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Protection Against Clinical Endotoxemia in Horses by Using Plasma Containing Antibody to an Rc Mutant *E. coli* (J5)

Sharon J. Spier, Jean-Pierre Lavoie, James S. Cullor, Bradford P. Smith, Jack R. Snyder, and William M. Sischo

Departments of Medicine (S.J.S., J.P.L., B.P.S.), Clinical Pathology (J.S.C), Surgery (J.R.S.), and Epidemiology and Preventive Medicine (W.M.S.), School of Veterinary Medicine, University of California, Davis

Thirty-two horses with clinicopathologic evidence of endotoxic shock were randomly selected for a double-blind trial of hyperimmune lipopolysaccharide (LPS) core antigen plasma. Horses were suffering from acute toxic enteritis (n=15), 360° volvulus of the large colon (n=9), proximal jejunitis/duodenitis (n=6), or strangulating obstruction of the small intestine (n=2). Plasma was harvested from suitable equine plasma donors (preimmune plasma) and horses were immunized with a whole-cell bacterin of an Rc mutant *E. coli* (J5). Plasma was again harvested from these horses when IgG ELISA titers recognizing LPS core antigen were ≥1:32,000. All horses included in the trial received either preimmune or hyperimmune plasma in addition to traditional therapy (fluids, antimicrobials, antiinflammatory agents, etc.) as dictated by the attending clinician. The mortality rate in the group of horses receiving hyperimmune plasma was 13%; in the control group receiving preimmune plasma, the mortality rate was 47% ($P=.045$). Horses receiving J5 hyperimmune plasma had a significantly improved clinical appearance 48 hours after plasma administration ($P<.05$) and a shorter period to recovery than control horses ($P=.069$).

Key words: endotoxin, gram-negative core antigen, passive immunotherapy, equine colic, plasma transfusion

INTRODUCTION

Gram-negative septicemia and endotoxic shock associated with equine diseases such as acute toxic enteritis and strangulating obstructions of the intestine are costly problems. A high mortality rate occurs despite vigorous antimicrobial therapy due to the inability to counteract the lethal effects of bacterial endotoxins once cascades of immunologic mediators are fully initiated [1–6]. The frequent sequelae to disease,

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Address reprint requests to Dr. Spier, Dept. of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616.

including laminitis, venous thrombosis, or multiple organ failure, add to economic losses [6].

Endotoxin, also referred to as lipopolysaccharide (LPS), is a cell wall component of gram-negative bacteria and present in the intestinal contents of normal animals. Endotoxin is released by bacterial multiplication or bacteriolysis [2] and is absorbed into the circulation when the intestinal mucosal barrier is disrupted by intestinal obstruction, ischemia, or enteritis [3,7–9]. Gram-negative septicemia, secondary to inadequate colostral absorption or immunosuppression, offers another means for endotoxin to access circulation [6]. When a critical amount is absorbed or released, a shock state is produced, which includes tachycardia, reduced pulse pressure, prolonged capillary refill time, injected mucous membranes, cold extremities, and abdominal discomfort. Laboratory data observed with endotoxemia reveal numerous alterations, including hemoconcentration, leukopenia due to neutropenia and lymphopenia, hyperglycemia followed by hypoglycemia, elevated hepatic enzymes, elevated blood lactate, and coagulopathy suggestive of disseminated intravascular coagulation (DIC) [1–3,5]. Treatment for gram-negative sepsis and endotoxemia is costly in large animals and includes intense fluid administration, antimicrobials, corticosteroids, nonsteroidal antiinflammatory drugs, and other therapeutic agents [3,5,6].

A recent approach to therapy of endotoxic shock is the administration of plasma or serum products containing antibody directed toward endotoxins. Passive administration of antisera to a genetically stable rough mutant of *E. coli* 011:B4 (J5) [10,11] and of polyclonal antisera against LPS [12] has been shown to improve the survival rate of humans suffering from gram-negative bacteremia and endotoxemic shock. J5 is a mutant strain of *E. coli* which lacks specific oligosaccharide side chains, so the core polysaccharide, which is similar among gram-negative bacteria, is exposed. Antibody to these core polysaccharides may thus be cross protective, binding to many gram-negative organisms or endotoxins [13–15]. The host immune reaction to endotoxin is believed to be an important determinant of mortality in diseases such as acute toxic enteritis and strangulating obstructions of the large and small intestine [3,5,6]. Previous studies utilizing antiendotoxin antibody in horses with clinical evidence of endotoxemia reported decreased mortality and recovery periods [16,17]. However, some studies in horses failed to show protection of LPS core antigen antibody against endotoxin challenge [18] or neonatal septicemia [19]. While the protective effects of LPS antibody are still debated [18–26], strong evidence exists for their application in therapy for gram-negative disease. The purpose of this study was to perform a controlled clinical trial to determine if hyperimmune plasma containing antibody to gram-negative core antigens would reduce mortality in horses with clinical and laboratory evidence of gram-negative septicemia or endotoxemia.

MATERIALS AND METHODS

Preparation of Plasma

Three healthy horses were selected as plasma donors based on blood typing and absence of alloantibody.[1] All donors were negative for A, C, and Q red blood cell

[1]Serology Laboratory, University of California, Davis.

antigens, antierythrocyte antibodies, and equine infectious anemia. Horses were dewormed every 3 months and were vaccinated every 6 months against eastern and western encephalomyelitis, tetanus, and equine influenza A1 and A2. Donors were monitored for anemia by hematocrit determination prior to collection, and volume was replaced with lactated Ringer's solution. Eight liters of blood was collected aseptically at approximately 30-day intervals. Sterile acid citrate dextrose (10%) was used as the anticoagulant and plasma was harvested after centrifugation (221G for 40 minutes). All plasma was collected in 1 L plastic bags,[2] coded by number, and stored at −20C. Once sufficient plasma was banked to serve as preimmune (control) plasma, the horses were immunized with a heat-killed *E. coli* J5 bacterin with incomplete Freund's as adjuvant. The *E. coli* J5 bacterin was prepared as described by Ziegler et al. [10]. Horses were immunized intramuscularly with 3.5 ml of J5 (5×10^9 CFU/ml) mixed with 1 ml of Freund's incomplete adjuvant. Plasma was harvested when the anti-J5 IgG ELISA titer was ≥1:32,000. Horses were boostered every 30 days with bacterin to maintain elevated titers. J5 antibody titers were measured prior to all collections. The number of immunizations required to reach a titer of ≥1:32,000 was variable between donors, usually requiring at least four injections at 10-day intervals and two injections at monthly intervals. All preimmune plasma had an anti-J5 IgG titer of <1:500; the geometric mean IgG titer of hyperimmune plasma units was 1:41,499. All plasma batches (approx. 5 L plasma/harvest) were coded with a three-digit number picked at random and recorded by a research assistant. Horses were administered plasma from one code number. The plasma was stored in a freezer accessible to the large-animal clinicians. Horses were not preassigned to treatment groups; the selection was arbitrary based upon the coded plasma selected for the patient. Neither the clinicians or the investigators knew if the plasma was hyperimmune or control until the end of the trial. Equal amounts of hyperimmune and control plasma were harvested. The trial was terminated when the plasma stores were exhausted. No attempts were made to achieve equal division of hyperimmune and control plasma between disease groups.

Serology

An indirect enzyme-linked immunosorbent assay (ELISA) was used to determine anti-J5 IgG antibody activity [38]. In brief, 96-well flat-bottom microtiter plates[3] were coated with heat-killed, washed whole-cell *E. coli* strain J5 (100 μl/well) that had been adjusted spectrophotometrically with 0.9% sterile saline to an optical density of 0.854 at 610 nm. Plates were incubated overnight at 37°C, washed, and sequentially incubated with 100 μl of diluted test plasma, conjugate (peroxidase-labelled antiequine IgG[4]), and substrate (40 mM ABTS, 0.05 M citrate buffer, 0.5 M H_2O_2). Stopping reagent (5 mM Na azide) was added and plates were read immediately with an automated plate reader.[5] All samples were processed in triplicate with titers defined as the reciprocal of the highest dilution in which a

[2]Transfer pack unit with coupler, Fenwal Laboratories, Deerfield, IL.

[3]Falcon Probind 3916, Becton Dickenson Labware, Oxnard, CA.

[4]Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD.

[5]Dynatech Laboratories, Alexandria, VA.

minimum of two of three identical wells exceeded 0.05 optical density after blanking on the arithmetic mean of eight control wells with no serum.

Horses Chosen for Study

All horses were clinical cases at the Veterinary Medical Teaching Hospital, University of California, Davis. All horses were hospitalized in the Large Animal Intensive Care Unit (ICU) or isolation facility with 24-hour supervision. Adult horses (>1 year age) were considered suitable for the clinical trial if there was owner consent and they met the following criteria: horses had to be severely ill with acute onset of severe diarrhea, proximal jejunitis/duodenitis, or strangulating obstruction of the intestine, and a high suspicion of gram-negative septicemia or endotoxemia. The diagnosis of proximal jejunitis/duodenitis was made either at exploratory laparotomy or based upon the clinical findings of abdominal pain, continuous gastric reflux for >24 hours, and results of rectal palpation. In addition, all horses had to display signs of acute toxemia or shock (altered mucous membrane color, increase in capillary refill time, tachycardia with weak pulse pressure, weakness, depression [i.e., lethargy, unresponsive to surroundings], cold extremities) to be included in the study. All nonsurviving horses had complete necropsies performed to confirm diagnoses. Each horse was administered either preimmune control plasma or hyperimmune plasma, intravenously, over 1–2 hours. Plasma was warmed to body temperature and administered through a blood filtration system.[6] Horses weighing <250 kg received 1 L, while horses over 250 kg received 2 L. All plasma was coded by a research assistant, and neither the investigators or the attending clinicians knew whether J5 hyperimmune plasma or preimmune control plasma had been given. All plasma was stored at −20°C in a freezer accessible to hospital clinicians. Clinicians were asked to select the coded plasma and record the code number on the patient's record. If additional plasma was needed to correct hypoproteinemia, normal plasma was administered from the hospital plasma bank. All horses received conventional therapy as dictated by attending clinicians. All horses received nonsteroidal antiinflammatory drugs, parenteral antimicrobials, and intravenous crystalloid supportive therapy. In addition, a reasonable commitment to support the patient following plasma administration was made. If euthanasia was performed, it was for humane reasons only (severe laminitis with uncontrollable pain or if death was imminent). If economic constraints were present, the horse was not included in the study.

Laboratory Data

Horses with acute toxic enteritis had at least three serial fecal cultures for *Salmonella* spp., *Campylobacter*, and *Clostridium perfringens* type A. Serology for *Ehrlichia risticii* was performed only on selected cases as the test was not available when the study was started. Laboratory data collected included complete blood count, platelet count, blood chemical analysis, blood gases, electrolytes, osmolality, and coagulation panel (PT, PTT, FDP). An attempt was made to collect and record these values daily for 3 days and at the time of recovery (discharge from the hospital) or death.

