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Rotavirus Serotype G3 Predominates in Horses

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Foal fecal group A rotavirus strains were characterized by electropherotype, serotype, and subgroup and shown to be distinctly different from rotaviruses of other mammals. Of 86 strains that were electropherotyped, 98% had similar profiles, with gene segments 3 and 4 close together and segments 7, 8, and 9 widely spaced. Of 70 strains that had sufficient detectable VP7 antigen to be serotyped by enzyme-linked immunosorbent assays (ELISAs), 63% were serotype G3 (39% were subtype G3A and 24% were subtype G3B), 4% were serotype G13, and 33% were untypeable. Serotypes G1, G2, G4, G5, G6, G9, G10, and G14 were not detected, although G5 and G14 strains have been identified among cultivable equine strains. Of 50 strains that had sufficient detectable VP6 antigen to be subgrouped by ELISAs, only 12% were able to be assigned to either subgroup I or II, with the remaining 88% belonging to neither subgroup.

Group A rotaviruses are a major cause of diarrhea in foals of up to 3 months of age (6, 12). An effective vaccine design is dependent on a knowledge of the prevalence of different rotavirus serotypes circulating within the horse population. Additionally, aspects of the epidemiology of rotavirus infections can be further investigated by determining the serotype and subgroup of the infecting virus.

The major neutralization antigen on group A rotaviruses is VP7, variants of which determine G serotypes. This distinguishes them from variants of the minor neutralization antigen, VP4, which determine P serotypes (24). Fourteen G serotypes have been described (5, 8, 10, 21, 26, 28, 31, 32), with serotypes G3, G5, G13, and G14 being found among equine rotavirus isolates, including two subtypes of G3 (G3A and G3B) identified among equine viruses (5, 7, 8, 20, 22, 23). Thirteen cell culture-adapted equine rotavirus isolates from the United States reacted with antiserum to equine G3 strain H2, suggesting that they may all belong to serotype G3 (18). However, the relative prevalence of these G serotypes in field samples from the horse population has not been investigated.

Further epidemiological information can be derived from the determination of the two rotavirus subgroup antigens on VP6. Equine rotaviruses which carry neither subgroup I nor II, both subgroup I and II, or only subgroup I specificities have been identified (7, 20, 22, 23), but again, the relative prevalence of these specificities among rotaviruses in the field has not been determined.

The determination of rotavirus G serotypes has been facilitated by the production of G serotype-specific neutralizing monoclonal antibodies (MAbs), which have been used in enzyme-linked immunosorbent assays (ELISAs) to determine rotavirus serotypes in feces and thus to determine the prevalence of various serotypes among rotaviruses infecting children and calves (1–4, 11, 14, 27, 31, 33, 34). Similarly, subgroup-specific MAbs have been used in ELISAs to determine the subgroup of rotaviruses in fecal samples (1, 16).

The aim of this study was to determine the prevalence of different rotavirus subgroups and G serotypes in fecal samples from diarrheic foals and to examine the consequences of this for future vaccine development.

MATERIALS AND METHODS

Fecal samples. Feces were collected from diarrheic Thoroughbred foals in Britain and Ireland from 1987 to 1989 (6). Diagnosis and electropherotyping of rotaviruses were performed by silver staining of the double-stranded RNA genome separated in polyacrylamide gels (19).

MAbs. Assays were performed by using VP7-specific MAbs 2C9 (recognizes G1), IC10 (recognizes G2), 4F8 (recognizes G3A), 159 (recognizes G3, G10, and G13), 5B8 (recognizes G5), UK7 (recognizes G6), B223/3 (recognizes G10), B223/4 (recognizes G3A and G10), 57/8 (recognizes G3, G4, G6, G9, G10, and G14), and 60A1 (recognizes all G types) (7, 17, 25, 29–31). VP6-specific MAbs 255/60 (recognizes subgroup I), 631/9 (recognizes subgroup II), and UK1 (produced by immunization with UK virus and recognizes common VP6 epitope) were also used (9, 10).

Standard serotype and subgroup strains. Reference rotavirus serotype strains used were Wa (G1), DS-1 (G2), RRV (G3), ST-3 (G4), OSU (G5), UK (G6), and B223 (G10) (21, 31). DS-1 (subgroup I) and Wa (subgroup II) were used as reference subgroup strains (17).

