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# Effects of intravenous administration of polymyxin B in neonatal foals with experimental endotoxemia

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## Abstract

**Objective**—To evaluate the effect of IV administration of polymyxin B on clinical and serum biochemical variables in foals with experimental endotoxemia.

**Design**—Prospective experimental study.

**Animals**—14 healthy neonatal foals.

**Procedures**—Foals were randomly assigned to a treatment or control group and were administered a single dose of lipopolysaccharide (0.5 µg/kg [0.23 µg/lb]) IV over 30 minutes. The treatment group received polymyxin B (6,000 U/kg [2,727 U/lb], IV) immediately after completion of lipopolysaccharide infusion; the control group was administered an equal volume of saline (0.9% NaCl) solution. Subsequent doses of polymyxin B or saline solution were administered IV at 8 and 16 hours. Blood was collected at various time points, and outcome variables, including heart rate, respiratory rate, rectal temperature, attitude score, WBC count, neutrophil count, lymphocyte count, monocyte count, platelet count, Hct, blood lactate concentration, blood glucose concentration, serum tumor necrosis factor-α concentration, and plasma thromboxane B<sub>2</sub> concentration, were measured. Urine was collected prior to and after experimentation to determine whether nephrotoxicosis was associated with treatment.

**Results**—The treatment group had significantly lower blood lactate concentration and serum tumor necrosis factor-α and plasma thromboxane B<sub>2</sub> concentrations and had higher blood glucose concentrations and better attitude scores, compared with the control group, at various time points during the study. No other significant differences and no evidence of overt nephrotoxicosis were detected.

**Conclusions and Clinical Relevance**—Administration of polymyxin B IV in healthy neonatal foals challenged with lipopolysaccharide attenuated some clinical and serum biochemical derangements associated with endotoxemia.

## Disciplines

Comparative and Laboratory Animal Medicine | Large or Food Animal and Equine Medicine | Veterinary Physiology | Veterinary Preventive Medicine, Epidemiology, and Public Health | Veterinary Toxicology and Pharmacology

## Comments

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**Conclusions and Clinical Relevance**—Administration of polymyxin B IV in healthy neonatal foals challenged with lipopolysaccharide attenuated some clinical and serum biochemical derangements associated with endotoxemia. (*J Am Vet Med Assoc* 2013;243:874–881)

Septicemia is a common clinical entity in neonatal foals, and this disease process and its associated complications (eg, septic arthritis) are one of the leading causes of morbidity and death in neonatal foals.<sup>1,2</sup> Although various bacterial organisms have been recognized in equine neonatal septicemia, gram-negative bacteria account for 70% to 95% of the microorganisms isolated from bacteriologic cultures of blood, with *Escherichia coli* being most commonly identified.<sup>2–4</sup> Incorporated in the outer membrane of gram-negative bacteria is LPS (endotoxin), which plays a pivotal role in host response to bacterial invasion by promoting inflammation via production of prostaglandins, serotonin, kinins, inflammatory interleukins, and other mediators.<sup>5,6</sup> Clinically, endotoxemia has been reported in 10% to 50% of neonatal foals ( $\leq 14$  days of age) evaluated at equine referral hospitals for treatment of presumed septicemia.<sup>7,8</sup> Possible sequelae of gram-negative septicemia and associated endotoxemia include exag-

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## ABBREVIATIONS

GGT	$\gamma$ -Glutamyltransferase
LPS	Lipopolysaccharide
TNF	Tumor necrosis factor
TXB <sub>2</sub>	Thromboxane B <sub>2</sub>

gerated activation of the inflammatory response, activation of the coagulation cascade, and derangements in multiple organ systems (eg, cardiovascular, respiratory, or hemostatic).<sup>7,9</sup> Subsequently, derangements in organ function and biochemical alterations may progress to shock, multiorgan failure, and death.<sup>7,9</sup>

Scientific investigation has elucidated the mechanisms of cellular initiation and activation of inflammation induced by LPS, which has provided targets for therapeutic intervention.<sup>10,11</sup> As such, drugs that interfere with the molecular sequence of events of cellular activation of inflammation may lessen the exaggerated activation of inflammation and improve clinical severity and outcome in patients with septicemia or endotoxemia.<sup>10–12</sup> Polymyxin B sulfate is a readily available and affordable cationic antimicrobial with a broad range of activity against gram-negative bacteria. In addition to having antimicrobial properties, polymyxin B functions as a chelating agent by binding the

lipid A portion of LPS in a 1:1 ratio, thus neutralizing LPS and negating the interaction of LPS with cellular receptors that results in inflammation.<sup>13-15</sup> Positive effects of IV administration of polymyxin B have been determined in experimental endotoxemia studies<sup>15-19</sup> in adult horses, with significant decreases in concentrations of inflammatory mediators (TNF- $\alpha$  and TXB<sub>2</sub>) as well as improved clinical attitude (eg, activity level, appetite, and mentation) scores in horses treated with polymyxin B, compared with control horses. Treatment with polymyxin B to attenuate clinical endotoxemia is frequently used in adult horses for conditions such as strangulating intestinal lesions, pleuropneumonia, and postpartum metritis, with purported beneficial effects.<sup>20</sup> In light of the high prevalence of gram-negative septicemia and endotoxemia in neonatal foals, a search for a safe, affordable, and effective treatment is warranted to improve the clinical course and outcome of equine neonatal septicemia. To our knowledge, no studies have been reported regarding the IV use of polymyxin B for equine neonatal septicemia or endotoxemia; therefore, the objective of the study reported here was to determine whether beneficial clinical and serum biochemical effects are observed in neonatal foals with experimental endotoxemia administered polymyxin B IV.

