PBRG Sub-project Completion Report (PCR)

A. Sub-project Description

1. **Title of the PBRG sub-project:** Preparedness for the control of PPR in Bangladesh
2. **Implementing organization (s):**

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4. Sub-project budget (Tk.)

4.1 **Total:(in Tk. as approved):** 1,97,73,142.00

4.2 **Latest Revised (if any)**

5. Duration of the sub-project

5.1 **Start date (based on LoA signed):** 24.06.2018

5.2 **End date:** 15.02.2022

6. Background of the sub-project

Peste des Petits Ruminants (PPR) is popularly known as goat/sheep plague, an important OIE listed transboundary animal disease (TAD) of small ruminants in Bangladesh. PPR is a highly contagious animal disease affecting domestic and wild small ruminants. The disease is caused by a virus called PPR virus (PPRV) or "*small ruminant morbillivirus*", belonging to the genus *Morbillivirus*, family *Paramixoviridae*. PPR in small ruminants causes much devastation in village farmers due to high morbidity (10-100%), high mortality (up to 100%), and heavy economic losses (due to the death of infected goats).PPR infection was first detected and reported in Côte d'Ivoire in 1942 and now countries in Africa, Asia and the Middle East have reported PPR infections covering more than 70 countries in the world (FAO, 2021). In Bangladesh, PPR was first detected in 1993 in Meherpur district. Since then, the disease has spread all over the country resulting in severe socioeconomic consequences (Chowdhury et al., 2014). The economic impact of PPR through its spread to some 70 countries in Africa, the Middle East and Asia has been estimated to be at $1.4 to $2.1 billion (Anon 1). PPRV is

transmitted directly via aerosols and close contact (Munir et. al., 2013). In total, PPRV currently impacts 80% of the world's sheep and goat population (FAO, 2015). The virus causes high morbidity and mortality among sheep and goats and causes an estimated $1.45–2.1 billion USD in global annual losses due to mortality, impaired production, and treatment of infected animals (FAO/OIE, 2015). Bhuiyan et al. (2012) reported an overall seroprevalence of PPR in goats to be 21% in Bangladesh that varied greatly in the different districts. According to them, prevalence was highest in Jashore (49.4%) and lowest (6.3%) in Chattogram. Sero-prevalence was 26.7% in Rajshahi, 20.0% in Sylhet, 12.5% in Mymensingh, and 10.5% in Dhaka. In addition, the overall seroprevalence of PPR in goats was 37.5% in Saint Martin's Island of Bangladesh (Siddiqui et al., 2014). Balamurugan et al. (2014) reported seroprevalence of 21.8% against PPRV in five states (Andhra Pradesh, Gujarat, Jammu and Kashmir, Maharashtra and Rajasthan) of India. An overall seroprevalence of PPR to be 21.3% in Somalia, Amhara region of Ethiopia was reported by Waret-Szkuta et al. (2008). Megersa et al. (2011) reported an overall seroprevalence of PPR to be 30.9% from sheep and goat in pastoral and agro-pastoral area of Afar and Gambella region of Ethiopia. Saha et al. (2005) and Agrawal et al. (2006) found highest seroprevalence of PPR in animals of the age group of six months to one year and absence of seroprevalence in goats below 6 months of age that they reasoned may be due to the presence of colostral antibodies in the kids.

Like the rinderpest virus which has long been eradicated from the world, the PPR virus has many of the characteristics of an eradicable disease. PPR vaccines currently in use are able to induce protective immunity against all known serotypes; immunity is lifelong, whether due to natural infection or vaccination; infection is transmitted primarily by direct contact and the virus does not persist in the environment; infected animals are infectious for a short period of time and there is no carrier state; while a number of different wildlife ungulate species can be infected, there is no evidence to indicate that wildlife populations play an important role in virus maintenance; an effective, robust, safe and affordable vaccine is available and sensitive and specific diagnostic tests are available. Availability of an effective vaccine against PPR along with the availability of sensitive and specific diagnostic tests are therefore, considered as the main tools for controlling and eradicating PPR from the areas infected with PPRV. The Food and Agriculture Organization (FAO) of the United Nations and the World Organization for Animal Health (OIE) jointly launched a $996.4 million plan, the first phase of what will be a 15-year effort to eradicate PPR by 2030. Among the five technical elements of the PPR eradication plan, proper diagnosis and

prevention & control of the disease using an effective vaccine have been given emphasis. PPR vaccines currently in use in Bangladesh was derived from clade 3 of PPRV but our circulating virus belongs to clade 4 ; therefore, clade specific viral vaccine is necessary to obtain protective immunity and protect infections against circulating viruses. Thus, for the development of an effective PPR vaccine there is no alternative of using PPRV (clade 4) that is currently circulating in the country. Moreover, specific diagnostics for PPRV are commercially available but are expensive; thus, it needs to develop diagnostics for PPRV using circulating viruses in ELISAs.

To isolate PPRV from naturally infected field cases, primary lamb kidney (LK) cells were used (Roozbeh, 2017). Once the virus was adapted in either LK cells (following 4-5 passages) or goat kidney (GK) cells (Begum et al., 2020; Zahur et al., 2014) they were grown in Vero cells. Vero cells were routinely used (Zahur et al., 2014; Sreenivasa et al., 2000) because of their continuity and lesser contamination chances. The Vero cells appeared suitable for the isolation of PPRV with the characteristic CPE. The CPE observed in the infected LK and Vero cells were cell rounding, surface detachment, retraction, vacuolation and formation of multi-nucleate syncytia (Zahur et al., 2014; Sreenivasa et al., 2000; Anderson et al., 1996 and Nanda et al., 1996). By serial passage of the Nigeria/75/1 isolate in Vero cells, Diallo et al. (1989) could generate a live-attenuated PPR vaccine. The Vero cell adapted PPRV was also used in antigen formulation in indirect ELISA. This antigen may be used to develop polyclonal antibodies in goats and rodents towards using in antibody-based indirect ELISA (Singh et al., 2004).

The OIE and FAO guideline for Global PPR control strategy with the Step-wise approach towards eradication of PPR virus is given below.

Step-wise approach towards eradication of PPR virus

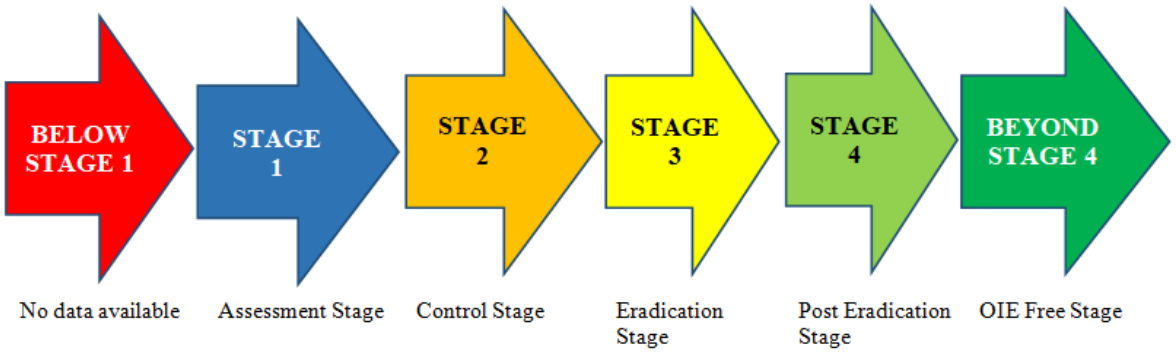

Figure 1: From Stage 1 (assessment of the epidemiological situation) to Stage 4 (absence of circulating virus) countries are ready to submit their dossier for official recognition of country freedom to PPR.

	STAGE 1	STAGE 2	STAGE 3	STAGE 4
	Assessment Stage	**Control Stage**	**Eradication Stage**	**Post-eradication Stage**
FOCUS	Improved knowledge about PPR situation	Disease control in part of or the entire country	Achieve the eradication of the disease in the entire country	Demonstrate that there is no more virus circulation

Five technical elements characterise each stage

1. Diagnostics
2. Surveillance
3. Prevention and control
4. Legal framework
5. Stakeholder involvement.

Progressivity of each technical element along the stages

PPR Stages Elements	Stage 1 (Assessment)	Stage 2 (Control)	Stage 3 (Eradication)	Stage 4 (Post-eradication)
Diagnostic	To establish laboratory diagnostic capacity mainly based on ELISA methods	To strengthen the laboratory capacity through the introduction of bio-molecular methods for a better characterization of field strains	To further strengthen laboratory capacity to support eradication through the introduction of a laboratory quality assurance system	To maintain laboratory capacity as in the previous Stage and strengthen the differential diagnostic pathways. To start implementing PPRV sequestration activities

PPR Stages Elements	Stage 1 (Assessment)	Stage 2 (Control)	Stage 3 (Eradication)	Stage 4 (Post-eradication)
Surveillance	To implement monitoring activities and evaluate socio-economic impacts	To implement surveillance incorporating a response mechanism and risk mitigation measures	To strengthen surveillance incorporating an emergency response mechanism	To shift the goal of surveillance to proving the absence of PPR

PPR Stages Elements	Stage 1 (Assessment)	Stage 2 (Control)	Stage 3 (Eradication)	Stage 4 (Post-eradication)
Prevention & Control	No vaccination	Targeted vaccination	Either mass vaccination or vaccination of the remaining non vaccinated zone (depend on the result of Stage 2 and monitoring system in place)	No vaccination (or emergency vaccination with back in Stage 3)
			Focus on the vaccination	

The eradication plan of PPR from Bangladesh is yet to start as effective way and it needs massive investigation and activities. There are limited studies onto the epidemiology of PPR in Bangladesh (Siddiqui et al., 2014). The epidemiological investigation is needed to develop a progressive preventive/control strategy. A progressive control strategy using intensive surveillance and mass vaccination program over 10±05 years may ensure the attainment. Regular and routine immunization against PPR viruses may generate an immune population of small ruminants in Bangladesh, which is necessary to block the transmission cycle of the virus. OIE and FAO have given priorities towards eradication of PPR from the world like the case of Rinderpest. This is a rare window of opportunity for PPR eradication in Bangladesh. This intervention will contribute to increased household income, food and nutritional security, women empowerment, and significantly reduce poverty which will contribute towards achieving the MDGs. The proposed project on “Preparedness for the control of PPR in Bangladesh” comprised a nationwide sero-survey, disease surveillance and outbreak investigation for epidemiological information, development of PPR-free zone with the locally produced vaccine, development of

biologics and vaccine seeds along with socioeconomic studies. It was hoped that the above studies and outputs will give solid foundation to formulate a disease control plan in Bangladesh.

7. Sub-project general objective (s)

To help increase the availability of safe and high quality livestock protein through controlling PPR in Bangladesh and to meet global control strategy.

8. Sub-project specific objectives (component wise)

a. Coordination Component (BARC)

i. To coordinate, supervise, strengthening capacity, monitor and evaluate the activities of the two research components (BLRI and BAU).

b. Component-1 (BLRI)

i. To conduct sero-epidemiology study of PPR in small ruminants (goats and sheep)

ii. To conduct outbreak investigation and monitoring of circulating PPR virus using molecular technique

iii. To develop PPR free zoneand study of its socio-economic benefit

c. Component-2 (BAU)

i. To develop biologics (diagnostic kits) for the diagnosis of PPR.

ii. To develop vaccine seed using local isolates of PPR virus.

9. Implementing location (s)

a. Bangladesh Agricultural Research Council (BARC), Dhaka

b. Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka

c. Bangladesh Agricultural University (BAU), Mymensingh

10. Methodology in brief

Coordination component (BARC)

The sub-project “Preparedness for the control of PPR in Bangladesh” was being coordinated by the Livestock Division of BARC. The sub-project had two research components; one at BLRI and the other at BAU. Livestock Division of BARC being the Coordination body of the sub-project, for successful implementation and to guide the activities towards a successful completion, organized (i) Annual review workshops; (ii) Arranged coordination meetings; (iii) Conducted monitoring and evaluation programs and (iv) Arranged capacity enhancement training programs as part of the coordination activities of the sub-project. Livestock Division,

BARC also compiled annual progress reports and submitted monthly SoE to PIU-BARC, NATP-II authority regularly.

Component- 1 (BLRI)

a) Sero-epidemiology study of PPR in small ruminants (goats and sheep)

A total of 3933 sera samples were collected from unvaccinated goats and sheep, in the selected 27 districts (Table 2), for sero-epidemiology study of PPR in the goats and sheep. Among the samples 3445 were from goats and 488 from sheep. The age groups considered for sample collection were up to 6 months, >6-12 months, >1-2 years, and above 2 years. All the serum samples were transported (maintaining proper cool chain and avoiding contamination) to the SAARC Regional Leading Diagnostic Laboratory for PPR (SAARC-RLDL) and tested immediately with cELISA (Figure 1) or stored at -20°C for a maximum of 1 month and at -80°C for longer terms. In the case of delayed shipment, the samples were stored in refrigerators at different sample collection areas.

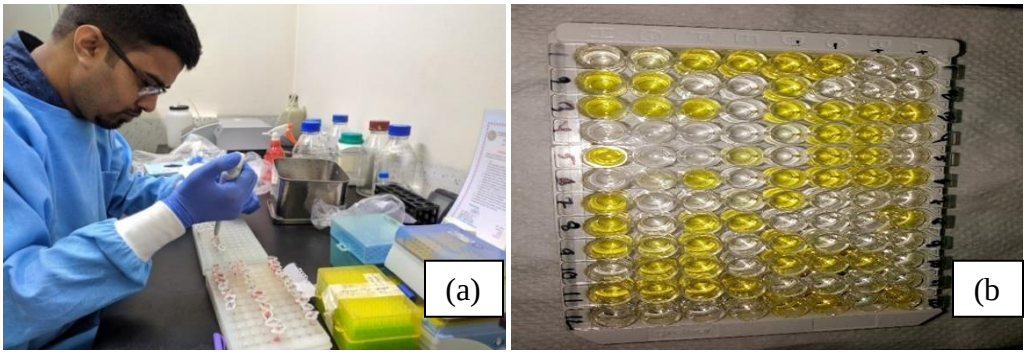

Figure 2: Photo showing sample preparation for cELISA (a) and ELISA plate showing sero-positive cases (b)

b) Outbreak investigation and monitoring of circulating PPR virus using molecular detection technique

Outbreak investigation of the PPR in goats and sheep was conducted in 23 selected districts (Table 4). For the outbreak investigation, necessary PPR suspected samples were collected along with related epidemiological and economic impacts information using appropriate questionnaire. Overall 403 Nasal swab samples were collected individually from a total of 403 goats and sheep suspected to PPR virus infection, for the molecular detection of N gene using Reverse Transcription Polymerase Chain Reaction (RT-PCR) technique. The samples were collected aseptically and were placed separately in sterile falcon tubes containing viral transport medium (VTM) with proper labeling, and then transported to the SAARC-RLDL for PPR, maintaining

cool chain properly. The field samples were either processed immediately or stored at -20°C or -80°C until used.

