Prevalence of G and P serotypes among equine rotaviruses in the faeces of diarrhoeic foals

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Summary. Variant types of VP4 and VP7 gene segments of faecal rotaviruses from diarrhoeic foals were identified by restriction endonuclease digestion of reverse transcription/polymerase chain reaction (RT/PCR) products. The variants observed were correlated with serotypes by determination of the sequence of representative RT/PCR products (entire coding sequence for VP7 and the VP8 region of VP4) and comparison to published sequences of equine G and P serotype genes. Both G and P serotypes could be predicted for 95/116 (82%) strains, P serotype only for a further 8 (7%) strains and G serotype only for 1 (1%) strain. All characterised strains belonged to the same P serotype, P12, although minor sequence variations were observed. Of those strains able to be assigned to G serotypes, 84/96 (87.5%) belonged to serotype G3A, and 12/96 (12.5%) belonged to serotype G14. Comparison of G serotyping by ELISA to the RT/PCR method showed that serotyping equine rotaviruses by currently available ELISA methods was prone to error. This study establishes the restricted serotypic diversity of equine rotaviruses, and the significance of serotype G14.

Introduction

Rotavirus infections are the most common cause of diarrhoea in young foals, with microbiological surveys incriminating them in at least 38% of cases in foals younger than 3 months of age [5, 7, 9, 28, 41]. Epidemiological and immunological studies of these infections are enhanced by the ability to determine the serotypes of the viruses involved. Rotaviruses have two distinct outer virion proteins, VP4 and VP7, which are encoded by separate genomic segments, and hence can segregate independently. Fourteen different serotypes of the VP7 (G serotypes) have been defined, and all equine rotaviruses have been assigned to serotypes G3 (subtypes G3A or G3B), G5, G13 or G14 [2–4, 6–8, 21, 24, 25, 28, 41, 53]. The serotyping of VP4 (P serotypes) is less established, but at least 20
distinct serotypes have been reported, with all characterised equine rotaviruses assigned to P7, P12 or P17 [20, 30, 53, 54].

While G serotypes are relatively easy to determine on cell culture adapted strains using reciprocal cross neutralization assays [23], P serotype definition has depended upon the production of baculovirus expressed VP4 segments, or the generation of reassortant viruses [13, 15, 30, 32, 33, 38, 48, 51]. Similarly while determination of G serotypes of rotaviruses in faeces has been facilitated by the availability of serotype specific monoclonal antibodies [18, 34, 49], which enabled the development of serotyping ELISAs, the lack of similar reagents for P serotypes has hampered examination of their distribution in the field. As a result, while the prevalence of G serotypes among equine rotaviruses has been examined, and subtype G3A found to be the most prevalent [2, 7], the prevalence of P serotypes is unknown. Thus the association, if any, between particular G and P serotypes, as occurs in rotaviruses of other species [31, 42, 45-47, 52], is unknown.

Polymerase chain reaction based assays have been developed for P serotype prediction and have been applied to human isolates [11], but they have depended upon a knowledge of the sequence of the genes encoding all expected serotypes. This approach is not appropriate for species in which the range of serotypes has not been established. A more suitable approach in this situation is to amplify a portion of all VP4 genes and then differentiate variants by restriction endonuclease polymorphisms [27]. However, this approach has not been used on faecal samples.

Accordingly, the aim of this study was to develop polymerase chain reaction based assays which would function independently of the serotypes of the rotavirus examined, yet enable prediction of the serotypes of the virus. These assays would then be used to examine the prevalence of different G and P serotypes amongst equine rotaviruses in the faeces of diarrhoeic foals, and the association, if any, between particular G and P serotypes.

Materials and methods

Faecal samples
Faecal samples from 116 diarrhoeic foals excreting rotaviruses were used in this study. Rotaviruses were detected in faeces by separation of faecal nucleic acid in polyacrylamide gels and staining with silver [22]. The samples were collected over the period 1990–1995 and came from foals on 22 different Australian stud farms, all but one of which bred exclusively Thoroughbreds.

Polymerase chain reaction assays
The entire VP7 gene sequence and the VP8 section of the VP4 gene sequence were amplified essentially as described by Gouvea et al. and Gentsch et al. [11, 16]. The VP7 primers (5' to 3') were GGTCTTTAAAAGAGAGAATTTCCGTCTGG and GGTCACATCATAC-AATCTAAG, corresponding to bases 1 to 28 and 1036 to 1062, respectively, of the VP7 gene sequence. The VP4 primers (5' to 3') were TGCTGCGCCAATTTTATAGACA and