Detection of mammalian-like group A rotavirus in diarrhoeic poultry using RNA-PAGE In Kerala, India

Rinsha Balan, M. Mini*, P.M Priya, Siju Joseph and Surya Sankar

Department of Veterinary Microbiology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680 651, Kerala, India.

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ABSTRACT

The present work was conducted to determine the prevalence of rotavirus infection among poultry birds having diarrhoea. A total of 143 faecal samples were collected from different parts of Kerala and screened for the presence of rotavirus using RNA polyacrylamide gel electrophoresis (RNA-PAGE). Out of 143 samples, 5 (3.49%) were found to be positive in RNA PAGE with a migration pattern 4:2:3:2 of a mammalian-like electropherogroup A rotavirus. The study records the first evidence of rotavirus detection from avian species in Kerala.

Key words: Diarrhoea, Kerala, Poultry, RNA-PAGE, Rotavirus.

INTRODUCTION

Rotavirus (RV) is one of the leading causes of acute gastroenteritis affecting several mammalian and avian species. Economic losses are associated with diarrhoeal syndrome in birds, making this a major concern to poultry industry. Like mammalian rotaviruses, they contain 11 segments of double stranded (ds) RNA genome. Rotaviruses isolated from different species can be distinguished by the electrophoretic migration patterns (electropherotypes) of their ds RNA upon polyacrylamide gel electrophoresis (PAGE) (Kalica et al., 1978; Yason and Schat, 1985). The group specific antigenic determinants located within the inner capsid protein (VP6) classify the virus into seven groups (A-G). Among these seven groups, rotavirus type A (RVA) strains predominates and affects both mammals and birds, while type D, F, and G are reported only in birds.

For diagnosis, RNA PAGE is an important technique, which is more commonly used for confirmation of avian RV infection.

Avian rotaviruses are morphologically similar and antigenically related to mammalian rotaviruses (McNulty et al., 1978). The present communication documents the detection of a mammalian-like electropherogroup A rotavirus in poultry having diarrhoea. This is the first record of detection of rotaviruses from birds in Kerala.

MATERIALS AND METHODS

One hundred forty three samples were collected from poultry with diarrhoea, from various regional poultry farms in Kerala. Samples were collected in RNA later solution and transported to the laboratory under cold chain and preserved at -20°C until use. The RNA was extracted from diarrhoeic faecal samples as per the method prescribed by Chomczynski (1993). Briefly, 1 ml of TRI reagent was added to 0.2 ml of faecal sample taken in a sterile RNase free tube followed by homogenization. The insoluble material from the homogenate was removed by centrifugation at 10,000 rpm for 10 minutes at 4°C. The clear supernatant was transferred to a fresh tube and 0.2 ml of chloroform was added and vigorous shaking was done for 15 seconds. The resulting mixture was kept at room temperature for 10 minutes and centrifuged at 10,000 rpm for 15 minutes at 4°C in a refrigerated centrifuge. The upper aqueous phase containing RNA was transferred to an RNase free tube. The RNA was precipitated using isopropanol and pellet was washed with 75% ethanol. The pellet was dissolved in 20 μl sterile nuclease-free water. The extracted RNA was subjected to electrophoresis in 1% agarose gel in TAE buffer. The results were documented in a gel documentation system (Bio-Rad Laboratories, USA).

RESULTS AND DISCUSSION

In the present study, electrophoretic pattern of the 11 segmented double-stranded RNA genome of rotavirus was detected by RNA-PAGE. Five samples out of 143 (3.49%), were found to be positive for rotavirus which showed a migration pattern of 4:2:3:2 which is the characteristic of group A rotaviruses of mammalian species. (Fig. 1). None of the samples displayed the avian type electropherogroup A rotavirus migration pattern of 5:1:3:2. Wani et al. (2003) studied the prevalence of group A rotavirus in chickens in Srinagar using RNA-PAGE and a prevalence of 4% could be detected. The authors observed an electropherotype similar to that of mammalian group A rotaviruses, i.e. a 4:2:3:2 migration pattern. Similar results had been documented by Kattoor et al. (2013). Whether this strain represents a mammalian rotavirus causing inter-species transmission to
chickens or a mammalian–avian reassortant is not clear. Since species barrier is not an absolute concept and many animal viruses are being detected in humans, the findings in present study may be attributed due to a break in species barrier (Wani et al., 2003). For confirmatory evidence, a more comprehensive study of the virus in different animal species and humans followed by genome sequencing is required.

The conventional diagnostic methods for virus detection like isolation in cell cultures, electron microscopy (EM) and serological tests like ELISA were found to be tedious and time consuming and requires skilled personnel (Suresh et al., 2014). Nowadays, the diagnosis is mostly based on rapid molecular methods like RNA-PAGE and RT-PCR (Wani et al., 2004). RNA-PAGE can be employed as a simple method for the detection and characterization of rotavirus and the advantage of the technique lie in its simplicity and rapidity in obtaining results, thereby making it a suitable method for detection of rotavirus from faecal samples.

REFERENCES