For molecular detection of PPR virus the samples collected during the suspected outbreak of PPR and confirmed individual samples by RT-PCR, viral RNA was extracted from each samples using the PureLink^{TM} RNA extraction mini kit (invitrogen by Thermo Fisher Scientific, USA), following the instructions of the kit manufacturer. The extracted RNA was evaluated both quantitatively and qualitatively using Nanodrop machine (Sp). The forward and reverse primers used were to detect PPR virus and the cDNA was amplified using PPRV specific NP3: (5′- GTC TCG GAA ATC GCC TCA CAG ACT - 3′) and NP4: (5′ CCT CCT CCT GGT CCT CCA GAATCT 3) primers, as described by Couacy- Hymann et al. (2002). The single-step conventional RT-PCR was conducted by using the Ambion® Kit (AgPath-IDTM One-step RT-PCR kit, USA) as per the manufacturer's instructions. A 25µl scale of reaction mixture consisted of 2× RT-PCR buffer 13µl, forward and reverse primers (100 pmole/µl each) 0.5µl, 2× RT-PCR enzyme mix 1.0µl, template RNA 5µl, and the rest 5µl was nuclease-free water (Figure 2). The primers used for amplification of N gene of PPRV were NP3 (5′- GTC TCG GAA ATC GCC TCA CAG ACT - 3′) and NP4: (5′ CCT CCT CCT GGT CCT CCA GAATCT 3) targeting an amplicon of 351bp, as described by Couacy-Hymann et al. (2002). The oligonucleotide primers were obtained from Integrated DNA Technologies (Japan). The thermal profile for the RT-PCR reaction was followed by the conditions mentioned by Couacy-Hymann et al. (2002). Agarose gel electrophoresis was done for the visualization of the RT-PCR products after staining with ethidium bromide (10 mg/ml) (Sigma®, USA).

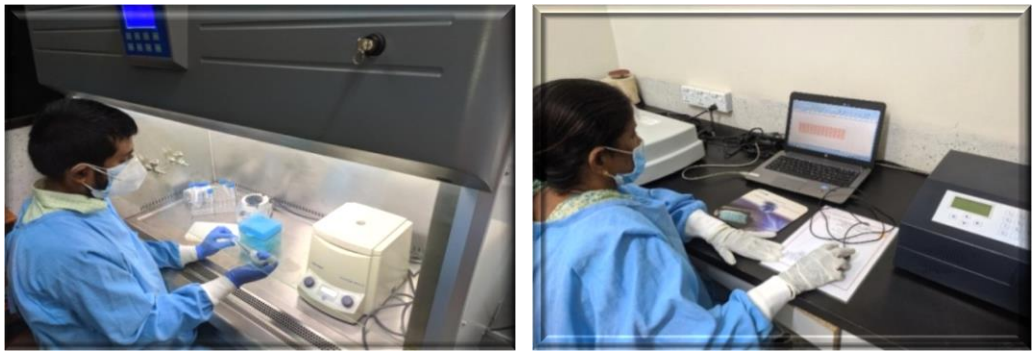

Figure 3: Photo showing sample preparation for conducting RT-PCR

c) Development of PPR free zone with vaccination and study of its socio-economic benefit

Three upazilas namely Debhata (Satkhira), Gangni (Meherpur) and Badarganj (Rangpur) were selected for the development of a PPR-free zone. Required PPR live homologous vaccine was collected directly from vaccine production center, LRI, Mohakhali and vaccinated in goats and sheep of above mentioned three upazilas and, deworming was performed at least 7 days before vaccination. A total of 927 serum samples were also collected from goats (806 samples) and sheep (121 samples) at 2 months, 6 months, and 1 year of post vaccination covering the three selected upazilas of Debhata (Satkhira), Gangni (Meherpur) and Badarganj (Rangpur). In this study, we also kept unvaccinated control villages from the respective upazilas and serum samples were collected from those villages considering above mentioned different age groups. Training programs were conducted in these selected upazilas for awareness development and vaccination campaign regarding PPR diseases in small ruminants. A socio-economic study was conducted among 300 farmers with the help of a pre-designed structured questionnaire. The farmers were selected using a simple random sampling technique. The information collected includes the demography of farmers with the emphasis on goat and sheep disease, farming system and management issues, economic impacts of PPR disease, problem and prospects of goat farming and other related issues. Some information like, shed conditions of goat were collected through visual observation of farms and the sheds. Some data were also collected from secondary sources like government documents, related literature, books, journals, newspaper, articles, and websites. A pictorial view of the activities in the selected upazilas is shown under Figure 4.

a. Vaccination Campaign at Gangni, Meherpur

b. Blood sample collection at Gangni, Meherpur

c. Vaccinating animal at Debhata, Satkhira

d. Data collection using questionnaire

Figure 4: Pictures of field activities in the areas selected for PPR free zone

Component- 2 (BAU)

a) Development of biologics (diagnostic kit) for the diagnosis of PPR

The PPR viral antigen and or anti PPRV antibodies can best be detected by using immunological test methods such as ELISA. Detection of viral genome however, requires nucleic acid based technologies and most commonly practiced technology is RT-PCR. RT-PCR assay can be thousand times more sensitive than traditional titration methods of virus replicating in Vero cells (Couacy-Hymann *et al*., 2002).

This study developed RT-PCR technique for the detection of PPRV following isolation of the virus in Vero cells (described earlier). This study, therefore, developed rabbit and goat anti PPRV antibodies using the PPRV isolate in Vero cells. These rabbit and goat anti PPRV antibodies and PPR viruses were used in sandwich ELISA to detect goat anti PPRV antibodies in naturally infected and vaccinated goats.

i. **Development of goat and rabbit anti PPRV antibodies:** As described under the topic "Propagation of PPRV in Vero cell culture" the locally isolated PPRV, after confirmation by RT-PCR of N protein gene, was initially propagated on adult goat kidney (GK) cells for five passages. After getting adapted on GK cells and the infected cells showed cytopathic effect (CPE), they were propagated in Vero cells to minimize contamination. After each passage in GK cells, the existence of the Virus in culture was tested by using RT-PCR, and the cell lysate yields desired amplicon (402bp). Following sequencing of the amplified cDNAs, the N gene specific response was noted. After five passages of PPRV in GK cells, the virus was grown in Vero cells. Bulk culture following five passages of PPR virus in Vero cells was produced in five 150cm^2

flasks (5-7 days). After five freezing-thawing cycles of the infected cells in 150cm^2 flask, 500ml culture fluid was taken in 50ml falcon tube for centrifugation at 3000rpm for 10mins to remove the cell debris. The supernatant was collected aseptically and subjected to precipitation using 8% (w/v) polyethylene glycol (PEG) along with 2.3% (w/v) sodium chloride. This mixture was incubated overnight at 4°C. Then the cultured fluid was centrifuged at 32000 rpm for 2hrs. Then the pellet was collected and added 10ml PBS. 70% sucrose suspension was prepared and took into the bottom of the ultracentrifuge tube. 30% sucrose solution was overlaid and top of which viral pellet suspension was added. This mixture was centrifuged for 50000 rpm for 1hr. The white band above the 70% sucrose solution was collected by aspiration.

Thus, crude virus was taken into a cryovial, and the N protein gene of the PPRV was confirmed by RT-PCR. The PPR viral pellet was reconstituted in 10 ml PBS. The infective viral particles was inactivated by using binary ethylenimine (BEI), at a concentration of 1mM and 2mM. This concentration of BEI was generated by treating 0.1M 2-bromoethylamine hydrobromide with 0.2N NaOH for 1 hour at 37°C. This solution was then be added to the virus suspension to give the desired concentration of BEI, i.e. 1% v/v for 1mM BEI and 2 % v/v for 2mM BEI. Periodically, the virus-containing bottle was inverted in order to inactivate virus in the air space above the liquid level. Excess BEI was neutralized at the end of the inactivation period with 0.1 % w/v sodium thiosulphate. The extent of viral inactivation was determined by growing the inactivated samples in Vero cells, studying the CFE and detecting the viral component using RT-PCR. The flask containing Vero cells and inoculated with BEI inactivated viruses was not infected and the cell lysate did not show any amplicon in RT-PCR indicating complete inactivation of the viruses. The inactivated viral suspension (1ml) was admixed with (1:1, v/v) 1ml Freund's complete adjuvant. Primary immunization was carried out with the Freund's complete adjuvant and goats (n=03, 0.5ml, Figure 4a)) and rabbit (n=05, 0.1 mL, Figure 4b) were immunized subcutaneously with the antigen. Subsequent boost was carried out using Freund's incomplete adjuvant (1:1, v/v) following day7 and day 15 of immunization. Serum samples (Figure 4c) from the vaccinated rabbit and goats were collected by cardiac puncture following 30 days of immunization. This antibody was used in sandwich ELISA and indirect ELISA to detect anti PPRV antibodies.

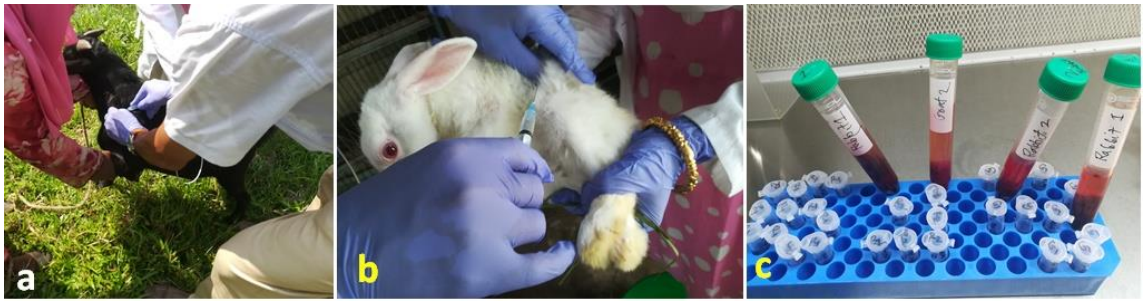

Figure 5: Subcutaneous injection of PPR virus replicated in Vero cells for raising hyper immune serum in goat (a) and rabbit (b). Blood from immunized goats and rabbit in Falcon tubes for antisera collection (c).

ii. **Detection of PPRV and anti PPRV antibodies:** The anti PPRV antibodies in bloods from the experimentally vaccinated or naturally infected and PPR virus from infected goats were detected by using sandwich ELISA. Locally developed rabbit anti PPRV antibodies, goat anti PPRV antibodies and locally isolated PPR virus, replicated in Vero cells, were used in this study as an antigen. Briefly, for sandwich ELISA, the well of the polystyrene ELISA plates were incubated overnight at 4°C with rabbit anti PPRV antibodies. The charged sites onto the plates were blocked with 150µl milk protein (Marvel, 3% solution in PBS Tween 20) by incubating the well at 37°C for an hour. Washed the well of the plates 3x with wash buffer and the well were filled up with 100µl (1:1000 dilution) of purified PPRV to bind with the rabbit anti PPRV antibodies. The plates were incubated at 37°C and washed 3x with wash buffer. The well of the plates were incubated with 100µl/well (1:1000 dilution) of sera collected from known vaccinated goats or suspected goats (previously infected with PPRV). Following incubation at 37°C for 45 minutes the wells were washed 3x with wash buffer and incubated at 37°C for 45 minutes with 100µl/well (1:1000 dilution) of mouse monoclonal anti goat IgG labelled with HRP (Santa Cruz Biotechnology, sc-2354-CM). The well was washed 3x with PBS and incubated with 100µl/well 3,3',5,5'-Tetramethylbenzidine Dihydrochloride (TMB) substrate in the dark at room temperature for 15 minutes. The enzymatic reaction was terminated by adding 50µl/well of 10% sulfuric acid. The optical signals in the wells were detected by reading the plate in the ELISA plate reader at A^{450} (Figure 5a). The polystyrene ELISA plates were also coated with purified PPRV (known antigen) for conducting indirect ELISA (Figure 5b) to detect both the acute infectivity due to PPRV or anti PPR antibodies following chronic infectivity in goats.

iii. OD values considered for calculating positive response include the following:

Blank well (as background): mean OD 0.109 and SD 0.0056
Used PBS as negative control: mean OD 0.163 and SD 0.0035
Lower limit of Detection: mean OD 0.272 and SD 0.0045

A base line value of 0.329±**SD 0.0045** and above was considered as positive response and used in this study.

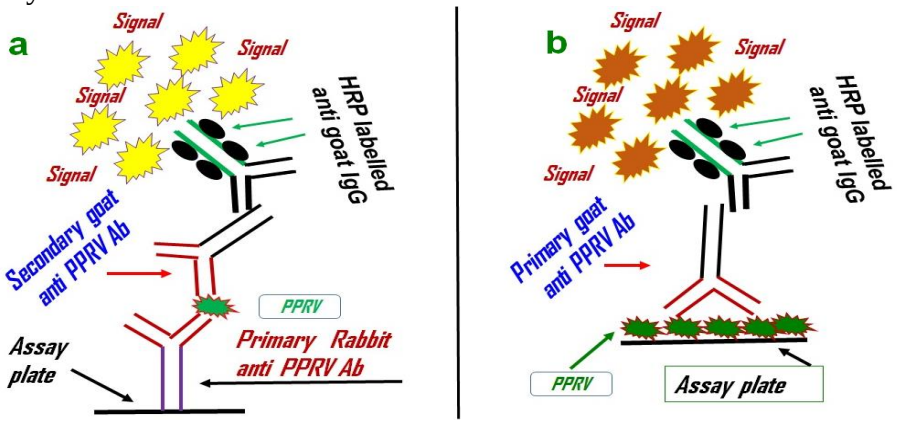

Figure 6: Schematic diagram for the detection of anti PPRV antibodies and PPR viruses from infected goats by using sandwich ELISA (a). The PPRV isolated in this study was also used in an indirect ELISA to detect polyclonal (anti sera) antibodies against PPRV (b).

iv. **Development of PCR/RT-PCR protocol for the detection of PPR virus in the samples co-infected with other viruses:** In Bangladesh PPR infected goats are usually get co-infected with other commonly found viruses like FMD, Orf and Pox viruses. These co-infecting viruses, if present in a sample used for propagation of Vero cells, may create trouble during isolation process of PPR viruses. Therefore, conventional reverse transcription polymerase chain reaction (RT-PCR) technique was designed to detect N protein gene of PPRV and Lpro gene of FMD viruses. Similarly conventional PCR technology was designed to detect envelop protein gene of goat pox viruses and major envelop gene of Orf viruses. Primer pairs against selective genes of PPRV, goat pox viruses, Orf viruses and FMD viruses were designed from the published sequences available in Genbank (Table 1). The samples used for isolation and detection of PPRV were the nasal discharges, bronchial and pharyngeal lymph nodes. Viral RNA or DNA was extracted from the lymph nodes and used in RT-PCR or PCR amplification of the specific viral genomes. The RT-PCR and PCR protocol successfully detected PPRV and FMDV along with the pox and Orf viruses respectively, as co-infecting viruses in the field samples. Lymph nodes free

from co-infectivity with any of the viruses were used in the isolation of PPR viruses in culture in order to develop PPR viral vaccine seed.