## Materials and Methods

**Animals**—Fourteen healthy neonatal foals (Thoroughbreds and Quarter Horses) with a mean age of 3.9 days (range, 3 to 5 days) and mean body weight of 60.5 kg (133.1 lb; range, 46 to 65 kg [101.2 to 143 lb]) were used for this study after approval by the Institutional Animal Care and Use Committee of Iowa State University. The 8 fillies and 6 colts were assigned to 1 of 2 groups via block randomization and drawing of a piece of paper identifying the foal as receiving either polymyxin B or saline (0.9% NaCl) solution (control). Experimentation was performed on each foal individually, with no overlap among foals; foals remained with their dams during the study period.

**Initial evaluation and instrumentation**—Twenty-four hours prior to administration of LPS, all foals were determined to be healthy on the basis of a complete physical examination, evaluation of adequate transfer of maternal antibodies (at  $\geq$  24 hours of age) with a commercial ELISA,<sup>a</sup> and CBC results. Subsequently, the foals were sedated with xylazine<sup>b</sup> (1 mg/kg [0.45 mg/lb], IV) and an 18-gauge, 3-inch IV catheter<sup>c</sup> was placed by means of an aseptic technique in each jugular vein (2 catheters/foal). In addition, urine was collected via urinary bladder catheterization with a sterile catheter.<sup>d</sup> Heparinized saline solution (3 mL) was flushed through each IV catheter every 6 hours to maintain patency.

**Administration of LPS and polymyxin B**—The following day, all foals were administered LPS (O55:B5 *E coli*)<sup>e</sup> diluted in 50 mL of saline solution (dose, 0.5  $\mu$ g/kg [0.23  $\mu$ g/lb]), via the IV route (left jugular catheter), as described,<sup>21-23</sup> over a 30-minute period with an electronic syringe pump (start of LPS infusion = time 0). Seven foals were randomly assigned to the treatment group and were administered 6,000 U of polymyxin B/

kg (2,727.3 U/lb) diluted in 12 mL of saline solution, IV, as a bolus immediately after the end of the LPS infusion (0.5 hours). Similarly, 7 foals were randomly assigned to the control group and were administered an equal volume of saline solution IV at 0.5 hours. Subsequent doses of polymyxin B (6,000 U/kg; treatment group) or saline solution (control group) were administered IV at 8 and 16 hours.

**Sample collection and clinical evaluation**—Twelve milliliters of blood was collected from the right jugular catheter at -24, -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 48 hours and placed in EDTA and serum clot tubes. Serum was harvested from samples not used for CBC, blood lactate analysis, or blood glucose analysis and stored at -80°C until samples from all foals had been collected. Physical examination variables including heart rate, respiratory rate, and rectal temperature were recorded at the same time as blood collection. Each time blood was obtained, a subjective attitude score was determined by an observer unaware of group assignments on the basis of the following criteria: 1 = bright and alert; 2 = signs of depression (head hanging and lethargic), but suckling; 3 = signs of depression and not suckling; and 4 = comatose (recumbent and unable to rise).<sup>21-23</sup> Urine was obtained at 24 hours either via free catch (9 foals) or urinary bladder catheterization (4 foals) by use of light sedation (xylazine [1 mg/kg, IV]).

**Assays**—A CBC including a WBC count, neutrophil count, lymphocyte count, monocyte count, platelet count, and Hct was performed immediately after blood collection with an automated hematology machine.<sup>g</sup> Blood lactate concentration<sup>h</sup> and blood glucose<sup>i</sup> concentration were also measured immediately after blood collection with point-of-care monitors validated for horses.<sup>24,25</sup> Urine creatinine concentration and GGT activity were measured (-24 and 24 hours) by use of an automated chemistry analyzer<sup>j</sup> with commercially available reagents and standards. Urine GGT (U/L)-to-creatinine (mmol/L) ratio was calculated.<sup>26</sup> After samples were collected from all 14 foals, serum was thawed and purified by removing interfering substances with C-18 solid-phase extraction columns.<sup>k</sup> Tumor necrosis factor- $\alpha$  and TXB<sub>2</sub> were measured via commercially available assays<sup>l,m</sup> following the manufacturer's instructions.

**Statistical analysis**—Data for response variables (heart rate, respiratory rate, rectal temperature, attitude score, WBC count, neutrophil count, lymphocyte count, monocyte count, platelet count, Hct, blood lactate concentration, blood glucose concentration, serum TNF- $\alpha$  concentration, and plasma TXB<sub>2</sub> concentration) from the treatment and control groups were reported as mean  $\pm$  SD values. Effects of treatment and time on response variables were analyzed via repeated-measures ANOVA. Tumor necrosis factor- $\alpha$  and TXB<sub>2</sub> concentration measurements were log transformed to facilitate analysis via ANOVA. Treatment, time, and their interaction were used as fixed effect, whereas the foal was the subject of repeated measures. Baseline outcome values were used as covariates in analyses. Significance was set at  $P \leq 0.05$ .

## Results

**Physical examination variables and attitude score**—All foals developed mild to moderate signs of colic as well as diarrhea within 1 hour after LPS administration. Mucous membranes became extremely congested, and scleral vasculature was prominent. Additionally, marked lethargy, lack of awareness of their environment, and lack of suckling between 1 and 2 hours (range, 0.5 to 5 hours) were observed in all foals. In the control group, a significant increase in heart rate (at 8 hours) and decrease in respiratory rate (at 2 hours) were observed, compared with time 0. In the polymyxin B group, a significant increase in rectal temperature was observed at 4, 5, and 6 hours, compared with time 0. No difference in rectal temperature, heart rate, or respiratory rate was observed at any time point when the control group was compared with the polymyxin B group (Table 1).

