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Epidemiology of Equine Herpesvirus 2 (Equine Cytomegalovirus)

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The epidemiology of equine herpesvirus 2 was examined by using restriction endonuclease DNA fingerprints to distinguish viruses isolated from two groups of horses. The first group consisted of three yearlings isolated from other horses but in contact with each other for 418 days, whereas the second comprised seven mares and their foals, which were sampled at monthly intervals from parturition until the foals were about 180 days old. There was a complex pattern of transmission, with 15 different viruses isolated from both groups. Four distinguishable viruses were isolated from the three yearlings by day 16 of quarantine, and by day 141 an additional two viruses were isolated. Up to five different viruses were isolated from one yearling. Although four repeat isolations of one virus from the nasal cavity of one yearling over 54 days indicated that equine herpesvirus 2 established persistent infection with constant shedding, most repeat isolations yielded distinguishable viruses. Identical viruses were isolated from the nasal cavity and leukocytes of one yearling and the nasal cavity and vagina of another, indicating that a particular equine herpesvirus 2 strain was not site specific. Although seven different viruses were isolated from the three yearlings throughout the quarantine period, two appeared to establish latent infections; one virus was not isolated until 141 days after quarantine, whereas the second was first isolated 16 days after quarantine and then for the second time, from the same horse, 402 days later. Multiple concurrent local infections were demonstrated by the isolation of two or more viruses from the same nasal swab.

Equine herpesvirus 2 (EHV 2), also called equine cytomegalovirus (8, 23), is slowly cytopathic, highly cell associated, and relatively host cell specific in vitro (7, 12, 18, 23). EHV 2 can be isolated from nasal swab filtrates, presumably as cell-free virus, from many horses (7, 21, 24) and from leukocytes of 89% (11,16) of normal horses, presumably as cell-associated virus, when samples are inoculated onto "feeder" monolayer cultures of equine fetal kidney or primary rabbit kidney cells (3–5). EHV 2 has also been isolated from the conjunctiva, mammary gland, vagina, kidney, spleen, and bone marrow (3, 6–8, 12, 13, 18, 21). It can be isolated from nasal swabs or leukocytes for extended periods (1, 6, 7). Although a wide variety of diseases have been associated with EHV 2 (1, 18), only mild conjunctivitis and rhinitis and chronic follicular pharyngitis have been reproduced experimentally (1, 6). The role of EHV 2 in disease is confounded by the high prevalence of infection in apparently normal horses, and, by analogy with human cytomegalovirus, where symptoms of disease are often subtle and protean (22), the difficulty of detection of subjective manifestations of disease in a dumb animal (18).

There is evidence based on growth characteristics in cell culture and cross-neutralization studies (7, 15, 18, 21, 24) that EHV 2 isolates are heterogeneous, although common antigens have been recognized by complement fixation and immunofluorescence (12, 14). The significance of the heterogeneity in the pathogenesis and epidemiology of EHV 2 has not been determined.

The purpose of this study was to examine the degree of heterogeneity among EHV 2 isolates by using restriction endonuclease fingerprints of the virus DNA and to correlate the findings with available epidemiologic data.

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MATERIALS AND METHODS

Horses and viruses. Viruses (Table 1) were isolated from two groups which represented epidemiologically linked cohorts. One group comprised three yearlings that were stabled together, in isolation from other horses for 141 days and that remained together for 418 days (21). The second group consisted of seven mares and their foals that were sampled at monthly intervals from parturition, until the foals were about 180 days old (24). The site and time of isolation and the number of passages in equine fetal kidney cell culture are also shown in Table 1.

Plaque size determination. Viral stocks were diluted, inoculated onto confluent fifth-passage equine fetal kidney cell monolayers, and overlaid with methyl cellulose medium as described previously (20). After 14 days of incubation the overlay was removed, and the monolayer was stained with 1% crystal violet in 10% Formalin. The average plaque diameter was determined by measuring at least 15 well-isolated plaques.

Restriction endonuclease analysis. DNA was prepared from mock-infected and virus-infected equine fetal kidney cells, digested to completion with restriction endonucleases, separated by electrophoresis through 0.5% agarose submersion gels, stained with ethidium bromide, and photographed (19).

