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Passive Transfer of Mucosal Antibody to *Streptococcus equi* in the Foal

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Passive transfer of mucosal antibody to *Streptococcus equi* was studied in foals during the first 2 months of life. Immunoglobulin G (IgG) and IgA antibodies were found in sera and nasal secretions of foals shortly after colostrum intake. Titers were highest 2 days after birth; IgG predominated in sera, and IgA predominated in nasal washes. Intragastrically administered ^{99m}Tc-labeled IgA was transported from the bloodstream to the nasal mucosa of a newborn foal within a few hours of colostrum intake. Western blot analysis of the specificities of colostral and serum antibodies showed that selective transfer of immunoglobulins of defined specificity did not occur. Antibodies from milk samples taken a month or more into lactation had different specificities than those of colostrum or serum samples. Acid-extracted M protein fragments of *S. equi* recognized by milk antibodies were the same as those recognized by IgG and IgA from nasopharyngeal mucus of horses recently recovered from strangles. We postulate that passive antibody protection of the foal is derived both by secretion of colostral immunoglobulins onto the nasopharyngeal mucosa and by immunoglobulins ingested in milk that directly coat the upper respiratory and oral mucosa during the first months of life.

Local antibody responses of the upper respiratory mucosa are closely correlated with protection of horses against strangles, an infectious disease of the upper respiratory tract and associated lymph nodes caused by *Streptococcus equi*. These antibodies have different specificities than those of serum origin, a finding that clearly establishes the independence of the mucosal and systemic immune response of horses to *S. equi* (2). Foals up to 3 month of age, born from immune mares, are known to be resistant to strangles, though little information is available on the basis of this protection. It has been proposed that young foals lack receptors for *S. equi*, but adhesion studies do not support such a hypothesis (14).

Owing to the anatomical conformation of the equine placenta, foals are born with a virtual absence of circulating immunoglobulins (3, 5) and must receive these protective proteins by passive transfer from colostrum. Failure of passive transfer of immunoglobulins predisposes foals to a variety of infections (3, 6). At 24 h after birth there is a rapid decrease in intestinal absorption of immunoglobulins accompanied by a simultaneous decrease in the immunoglobulin concentration of colostrum. Throughout lactation, milk, however, continues to have moderate concentrations of immunoglobulins that are mostly locally synthesized in the mammary gland (6).

Our studies presented here focused on colostrum and milk in passive protection of the foal against *S. equi* infection. Our data suggest that antibodies similar to those observed in horses vaccinated intranasally with live vaccine or in horses recently recovered from experimentally induced strangles (2, 16) are present on the respiratory mucosa of foals during the first 2 months of life.

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MATERIALS AND METHODS

Animals. Four pony mares recently recovered from strangles were separately housed in isolation before foaling and remained there with their foals throughout the study.

Sample collection. Serum and colostrum or milk samples were taken from mares just before foaling and at 15, 30, and 60 days after that. Serum samples and nasal washes were taken from foals before suckling and 1, 2, 3, 4, 8, 19, and 60 days afterward. Nasal washes were performed by flushing 30 ml of phosphate-buffered saline into each nostril with a rubber urinary catheter attached to a 50-ml syringe. Samples thus obtained were centrifuged at 3,000 × *g* for 15 min. Supernatants were saturated to 50% ammonium sulfate, and the precipitated proteins were recovered by centrifugation at 10,000 × *g* for 30 min. Pellets were suspended in 2 ml of phosphate-buffered saline, dialyzed overnight against several volumes of the same buffer, and stored at −70°C until analyzed. Milk and colostrum samples were clarified by centrifugation at 28,000 × *g* for 90 min and stored at −70°C until used in different antibody assays.

Antisera to horse IgA and IgG. Antisera to horse immunoglobulin A (IgA) and IgG were prepared as described elsewhere (2).

Radioimmunoassay. The radioimmunoassay to detect *S. equi*-specific IgG and IgA was performed as described previously (2). Serum, colostrum samples, and nasal washes were assayed at dilutions of 1:1,000, 1:1,000, and 1:500, respectively.