The attitude score was significantly increased in both groups at various time points, compared with time 0 (Table 1). A significantly lower (ie, improved) attitude score was observed in the polymyxin B group, compared with the control group, at 4, 8, and 12 hours; attitude score was not significantly ( $P = 0.06$ ) lower in the polymyxin B group at 1.5, 5, and 6 hours, compared with the control group.

**Blood glucose concentration**—A significant decrease in blood glucose concentration was detected in the control and polymyxin B groups at various time points, compared with time 0 (Figure 1). The mean blood glucose concentration reached a nadir between 3 and 4 hours after initiation of LPS infusion. Blood glucose concentrations

were significantly higher in the polymyxin B group at 1.5, 4, and 5 hours, compared with the control group. Five hundred milliliters of 5% dextrose in water was administered at 4 hours to 1 foal in the polymyxin B group and 5 foals in the control group because the blood glucose concentration decreased to  $< 35$  mg/dL; this treatment was instituted because the investigators were concerned that the blood glucose concentration would decrease to a life-threatening concentration without intervention. Of note, foals administered 5% dextrose were included in the statistical analysis, although administration of dextrose likely affected blood glucose concentrations at subsequent times ( $\geq 5$  hours).

**Blood lactate concentration**—A significant increase in blood lactate concentration was detected in

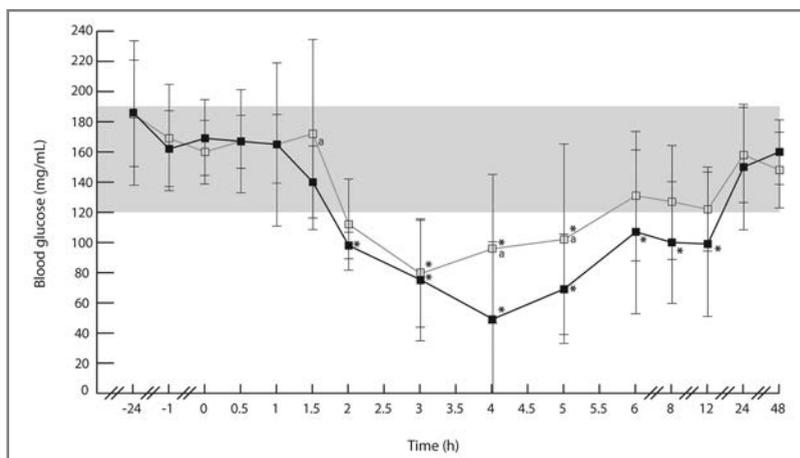


Figure 1—Mean  $\pm$  SD blood glucose concentrations of foals administered LPS (0.5  $\mu$ g/kg [0.23  $\mu$ g/lb], IV, over 30 minutes; start of infusion = time 0) and then given saline (0.9% NaCl) solution, IV, every 8 hours (control; black squares) or polymyxin B (6,000 U/kg [2,727.27 U/lb], IV, q 8 h; white squares) beginning 30 minutes after the start of LPS infusion. <sup>a</sup>Significant ( $P < 0.05$ ) difference between groups. \*Significant ( $P < 0.05$ ) difference from baseline (time 0) value within a group. Shaded area denotes reference interval in healthy foals.<sup>27</sup> At 4 hours, 1 foal in the polymyxin B group and 5 foals in the control group were administered 500 mL of 5% dextrose, IV, as a bolus to treat severe hypoglycemia.

Table 1—Mean  $\pm$  SD values for variables measured at various times in foals given LPS (0.5  $\mu$ g/kg [0.23  $\mu$ g/lb], IV) over 30 minutes (start of infusion = time 0) and then given saline (0.9% NaCl) solution, IV, every 8 hours (control), or polymyxin B (6,000 U/kg [2,727.27 U/lb], IV, q 8 h) beginning 30 minutes after the start of LPS infusion.