Table 1: List of primers used for the detection of PPRV (RT-PCR), Goat pox virus (PCR), Orf virus (PCR) and FMD virus (RT-PCR).

Primers Name	**Sequences** (5´-3´)	**Genes of the viruses**	**Size**	**Genbank accession No.**
PPRV FF1	gctctgtgattgcggctgagc	Nucleocapsid (N) gene (PPR)	402bp	GQ122187.1
PPRVFR1	Cctggtcctccagaatcttggcc			
PoxF1	Gcgaaatttcagatgtagttc	Envelop protein gene (Goat Pox)	287bp	KY389314.1
PoxR1	Ccgcatcagcatacgatttcc			
ORFF1	Cagcttctgctgcaacctgag	Major envelop protein gene (Orf)	587bp	KX129982.1
ORFR1	Gcttgatcaccggcaccatcg			
FMDVF1	ctctctgttacacgctctcag	L pro gene (FMD)	430bp	Islam et al., 2016 (Designed in the lab)
FMDVR1	Cgaacacagcgtgttcttgcc			

**The primers were designed from the sequences available in Genbank*.

b) Development of PPR viral vaccine seed

- **Isolation and molecular identification of PPRV from infected animals:**

A total of 36 clinically PPR suspected goats (2 months to 2years of age) were collected during January-April, 2019, brought to the department of Pathology, BAU, Mymensingh. Goats were externally examined and a systemic necropsy was carried out following sacrifice. Then gross tissue changes were unveiled by systematic dissection (Figure 6a), identifying characteristics lobar pneumonia (Figure 6b) and hemorrhagic & congested lymph node (Figure 6c). Representative tissue samples from each goat like prescapular, mesenteric and bronchial lymph nodes, liver, spleen, intestine, lungs, trachea, heart, and kidney were collected and preserved in 10% neutral buffered formalin. Portion (10 mg) of the suspected PPR infected pharyngeal lymph nodes (Figure 8a) was crushed in liquid nitrogen (sterile mortar and pestle were placed in a Styrofoam tub where liquid nitrogen was poured or dispensed onto the mortar and pestle. After several minutes, when the set was cooled, indicated by the fog being settled over the apparatus, the snap frozen sample was placed in the apparatus and grind by holding the pestle with a gloved hand and firmly pressing on the sample while twisting) and made homogeneous suspension in 10ml PBS. The suspension was spun down at 10000g for 5 minutes; supernatant containing the virus collected, treated with anti-bacterial and antifungal agents and allowed to replicate in Vero cells (Figure 8b). The supernatant obtained from the pharyngeal lymph nodes and from the PPR

virus infected Vero cell culture, were used in PPR viral RNA extraction and amplification of PPR viral N gene in a thermal cycler using RT-PCR (Figure 8c). The viral RNA from the lymph nodes was extracted using SV Total RNA Isolation System (Promega, USA).The purity and concentration of extracted RNA was measured at 260nm/ 280nm in a Nanodrop^{TM} spectrophotometer (IAEA, Scibersdoff, Vienna). A 260nm/280nm ratio of~2.0 was generally considered as "pure" and used for reverse transcriptase polymerase chain reaction (RT-PCR) detection of N protein gene of PPRV. The RT-PCR was carried out using the primer sequences obtained from commercial source (AIT Biotech, Singapore, Table 1). An RT-PCR amplicon of 402bp was considered as positive. Differential diagnosis from pox virus (PCR test), Orf virus (PCR test) and FMD virus (RT-PCR) along with PPRV (RT-PCR) were carried out using the designed primers (Table 1). The PCR test protocol (GoTaq G2 Green master mix kit, Promega, USA) for the differential diagnosis of pox and Orf viruses were carried out with the DNA extracted by using commercially available DNA extraction kit (Wizard Genomic purification kit, Promega, USA). The co-infected samples were discarded. The cDNAs from the test positive samples were sequenced and phylogenetic analysis was carried out to know the viral clade position and orientation of PPR virus.

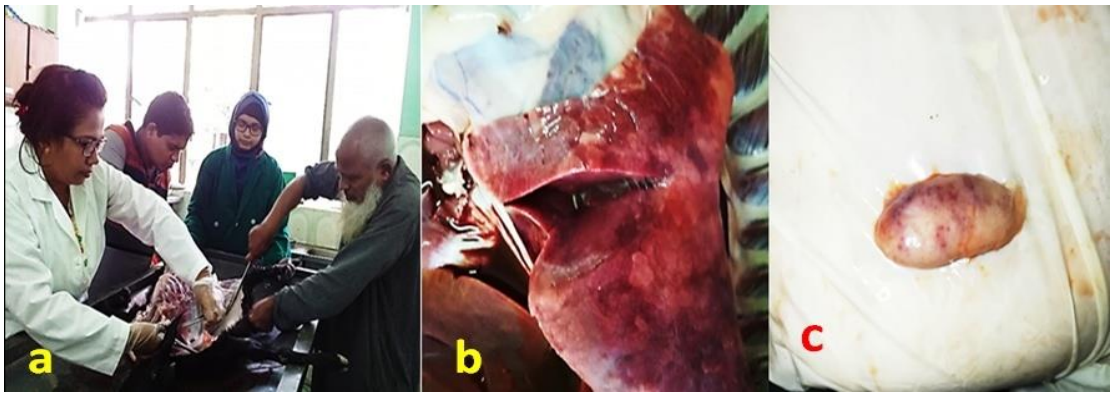

Figure 7: Infected goat was subjected to systemic investigation (a) to identify characteristics lobar pneumonia (b) and hemorrhagic & congested lymph nodes (c).

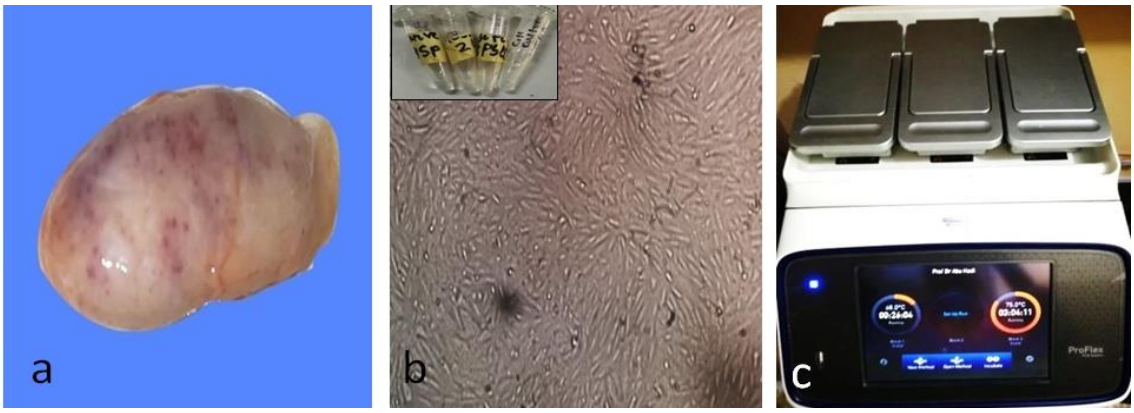

Figure 8: Pharyngeal lymph node collected from the suspected PPR infected goat (a); PPR virus infected Vero cells at 48hpi (b); Oil free thermal cycler (gradient PCR machine) for RT-PCR amplification of N gene of PPR virus (c).

Propagation of PPRV in Vero cell culture

- **Preparation of cell culture media**
  - Single strength (1X) growth/working medium was prepared by using 10X MEM liquid using L-glutamin, penstrep, kenamycin, anti-anti (antifungal), 7.5% sodium bicarbonate solution, 1M HEPES buffer.
  - Shaken well after addition of each ingredient.
  - Maintained PH of the media between 7.2-7.4.
  - Stored 1X medium at 4°C and used within seven days of preparation.
  - Foetal bovine serum (FBS) was added to the medium as per requirement immediately before use. Medium containing serum was not stored any more.
- **Revival of frozen Vero cell**
  - The cryovial containing Vero cells (10^7 cells/ vial) was taken out from liquid Nitrogen tank (Liquid N2-192C)
  - The vial was placed directly into warm water bath (37°C)
  - Agitated the cryovial contents until cell suspension thawed completely
  - The cryovial was immersed in 70% alcohol at room temperature
  - Using a laminar airflow cabinet, the cell suspension from the cryovial was transferred in 25sqcm tissue culture flasks containing 5ml growth medium (medium with 10% FBS)

- The flasks with vented cap were also covered with parafilm and then incubated at 37°C for 3/4 days until monolayer is formed.

➢ **Subculture and maintenance of Vero cells**

- The growth medium, 0.25% trypsin and 1X PBS was pre-warmed to 37°C before contacting cells.
- When cells in the flask reached a growth of 90% confluent monolayer, cells were sub cultured into new tissue culture flasks for maintenance of Vero cell.
- At first growth medium was removed from the flasks.
- Then the cells were washed with 1XPBS (3ml for 25cm flask, 5ml for 75cm flask)
- Added 1ml of 0.25% trypsin in 25cm flask (2ml for 75cm flask) and incubate at 37°C for 3-5mins until the cells were detached from the flask. Observed under microscope to ensure detachment.
- 5ml growth medium containing 10% FBS (7ml for 75%cm flask) was added to the flask to inactivate the trypsin activity. The flasks were then pipetted gently to break up any clumps of cells.
- Desired dilution of cells was prepared (10^7 cells/ml) and the cell fluids were transferred into two or three new tissue cultures flasks containing growth medium (5ml for 25cm flask, 25ml for 75cm flask) with vented cap.
- Then the flasks were incubated at 37°C in an incubator and monitored daily.
- When cells reached about >90% confluent monolayer, cells were passaged by repeating the procedure.

➢ **Cryopreservation**

- The flasks containing confluent monolayer cells were taken and removed the growth medium from the flasks.
- The flasks were washed 3x with 1X PBS.
- Trypsinization of the flasks was carried out.
- Added 5ml growth media and pipetted the cells gently.
- Collected cell culture solution in 15ml falcon tube.
- The tube containing cells was centrifuged at the rate of 2500 rpm for 7mins.
- The supernatant was discarded without disturbing cell pellets.

- Storage medium (MEM +10% FBS+7% DMSO) was added and pipetted carefully.
- Resuspended cells were then used to aliquot into 1ml volumes (10^7 cells/ vial) in the cryovials.
- Cryovials were snap frozen and stored in vapor phase of LN2.

➢ **Preparation of viral inoculums from field sample**

- For viral inoculums preparation, selected PPR positive field sample which have not been co-infected with Pox, Orf and FMD viruses as identified by RT-PCR and PCR.
- Collected prescapular/ pharyngeal lymph node rinsed with 95% ethanol and put onto the sterile mortar. A small piece of tissue (about 10mg) was macerated in sterile mortar and pestle. PBS was added (1x) to make 20% (W/V) suspension and collected in a sterile 15ml Falcon tube.
- Tissue suspension was centrifuged at 2500rpm for 8minutes.
- The suspensions was filtered using Acrodisc syringe filter (0.2µm pore size)
- The filtrate was aliquoted at 500µl(250 µl viral suspension+250 µl 50% buffered glycerol) in each eppendorf tube for future use as inoculums.

➢ **Preparation of 50% buffered glycerol**

- With the 5ml absolute glycerol in a sterile 15ml falcon tube added equal part of sterile PBS(1X).
- Then added 25 µl Penstrep, 25 µl Kanamycin and 50µl anti-anti (antifungal) in the tube and mixed well.
- This mixture was then used to create 5ml volume aliquots in the falcon tubes.
- The tubes were stored at –20°C until used.

➢ **Inoculation of PPR virus in Vero cell culture**

- The fresh filtrate viral suspension of 250µl was used as 1st inoculums in Vero cell line
- When the Vero cells reached a >80% confluent monolayer, the spent medium was removed from the flask
- Washed the cells with pre-warmed PBS
- Removed the PBS and inoculated the cells with virus suspension @250µl per 25sq.cm. flask and 1ml per 75sq.cm. flask
- Only PBS was used instead of virus suspensions in the control flasks as mock infection
- Spread the inoculums over the monolayer cells by tilting the flask to and fro

- Allowed the virus to adsorb on to the cells at 37°C in the incubator for an hour, but spreading the inoculum over the monolayer cells as above, at 15minutes interval
- After adsorption, pre-warmed maintenance medium containing 5% foetal bovine serum was added
- The flask was returned to the incubator and examined daily for the development of cytopathic effects (CPE) if any up to 7 days of inoculation
- When the maximum CPE appeared, the flask containing cells were preserved at -20°C.

➢ **Growth of Vero cells on coverslips and study of the cytopathic effect**

Cytopathic effect or cytopathogenic effect (CPE) refers to structural changes in host cells that are caused by viral invasion. Therefore, appearance of CPE in virus infected host cells is considered as the primary sign establishment and replication of a particular virus in host cells. CPE in general involves lysis of host cells, cell aggregation, cell sheet separation, rounding of cells, syncytia formation, cytoplasmic vacuolation, nuclear aggregation, appearance of nuclear or cytoplasmic inclusion bodies, etc. The CPE in host cells caused by any virus can be studied by growing infected cells in flasks and examining the flasks under microscope. However, detection of CPE in the flask under microscope is difficult. To help solve this problem Luna (1968) described a technique of growing infected Vero cells on coverslips and visualizing the CPE under microscope after staining with hematoxylin and eosin (H&E). Hegde et al. (2009) also reported using H&E and May & Grunwald's Giemsa stains for staining coverslip culture of Vero cells, to detect CPE in PPR virus infected cells. Since then, as reported by FAO (http://www.fao.org › A050-ppr › mod0 › 0333-virus), the technique of coverslip culture of PPRV infected Vero cells and visualization after staining the coverslip culture with H&E is being used by different institutes for detecting CPE in the form of small syncytia earlier than the day 5. In this study our team adapted, for the first time in Bangladesh, the technique of growing Vero cells on coverslips and detection of CPE in PPRV infected cells after staining with H&E.

The technique of growing Vero cells on coverslips and detection of CPE in PPRV infected cells after staining with H&E in brief is as follows:

- Vero cells were grown at 37°C temperature on 40x25mm coverslips in 75cm3 tissue culture flasks containing 5ml growth medium with 10% fetal bovine serum (FBS).