Isolation of IgA. Horse IgA was isolated from colostrum by a procedure described elsewhere (2).

Radioactive labeling of IgA. Radioactive labeling of horse IgA with ^{99m}Tc was performed as described elsewhere (20), with several modifications. Briefly, 40 μg of purified IgA in 10 μl of 10 mM phosphate buffer was added to 400 μl of solution 1 (65 mM sodium chloride, 200 mM succinic acid, 20 mM sodium acetate [pH 5] with sodium hydroxide) and 100

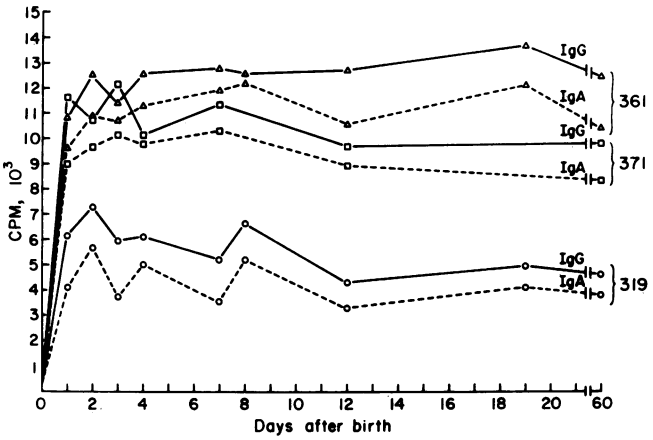

FIG. 1. *S. equi* antibody levels of serum samples taken from foals 361 (△), 371 (□), and 319 (○) before suckling (day 0) and at 1, 2, 3, 4, 8, 19, and 60 days after birth. Titers are expressed in counts per minute in a solid-phase radioimmunoassay and represent the mean of three determinations. The standard error of the mean for each time point was less than 10% and is not shown.

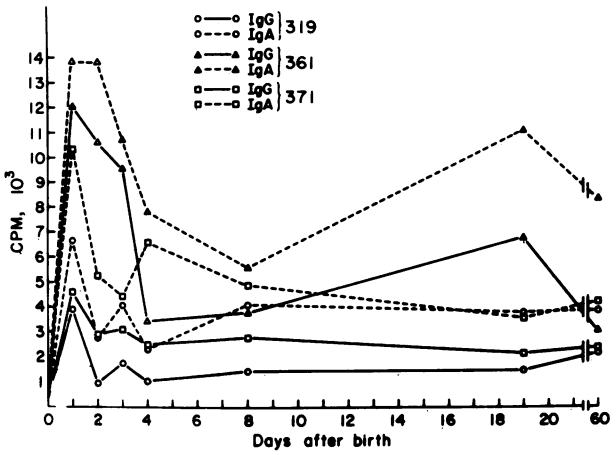

FIG. 3. *S. equi* antibody levels of nasal washes taken from foals 361 (△), 371 (□), and 319 (○) before suckling (day 0) and at 1, 2, 3, 4, 8, 19, and 60 days after birth. Titers are expressed in counts per minute in a solid-phase radioimmunoassay and represent the mean of three determinations. The standard error of the mean for each time point was less than 10% and is not shown.

μl of freshly prepared 10 mM stannous tartrate in solution 1. After incubation of the mixture for 15 min at room temperature, 1 mCi of ^{99m}Tc was added. The reaction mixture was incubated for 45 min at room temperature, applied to a Sephadex G25 column, and eluted with 50 mM phosphate buffer.

Antigen preparations. Acid extracts of *S. equi* and *S. zooepidemicus* and culture supernatant proteins of *S. equi* were prepared as previously described (2).

Immunoblots. Immunoblot analysis was performed as described elsewhere (2).

Experimental procedures. ^{99m}Tc-labeled IgA was administered intragastrically to one foal immediately after birth. Nasal washes and serum samples were taken 2, 5, and 10 h after that. The radioactivity in 250-μl serum samples and 750-μl nasal washings was measured in a gamma counter. Samples were then precipitated with an equal volume of a 30% solution of trichloroacetic acid, and the precipitates were collected by centrifugation at 13,000 × *g*. The pellets were washed three times with a 30% solution of trichloroacetic acid, and the radioactivity was measured in a gamma counter.

