

PRODUCT EVALUATION/PHARMACOLOGY

Normal ponies were passively immunized with antiserum. The antiserum contained an endotoxin-specific IgG(t) antibody titer of > 1:16000 and a total IgG antibody level of > 3000 mg/dl, which was harvested from horses hyperimmunized with a Salmonella typhimurium bacterin-toxoid. An intravenous bolus challenge of Escherichia coli 055:B5 endotoxin was used to test whether or not ponies were protected from the effects of endotoxemia. Clinical responses were evaluated by an endotoxin colic index and the results were correlated with serum titer measurements of the specific anti-endotoxin antibody IgG(t) levels. Placebo-treated and antiserum-treated groups were significantly different (P < 0.05), as measured by both the endotoxin colic index and serum IgG(t) antibody levels. It was concluded that antiserum, produced from horses hyperimmunized with a recently developed Salmonella typhimurium bacterin-toxoid, successfully treated ponies from the heterologous challenge of Escherichia coli 055:B5 endotoxin.

Cross-Protection of Ponies from Sublethal *Escherichia Coli* Endotoxemia by *Salmonella Typhimurium* Antiserum

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Introduction

Gram negative endotoxins often complicate diseases of adult horses and foals.¹⁻⁴ Enteritis/diarrhea, dystocia/metritis, bacteremia/septicemia, carbohydrate overload, colic and failure of passive transfer of maternal antibodies to foals are all conditions often associated with Gram negative endotoxemia. Endotoxin activates biologic mediators of shock and inflammation. The resulting immuno-inflammatory cascade can lead to death from endotoxic shock, despite aggressive therapy.

As previously reported, endotoxin-specific antibodies provide significant protection from the effects of endotoxin and improve survival rates in patients with Gram negative septicemia.⁵ Antibodies produced to combined lipopolysaccharide (endotoxin) O-side chain complex are directed primarily against the O-side chains. The resulting protection is limited to the particular strain of immunizing bacteria due to the high specificity of the O-side chains.

In contrast, cross protection among many antigenically diverse strains of Gram negative bacteria can be provided by the unique *Salmonella typhimurium* antiserum. Contained in this vaccine is an Re mutant strain of *Salmonella typhimurium* that markedly lacks O-side chains. Thus, only the "naked core" portion of the cell wall, KDO (3-deoxy-D-mannoctulosic acid, approximately 17% and lipid A, approximately 70%) is presented to the immune system for subsequent specific antibody production.⁵⁻⁷ The "core" or cell wall structure is very similar in all Gram negative organisms.^{5,8-10} Therefore, antibodies produced in response to the core antigen will theoretically provide cross protection against endotoxins from all Gram negative bacteria.^{6,7,11-13} Cross protection elicited by this vaccine in horses was demonstrated in previous studies in which vaccinated horses were challenged intravenously with homologous and heterologous endotoxins.³

The J-5 Rc mutant of *Escherichia coli* has been utilized in numerous human and animal studies. This distinct bacterial strain lacks an enzyme necessary for attachment of side chains to the core polysaccharide. Because of undesirable results with the J-5 *E. coli* mutant in another laboratory¹¹ and in this laboratory, the

development of a more desirable Gram negative mutant bacterium completely devoid of O-side chains was pursued.^{3,5}

An Re *Salmonella typhimurium* mutant was produced by radiation of a strain previously isolated from a horse suffering from Salmonellosis. A bacterin made from this mutant was combined with a toxoid and aluminum trioxide to formulate a vaccine free of the O-side chains.^{3,5} this vaccine was used for hyperimmunizing healthy antiserum donors.⁵ Gram negative endotoxin challenge of ponies passively immunized with serum harvested from horses hyperimmunized with this vaccine (Salmonella Typhimurium Bacterin-Toxoid)^{3,5} is described in the paper.