- When the cells become 70-80% confluent on coverslips, the growth medium was removed with a pipette and the cell sheets on the coverslips were inoculated with 100µl of virus isolate.
- Usually the virus isolates of 5^{th}, 10^{th}, 15^{th}, 20^{th} and 28^{th} passages were used for infecting coverslip culture of Vero cells.
- After an hour of incubation, 4ml of maintenance medium containing 5% fetal bovine serum was added to each of the flasks.
- There were control flasks with coverslips for using as uninfected control where, only the maintenance medium was used for the growth of Vero cells.
- The tissue culture flasks (both infected and control) with the coverslips were then incubated at 37°C for 3-5 days.
- After 3 and 5 days of incubation, infected and uninfected control coverslips containing cell sheets were removed from the flasks.
- For staining with H&E the coverslips were rinsed in PBS and fixed in ice cold methanol for 40 minutes.
- Vero cells on coverslips were then air dried and stained with Mayer's Hematoxylin for 8mins.
- Stained Vero cells were rinsed in warm running water for 10mins and in distilled water for 2mins.
- The cells were then stained with Eosin for 2mins and rinsed briefly in 95% ethanol and absolute alcohol.
- The coverslips were dipped for 4mins in xylene and alcohol solution (1:1).
- Stained Vero cells on coverslips were dipped 2x for 2mins in xylene.
- The coverslips were air dried and mounted on a glass slide using DPX (dibutylphthalate polystyrene xylene).
- Stained and unstained Vero cells were examined under microscope at low and high-power microscopic fields and examined cytopathic effect relevant to the infectivity of PPRV.

- **Harvesting the virus**
    - The flask containing infected Vero cells was took out of the freezer and allowed to thaw onto the clean bench placing the flask in upright position
    - When the frozen culture started to thaw, the flask containing the frozen cells was vigorously shaken
    - As soon as thawing is completed, the flasks was returned in the freezer by placing in horizontal position
    - The freezing-thawing cycle was repeated for three more times
    - The culture fluid from the flask was collected in falcon tubes and centrifuged at 3000 rpm for 10 minutes
    - The TCFs were aliquoted into 500 µl volumes in 4-5 eppendorf tubes.
    - With the 500 µl of culture fluid 500 µl of 50% buffered glycerol was added and stored at -20°C for future use
    - The freshly derived TCF was used as viral inoculums for further passages.
- **Detection of PPRV in cell culture**

  Following three freeze-thaw cycles of infected Vero cells in flasks, collected the culture fluid in Falcon tubes and centrifuged at 3000rpm for 10mins. Using the technique described above for RT-PCR the PPR viral RNA was extracted from the harvested tissue culture fluid (TCF) and cell pellet. The extracted viral RNA was used in RT-PCR to confirm the presence of PPR virus in culture condition.

  The whole process of the development of PPR viral vaccine seed starting from preparation of virus inoculum, propagation in Vero cells, identification of virus and attenuation of PPRV in Vero cell culture through to serial passages is shown in Figure 9 as a flow diagram.

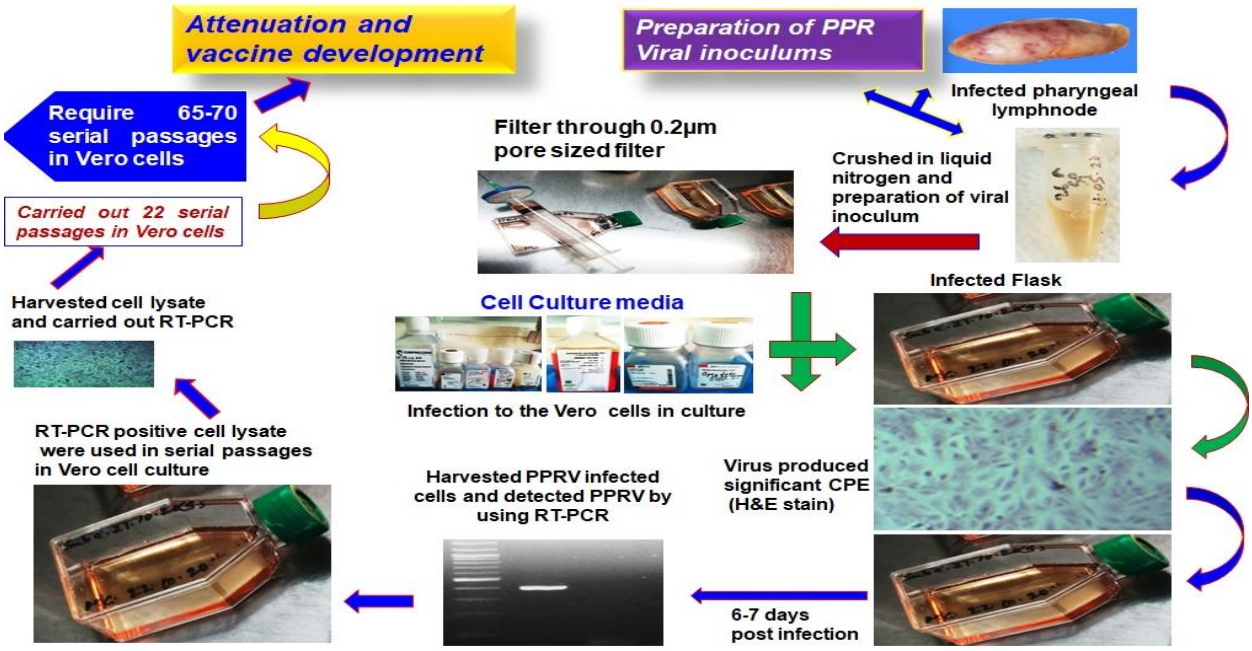

Figure 9: A flow diagram for propagation and attenuation of PPR virus in Vero cells.

For isolation of PPR virus, pharyngeal lymph nodes from the infected goats were collected and crushed on pestle and mortar with the aid of liquid nitrogen to make a fine suspension in 10ml PBS. The suspension was centrifuged at 4000g for 5 minutes and the supernatant was used to infect 75-80% confluent monolayer of goat kidney cell culture followed by Vero cells in flasks. The CPE in the infected Vero cells was examined under the microscope following 5-7 days of infection. Alternatively, infected Vero cells in the cover slips were stained with H&E and examined under microscope to visualize the cytoplasmic and nucleus specific CPE. The cell culture flask infected with PPRV was used in viral RNA extraction and detection of N protein gene of the virus by using RT-PCR. The RT-PCR positive cell lysate were used in serial passages for attenuation of PPR virus. A total of 65-70 serial passages are needed for attenuation of PPR virus in Vero cells for the development of a vaccine seed. Until now 32nd passage of PPRV in Vero cells are being carried out. Tissue culture infective dose of the virus in Vero cells could not be established in this study due to slow progressing CPE of PPRV culture in Vero cells. However, RT-PCR protocols were adapted following every passages of virus propagation in Vero cells as describe by Couacy-Hymann (2002).

- **Sequencing and phylogenic analysis of N gene of PPRV**

To confirm the genetic content and relatedness of PPR viruses, RT-PCR amplification of N protein gene of PPRV of six field samples (coinfection free) and five cell culture attenuated

virus (every five passages intervals- 1st, 5th, 10th, 15th and 20th passages) were amplified and sequenced (carried out in Apical Scientific laboratory, Malaysia). Designed primers for the detection of PPRV (Forward: gctctgtgattgcggctgagc and Reverse: cctggtcctccagaatcttggcc) were used in sequencing of partial N protein gene.

11. Results and discussion

Coordination component (BARC)

A. Inception and Annual Review Workshops

Livestock division, BARC organized a day-long Inception and Annual Review Workshop on PBRG sub-project "Preparedness for the control of PPR in Bangladesh (ID-139)" held on 31 October 2019 at BARC Conference room-1 (Figure 10). Participants from different organizations like DLS, BLRI, FAO, CVH, CDIL, NIB, ICDDRB, KGF, PIU-BARC, Universities and other organizations were participated in the workshop. The inaugural session was held under the chairmanship of Dr. Nazmun Nahar Karim, Member Director (Livestock) & Coordinator of PBRG sub-project. Mr. Kazi Wasi Uddin the Additional Secretary (Livestock-2) of the Ministry of Fisheries and Livestock was present in the workshop as the Chief Guest. Dr. Md. Kabir Ikramul Haque the Executive Chairman, BARC and Dr. Mian Sayeed Hassan the Director, PIU-BARC, were present in the workshop as Special Guests. Welcome address was delivered by the Associate Coordinator of the sub-project Dr. Mohammad Rafiqul Islam, CSO, Livestock Division, BARC. He presented, in brief, about the BARC component activities. Technical session-1 was chaired by Dr. Shah Md. Ziqrul Haq Chowdhury, Ex. Member Director, Livestock Division, BARC and Technical session-2 was chaired by Dr. Kazi M. Kamaruddin, Consultant, NATP-2, BARC. The annual progress activities of 2018-2019 were presented by Principal Investigators of the project of BAU and BLRI component.

Figure 10: Pictorial view of first Annual Review workshop on the sub-project at BARC

The second Annual Review Workshop of the sub-project “Preparedness for the control of PPR in Bangladesh” was organized by the Livestock Division, BARC on 05 November 2020 at BARC Conference room-1 (Figure 11). Participants from different organizations like BARC, BLRI, IEDCR, DGHS, NIB, DLS, KGF, Universities and other organizations participated in the workshop. The inaugural session was started at 10:00 A.M with the welcome remarks as well as short briefing of the projects by the Associate Coordinator of these sub-projects Dr. Mohammad Rafiqul Islam, CSO, Livestock Division, BARC. The inaugural session was graced by Dr. Md. Aziz Zilani Chowdhury, Member Director (Crops), BARC as the Chief Guest while Dr. Harunur Rashid, Director, PIU-NATP, BARC was the special guest in the workshop. Dr. Nazmun Nahar Karim, Member Director (Livestock), BARC chaired the inaugural session. The annual progress activities of 2019-2020 were presented by the Principal Investigators of the sub-project coming from BAU and BLRI. The concluding session was graced by Dr. Md. Aziz Zilani Chowdhury, Member Director (Crops), BARC. Member Director, Livestock, BARC concluded the workshop with thanks to the participants for their spontaneous contribution.

Figure 11: Photographic view of second Annual Review Workshop on the sub-project at BARC

B. Arranging meetings

Coordination meetings

The first coordination meeting of the PBRG sub-project "Preparedness for the control of PPR in Bangladesh" was held at Livestock Division, BARC on 08 December 2019 with the presence of the PIs and Co-PIs of the sub-project (Figure 12). Member Director, Livestock division chaired the meeting where the scientists of the Division were also present. PIs of BLRI and BAU components of the sub-project presented their research activities. The recommendations taken in the meeting were sent to the PIs and Co-PIs for necessary actions.

Figure 12: A view of first coordination meeting of the sub-project at BARC

The second coordination meeting of the PBRG sub-project "Preparedness for the control of PPR in Bangladesh" was held online, due to COVID- 19 pandemic situation, on 25 June 2020. The PIs and Co-PIs of the sub-project from BAU and BLRI components attended the meeting (Figure 13). Member Director, Livestock Division chaired the meeting where the scientists of the Division were also present. After a thorough discussion on the activities of the sub-project some recommendations were taken in the meeting that was sent to the PIs and Co-PIs for necessary actions.

Figure 13: A view of online coordination meeting of the sub-project

c) Monitoring and Evaluation of the sub-project

BAU component of the PBRG sub-project: Preparedness for the control of PPR in Bangladesh was monitored by the BARC monitoring team on 13 February 2020 (Figure 14). Co-PI and PhD student under the sub-project narrated the ongoing activities (cell culture and PPR virus propagation) of the sub-project to the monitoring team. The team after detailed discussion on the various activities suggested the BAU component to start the activities for monoclonal antibody development as soon as possible.

Figure 14: A view of field monitoring & evaluation of the project (BAU component)

d) Organizing training

Training Program on "Control of Peste des Petits Ruminants (PPR) in Bangladesh":

Livestock Division, Bangladesh Agricultural Research Council organized four Training Programs on "Control of Peste des Petits Ruminants (PPR) in Bangladesh", one each at Rangpur and Dinajpur Divisions, and two at Rajshahi Division. The training program of Rangpur Division was held on 24thFebruary 2021 at the Conference Room of the Divisional Livestock Office, Rangpur Division, Rangpur (Figure 15). In this training program, thirty officers from the

Department of Livestock Services (DLS) were present as participants.Dr. Mohammad Rafiqul Islam, Chief Scientific Officer, Livestock Division, Bangladesh Agricultural Research Councilwas present as the Chief Guestin the inaugural session. Shah Jamal Khondokar, Director, Divisional Livestock Office, Rangpur Division, Rangpur was present as the Special Guest. Welcome address was delivered by Dr. Md. Ismail Hossen, SO, Livestock Division, BARC. The training program included lectures on Recognition, Epidemiology and Geographical Distribution of PPR; Global PPR Control Strategy; Molecular Diagnosis of PPR; Treatment, Prevention, and Control of PPR and PPR Control Model for Bangladesh. After successfully completion of the course, certificates were awarded to the participants.

Figure 15: Photographic view of training Program onControl of Peste des Petits Ruminants (PPR) in Bangladesh at Divisional Livestock Office, Rangpur Division, Rangpur.

The training programof Dinajpur Division was held on 25th February 2021 at the Conference Room of District Livestock Office, Dinajpur (Figure 16). In this training course, a total of thirty participants, twenty officers from the Department of Livestock Services (DLS) and ten teachers from the Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur were present. Dr. Mohammad Rafiqul Islam, Chief Scientific Officer, Livestock Division, Bangladesh Agricultural Research Council was present as Chief Guestin the inaugural session. Dr. Md. Shahinur Islam, District Livestock Officer, Dinajpur and Prof. Dr. Md. Khaled Hossain, Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur were present as Special Guest. Welcome address was delivered by Dr. Md. Ismail Hossen, Scientific officer, Livestock Division, BARC. The training program included lectures on

Recognition, Epidemiology and Geographical Distribution of PPR; Global PPR Control Strategy; Molecular Diagnosis of PPR; Treatment, Prevention, and Control of PPR and PPR Control Model for Bangladesh. After successfully completion of the course, certificates were awarded to the participants.

Figure 16: Photographic view of training Program on"Control of Peste des Petits Ruminants (PPR) in Bangladesh" at District Livestock Office, Dinajpur

At Rajshahi Division, in total two training programs were organized by the Livestock Division of Bangladesh Agricultural Research Council, one on 27th and the other on 28th February 2021. Both the training programs were held at the Conference Room of the Divisional Livestock Office, Rajshahi Division, Rajshahi. In the first training program, held on 27th February (Figure 17), twenty officers from the Department of Livestock Services (DLS) and ten teachers from the University of Rajshahi (RU), in total thirty officers/teachers, were present as participants. The inaugural session was held under the chairmanship of Dr. Mohammad Rafiqul Islam, Chief Scientific Officer, Livestock Division, Bangladesh Agricultural Research Council. Dr. Shaikh Azizur Rahman, Director, Divisional Livestock Office, Rajshahi Division, Rajshahi was present in the inaugural sessionas the Chief Guest. Prof. Dr. Md. Jalal Uddin Sarder, Dept. of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi and Dr. Md. Ruhul Amin Al Faruque, Assistant Director, Divisional Livestock Office, Rajshahi Division, Rajshahi were present as special guests. Welcome address was delivered by Dr. K. M. Mozaffor Hossain, Professor, Dept. of Veterinary and Animal Sciences, University of Rajshahi. The training program included lectures on Recognition, Epidemiology and Geographical Distribution of PPR; Global PPR Control Strategy; Molecular Diagnosis of PPR; Treatment, Prevention, and Control of PPR and

PPR Control Model for Bangladesh. After successfully completion of the course, certificates were awarded to the participants.

