Epidemiology of Mexico Virus, a Small Round-Structured Virus in Yorkshire, United Kingdom, between January 1992 and March 1995

David C. Lewis, Antony Hale, Xi Jiang, Roger Eglin, and David W. G. Brown

The epidemiology of the small round-structured virus, Mexico virus (MxV), was investigated in North and West Yorkshire, United Kingdom, between January 1992 and March 1995 using a type-specific antigen ELISA. The results indicate that an epidemic of MxV occurred during the winter of 1993–1994, when this strain was associated with 45 of 99 outbreaks and sporadic childhood cases of gastroenteritis. Only 4 MxV-like isolates were found during the 1992–1993 winter season and none in the 1994–1995 season. This descriptive epidemiologic study suggests that MxV has an epidemic pattern of infection.

Materials and Methods

Specimen preparation and EM. Fecal suspensions were made as 20% wt/vol in PBS, pH 7.2, and extracted by the addition of 1 mL of arkone (1,1,2 trichlorotrifluoroethane) to 2–5 mL of fecal suspension and vortexing for 20 s. Suspensions were then centrifuged at 2500 g for 30 min, and the supernatant was used to make antigen-capture ELISA for MxV has been used to survey the occurrence of MxV-like strains in the United Kingdom (UK), showing the sporadic appearance of these strains in adult outbreaks and sporadic cases in infants since 1983 [9]. However, this ELISA has not yet been applied to intensively survey the epidemiology of MxV in a discrete time period and region. We now report the use of this ELISA, as a type-specific assay [5], to describe the epidemiology of MxV using a collection of SRSV strains identified by EM from outbreaks and sporadic childhood cases of gastroenteritis submitted to a large regional virus laboratory covering North and West Yorkshire between January 1992 and March 1995.

Received 22 July 1996; revised 23 October 1996.
Reprints or correspondence: Dr. David C. Lewis, Public Health Laboratory, Bridle Path, York Road, Leeds LS15 7TR, UK.
The Journal of Infectious Diseases 1997;175:951–4
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0022-1899/97/7504–0033$01.00
fecal supernatants were available from 198 of 225 outbreaks for testing in the rMxV ELISA, all of which contained virus with typical SRSV morphology by EM. Of the outbreaks, 188 involved adults only and 10 involved children; 91 (46%) were on hospital wards, 83 (42%) were in residential homes for the elderly, 10 were in hotels and restaurants, 4 were in schools, 4 were family outbreaks, and 6 occurred in other situations. From 1 to 3 specimens were tested per outbreak (mean, 1.9) as availability allowed. SRSV-positive extracts were also tested from 109 children <10 years of age (mean, 1.9 years; range, 1 month to 10 years) who had sporadic cases of gastroenteritis. Thus, a total of 489 SRSV EM-positive extracts were tested, 380 from outbreaks and 109 from children.

**Detecting MxV-positive fecal specimens by ELISA and reverse transcription–polymerase chain reaction.** SRSV-positive fecal extracts were diluted to ~10% in 1% skim milk in PBS and tested in the rMxV ELISA as previously described [5]. The positive control (specimen RBH/93/UK previously characterized as MxV-like by sequencing) [5] and negative control (1% skim milk/PBS) were included with each test run. Samples were considered positive if the optical density (OD) in the well coated with hyperimmune rabbit antiserum was >0.1 and the ratio of the ODs in the wells coated with hyperimmune rabbit antiserum and preimmune rabbit antiserum (P/N) were >2. All specimens initially reactive in the assay were retested. All positive specimens with P/N values >6 were considered to contain MxV-like strains, and those giving a low reactivity in the assay (P/N value 2–6) were genetically characterized by performing reverse transcription–polymerase chain reaction using primer pairs N1/E3 and 36/E3 followed by sequencing of the interprimer regions [5]. Strains with >90% sequence similarity to MxV were classified as MxV-like; otherwise, strains were classified as either SRSV genogroup 2 or genogroup 1 strains following phylogenetic analysis [6].

**Results**

The distribution of SRSV-positive fecal specimens from persons involved in outbreaks in North and West Yorkshire indicates a pattern of winter epidemics with three periods of seasonal SRSV peak activity observed during this 39-month study (figure 1). Of 489 fecal samples tested in the rMxV ELISA, 25 (23%) of 109 children’s specimens were highly reactive (P/N >6), confirming these strains as MxV-like. Specimens from 26 (13%) of 198 outbreaks were also identified as MxV-like. Samples from 23 outbreaks were highly reactive, and specimens from 3 outbreaks gave a low reactivity (P/N values of 2–6) but were confirmed to be MxV-like by sequencing of polymerase chain reaction amplicons. Of the remaining specimens, only samples from 2 outbreaks were positive in the MxV assay, with P/N <6, but these were identified as non-MxV SRSV genogroup 2 viruses by genetic characterization.

The 26 MxV-positive outbreaks identified did not show any geographic clustering likely to indicate a common source but occurred in eight different urban areas distributed throughout the region. However, 3 outbreaks affected wards of hospitals where there had been MxV outbreaks 3–12 weeks earlier. There were 15 hospital ward outbreaks, 7 in homes for the elderly, 2 in hotels, 1 in a university, and 1 in an adult training center. This mix of locations reflects the range of outbreaks tested.