Materials and Methods

Three mares, 15 geldings, and two stallions were randomly divided into two groups of 10 each. Members of one group each received 1.2 ml/kg body weight, 80% of the 1.5 ml/kg recommended dose, of hyperimmune serum diluted 1:1 in lactated Ringer's solution, administered 12 hours prior to receiving an intravenous bolus dosage of 50 ng/kg body weight of the *Escherichia coli* 055:B5 endotoxin. Each member of the control (placebo) group was administered 2.4 ml/kg body weight of lactated Ringer's solution 12 hours prior to receiving an intravenous bolus dosage of 50 ng/kg body weight of the *Escherichia coli* 055:B5 endotoxin.

Sera harvested from previously hyperimmunized horses was prepared according to previously published methods⁵ and administered to normal ponies in order to passively immunize them against the heterologous endotoxin challenge.^{5,7,8,13,14}

Clinical responses evaluated via the endotoxin colic index and observations of anorexia were conducted on a double blind basis, *i.e.*, neither the person observing or scoring was privy to the treatment given a particular animal. The endotoxin colic index is a quantitation of visceral pain and central nervous depression signs utilized to differentiate clinical responses to intravenous endotoxin challenges of placebo versus antiserum-treated ponies. It was previously developed following the observation of endotoxin-challenged horses and ponies, and drawing on the observations of other investigators as well.^{2,3,12,15,16} All clinical responses were recorded by an observer and subsequently scored by another person.³

The ponies in the present study were evaluated for clinical responses by previously established methods.¹⁶ However, during the second hour following endotoxin administration, each pony was released in a box stall and allowed free choice alfalfa hay to determine the degree of appetite suppression or anorexia that may have occurred as an endotoxin colic response.

The antiserum used in this study was analyzed for IgG(t) titer by an ELISA assay adapted from a previously developed radioimmunoassay (RIA).¹⁷ Serum samples from both groups of ponies were collected before and 12 hours after placebo or antiserum administration but prior to endotoxin challenge. These samples were analyzed for IgG(t) titers.¹⁷ Total IgG levels in the antiserum were measured via two radial immunodiffusion assays (VMRD, BSI).

Data were analyzed using ANOVA statistical techniques. The acceptable probability level of $P > 0.05$ or less was determined prior to the study.

Results

Antiserum-treated compared to placebo-treated animals challenged with intravenous *Escherichia coli* 055:B5 endotoxin were significantly ($P < 0.001$) different in terms of the endotoxin colic index (Fig. 1), antibody levels (Fig. 2), and anorexia levels (Table 1). The mean endotoxin colic index score was significantly ($P < 0.001$) higher than the mean index scores of the antiserum treated ponies (Fig. 1). The mean serum IgG(t) titer of 1:1393 of the antiserum-treated ponies 12 hours following antiserum administration and prior to endotoxin challenge was significantly ($P < 0.001$) higher than their own control mean titer or 1:597 or the placebo-control mean titer or 1:724 or the post-placebo mean titer of 1:661. The antiserum IgG(t) antibody titer was $> 1:16000$ and the total IgG antibody levels > 3000 mg/dl. Anorexia was exhibited for a significantly ($P < 0.001$) longer period in the unprotected animals than in the protected ponies (Table 1). Antiserum-protected ponies given free choice alfalfa were anorexic for an average of 9.2 minutes during the second hour following endotoxin administration, while ponies receiving no antiserum were anorexic for an average of 43.5 minutes.

Somnolence or central nervous depression was one of the clinical signs evaluated within the endotoxin colic index. Episodes of somnolence were observed in the unprotected animals, but it was not characteristic of the protected animals.

Pyrexia or increase in body temperature was not a consistent response to endotoxin in either the placebo- or antiserum-treated group.

Discussion

Following intravenous administration of Gram negative endotoxin, somnolence and intense, somnolence and intense colicky pain were attenuated in the antiserum-protected ponies as measured by the endotoxin colic index. The increase in serum antibodies provided by the antiserum apparently attenuated the more severe endotoxemic responses to endotoxin challenge exhibited by the control ponies.