[6]Y-type surgical blood-solution administration set, Travenol Laboratories, Inc., Deerfield, IL.

garis, antimicrobial-resistant strains of *E. coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Salmonella* spp., and *Serratia marcescens* [13,15,21,29]. The mechanisms of protection provided by antibody to gram-negative antigens are likely multifactorial. Mechanisms include 1) targeting intact gram-negative bacteria or endotoxin for cellular effectors or opsonization and RE clearance [10,23] or 2) blocking of pharmacologically active lipid A epitopes [15]. Antibody to LPS has been shown to enhance bacterial clearance [22,30] and phagocytosis [22] and to decrease levels of detectable endotoxin [33]. Alteration in coagulation or reduction in the thrombogenic capacity of multiplying bacteria by antibody to core antigen has been suggested by experimental work in swine [23]. Protection against the hemodynamic sequelae following heterologous bacterial challenge was observed in dogs, suggesting an antitoxin effect of core antibody [35]. The immunoglobulin isotype (IgM or IgG) which provides protection is controversial [10,22,32,34–36]. The bulk of gram-negative core antibody in normal animals or patients recovering from gram-negative bacteremia is IgG [36,37]. Previous studies performed in our laboratory [23,39,40] have agreed with others [35] that IgG may be more strongly associated with protection than IgM. Until a broader understanding of the protective isotype and titer is known, specific recommendations for immunization schedules and dosages of plasma or serum cannot be made. From this study, it appears that 4.4 ml/kg of high-titer plasma (≥1:32,000 anti-J5 IgG) improves clinical appearance and survival in adult horses with clinical evidence of endotoxemia.

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REFERENCES

1. Burrows GE: Endotoxemia in the horse. Equine Vet J 13:89–94, 1981.
2. Morrison DC, Ulevitch RJ: The effects of bacterial endotoxins on host mediation systems: A review. Am J Pathol 93:527–617, 1978.
3. Moore JA, Garner HE, Shapland JE, Schaub RG: Equine endotoxemia: An insight into causes and treatment. J Am Vet Med Assoc 179:473–477, 1981.
4. Kreger BE, Craven DE, McCabe WR: Gram-negative bacteremia IV. Re-evaluation of clinical features and treatment in 612 patients. Am J Med 68:344–355, 1980.
5. McCabe WR: Gram-negative bacteremia. Adv Intern Med 19:135–158, 1974.
6. Smith BP: Understanding the role of endotoxins in gram-negative septicemia. Vet Med 12:1148–1160, 1986.
7. White NA, Moore JN, Trim CM: Mucosal alterations in experimental intestinal strangulation obstruction in ponies. Am J Vet Res 41:193–198, 1980.
8. King JN, Gerring EL: Detection of endotoxin in clinical cases of colic. In "Proc. Third Equine Colic Research Symposium." Athens: Univ. of Georgia, 1988, p 54 (Abst.).
9. Fessler JF, Bottoms GD: Plasma endotoxin concentrations in experimental and clinical equine and canine subjects. Vet Surg (Abst.) 17:32, 1988.
10. Ziegler EJ, McCutchan JA, Fierer J, Glauser MP, Sadoff JC, Douglas H, Braude AI: Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. N Engl J Med 307:1225–1230, 1982.

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Prognostic Value of Clinicopathologic Variables Obtained at Admission and Effect of Antiendotoxin Plasma on Survival in Septic and Critically Ill Foals

Simon F. Peek, Sue Semrad, Sheila M. McGuirk, Ase Riseberg, Jo Ann Slack, Fernando Marques, Dane Coombs, Laura Lien, Nicholas Keuler, and Benjamin J. Darien

This prospective study compared survival rates of critically ill and septic foals receiving 1 of 2 different types of commercial equine plasma and analyzed admission variables as possible predictors of survival. Standardized clinical, hematologic, biochemical, and hemostatic admission data were collected and foals received either conventional commercially available hyperimmune equine plasma or equine plasma specifically rich in antiendotoxin antibodies in a double-blinded, coded fashion. Sepsis was defined as true bacteremia or sepsis score >11. Overall survival rate to discharge was 72% (49/68). Foals that were nonbacteremic and demonstrated a sepsis score of ≤11 at admission had a 95% (18/19) survival rate. The survival rate to discharge for septic foals was 28/49 (57%), with truly bacteremic foals having a survival rate of 58% (14/24), whereas that for nonbacteremic, septic foals was 56% (14/25). Sensitivity and specificity for sepsis score >11 as a predictor of bacteremia were 74 and 52%, respectively. For the entire study population, a higher survival rate to discharge was documented for those foals receiving hyperimmune plasma rich in antiendotoxin antibodies ($P = .012$, odds ratio [OR] 6.763, 95% confidence interval [CI]: 1.311, 34.903). Administration of plasma rich in antiendotoxin antibodies also was associated with greater survival in septic foals ($P = .019$, OR 6.267, 95% CI: 1.186, 33.109). Statistical analyses demonstrated that, among 53 clinical and clinicopathologic admission variables, high sepsis score ($P < .001$), low measured IgG concentration ($P = .01$), high fibrinogen concentration ($P = .018$), low segmented neutrophil count ($P = .028$), and low total red blood cell numbers ($P = .048$) were the most significant predictors of overall mortality.

Key words: Plasma; Sepsis; Systemic inflammatory response syndrome.

Neonatal septicemia is the most common cause of death in foals during the 1st week of life.[1–5] A strict consensus definition for sepsis in horses has not been established, but the terms sepsis, septic, and septicemia often are used in the literature interchangeably. In human medicine, sepsis, severe sepsis, systemic inflammatory response syndrome (SIRS), multiple-organ dysfunction (MODS), and septic shock are terms that have been developed to describe the sequentially worsening systemic pathology and impaired organ homeostasis that occurs as a consequence of an infectious process that causes the release of inflammatory mediators into the systemic circulation.[6,7] Similar criteria have been used to describe sepsis in adult horses and foals.[8] In the majority of cases, the inciting cause is a bacterial infection, but it can occur due to viral, fungal, or parasitic infections.[7,8] In foals, bacterial sepsis, commonly associated with failure of passive transfer, and primary gastrointestinal and pneumonic conditions are the most likely causes of sepsis and septic shock.[4,8] Although much of the available literature focuses on the role of gram-negative bacterial infections and endotoxin in the pathogenesis of sepsis, clinical signs of disease and multiple-organ dysfunction are seen with both gram-positive and gram-negative infections.[2,3,4,8] Previous studies examining survival rates in critically ill foals admitted to referral hospitals in the United States have documented survival rates that vary widely.[1–5] These studies have been predominantly retrospective in design and, in those instances in which prospective information was collected,[2,3] it was done to examine the prognostic value of specific clinicopathologic variables rather than approaches to treatment. Several referral practices and university hospitals throughout the world devote considerable resources, time, and effort to the treatment of neonatal foals at considerable financial cost to owners, but there is very little data from controlled studies to validate or confirm many of the commonly used components of therapy for critically ill foals. Although the use of equine plasma of varying types in the treatment and prevention of septicemia in foals is commonplace, only 1 prospective study compared the efficacy of different plasma types in hospitalized foals.[9] Consequently, we designed a double-blinded, prospective clinical study to examine the effect of 2 different plasma types in the treatment of neonatal septicemia in foals. One of the plasma types was a commonly used, commercially available hyperimmune equine plasma product,[a] and the 2nd was a product obtained from mares that had been hyperimmunized with a core mutant gram-negative *Escherichia coli* isolate and which contained much higher concentrations of antilipopolysaccharide (anti-LPS) antibody compared with the

From the Departments of Medical Sciences, University of Wisconsin-Madison, School of Veterinary Medicine, 2015 Linden Drive, Madison, WI (Peek, Semrad, McGuirk, Riseberg, Slack, Marques, Coombs, Lien, Darien); and the Department of Computing and Biometry, University of Wisconsin-Madison, College of Agriculture and Life Sciences, 1210 West Dayton Street, Madison, WI (Keuler). *Results from this study were presented in part at the 22nd Annual Forum of The American College of Veterinary Internal Medicine, in Minneapolis, MN, June 2004, and the 50th Annual Convention of The American Association of Equine Practitioners in Denver, CO, December 2004.*

Reprint requests: Simon F. Peek, BVSc, MRCVS, Diplomate ACVIM (Internal Medicine), PhD, Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706; e-mail: peeks@svm.vetmed.wisc.edu.

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conventional product.[b] The hypothesis was that survival of critically ill and septic foals would be enhanced by the administration of plasma rich in anti-LPS antibodies. Furthermore, by obtaining a standardized clinicopathologic data set from each foal at admission, we were able to analyze 53 variables as possible predictors of survival for 96 hours after hospitalization as well as to discharge from the hospital.

Materials and Methods

Animals

Foals referred for treatment to the Veterinary Medical Teaching Hospital (VMTH) at the University of Wisconsin during the 2002, 2003, and 2004 foaling seasons provided the case material for this study. All foals <7 days of age admitted to the neonatal intensive care unit (ICU) were eligible for the study.

Sample Collection

Each foal underwent a standardized series of clinicopathologic tests at admission, including CBC, serum sodium, potassium, chloride, calcium, total protein, phosphorus, magnesium, aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (AP), glucose, total CO_2, anion gap, creatine kinase (CK), creatinine, blood urea nitrogen (BUN), antithrombin III (ATIII), fibrinogen degradation products (FDP), 1 stage prothrombin time (OSPT), activated prothrombin time (APTT), endotoxin concentration assay,[c] and selenium concentration. An individual sepsis score was calculated for each individual according to the modified system of Brewer and Koterba.[1] Passive transfer of humoral immunity was assessed at the time of admission by a foal-side semiquantitative assay[d] and specifically quantitated by radioimmunodiffusion of IgG using a simultaneously acquired serum sample. Both aerobic and anaerobic blood cultures[e] were obtained at admission. In cases in which antimicrobials had been administered before referral, antibiotic filtration resins[f] were used to maximize chances for a positive culture.

Each foal received 1 of 2 commercial equine hyperimmune plasma products[a,b] immediately after initial admission tests were performed regardless of passive immunity status. Foals >25 kg in body weight received 2 L administered consecutively over the 1st 6–24 hours of therapy, whereas foals <25 kg received 1 L. Plasma bags were heparinized with 2,000 IU/L of heparin[g] before administration. Plasma was stored frozen at 0°C and thawed in warm water (approximately 25°C) immediately before use. Plasma administration was performed in a double-blinded fashion under code, with the coding system preserved for the entire 3 years of the study.

Initial antimicrobial treatment was standardized as follows:

(a) If serum creatinine concentration was <5 mg/dL at admission: potassium penicillin at 22,000 IU/kg IV q6h, and amikacin at 20 mg/kg IV q24h.
(b) If serum creatinine concentration was >5 mg/dL or the foal was anuric at admission: potassium penicillin at 22,000 IU/kg IV q6h and cefazolin at 20 mg/kg IV q8h.

Flunixin meglumine also was administered at 0.25 mg/kg IV q8h for the 1st 24 hours of hospitalization. All other treatments (eg, fluids, pressor agents, anticonvulsants) varied during hospitalization on a case-by-case basis.

Survival was described in 2 ways: 1st as survival to 96 hours from hospitalization and 2nd to discharge from the hospital.

Statistical Analyses

Analysis of Sepsis Score. For each foal in the study, the sepsis score on admission was determined as previously described.[1] The sensitivity and specificity of a sepsis score >11 compared with a gold standard of positive blood culture was calculated.

Survival Data and Comparisons between Plasma Types. A total of 53 variables were examined for prognostic value between surviving and nonsurviving foals. The following clinical and clinicopathologic variables were examined using admission data from each foal: pulse rate, rectal temperature, respiratory rate, total white blood cell count, segmented neutrophil count, band neutrophil count, presence or absence of metamyelocytes, lymphocyte count, monocyte count, eosinophil count, basophil count, presence or absence of toxic changes in neutrophils, red blood cell count, hemoglobin concentration, hematocrit, platelet count, packed cell volume, presence or absence of abnormal erythrocyte morphology, anion gap, sodium, potassium, chloride, calcium, phosphorus, magnesium, carbon dioxide, glucose, BUN, creatinine, albumin, globulin, total bilirubin, total protein, fibrinogen, as well as serum enzyme activities for AP, CK, AST, and GGT. In addition, AT III, OSPT, APTT, FDP concentration, whole-blood selenium concentration, IgG quantitation (by radial immunodiffusion [RID]), and endotoxin concentration also were examined for prognostic value using admission samples. Additional variables included from admission data were volume of plasma administered, body weight, sepsis score, presence or absence of a positive blood culture, gram-positive or gram-negative organism, year of the study, and plasma type administered.