The MxV-like strains identified were concentrated in a 10-month period from September 1993 to June 1994, during which 40% of outbreak strains and 59% of strains from sporadic cases were MxV-like (figure 1). All MxV-like outbreaks occurred during this period and most of the sporadic cases, with the exception of 4 sporadic cases that occurred during the summer prior to the MxV epidemic and 2 isolates from children obtained early in 1992.

Sequences of a 359-base region of the RNA-dependent RNA polymerase, corresponding to the interprimer sequence of the 36/E3 amplification product, of 12 MxV-like strains obtained throughout the MxV epidemic show >97% sequence similarity to each other and to the prototype MxV strain. There were, however, minor sequence differences between outbreaks (2–8 nt changes from the prototype MxV), indicating that a single common source of infection was unlikely.

**Discussion**

Antigenic typing methods for SRSVs have been described using IEM and SPIEM [4, 10]. However, little descriptive epidemiology exists, as these methods are time-consuming, require a great deal of expertise, and need well-characterized acute and convalescent human sera. An antigen-detection ELISA based on recombinant Norwalk virus has been extensively used but has not revealed epidemiologically interesting results because Norwalk virus is not circulating widely [11]. Use of a similar assay based on rMxV indicates that MxV is currently circulating [5, 8, 9]. The rMxV antigen ELISA has been shown to be specific for MxV-like strains if a sufficiently high cutoff is used. Low reactive strains are either MxV-like strains with a low virus load or other SRSV genogroup 2 viruses that exhibit low-level cross-reactivity [5]. The precise sensitivity of the MxV ELISA has yet to be established. In this evaluation, specimens were selected that had a relatively high virus load as determined by direct EM. This, together with the low number (5 of the 53 positive) of strains producing a low reactivity in the assay, of which 3 were MxV-like, suggests a high sensitivity of the assay with this collection of specimens. The precise sensitivity of the assay could only be established by genetic characterization of all 252 strains unreactive in the test.

This retrospective analysis of 307 SRSV strains obtained from outbreaks and sporadic childhood cases of gastroenteritis over a 3-year period indicates that an epidemic of MxV occurred in North and West Yorkshire in the winter of 1993–1994. At the height of this epidemic in December 1993, 15 (94%) of 16 SRSVs were MxV-like. The MxV epidemic coincided with the winter peak of SRSV activity, and although MxV is the predominant strain during this SRSV season, it
is apparent that other strains were cocirculating. SPIEM and sequence analysis of a number of non-MxV SRSVs submitted during this period indicate that both SRSV UK3 and UK2 viruses were also present (data not shown); however, the precise contribution of other SRSV genotypes during this season remains to be established. The prevalence of SRSVs increased over the study period (figure 1), a trend that was observed throughout the UK [12]. Whether this reflects a true increase in the numbers of SRSV-associated outbreaks or increased awareness or investigation of outbreaks of gastroenteritis is unknown.

The SRSV season preceding and that directly following the MxV epidemic are notable by the complete absence of MxV. Four MxV-like isolates were obtained from children during the summer of 1993, heralding the onset of the MxV epidemic, and 2 others were identified in early 1992, when only a handful of SRSV strains were available for testing. A similar epidemic pattern of MxV was observed in the Netherlands at the same time [13]. Additional UK MxV-like strains were also identified from children in 1987, 1990, and 1992 (data not shown). MxV was originally obtained from a child with gastroenteritis in 1989 [14], and similar strains have been isolated from other

![Figure 1. Distribution of small round-structured viruses by month from outbreaks (198; lower axis) and from sporadic cases (109; upper axis) in children. Solid bars, MxV ELISA-positive outbreaks or sporadic cases; hatched bars, ELISA-negative outbreaks or cases; open bars, untested outbreaks.](image-url)
parts of the world, including the United States, Canada, UK, and Japan [6, 7, 9, 15]. Clearly, MxV circulated in the UK and elsewhere prior to the 1993–1994 epidemic [9]. Two epidemiologic patterns can be put forward to explain these observations: frequent UK MxV epidemics (to account for the isolation of MxV-like strains in 1987, 1990, and 1992), with the virus maintained by global circulation, and alternatively, endemic MxV in the UK, possibly maintained in children, with epidemics occurring following spread into the adult population. Volunteer studies indicate that immunity to SRSVs is short-lived; thus, herd immunity following an epidemic with subsequent decline of protective antibodies may play a role in the dynamics of MxV epidemics. The periodicity of these epidemics has yet to be ascertained, and it will be interesting to prospectively monitor SRSVs to identify the reappearance of MxV.

The sequence data indicate that MxV remained genetically stable throughout the epidemic and, indeed, the UK strains are very similar to prototype MxV. Although SRSVs are genetically diverse, these data suggest that individual genotypes are relatively stable, and this diversity more likely reflects the existence of a large number of distinct genotypes rather than genetic drift within genotypes. Whether the epidemic pattern we describe for MxV in the UK is typical of other SRSV antigenic types remains to be determined. This study describes the use of a rapid method for typing MxV based on hyperimmune animal antisera raised to recombinant capsid proteins. It is likely that similar assays will be developed for other SRSV antigenic types that will allow investigation of their epidemiology and eventually allow a fuller understanding of the circulation of this group of viruses. Without such an understanding, the precise role, use, and likely impact of potential vaccines based on recombinant capsids will be impossible to establish.

References