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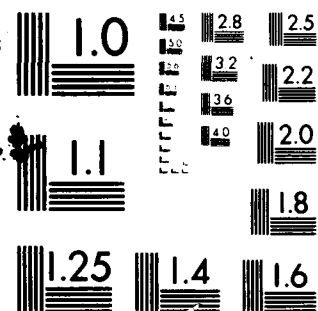
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Shaker Foal Syndrome	Immunization	Antibody
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Shaker foal syndrome (SFS) is a highly fatal toxicoinfectious form of botulism occurring most often in horses 2 to 8 weeks of age. Clinically SFS is an acute neuromuscular paralytic disease having an incidence among thoroughbred foals in the area around Lexington, Kentucky of 25-30 cases per year. <u>Clostridium botulinum</u> , type B, has been isolated repeatedly from the feces of affected foals and the symptoms of SFS have been reproduced in foals experimentally inoculated with type B botulinum toxin. Fourteen thoroughbred broodmares, stabled in an SFS endemic area and on farms having a history of repeated instances of SFS, were		

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immunized during the third trimester of gestation with 3 doses of an experimental lot of type B botulinum toxoid. The standard mouse bioassay was used to determine the type B botulinum toxin neutralizing titer of colostrum and sera (mares and foals). Each immunized mare seroconverted before foaling. On the day of foaling, broodmare colostrum titers exceeded serum titers by 3-15 fold. Toxin neutralizing activity was not detected in serum collected from foals at birth. However, substantial titers were demonstrated in sera collected from the same foals at 7, 21, and 42 days after birth, suggesting strongly a rapid passive acquisition of antibodies via the colostrum. SFS did not occur in foals of immunized mares, while SFS did occur among foals of nonimmunized mares stabled on the same and adjacent farms.

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EVALUATION OF A BOTULINUM TOXOID, TYPE B,
FOR THE PREVENTION OF SHAKER FOAL SYNDROME

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. USAMRIID facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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Shaker foal syndrome (SFS) is a highly fatal toxicoinfectious form of botulism occurring most often in foals 2 to 6 weeks of age. The syndrome is characterized by signs of symmetric motor paralysis, stilted gate, muscular tremors and progressive muscular weakness.^{9,12} Clostridium botulinum, type B, has been isolated repeatedly from the feces of affected foals⁴ and the disease can be reproduced experimentally with type B botulinal toxin.¹³ The incidence is 25 to 30 cases per year among thoroughbred foals stabled in central Kentucky.

Shaker foal syndrome, like human infant botulism, seems to stem from colonization of the neonatal intestine, probably the large bowel with C. botulinum and the subsequent production and absorption of botulinal neurotoxin.¹³ The neurotoxin induces paralysis by blocking release of the neurotransmitter acetylcholine at cholinergic synapses.

Apparently the humoral immune system of the newborn foal is deficient in the production of protective neutralizing antibodies in response to challenge with colonizing clostridia or their lethal neurotoxins. Therefore, protection, if available during the early weeks of life is afforded only by passive transfer of maternal antibodies in the colostrum. Before parturition, immunoglobulins, in particular IgG, derived from the dam's serum are selectively concentrated in the mammary gland for colostrum formation.

Active immunization of thoroughbred broodmares with tetanus toxoid late in pregnancy has been shown to be an efficient means of passively immunizing foals against the tetanus neurotoxin produced by Clostridium tetani.¹⁰

The purpose of this study was to evaluate the feasibility of passively immunizing thoroughbred foals against type B botulinal toxin

by actively immunizing their dams with the specific toxoid. In doing so it was important to determine if substantial and persistent serum levels of antibody, capable of neutralizing type B botulinal toxin, could be passively established in foals born to and ingesting the colostrum of actively immunized broodmares. It was believed that this study would provide valuable data on the role of botulinal toxoid in the prevention of toxicoinfectious botulism (SFS) in thoroughbred foals.