For each of the variables measured, the 68 study foals initially were tested to determine whether recipients of the 2 different plasma types were approximately identical in all measurable respects. Continuous and categorical variables were screened using *t*-tests and chi-squared tests, respectively. The treatment groups were found to be significantly different for 3 of the 53 variables screened (sodium higher by 3.33 mEq/L in the group receiving plasma rich in anti-LPS antibody, $P = .049$; temperature higher by 1.09°F in the group receiving conventional hyperimmune plasma, $P = .053$; and anion gap, higher by 2.98 mEq/L in the group receiving plasma rich in anti-LPS antibody, $P = .083$). Because of the existence of these possible confounding variables, the evaluation of differences in the 2 plasma treatment groups was corrected by fitting a logistic regression model on survival that included all 3 confounders in addition to the plasma effect. For comparison purposes, the uncorrected analyses were performed as well using Fisher's test. Survival rates to 96 hours of hospitalization and discharge from the hospital for foals receiving the 2 different plasma types were compared for the following groups:

(a) The entire study population (n = 68).
(b) Foals that were bacteremic at admission (n = 24).
(c) Foals that either were bacteremic or had sepsis scores >11 at admission (septic foals; n = 46).
(d) Foals that were not bacteremic and that had sepsis scores ≤11 at admission (n = 22).

Analysis of Admission Variables as Potential Predictors of Survival. This analysis was performed as a 2-step procedure:

(a) All 53 variables initially were screened by comparing the mean values for foals that survived versus foals that died. Each variable was examined independently using a *t*-test for continuous variables and a chi-squared test for categorical variables. The necessary *P*-value to progress to the next step was arbitrarily set at .4.
(b) The pool of variables with *P*-values <.4 from step (a) was analyzed by logistic regression in a forward-selection procedure. The 1st term added to the survival model was that which had the lowest associated *P*-value from step (a). The next term added was that which achieved the smallest *P*-value when added to the

Table 1. Survival data for foals receiving hyperimmune equine plasma rich in anti-lipopolysaccharide antibodies (plasma a) or conventional equine hyperimmune plasma (plasma b).

	Overall Survival						
	Lived			Died			
	Plasma a	Plasma b	Total Lived (%)	Plasma a	Plasma b	Total Died	Grand Total
Septic foals	17	11	28 (61%)	8	10	18	46
Not septic foals	10	11	21 (95%)	1	0	1	22
All foals	27	22	49 (72%)	9	10	19	68
Bacteremic foals	8	6	14 (58%)	5	5	10	24
Gram-negative foals	4	4	8 (57%)	2	4	6	14
	Survival to 96 hours						
	Lived			Died			
	Plasma a	Plasma b	Total Lived (%)	Plasma a	Plasma b	Total Died	Grand Total
Septic foals	18	12	30 (65%)	7	9	16	46
Not septic foals	11	11	22 (100%)	0	0	0	22
All foals	29	23	52 (76%)	7	9	16	68
Bacteremic foals	8	6	14 (58%)	5	5	10	24
Gram-negative foals	4	4	8 (57%)	2	4	6	14

model already containing the previously added term. This process was continued until no addition resulted in a *P*-value <.05. *P*-values for addition to the survival model were calculated using the likelihood ratio test.

Results

Sixty-eight foals were enrolled in the study. Forty-six foals were defined as septicemic on the basis of positive blood culture or sepsis score >11 using clinicopathologic data and samples obtained at admission. There were 24 blood culture-positive foals, 14 of which had single gram-negative infections. There were 39 foals with sepsis score >11, 17 of which also had positive blood cultures. Seven foals with sepsis score of ≤11 had positive blood cultures. Sensitivity and specificity for sepsis score (>11) as a predictor of true bacteremia were 74 and 52%, respectively.

Data describing survival to 96 hours of hospitalization as well as to discharge from the hospital are summarized in Table 1. Of the 19 foals that did not survive to discharge, 8 died, 7 were euthanized primarily for financial concerns, and 4 were euthanized primarily based on a hopeless prognosis. Only 3 foals were alive at 96 hours of hospitalization that did not survive to discharge, all of which were euthanized due to financial concerns. Survival rates to 96 hours of hospitalization and discharge from the hospital did not differ significantly for each category of foal analyzed.

Statistical analyses demonstrated significant differences in survival for foals that received plasma that was rich in anti-LPS antibodies (Table 2). Twenty-seven of the 36 foals that received plasma that was rich in antiendotoxin antibodies survived (75% survival) to discharge, compared with 22/32 (68% survival) of the foals that received the other plasma type. For the entire study population, a significant

Table 2. Determination of differences in survival due to plasma type.

	Overall Survival						
	n	Deviance	df	OR a/b[a]	95% Lower	95% Upper	LR *P*-Value[b]	Fisher's *P*-Value
All foals	68	6.305	1	6.763	1.311	34.903	.012	.599
Septic foals	46	5.535	1	6.267	1.186	33.109	.019	.367
Bacteremic foals	24	0.990	1	3.382	0.279	40.972	.320	1.000
Gram-negative foals	14	3.693	1	59.597	0.137	25,867.9	.055	.627
	Survival to 96 hours						
	n	Deviance	df	OR a/b	95% Lower	95% Upper	LR *P*-Value	Fisher's *P*-Value
All foals	68	4.119	1	4.119	0.963	20.806	.042	.568
Septic foals	46	3.559	1	4.137	0.875	19.566	.059	.360
Bacteremic foals	24	0.990	1	3.382	0.279	40.972	.320	1.000
Gram-negative foals	14	3.693	1	59.597	0.137	25,867.9	.055	.627

[a] Odds ratio for probability of survival for foals receiving hyperimmune equine plasma rich in antilipopolysaccharide antibodies (plasma a) compared with foals receiving conventional equine hyperimmune plasma (plasma b).

[b] Likelihood ratio test *P*-value.

difference was seen in the survival to 96 hours ($P = .042$) as well as to discharge from the hospital ($P = .012$) for foals that received hyperimmune plasma that was rich in antiendotoxin antibodies compared with those that received the other plasma type. Statistical analyses demonstrated that these differences were independent of foal body weight and the volume of plasma administered. Administration of plasma rich in antiendotoxin antibodies also was associated with greater survival to discharge in the 46 septic foals ($P = .019$). No difference was seen in overall survival rate for those foals that were bacteremic at admission according to plasma type administered ($P = .320$). When considering only foals with gram-negative infection (n = 14), the survival rates were not statistically significantly different for the 2 different plasma types ($P = .055$).

For survival to discharge, statistical analyses demonstrated that of the 53 clinicopathologic admission variables examined, only 5 were retained in the final survival model. Specifically, sepsis score ($P < .001$) and fibrinogen concentration ($P = .018$) were negatively related to survival, whereas measured IgG concentration ($P = .01$), segmented neutrophil count ($P = .028$), and total red blood cell numbers ($P = .048$) were positively related to survival. When considering survival to 96 hours, the retained variables were sepsis score ($P < .001$) and fibrinogen concentration ($P = .007$), which again were negatively associated with survival, and segmented neutrophil count ($P = .055$) and blood glucose concentration ($P = .024$), which were positively associated with survival. The details of the model's fit are presented in Table 3. The logistic regression equations for survival to discharge and survival to 96 hours of hospitalization are given below:

logit (survival to discharge) = 0.019 + (−0.456)sepsis score + (0.519)segmented neutrophil count + (0.003)IgG + (−0.004)fibrinogen + 0.563(RBC).

Table 3. Details of logistic survival model.

Overall Survival			
Variable	Coefficient	SE	LR *P*-Value
Intercept	0.019	3.543	NA
Sepsis score	−0.456	0.185	<.001
SEGS	0.519	0.208	.028
RID	0.003	0.001	.010
FIB	−0.004	0.002	.018
RBC	0.563	0.309	.048
Survival to 96 hours			
Variable	Coefficient	SE	LR *P*-Value
Intercept	5.852	2.544	NA
Sepsis score	−0.346	0.151	<.001
SEGS	0.270	0.130	.055
FIB	−0.006	0.002	.007
Glucose	0.017	0.008	.024

[a]LR, likelihood ratio test *P*-value; SEGS, segmented neutrophil count; RID, radial immunodiffusion; RBC, red blood cell; FIB, fibrinogen concentration.

logit (survival to 96 hours) = 5.582 + (−0.346)sepsis score + (0.270)segmented neutrophil count + (−0.006)fibrinogen + 0.017(glucose).

Discussion

Our finding that the administration of plasma harvested from mares hyperimmunized with LPS was associated with greater survival in all foals admitted to our ICU as well as the septicemic foal subpopulation is a unique observation. It contrasts with findings in a prior study by Morris and Whitlock[9] that also prospectively looked at anti-LPS antibody-rich plasma in comparison with lower anti-LPS titer plasma. That study was a multicenter study that did not demonstrate any significant difference in survival for critically ill neonates receiving either plasma type. We were able to demonstrate enhanced survival for the entire study population as well as for foals that we defined as septic by virtue of positive blood culture or sepsis score >11 at admission. Although statistical significance was identified both for overall short-term survival (96 hours of hospitalization, $P = .042$) and survival to discharge ($P = .012$) for those foals receiving hyperimmune plasma that was rich in anti-LPS antibody, the clinical relevance was less compelling, with 75% survival to discharge versus 68% for the 2 different plasma types. Statistical significance does not necessarily imply clinical relevance. From a statistical standpoint, it can be seen from the higher values for Fisher's *P*-values in Table 2 that, if corrections had not been made in our data analysis for those factors that were not randomly distributed between foals receiving the 2 plasma types (sodium, temperature, and anion gap), we would not have identified a significant difference at all. There were no significant differences noted in survival between foals that were bacteremic and those that were not nor between those that had a gram-negative isolate obtained compared with a gram-positive isolate from samples obtained at admission. The overall difference in survival rate suggests that administration of plasma rich in anti-LPS conferred the greatest benefit on nonbacteremic foals. It is not clear from this study whether anti-LPS antibody-rich plasma may confer a therapeutic or survival benefit on foals with gram-negative sepsis.

Another important fact to consider is that, although 1 of our plasma types was obtained from donors that were hyperimmunized with the J5 vaccine[h] product, it would be incorrect to assume that the other plasma type used in our study was devoid of anti-LPS antibody. Precise anti-LPS antibody titrations were not performed on every bag or batch of plasma used in this study. All of the donors for the anti-LPS antibody-rich plasma had titers of >1 : 12,000, but approximately 10% of the donors for the other plasma type also had titers this high.[i] However, 50% of donors of the other plasma type had negative anti-LPS antibody titers at a dilution of 1 : 1,600. Therefore, our data actually may underestimate the potential beneficial effect of anti-LPS antibody in treating sick foals.