RESULTS

Antibodies were not detected in serum samples taken from foals before colostrum ingestion but were readily detected

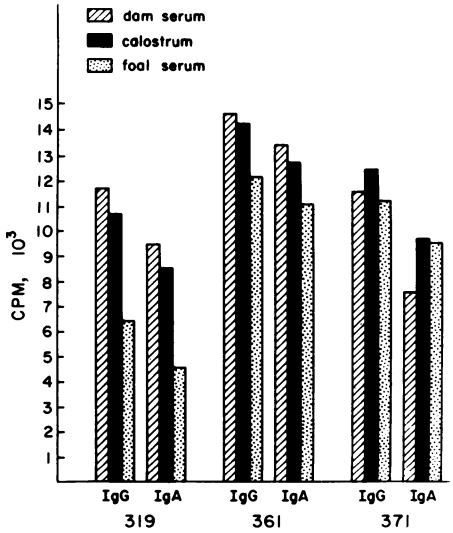

FIG. 2. IgG and IgA *S. equi* antibody levels of colostrum and mare and foal sera. Colostrum and serum samples were obtained from mares shortly before foaling. Foal serum samples were obtained 2 days after birth. Titers are expressed in counts per minute in a solid-phase radioimmunoassay and represent the mean of three determinations. The standard error of the mean for each sample was less than 10% and is not shown.

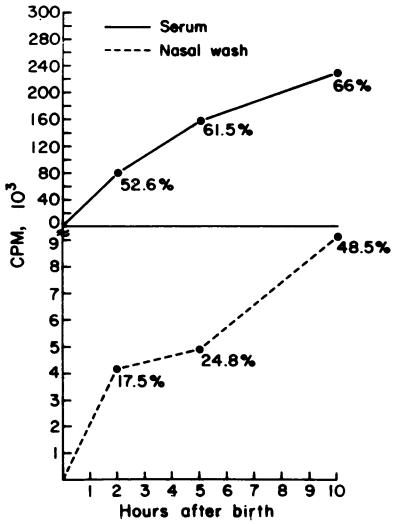

FIG. 4. Radioactivity detected in foal serum samples and nasal washes at different intervals after administration of ^{99m}Tc-labeled colostral IgA. The percentage value at each data point represents the percentage of trichloroacetic acid-precipitable counts. Activity is expressed in counts per minute and was corrected for decay.

after its intake (Fig. 1). The highest values were observed 2 days after birth, but levels remained high throughout the experiment. IgG was detected at higher levels than IgA. Antibody titers in the sera of foals 361 and 371 were similar to those of colostrum and serum from their dams (Fig. 2). The serum titer of foal 319 did not reach the level of its dam. Antibody levels in nasal washes of foals appear in Fig. 3. As in the case of serum samples, antibodies were not detected before colostrum intake but were observed 24 h after that and at various levels throughout the study. IgA titers in nasal washings were higher than those of IgG. As was observed for its serum, foal 319 had nasal wash titers lower than those of foals 361 and 371.

Radioactivity was readily detected in serum and nasal washes as early as 2 h after administration of ^{99m}Tc-labeled IgA and at increasing levels at 5 and 10 h (Fig. 4). Percentage of trichloroacetic acid-precipitable counts, a measure of the radioactivity directly associated with IgA, increased over time in both serum and nasal washes. In the case of nasal washes, the majority of the radioactivity detected at 2 h was associated with free pertechnetate that may have passively diffused from the blood to the different compartments. Over time, more IgA was actively transported onto the nasal mucosa and therefore more activity was associated with it. In the case of serum samples, as free pertechnetate passively diffused from the serum to the different compartments, progressively more activity was associated with IgA.