Figure 17: Photographic view of first training Program on "Control of Peste des Petits Ruminants (PPR) in Bangladesh" at Divisional Livestock Office, Rajshahi Division, Rajshahi.

In the second training program for Rajshahi Division, held on 28th February 2021 (Figure 18), twenty officers from the Department of Livestock Services (DLS) and ten teachers from University of Rajshahi (RU), in total thirty officers/teachers were present as participants. The inaugural session was held under the chairmanship of Dr. Mohammad Rafiqul Islam, Chief Scientific Officer, Livestock Division, Bangladesh Agricultural Research Council. Dr. Shaikh Azizur Rahman, Director, Divisional Livestock Office, Rajshahi Division was present as the Chief Guest in the inaugural session. Prof. Dr. Md. Shah Md. Abdur Rauf, Dept. of Veterinary and Animal Sciences, University of Rajshahi and Dr. Md. Ruhul Amin Al Faruque, Assistant Director, Divisional Livestock Office, Rajshahi Division were present as Special Guests. Welcome address was delivered by Dr. K. M. Mozaffor Hossain, Professor, Dept. of Veterinary and Animal Sciences, University of Rajshahi. The training program included lectures on Recognition, Epidemiology and Geographical Distribution of PPR; Global PPR Control Strategy; Molecular Diagnosis of PPR; Treatment, Prevention, and Control of PPR and PPR Control Model for Bangladesh. After successfully completion of the course, certificates were awarded to the participants.

Figure 18: Photographic view of training Program on "Control of Peste des Petits Ruminants (PPR) in Bangladesh" at Divisional Livestock Office, Rajshahi Division, Rajshahi.

Component- 1 (BLRI)

a) Sero-epidemiology study of PPR in goats and sheep

A total of 3933 blood samples were collected from unvaccinated goats and sheep and the sera were tested using c-ELISA for the presence of PPRV antibody. Detail of the result is shown in Table 2. The overall sero-prevalence was found to be 44.95% (out of 3933 sera samples tested a total of 1768 were positive for PPRV antibody) in unvaccinated goats and sheep with the lowest (29.36%) being found in Badarganj, Rangpur and highest in Sirajganj (65.60%). The study indicated that around 55% of the goat and sheep population in Bangladesh are at risk of contracting PPR. Using the competitive ELISA method similar sero-prevalence was reported by Khan *et al.,* 2008 (51%) and by Misbah *et al.,* 2009 (50%) in Pakistan. Sero-prevalence rates as high as 45.5%, 78% and 92.5% have been reported in Cameroon, Nigeria and Sudan respectively (Ekue *et al.,* 1992; Obidike *et al.,* 2006; Osman *et al.,* 2008) using neutralisation and haemagglutination tests. Megersa *et al.* (2011) reported 30.5% sero-prevalence of PPRV in their study which was lower than the present finding. Lower rates of sero-prevalence of PPRV have also been documented in other countries including Bangladesh like 22.4% in Turkey (Özkul *et al.,* 2002); 33% in India (Singh *et al.,*2004a); 26% in Bangladesh (Banik *et al.,* 2008); 32.8% in India (Balamurugan *et al.,* 2012); 22.1% in Tanzania (Kivaria *et al.,* 2013) and 34.2% in Pakistan (Munir *et al.,* 2013). The inconsistency in the sero-prevalence of antibodies to PPRV in different areas is attributed to variations in a number of factors including the management system, levels of immunity, diagnostic test, sampling procedures used and technical know-how of the researchers (Singh *et al.,* 2004b; Waret-Szkuta *et al.,* 2008).

Table 2: Sero-prevalence of PPR in unvaccinated goats and sheep

Serial No.	Name of the district	Number of sera samples tested	No of the positive sample	Percentage (%)
1.	Cumilla	103	42	40.78
2.	Brammanbaria	105	52	49.52
3.	Rangpur (Badarganj*)	470	138	29.36
4.	Natore	125	57	45.60
5.	Sylhet	130	68	52.30
6.	Manikganj	146	63	43.15
7.	Gazipur	112	48	42.86
8.	Thakurgaon	225	73	32.44
9.	Bogura	116	50	43.10
10.	Meherpur (Gangni*)	232	86	37.06
11.	Pabna	123	62	50.41
12.	Sirajganj	125	82	65.60
13.	Tangail	109	45	41.28
14.	Jhenaidah	202	114	56.44
15.	Chattogram	100	63	63
16.	Habiganj	102	60	58.82
17.	Dhaka	270	106	39.26
18.	Satkhira (Debhata*)	230	100	43.71
19.	Bhola	103	42	40.77
20.	Netrokona	100	49	49
21.	Rajshahi	100	34	34
22.	Jashore	100	45	45
23.	Mymensingh	102	41	41.17
24.	Bandarban	103	42	40.77
25.	Patuakhali	100	49	49
26.	Barishal	100	39	39
27.	Moulovibazar	100	41	41
Total		3933	1691	44.95 (Avg.)

*Upazilas selected for PPR free zones

b) Outbreak investigation and monitoring of circulating PPR virus using molecular detection technique

Outbreak of PPR was investigated in the villages under vaccination and in the control villages with no vaccination using molecular technique. Samples were collected at different time intervals from the goats and sheep suspected of suffering from PPR and tested using RT-PCR technique for confirmation of the disease. Table 3 shows the pattern of PPR outbreak in the

treatment villages (with vaccination) and control villages (without vaccination). Before vaccination, PPR disease outbreak pattern in the treatment (villages selected for vaccination) and control villages (with no vaccination) was 21% and 25% respectively. But, one year after vaccination the outbreak of PPR came down to 3% in the vaccinated villages that was 19% in unvaccinated villages indicating that vaccination of small ruminants with PPR vaccine was able to reduce the outbreak of PPR disease.

Table 3: Pattern of PPR outbreak in the Treatment Villages (TV) and Control Villages (CV)

Particular	Before vaccination	1 month after vaccination	2 months after vaccination	6 months after vaccination	1 year after vaccination
TV	21%	30%	19%	15%	3%
CV	25%	42%	27%	31%	19%

For the molecular detection and confirmation of PPRV, a total of 403 samples were tested using RT-PCR test. The fragments of the RT-PCR amplified products of the test samples migrating alongside the 351bp PPR positive control (Figure 19) was considered as the PPR positive cases.

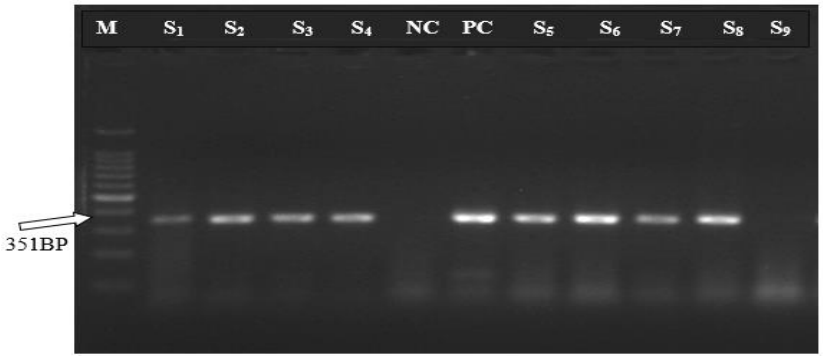

Figure 19: Agarose gel electrophoresis of PCR products targeting N gene (351bp) with NP3 and NP4, PPR specific primers (Couacy- Hymann *et al.* 2002). Lane M: 100bp DNA molecular weight marker; Lane PC: Positive control; Lane NC: Negative control; Lane S_1-S_9: Field samples.

Table 4 shows the prevalence rate of PPR disease in the study areas. Using molecular detection technique of RT-PCR, the highest percentage (78.84%) of PPR virus was detected in the samples collected from goats and sheep suspected of suffering from PPR at Savar region, Dhaka; where, out of 52 samples tested 41 were positive for PPR. Whereas, in Mymensingh the prevalence was lowest (20%) where, out of 10 samples tested only 2 were positive for PPR. The overall prevalence of PPR was found to be 54.59% (220/403). The finding of the current study was comparable with the findings of Abu Bakar et al. (2008), 40.98% and Ahamed et al. (2019),

40%. Other authors like Munir *et al.* (2009), Abu Bakar et al. (2011) and Munir et al. (2013) found less prevalence rates of 21.4%, 34.3% and 25.7% respectively in their studies. This type of heterogeneity in the findings may be attributable to population size, area of the examination, season of the examination, sampling procedure, vaccination status, test procedure, etc.

Table4: District wise prevalence of PPR (PPRV was detected in the samples using RT-PCR)

SL No.	Name of the districts	Number of samples	Positive	Prevalence (%)
1.	Brammanbaria	05	02	40
2.	Rangpur	19	13	68.42
3.	Natore	12	05	41.67
4.	Sylhet	07	03	42.85
5.	Manikganj	17	09	52.94
6.	Tangail	05	02	40
7.	Gazipur	19	11	57.89
8.	Bagura	11	07	63.64
9.	Dhaka (Savar)	52	41	78.84
10.	Dhaka (Dhamrai)	69	36	52.17
11.	Pabna	19	09	47.37
12.	Chattogram	12	07	65.34
13.	Meherpur	17	09	41.28
14.	Jhenaidah	07	03	56.58
15.	Sirajganj	10	05	63.33
16.	Thakurgaon	09	06	58.12
17.	Satkhira	09	07	39.59
18.	Bhola	15	08	66.67
19.	Munshiganj	17	08	47.06
20.	Netrokona	21	09	42.85
21.	Rajshahi	13	03	23.07
22.	Jashore	28	15	53.57
23.	Mymensingh	10	02	20
	Total	**403**	**220**	**54.59 (Avg.)**

c) Development of PPR free zone and the study of socio-economic benefits of vaccination

The area wise goat and sheep population in the Unions under the Upazilas (Debhata, Gangni and Badarganj) selected for development of PPR free zone by vaccination is depicted in Table 5. Out of the total population of 37,709 goats and sheep in the selected Unions a total of 30,615 were vaccinated with PPR vaccine covering more than 80% of the population. Required amount of

PPR vaccine was collected directly from vaccine production centre, LRI, Mohakhali. Sera samples were collected from goats and sheep of the treatment areas, before vaccination (at 0 day) and then at 2 months, 6 months and at 1 year of post vaccination (PV). There were control villages where the animals were not vaccinated and sera were collected at similar intervals.

Table 5: Area wise goat and sheep population

District	Upazila	Union	Goat and Sheep population	Vaccinated animals	Percent vaccinated
Satkhira	Debhata	Nowapara	7,717 (Goat-6,541, Sheep-615)	6,275	81.30
Meherpur	Gangni	Raypur	13,595 (Goat-12,143, Sheep-1,452)	10,892	80.10
Rangpur	Badarganj	Damodarpur	16,397 (Goat-14,258, Sheep-2,139)	13,448	82.00
Total			37,709	30,615	81.13

Table 6 shows that before vaccination, anti PPR sero-positivity were 43.71%, 37.02% and 29.30% in goats and sheep from Nowapara (Debhata, Satkhira), Raypur (Gangni, Meherpur) and Damodarpur (Badarganj Rangpur) respectively. The sero-positivity was found to increase steadily to 85.72%, 90.82% and 81.89%; 90.76%, 83.54% and 95.45%; 86.14%, 93.06% and 88.11% at 60 days (2 months), 6 months and 1 year of post vaccination in Nowapara (Debhata, Satkhira), Raypur (Gangni, Meherpur) and Damodarpur (Badarganj, Rangpur) respectively. Regmi et al. (2019) found 75.2% sero-positivity after vaccination which was slightly lower than the present findings. The proportions of sero-positive animals may differ significantly between sex, age groups, grazing management, introduction of new animal in the herd, etc. There may be several other factors contributing for successful outcome of a vaccination campaign like, cold chain maintenance, time spent between reconstitution of vaccine and vaccination, skill of vaccinators, area coverage and the health condition of animals during the immunization time. Rossiter and Jame (1989) estimated that minimum of 75-80% herd immunity is essential for controlling the rinderpest and its related disease (PPR). Other study showed that such antibody titers are completely protective from infection upon virulent PPRV challenge (Balamurugan et al., 2012). In unvaccinated control villages, the highest and lowest sero-positivity was found to be 48.51 and 34.65% respectively after one year (Table7). Rahman et al. (2016) and Regmi et al.

(2019) also reported sero-positivity pattern against PPR to be 38.77% and 25.8% respectively in unvaccinated goats. The present study revealed that herd immunity (minimum of 75-80% immunity in a population) could be developed using the PPR vaccine produced at LRI, Mohakhali. However, for declaring the areas as PPR free zone the present high level of sero-positivity has to be maintained at least for 3 years and beyond through vaccination of new generation of animal population and stringent sero-surveillance study. This study also indicates that if the production of PPR vaccine could be increased at a scale to cover 80% of the total population of goat and sheep in the country then we could go for lunching mass vaccination program to achieve PPR free status as per OIE.

Table 6: Presence of antibody against PPR virus in the goats and sheep from vaccinated areas

Locations		**Sero-positive animals (percent) at different time intervals of vaccination**			
Upazilas	**Unions**	**At 0 day (%)**	**After 2 months (%)**	**After 6 months (%)**	**After 1 year (%)**
Debhata	Nowapara	43.71	85.72	90.76	86.14
Gangni	Raypur	37.02	90.82	83.54	93.06
Badarganj	Damodarpur	29.3	81.89	95.45	88.11

Table7: PPR sero-positivity in goats and sheep from unvaccinated control areas

Locations			**Sero-positive animals (percent) at different time intervals**			
Upazilas	**Unions**	**Villages**	**At 0 day (%)**	**After 2 months (%)**	**After 6 months (%)**	**After 1 year (%)**
Debhata	Nowapara	Askara	27.89	42.02	38.12	48.51
Gangni	Raypur	Koroigachi	32.25	29.49	37.91	41.20
Badarganj	Damodarpur	Jelepara	30.8	39.14	26.32	34.65

Participatory epidemiological study of PPR

Clinical signs of PPR in animals: During collection of samples from the PPR suspected goats and sheep clinical signs and symptoms of PPR were recorded from a total of 300 respondents. Different clinical signs and symptoms like nasal discharge, watery diarrhea, severe dehydration followed by death were reported by the livestock keepers in suspected PPR cases during the participatory epidemiological study (Figure 20). The highest rate of clinical symptoms was recorded to be the nasal discharge (32%) followed by diarrhea (27%), oral ulcer (21.67%),

respiratory distress (10%), ocular discharge (8%) and abortion (1.66%); shown in Figure 21. The study findings were closely related to the findings reported by others (El-Hakim, 2006; Abu Bakar et al., 2011). The clinical findings were confirmed by identification of PPRV in the samples, using RT-PCR test.

Figure 20: Clinical symptoms of PPR in goats; nasal discharges (a), watery diarrhea (b) and severe dehydration (c).