Another relevant difference between our study and that of Morris and Whitlock[9] was the fact that all plasma was heparinized before administration to the

foals in our study. Some hemostatic variables differ between newborn and 1-month-old foals[10] as well as between newborn foals and adults,[11,12] but clinically relevant disorders of hemostasis only become apparent during acquired conditions, such as septicemia.[13,14] Hemostatic abnormalities in association with neonatal septicemia are well established in foals,[5,15] but our findings are in accordance with those of Barton et al,[5] who observed that survival did not significantly correlate with coagulopathy. The heparin used in our study was of porcine origin[g] and of mixed molecular weight and may have conferred a beneficial effect on the treatment of sepsis when combined with plasma by virtue of both its anticoagulant and antithrombotic properties.[13] Heparinization of plasma alone, however, could not have been the only factor involved in enhancing survival rates compared with previous studies because both plasma types were treated with 2,000 IU/L of sodium heparin, and statistically significant differences were seen between the 2 plasma types administered. All foals in this study received flunixin meglumine in addition to heparinized plasma and antibiotics on the 1st day of hospitalization, even those for which the sepsis score was ≤11 on admission. Arguably, the use of this drug was not justifiable in all study foals, but in designing a prospective study, we sought to provide a standardized initial therapeutic regimen for comparative reasons and to ensure that the quality of care for the sickest client-owned foals was not compromised. Because of concerns regarding ulcerogenesis and renal toxicity associated with the use of nonsteroidal anti-inflammatory medications, especially in compromised neonates, other clinicians may reasonably elect not to use flunixin meglumine in all cases.

Of the 53 admission variables measured on every foal in this study, 4 were significantly associated with survival to discharge from the hospital. In terms of *P*-value, sepsis score was the most significant ($P < .001$), with higher sepsis score foals having a poorer chance of survival. No metabolic variables were retained as being significantly associated with survival to discharge from the hospital. This finding is in contrast with previous studies that have demonstrated high anion gap and low venous oxygen tension,[2] or low glucose, low albumin, and low pH,[4] to be associated with poorer survival rates for foals admitted to ICUs at different institutions. Interestingly, low blood-glucose concentration at admission was associated with poorer survival to 96 hours of discharge in our study but was not retained in the model examining survival to discharge. Other admission variables that were significantly associated with survival to discharge were IgG concentration (as measured by RID), fibrinogen concentration, total neutrophil count, and total red blood cell (RBC) count. Low IgG concentration, as a consequence of failure of passive transfer, potentially compounded by antibody consumption in the early stages of infection, was not a surprising risk factor for failure to survive in critically ill neonates. Three studies comparable with ours examined factors potentially associated with survival in septicemic[4] foals or foals admitted to referral hospitals for intensive care,[2,3] and in 2 of those,[3,4] low neutrophil count was documented as a variable that was significantly different between survivors and nonsurvivors. Although low neutrophil count was significantly associated with failure to survive in our study ($P = .028$) it was not as statistically significant as sepsis score, IgG concentration, and fibrinogen concentration. The finding that a high fibrinogen concentration was significantly associated with failure to survive ($P = .018$) is a novel finding, and one that suggests that foals with evidence of acute-phase inflammation, in conjunction with failure of passive transfer and neutrophil sequestration, are the poorest survival candidates. The admission variable with significance ($P = .048$) as a predictor of survival that potentially is the hardest to explain is the total RBC count. Although it demonstrated the lowest *P*-value in the final set of retained variables, its persistence in our analyses indicates that low RBC numbers are somehow related to survival. Whether this observation reflects anemia, low-grade hemolysis, or both granulocytic and erythropoetic precursor suppression is an area worthy of further study.

Our final survival models included both sepsis score and several of its components (ie, segmented neutrophil count, fibrinogen, and IgG for overall survival and segmented neutrophil count, fibrinogen, and glucose for survival to 96 hours). This finding is evidence that a component measure may provide information on survival that is not captured by that component's contribution to the sepsis score. This result may occur because of the inherent loss of information that occurs when a continuous predictor is categorized, as is the case for most of the other components of the sepsis score. It therefore seems reasonable and desirable to allow some predictors to be included twice, once as a component of the sepsis score and once as a separate predictor. When performing multiple logistic regression, correlation between the potential predictor variables (also known as multicolinearity) is always a major concern. Multicolinearity can lead to misleading or inaccurate estimates, standard errors, or *P*-values. In most cases, highly correlated predictors will not appear together in a model formed through a forward-selection procedure such as the one used in this study. This is because correlated predictors explain roughly the same portion of the response and generally will not both show as significantly predictive when included together, even if they may be predictive when included alone. Because forward selection implicitly protects against multicolinearity, correlations among all 53 potential predictors were not calculated. However, because it was known that sepsis score is composed of several of the other measured variables, it seemed prudent to explicitly check for correlations between sepsis score and those variables. Correlations were quite small for all such variables and thus should not have caused problems in the model fitting.

Although the precision of sepsis scoring with respect to accurately identifying truly septic foals has been the subject of recent debate,[16] our data suggest sepsis scoring can be useful prognostically in a referral population. When defining the sensitivity and specificity of sepsis

score in our hospital, we used a gold standard of true bacteremia, which is more stringent and arguably too narrow by comparison with the definition originally used by Brewer and Koterba.[1] Consequently, our calculated values for sensitivity and specificity are low (74% and 52% compared with 93% and 86%, respectively). Undoubtedly, there are many non-bacteremic foals that could reasonably be described as septic and there is variation in acceptance of sepsis score among institutions and individual clinicians. However, our findings suggest that the calculation of sepsis score using the system and criteria described by Brewer and Koterba[1] gave the most prognostically useful index of survival for neonatal foals in our hospital.

The overall survival rate for foals admitted to our ICU was 72%, with the septicemic population and truly bacteremic foals having lower survival rates of 58 and 57%, respectively. The survival rate for all ICU admissions in our study was comparable with those documented by Hoffman et al[2] (66%) and Furr et al[3] (74%), but our survival rate for septicemic foals was higher than those previously published for comparably defined populations by Gayle et al[4] (45%) and Morris and Whitlock[9] (52.5%). Comparisons among referral hospitals are obviously of limited value when criteria for admission to neonatal intensive care units likely vary among institutions. In our hospital, not all foals <7 days of age are admitted to the ICU, several categories of foals (eg, angular limb deformities and other neonatal orthopedic problems in which foals can still nurse, mild enteric and respiratory disease cases) seen by the surgery and medicine services remain in the main hospital population. Although all foals that require IV fluid support and close monitoring are admitted to our ICU, the ultimate decision is clinician dependent and empirical, such that we may have different criteria compared with other studies and therefore a slightly different population base for comparative purposes. Our observation that approximately equal proportions of truly bacteremic and septicemic foals survived to discharge from the hospital was somewhat surprising; however, the fact that we performed blood cultures routinely, using antimicrobial retrieval systems for those foals that had been pretreated with antibiotics, may have increased the numbers of foals from which positive cultures were obtained. Survival rates for septicemic and bacteremic foals likely have increased over the last decade given improvements in critical care, therapeutics, and intensive nursing care available at university and private referral practices.

For ethical reasons, it is unlikely that a controlled, prospective study establishing whether plasma administration to critically ill foals confers a survival advantage over antibiotics and intensive supportive therapy alone will be conducted. However, we have been able to demonstrate that, within our referral population, the administration of anti-LPS-rich antibody plasma is associated with higher survival rates. Furthermore, the combination of failure of passive transfer, neutropenia, high fibrinogen concentration, and low RBC counts as measured at admission, warrants a graver prognosis.

Footnotes

[a] Polymune, Veterinary Dynamics, Templeton, CA
[b] Polymune Plus antiendotoxin Ab, Veterinary Dynamics, Templeton, CA
[c] Limulus Amebocyte Assay, BioWhittaker Co, Wlakersville, MD
[d] Snap IgG Test, Idexx Laboratories, Westbrook, ME
[e] BBL Septi-Chek Columbia Broth, Becton Dickinson and Company, Sparks, MD
[f] BBL Septi-Chek TSD With Resins, Becton Dickinson and Company, Sparks, MD
[g] Heparin sodium injection USP, Elkins Sinn Inc, Cherry Hill, NJ
[h] J5 Hygeica Biological Corporation, Woodland, CA
[i] Personal communication, Dennis Brook, Veterinary Dynamics, Templeton, CA

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References

1. Brewer BD, Koterba AM. Development of a scoring system for the early diagnosis of equine neonatal sepsis. Equine Vet J 1988;20(1):18–22.
2. Hoffman AM, Staempfli HR, Willan A. Prognostic variables for survival of neonatal foals under intensive care. J Vet Intern Med 1992;6:89–95.
3. Furr M, Tinker MK, Edens L. Prognosis for neonatal foals in an intensive care unit. J Vet Intern Med 1997;11:183–188.
4. Gayle JM, Cohen ND, Chaffin MK. Factors associated with survival in septicemic foals: 65 cases (1988–1995). J Vet Intern Med 1998;12:140–146.
5. Barton MH, Morris DD, Norton N, Prasse KW. Hemostatic and fibrinolytic indices in neonatal foals with presumed septicemia. J Vet Intern Med 1998;12:26–35.
6. Hotheckiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;348:138–150.
7. Bone RC. Sir Isaac Newton, sepsis, SIRS and CARS. Crit Care Med 1996;24:1125–1128.
8. Roy MF. Sepsis in adults and foals. Vet Clin North Am Equine Prac 2004;20:41–61.
9. Morris DD, Whitlock RH. Therapy of suspected septicemia in neonatal foals using plasma-containing antibodies to core lipopolysaccharide (LPS). J Vet Intern Med 1987;1:175–182.
10. Barton MH, Morris DD, Crowe N, et al. Hemostatic indices in healthy foals from birth to one month of age. J Vet Diag Invest 1995;7:380–385.
11. Darien BJ, Carleton C, Kurdowska A, et al. Haemostasis and antithrombin III in the full term newborn foal. Comp Haematol Inter 1991;1:161–165.
12. Clemmons RM, Dorsey Lee MR, Gorman NT, et al. Haemostatic mechanisms of the new born foal; reduced platelet responsiveness. Equine Vet J 1984;16:353–356.
13. Darien BJ. Heparin therapy: Rationale and clinical implications. Comp Cont Educ Pract Vet 1993;15:1273–1276.
14. Weiss DJ, Rashid J. The sepsis-coagulant axis: A review. J Vet Intern Med 1998;12:317–324.
15. Darien BJ, Williams MA. Possible hypercoagulation in 3 foals with septicemia. Equine Vet Educ 1993;5:19–22.
16. Corley KT, Furr MO. Evaluation of a score designed to predict sepsis in foals. J Vet Emerg Crit Care 2003;13:149–155.

Endotoxemia in horses: Protection provided by antiserum to core lipopolysaccharide

Debra Deem Morris, DVM; Robert H. Whitlock, DVM, PhD; Lynette B. Corbeil, DVM, PhD

SUMMARY

An equine antiserum to core lipopolysaccharide was produced by inoculation of 6 horses with a boiled cell bacterin made from the J-5 mutant of *Escherichia coli* O111:B4. The antiserum immunoglobulin G titer to J-5 mutant *E coli*, as determined by enzyme-linked immunosorbent assay, was 1:15,006. Pooled serum prepared before inoculation (preimmune serum) had a J-5 immunoglobulin G titer of 1:350.

The J-5 antiserum was tested for its protective efficacy in sublethal endotoxemia in 14 horses. Four horses served as nontreated controls and were given nothing before endotoxin challenge exposure (10 μg/kg of body weight, IV). Pooled preimmune serum (3 ml/kg, IV) was administered to 5 horses and J-5 antiserum (3 ml/kg, IV) was administered to 5 other horses 2 to 15 hours before endotoxin challenge exposure. During the 24 hours postendotoxin challenge exposure, endotoxemia was accompanied by significant ($P < 0.05$) time-related changes in temperature, heart rate, pulse character, respiratory rate and character, capillary refill time, mucous membrane color, fecal composition, attitude, PCV, total plasma protein, WBC count, platelet count, plasma fibrinogen, prothrombin time, activated partial thromboplastin time, fibrinolytic degradation products, plasma glucose, and plasma lactate in all horses. There were no apparent treatment vs time interactions ($P > 0.05$). Two horses (1 control and 1 given J-5 antiserum) died suddenly from unknown causes immediately after endotoxin challenge exposure. Seemingly, equine antiserum to core lipopolysaccharide did not provide protection from the adverse effects of experimental endotoxemia produced by bolus IV infusion of 10 μg of endotoxin/kg.