RESULTS

The means and standard deviations of the plaque diameters (Table 1) show that all isolates were slowly cytopathic and distinguishable from EHV 1, 4, and 3 (18). *Hin*dIII restriction endonuclease patterns (Fig. 1) indicated that many isolates are genomically distinct. Four isolates (1-34N, 1-57N, 2-397N, 3-16N; Fig. 1, lanes 3, 4, 10, and 13, respectively) produced more complex patterns than would be expected, and close examination revealed them to be composites of the patterns of at least two viruses. That these

TABLE 1. EHV2 isolates from three quarantined yearlings and seven mares and their foals

Fig. 1 lane no.	Sample designation[a]	Passage no.	Plaque diam (mm)
1	1-3B	2	2.0 ± 0.5
2	1-6N	2	2.1 ± 0.5
3	1-34N	2	2.9 ± 0.6
4	1-57N	2	3.4 ± 0.7
5	1-141N	9	2.5 ± 0.6
6	2-16V	5	3.3 ± 0.6
7	2-16N	3	3.0 ± 0.8
8	2-57N	5	3.1 ± 0.7
9	2-141N	4	2.2 ± 0.5
10	2-397N	4	2.0 ± 0.4
11	2-412N	4	2.4 ± 0.5
12	2-418N	4	2.8 ± 0.6
13	3-16N	3	1.9 ± 0.5
14	3-141N	5	2.0 ± 0.5
15	F1-N60	2	2.0 ± 0.6
16	F1-N150	3	2.1 ± 0.6
17	F1-N180	3	2.5 ± 0.5
18	M2-B0	4	1.7 ± 0.6
19	F2-N60	2	2.5 ± 0.5
20	M3-B30	4	2.0 ± 0.4
21	F4-N30	4	1.5 ± 0.4
22	F4-N120	2	2.6 ± 0.6
23	F4-N150	2	2.0 ± 0.5
24	M6-N30	2	1.5 ± 0.4
25	F7-B150	3	2.7 ± 0.4

[a] Prefix indicates horse number: F, foal; M, mare. Suffix indicates day of isolation and site of isolation: N, nasal cavity; B, buffy coat; and V, vagina.

isolates contain more than one distinguishable strain was verified by examination of the DNA of plaque purified 1-57N (Fig. 2, lane 4pp) (C. R. Wilks, MVSc thesis, University of Melbourne, 1973), which reproduced only part of the pattern seen in the initial isolate (Fig. 2, lane 4). Indistinguishable strains were isolated from one horse on two occasions (samples 1-3B and 1-6N; Fig. 1, lanes 1 and 2) and appeared to be among the mixed isolations on two subsequent occasions (1-34N and 1-57N; Fig. 1, lanes 3 and 4), covering a period of 54 days, but most other repeat isolations yielded distinct strains. One horse yielded five different strains over a 402-day period (2-16N and 2-16V, 2-57N, 2-141N, 2-397N, and 2-412N; Fig. 1 lanes 6 and 7, 8, 9, 10, and 11). Identical viruses were isolated at about the same time from the buffy coat and nasal passages of one horse (1-3B and 1-6N; Fig. 1, lanes 1 and 2) and the nasal passages and vagina of another (2-16V and 2-16N, Fig. 1, lanes 6 and 7). One virus was reisolated from the same horse after an interval of 402 days (2-16V, 2-16N, and 2-418N; Fig. 1 lanes 6, 7, and 12), during which it was not among the viruses isolated from the other two horses. Similarly one virus isolated from two horses 141 days after the horses were placed in isolation had not been isolated previously (2-141N and 3-141N; Fig. 1 lanes 9 and 14.)

Whereas most isolates from the mares and their foals are distinct, a single strain was isolated from two foals on the same day (F1-N60 and F2-N60; Fig. 1, lanes 15 and 19) and from one of these 3 months later (F1-N150; Fig. 1, lane 16).

DISCUSSION

The features of the epidemiology of EHV 2 revealed in these studies show some contrast to those of other betaherpesviruses, including human cytomegalovirus. Multiple concurrent infections by human cytomegalovirus have only been documented in severely immunosuppressed hosts (2, 17), whereas in immunocompetent persons reinfection by different strains is less common than persistent infection by a single virus (9, 10). In contrast, at least two different EHV 2 viruses were excreted concurrently from the same site in a normal horse, and there was also evidence that reinfection, or perhaps reactivation of different viruses, was much more common than persistent infection and constant shedding of a single virus. It was not established whether concurrent infection and reinfection by different viruses occurred systemically, assessed by buffy coat cell isolation, as well as locally.