ELISAs. Both serotyping and subgrouping ELISAs were performed essentially as described previously (13). Briefly, separate Nunc Maxisorb ELISA microplates were coated with mouse ascitic fluid containing each MAb diluted in carbonate-bicarbonate buffer overnight at 4°C. The plates were then incubated with dilution buffer alone for 1.5 h, and cell culture lysates of standard rotavirus strains and test fecal suspensions were added to each plate. Next, a diluted polyclonal rabbit antiserum to calf rotavirus was added (the same serum was used for all tests), and finally, a sheep anti-rabbit immunoglobulin G conjugated with alkaline phosphatase was added before development with an appropriate substrate. Phosphate-buffered saline containing 10% ultra-high-temperature-treated skim milk, 0.05% Tween 20, and 0.5 mM $CaCl_2$ was used as a reagent diluent, and phosphate-buffered saline containing 0.05% Tween 20 and 0.5 mM $CaCl_2$ was used as a washing buffer throughout.

The results were expressed as the mean optical density at

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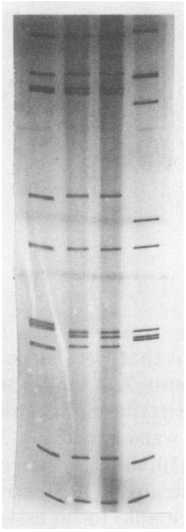

FIG. 1. Electropherotype of fecal equine rotaviruses showing the pattern typical of 84 of 86 of the viruses examined. Lanes, from left to right: the standard equine rotavirus isolate H2; equine field strains R2437/6 and R2437/3; porcine isolate OSU, showing typical nonequine mammalian electropherotype similar to that observed in the two unusual equine strains.

405 nm (OD_{405}) in two wells. These means for each sample for each serotype were divided by the lowest serotyping OD_{405} obtained with that sample: a sample with a value of >5 and with a test OD_{405} of >0.10 was considered positive for that MAb.

On the basis of reactivity patterns observed with MAbs to well-characterized cultivable strains of equine rotavirus (7), samples were assigned to different G serotypes. G3A viruses were positive with MAbs 4F8, 159, and 57/8; G3B viruses were positive with MAbs 159 and 57/8; G13 viruses were positive with MAb 159 only; and G14 viruses were positive with MAb 57/8 only. Viruses were regarded as untypeable when they were positive with MAb 60A1 but not with any other VP7-specific MAb.

In the subgrouping assays, the ELISA result for each sample was compared with those obtained with the reference strains. When the OD_{405} obtained with the sample was >0.1 and at least three times greater than the value for the negative control, the virus was considered positive for that subgrouping MAb. Those samples which were positive (OD_{405}, >0.1) for MAb UK1 but not for either subgrouping MAb were classified as belonging to neither subgroup.

RESULTS

Eighty-six fecal samples from different diarrheic foals were identified by polyacrylamide gel electrophoresis as containing group A rotaviruses. Eighty-four of these rotaviruses had similar electropherotypes which were distinctive from those usually found in other species, with gene segments 3 and 4 close together and segments 7, 8, and 9 widely spaced (Fig. 1). Within this overall pattern, those from epidemiologically distinct outbreaks had distinguishable electropherotypes.

All 86 samples were examined in serotyping ELISAs, and there was a sufficient amount of 64 samples to test them in subgrouping ELISAs. Those samples with insufficient antigen to be successfully serotyped or subgrouped were identified by their failure to react with MAb 60A1 (OD_{405}, <0.1), which recognizes a VP7 epitope common to all group A rotaviruses, and MAb UK1, which recognizes a VP6 epitope common to all group A rotaviruses, respectively. A total of 64 fecal rotavirus samples were tested against MAb UK1, with 50 (78%) reacting and 14 (22%) failing to react. A total of 86 fecal rotavirus samples were tested against MAb 60A1, with 70 (81%) reacting and 16 (19%) failing to react.

TABLE 1. Serotype reactions of 70 equine rotaviruses with sufficient VP7 antigen

MAb(s) (serotype)	No. reacting in ELISA (% of total)
4F8, 159, 57/8 (G3A)	27 (39)
159, 57/8 (G3B)	17 (24)
159 only (G13)	3 (4)
57/8 only (G4, G9, or G14)	0 (0)
2C9, IC10, 5B8, UK7, B223/3 (G1, G2, G5, G6, G10)...	0 (0)
None (untypeable)	23 (33)

In the serotyping ELISAs, the 70 samples with detectable VP7 antigen exhibited four differing reactivity patterns. Thus, 27 samples reacted with MAbs 4F8, 159, and 57/8, suggesting that they were serotype G3A; 17 reacted with MAbs 159 and 57/8 only, suggesting that they were serotype G3B; 3 reacted with MAb 159 only, suggesting that they were serotype G13; and 23 did not react with any of the serotype-specific MAbs (Table 1).