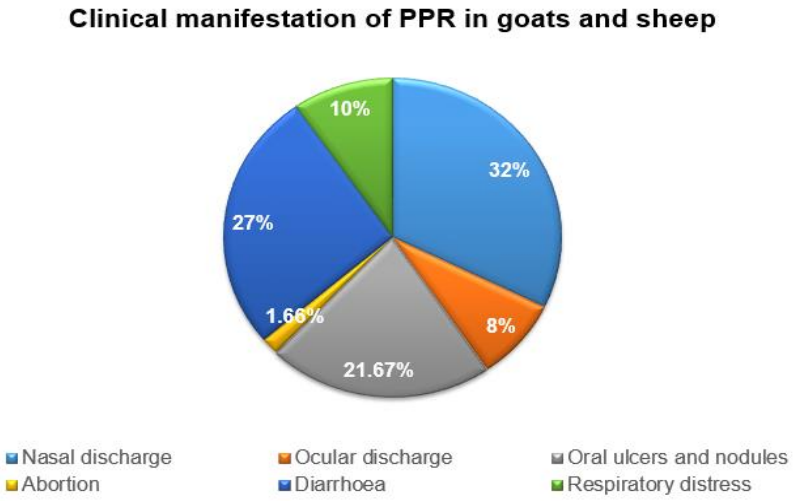

Figure 21: Clinical manifestation of PPR in goats and sheep

Sex of animals: Table 8 shows the sex-wise prevalence of PPR sero-positive cases in unvaccinated and vaccinated groups of animals. In this study, serum samples collected from the animals before vaccination showed higher PPR sero-prevalence rate in the males (48.46%) compared to the female (46.36%). In the same animals, after 365 days (1 year) of vaccination, sero-prevalence was also found to be higher in the male (90%) compared to the female (88.74%).This could be related to the physiological differences between male and female where,

females are more prone to production and reproduction related stresses resulting in lowered immune response against PPR. However, present study differed from the study of Megersa et al. (2011). They reported a significantly higher sero-prevalence of PPR in unvaccinated females compared to unvaccinated males and reasoned the case to be related to the same physiological differences between the two subjects where, according to them, females reveal some degree of infection preponderance as a result of production and reproduction related stresses.

Table 8: Effect of sex on the prevalence of PPR sero-positive cases in unvaccinated and vaccinated groups of animals

Sex	Unvaccinated animals (serum titre at day 0)		Vaccinated animals (serum titre at day 365)	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Male	48.46%	51.54%	90%)	10%
Female	46.36%	53.64%	88.74%	11.26%

Age of animals: Table 9 shows the effect of age on the prevalence of PPR sero-positive cases in unvaccinated/pre-vaccinated and post vaccinated groups of animals.PPR sero-positivity in unvaccinated animals was found to vary from 35.50% up to 6 months'age group to 46.87% in >1-2 year and 46.50% in >2 year and above age groups. Accordingly, after 365 days (1 year) of post vaccination, the sero-positivity was 85.26%, 92.31% and 86.36% in the same age group of animals respectively. These findings are in agreement with previous reports from Pakistan (Abu bakar et al., 2011) and Turkey (Ozkul et al., 2002); where they reported higher rates of PPR sero-prevalence in adults. It has been documented that sheep and goats exposed to natural infection to PPRV at a very young age may carry antibodies for 1-2 year following exposure and remains sero-positive for a long time (Dhar et al., 2002; Ozkul et al., 2002; Singh et al., 2004a).

Table 9: Effect of age on the prevalence of PPR sero-positive cases in pre-vaccinated and post vaccinated groups of animals

Age	Unvaccinated animals (serum titre at day 0)		Vaccinated animals (serum titre at day 365)	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Up to 6 Month	35.50%	64.50%	85.26%	14.74%
>6-12 Month	38.89%	61.11%	86.67 %	13.33%
>1-2 Year	46.87%	53.13%	92.31%	7.69%
Above 2 Year	46.50%	53.50%	86.36%	13.64%

Demographic analysis of goat and sheep farmers and their knowledge of disease management

The study was conducted on the sheep and goat farmers coming from the upazilas selected for establishment of PPR free zone. Three Unions were selected for the establishment of PPR-free zone. The selected Unions were Nowapara (Debhata, Satkhira), Raypur (Gangni, Meherpur) and Damodarpur (Badarganj Rangpur). Before the selection of these Upazila, a baseline survey was conducted with a pre-tested questionnaire. Questionnaires were designed giving emphasis on socio-economic information of goat and sheep farmers, the prevalence of PPR and other diseases in the areas including the different animal health management issues.

Demographic analysis

Table 10 shows the analysis of different demographic parameters of the sheep and goat farmers.

Age of farmers

Most of the goat and sheep farmers (45.33%) in the areas were from the middle-aged group with the age range of 31 to 50 years followed by young age (40.33%) of up to 30 years. Only 14.33% of the farmers were in the old age group (above 50 years). These findings was found to support the findings of Nipane et al. (2016) but differed with the findings of Tanwar et al. (2008) who reported that the majority of the people under old age group in Rajasthan, preferred goat farming as the animals are easy to manage.

Education

Education is one of the most important socioeconomic variables which influence the overall pattern for a better livelihood. In general, a farmer with a higher level of education has more potential than a lower one especially in the situation where more technical knowledge is required. Most of the goat and sheep farmers in the study area were illiterate (47.67%) and the formal education in terms of primary, secondary, and upper than secondary education was 27.67%, 22.67%, and 3.00% respectively. The outcome of the study was found to be in accordance with the findings of Praveena et al. (2014).

Main occupation

This was clear from the study that goat and sheep keeping is generally practiced in the study area by poor homestead workers to acquire extra income. Most of the farmers were engaged in agricultural work (50.33%) followed by housewife (29.33%), business (12.33%) and others profession (8.01%). The findings of the study were in accordance with the findings of Sharma et

al. (2007), Deshpande et al. (2010) and Thombre *et al.* (2010) who reported that a major proportion of the goat keepers were rural workers with goat keeping as their secondary occupation.

Annual income

The annual income of the farmer was found to vary from person to person that ranged from Tk. 40,000 to Tk. 500,000. The farmers were divided in to three groups depending on their annual income as low (Tk. 40 to 100 thousand), medium (Tk. 101 to 1150 thousand), and high (Tk. 151 to 500 thousand). Most of the farmers in the study was found to belong with the low income group (83%) followed by medium (12%) and high (5%) income group. Our findings agreed with the findings of Raghavan and Raja, 2012 who reported the majority of goat farmers (65.89%) were from the low income group with the monthly income of 500 to 2000 Rs. and only 8.7% of the farmers had an average monthly income of >5000 Rs.

Table 10: Demographic profile of goat and sheep farmers of the study area

SL No.	Parameter	Categories	Respondent number (300)	Percentage of respondents
1.	Age	Young age (up to 30 years)	121	40.33
		Middle age (31-50 years)	136	45.33
		Old age (above 50 years)	43	14.33
2.	Education	Illiterate	143	47.67
		PSC	83	27.67
		SSC	68	22.67
		HSC	09	3.00
3.	Main Occupation	Agriculture	151	50.33
		Housewife	88	29.33
		Business	37	12.33
		Others	24	8.01
4.	Annual income (Thousand taka)	Low (40-100)	249	83
		Medium (101-150)	36	12
		High (151-500)	15	5

Analysis of the knowledge of farmers about goat and sheep diseases and their management

Table 11 shows the results of the analysis of the knowledge of farmers about goat and sheep diseases and their management.

Disease occurrence

When the farmers from the study areas were asked about the occurrence of different diseases of goat and sheep a total of 35.81% of the farmers responded to have experienced PPR in their animals followed by Digestive disorder (21.62%), Non-specific Fever (10.81%), Enterotoxaemia (10.14%), Non-specific Diarrhoea (8.11%), Non-specific Pneumonia (6.08%), HS (4.05%), FMD (2.02%) and Tetanus (1.35%).

Idea about disease

Out of the total respondents of 300, about two-thirds of the farmers (66%) were found to have a partial idea about goat and sheep diseases. However, there were some farmers (20%) who even responded not to have any knowledge about the diseases of goat and sheep. Only 14% farmers had responded have a clear idea of goat and sheep disease. The main reason behind the partial or no idea about the diseases was lack of training on goat keeping and disease management, as mentioned by the respondents. The findings of the study indicate that for implementation of PPR eradication program in the country farmers should be provided with some basic training on goat diseases with their prevention and control measures.

Vaccination

Prevention is always better than the cure of diseases and vaccination is the only way to keep disease away from the farm. PPR is a fatal disease for sheep and goats but, 79% of the interviewed farmers didn't follow any proper vaccination schedule. This study indicates that along with making PPR vaccine available at the doorsteps of farmers, they need to be educated through motivational training about the impact of vaccination in their animals.

Deworming

Parasitic infestation induces economic losses through reduced growth, decreased milk production and even causing death of animals. Out of the 300 respondent farmers, 59% were found to practice deworming of their animals using anthelmintics indicating that farmers need motivational training on the use of anthelmintics to control parasitic infestation and keeping the animals healthy and fit for production.

Veterinary services

Only a small proportion of the farmers (26%) reported to get animal health and management related help directly from Veterinarians. This indicates a shortage of veterinarians at the rural level that affects more than just the health and welfare of animals but, it can also negatively affect the health of their owners and other members of the community.

Disposal of carcass

Improper disposal of carcasses is the source of different diseases. Proper hygienic management of carcasses help reducing the occurrence of different diseases. The good sign is that 89% of the farmers responded to bury the dead animals deep into soil although, 11% of the farmers reported that they throw the dead bodies into the canals, rivers, or outside of the village which is still posing threat of spreading infections in the animals.

Table 11: Farmers' perception about goat and sheep diseases and their management

SL No.	Parameter	Categories	Respondent number*	Percentage of respondents
1.	Disease prevalence	PPR	106	35.81
		Digestive disorder	64	21.62
		Enterotoxaemia	30	10.14
		Non-specific Fever	32	10.81
		Non-specific Diarrhea	24	8.11
		Non-specific Pneumonia	18	6.08
		HS	12	4.05
		Tetanus	4	1.35
		FMD	6	2.02
		Total respondents	296	
2.	Idea about disease	Clear Idea	42	14
		Partial Idea	198	66
		No Idea	60	20
3.	Vaccination	Idea about Vaccination	63	21
		No clear idea about vaccination	237	79
4.	Deworming	Practiced by farmers	177	59
		Not practiced by farmers	123	41
5.	Veterinary services	Directly from veterinarians	78	26
		From other livestock personnel	222	74
6.	Disposal of Carcass	Burying of carcass	267	89
		Other means of disposal	33	11

** For all the parameters where the number is not mentioned the total respondents were 300.*

Socio-economic impact of PPR vaccination in goat and sheep

To determine the socio-economic impact of PPR vaccination on goat and sheep a survey was conducted after 1 year of vaccination. Table 12 below shows the socio-economic impact of vaccination in terms of mortality rate in goats and sheep, income from goat farming, feeding pattern, housing system and marketing pattern.

Impact on mortality rate

At pre-vaccination, the mortality of goat and sheep due to PPR was 23.54% whereas, after one year of post-vaccination, the mortality gradually reduced to 3.23%, as indicated by the farmers. Vaccinations, along with other animal health interventions, are undeniably responsible for improving health outcomes of animals.

Impact of income from goat farming

At pre-vaccination, according to a big group of farmers (89.33%), the annual income from goats and sheep was low (Tk. 20-50 thousands only).Only 9.67% and 1.0% of the farmers reported the annual income from goat and sheep keeping to be medium (Tk. 51-100 thousands) and high (Tk. 101-200 thousands). However, the study after one year of vaccination indicated a change in the income status of the farmers due to reduced mortality and increased flock size of the animals. Consequently at one year post-vaccination, the number of low income group of farmers was reduced from 89.33% to 42.66%, and the number of medium income group of farmers was increased from 9.67% to 52.67% along with the increase in high income group of farmers from 1% to 4.67%.

Impact on feeding pattern

Feeding management is one of the most important factors in goat farming as feed cost is the highest cost among all other production costs. Two types of feeding practices prevailed dominantly, one was grazing only and the other was grazing with supplement feeding. Feeding pattern, when surveyed at pre-vaccination period grazing only was found to be practiced by 39% of the farmers along with the 61% practicing grazing with supplement feeding. The dominancy in grazing with supplement feeding practices was found to be increased from 61% to 65.33% (an increase of 4.33%) after vaccination of the animals. Consequently, the number of free range grazing farmer was reduced from 16.23% to 11.11% (5.12% reduction) and the number of grazing in confined area farmers was increased from 13.67% to 23.07% (an increase of 9.40%) for pre-vaccination and post-vaccination survey period respectively. Farmers from the study areas also expressed their willingness to rear goats and sheep in the confined areas which is a positive sign for the land scarce country like Bangladesh. Respondents gave their opinion that grazing in the confined areas also helped to take close care of the animals avoiding dog biting problem.

Impact on housing system

The survey during pre-vaccination period revealed that 64% of the farmers had tin-shed housing system followed by 21% straw-covered and 15% brick-built. After one year of PPR vaccination, the number of tin-shed housing was increased by 2.33% (from 64% to 66.33%) with 3.33% decrease in the number of straw-covered sheds (from 21% to 17.67%). The number of brick-built sheds was also increased by 1.0%. This type of changes in housing system is attributable to the increase of income of sheep and goat farmers through decreased mortality due to vaccination of the animals against PPR, better animal health management and improved feeding practices.

Impact on Marketing Pattern

The marketing pattern before vaccination of goat and sheep was selling the animals at the local markets to be 38.33% to fetch higher price, followed by selling to the middle man (36%) and then to the neighbours (22.67%),for easy access as well as for saving transport cost. However, the survey after one year of vaccination revealed an unusual change in marketing pattern with the increase in selling animals to middle man by 9% (an increase from 36% to 45%) and to neighbours by 1.33% (an increase from 22.67% to 24%) with the consequence of decreasing of sells at the local market by 7.33% (a decrease from 38.33% to 31%). This type of unusual decrease in selling the animals at the local market that usually fetch higher prices is thought to be due to COVID- 19 pandemic which made farmers reluctant to go to the local market to sell their animals rather encouraged them to sell their animals to the middle man at the door steps or to the close neighbours to maintain the COVID- 19 health regulation of social distancing.