Isolation of the same virus from various sites in the same horse indicated the absence of site specificity for a particular

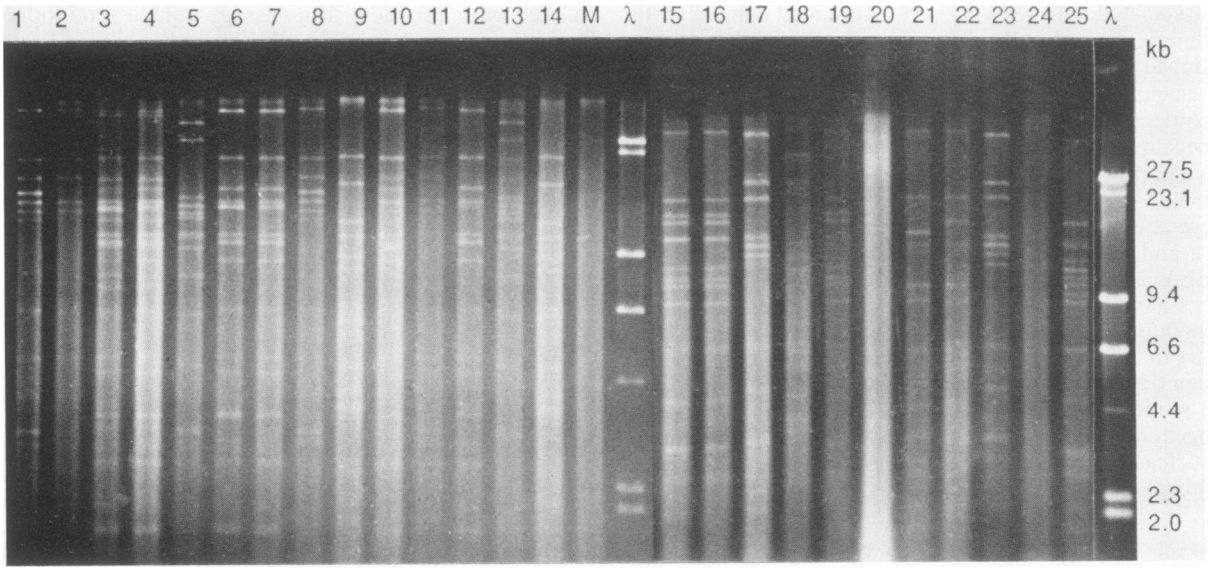

FIG. 1. *Hin*dIII restriction endonuclease fingerprints. Lanes are numbered as in Table 1. λ DNA cut in *Hin*dIII was used as a kilobase marker. M, Mock-infected cell DNA.

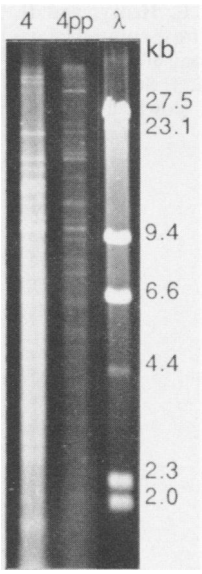

FIG. 2. *Hin*dIII restriction endonuclease fingerprints of isolate 1-57N and a plaque-purified virus obtained from 1-57N showing that the original isolate was a mixture of at least two viruses. λ DNA cut with *Hin*dIII was used as a kilobase marker.

virus. EHV 2 established persistent infections with sustained shedding and latent infections with intermittent shedding, and different viruses were excreted from the nasal passages during these persistent or latent infections. The isolation of identical EHV 2 viruses from two young foals on the same day demonstrated the importance of horizontal transmission. The contrast with human cytomegalovirus infection of neonates, where vertical transmission predominates (9, 10), may be attributable to the more gregarious behavior of neonatal foals and the apparent absence of intrauterine infection by EHV 2 (7, 19).

The pattern produced by the plaque-purified 1-57N isolate differed from those of the other viruses isolated from the horses in contact. The plaque-purified virus may be a recombinant of the different viruses, produced by in vitro passage at relatively high multiplicity of infection. However, heterogeneity data recorded in these studies may result at least in part from frequent in vivo recombination. It is not possible to determine whether the three yearlings were infected by seven different viruses when first quarantined, or whether some of these viruses are the result of mutation or recombination of a smaller number of original viruses. However the differences observed in the restriction patterns are not trivial; they correlate with plaque diameters and antigenic differences (15, 21). Frequent reinfection by antigenically different EHV 2 viruses may have pathogenetic implications. Some viruses may be sufficiently different antigenically from those encountered previously to allow systemic infection and possibly recurrent disease. Repeated infection by partially related strains may result in a chronic local pathological response, as has previously been reported (1). Three isolates (2-141N, 3-141N, and M2-BO; Fig. 1, lanes 9, 14 and 18) share no comigrating restriction fragments and show less homology with other EHV 2 isolates as assessed by Southern blot hybridization under stringent conditions (G. F. Browning and M. J. Studdert, unpublished data). However, both the in vivo mutability of EHV 2 and the clinical consequences of reinfection by different EHV 2 viruses have yet to be elucidated.

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