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Vandana Gupta

Assistant Professor, Department
of Veterinary Microbiology,
College of Veterinary Science &
A.H., Jabalpur, Madhya
Pradesh, India

Megha Pandey

Assistant Professor, Department
of Veterinary Pathology, College
of Veterinary Science and
Animal Husbandry, Anjora
Chhattisgarh, India

Anju Nayak

Associate Professor, Department
of Veterinary Microbiology,
College of Veterinary Science &
A.H., Jabalpur, Madhya
Pradesh, India

Shweta Rajoriya

Ph. D. Scholar, Department of
Animal Biochemistry, Ph. D.
Scholar, ICAR- IVRI, Izatnagar,
Bareilly, Uttar Pradesh, India

Smita Bordoloi

Ph. D. Scholar, Department of
Veterinary Microbiology, College
of Veterinary Science & A.H.,
Jabalpur, Madhya Pradesh,
India

Correspondence**Shweta Rajoriya**

Ph. D. Scholar, Department of
Animal Biochemistry, Ph. D.
Scholar, ICAR- IVRI, Izatnagar,
Bareilly, Uttar Pradesh, India

Polyacrylamide gel electrophoresis and silver staining for detection of rotavirus in goat fecal samples

Vandana Gupta, Megha Pandey, Anju Nayak, Shweta Rajoriya and Smita Bordoloi

Abstract

The present study was undertaken to detect the presence of Rotavirus in non-diarrhoeic goat fecal sample by RNA-PAGE and Silver staining. Around 70 fecal samples from non-diarrhoeic goats aging between 2 months to 2 year were subjected to RNA- PAGE after nucleic acid extraction, of which none of the sample was found to show 11 segments migration pattern typical of Rotavirus. RNA-PAGE serves as a simple and inexpensive technique, for detection of rotaviral double-stranded RNA genome in faecal specimens by PAGE in combination with silver staining.

Keywords: Rotavirus, RNA-PAGE, Non-diarrhoeic and silver staining

Introduction

Rotaviruses (RVs) are major enteric pathogens of humans and a wide variety of animals [1]. Clinical presentations range from asymptomatic infection to acute diarrhea that may lead to death due to severe dehydration or other complications. In livestock, RVs are commonly detected in the feces of diarrheic young animals, leading to either epizootic or enzootic forms of gastroenteritis, particularly in young calves, piglets and foals [2]. RV is a member of the Reoviridae family. Officially, the genus is divided into five species (Rotavirus A to E) and historically two additional species (Rotavirus F and G) have been distinguished based on genetic and phenotypic features. RVA, RVB, RVC and RVE have been found to infect various animal species and humans, whereas RVD, RVF, and RVG have been isolated only from avian species [3]. In ruminants, most commonly identified RV strains belong to RVA, but in some settings RVB and RVC are also frequently found and implicated in severe diarrhea, particularly in young lambs and goats⁴. The RV genome is composed of 11 double-stranded RNA (dsRNA) segments, each encoding one protein, except segment 11, which encodes two non-structural proteins (NSP5/6). The RVA genome encodes six structural proteins (VP1-4, VP6, VP7), and five or six non-structural proteins (NSP1-NSP5/6) [1]. A binary classification system has been widely used for RVs, which is based on the configuration of the outer capsid antigens, VP7 or G types (G for glycoprotein) and VP4 or P types (P for protease sensitive protein). The system has been adopted for all RV species. Within RVA, at least 27 G and 37 P genotypes have been reported in mammalian and avian strains [5].

The rotaviruses contain a genome of 11 segments of double stranded RNA (dsRNA) which can be separated into distinct bands by electrophoresis. The migration pattern of the 11 genome segments following electrophoresis of the viral RNA in polyacrylamide gel is called the RNA electropherotype [6]. The RNA patterns of rotaviruses can be classified into two major distinctive groups; i.e., 'long' and 'short' RNA patterns in which the migration of segment 11 is rapid and slow, respectively [7]. Rotavirus strains can be distinguished on the basis of RNA electro-phenotype because this marker is both characteristic and constant for a given virus strain [6, 8]. Because of difficulty in the propagation of rotavirus in tissue culture, comparison of the migration patterns of RNA during polyacrylamide gel electrophoresis (PAGE) became an important laboratory technique for characterization of strains [9]. Polyacrylamide gel electrophoresis procedure provides a rapid, simple, reproducible method of obtaining rotaviral double stranded RNA preparations suitable for electrophoretic analysis in polyacrylamide gels.

