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Prospective study of progeny of inapparent equine carriers of equine infectious anemia virus

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SUMMARY

Progeny of a band of horses, positive by the agar-gel immunodiffusion (AGID) test for equine infectious anemia (EIA) antibody, were observed through their weaning over a 4-year period. Sentinels (AGID test-negative) were allowed to mingle with EIA-infected mares and their foals in pasture situations in an area with high populations of potential vectors. Of 27 adult sentinels, 8 (30%) seroconverted in annual rates ranging from 0% to 75%. In contrast, only 2 of 31 (6%) foals weaned became infected. Difference in infection rates between adult sentinels and foals was significant (χ^2, $P < 0.05$). Possible explanations for differences included protective value of colostral immunity and differences in attractiveness to blood feeding vectors. Detectable colostral immunity to EIA virus in the AGID test persisted for 25 to 195 days, with a mean of 124 days.

Equine infectious anemia (EIA) is caused by a retrovirus that induces a persistent infection in horses. Only a small percentage of infected horses have overt clinical signs of disease, ie, most infected horses are inapparently infected.[1] This infection can be detected indirectly by the use of an agar-gel immunodiffusion (AGID) test (Coggins test) for antibodies to the major core protein of EIA virus.[2,3] Control regulations that are based on this serotest assume that all horses infected with EIA virus develop detectable immune responses to EIA viral proteins and that horses are the only reservoir for EIA virus in nature.

In the United States, EIA virus infection is most commonly observed in the states bordering the Gulf of Mexico,[4] presumably because the semitropical environment favors a long season for blood-feeding vectors that mechanically transmit EIA virus. We estimate that > 30% of AGID test-positive horses in the southern parishes of Louisiana are without obvious clinical disease associated with EIA virus.[4] This high prevalence is partially indicative that many owners have maintained their AGID test-positive horses. Therefore, we have attempted to gather data to better advise producers of risks associated with maintenance of inapparently infected AGID test-positive horses.[5] The purpose of the present report was to observe the infection rate in foals from 1 breeding ranch in south-central Louisiana over a 4-year period.

Materials and Methods

Horses—The AGID test-positive mares (5 to 22 registered Thoroughbreds and American Quarter Horses) were assembled from various sources by a breeder in south-central Louisiana. Over a 4-year period, the herd was aggressively managed. Mares determined to be not pregnant by rectal palpation in the fall were usually removed from the band, and replacement mares were added during the next breeding season. Yearly, a fair percentage of principals were changed; however, the breeder attempted to add only mares in good physical condition with no known history of clinical disease compatible with EIA.

Five to 8 of the AGID test-negative adult sentinel horses were added to the band early each spring and were allowed to mingle with infected mares and their foals in pasture situations. They were removed from the band after the first killing frost each year and were isolated for at least 60 days to verify their AGID test status.

During the vector season, blood samples were collected at biweekly intervals from all horses at risk, ie, AGID test-negative sentinels and all foals from AGID test-positive mares. Samples were collected at less frequent intervals after the first killing frost. Samples were collected in evacuated glass tubes and were held for at least 2 hours for clot formation and retraction. Subsequently, serum was harvested, tested, and stored in (3.7-ml) sterile glass vials at −20 C until retested. When possible, blood samples were collected from newborn foals before they suckled for the first time and/or about 24 hours after birth, but pasture foaling precluded the collection of many of these samples.

Foals were usually weaned at 3 to 5 months old, depending on the physical development of the foal in question and the status of the AGID test reaction. During the weaning period, foals were held indoors in separate stalls at least 50 m from AGID test-positive horses until 2 negative AGID tests were obtained at 30-day intervals. At that time, foals were added to the foal crop from AGID test-negative mares (20 km from the infected herd) and were monitored for an additional 30 days for a confirmatory negative AGID test.

Serotesting—Serum samples were tested from all horses at risk at biweekly intervals, using the AGID test during the vector season. Commercially available reagents[a] were used.[3] All samples were stored. The appropriate series of samples were tested if AGID test status of the horse appeared to change. Sentinels and foals

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[a] Pitman Moore Inc, Washington Crossing, NJ.

were considered to have seroconverted according to the AGID test if they had 2 consecutive positive tests (sentinels) or if the intensity of their AGID test reactions obviously increased over 3 consecutive sample collections (foals). To determine extinction of AGID test-positive reactions in foals, all serum samples were tested side by side. The first negative sample was that which was the first to be determined to be unequivocally negative in the opinion of ≥ 2 or more individuals qualified to read the AGID test.

Results

Of 34 foals born to AGID test-positive mares, 31 survived to weaning age. Cause of death was not determined in the 3 foals that succumbed, but all 3 had declining titers in the AGID test (Table 1) and no clinical signs suggestive of EIA (ie, fever, weakness, listlessness, ataxia) were observed. The 2 foals that seroconverted had stronger reactions in the AGID test for the first time at 80 and 160 days of age, respectively. Detectable colostral immunity to EIA virus in the AGID test persisted in the 29 other foals for 25 to 195 days with a mean persistence of 124 days (Fig 1).

Collection of precolostral samples from 3 foals was possible, and all 3 proved negative on the AGID test. Twenty-four hour blood collections were made from these 3, and another 2 foals, and these samples proved to be the strongest AGID test reactions that were observed in these foals.

When we compared persistence of colostral immunity in successive foals from AGID test-positive mares, 7 of the 8 first AGID tested foals had AGID test-positive reactions that persisted about 30 days longer than those of the 2nd group of foals tested (mean values of 140 days and 103 days for the 1st and 2nd group of foals, respectively).