In the subgrouping ELISAs, three reactivity patterns were observed among the 50 samples with detectable VP6 antigen. A total of 2 samples (4%) were detected only by the subgroup I MAb (MAb 255/60), 4 (8%) were detected only by the subgroup II MAb (MAb 631/9), and 44 (88%) were not detected by either MAb.

DISCUSSION

Of the four G serotypes among equine rotaviruses which have been characterized, only G3 and G13 were identified in field samples in this study. Of the two, G3 was predominant, representing 63% of the samples with detectable VP7 antigen, with monotype G3A more prevalent than G3B. G13, the only other serotype identified, accounted for only 4% of the samples.

The serotyping ELISAs used in this study were able to detect all rotavirus serotypes except G7, G8, G11, and G12, yet 33% of the samples were untypeable. It is possible that the untypeable viruses may belong to novel G serotypes or to G8, G11, or G12, none of which would have been detectable by these assays. As G7 viruses have been identified only in poultry (21), it is unlikely that this serotype is prevalent in horses. This observation suggests that G serotypes, other than the four which have been described, are circulating in the Thoroughbred horse populations of Britain and Ireland.

The prevalence of G13 and G14 equine rotaviruses appears to be low. However, as only single isolates of each of these G serotypes have been characterized, some caution must be exercised in interpretation of these results. It may be that most G13 and G14 viruses are not recognized by the MAbs used in this study, so definitive evidence of their prevalence must await the derivation of G13- and G14-specific MAbs.

In subgrouping assays, only 12% of the samples were able to be assigned to either subgroup I or II, with the remainder

reacting with neither subgroup MAb. In a study of cultivable equine rotaviruses, the majority of isolates reacted with both subgroup MAbs at a low level. It is possible that many of the fecal samples examined here had low-level reactivity with both subgroup MAbs, as the OD_{405} values observed in assays with the common VP6 MAb were much lower than those seen in assays with cell culture-adapted rotaviruses (data not shown). Additionally, the OD_{405} values for the two samples detected by the subgroup I MAb were low (<0.12), suggesting that these viruses may also be detected by both subgrouping MAbs at a level beyond the sensitivity of the assay.

Although samples from any one outbreak were the same serotype, there was no evidence of geographical restriction of any serotype. On one stud farm, G3A, G3B, and untypeable rotaviruses were identified in the same year. Similarly, there was no apparent correlation between the age of the foal and the rotavirus serotype or between the subgroup and the serotype of the virus (data not shown).

An association between outbreaks of rotavirus infections on stud farms and the introduction of foals has been made (12). The lack of geographical restriction of serotypes suggests that all serotypes are probably endemic and that the association with introduction is probably due to multiplication and dissemination of an endemic rotavirus strain by a stressed foal rather than to the introduction of a novel serotype.

It is notable that two G serotypes which have been identified among cultivable equine rotaviruses were not detected in this survey. The G14 equine rotavirus isolate FI23 originated in the United States (7, 15), so it may be that this serotype is more common among North American horses or breeds other than Thoroughbreds. The absence of G5 viruses is less explicable. The equine G5 isolate H-1 originated in the same area as many of the samples in this survey (7, 22). Furthermore, unlike FI23, its electropherotype is quite different from those of other equine rotaviruses, and it reacts strongly with the subgroup I MAb in ELISA. These observations suggest that H-1 is either unusual or possibly a laboratory contaminant of porcine origin. A second cultivable equine rotavirus, P9, which appears to be serotype G10 also has an unusual electropherotype and a strong subgroup I reaction (6). Like H-1, it was originally isolated from the area surveyed in this study, so it may also be a laboratory cross contaminant.

This study confirms that rotavirus serotypes are relatively host restricted. In studies of serotypic prevalence among rotavirus isolates from human infants, G1 generally predominates, although G2, G3, G4, G8, G9, and G12 also occur (1–3, 11, 14, 27, 33, 34). In calves, the serotypic diversity is even more restricted, with G6 being the most predominant and only G10 and G8 being found among the remainder of isolates (4, 31). This report, together with the previous study in the United States (18), shows that G3 is the predominant rotavirus serotype in horses and that equine rotaviruses form an epidemiologically separate population, with a distinctive electropherotype and unusual subgroup reactivity.

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