The specificities of antibodies from colostrum and milk and maternal and foal sera were determined by Western blot analysis. There was no difference in the specificities of IgG from colostrum and mare and foal sera when checked against different streptococcal preparations (Fig. 5). Several polypeptides of different molecular weights were recognized in acid extracts of *S. zooepidemicus* and *S. equi* and in culture supernatants of *S. equi*. The specificities of serum IgA from colostrum and dam and foal sera were the same as those of IgG and therefore are not shown. IgA antibodies from milk samples taken a month or more into lactation had different specificities than those of colostrum or serum (Fig. 6). Their specificities were more restricted, since they recognized only a 64,000-molecular-weight polypeptide in acid extracts of *S. zooepidemicus*, 46,000- and 41,000-molecular-weight polypeptides in acid extracts of *S. equi*, and an approximately 130,000-molecular-weight polypeptide in acid extracts and culture supernatants of *S. equi*. IgG antibodies from milk and serum samples had the same specificities and therefore are not shown.

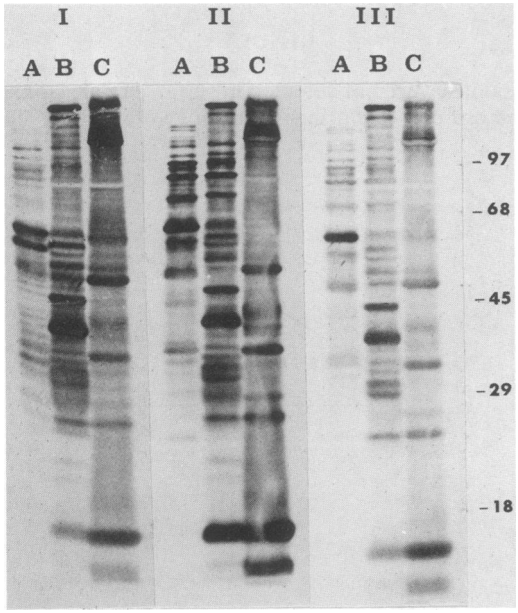

FIG. 5. Reactivities of IgG of colostrum (I), mare (II), and foal (III) sera. Acid extracts from *S. zooepidemicus* (A), *S. equi* (B), and culture supernatant proteins of *S. equi* (C) were run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, transferred to nitrocellulose sheets, and treated with colostrum or mare or foal sera. The sheets were subsequently treated with guinea pig antiserum to horse IgG. After treatment with peroxidase-labeled rabbit anti-guinea pig antiserum, the blots were developed with 4-chloro-1-naphthol.

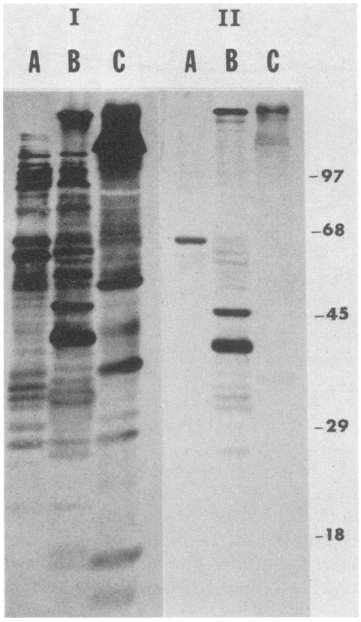

FIG. 6. Reactivities of IgA of milk (I) and serum (II) taken from the mare described in the legend to Fig. 5 45 days into lactation. Acid extracts from *S. zooepidemicus* (A) and *S. equi* (B) and culture supernatant proteins of *S. equi* (C) were run on sodium dodecyl sulfate-polyacrylamide electrophoresis gels, transferred to nitrocellulose sheets, and treated with milk or serum. The sheets were subsequently treated with guinea pig antiserum to horse IgA. After treatment with peroxidase-labeled rabbit anti-guinea pig antiserum, the blot was developed with 4-chloro-1-naphthol.

