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Specific Immune Response of Mares and their Newborn Foals to *Actinobacillus* spp. Present in the Oral Cavity

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Sternberg S: Specific immune response of mares and their newborn foals to *Actinobacillus* spp. present in the oral cavity. Acta vet. scand. 2001, 42, 237-242. – Oral swab samples, serum and colostrum was taken from 15 mares and 14 of their foals, within 24 h of birth. The presence of antibody against *Actinobacillus* spp. isolated from the oral cavity was investigated using agar gel immunodiffusion. Antibodies against 48 out of the 77 *Actinobacillus* isolates from all horses in the study were present in the respective sera of 13 mares and 9 foals. In 11 mother-foal pairs, the antibody content of the foal serum was similar to that of the mare, and in 9 cases this was reflected in the antibody content of colostrum from the mare. The results indicate that an immune response to *Actinobacillus* spp. colonising the oral cavity is present in many adult horses and that this immune response can be transferred from mother to foal via colostrum.

horse; foal; Actinobacillus; immune response; immunodiffusion; bacteria.

Introduction

Foal septicaemia due to *Actinobacillus equuli* infection is a common cause of illness and death in newborn foals (*Baker* 1972, *Deem Morris* 1984, *Brewer & Koterba* 1990, *Raisis et al.* 1996), but other *Actinobacillus* spp. have also been associated with neonatal septicaemia (*Carter et al.* 1971, *Carman & Hodges* 1982, *Nelson et al.* 1996). The taxonomy of equine actinobacilli is unclear. Historically, all *Actinobacillus* spp. isolated from horses have been named *A. equuli,* but further taxonomical studies have revealed several distinct types (*Bisgaard et al.* 1984, *Jang et al.* 1987, *Samitz & Biberstein* 1991) of equine actinobacilli, although a definite classification of this group of bacteria is not yet available. Consequently, the pathogenic potential of various subtypes has not been fully determined. Generalised infections with *Actinobacillus* spp. are extremely rare in adult horses, unless some other underlying disease or other predisposing factor is present. The foal is usually believed to be infected during, or shortly after, birth. Failure of passive transfer, i.e. colostrum deficiency, has sometimes been specifically associated with equine actinobacillosis (*Kamada et al.* 1985, *Vaissaire et al.* 1988, *Robinson et al.* 1993), but the presence or absence of specific antibodies against the infecting strain were not investigated in these studies. The presence of serum antibodies in the mare against the strain infecting the foal has been reported in clinical cases (*Farrelly & Cronin* 1949, *Harbourne et al.* 1978, *Rycroft et al.* 1998), but it is not clear whether all these cases were subject to failure of passive transfer. In some cases of neonatal actinobacillosis, *A. equuli* has been isolated from both the healthy mother and the sick foal (*Platt* 1973). *A. equuli,* as well as other *Actinobacillus* spp., are commonly isolated from the oral cavity of healthy

horses (*Bisgaard et al.* 1984, *Sternberg* 1998), and sometimes the same strain is present in both the mare and her foal (*Sternberg* 1998). It is likely that foal actinobacillosis is caused by one of the strains present in the dam's normal flora. The uptake via colostrum of specific antibodies against actinobacilli present in the oral cavity of the mare would provide the foal with protection against infection with these strains. The aim of this study was to establish whether specific antibodies against actinobacilli present in the oral cavity of healthy mares could be detected in their serum and colostrum and if such antibodies could also be found in the serum of their newborn foals.

Materials and methods

Sampling

Serum, colostrum and culture samples were taken from 15 mares and 14 of their newborn foals, within 24 h of birth. One foal died, due to non-infectious disease, and was therefore not available for sampling. From 2 mares, colostrum samples were not available. With one exception, sampling was made at least 10 h after intake of colostrum. From 1 foal, the blood sample was taken only 1 h after intake of colostrum. Blood samples were collected in Vacutainer® (Becton Dickinson, Meylan Cedex, France) tubes and centrifuged at 150 × g for 5 min, after which aliquots of serum were stored at -70 °C. Colostrum samples were divided into aliquots and kept at -70 °C until further analysis. For the swab samples, a commercial swab-and-transport system (Transystem, Copan, Bovezzo, Italy) was used, and sampling from the buccal part of the oral cavity of both mares and foals was performed as earlier described (*Sternberg* 1998). With one exception, all samples were kept at 8 °C until transported to the laboratory, within 24 h of sampling. The samples from one mare and one foal were accidentally kept at a temperature of 20-30 °C overnight. One mare had been systemically treated with a combination of penicillin and streptomycin before sampling.