Table 12: Socio-economic impact of PPR vaccination on goat and sheep farmers

Sl. No.	Parameter	Categories	Pre-vaccination		Post-vaccination		Change in Percentage (%)
			Respondents*	Percentage of respondents (%)	Respondents	Percentage of respondents (%)	
1.	Mortality	PPR mortality rate	23.54 %		3.23%		-20.31
2.	Income from goat and sheep rearing (Thousand Tk.)	Low (20-50)	268	89.33	128	42.66	-46.66
		Medium (51-100)	29	9.67	158	52.67	43
		High (101-200)	03	1.0	14	4.67	3.67
3.	Feeding pattern	Grazing	117	39	104	34.67	-4.33
		a) Free range	19	16.23	13	11.11	-5.12
		b) Confined area	16	13.67	27	23.07	9.40
		c) Free range and confined area	82	70.08	77	65.81	-4.27
		Only feed supplement	0	0	0	0	0
		Grazing & feed supplement	183	61	196	65.33	4.33
4.	Housing pattern	Tin-shed	192	64	199	66.33	2.33
		Straw made	63	21	53	17.67	-3.33
		Brick and others made	45	15	48	16	1.0
5.	Marketing pattern	Local market	115	38.98	93	31.0	-7.98
		Neighbor	68	23.05	72	24.0	0.95
		Middle man	112	37.97	135	45.0	7.03
		Total respondents	295				

** For all the parameters where the number is not mentioned the total respondents were 300.*

Component- 2 (BAU)

a) Development of (diagnostic kit) for the detection PPR

This study mainly focused onto the development of mouse monoclonal anti PPR antibodies, polyclonal goat/ rabbit anti PPRV antibodies and isolation of PPRV in culture and designing RT-PCR protocols to detect PPR viruses. Due to time constraints however, mouse monoclonal anti PPRV antibodies could not be developed but polyclonal goat and rabbit anti PPRV antibodies were developed for the purpose of the study. This study also isolated PPR virus in Vero cells and this virus was used in viral antigen formulation and detection of anti PPR antibodies in ELISA.

Detection of PPRV and Development of anti PPRV antibodies

PPR viruses were isolated from PPR infected goats and used in raising polyclonal antibodies against PPR viruses in rabbits and goats. The freshly isolated PPR viruses were also used for vaccine seed development under this sub-project. The polyclonal antibodies and the viruses were used in two types of ELISAs like, sandwich ELISA and indirect ELISA to detect anti PPRV antibodies in vaccinated and PPR infected goats. In sandwich ELISA, rabbit anti PPRV antibodies were used as a primary coating agent to coat the polystyrene ELISA plate. The PPRV was, therefore, used to bind with the polyclonal rabbit anti PPRV antibodies. The known polyclonal goat anti PPRV antibodies and test serum (1: 1000 dilutions) were used as secondary anti PPRV antibodies and used in sandwich ELISA. The secondary antibodies were detected by using a commercially available mouse monoclonal anti goat IgG tagged with horseradish peroxidase (HRP). The enzyme system was developed with 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) substrate. An average OD value 0.350±0.071 and above was considered as positive immune response in the field. Randomly selected 10 vaccinated goat and 10 apparently healthy goat sera were tested in sandwich ELISA. All of the vaccinated goats showed moderate to high anti PPRV antibody response and six out of 10 nonimmunized goats showed anti PPRV antibody response at above baseline level (Figure 22). Similarly, an indirect ELISA protocol was developed by coating ELISA plates with PPR viruses and tested with the known polyclonal goat anti PPRV antisera and tests goat sera (n=10). Similar to sandwich ELISA, indirect ELISA protocols also generated similar response and can be used in the field to test anti PPR anti bodies response either following recovery, subclinical infection or to measure the level and duration of immune response following vaccination.

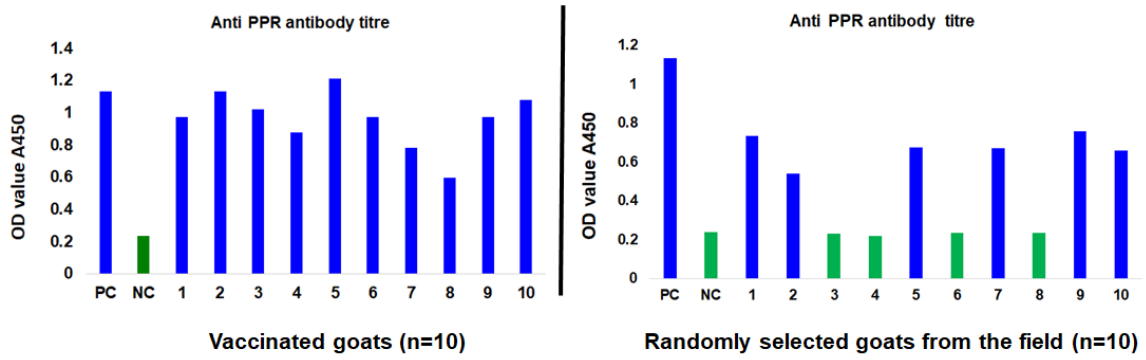

Figure 22: Anti PPRV antibody response as seen in vaccinated goats (left) and randomly selected goats collected from the field. All of the vaccinated goats (vaccine manufactured by the DLS) and six goats (free range) showed anti PPRV antibodies responses as detected by sandwich ELISA.

Development of PCR/RT-PCR protocol for the detection of PPR virus in the samples co-infected with other viruses

Current control of PPR mainly includes isolation of circulating viruses in culture and immunizing animals using a live-attenuated vaccine, which provides a strong immunity. Nucleotide sequence-based phylogenetic analyses of the structural gene (nucleocapsid or N gene) of currently used vaccine showed that the vaccine strain used in Bangladesh is phylogenetically similar to the lineage IV PPRV strains; showing particularly strong affiliation with the Tibetan virus. It was therefore needed to isolate a native virus and attenuated in Vero cells to develop a vaccine seed from local isolate. This study has successfully adapted PPR virus of local strain in goat kidney cells and thereafter in Vero cells. To achieve this, 36 clinically infected goats were collected from various locations of Bangladesh and systematic investigation was carried out including histopathological investigation of spleen, intestine and lungs of PPRV infected goats. There were massive hemorrhages in the spleen samples (Figure 23: left), severe hemorrhagic and necrotizing enteritis in the intestinal samples (Figure 23: middle) and massive congestion and hemorrhages in the lungs sample (Figure 23: right). Pharyngeal lymph nodes from the infected animals were collected aseptically. A portion of the gland was macerated using liquid nitrogen, extracted PPR viral RNA and used in RT-PCR detection of N gene of PPR viruses.

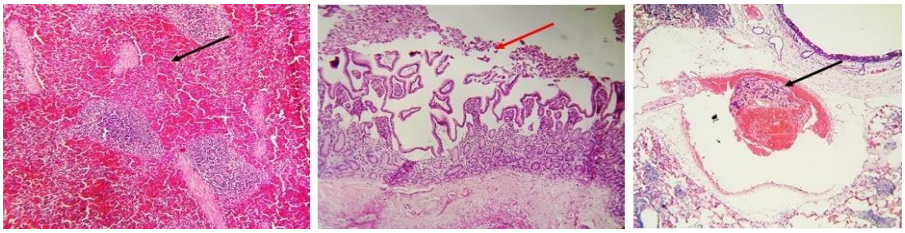

Figure 23: Histopathological investigation of spleen (left), intestine (middle) and lungs (right) of PPRV infected goats. There were massive hemorrhages in spleen (left, arrow), severe

hemorrhagic and necrotizing enteritis (middle, arrow) and massive congestion and hemorrhages (right, arrow) in lungs.

The cDNAs as obtained from the RT-PCR were subjected to gel electrophoresis and images were captured using an automated gel image capturing software. In positive cases 402bp fragment of N gene of PPR virus were detected (Figure 24). Viral RNAs from the lymph nodes of 20 naturally infected goats were used in RT-PCR and 402bp fragment of N gene were generated in 16 cases.

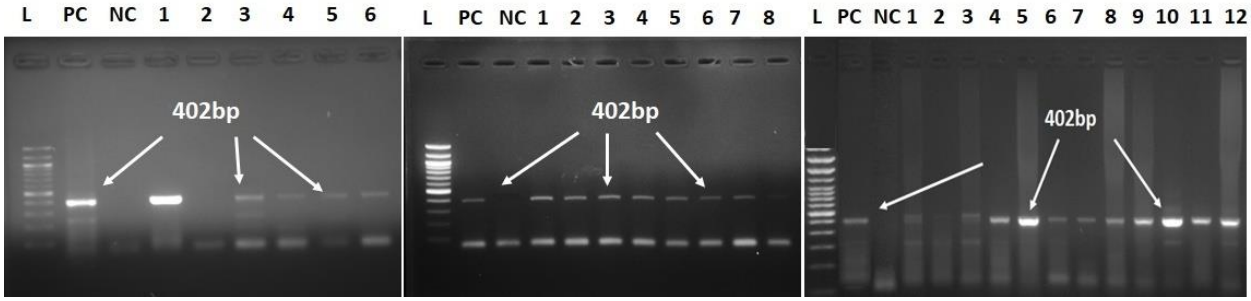

Figure 24: RT-PCR amplification of N gene of PPR virus with the RNA extracted from the lymph nodes of infected goats. In positive cases 402bp amplicon was generated. The lane L is for 100bp ladder, PC is for positive control, NC is for negative control, lane 1 to 6 (left), lane 1 to 8 (middle) and lane 1 to 12 (right) are for test samples.

Detection of contaminating viruses in lymph nodes

Viral RNA and DNA were extracted from the lymph nodes of 36 goats and used in PCR and RT-PCR amplification of selected genes of pox virus, Orf virus, FMD virus and PPR virus. The partial N gene of PPR virus was amplified (402bp amplicon) in 30 cases. Out of 30 PPR positive samples, only 02 goats (lymph nodes) showed Pox virus specific response (287bp amplicon), a goat showed Orf virus positive response (587bp), and two goats showed FMD virus specific response (430bp). Partial N gene of six (06) PPR positive samples was amplified and sequenced before isolating viruses in Vero cells. The co-infected samples were discarded and hence were not documented in this report.

Sequencing and phylogenetic analysis of N gene of PPRV: The sequence data were first identified by online NCBI Basic Local Alignment Search Tool (BLAST) to the gene of Peste des Petits Ruminant virus isolated from different countries in different years were retrieved from NCBI gene bank. Sequences were aligned using BioEdit 7.0.5 (Hall 1999) and phylogenetic tree were constructed using MEGA software (Hall BG, 2103). The sequence data of N gene of PPR virus were analysed, edited and submitted to the GenBank (having the accession no. of MW444788, MW525117, MW525118, MW525119, MZ028630 and MZ028631).The PPRV partial N gene sequences obtained in this study and other viruses representing PPRV lineages

downloaded from GenBank and used to construct phylogenetic tree. The six isolates (N gene sequences of PPRV) was clustered into Lineage IV together with other PPRV previously reported from Bangladesh, China, India, Tibet, Pakistan and other Asian and African isolates. The obtained partial N gene fragment searched in BLAST revealed that these isolates were very close relationship with China (KX421388), Tibet (FJ905304) and Indian (KT860065) isolates with nucleotide percent identity 96.73% to 98.19%. The partial N gene of an original virulent field isolate (MW525119) following 1st passages in Vero cells showed 97.87% percent identity with the Chinease isolates KX421388. The PPRV isolate recently adapt in Vero cell culture were made a seperate subcluster and quite dintinct from previous other Bangladeshi isolates. These results suggest that the N gene of Bangladeshi PPRV has evolved somewhat (Figuire 24).

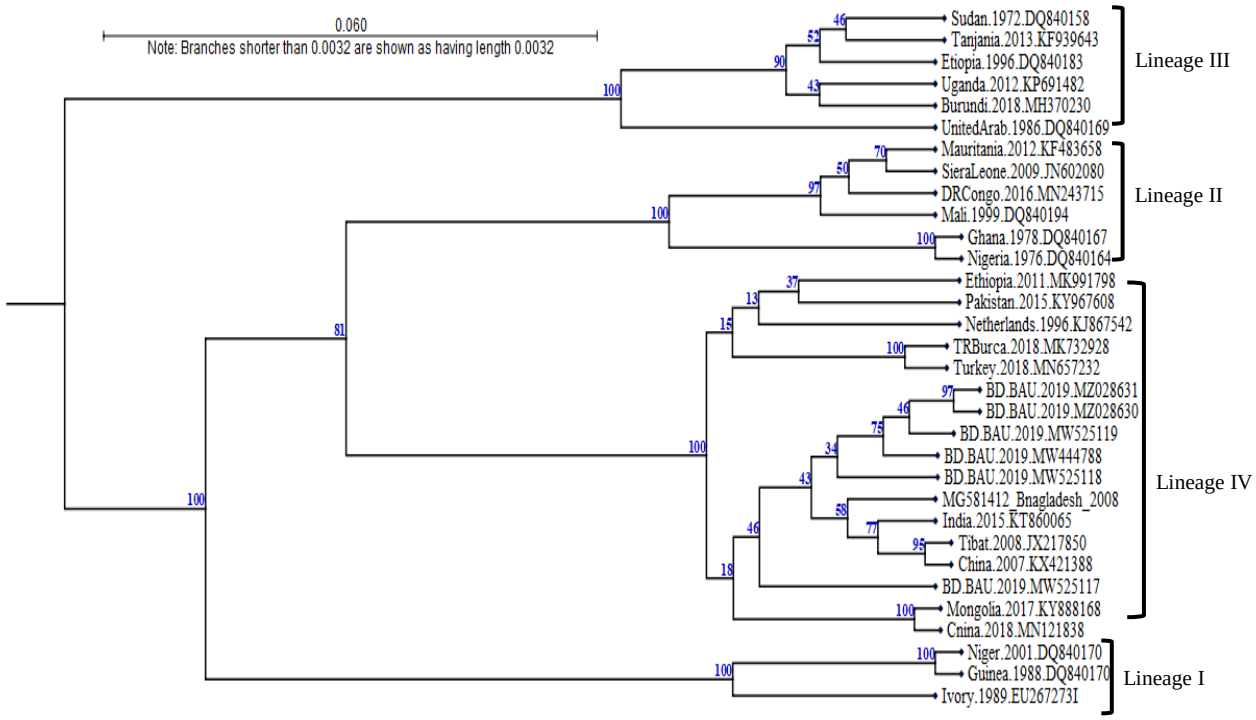

Figure 25: Phylogenetic analysis of PPRV N gene of six isolates BD.BAU showed that the viruses share 96.73% to 98.19% homology with viruses in India and China forming a separate sub-cluster.

b) Development of PPR viral vaccine seed

Isolation and attenuation of PPR Virus in Cultures

Out of the 06 selected isolates of PPR virus, the field isolates of PPR virus, GenBank Acc. No. MW525119 was found to show maximum antigenic similarity with the circulating viruses consequently, when passaged in goat kidney cells and Vero cells for attenuation resulted in significant CPE such as cell rounding up, cell aggregation syncytia formation and detachment from the flasks during isolation procedure (Figure 26). Flask containing Vero cells with other isolates of PPR virus had less adaptability and did not show distinct CPE. Therefore, serial

passage was continued with the PPR isolate MW525119 to attenuate the virus. A total of 65-70 serial passages are needed to attenuate PPR virus in Vero cells to develop a vaccine seed. Due to time constraints, the total number of serial passages in Vero cells required (65-70) for attenuation of PPRV could not be completed. However, during the submission of this report a total of 28 passages of the isolate in Vero cells were carried out. Presence of the virus in the passage was confirmed by RT-PCR amplification of the N gene of the PPR virus.