The endotoxin colic index is a system that utilizes progression of clinical signs to evaluate equine responses to intravenous bolus dosage of Gram negative endotoxin. Other investigators have used similar methods to evaluate calf responses to Gram negative septicemia¹ and pony responses to cecal pressure increases.¹⁶ The evaluation of somnolence responses was added to observations related to visceral pain responses in the present study to account for the CNS depression that resulted, following intravenous administration of Gram negative endotoxins.³

A significant difference in mean anorexia time intervals was evident when control ponies were compared to antiserum-treated ponies. Appetite suppression may have been related to both the colic and central nervous responses via the release of cachectin, interleukin 1, and possibly other intermediates following endotoxin administration. Confirmation of the involvement of these endotoxin intermediates in equine endotoxemia awaits further investigation. Laboratory animal studies, however, have documented that endotoxin causes the release of cachectin and interleukin 1.⁹⁻¹¹

Pyrexia, along with anorexia and somnolence, is a consistent clinical response to interleukin 1 and endotoxin in laboratory animals.^{9-11,15} However, in laboratory animals it requires 10 times the amount of interleukin 1 to cause pyrexia as it takes to cause somnolence and anorexia.^{11,15} It is not surprising, therefore, that neither the control nor antiserum-protected ponies in these studies demonstrated consistent body temperature rises in response to endotoxin even though both anorexia and somnolence responses were evident among the control ponies.

The total IgG measurements compare with 1000 to 1500 mg/dl contained in normal plasma and 5000 to 6000 mg/dl in colostrum. Therefore, the *Salmonella typhimurium* antiserum in properly calculated doses can be considered for treatment of failure of passive transfer of maternal antibodies in foals.

In this study, only 80% of the recommended dose of the antiserum was used to determine protection of the ponies from the endotoxin challenge. Because of this requirement by USDA for establishing efficacy of a biological product, protection similar to that demonstrated in this study can certainly be expected when the recommended dose of 1.5 ml/kg of antiserum is used in management of clinical endotoxemia cases that are challenged by endotoxin levels similar to those employed in these studies. Because IgG(t) antibodies may be rapidly consumed during endotoxemia episodes, re-administration of the antiserum on the basis of clinical signs may be necessary for successful results.

This study was designed to demonstrate the cross-protective prophylactic and passive immunity capabilities of the antiserum.

Based on the results of this study, it can be concluded that the antiserum may be used therapeutically or in the face of clinical endotoxemia. Because endotoxins are probably released on an intermittent or continual basis during clinical episodes of endotoxemia, antiserum administered therapeutically could be expected to block the endotoxin subsequently gaining access to the circulating blood. The antiserum administered after clinical signs of endotoxemia are manifested, however, could not be expected to block the mediators responsible for the clinical signs of endotoxemia.

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TABLE 1

Comparison of Mean Endotoxin Colic Index Scores, Serum IgG(t) Antibody Titers and Anorexia Time Intervals of *Escherichia coli* Endotoxin Challenged Placebo- and Antiserum-Treated Ponies

Parameter	Endotoxin Challenged Ponies			
	Placebo (control) n = 10		Antiserum n = 10	
<u>Mean Endotoxin Colic Index Score^a</u>				
Mean	3.41		1.71 ^c	
SD	± 0.76		± 0.32	
Range	2.5 – 4.6		1.0 – 1.8	
<u>Mean Anorexia Time Interval (minutes)^b</u>				
Mean	43.5		9.2 ^c	
SD	± 14.5		± 11.5	
Range	20 - 60		0 - 35	
<u>Mean Serum IgG(t) Titer (Log 2)^b</u>				
	Pre-	Post-	Pre-	Post-
Mean	9.50	9.40 ^d	9.2	10.40 ^c
SD	± 0.71	± 0.52	± 0.42	± 0.84
Range	8 - 10	9 - 10	9 - 10	10 - 12

^a- Endotoxin colic index scores were analyzed via three-factor analysis of variance techniques with repeated measurements of one factor.

^b- Serum IgG(t) antibody and anorexia time interval measurements were analyzed via two-factor analysis of variance techniques with repeated measurements on one factor.

^c- Mean value significantly (P < 0.05) different from control or pretreatment mean values.

^d- Mean value not significantly (P > 0.05) different from control or pretreatment mean values.

SD = Standard Deviation