Variable	Time (h)														
	-24	-1	0	0.5	1	1.5	2	3	4	5	6	8	12	24	48
Temperature ( $^{\circ}$ C [reference range, 37.22–38.89 $^{\circ}$ C])															
Control	38.67 $\pm$ 0.7	38.61 $\pm$ 0.5	38.61 $\pm$ 0.9	38.667 $\pm$ 0.7	38.67 $\pm$ 1	38.61 $\pm$ 1	38.39 $\pm$ 2	39.00 $\pm$ 2.3	39.22 $\pm$ 1	39.22 $\pm$ 1	39.17 $\pm$ 1	38.5 $\pm$ 0.5	38.72 $\pm$ 0.9	38.39 $\pm$ 0.7	38.5 $\pm$ 0.9
Polymyxin B	38.5 $\pm$ 0.7	38.61 $\pm$ 0.9	38.67 $\pm$ 0.9	38.72 $\pm$ 1	38.89 $\pm$ 0.9	38.89 $\pm$ 2	38.72 $\pm$ 2	39.33 $\pm$ 1	39.56 $\pm$ 1	39.56 $\pm$ 1	39.39 $\pm$ 1	38.5 $\pm$ 0.9	38.5 $\pm$ 2	38.28 $\pm$ 2	38.61 $\pm$ 1
Heart rate (beats/min [reference range, 70–100 beats/min])															
Control	103 $\pm$ 9	107 $\pm$ 8	110 $\pm$ 10	84 $\pm$ 14	100 $\pm$ 21	121 $\pm$ 47	102 $\pm$ 25	99 $\pm$ 15	101 $\pm$ 17	125 $\pm$ 20	137 $\pm$ 28	150 $\pm$ 14*	134 $\pm$ 34	125 $\pm$ 20	111 $\pm$ 14
Polymyxin B	95 $\pm$ 10	103 $\pm$ 23	101 $\pm$ 13	85 $\pm$ 12	115 $\pm$ 14	126 $\pm$ 28	98 $\pm$ 9	93 $\pm$ 14	104 $\pm$ 22	115 $\pm$ 25	128 $\pm$ 9	131 $\pm$ 15	125 $\pm$ 13	103 $\pm$ 5	96 $\pm$ 12
Platelet count ( $\times 10^3$ platelets/ $\mu$ L [reference range, 105–353 $\times 10^3$ platelets/ $\mu$ L])															
Control	130 $\pm$ 42	103 $\pm$ 38	103 $\pm$ 40	84 $\pm$ 32	68 $\pm$ 28*	71 $\pm$ 32*	72 $\pm$ 27*	72 $\pm$ 28*	67 $\pm$ 23*	60 $\pm$ 20*	52 $\pm$ 21*	49 $\pm$ 19*	36 $\pm$ 15*	38 $\pm$ 17*	65 $\pm$ 15*
Polymyxin B	191 $\pm$ 37	152 $\pm$ 44	152 $\pm$ 39	120 $\pm$ 40*	114 $\pm$ 44*	119 $\pm$ 33*	98 $\pm$ 33*	102 $\pm$ 31*	100 $\pm$ 35*	93 $\pm$ 32*	90 $\pm$ 39*	86 $\pm$ 37*	75 $\pm$ 36*	62 $\pm$ 28*	91 $\pm$ 32*
Hct (% [reference range, 30%–44%])															
Control	33 $\pm$ 3	32 $\pm$ 4	32 $\pm$ 3	36 $\pm$ 4*	38 $\pm$ 4*	37 $\pm$ 3*	37 $\pm$ 4*	37 $\pm$ 5*	37 $\pm$ 4*	37 $\pm$ 5*	36 $\pm$ 4*	36 $\pm$ 4*	36 $\pm$ 4*	32 $\pm$ 4	30 $\pm$ 4
Polymyxin B	34 $\pm$ 5	34 $\pm$ 5	33 $\pm$ 5	38 $\pm$ 6*	39 $\pm$ 4*	39 $\pm$ 4*	37 $\pm$ 4*	38 $\pm$ 4*	37 $\pm$ 5*	37 $\pm$ 4*	36 $\pm$ 4*	36 $\pm$ 4*	37 $\pm$ 4*	32 $\pm$ 4	30 $\pm$ 4
Attitude score															
Control	1	1	1	3.1 $\pm$ 0.7*	3.4 $\pm$ 0.5*	3.9 $\pm$ 0.4*	3.3 $\pm$ 0.5*	2.9 $\pm$ 0.7*	3.3 $\pm$ 1*	2.4 $\pm$ 0.8*	2.1 $\pm$ 0.4*	2 $\pm$ 0.6*	1.9 $\pm$ 0.4	1.4 $\pm$ 0.5	1
Polymyxin B	1	1	1	2.9 $\pm$ 1.2*	3.4 $\pm$ 0.5*	3.3 $\pm$ 0.5*	3.1 $\pm$ 0.4*	2.6 $\pm$ 0.5*	2.3 $\pm$ 1.1†	1.9 $\pm$ 0.7	1.6 $\pm$ 0.5	1.1 $\pm$ 0.4†	1†	1	1

Reference ranges for healthy foals.<sup>27</sup>

\*Significantly ( $P < 0.05$ ) different from time 0 within a group. †Significantly ( $P < 0.05$ ) different between groups.

both groups at various time points, compared with time 0 (Figure 2). The blood lactate concentration was significantly lower at 4 and 5 hours in the polymyxin B group, compared with the control group (Table 1).

**Hematologic evaluation**—A significant change in the WBC and neutrophil counts was detected in both groups at various time points, compared with time 0 (Figures 3 and 4). The WBC and neutrophil counts were significantly higher in the polymyxin B group at 8 hours, compared with the control group. In comparison, the WBC (48 hours) and neutrophil (24 and 48 hours) counts were significantly lower in the polymyxin B group, compared with the control group. A significant decrease in platelet count and increase in Hct were observed in both groups at various time points (Table 1). No significant difference in platelet count or Hct was detected between groups. No significant differences in lymphocyte or monocyte counts were detected within or between groups.

**Serum TNF- $\alpha$  concentration**—A significant increase in serum TNF- $\alpha$  concentration was detected between baseline (time 0) and 0.5 to 24 hours after LPS infusion in the control group and between baseline (time 0) and 0.5 to 6 hours ( $P < 0.001$ ) in the polymyxin B group (Figure 5). Peak TNF- $\alpha$  concentration was detected at 1.5 hours in both groups. Foals in the polymyxin B group had significantly lower TNF- $\alpha$  concentrations at 8 and 24 hours, but not ( $P = 0.06$ ) at 12 hours, compared with the control group.

**Serum TXB<sub>2</sub> concentration**—A significant increase in plasma TXB<sub>2</sub> concentration was detected between baseline (time 0) and 0.5 to 8 hours in both groups (Figure 6). Peak TXB<sub>2</sub> concentration was observed at the completion of the LPS infusion (0.5 hours) in both groups. Foals in the polymyxin B group had significantly lower TXB<sub>2</sub> concentrations at 1, 2, and 3 hours, compared with the control group.

