Short Communication

Evidence of Rotavirus Associated with Neonatal Lamb Diarrhoea in India

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; N, negative; OPD, o-phenylenediamine; P, positive; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline–Tween; RNA, ribonucleic acid

INTRODUCTION

Rotaviruses are an antigenically complex group of viruses that cause diarrhoea in humans and livestock (Saif, 1990; Theil, 1990). These viruses are subdivided into seven antigenically distinct groups, designated A to G, with members in a group sharing common antigens (Kapikian and Chanock, 1996). Most rotaviruses detected in clinical specimens belong to group A. Ovine rotaviruses, in contrast to the globally distributed rotaviruses infecting other livestock, have only been reported from a few countries, including Morocco, UK, USA, Australia (Chasey and Banks, 1984; Fassi-Fehri et al., 1988; Ellis and Daniels, 1988; Fitzgerald et al., 1995; Theil et al., 1995). Group A and group B rotaviruses both infect lambs (Snodgrass et al., 1976), but group B rotaviruses have been shown to be a more common cause of rotavirus diarrhoea in neonatal lambs (Chasey and Banks, 1984). Although rotaviruses have been isolated from the calves of cattle and buffaloes and from pigs in India, there seem to be no reports on ovine rotavirus from India. The present communication, which describes the detection of a group A rotavirus from an outbreak of diarrhoea in lambs in the Kashmir valley, is therefore the first report on ovine rotavirus from India.
MATERIALS AND METHODS

An outbreak of acute diarrhoea occurred in 30–45-day-old suckling lambs born and raised in an organized sheep-breeding farm in Kashmir valley. The outbreak began in the third week of April 2001, about 2 months after the start of the lambing season, and continued for 2 weeks. The local ambient temperature and relative humidity during the outbreak ranged from 7°C to 31°C and from 39% to 85%, respectively. Affected lambs were diarrhoeic for 3–4 days and voided milky white, liquid, malodorous faeces. Other signs included lethargy and mild dehydration. Morbidity was around 11% (7 out of 65 lambs were affected). One of the affected lambs died.

Faecal samples from two of the diarrhoeic lambs in the outbreak were referred to this laboratory for investigation. Both diarrhoeic faecal samples were screened to determine the presence of any rotavirus or pathogenic *Escherichia coli*. Detection of rotavirus was carried out by sandwich ELISA and RNA-PAGE, followed by silver staining. For detection of *E. coli*, both the faecal samples were subjected to cultural examination.

*Extraction of RNA*

To extract rotavirus RNA, the technique of Svensson and colleagues (1986) was followed with minor modifications. In brief, a 20% faecal suspension in PBS was clarified by centrifugation at 10,500g for 20 min. Then, 450 μl of the supernatant was vortex-mixed with 50 μl of 10% sodium dodecyl sulphate (SDS) and 4 μl of 20 mg/ml proteinase K (Bangalore Genei Pvt. Ltd, Bangalore, India) and incubated at 56°C in a water bath for 1 h. After incubation, it was treated with an equal volume of a phenol–chloroform–isoamyl alcohol mixture (25:24:1), vortexed twice and centrifuged at 12,000g for 15 min. The upper aqueous layer was collected in a fresh tube and the extraction was repeated with an equal volume of a chloroform–isoamyl alcohol mixture (24:1). The aqueous phase so obtained was precipitated with 3 mol/L sodium acetate and 1 ml of cold ethanol at −20°C overnight. Next day, the RNA was sedimented at 12,000g for 15 min. The supernatant was discarded and the pellet was dried in an incubator at 37°C for 1 h. The dried pellet was dissolved in 10 μl of 2× Laemmli’s sample buffer (0.12 mol/L Tris-HCl, 4.0%, SDS, 20% glycerol, 0.002% bromophenol blue; pH 6.8; Laemmli, 1970). This served as the source of rotavirus RNA for the RNA-PAGE.

*RNA-PAGE*

Electrophoresis of the RNA samples was performed in a 100 mm × 105 mm, 1.5 mm thick, 7.5% polyacrylamide gel, using Laemmli’s discontinuous buffer system (Laemmli, 1970). Electrophoresis was done at 20 mA in a Hoefer miniVE electrophoretic unit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) for 8 h. Silver staining of the gel was done by the method described by Svensson and colleagues (1986).