Endotoxin, a lipopolysaccharide (LPS) cell wall component of gram-negative bacteria, has been implicated as a primary pathologic factor in equine gastrointestinal disorders[1-7] and gram-negative bacterial infections.[8-12] Clinical and laboratory abnormalities that occur during intestinal strangulation obstruction, colitis X, acute salmonellosis, or neonatal septicemia strongly resemble the syndrome of shock and coagulopathy produced by endotoxin administration to horses.[13-19] The concentration of endotoxin within the equine large intestine increases rapidly when there are changes in gram-negative flora, such as occurs with an increase in dietary carbohydrate[20] or intestinal strangulation.[21] Luminal lactic acid accumulation, which also accompanies high carbohydrate intake,[20,22,23] intestinal ischemia,[3,6,24] and severe inflammation of the intestinal wall[25] are thought to cause loss of mucosal integrity, allowing transmural movement of endotoxin into the peritoneal cavity and systemic circulation. Endotoxin has been demonstrated in the peritoneal fluid and peripheral blood of ponies coincident with loss of villous epithelial cells after experimental small intestinal strangulation obstruction.[26,27] Endotoxin-like substances have been isolated from the blood of horses with spontaneous gastrointestinal disease, and this finding was associated with a high mortality.[7] Endotoxemia also accompanied gram-negative bacteremia.[10,12,15]

Most current research on equine endotoxemia is aimed at pharmacologic means to prevent endotoxin-induced cardiovascular derangements and formation of reactive metabolites.[17,28-32] The use of flunixin meglumine, an inhibitor of prostaglandin synthesis, before endotoxin administration reduces vasodilation and enhances perfusion to vital organs,[28,30] but does not prevent general tissue damage and coagulopathy during endotoxemia.[31] The pathophysiologic mechanisms of the numerous variable effects of endotoxin on thermoregulation, the cardiovascular system, coagulation mechanism, complement cascade, cellular constituents of the blood, and other body systems have not been elucidated completely, but probably involve more than one mechanism of action.[30,33] Therefore, direct destruction or inactivation of endotoxin seems most appropriate in treatment of endotoxin-mediated diseases.

There has been extensive research to develop an antiserum against endotoxin that could be used as an adjunct to antibiotics in the treatment of gram-negative bacteremia in human beings.[34] The 3 major structural components in endotoxin that are theoretic targets for protective antibody include: lipid A, the innermost region of the LPS molecule and the toxic moiety; core polysaccharide, composed of inner and outer parts linked to lipid A by 2-keto-deoxyoctonate; and the O polysaccharides, repeating short polysaccharide side chains located in the

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From the Department of Clinical Studies, Section of Medicine (Morris, Whitlock), University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, Kennett Square, PA 19348; and the Department of Veterinary Microbiology and Pathology (Corbeil), Washington State University, School of Veterinary Medicine, Pullman, WA 99164-7040.

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outermost region of LPS. Although the O antigens vary widely between species and serotypes of bacteria, the lipid A and core polysaccharide regions of endotoxin from most gram-negative organisms share a similar structure.[35] Antibodies to a common region could cross-react with heterogenous endotoxins and circumvent the problem of antigenic diversity.[36]

Antibodies to endotoxin are produced after natural infection and inoculation with gram-negative organisms, and these antibodies can be used to protect animals passively against morbidity and mortality induced by the homologous endotoxin.[34,37-42] Cross protection from heterologous gram-negative organisms was minimal, indicating that the protective antibodies were likely directed against the O side chains. To achieve cross protection, it seemed necessary to produce antibodies to the core region of LPS, which is similar in all gram-negative endotoxins. Attempts at antiserum production used rough gram-negative mutants that had lost the ability to produce the O polysaccharides or to attach them to the core. Antisera to rough mutants of *Salmonella typhimurium* protected mice against lethal challenge exposure with *Klebsiella pneumoniae*, whereas smooth *Salmonella* antisera were ineffective.[36]

Antiserum to the J-5 mutant of *Escherichia coli* O111:B4, produced in rabbits or people, protected animals against adverse effects of endotoxins from many unrelated gram-negative organisms[40,41,43] and improved survival from bacteremia caused by enteric gram-negative rods,[44] *Pseudomonas aeruginosa*,[45] and *Haemophilus influenzae* type B.[46] The J-5 mutant lacks the enzyme uridine 5'-diphosphate galactose 4-epimerase and this enzyme deficiency prevents the attachment of O side chains to core polysaccharides.[47] Antiserum against the parent strain of J-5, the core of which is obscured by O side chains, did not provide protection against heterologous organisms; this indicated that O antigens mask core determinants and impede development of cross-reactive core antibodies. Absorption of J-5 antiserum with J-5 LPS removed the beneficial effect, and antiserum prepared by inoculation with purified, protein-free lipid A-rich J-5 LPS was fully cross protective, indicating that antibodies to J-5 were directed against the endotoxin core.[46] When human J-5 antiserum or preimmune control serum was given to human patients with culturally proven gram-negative bacteremia and septic shock, overall mortality was significantly lower in patients given J-5 antiserum ($P < 0.05$).[48] The prophylactic use of J-5 antiserum reduced febrile morbidity in neutropenic human patients given cancer chemotherapy for hematologic malignancy.[49]

Horses have produced antiserum against rough gram-negative organisms, which has been used in horses, as well as other species.[36] An antiendotoxin hyperimmune serum produced in ponies was efficacious in treating horses with colitis and peritonitis, although specific information concerning its preparation was not provided.[50] The purpose of the present report was to test the protective effect of equine J-5 antiserum in horses with endotoxemia.

Materials and Methods

Antiserum production—Six healthy horses (5 mares and 1 gelding) between the ages of 7 and 14 years and weighing between 450 and 500 kg were used for antiserum production. The horses were fed a diet of timothy-alfalfa hay (0.45 to 0.90 kg/45 kg of body weight/day) and were given free access to grass pasture (5 horses/ha), water, and salt-mineral mix. Horses were dewormed, tested negative for equine infectious anemia antibodies, and inoculated with tetanus toxoid 1 month before the start of the experiment.

Samples of blood were collected from the jugular vein for determination of preinoculation serum titer against the J-5 mutant of *E coli* O111:B4. Jugular venous blood (6 L) was collected from each horse into sterile, evacuated, 0.5-L bottles for preparation of preimmune serum. The blood was allowed to clot for 2 hours in a 37-C waterbath, the bottles were centrifuged, and sera were harvested and pooled into a sterile 20-L plastic carboy. After samples were taken for bacterial culture, the pooled serum was partitioned into sterile, evacuated 1-L and 0.5-L bottles, frozen at −20 C, and stored until later use in the endotoxin trials.

The 6 horses were inoculated on postinoculation days (PID) 1, 3, 31, 59, and 87, with 2 ml of J-5 boiled cell bacterin. The bacterin was prepared from J-5 mutant *E coli* O111:B4 bacterial cells in a stationary growth phase of an 18-hour Trypticase soy-broth culture.[48] Bacterial cells were removed by centrifugation, washed 3 times in sterile 0.9% NaCl, and boiled for 2.5 hours. The concentration was adjusted spectrophotometrically, by the addition of 0.9% sterile saline solution, to approximately 5×10^9 bacteria/ml (22% light transmission at 510 nm), and phenol was added to a final concentration of 0.5%. The first dose of bacterin, mixed with an equal volume of Freund's incomplete adjuvant, was administered in 2 IM sites. Subsequent doses without adjuvant were administered subcutaneously in 2 different sites.

Because the serologic response to J-5 bacterin administration was not known, samples of blood were collected for the determination of titer to J-5 at PID 10, 17, 31, 38, 45, 59, 66, 73, 87, and 94. Jugular venous blood (6 L) was collected and handled as previously described, on PID 10, 38, 66, and 94, for preparation of postimmunization serum from these dates. It was planned

TABLE 1—Abnormality score grading scale for pulse character, respiratory character, fecal composition, and attitude of horses given endotoxin

Abnormality score	Pulse character	Respiratory character	Fecal composition	Attitude
0	Normal	Normal	Normal	Normal
1	Bounding	Expiratory abdominal lift	Soft balls	Mild colic
2	Weak	Flaring nostrils	Cow-like consistency	Mild depression
3	Imperceptible	Expiratory abdominal lift and flaring nostrils	Fluid with fiber	Marked depression
4	...	Dyspnea	Fluid without fiber	Ataxia
5	...	...	...	Recumbency

TABLE 2—Serum immunoglobulin G titers to J-5 mutant *E coli* in horses, inoculated with J-5 boiled cell bacterin

	PID				
Horse	0	10	38	66	94
D	350	52,521	4,287	4,287	4,287
K	350	15,006	15,006	4,287	15,006
S	350	1,225	15,006	15,006	15,006
C	350	4,287	15,006	15,006	15,006
T	100	1,225	1,225	1,225	1,225
C	350	15,006	4,287	4,287	4,287
Geometric mean	284	6,510	6,509	5,283	6,509

Horses were inoculated on PID 1, 3, 31, 59, and 87 with 10^{10} J-5 boiled cells. The 1st inoculation included Freund's incomplete adjuvant.
PID = postinoculation days.

TABLE 3—Mean values (± SD) for selected clinical data in horses after endotoxin administration

Values	PIH 0	0.25	0.5	0.75	1	1.5	2
Temperature (C)	37.8 ± 0.3[a]	37.8 ± 0.4[a]	37.9 ± 0.5[a]	37.8 ± 0.8[a]	38 ± 0.5[a]	38.2 ± 0.6[a,c]	38.3 ± 0.4[a]
Heart rate (beats/min)	41 ± 4[a]	51 ± 13[b]	50 ± 10[b]	57 ± 12[b,c]	57 ± 11[b,c]	60 ± 12[b,c]	62 ± 15[b,c]
Respiratory rate (breaths/min)	17 ± 9[a,c]	31 ± 12[b,d]	28 ± 10[a,b,c]	25 ± 10[c,b,c]	28 ± 12[a,b,c]	30 ± 14[a,b]	21 ± 8[a,c,d]
Capillary refill time (s)	2[a]	3 ± 0.9[a,b]	3 ± 0.8[a,b]	3 ± 1.6[b]	4 ± 1.1[b]	4 ± 1.6[b,e]	4 ± 1.7[b,e]
Fecal composition*	0.8 ± 0.6[a]	1.2 ± 0.6[a,b]	1.5 ± 0.5[a,b,c]	1.7 ± 0.5[b,c,e]	2.3 ± 1.0[c,e]	2.3 ± 2.6[c,d]	2.2 ± 0.7[c,d]
Attitude†	0[a]	2.5 ± 1.1[b]	3.3 ± 1.1[c]	3.8 ± 1.1[c,d,e]	4.4 ± 0.8[d]	4.7 ± 0.7[f]	4.7 ± 0.7[f]

* Fecal composition abnormality score: 0 = normal; 1 = soft balls; 2 = cow-like consistency; 3 = fluid with fiber; 4 = fluid without fiber. † Attitude abnormality score: 0 = normal, bright; 1 = mild colic; 2 = mild depression; 3 = marked depression; 4 = ataxia; 5 = recumbency.
There were no significant treatment (group) effects ($P > 0.05$). Values without common superscripts are significantly ($P < 0.05$) different.

to mix equal volumes of pooled PID 10 serum (probably high in J-5 specific immunoglobulin [Ig] M) and pooled serum from the subsequent day with the highest IgG titer to J-5 to produce the postimmune serum to be used in endotoxin trials.