**Urine GGT-to-creatinine ratio**—Urine was not obtained from 1 foal in the treatment group because the foal returned to its normal housing environment prior to collection. The mean GGT-to-creatinine ratio before or after LPS administration for the polymyxin B group was  $0.015 \pm 0.018$  or  $0.007 \pm 0.003$ , respectively, whereas the mean GGT-to-creatinine ratio before or after LPS administration for

the control group was  $0.008 \pm 0.005$  or  $0.008 \pm 0.003$ , respectively. No significant difference was found in the GGT-to-creatinine ratio before or after LPS administration within or between groups.

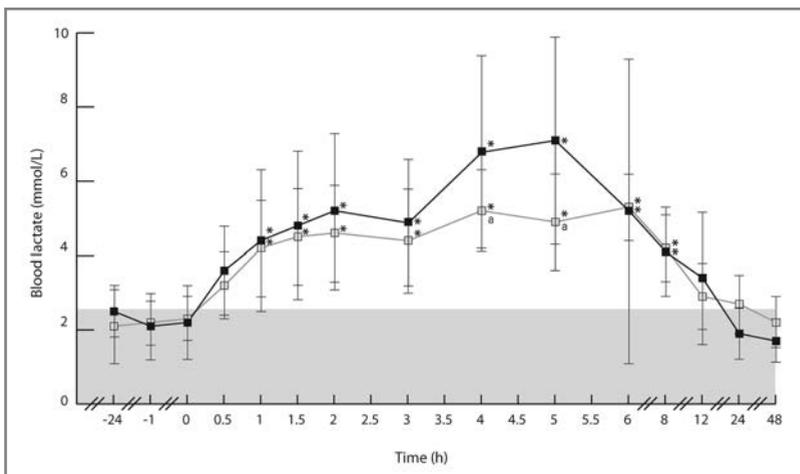


Figure 2—Mean  $\pm$  SD blood lactate concentrations of the same foals as in Figure 1. See Figure 1 for remainder of key.

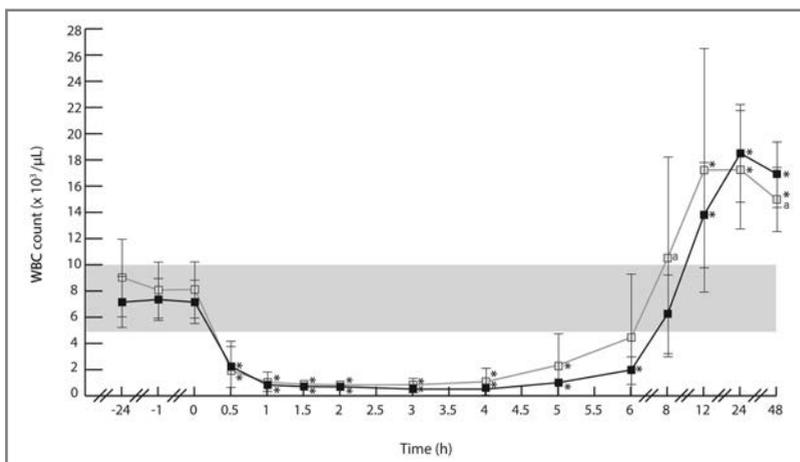


Figure 3—Mean  $\pm$  SD total WBC counts of the same foals as in Figure 1. See Figure 1 for remainder of key.

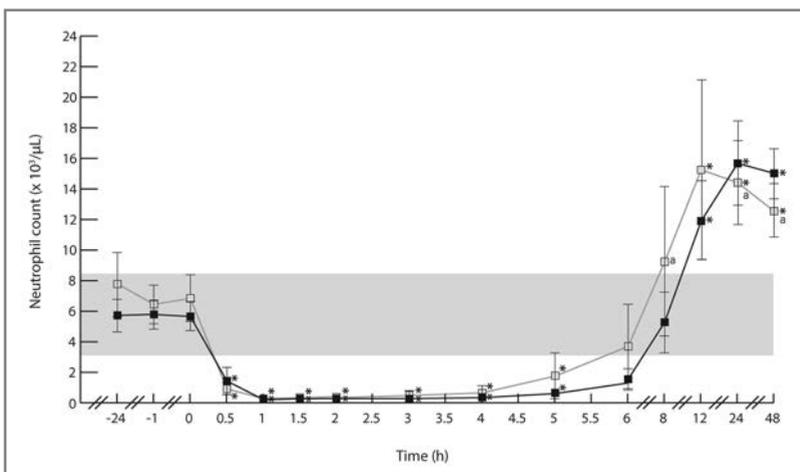


Figure 4—Mean  $\pm$  SD neutrophil counts of the same foals as in Figure 1. See Figure 1 for remainder of key.