The causes of diarrhea in goats include infectious agents including bacteria, viruses, parasites as well as management practice. Caprine RVs have been commonly implicated in diarrhea in 2-3 days old kids and the identified goat RV strains were found to belong to RVA, RVB, or RVC. A submission of single Indian goat RVA strain typed G8 have been seen from India [10]

Detection, surveillance of rotavirus and circulating strains in small ruminants (i.e. lambs and goats) has been a neglected research area in the past. So, the present study had been designed to study the presence of rotavirus infection in non-diarrhoeic goat faecal samples, to identify the presence of rotavirus infection, using RNA-Polyacrylamide gel electrophoresis.

Material and methods

Collection of samples

The freshly voided faecal samples were collected in sterile screw capped containers and in possible cases the samples collected directly from the rectum of goat by inserting fingers equipped with thin rubber hand gloves. The faecal materials were kept in a sterile, screw capped container. In all cases 5-10gms of sample were collected and precautions were taken to avoid contamination of one specimen with other. During sample collection, date, age, sex, clinical signs, important clinical history and environmental situations were recorded. The samples were transported to the laboratory in container containing ice bag and stored at -20 °C (until used for electrophoresis) as quickly as possible.

Preparation of 10% faecal suspension

A 10% faecal suspension of the faecal material was prepared with phosphate buffer saline (PBS; pH 7.2) by dissolving 1 gram of faeces in 10ml PBS. Centrifugation was carried out, after thorough vortexing, at 7500 rpm for 20 min to remove the coarse debris. A positive sample was created by spiking the freshly voided faeces with a standard concentration of Rota-Viral RNA for checking downstream processes.

Extraction of Viral RNA

Total RNA was isolated using Tri reagent according to the manufacturers protocol. The protocol is as follows- To 500µl of faecal suspension, a 500µl of Tri reagent was added in a 1.5 ml eppendorf tube, thoroughly vortexed and kept at room temperature for 15 min. A volume of 200µl of chloroform or 100µl of 1-Bromo-2-chloropropane was then added to the tube, followed by vortexing and kept at room temperature for 10 min. The mixture was then centrifuged at 12,000 x g for 15 min. The upper aqueous layer thus obtained after centrifugation was carefully collected in a new 1.5 ml microcentrifuge tube. A volume of 500µl of Isopropanol was added to it and kept at -20 °C for 1 hour for precipitation of RNA. RNA was pelleted at 12,000 x g for 15 min. Supernatant was discarded and the pellet was washed with prechilled 70% ethanol by centrifuging at 7500xg for 10 min and then air-dried. Final RNA pellets were dissolved in 20µl nuclease-free water (NFW). Quality and purity was checked by Nanodrop.

RNA-Polyacrylamide gel electrophoresis (RNA-PAGE)

RNA-PAGE was used for all the caprine faecal samples for RV presence by observing the 11 genome segments and their typical migration pattern. For electrophoresis, resolving gel (7.5%) and staining gel (5%) were prepared as per the method of Laemmli (1970) [11].

Staining of the gel

The silver staining of the gel was carried out as described by Severson *et al.* (1986) [12]. The gel was removed from the plates and marked at the corner. Subsequently, the gel was put in the staining box containing fixative solution (0.5% glacial acetic acid and 10% ethanol) for 20 minutes at room temperature with intermittent gentle shaking. After discarding the fixative solution, silver nitrate solution (0.185 g AgNO₃/100ml distilled water) was poured in the box and the box was put on a shaker for 30 minutes at room temperature. To minimize the background staining, silver nitrate solution was drained off and the gel was quickly washed three times with distilled water. Then the gel was put in the Developer Solution (0.75M NaOH and 0.1M formaldehyde) for 5-10 minutes with gentle intermittent shaking till bands were visible. Finally for preventing the darkening of the gel, the reaction was stopped by adding a freshly prepared stopper solution (10% acetic acid). The stained gel was photographed and stored in 10% ethanol.

Result and Discussion

Rotaviruses are one of the most important causes of calf mortality during the early weeks of life. The etiology of diarrheal syndrome is quite complex, involving many infectious agents like rotavirus, *E. coli*, *Salmonella* etc. Rotaviruses are considered as the most significant cause of acute viral gastroenteritis. The study was undertaken with the objectives to screen 70 caprine faecal samples by RNA-PAGE. In the present study, survey was conducted for detection of rotavirus in non-diarrheal goats. All the 70 faecal samples were subjected to PAGE after nucleic acid extraction, of which, none of the sample was found to show 11 segments migration pattern typical of Rotavirus. (Figure: 1). This is in accordance with an Indian report showed the absence of RV infection on screening of 25 diarrheic goats [13]. This is contradictory to the finding by Munoz *et al.*, 1995, where they reported the presence of RVB in non-diarrhoeic goats along with diarrhoeic goats as well [4]. Very few epidemiologic reports of RV in goats have been noted in Africa or Asia. Munoz *et al.*, 1996 found lower prevalence of RVB in diarrheic goats (13.5%) and reported RVA in 8% of samples [14]. However, RVs could also be detected in non-diarrheic goat samples (RVA, 15%; RVB, 1%; RVC, 4%) [14]. In our study we collected samples of non diarrhoeic goats above 2 months of age. Microbiological colonization is achieved at one month of age while rumen becomes fully functional at two months of age. Rotavirus is considered to be one of the most important pathogens of gastrointestinal tract, associated with acute diarrhoea cases mainly in young animals and children [15, 16]. As a disease causing agent Rotaviruses have a short viraemia phase or none at all. For this reason antigen can mostly be found in animals with diarrhoea [17]. However, the severity of disease decreases as the age progress, probably because of the secretion of gastric acids into the stomach, causing viruses inactivation, as well as the development of adaptive immunity due to previous exposures [18, 1].