Serum titers to the J-5 mutant *E coli* were quantitated by an enzyme-linked immunosorbent assay (ELISA), using an automatic analyzer.[a] The system originally designed for LPS was modified to use boiled cells as the heat-stable antigen in the detection system.[51] The J-5 bacterial cells were boiled 2.5 hours in 0.9% saline solution, washed, and diluted in phosphate-buffered saline solution to a suspension of 75% light transmission at 610 nm. The antigen suspension was incubated overnight in plastic cuvettes that were then washed and overlaid with 10% bovine fetal serum buffer to cover nonspecific binding sites. After incubation and washing, 3.5-fold dilutions of test serum in 10% bovine fetal serum buffer was added, and the serum was incubated for 2 hours at 37 C. The wells were washed and peroxidase-conjugated rabbit anti-equine IgG,[b] diluted in bovine serum albumin buffer, was added. After incubation, the wells were washed extensively (7 times) and the H_2O_2 substrate, containing *o*-phenylenediamine, was added. The optical density (OD) at 450 nm was recorded at 0 and 30 minutes. Each sample was run in 7 dilutions (3.5-fold each) with a positive-control serum, a negative-control serum, and a blank (no serum) in wells containing the antigen suspension and concomitantly in wells without antigen (no antigen wells). Equine IgG titers to J-5 mutant *E coli* were calculated as follows: (i) the OD at 0 minutes was subtracted from the OD at 30 minutes for each sample dilution, negative and positive controls, and no serum well to generate the OD change; (ii) the 0- to 30-minute OD change in the no antigen well was subtracted from this change in the corresponding well, containing antigen, to produce a net OD change; and (iii) the titer was considered to be the reciprocal of the last serum dilution that produced a net OD change > 3 times the mean net OD change of 10 no serum wells.

Endotoxemia in horses—Fourteen healthy horses (6 mares and 8 geldings) between 3 and 19 years of age and weighing between 412 and 555 kg were used. Horses were conditioned as described previously. Horses were randomly assigned to 1 of 3 groups. Group I (controls)—1 mare and 3 geldings given nothing before endotoxin administration; group II—2 mares and 3 geldings given 1.5 L of preimmune serum IV 2 to 15 hours before endotoxin administration; and group III—3 mares and 2 geldings given 1.5 L of postimmune serum IV 2 to 15 hours before endotoxin administration. The serum was negative for hemagglutinins and hemolysins to recipient RBC before administration. Only 1 horse was used at a time.

Horses were acclimated to a padded box stall for 12 to 15 hours before the start of the trial. An indwelling jugular venous catheter was placed for administration of drugs and obtaining blood samples for laboratory analyses. Between 2 and 15 hours after serum had been administered and immediately after base line (time 0), blood samples were obtained, *E coli* endotoxin[c] (serotype O111:B4, phenolic extraction), at a dosage rate of 10 μg/kg in 50 ml of 0.9% sterile saline solution, was administered IV over 2 minutes.

The horses were monitored from 2 hours before through postinoculation hours (PIH) 24. The following clinical values were recorded immediately before endotoxin administration and then every 15 minutes during the first 3 hours after endotoxin and at PIH 4, 6, 8, 10, 12, and 24: temperature, heart rate and pulse character, respiratory rate and character, mucous membrane color and capillary refill time, fecal composition, and attitude. Pulse character, respiratory character, fecal composition, and attitude were assigned an abnormality score, subjectively based on degree of deviance from normal (Table 1).

Venous blood was drawn for the following laboratory tests immediately before endotoxin administration (0 hour) and at PIH 0.5, 1, 1.5, 2, 3, 6, 12, and 24: PCV, total plasma protein (TPP), WBC count and differential, platelet count, plasma fibrinogen, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinolytic degradation products (FDP), plasma glucose, and plasma lactate. The PCV and TPP were measured by microhematocrit centrifugation and refractometry,[d] respectively. The WBC and platelet counts were determined on a hemacytometer, with commercial dilution reagents,[e] according to manufacturer's recommendations. Plasma fibrinogen was determined by the microhematocrit heat-precipitation method.[52] The PT and APTT were determined on a single-channel photooptical clot-timing device,[f] using commercial thromboplastins.[f] The FDP were determined by a latex agglutination reaction.[g] Plasma glucose was measured by the hexokinase method[h] and plasma lactate by an enzymatic pyruvate trapping reaction.[i] Blood was drawn for determination of IgG titers to J-5 mutant *E coli* by ELISA, as previously described: before serum administration (groups II and III) and immediately before endotoxin administration (0 hour) and at PIH 24.

Prausniz-Kustner (P-K) test—A P-K test to detect type I (mast cell-mediated) hypersensitivity was performed on 2 clinically normal horses as follows[53]: A serum sample from each of 2 horses inoculated 10 or 14 days previously with J-5 boiled cell bacterin was divided, and half was heated at 56 C for 120 minutes to inactivate IgE.[54] Aliquots (0.1 ml) of heated and unheated sera were injected intradermally in the cervical region of 2 healthy horses. Twenty-four hours later, 0.1 ml of endotoxin[c] (1 mg/ml) was injected intradermally at each site of serum injection. Presence and size of a wheal at each injection site were evaluated 30 minutes later. A positive reaction was characterized by wheal formation within 30 minutes. A positive reaction in the heat-treated serum would indicate the presence of cytophilic IgG to endotoxin, whereas positive in the unheated serum indicated the presence of IgE to endotoxin. Positive in both serum sites would indicate the presence of endotoxin-specific IgG and IgE

[a] Gilford Instrument Laboratories Inc, Burlington, Mass.
[b] Miles Laboratories, Elkhart, Ind.
[c] Difco Laboratories Inc, Detroit, Mich.
[d] American Optical Corp, Keen, NH.
[e] Unopipette, Becton, Dickinson & Co, Rutherford, NJ.
[f] General Diagnostics, Morris Plains, NJ.
[g] Thrombo-Wellcotest, Burroughs-Wellcome Co, Research Triangle Park, NC.
[h] Sigma Chemical Co, St Louis, Mo.
[i] Rapid Lactate Reagents, Calbiochem-Behring Corp, LaJolla, Calif.

TABLE 3—Continued

PIH							
2.5	3	4	6	8	10	12	24
38.3 ± 0.5[a]	38.3 ± 0.7[a]	38.4 ± 1.1[a]	39.9 ± 0.7[b,f]	39.6 ± 0.7[c]	38.9 ± 0.9[c]	38.7 ± 0.9[a,c]	37.7 ± 0.7[a]
55 ± 14[b,d]	56 ± 14[b]	56 ± 14[b]	67 ± 16[c,d]	67 ± 14[c,d]	64 ± 15[c,d]	68 ± 22[c]	63 ± 10[c]
18[f] ± 7[a,c,d]	15 ± 4[c]	15 ± 7[c]	22 ± 14[a,c,f]	38 ± 21[b]	27 ± 9[a,b,c]	30 ± 14[a,b,c]	14 ± 8[a,b,c]
4 ± 1.4[b,e]	4 ± 1.0[b,e]	4 ± 1.0[b,e]	6 ± 2.2[c,d]	6 ± 2.2[c]	4 ± 1.8[b]	6 ± 2.3[c,e]	4 ± 1.5[b]
2.3 ± 0.7[c,d]	2.5 ± 2.6[c,d]	2.6 ± 0.9[d,e]	2.4 ± 1.0[c,d]	2.3 ± 1.0[c,d]	1.9 ± 1.2[b,c,e]	1.5 ± 0.8[a,b,c]	2 ± 0.9[c,d]
4.4 ± 0.7[d]	4.3 ± 0.7[d]	4.3 ± 0.7[d]	3.6 ± 0.7[c,e]	3.3 ± 0.7[c]	3.2 ± 0.6[c]	3.2 ± 0.9[c,d]	3 ± 0.7[c,d]

in the serum, which had tagged tissue mast cells. Saline solution (0.9%) and histamine served as negative and positive controls, respectively. Endotoxin, injected intradermally in the croup region, was another negative control.

Effects of treatment and time on clinical and laboratory values in horses after endotoxin administration were evaluated, using 2-way analysis of variance for repeated measurements. The Student's-Newman-Keuls' test[55] was used to make comparison, where appropriate.

Results

Antiserum production—The serum J-5 IgG titer in the 6 horses used for antiserum production peaked at PID 10 to 38 (Table 2) and did not increase with subsequent doses of the bacterin. Four other horses inoculated with a larger antigenic mass (10^{11} J-5 organisms) in Freund's incomplete adjuvant did not achieve higher titers. Because the geometric mean titer on PID 38 and 94 were similar and greater than those on PID 66, sera collected on PID 38 were selected for combination with an equal volume of that collected on PID 10 for preparation of the pooled postimmune serum later used in the endotoxin trials. The serum IgG titer against J-5 mutant *E coli* was 350 to 1,225 in the pooled preimmune serum and was 15,006 to 52,521 in the pooled postimmune serum.

Endotoxemia in horses—There were no significant treatment effects on clinical values with the exception of fecal composition, which was increasingly abnormal in groups I, III, and II ($P < 0.05$; Table 3). Because only fecal composition was statistically different among the groups, data are presented as means for all horses. There was a significant effect of time on all clinical values after endotoxin administration, including temperature, heart rate, pulse character, respiratory rate and character, capillary refill time, mucous membrane color, fecal composition, and attitude (Table 3; $P < 0.05$). There were no apparent treatment group/time interactions ($P > 0.05$). The pulse was bounding immediately after endotoxin administration, then became weak and imperceptible, remaining abnormal through PIH 24. An abnormal respiratory pattern, characterized by marked abdominal effort, nostril flaring, or obvious dyspnea, developed within 15 minutes of endotoxin administration, and the respiratory character remained abnormal during 24 hours after endotoxin. All horses developed congested, hyperemic mucous membranes within 15 minutes after endotoxin administration. During the time when capillary refill time was most prolonged (PIH 6 to 12), some horses had cyanotic oral mucosae. Fecal composition after endotoxin administration ranged from soft fecal balls to fluid (without form) and feces remained softer than normal in most horses through PIH 24. Horses in all groups developed an abnormal attitude after endotoxin administration, characterized by mild colic, depression, and/or ataxia. Most horses became recumbent between PIH 1 and 4, but had again risen by PIH 6. Sweating, cool extremities, and salivation were shown by most horses during the first 3 hours after endotoxin. Five horses had an abnormal head motion, with the muzzle thrust forward and the poll directed laterally. Anal dilatation developed in 8 horses. Depression persisted through PIH 24.

One horse in group I was euthanatized at PIH 12 when showing signs of severe endotoxic shock. Other horses in the experiment were not so severely affected by endotoxemia. One horse each from groups I and III died unexpectedly within 5 minutes of endotoxin administration. Postmortem examination of these horses revealed splenic and/or hepatic congestion with edema and/or petechial hemorrhages of the respiratory and gastrointestinal tracts.

Similar to the clinical observations, there were no significant treatment effects on laboratory values ($P > 0.05$; Table 4). There was a significant effect of time on all laboratory values after endotoxin administration, including PCV, TPP, WBC, platelet count, plasma fibrinogen, PT, APTT, FDP, plasma glucose, and lactate (Table 3; $P < 0.05$). There was a significant linear increase in total number of band neutrophils after endotoxin ($P < 0.05$), which corresponded to the increase in total WBC count (data not shown). Although the TPP increased in each individual horse after endotoxin administration, the variability among horses resulted in a nonsignificant time effect on overall means. There were no interactions between treatment group and time ($P > 0.05$).