DISCUSSION

S. equi IgA and IgG antibodies were observed in sera and nasal secretions of foals after colostrum ingestion (Fig. 1 and 3). IgG titers were predominant in serum samples of the newborn foals, in contrast to nasal secretions, in which IgA was the most abundant immunoglobulin. Titers were similar in mare sera, colostrum samples, and foal sera (Fig. 2), findings in accord with those reported for the passive transfer of antibodies to herpesvirus 1 and 2 (5). Serum titers in two foals peaked 2 days after birth and slowly declined after that. Titers remained high in foal 361 for the period of study. IgG and IgA antibody titers in nasal secretions peaked at 2 to 3 days after birth and then sharply declined. The increase of antibody noted in foal 361 at 20 days after birth was probably due to the fact that its dam was shedding *S. equi* from a mandibular abscess at the time of foaling and continued to do

so for the following 3 weeks. As previously demonstrated in other studies in foals (3, 5), antibody synthesis begins as early as 16 days after birth when the appropriate antigenic stimulation is provided. It is likely, then, that foal 361 began to synthesize antibodies after exposure to *S. equi* and that this accounts for the rise in antibodies in nasal secretions and the absence of a decline in serum antibody. The absence of any clinical evidence of *S. equi* infection in this foal during the first weeks of life argues convincingly for the protective effect of antibody received in colostrum and later in milk.

Bradley et al. (1) and Morgan et al. (7) have suggested that colostral acquired immunoglobulins are transported onto the nasal secretions of newborn piglets and based their conclusions on immunohistochemical studies. Smith et al. (12, 13) and Wells et al. (18) found that colostrum-deprived lambs did not have detectable immunoglobulins in nasal secretions until 3 weeks of age, whereas normally nursed lambs had detectable levels of immunoglobulins, mostly IgG, as early as 24 h after birth. Similar findings have been reported for bovines (10, 19). Our radiolabeling studies clearly showed that passively acquired IgA was rapidly transported from the blood stream onto the nasal mucosa of newborn foals (Fig. 4). As Sheldrake et al. (11) have demonstrated, selective transport of serum IgA occurs at a number of mucosal sites and depends on secretory component availability. Because local antibody synthesis in newborn foals has not yet begun, large quantities of secretory pieces would be available for the transport of passively acquired polymeric IgA, quantitatively the most important form of this immunoglobulin in colostrum (4). We offer this explanation to partially account for the rapid increase of IgA antibodies on foal nasal mucosa shortly after birth.

The specificities of IgG and IgA from mare and foal sera and colostrum were the same (Fig. 5). Antibodies of these isotypes recognized several polypeptides in the different streptococcal antigen preparations and were the same as those of IgG and IgA from sera of horses recently recovered from strangles (2). These data do not support selective transfer of immunoglobulins of defined specificity from mares to foals.

IgG and IgA from milk samples taken a month or more into lactation had different specificities. These immunoglobulins recognized a selected repertoire of streptococcal polypeptides (Fig. 6). Most of the activity was directed against M protein fragments of 46,000 and 41,000 molecular weight in acid extracts of *S. equi* and a 130,000-molecular-weight polypeptide in acid extracts and culture supernatants of *S. equi*. Only one polypeptide, of 62,000 molecular weight, was recognized in acid extracts of *S. zooepidemicus*. In contrast, the reactivities of antibodies from serum samples taken at the same time (Fig. 6) were the same as those of colostrum and prefoaling serum samples (Fig. 5). These findings are of great interest since the M protein fragments recognized by milk antibodies are the same as those recognized by IgG and IgA from nasopharyngeal mucus of horses recently recovered from strangles (2). We have previously shown that a very limited number of streptococcal M protein fragments are immunologically active on the nasopharyngeal mucosa of adult horses and that antibodies against these are crucial in protection (2, 16). These polypeptides, as we showed in this study, are the same as those recognized by milk antibodies a month or more into lactation. At this time, milk antibodies are largely locally secreted (4) and are in the main originally derived from lymphocytes primed in other mucosal sites, such as the nasopharyngeal and intestinal mucosa (15, 17), where the priming antigen is immunologically active. It seems fair to conclude that antibodies directed against specific streptococcal epitopes exert a protective effect in newborn foals by coating the nasopharyngeal mucosa, a mechanism shown by others to be important in protection (8, 9). Our data indicate that these antibodies are derived both by secretion of passively acquired colostral immunoglobulins onto the nasopharyngeal mucosa and by immunoglobulins passively trapped in the upper respiratory tract mucosa after ingestion of milk by foals during the first months of life.

ACKNOWLEDGMENT

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