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SHORT COMMUNICATION

Preparation of purified *peste des petits ruminants* (PPR) virus antigen from a local isolate of Bangladesh

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ABSTRACT

Peste des petits ruminants (PPR) is the number one killer disease of small ruminants especially sheep and goats. The disease is presently considered as one of the major threats to about 22 million small ruminant population of Bangladesh with 80-100% mortality in an outbreak and ultimately causes severe losses to small ruminant production. To control and eradicate PPR disease from Bangladesh quick detection and diagnosis is necessary. In field condition, PPR can be diagnosed by serological test and further confirmed by RT-PCR in laboratory. But all the tests are too expensive. The present study was performed to prepare purified *Peste des petits ruminants* virus (PPRV) antigen which can be used for the development of a quick and cheapest diagnostic ELISA kit. A recombinant tissue culture adapted strain of PPRV was propagated in Vero cell culture, the virus was pelleted by high speed centrifugation and the concentrated virus was purified by density gradient centrifugation using a discontinuous gradient of 30% and 60% sucrose. For further confirmation the purified virus was subjected to RT-PCR. The present method of preparing purified antigen appeared to be quite efficient as a distinct clear band of purified virus was found at the interface of 30% and 60% sucrose following density gradient centrifugation. In RT-PCR, a fragment of the Fusion (F) gene of purified PPRV was amplified with the expected band size of 448 bp. The purified PPR viral antigen may be used as potential diagnostic antigen.

Keywords: PPRV, antigen, density gradient centrifugation, ruminant

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1 Introduction

Peste des petits ruminants (PPR) is an economically important disease of small ruminants specially sheep and goats (Kamaruddin and Islam, 2005) and is of increasing importance in Africa and Asia wherever small ruminants form an important component of agricultural food production. In recent years, the disease has been recorded in several parts of the world such as Middle East (Lefevre et al., 1991), the Arabian Peninsula (Abu Elzein et al., 1990), and southern Asia (Shaila et al., 1989) including Bangladesh, India, Nepal, Pakistan and Afghanistan. PPR is a highly contagious viral disease of small ruminants, particularly in goats in Bangladesh (Islam et al., 2001) characterized by high fever, orinasal discharge, pneumonia, necrosis and ulceration of the mucous membrane and inflammation of gastro-intestinal tract leading to se-

vere diarrhoea (Gibbs et al., 1979; Chowdhury et al., 2014; Begum et al., 2018). The pneumonia is exacerbated by secondary bacterial infection. In Bangladesh, natural outbreaks of PPR in Black Bengal goats results in 75% flock morbidity and 59% mortality, with a case fatality rate of 74% (Chowdhury et al., 2014). In field condition, PPR can be diagnosed by serological test and can be confirmed by RT-PCR in laboratory. These tests are highly expensive and time consuming as the kits or reagents used are mostly imported and are not readily available. Thus the study was aimed to prepare a purified PPR virus antigen using a local PPRV isolate which might be helpful for the development of a quick and cheapest ELISA diagnostic kit for the diagnosis of PPR in Bangladesh.

2 Materials and Methods

2.1 Collection of samples

For the isolation of PPRV, PPR suspected dead goat was collected from field outbreaks and necropsy was performed under aseptic condition. Tissues from lung, bronchial and mesenteric lymph nodes were collected and processed for virus isolation. Initially, RT-PCR was performed from the tissue homogenate to confirm the presence of PPRV following an established protocol (Forsyth and Barrett, 1995).

2.2 Preparation of inoculum

Tissues (lungs and lymph nodes) collected from individual goat were pooled, weighed and macerated with sterile mortar and pestle while the tissues are still frozen. PBS was added to make 20% (w/v) suspension and then the tissue suspension was centrifuged at 3000 rpm for 10 min. Supernatant was collected in fresh sterile Falcon tubes and Gentamycin was added @ 500 μg mL^{-1} and stored at -70 °C. Prior to inoculation the suspension was filtered using Acrodisc® Syringe filter (13 mm diameter, 0.2 μm pore size) [Sigma-Aldrich, USA] and the filtrate was used as inoculum.