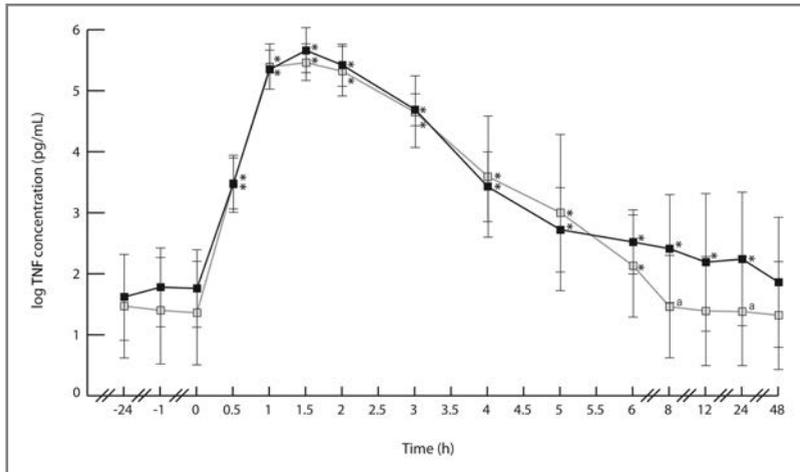


Figure 5—Mean  $\pm$  SD log of serum TNF- $\alpha$  concentrations in the same foals as in Figure 1. Serum TNF- $\alpha$  concentration was 0 pg/mL in 31 clinically normal foals.<sup>28</sup> See Figure 1 for remainder of key.

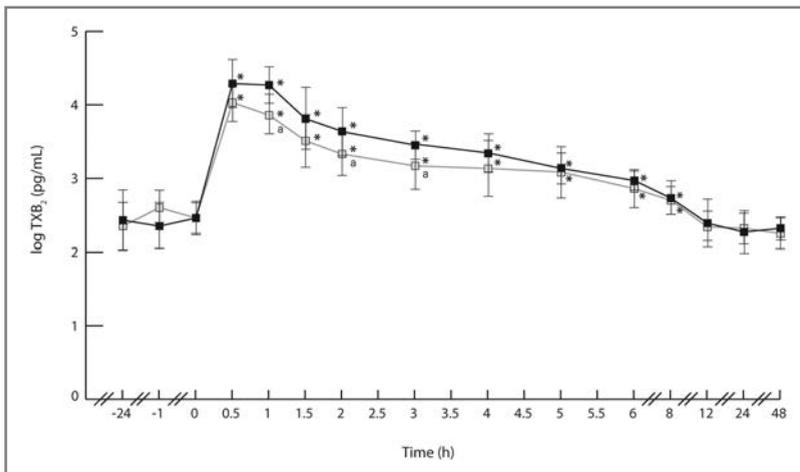


Figure 6—Mean  $\pm$  SD log of plasma TXB<sub>2</sub> concentrations of the same foals as in Figure 1. No published information is available for plasma TXB<sub>2</sub> concentrations in healthy 4-day-old foals. See Figure 1 for remainder of key.

## Discussion

Septicemia and endotoxemia remain common clinical entities in humans and veterinary species, driving investigations into treatments that improve patient welfare, recovery, and outcome but have minimal adverse effects. Administration of antimicrobials is the cornerstone of treatment for septicemia, but medications that attenuate the exaggerated inflammatory response associated with septicemia can decrease patient morbidity and mortality rates.<sup>12</sup> In the present study, IV administration of polymyxin B resulted in improvement in attitude score as well as some clinicopathologic variables and concentrations of inflammatory mediators without evidence of overt nephrotoxicosis. This information suggested that polymyxin B may be a beneficial ancillary treatment for equine neonatal endotoxemia and septicemia.

The observations of lethargy, failure to suckle, colic, diarrhea, alterations in vascular perfusion, tachycardia, and fever were consistent with other experimental

studies<sup>21–23,28,29</sup> of endotoxemia in foals as well as naturally occurring cases of equine neonatal septicemia. The attitude score was a subjective assessment assigned by an observer unaware of group assignments; this score was significantly better in the polymyxin B group at 4, 8, and 12 hours and approached significance ( $P = 0.06$ ) at 1.5, 5, and 6 hours in this study, suggesting some clinical benefit. In addition, at various time points during this study, significant improvement in blood glucose concentration and blood lactate concentration was detected in foals that received polymyxin B, compared with control foals. Neonatal foals are susceptible to hypoglycemia because of low fat and glycogen reserves; thus, a neonatal foal that is not suckling can develop hypoglycemia rapidly.<sup>30</sup> Hypoglycemia is a common feature in ill neonatal foals evaluated at referral hospitals, with 1 large retrospective study<sup>31</sup> detecting hypoglycemia at admission in 34% of 515 neonatal foals. Furthermore, foals with blood glucose concentrations  $< 50$  mg/dL at admission in that study<sup>31</sup> were associated with septicemia and positive results of bacteriologic culture of blood and were less likely to survive. In the present study, the mean blood glucose concentration reached its nadir 3 to 4 hours after initiation of the LPS infusion, and hypoglycemia likely developed as a result of decreased to absent suckling behavior from 0.5 to 4 hours after LPS administration. As reflected in the attitude score, the foals in the polymyxin B group started to suckle sooner after LPS administration, compared with the control foals, thus increasing their blood glucose concentrations earlier and to a greater extent. In this study, 500 mL of 5% dextrose in water was administered IV to 1 foal in the polymyxin B group and 5 foals in the control group because of extremely low blood glucose concentrations ( $< 35$  mg/dL) measured at 4 hours. This intervention may have compromised the ability to detect significant differences in blood glucose concentrations between the polymyxin B-treated and control foals at subsequent times (5 hours and after); however, concerns regarding severe hypoglycemia warranted intervention. Interestingly, the blood glucose concentration at 5 hours was not markedly increased after dextrose administration, as would be suspected in a healthy foal, suggesting that an increased catabolic state secondary to endotoxemia and the systemic inflammatory response syndrome could have also been a factor in the development of hypoglycemia. Other potential causes of hypoglycemia include altered metabolism or function of endocrine glucoregulatory mechanisms (eg, insulin, glucagon, cortisol, and epinephrine), which has been observed in critically ill foals.<sup>32,33</sup>