There was a significant interaction between treatment group and time on the mean titers ($P < 0.05$; Table 5). There were no group differences at any time and groups I and II had no difference over time ($P > 0.05$). There were significant time differences within group III: geometric mean titers were significantly greater after administration of postimmune serum and at PIH 24 than before administration of postimmune serum ($P < 0.05$).

Four horses inoculated with J-5 boiled cell bacterin by the protocol used to produce antiserum were given a sublethal dose of endotoxin (10 μg/kg) on PID 10 or 14. Two horses died of anaphylaxis within 5 minutes after endotoxin administration, and 2 others had endotoxemia with clinical signs and laboratory abnormalities indistinguishable from horses in groups I to III. Three horses had a titer of 15,006 and the other had a titer of 1,225 to J-5 at the time of endotoxin administration (geometric mean = 8,021). Prausniz-Kustner tests, using serum from the 2 inoculated horses that died suddenly after the administration of endotoxin, were negative.

TABLE 4—Mean (± SD) values for laboratory data in horses after endotoxin administration

Values	PIH 0	0.5	1	1.5	2	3	6	12	24
PCV (%)	34 ± 3[a]	44 ± 6[b]	49 ± 6[cd]	51 ± 6[cd]	52 ± 7[c]	51 ± 6[c]	48 ± 8[cd]	46 ± 8[b,d]	44 ± 7[a,b,c]
TPP (g/dl)	7.3 ± 0.5[a]	7.6 ± 0.5[a]	7.7 ± 0.6[a]	7.8 ± 0.7[a]	8.0 ± 0.8[b]	8.0 ± 0.8[b]	7.8 ± 0.8[b]	7.6 ± 0.6[a]	7.6 ± 0.6[a]
WBC (μl)	10,066 ± 2,199[a,b]	3,795[a] ± 1,632[a,b]	2,823 ± 1,121[a]	2,351 ± 978[a]	2,255 ± 919[b]	2,631 ± 1,303[b]	4,367 ± 5,373[b]	14,762 ± 6,847[c]	21,574 ± 7,100[d]
Platelets (× 10^3/μl)	212.4 ± 39.3[a]	127.7 ± 50.1[b]	140.5 ± 40.1[b]	130.9 ± 28.4[b]	130.7 ± 28.4[b]	135.8 ± 24.2[b]	135.8 ± 23.7[b]	118.5 ± 31.0[b]	109.8 ± 30.4[b]
Fibrinogen (mg/dl)	456 ± 88[a]	438 ± 128[a]	459 ± 102[a]	446 ± 110.5[a]	481 ± 114[a]	524 ± 133[a]	468 ± 116[a]	550 ± 120[a,b]	637 ± 152[b]
Prothrombin time (s)	12.8 ± 1.8[a]	13.7 ± 1.9[a,c]	14.2 ± 3.2[a,c,d]	14.4 ± 2.9[a,b,c]	15.3 ± 3.1[b,c]	15.6 ± 3.5[b,c]	16.3 ± 5.0[b,d]	16.6 ± 4.1[b]	15.8 ± 2.6[b,c]
Activated partial thromboplastin time (s)	56.8 ± 11.3[a]	62.7 ± 16.5[a]	59.5 ± 18.1[a]	62.8 ± 21[a]	64.0 ± 14.7[a]	60.9 ± 12.0[a]	66.2 ± 10.6[a,b]	74.3 ± 23.1[b]	79.6 ± 15.9[b]
FDP score*	1.0[a]	1.0[a]	1.0[a]	1.0[a]	1.0[a]	1.1 ± 0.3[a]	1.2 ± 0.6[a,b]	1.4 ± 0.7[b]	2.2 ± 0.4[c]
Glucose (mg/dl)	128 ± 40[a]	151 ± 51[a,b]	195 ± 56[b,d]	210 ± 73[c,d]	194 ± 87[b,c]	149 ± 87[a,b]	121 ± 87[a]	128 ± 43[a]	121 ± 43[a]
Lactate (mg/dl)	6.2 ± 1.7[a]	17.1 ± 5.1[b]	25.0 ± 8.7[c]	31.1 ± 9.3[d]	36.0 ± 9.3[d]	35.5 ± 12.1[d]	25.2 ± 12.9[c]	16.0 ± 10.5[b]	8.4 ± 3.1[a]

* Fibrinolytic degradation product score: 1 = < 10 μg/ml; 2 = 10 to 40 μg/ml; 3 = > 40 μg/ml.
There were no significant treatment (group) effects ($P > 0.05$). Means in a row without common superscripts are significantly ($P < 0.5$) different.
TPP = total plasma protein.

TABLE 5—Geometric mean serum IgG titers to J-5 mutant *E coli* in horses treated with preimmune or J-5 postimmune serum before endotoxin administration

Treatment (group)	n	Time in relationship to sera and/or endotoxin administration: Preserum	Postserum and/or preendotoxin (0 hr)	Postendotoxin (24 hr)
Nothing (I)	3	350 (2.54 ± 0.54)	350 (2.54 ± 0.54)	350 (2.54 ± 0.54)
Preimmune serum (II)	4*	479 (2.68 ± 0.27)	350 (2.54 ± 0.44)	350 (2.54 ± 0.44)
Postimmune serum (III)	4	137 (2.14 ± 0.27)	1225† (3.09)	655† (2.82 ± 0.31)

* One horse was excluded from calculations because of missing values. † Geometric means in group III, with superscripts, are significantly different from unmarked mean over time ($P < 0.05$).
Data in parentheses are the log mean ± SD.
n = No. of horses.

Discussion

Administration of equine antiserum to the rough mutant of *E coli* O111:B4 (J-5) was not protective against equine endotoxemia produced by smooth *E coli* O111:B4 endotoxin. Possible reasons for lack of protective efficacy of the equine J-5 antiserum include: failure of inoculated horses to produce sufficient cross-protective antibodies, ie, poor quality postimmune serum; insufficient dose of antiserum given to endotoxin challenge-exposed horses; failure of the J-5 boiled cell bacterin to elicit production of antibodies capable of protecting against heterologous endotoxemia.

The present inoculation protocol was similar to that described for production of protective J-5 antiserum in people,[48] and inoculation of horses with a larger antigenic mass (10^{11} organisms) did not result in higher titers. All inoculated horses had a 12 to 150 times increase in titer (1.2 to 1.8 log) at PID 10 to 38, which is greater than that in people.[48] In trials indicating efficacy of J-5 antiserum, antibodies within the antiserum have been measured by bacterial cell agglutination and/or by passive hemagglutination, using RBC sensitized with J-5 LPS.[44,48,56,57] Equine antibodies to J-5 were quantitated by an ELISA, designed to detect IgG. Rabbit titers of IgG for J-5 were similar in magnitude when determined by ELISA or hemagglutination,[51] even though the latter measures predominantly IgM. Boiled bacterial cells were used as the antigen because there was not a pure source of J-5 LPS and to circumvent problems associated with adherence of rough endotoxin to the solid phase.[51] Although work with laboratory animals indicates that titers to J-5 boiled cells correspond with those against J-5 LPS,[51] it is possible that equine antibodies against nonprotective determinants or the wrong Ig class were being measured. Horses did attain a significantly higher J-5 titer after administration of the pooled postimmune serum.

Horses were given 1.5 L of preimmune or postimmune serum that corresponded to a dose of 3 to 4 ml/kg, the dose of human J-5 antiserum that was effective in reducing mortality in human beings with gram-negative sepsis.[48] Rabbits have been protected from the adverse effects of endotoxin and gram-negative bacteremia by doses of J-5 antiserum ranging from 5 to 20 ml/kg.[40-44] Neither the minimum J-5 titer nor the Ig class necessary for protection from the toxic effects of endotoxin[40-43] or gram-negative sepsis[44] has been established in the host or pro-

tective sera. Mortality from bacteremia was significantly less in human patients treated with J-5 antiserum with hemagglutination titers ≥ 128.[48] The 7-S (IgG) and 19-S (IgM) fractions of lapine J-5 antiserum protected rabbits against bilateral renal cortical necrosis, induced by 2 doses of heterologous LPS (Shwartzman reaction)[40,41]; therefore, pooled postimmune serum was made from sera collected at PID 10 and 38, with the intent to ensure high titers of IgM and IgG against J-5. However, the IgG titer peaked in 5 of 6 inoculated horses on PID 10. This indicated that the horses were responding anamnestically to the organism and that the pooled postimmune serum may have been low in IgM. Natural antibody to core glycolipid has been found in serum from human beings,[58,59] rabbits,[58] and dogs,[58] and the predominant Ig class is IgG.[60] Inoculation generally results in a ≥ 4-fold increase in IgG titer,[60] as seen in horses inoculated with J-5 bacterin. Therefore, it seems that horses responded appropriately to inoculation and that the J-5 antiserum should have been qualitatively similar to that produced in rabbits and people.

One endotoxin challenge-exposed horse given preimmune serum had a base-line titer to J-5 of 1,225 before and after serum administration. Titers obtained in group III horses by administration of the postimmune serum were all 1,225. Because titers were determined retrospectively, this horse with a high base-line titer produced an unpredicted inequality among individuals in the groups and minimized differences between treatment groups. Nevertheless, mitigation of the deleterious effects of endotoxin was not observed in any of the horses, even those that were actively inoculated and had a titer of 15,006 when challenge exposed.

The cause of sudden death in 2 horses after being given endotoxin is perplexing. Results of the P-K tests, using serum from the inoculated horses that died, were negative. The last horse that died had not been given serum, had not been inoculated, and had a low titer to J-5 (100). Although this horse and others may have had unmeasured cytophilic IgE to LPS, it is equally likely that the endotoxin preparation contained an antigen to which some of the horses had been previously sensitized or that an unknown mechanism caused death. The administration of the same endotoxin solution to mice did not result in sudden death.

The protective antigen in J-5 has not been characterized, but there is evidence to indicate that it lies in the lipid A-2-keto-deoxyoctonate portion of the LPS molecule; protection to heterologous endotoxemia and gram-negative sepsis has been reported using antiserum to the Re mutant of *Salmonella minnesota*, which lacks core polysaccharides.[57,61-63] A common determinant group retained by the lipid-deficient (carbohydrate) and lipid-rich (toxic) fractions of the LPS molecule when they are chemically isolated may represent the protective moiety.[64,65]

Protective antibodies induced by J-5 mutant *E coli* may bind some portion of the LPS core and prevent access of lipid A to mediators of toxicity.[44,48,59] Most naturally occurring antibodies induced by gram-negative infection belong to the IgM class; however, these are directed against the O side chains, function as opsonins and enhance complement-mediated bacteriolysis,[66] and are not active against heterologous organisms. Most research has proven that O-specific antiserum affords better protection against bacterial infections than does antiserum to rough mutants, even though both antisera show a similar protective activity against lethal challenge exposure with LPS.[57,60] Although the J-5 antiserum has protected against the toxicity of purified LPS, the equine J-5 antiserum, prepared in the present manner, did not protect horses from endotoxemia.