2.3 Inoculation in Vero cells

Vero cell was maintained in media M199 with bovine fetal serum (10% for growth and 5% for maintenance). When Vero cell monolayer became confluent in the culture flasks, the medium was discarded and the flasks were washed with 10 mL sterile pre-warmed PBS for 2 times. The cells were infected with the prepared sample suspension @ 200 μL 25cm^{-2} flasks and 200 μL PBS was added as mock infection in the control flask. The inoculum was spread all over the cell sheet and the flasks were incubated at 37 °C for 1 h for adsorption of the virus. The inoculum was spread by tilting the flasks at every 15 min. After one hour, maintenance medium was added to the flasks without removing the excess inoculum. The flasks were returned to the incubator and examined twice daily for the appearance of cytopathic effects (CPE).

2.4 Virus harvesting and purification

When maximum cytopathic effects (CPE) such as initial cell rounding, ballooning and aggregations of cells, syncytial development, detachment and floating of rounded cell manifested the infected cell culture fluid was harvested after 5 cycles of freezing and thawing. The culture fluid was clarified by centrifugation at 3000 rpm for 20 min. The supernatant culture fluid was collected and the virus was concentrated and purified from the culture fluid by density gradient ultracentrifugation as described for reovirus by Islam and Jones (1988) with some modifications. Briefly, the virus suspension was centrifuged at 50,000 g for 90 min at 4°C in an angle rotor, the supernatant was carefully discarded and the virus pellet was resuspended in 1 mL Tris-NaCl-EDTA buffer after allowing to soak overnight. The concentrated virus suspension was carefully layered onto two discontinuous layers of 60% (1.5 mL) and 30% (5 mL) w/w sucrose solution. The centrifuge tubes were topped up with Tris-NaCl-EDTA buffer and then centrifuged at 90,000 g for 4 h at 4°C in a swing-out rotor. The distinct band at the 30% and 60% sucrose solution interface was carefully collected and resuspended in Tris-HCl buffer, pH 7.8 at 100-fold concentration of the starting volume of tissue culture fluid. The concentrated and purified virus was clarified by low speed centrifugation (3000 rpm for 5 min) and divided into small aliquots, and then stored at -70 °C for further use.

2.5 Identification of the virus by RT-PCR

To confirm the presence of PPRV in prepared purified PPRV antigen viral RNA was isolated from the purified virus and PPR vaccine using RNeasy Kit (Qiagen, Germany) as recommended by the manufacturer instruction. RT-PCR was carried out with F gene-specific primer sets of PPRV as described by Forsyth and Barrett (1995) using Qiagen one step RT-PCR kit (Qiagen, Germany). The RT-PCR products were analysed by 1% (w/v) agarose gel electrophoresis.

3 Results and Discussion

The preparation of PPR virus antigen involved propagation of virus in Vero cell culture, pelleting virus from the cell culture fluid and purification of the virus by density gradient centrifugation. On inoculation, PPR virus grew in Vero cell culture and produced 70-80% CPE at day 5 post infection. Then the virus