The experimental design was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

Bacterial culture

The swabs were streaked onto agar plates (blood agar base no. 2, Oxoid, Basingstoke, UK), supplemented with 5% horse blood. Each sample was also cultured in parallel on a blood agar plate supplemented with 0.5 mg/l of clindamycin, as previously described for the selective culture of equine actinobacilli (*Sternberg* 1998). All plates were incubated at 37 °C for up to 24 h. After incubation, colonies matching the description of *Actinobacillus* spp. were selected and subcultured twice on blood agar. After subculture, isolates were identified as previously described (*Sternberg* 1998). For each mother-foal pair at least 2 isolates of each subtype, if present, were retained. All isolates were stored at -70 °C in trypticase soy broth supplemented with 15% glycerol (SVA BaktDia, Uppsala, Sweden).

Antigen preparation

Bacterial antigen was prepared by the use of Na-deoxycholate ($C_{24}H_{39}O_4Na$, Sigma Chemical Co., St. Louis, Missouri, USA), modified from the method described by *Kim* (1976). In short, 10 µl of colony material from a fresh overnight bacterial culture was suspended in 1 ml of PBS (SVA BaktDia, Uppsala, Sweden), in a sterile Eppendorf tube. Na-deoxycholate was added to a final concentration of 1% (w/vol) and after vigorous shaking the solution was incubated at 8 °C for 6 h. After incubation, the tubes were shaken, centrifuged at 90 × g for 4 min, and the supernatant was used for immunodiffusion.

Table 1. No. of *Actinobacillus* isolates identified and included in the study.

Mare-foal pair	*A. equuli sensu stricto* (ss)	L-arabinose positive *A. equuli* (A+)	Bisgaard's taxon 11 type 1 (tx 11)	Non-typable *Actinobacillus* spp. (spp)
A	2 from mare	none	1 from mare 2 from foal	none
B	none	1 from mare 2 from foal	none	1 from mare 1 from foal
C	1 from foal	2 from mare 1 from foal	1 from foal	1 from foal
D	none	1 from foal	none	2 from mare 4 from foal
E	none	none	3 from mare	none
F	none	1 from mare 4 from foal	none	1 from mare
G	none	none	none	4 from mare 1 from foal
H	none	3 from mare 2 from foal	none	3 from foal
I	none	1 from mare	1 from foal	2 from mare 3 from foal
J[1]	3 from mare	2 from mare	none	none
K	1 from foal	2 from foal	none	2 from foal
L	2 from mare	2 from mare	none	none
M[2]	1 from mare	none	none	none
N	none	1 from mare 2 from foal	none	1 from mare 1 from foal
O	none	none	none	2 from mare 3 from foal

[1]Mare treated with penicillin and streptomycin before sampling.
[2]Samples accidentally left at 20-30°C overnight.

Immunodiffusion

Agar gel immunodiffusion (AGID) was performed in Auto I.D.® plates (Immunoconcepts, Sacramento, California, USA). A volume of 20 μl of antigen solution or serum was added to the respective wells. Na-desoxycholate, at a final concentration of 1% was added to the colostrum samples before application, as this was necessary to achieve diffusion of the colostrum. All isolates from each mare-and-foal pair were tested against the sera of both mare and foal, as well as the colostrum. All AGID plates with serum samples were incubated at room temperature for up to 48 h and checked every 12 h for the presence of precipitation lines. Plates with colostrum samples were incubated at 37°C for the first 24 h, as this was found to improve the diffusion of colostrum from the wells, and subsequently at room temperature for another 24 h, with checking for precipitation lines every 12 h. Initially, for the first 2 mare-foal pairs, all analyses were performed in duplicate, but as no difference could be detected between the results from different runs of the same experiment, the subsequent analyses were generally performed only once. However, in the cases where differences between mare and foal serum were detected, the entire analysis, including antigen

Table 2. No. of *Actinobacillus* isolates against which antibody could be detected in serum and colostrum.

Mare-foal pair	Ab in mare serum	Ab in foal serum	Ab in colostrum
A	2 ss[1]	2 ss	none
	3 tx11	3 tx11	
B	2 spp	2 spp	none
C	1 tx11	1 A+	1 tx11
	1 spp	1 tx11	1 spp
		1 spp	
D	1 A+	not sampled	1 spp
	1 spp		
E[2]	3 tx11	none	3 tx11
F	3 A+	none	none
	1 spp		
G	4 spp	2 spp	none
H	4 A+	5 A+	2 A+
	3 spp	3 spp	1 spp
I	4 spp	4 spp	4 spp
J	2 ss	none	2 A+
	2 A+		
K	2 spp	2 spp	2 spp
L	none	none	none
M	none	none	none
N	2 A+	1 A+	not sampled
O	5 spp	2 spp	not sampled

[1] ss=*A. equuli sensu stricto,* A+=L-arabinose positive *A. equuli,* tx11=Bisgaard's taxon 11 subtype 1, spp=*Actinobacillus* spp., non-typable.
[2] Foal sampled 1 h after colostrum intake.

preparation, was repeated once, to ensure that the detected difference was not accidental.

