In the current study, the seasonal occurrence of EN previously reported was confirmed with over 1/3 of cases presented in August.[37]

Given that only clinically affected horses were evaluated in the present study, it is difficult to draw conclusions regarding vaccine efficacy; however, it is interesting that 82% of horses with a known history of vaccination were fully vaccinated against *N. risticii*. At the time of the study, 4 inactivated vaccines from 4 different pharmaceutical companies were available.[a-d] The vaccines used were not recorded in the study; however, available vaccines during the study period each contained only 1 strain of inactivated bacterin.[16] Interestingly, there was no significant difference between overall survival rate and the survival rate of vaccinates.

The apparent high rate of vaccination failure is consistent with previous reports of vaccine inefficacy.[16,38,39] Vaccine failure has been reported to be as high as 89%, and in some studies, vaccination was not found to reduce prevalence, cost, or severity of disease.[16,39] However, 1 study demonstrated resistance to homologous reinfection in vaccinated horses for at least 20 months postinoculation.[40] Incomplete protection from vaccination is due in large part to extensive variability in the major surface antigens such as the 51-kDa and 55-kDa major antigens, and there would not be any cross-protection between strains.[21,41] Strain variation is considered an important virulence mechanism in that amino acid sequencing of 51-kDa antigens suggest a geographic segregation of *N. risticii* strains.[20] In addition, sequence variation has been reported among strains isolated in the early 1980s compared with strains sequenced in the 1990s.[10] Taken together, these findings suggest that *N. risticii* strains lack conserved immunogenic surface proteins that are able to elicit a broadly reactive adaptive immune response in affected horses, and that a cross-protective vaccine will likely require multiple strains or conserved peptides that contain neutralizing antibody or T-cell epitopes. The diagnosis of EN was made by PCR of whole blood or feces. Polymerase chain reaction based on 16S rRNA gene provides a sensitive diagnostic test because of the conserved nature of 16S rRNA among strains, as well as a specific test given the lack of cross-reactivity among other rickettsial organisms.[19,42] Although diagnostic PCR testing in the current study utilized amplification of the conserved 16S rRNA gene, the relatively low apparent sensitivity of PCR in the current study might be explained by timing of bacteremia and fecal shedding of *N. risticii*, which is consistent with one study that found whole blood PCR to be 81% sensitive when compared with whole blood culture for *N. risticii*.[11] The organism is likely to be detected by PCR in whole blood before and for a longer period of time compared with feces. Experimental infection results in positive whole blood PCR from approximately 7–21 days postinfection, whereas fecal PCR is typically positive from approximately 11–16 days postinfection.[18,43] Considering that in most clinical cases the day of exposure is not known, false negative test results may occur if these "windows" are missed. Therefore, it may be prudent to test suspect cases more than once.

High variation in IFA titers has been reported in naturally and experimentally infected horses, although titers >2,560 are typically associated with clinical disease.[11,41] In the present study, the vast majority of horses failed to demonstrate a "high titer" response (2,560), although a single titer was performed in all but one horse. Similar results have already been described in previous reports showing that the amplitude of seroconversion can vary greatly between horses experimentally infected with *N. risticii*.[11,43] In one study, IFA was found to be poorly specific and poorly sensitive as 16% of healthy horses had evidence of an antibody response and only 12.5% of seropositive

horses with clinical signs of EN had a rise in antibody titer between acute and convalescent samples.[13] In the current study, wide variation in IFA titers among recently vaccinated horses supports the notion that IFA testing is not a reliable predictor of protection.

In conclusion, severity of colitis as reflected by electrolyte loss and prerenal azotemia was predictive of survival in horses diagnosed with EN. Treatment with oxytetracycline was associated with increased survival.

Footnotes

[a] PHF-Vax (Schering Plough/Merck), Whitehouse Station, NJ: available from 1987 to 2008
[b] PotomacGuard (Fort Dodge/Pfizer), Madison, NJ: available from 1993 to 2011
[c] PHF-Gard (Pfizer), Madison, NJ: available from 2000 to 2001
[d] Equine Potomavac (Merial), Duluth, GA: currently available
[e] SAS 9.2; SAS Inc, Cary, NC

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