MATERIALS AND METHODS

This study included 14 thoroughbred brood mares each in the third trimester of gestation and due to foal during 1981. The mares and subsequently their foals were stabled on a central Kentucky farm having an incidence over the past 15 years of 1 to 4 fatal cases of SFS per year.

Aluminum phosphate adsorbed botulinal toxoid used to immunize broodmares was originally prepared in bulk for human use by the Michigan Department of Public Health, Lansing, Michigan, in 1971. Lot 7006 of this bulk toxoid was bottled and packaged for human use in 1978 as "Botulinum Toxoid Adsorbed Monovalent B." It was subsequently evaluated in humans and found to be safe and efficacious.¹ Lot Ex 94 of the bulk toxoid was bottled in 1980 at a slightly higher antigen concentration than lot 7006 and labeled "Botulinum Toxoid Adsorbed, Type B" for veterinary use only.

Thoroughbred broodmares were inoculated with three 2-ml doses of Lot Ex 94 subcutaneously in the anterior one-third of the neck. The first inoculation was given during the first few weeks of the third trimester of gestation and the second dose was administered 28 days

later. The third dose was given 2 to 3 weeks before the estimated date of parturition.

Blood specimens were collected from the mares and subsequently from their foals for the determination of serum neutralizing activity to type B botulinum toxin. The blood was obtained from mares in 10-ml amounts by venipuncture immediately before the initial inoculation of toxoid and at 14 days after each inoculation. The same amounts were obtained by venipuncture from foals prior to nursing and on postpartum days 7 and 42. Sera were separated from the clotted specimens for testing.

The neutralization titer for type B botulinal toxin contained in serum was determined by the standard mouse neutralization test and expressed as international units (IU) per ml of serum.² One IU of activity to type B botulinal toxin in 1 ml of serum neutralized 10,000 mouse intraperitoneal (i.p.) median lethal doses (LD₅₀) of type B botulinal toxin.

Results

The initial and subsequent doses of monovalent B botulinum toxoid given to each broodmare were tolerated well. No adverse reactions were noted to occur in any of the immunized mares.

Neutralizing activity for type B botulinal toxin was detectable in the sera of all 14 mares 2 weeks after the third toxoid inoculation (Table 1). Titers ranged from 0.05 to 3.58 IU/ml with a mean of 0.84 IU/ml. All mares were negative on day 0.

Neutralization titers of sera collected from foals born in 1981 to these immunized broodmares are shown in Table 2. Neutralization activity for type B toxin was not detected in serum collected before consumption

of colostrum from any of the 14 foals tested. However, neutralizing activity was demonstrated in serum collected from each of 12 foals tested at 7 days of age. Day 7 serum was not collected from Foals 8 and 9. The titers of Day 7 sera ranged from 0.01 IU/ml (Foal 12) to 3.58 IU/ml (Foal 18), with a mean of 0.72 IU/ml.

Foal sera collected on Day 42 ranged in titer from 0 (Foals 7, 10 and 12) to 0.90 IU/ml (Foal 18). The mean was 0.21 IU/ml. During the 1981 foaling season, no case of SFS occurred among the passively immunized foals born to the 14 broodmares immunized with the investigational toxoid. Shaker foal syndrome did occur during this period in 24 thoroughbred foals of nonimmunized broodmares stabled on other central Kentucky farms.

Discussion

The diffuse epitheliochorial structure of the equine placenta allows little if any transplacental passage of large protein molecules, i.e., serum gammaglobulins. The foal at birth is essentially agammaglobulinemic and thus totally dependent upon colostrum transfer of immunoglobulins after parturition.⁸ Prior to parturition, serum immunoglobulins of the mare are concentrated in the mammary gland for colostrum formation. A thoroughbred foal probably ingests 2 to 3 liters of this antibody-enriched colostrum within the first 12 hours of life.³ These passively acquired immunoglobulins are rapidly absorbed from maternal colostrum in the neonatal intestine and then into the foal's vascular system.