Administration of LPS resulted in a significant increase in blood lactate concentration, compared with baseline values, in both groups within 0.5 hour after completion of the LPS infusion. Hyperlactatemia has been used as a prognostic guide in ill neonatal foals, with blood lactate concentration significantly higher in nonsurviving foals.<sup>34–36</sup> Hyperlactatemia can occur via a multitude of pathophysiologic mechanisms, with the most common mechanism arising from anaerobic tissue metabolism, which occurs with an imbalance between oxygen delivery and tissue demand (type A hyperlactatemia).<sup>37</sup> Hyperlactatemia may also occur under conditions of adequate oxygen delivery (type B hyperlactatemia) in the face of increased or impaired oxygen utilization secondary to the systemic inflammatory response syndrome, sepsis, neoplasia, or mitochondrial dysfunction.<sup>38</sup> Type B hyperlactatemia can also occur from reduced lactate clearance in conditions such as liver or renal failure.<sup>38</sup> Critically ill foals likely have both type A and type B hyperlactatemia.<sup>37,38</sup> In the study reported here, the mean blood lactate concentration was significantly lower at 4 and 5 hours in the polymyxin B group, compared with the control group. Although the exact metabolic alterations causing hyperlactatemia were not examined in this study, this information suggested that treatment of neonatal endotoxemia with polymyxin B potentially improves systemic oxygenation or abates factors associated with the development of hyperlactatemia.

Similar to previous studies<sup>15,16,18,21</sup> in adult horses and foals, LPS administration in this study resulted in marked leukopenia and neutropenia. In similar LPS studies<sup>15,18</sup> investigating polymyxin B in adult horses, significantly higher neutrophil counts in the polymyxin B–treated horses, compared with control horses, were detected 1 hour after LPS infusion only when polymyxin B was administered prior to LPS infusion. Although a significant increase in neutrophil counts in the study reported here was detected in the polymyxin B group at 8 hours, compared with the control foals, significantly lower neutrophil counts were reported at 24 and 48 hours. Considering the time (8, 24, and 48 hours) at which these differences were detected, it is likely that the differences in neutrophil counts were associated with varying degrees of rebound neutrophilia commonly observed after a severe neutropenic episode.<sup>15,39</sup> Thus, it is unlikely that polymyxin B administration had any clinically beneficial effect on the WBC or neutrophil count in this study.

Significantly lower concentrations of 2 inflammatory mediators, TNF- $\alpha$  and TXB<sub>2</sub>, were measured in the polymyxin B group, compared with the control group in this study. Tumor necrosis factor- $\alpha$  is a cytokine produced by activated macrophages and is an important proximal inflammatory mediator in the pathogenesis of endotoxemia.<sup>28</sup> Increased serum concentrations of TNF- $\alpha$  have been detected after experimental administration of endotoxin to foals as well as in clinical cases of equine neonatal septicemia; with the latter situation, higher TNF- $\alpha$  activity has been associated with a poor prognosis.<sup>29,40</sup> In adult horses, significantly lower serum TNF- $\alpha$  activity was observed in horses that received polymyxin B (5,000 U/kg [2,272.73 U/lb]) prior to LPS administration, 1,000 U/kg [454.5 U/lb]) prior to LPS

administration, and 5,000 U/kg after LPS administration), compared with control horses, with an earlier and more sustained decrease in TNF- $\alpha$  when polymyxin B was administered prior to LPS infusion.<sup>15</sup> Significantly decreased TNF- $\alpha$  concentration was observed in the polymyxin B–treated foals of the study reported here in a similar pattern as the adult horse group that received polymyxin B after LPS infusion.<sup>15</sup> Interestingly, differences in TNF- $\alpha$  concentration between the polymyxin B and control group were not observed until later time points (8 and 24 hours). The reason for this apparent delayed effect is uncertain, but timing of polymyxin B administration, dose of LPS, or faster recovery from LPS challenge may play a role. Likewise, TXB<sub>2</sub>, another marker of inflammation, is an inactive yet stable metabolite and surrogate marker of thromboxane A<sub>2</sub>. Thromboxane A<sub>2</sub> is produced by activated platelets and has prothrombotic properties with increased production during inflammation such as occurs with septicemia or host exposure to LPS.<sup>15,16,18,41</sup> In the present study, a significant decrease in plasma TXB<sub>2</sub> concentration was observed in foals administered polymyxin B, compared with the control group. Collectively, this suggests that polymyxin B may attenuate some of the inflammatory mediator production associated with endotoxemia.

No difference in rectal temperature or heart rate was observed between groups in this study; conversely, authors of previous studies in adult horses have reported significant improvement in heart rate and rectal temperature in polymyxin B–treated horses, compared with control horses. However, this observation occurred only in horses that were administered polymyxin B prior to LPS administration.<sup>16,18</sup> A previous study<sup>15</sup> did conclude that the effectiveness of polymyxin B against LPS-induced alterations is dose and time (relative to LPS exposure) dependent. Polymyxin B was administered after LPS infusion in the study reported here, as this would be the most likely clinical scenario encountered in neonatal foals. However, one should consider that naturally occurring equine neonatal sepsis likely results in intermittent and repeated bouts of endotoxemia rather than a solitary episode; thus, continued administration of polymyxin B (ie, every 8 hours) in clinical cases may attenuate clinical and biochemical alterations from endotoxemia as the temporal course of treatment progresses. Taken together, this information suggests that administration of polymyxin B should occur as soon as possible in clinical cases of suspected or confirmed neonatal septicemia. Furthermore, preemptive polymyxin B administration to neonatal foals in the prodromal stages of septicemia or to those foals at high risk of developing septicemia, such as foals with failure of passive transfer of maternal antibodies or infected umbilical structures, may be beneficial.