References

1. Rooney JR, Bryans JT, Doll ER. Colitis "X" of horses. *J Am Vet Med Assoc* 1963;142:510–511.
2. Carroll EJ, Schalm OW, Wheat JD. Endotoxemia in a horse. *J Am Vet Med Assoc* 1965;146:1300–1303.
3. Nelson AW, Collier JR, Grimer LA. Acute surgical colonic infarction in the horse. *Am J Vet Res* 1968;29:315–327.
4. Vaughn JT. The acute colitis syndrome: colitis "X." *Vet Clin North Am* 1973;3:301–313.
5. McClure JJ. Endotoxic shock. *Vet Clin North Am* 1976;6:193–202.
6. White NA, Moore JN, Trim CM. Mucosal alterations in experimental intestinal strangulation obstruction in ponies. *Am J Vet Res* 1980;41:193–198.
7. Meyers K, Reed S, Keck M, et al. Circulating endotoxin-like substance(s) and altered hemostasis in horses with gastrointestinal disorders: an interim report. *Am J Vet Res* 1982;43:2233–2238.
8. Bryans JR, Fallon EH, Shephard BP. Equine salmonellosis. *Cornell Vet* 1961;51:467–477.
9. Wenkoff MS. *Salmonella typhimurium* septicemia in foals. *Can Vet J* 1973;14:284–287.
10. Smith BP, Reina-Guerra M, Hardy AJ, et al. Equine salmonellosis: experimental production of four syndromes. *Am J Vet Res* 1979;40:1072–1077.
11. Sembrat R, Distazio J, Maley W, et al. Oxygen consumption changes in the septic pony. *Am J Vet Res* 1981;42:1944–1948.
12. Martens RJ. Pediatrics. In: Mansmann RA, McAllister ES, eds. *Equine medicine and surgery*. 3rd ed. Santa Barbara, Calif: American Veterinary Publications Inc, 1982;301–355.
13. Burrows GE, Cannon J. Endotoxemia induced by rapid intravenous injection of *Escherichia coli* in anesthetized ponies. *Am J Vet Res* 1970;31:1967–1973.
14. Burrows GE. Hemodynamic alterations in the anesthetized pony produced by slow intravenous administration of *Escherichia coli* endotoxin. *Am J Vet Res* 1970;31:1975–1982.
15. Burrows GE. *Escherichia coli* endotoxemia in the conscious pony. *Am J Vet Res* 1971;32:243–248.
16. Burrows GE. Hematologic alterations associated with acute *E coli* endotoxemia. *Proceedings*, 1st Equine Hematol 1975;1:505–515.
17. Beadle RE, Huber TL. Blood chemistry changes associated with rapid intravenous administration of *Escherichia coli* endotoxin in anesthetized ponies. *J Equine Med Surg* 1977;1:371–375.
18. Burrows GE. Equine *Escherichia coli* endotoxemia: comparison of intraperitoneal endotoxin administration. *Am J Vet Res* 1979;40:991–998.
19. Moore JN, Garner HE, Shapland JE, et al. Lactic acidosis and arterial hypoxemia during sublethal endotoxemia in conscious ponies. *Am J Vet Res* 1980;41:1696–1698.
20. Moore JN, Garner HE, Berger JN, et al. Intracecal endotoxin and lactate during the onset of equine laminitis: a preliminary report. *Am J Vet Res* 1979;40:722–723.
21. Linerode PA, Goode RL. The effects of colic on the microbial activity of the equine large intestine. *Proc Annu Meet Am Assoc Equine Pract* 1970;16:321–341.
22. Garner HE, Hutcheson DP, Coffman JR, et al. Lactic acidosis. A factor associated with equine laminitis. *J Anim Sci* 1977;41:1037–1041.
23. Garner HE. Laminitis modeling. *Proc Annu Meet Am Assoc Equine Pract* 1982;1:116–119.
24. Moore JN, Garner HE, Shapland JE, et al. Equine endotoxemia. An insight into cause and treatment. *J Am Vet Med Assoc* 1981;179:473–477.
25. Smith BP. Salmonella infections in horses. *Compend Contin Educ Pract Vet* 1981;3:S4–S12.

26. Moore JN, White NA, Berg JN, et al. Endotoxemia following experimental intestinal strangulation obstruction in ponies. *Can J Comp Med* 1981;45:330–332.

27. McClure JJ, McClure JR, Johnson DW. Detection of endotoxin in the blood and peritoneal fluid of ponies with experimentally created strangulation obstruction of the small intestine. *Proc Annu Meet Am Assoc Equine Pract* 1982;1:102–108.

28. Moore JN, Garner HE, Shapland JE, et al. Prevention of endotoxin-induced arterial hypoxemia and lactic acidosis with flunixin meglumine in the conscious pony. *Equine Vet J* 1981;13:95–98.

29. Bottoms GD, Fessler JF, Roesel OF, et al. Endotoxin-induced hemodynamic changes in ponies: effects of flunixin meglumine. *Am J Vet Res* 1981;42:1514–1518.

30. Bottoms GD, Templeton GB, Fessler JF, et al. Thromboxane, prostaglandin I_2 (epoprostenol), and the hemodynamic changes in equine endotoxin shock. *Am J Vet Res* 1982;43:999–1002.

31. Fessler JF, Bottoms GD, Roesel OF, et al. Endotoxin-induced changes in hemograms, plasma enzymes, and blood chemical values in anesthetized ponies: effects of flunixin meglumine. *Am J Vet Res* 1982;43:140–144.

32. Moore AB, Roesel OF, Fessler JF, et al. Effects of naloxone on endotoxin-induced changes in ponies. *Am J Vet Res* 1983;44:103–109.

33. Culbertson R, Osburn BI. The biologic effects of bacterial endotoxin: a short review. *Vet Sci Commun* 1980;4:3–14.

34. Tate WJ, Douglas H, Braude AI, et al. Protection against lethality of *Escherichia coli* endotoxin with "O" antiserum. *Ann NY Acad Sci* 1966;133:744–792.

35. Luderitz O, Staub AM, Westphal O. Immunochemistry of O and R antigens of *Salmonella* and related Enterobacteriaceae. *Bacteriol Rev* 1966;30:192–255.

36. Chedid L, Parant M, Parant P, et al. A proposed mechanism for natural immunity of enterobacterial pathogens. *J Immunol* 1968;100:292–301.

37. Braude AI, Jones JL, Douglas H. The behavior of *Escherichia coli* endotoxin (somatic antigen) during infectious arthritis. *J Immunol* 1963;90:297–311.

38. Nowotny A, Radvany R, Neale NL. Neutralization of toxic bacterial O-antigens with O-antibodies while maintaining their stimulus on nonspecific resistance. *Life Sci* 1965;4:1107–1114.

39. Radvany R, Neale NL, Nowotny A. Relation of structure to function in bacterial O-antigens. VI. Neutralization of endotoxin O-antigens by homologous O-antibody. *Ann NY Acad Sci* 1966;133:763–786.

40. Braude AI, Douglas H. Passive immunization against the local Shwartzman reaction. *J Immunol* 1972;108:505–512.

41. Braude AI, Douglas H, Davis CE. Treatment and prevention of intravascular coagulation with antiserum to endotoxin. *J Infect Dis [Suppl]* 1973;128:157–164.

42. Ziegler EJ, Douglas H, Braude AI. Human antiserum for prevention of the local Shwartzman reaction and death from bacterial lipopolysaccharides. *J Clin Invest* 1973;52:3236–3238.

43. Davis CE, Ziegler EJ, Arnold K. Neutralization of meningococcal endotoxin by antibody to core glycolipid. *J Exp Med* 1978;147:1007–1017.

44. Ziegler EJ, Douglas H, Sherman JE, et al. Treatment of *E coli* and *Klebsiella* bacteremia in agranulocytic animals with antiserum to a UDP-GAL epimerase-deficient mutant. *J Immunol* 1973;111:433–438.

45. Ziegler EJ, McCutchan JA, Douglas H, et al. Prevention of lethal *Pseudomonas* bacteremia with epimerase-deficient *E coli* antiserum. *Trans Assoc Am Physicians* 1975;88:101–108.

46. Marks MI, Ziegler EJ, Douglas H, et al. Induction of immunity against *Haemophilus influenzae* type b infection by *Escherichia coli* core lipopolysaccharide. *J Clin Invest* 1982;69:742–749.

47. Elbein AD, Heath EC. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. I. The biochemical properties of uridine diphosphate galactose 4-epimeraseless mutant. *J Biol Chem* 1965;240:1919–1925.

48. Ziegler EJ, McCutchan JA, Fierer J, et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med* 1982;307:1225–1230.

49. Wolf JL, McCuthan JA, Ziegler EJ, et al. Prophylactic antibody to core lipopolysaccharide in neutropenia. In: Nelson JD, Grassi C, eds. *Current chemotherapy and infectious disease*. Washington, DC: American Society for Microbiology, 1980;1439–1441.

50. Gaffin SL, Baker B, DuPreez J, et al. Prophylaxis and therapy with anti-endotoxin hyperimmune serum against gastroenteritis and endotoxemia in horses. *Proc Annu Meet Am Assoc Equine Pract* 1982;28:335–340.

51. Ito JI, Wunderlich AC, Lyons J, et al. Role of magnesium in the enzyme-linked immunosorbent assay for lipopolysaccharide of rough *Escherichia coli* strain J-5 and *Neisseria gonorrhoeae*. *J Infect Dis* 1980;142:532–537.

52. Millar HR, Simpson JG, Stalker AL. An evaluation of the heat precipitation method for plasma fibrinogen estimation. *J Clin Pathol* 1971;24:827–830.

53. Mansmann RA. Diseases of the immune system. In: Mansmann RA, McAllister ES, eds. *Equine medicine and surgery*. Santa Barbara, Calif: American Veterinary Publications Inc, 1982;427–436.

54. Gershwin LJ. Heterologous passive cutaneous anaphylaxis with bovine immunoglobulin E. *Am J Vet Res* 1981;42:1184–1187.

55. Winer BJ. *Statistical principals in experimental design*. New York: McGraw-Hill Book Co, 1971.

56. Neter E, Westphal O, Luderitz O, et al. Studies of enterobacterial lipopolysaccharide: effects of heat and chemicals on erythrocyte-modifying, antigenic, toxic and pyrogenic properties. *J Immunol* 1956;76:377–385.

57. Johns M, Skehill A, McCabe WR. Immunization with rough mutants of *Salmonella minnesota*. IV. Protection by antisera to O and rough antigens against endotoxin. *J Infect Dis* 1983;147:57–67.

58. Young LS, Hoffman KR, Stevens P. "Core" glycolipid of Enterobacteriaceae: immunofluorescent detection of antigen and antibody. *Proc Soc Exp Biol Med* 1975;149:389–396.

59. Gaffin SL, Ginberg Z, Abraham C, et al. Protection against hemorrhagic shock in the cat by human plasma containing endotoxin-specific antibodies. *J Surg Res* 1981;31:18–21.

60. Young LS, Stevens P, Ingram J. Functional role of antibody against "core" glycolipid of Enterobacteriaceae. *J Clin Invest* 1975;56:850–861.

61. McCabe WR. Immunization with R mutants of *S minnesota*. I. Protection against challenge with heterologous gram-negative bacilli. *J Immunol* 1972;108:601–610.

62. Young LS, Stevens P. Cross-protective immunity to gram-negative bacilli: studies with core glycolipid of *Salmonella minnesota* and antigens of *Streptococcus pneumoniae*. *J Infect Dis* 1977;136:S174–S180.

63. Kaas EH, Wolff SM, eds. Bacterial lipopolysaccharides. *J Infect Dis [Suppl]* 1973;128:1–304.

64. Ng AK, Chen CLH, Chang CM, et al. Relationship of structure to function in bacterial endotoxins: serologically cross-reactive components and their effect on protection of mice against some gram-negative infections. *J Gen Microbiol* 1976;94:104–116.

65. Abdelnoor AM, Johnston AG, Anderson-Imbert, et al. Immunization against bacteria- and endotoxin-induced hypotension. *Infect Immunol* 1981;32:1093–1099.

66. Penhale WJ, Logan EF. Studies on the immunity of the calf to colibacillosis. II. Preparation of an IgM-rich fraction from bovine serum and its prophylactic use in experimental colisepticaemia. *Vet Rec* 1971;89:623–628.