Jeffcott⁶ has demonstrated that peak titers of passively acquired gammaglobulin are attained in the foal by 18 hours after birth, and

that the circulating titer obtained is usually slightly less than that of the dam's serum. Absorption is thought to decline at a linear rate from 100% at birth to 0% by 24 hours.⁵ The foal's total serum protein then decreases from about 3 days of age through 42 days.⁸ The total concentration of any specific immunoglobulin in the broodmare's colostrum at parturition undoubtedly influences the serum concentration of the same immunoglobulin in the nursing foal during the first 6 weeks of life.¹¹

Rossdale and Scarnell¹⁰ induced the passive immunization of four thoroughbred foals by the active immunization of their dams with aluminum hydroxide-adsorbed tetanus toxoid (derived from the neurotoxin of C. tetani) given in 2-ml doses at 2 months and 1 month before parturition. This regime resulted in substantial serum neutralizing titers to tetanus toxin which persisted for several months in each of these foals. Active immunization of the mares induced higher serum titers in their foals than did the parenteral administration of tetanus antiserum to foals of nonimmunized dams. The postparturition transfer from dam to foal of antibody specific for another clostridial toxin, that of Clostridium welchii type A, and the persistence of this antibody in foals has been well-documented.⁷

The results of the present study were not unanticipated. Adverse reactions to administration of the investigational botulinum toxoid did not occur in any of the 14 broodmares immunized. Each seroconverted by 2 weeks after the third toxoid immunization. We thus confirmed in horses the immunogenicity of a toxoid previously found to be efficacious in man. Active immunization of thoroughbred broodmares with botulinum toxoid consistently induced titers of neutralizing

antibody specific for type B toxin which was effective in establishing passive immunization of foals consuming their colostrum.

Shaker foal syndrome is reported to occur most often in foals 2 to 6 weeks of age. Sera collected during our study from each foal born to and nursing on an immunized broodmare, contained neutralizing activity for type B botulinal toxin. Neutralization titers were lowest in the 7-day sera of foals 7, 10 and 12; the 42-day sera of these 3 foals did not neutralize toxin. However, sera collected at 42 days of age from 11 of 14 foals did neutralize type B toxin; thus a measure of protection was provided throughout the critical 6-weeks of age time period.

The immunization of thoroughbred broodmares with botulinum toxoid, type B, during the third trimester of gestation is both feasible and efficacious for the passive immunization of colostrum-consuming foals against toxico-infectious botulism, shaker foal syndrome.

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TABLE 1. Neutralization titers for type B botulinal toxin in
actively immunized* thoroughbred broodmares

Mare	Titer** (IU/ml)
1	0.20
2	0.05
5	0.64
7	0.28
8	2.85
9	0.22
10	0.22
12	0.28
13	0.28
14	1.13
17	0.90
18	3.58
20	0.45
21	0.70
Mean	0.84

* Immunization with three 2-ml doses of botulinum toxoid
adsorbed, type B, Lot Ex 94.

**Sera were collected 2 weeks after the third immunization.

TABLE 2. Passively acquired neutralization titers for type B botulinal toxin in foals born to actively immunized* thoroughbred broodmares

Foal No.	Titer (IU/ml)		
	Birth	Day 7	Day 42
1	0	0.56	0.45
2	0	1.42	0.56
5	0	0.45	0.22
7	0	0.06	0
8	0	NS	0.06
9	0	NS	0.02
10	0	0.03	0
12	0	0.01	0
13	0	0.45	0.06
14	0	0.22	0.22
17	0	0.90	0.19
18	0	3.58	0.90
20	0	0.90	0.24
21	0	0.10	0.02
Mean	0	0.72	0.21

* Immunization with three 2-ml doses of botulinum toxoid adsorbed, type B, Lot Ex 94.