During 1979 and 1981, seroconversion of adult sentinels was not observed. When transmission to adult sentinels was observed, seroconversion rates of 28% and 75% were noticed (Table 1). Overall seroconversion rate in adult sentinels significantly exceeded that observed in foals of AGID test-positive mares (30% vs 6%) (χ^2, $P < 0.05$). When transmission to adult sentinels occurred at a 75% rate in 1980, transmission to foals in the same pastures did not occur (Table 1).

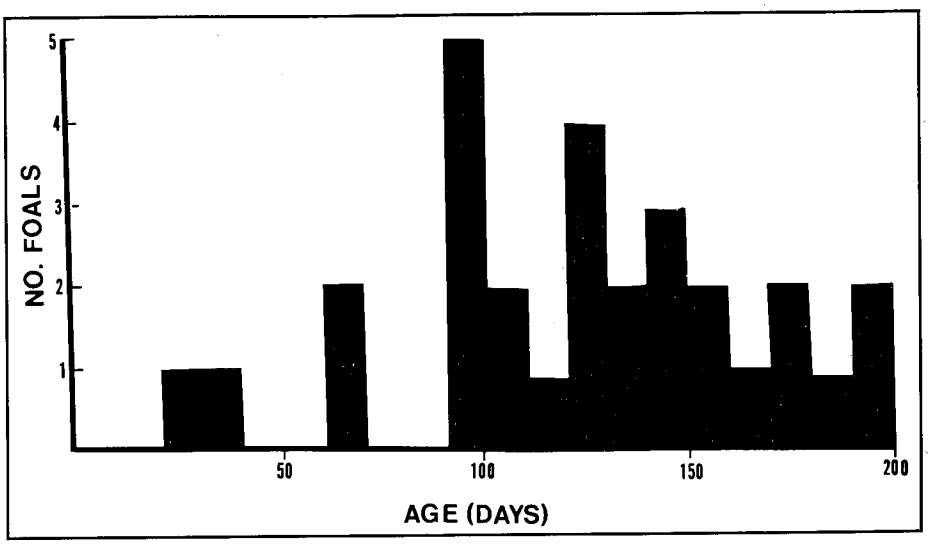

Fig 1—Persistence of detectable colostral antibody in the AGID test in foals of mares inapparently infected with EIA virus.

TABLE 1—Comparison of the seroconversion rate* of foals from AGID test-positive mares with adult sentinel horses

Horses	1979	1980	1981	1982	Total
INFECTED MARES					
No.	5	11	22	15	53
FOALS					
Total No.	5	8	6	15	34
No. weaned	5	8	5	13	31
No. seroconverting (%)	1(20)	0(0)	0(0)	1(7)	2(6)
ADULT SENTINELS					
No.	7	8	5	7	27
No. seroconverting (%)	0(0)	6(75)	0(0)	2(28)	8(30)

* Determined by repetitive biweekly testing with AGID.

Discussion

Foals are at lower risk of acquiring EIA virus infection than adult horses in constant pasture exposure in an area where potential mechanical vectors abound.[6] This was unexpected because the close physical contact and proximity of the foal with the mare would indicate a greater risk, especially for the refeeding of interrupted hematophagous insects.[7] Two explanations for the obvious protection appear most attractive. Colostral antibody transferred to the neonate could protect the foal against active infection with EIA virus. Although it is logical to assume that the neutralizing antibody to the strains of EIA virus isolate in the mare was transferred to the foal, its persistence at a protective amount for 4 months appears unlikely. In fact, the 2 foals that did seroconvert both had weak, but detectable, AGID test-positive reactions that markedly increased in intensity at 80 and 160 days of age, indicating a lack of protection from the colostral antibody. Furthermore, the AGID test-positive inapparent carriers in this study came from many different areas and were probably infected with several serologic strains of EIA virus. Although colostral immunity may protect against infection with a homologous strain for a period, it is unlikely that protection against infection with heterologous strains is afforded. This should be tested with appropriate subjects.

An alternative explanation is that foals could experience a lower burden of potential mechanical vectors than mares and consequently have a lower risk of acquiring infection by transmission via interrupted feeding of these vectors.[8]

Persistence of colostral immunity in any species is dependent on the degree of dam's immunity, the amount actively secreted into the milk, the amount transferred during the critical period before intestinal closure, and the amount absorbed by the newborn. Defects in any of these factors could result in a reduction or

a complete failure of passive transfer of immunoglobulins to the newborn, a phenomenon well documented in the horse.[9,10] Of the 31 foals raised, 2 had detectable AGID test-positive reactions for 25 and 36 days in contrast to the 67 to 195 day persistence in the other foals. Intensity of the AGID test reactions of dams of these foals was similar to that of other mares, indicating a decreased amount of passive transfer.

The question of persistence of AGID test reactions in foals of test-positive mares and in those negative or equivocal reactors must be reexamined in light of the ultrasensitive serologic procedures now available, eg, enzyme-linked immunosorbent assay. For example, we examined several of the same foal serum samples in enzyme-linked immunosorbent assay for anti-EIA virus p26 antibody and the samples were detected positive from 30 to 60 days longer when compared with that after the AGID test.[11] The sensitivity of the AGID test, as currently performed, could be increased by decreasing the strength of the commercially available reagents. However, under most conditions, with repeated testing, an adequate history, and correct interpretation of the AGID test reaction, samples with small amounts of antibody will not pose regulatory problems. Simultaneous testing of sequential samples with the same AGID test reagents are needed to compare accurately the AGID test reactions, because the AGID test is not a quantitative procedure as performed. This is important in determining the extinction point of colostral persistence, which controls the initiation of management recommendations to segregate and wean foals of sufficient age. Additionally, this practice can help identify foals with stronger AGID test reactions, suggestive of active immunity to EIA virus.

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