Older reports suggest that 1 detrimental effect of IV polymyxin B administration is nephrotoxicosis, although more recent studies<sup>13</sup> have found that toxicosis is less common and severe than originally believed. Renal toxicosis associated with polymyxin B administration is dose dependent, with the proposed mechanism of increasing membrane permeability resulting in increased influx of cations, anions, and water, leading to cell swelling and lysis.<sup>13</sup> Recent studies<sup>15,42</sup> in adult

horses suggest that a dose of 6,000 U/kg, IV, every 8 hours, maintains the ability to bind LPS yet remains devoid of nephrotoxic effects. Similarly, no evidence of overt nephrotoxicosis was detected in neonatal foals after IV administration of 3 doses of polymyxin B in the present study.

When the results of the present study are compared with those previously published, a crucial variable that must be considered is the various LPS doses used in different study protocols. In adult horses, the dose of LPS administered IV in experimental studies has varied from 20 to 250 ng/kg (9.1 to 113.6 ng/lb).<sup>15,16,18,28,43-45</sup> In the present study, an LPS dose of 0.5 µg/kg (500 ng/kg) was administered over 30 minutes; this dose was selected on the basis of reports<sup>21-23</sup> of experimental endotoxemia in neonatal foals. Presumably, a lower LPS dose would result in less clinical, serum biochemical, and inflammatory alterations in foals, as observed in adult horse studies. Another limitation of this study was the inability to control the individual foal's susceptibility and response to LPS administration. The host response to a foreign pathogen is highly variable and may be partially governed by genetic variables.<sup>46</sup> Notable individual variation in the concentrations of inflammatory markers was observed in the present study, which influenced results of statistical analysis. Finally, the experimental method of inducing endotoxemia used in this study, although widely accepted, does not perfectly mimic the global effects of naturally occurring septicemia in neonatal foals. Thus, results from this study should be viewed in light of the limitations of the experimental method.

In the present study, administration of LPS to neonatal foals resulted in clinical signs of lethargy, anorexia, colic, and diarrhea as well as hypoglycemia, hyperlactatemia, neutropenia, and increased concentrations of inflammatory mediators. Administration of polymyxin B (6,000 U/kg, IV, q 8 h) improved some variables, but multicenter studies investigating the clinical efficacy of polymyxin B in foals are necessary.

- a. SNAP Foal IgG Test, IDEXX Laboratories, Westbrook, Me.
- b. Xylazine, Lloyd Inc, Shenandoah, Iowa.
- c. 14-gauge, 3.5-inch IV catheter, Mila International, Erlanger, Ky.
- d. Feeding and urethral catheter, Tyco Healthcare, Mansfield, Mass.
- e. Endotoxin, Sigma-Aldrich, St Louis, Mo.
- f. Polymyxin B, X-Gen Pharmaceuticals, Northport, NY.
- g. Advia 120 Hematology System, Siemens, Tarrytown, NY.
- h. Accutrend, Roche Diagnostics, Mannheim, Germany.
- i. AlphaTRAK Blood Glucose Monitoring System Meter, Abbott Animal Health, North Chicago, Ill.
- j. Vitros Chemistry System, Ortho-Clinical Diagnostics Inc, Raritan, NJ.
- k. Strata-X SPE Tubes, Phenomenex, Torrance, Calif.
- l. Equine TNF alpha ELISA kit, Thermo Scientific Inc, Rockford, Ill.
- m. 2,3-dinor TXB2 ELISA kit, Caymen Chemical Co, Ann Arbor, Mich.

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## From this month's AJVR

### Effects of clopidogrel on the platelet activation response in horses

Marjory B. Brooks et al

**Objective**—To evaluate the platelet activation response before and after treatment with clopidogrel in horses.

**Animals**—12 healthy adult mares.

**Procedures**—In a masked study, horses (6/group) were randomly allocated to alternately receive placebo or clopidogrel via nasogastric tube at a loading dose of 4 mg/kg followed by 2 mg/kg every 24 hours. Blood samples were collected before and 72 hours after initiation of treatment for ADP- and collagen-induced light transmission aggregometry; determination of closure time in collagen-ADP cartridges; modified thrombelastography for comparison of maximal amplitudes generated by kaolin, reptilase, and reptilase plus ADP activation; and flow cytometric tests to detect platelet fibrinogen binding, P-selectin expression, and phosphatidylserine externalization before and after ex vivo stimulation with thrombin, convulxin, thrombin with convulxin, and calcium ionophore.

**Results**—Clopidogrel administration induced a significant decrease in mean aggregation response to 5 $\mu$ M and 10 $\mu$ M ADP stimulation; however, 2 horses had resistance to clopidogrel's inhibitory action. Significant differences after clopidogrel treatment were not found in any other tests of platelet function.

**Conclusions and Clinical Relevance**—Assays using commercially available reagents were configured to measure different variables of the platelet activation response; however, clopidogrel's platelet inhibitory action was only detected by ADP-induced light transmission aggregometry. Results also suggested that horses, like humans, have interindividual variability in response to clopidogrel that may influence the drug's clinical efficacy as an antiplatelet agent. (*Am J Vet Res* 2013;74:1212–1222)



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