Results

Bacterial isolates

All foals, with one exception, were judged to have an aerobic oral flora very similar to that of their respective dams. The sample from the foal of the dam treated with antibiotics yielded no bacterial growth. Various isolates of *A. equuli sensu stricto,* L-arabinose positive *A. equuli,* the subtypes of Bisgaard's taxon 11 (*Bisgaard et al.* 1984) and other non-typable *Actinbacillus* spp. were identified (see Table 1).

Antibody detection

Antibodies against 48 out of the 77 *Actinobacillus* isolates from all horses in the study were present in the respective sera of 13 mares and 9 foals. There was no species of *Actinobacillus* that appeared more likely to provoke an antibody response. One of the foals in which no antibodies could be detected was sampled only 1 h after intake of colostrum and another was the foal with no bacterial growth in the swab sample, where the dam had been treated with antibiotics. In 11 out of all mother-foal pairs, the antibody content of the foal serum was similar to that of the mare, although in some cases differing for 1-2 bacterial strains. In 7 colostral samples, some of the antibodies found in the serum of the mare and foal could be detected, but many of the colostral samples were difficult to analyse due to auto-precipitation. The details of the immune responses to different isolates are given in Table 2.

Discussion

The results in this study demonstrate the presence of an immune response in about 80% of the mares to actinobacilli normally present in the oral flora, and the transfer of this response to about 60% of their newborn foals. The presence of this immune response suggests that colostrum or serum from the mare could be used for the prevention of neonatal actinobacillosis in foals. Twenty-four out of 48 antibody reactions found in the serum of the mare and/or the foal were not detected in colostrum. This could be explained by the methodological problems encountered when using the AGID method on colostrum, something that may have impaired the detection of antibodies present in some of the colostrum samples. The absence of antibody detected in mare serum and colostrum in the foal serum that was taken only 1 h after intake of colostrum corresponds to the findings in other studies (*Jeffcott* 1974), in which it took

2-3 h for molecules absorbed via colostrum to reach the blood of the foal. In 2 foal samples, antibody that was not detected in the mare samples was found. This may be due to a true difference in immune response, or merely a difference in antibody concentration, with the mare serum falling below the detection level of the AGID test.

The presence in the mare sera of antibodies to some *Actinobacillus* strains indicates that these strains were a persistent part of the oral flora of the horses in question. The failure to detect antibodies against all strains does not necessarily prove the absence of such antibodies. The AGID method, although useful for preliminary studies on uncharacterised antigens, has limited sensitivity and the method used for antigen preparation may not have been optimal. However, it is not very likely that high concentrations of antibody against any strain would have remained undetected with the methods used in this study, provided that these antigens were expressed in vitro. The question whether all antigens expressed in vivo will be expressed in bacteria cultured in vitro remains and cannot be answered with the methods used.

In cases of adequate intake and absorption of colostrum, the foal would be expected to be protected against infection with *Actinobacillus* strains provoking a transferable immune response in the mare, while remaining unprotected against other strains. All foals sampled in the study remained healthy throughout foalhood and the failure to detect colostral antibodies against *Actinobacillus* spp. was not associated with neonatal infection. The pathogenic potential of the various strains present in the normal flora is not known. Moreover, this study only included the normal bacterial flora of the oral cavity and, although a common site for actinobacilli, this is only one of many reservoirs for opportunistic pathogens that can infect the newborn foal. The presence or absence of an antibody response is probably not the only factor involved in the development of neonatal actinobacillosis. Further studies on virulence factors of equine actinobacilli would be needed to determine whether the antibody response found in this study is correlated to the virulence of the various bacterial strains. Other aspects of the equine neonatal immune system are also of great interest in the study of this disease.

Conclusion

An immune response to the majority of actinobacilli colonising the oral cavity is present in most adult horses. This immune response, in the form of antibody, can be transferred to the newborn foal via colostrum and thus potentially protects against infection with some of the *Actinobacillus* strains carried by the mare.

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Sammanfattning

Specifikt immunsvar hos ston och deras nyfödda föl mot Actinobacillus spp. från munflora.

För att undersöka förekomsten av specifika antikroppar i serum och råmjölk mot *Actinobacillus* spp. togs munsvabbprover, serum och råmjölk från 15 ston och deras nyfödda föl inom 24 tim efter födelsen. Antikroppar mot isolerade *Actinobacillus* spp. påvisades med hjälp av immunodiffusion. Antikroppar mot 48 av 77 isolerade *Actinobacillus* spp. kunde påvisas i sera från 13 ston och 9 föl. Elva av fölen hade likartat serologiskt antikroppsmönster som sina mödrar och i nio fall återspeglades detta mönster i råmjölken. Resultaten visar att många vuxna hästar producerar serumantikroppar mot de *Actinobacillus* spp. som finns i deras munflora och att dessa antikroppar kan överföras från sto till föl via råmjölken.

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