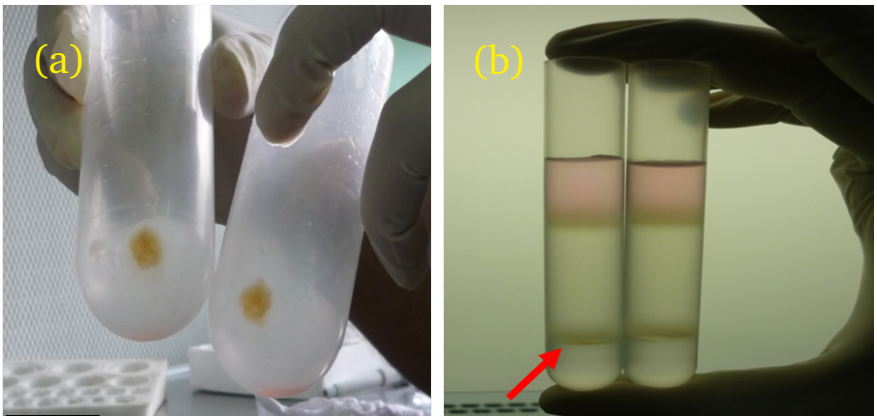

Figure 1. (a) Pellet of virus just after centrifugation at 50,000 g for 90 min at 4 °C, and (b) distinct layer of purified virus (red arrow) in between 30% and 60% sucrose gradient just after centrifugation at 50,000 g for 90 min at 4 °C.

was released and pelleted from the cell culture supernatant by high speed centrifugation (50,000 g for 90 min at 4 °C). An opaque gelatinous compact pellet was clearly visible at the bottom of the centrifuge tubes (Fig. 1a). The virus pellet was purified by density gradient centrifugation (90,000 g for 4 h at 4 °C) which gave a clear band between the two sucrose gradients against light (Fig. 1b). Furthermore, the PPR virus was confirmed from prepared purified antigen by amplifying 448 bp of F gene (Fig. 2).

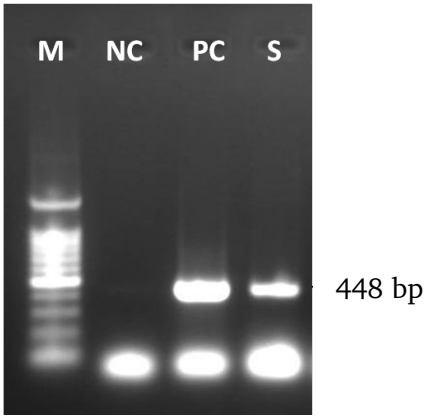

Figure 2. Amplification of the fragment of F gene of PPRV by RT-PCR. M, NC, PC, and S designate marker, negative control, positive control (vaccine), and sample, respectively.

Now-a-days PPR is a great threat to goat rearing in Bangladesh. For effective implementation of control measures of PPR requires a proper and rapid diagnosis of the diseases. ELISA is important to diagnose the disease as well as for seromonitoring. In Bangladesh, commercial diagnostic kits for the detection of PPR are available. However, the kits are highly expensive as the kits or reagents used are mostly imported and are not readily available. Here we prepared a purified concentrated antigen of local PPRV isolate. A combination of procedures is usually employed to attain final concentration and purification of virus (Lerner, 1964). Density-gradient centrifugation has become one of the most popular of these methods (Mould, 1962). Although several methods were used to concentrate and purify viral antigens, here we used density gradient centrifugation with a discontinuous gradient of 30% and 60% sucrose solutions as described by Islam and Jones (1988) for avian reovirus. The method appeared to be quite efficient for purification of PPRV as a very distinct band was observed in the interface of two sucrose solutions after centrifugation. Ayad et al. (2004) purified PPRV antigen from infected tissue culture fluid using polyethylene glycol precipitation and sonication technique and the antigen proved to be efficient for the detection of antibodies to PPR by serological tests like including ELISA, AGID. On the other hand, Obi et al. (1990) purified Nigerian PPRV isolate using the Vero cell and standardized indirect ELISA for titration of hyperimmune serum raised in rabbit. The prepared Bangladeshi PPRV antigen needs to be tested by diagnostic tests (ELISA and AGID) to detect PPR virus in serum sample of goat and sheep in Bangladesh.

4 Conclusions

The PPRV antigen was purified and concentrated using density gradient centrifugation and this antigen may offer an opportunity to develop a cheapest diagnostic kit for the serodiagnosis of PPR. This purified antigen can replace the expensive commercial antigens in Bangladesh. Furthermore, purified PPRV antigen will also become increasingly important in production of vaccines free from materials with possible harmful effects.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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