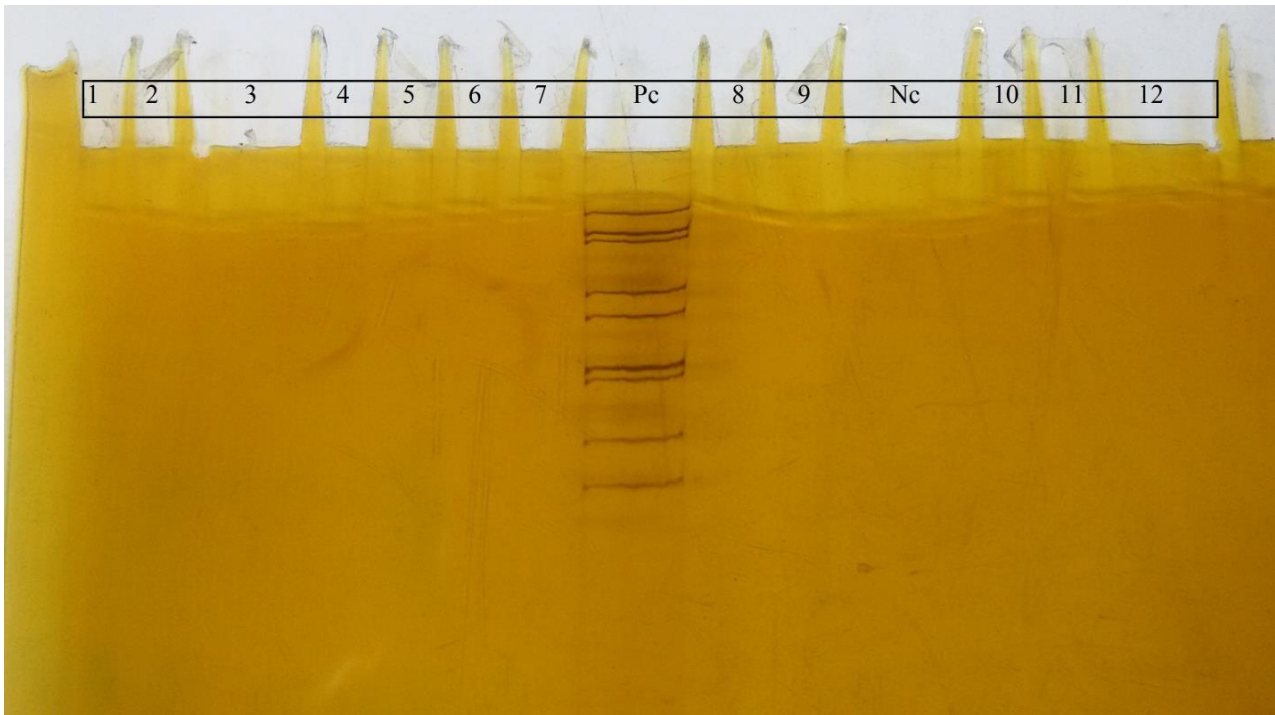


Fig 1: RNA-PAGE for Rotavirus detection
 Lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 & 12- Negative samples for rotavirus
 Pc: Positive control showing 4:2:3:2 pattern
 Nc: Negative control

Conclusion

The study indicates absence of Rotavirus in the studied goat population. The absence of the virus may also be due to selection of non-diarrhoeic faecal samples. Rotaviruses are generally associated with neonatal diarrhea, and hence sample collection during the first month after birth is crucial in establishing their role in causing diarrhea. As the samples in the present study were collected from goats aged above 2 months, this also might have resulted in negative results. However, further study is required including larger sample size from the diarrhoeic and non diarrhoeic goats aged below one month. The technique for detection of the rotaviral double-stranded RNA genome in faecal specimens by PAGE in combination with silver staining is simple and inexpensive and can be established in small diagnostic laboratories.

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