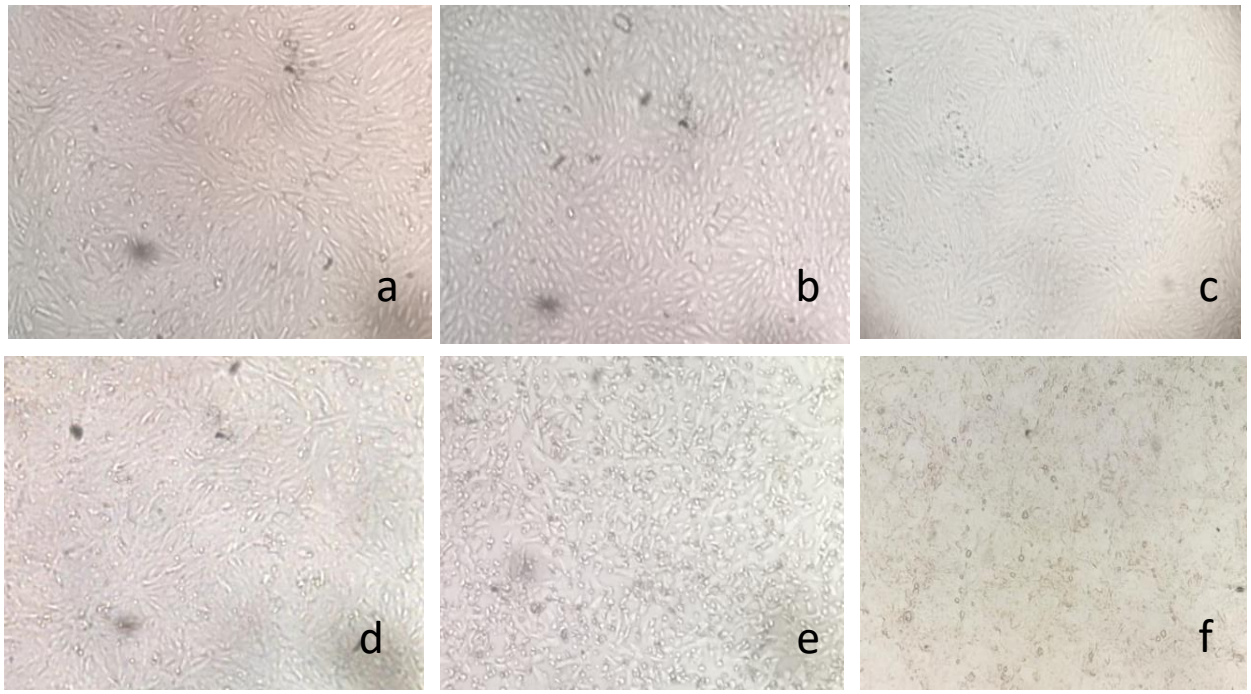

Figure 26: Field virulent strain PPR virus infection on Vero cell culture showed progrssive cytopathic change in different times. Unstained micrograph (a) was control Vero cell, (b) Virus inected cell at 1 hour, (c) Cells were elongated, few cells showed rounded at 2nd dpi, (d) CPE found at 3rd dpi, (e) Sinificant CPE's found on Vero cell at 4th dpi and (f) about 80-90% cell detachment occured at 5th dpi.

Sequencing of N gene and evaluating the way of mutation following subculture:

RT-PCR amplicons of N protein gene of PPRV of five cell culture attenuated virus (1st, 5th, 10th, 15th and 20th passages) was amplified in RT-PCR and the cDNAs were sequenced (carried out in Apical Scientific laboratory, Malaysia). This obtained gene sequence data showed nucleotide replacement (point mutation) as analyzed (Figure 27) based on partial N gene sequencing. From this analysis, it has been shown that a limited extent of evolution occurred in adaptation in Vero cell culture. The nucleotide percent identities and number of nucleotide substitution of cell passages PPRV's partial N gene compared with field isolates N gene revealed that 1% nucleotide substitution occurred in cell culture adaptation (Table 13).

Table 13: The nucleotide percent identities and number of nucleotide substitution of cell passages PPRV's partial N gene compared with field isolates N gene

SL No	ID. No. Of cell-cultured passages	Nucleotide percent identities	No. of Nucleotide substitution
01	1st passages of PPRV	370/376 (98%)	06
02	5th passages of PPRV	383/386 (99%)	03
03	10th passages of PPRV	394/397 (99%)	03
04	15th passages of PPRV	394/397 (99%)	03
05	20th passages of PPRV	393/396 (99%)	03

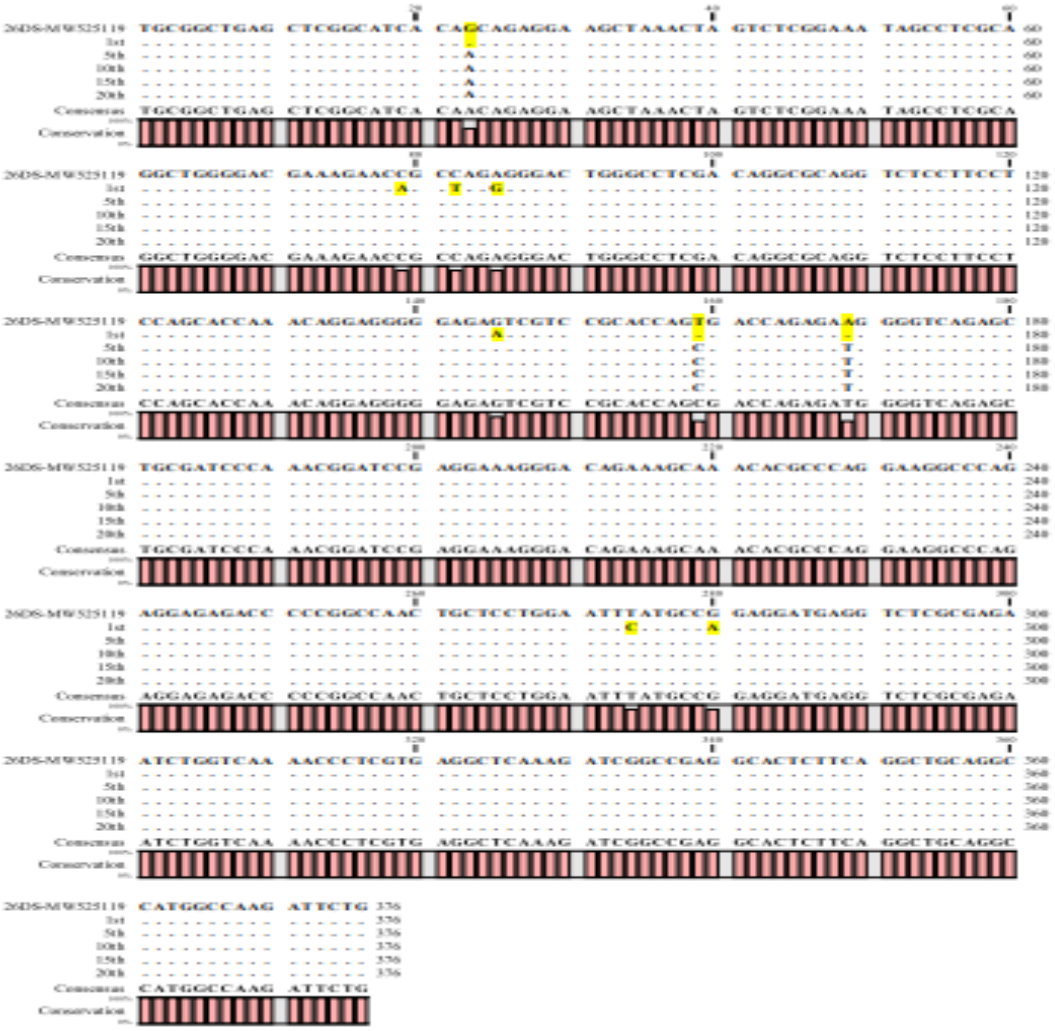

Figure 27: Point mutations were seen at nucleotide level 23 (G/A), 159 (T/C) and 169 (A/T) of the fragment of N gene as obtained following serial passages in Vero cells.

Detection of CPE in PPRV infected Vero cells grown on coverslips

In Vero cells, it is sometimes difficult to see the syncytia. If they exist, they are very small. However, in stained, infected Vero cells, small syncytia are always seen and Cover-slip cultures may give a CPE earlier than the day 5 (Hedge et al., 2009). Therefore, for the ease of detection of cytopathic effect (CPE) in Vero cells infected with PPR virus the Vero cells were grown at 37ºC temperature on 40x25mm coverslips in 75cm3 tissue culture flasks containing 5ml growth medium with 10% fetal bovine serum (FBS). Cells were stained with Mayer's hematoxylin & eosin at 24 hours, 72 hours (3 days) and 120 hours (5 days) of infection. The coverslips with stained and unstained Vero cells were then examined under microscope after mounting on glass slides for the presence of CPE. The CPE, evidenced by cell rounding, cell aggregation and syncytia formation was more pronounced in coverslips stained with H&E compared to unstained coverslips (Figure 28).

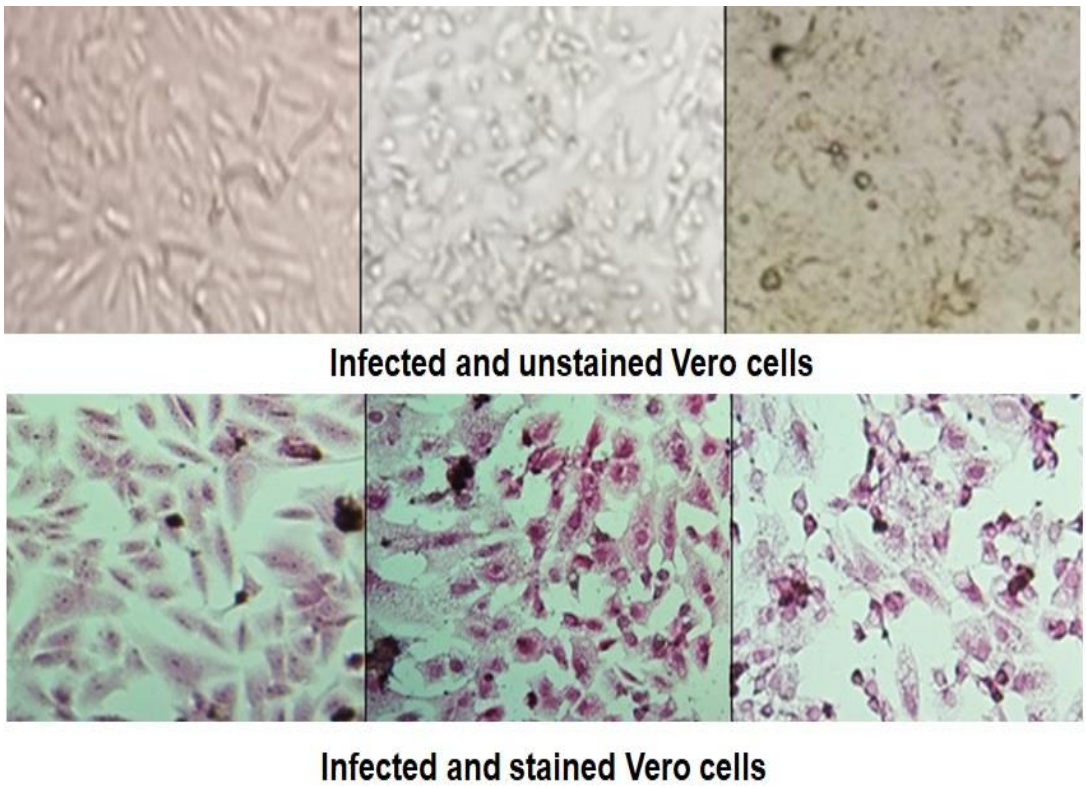

Figure 26: Pictures comparing infected and unstained Vero cells with the infected and stained Vero cells on coverslips. Lack of CPE of non infected cells (left), PPR viral infected cells following 72 hrs (middle) and 120 hrs (right) of infection as seen onto the infected Vero cells on cover slips stained with H&E.

12. Research highlights

Title of the sub-project: Preparedness for the control of PPR in Bangladesh

Background

Globally the disease Peste des Petits Ruminants (PPR), popularly known as goat or sheep plague is an important OIE listed transboundary animal disease (TAD) of small ruminants. PPR in Bangladesh is much devastating in goats in rural areas due to high morbidity (10-100%) and mortality (up to 100%) that in turn causes heavy production losses (due to death of infected goats). To control the spread of PPR virus regular and routine immunization using a potent PPR vaccine is needed for blocking the transmission cycle of the virus through generation of an immune population of small ruminants in the country. Commercially available diagnostics are much expensive and there are controversies about the effectiveness of the vaccine presently used in Bangladesh.

Objectives

i. To conduct sero-epidemiology study of PPR in small ruminants (goats and sheep)
ii. To conduct outbreak investigation and monitoring of circulating PPR virus using molecular technique
iii. To develop PPR free zone and study of its socio-economic benefit
iv. To develop biologics (diagnostic kits) for the diagnosis of PPR
v. To develop vaccine seed using local isolates of PPR virus

Methodology

- Sero-epidemiology study was conducted by using competitive-ELISA encompassing 27 districts of Bangladesh during the period from July 2018 to June 2021.
- Outbreak study of PPR was conducted using RT-PCR by amplification of the partial N gene of PPRV covering 23 districts of Bangladesh.
- For the study of development of PPR free zone, three unions namely Debhata (Satkhira), Gangni (Meherpur) and Badarganj (Rangpur) were selected where more than 80% (30,615 out of the total population of 37,709 sheep and goat) of the goats and sheep were vaccinated using locally produced PPR vaccine.
- A socio economic study was also conducted by BLRI among 300 farmers at the selected three upazilas.
- For polyclonal antibody preparation, rabbits and goats were immunized using locally isolated purified Vero cell adapted PPR virus.
- The anti-goat and the anti-rabbit polyclonal antibodies were then used in sandwich ELISA and in indirect ELISA for detection of both the disease PPR and the PPR virus.

- For genome-based diagnosis, specific primers of N protein gene of PPRV was designed and used in RT-PCR.
- In an effort to develop PPR viral vaccine seed, PPR infected goats (n=36) were collected from the outbreak areas; PPR infection was confirmed using RT-PCR of the samples collected from the bronchial and pharyngeal lymph nodes of infected goats.
- Co-infectivity with other viral diseases (like pox, FMD and Orf) in the lymph node samples were confirmed by using either PCR or RT-PCR.
- The pure isolates of PPR virus were then used for propagation in Vero cells.
- The cell adopted PPR viruses were then used for attenuation and development of vaccine seed by serial passaging in Vero cells.

Key findings

- Sero-prevalence study using cELISA, covering 27 districts of Bangladesh, revealed overall prevalence of PPR to be 44.95% in unvaccinated goats and sheep (out of a total of 3933 sera samples tested 1768 were positive for PPRV antibody) with the lowest being 29.36% found in Badarganj, Rangpur and highest 65.60% in Sirajganj.
- The highest rate of clinical symptoms of PPR was recorded to be the nasal discharge (32%) followed by diarrhea (27%), oral ulcer (21.67%), respiratory distress (10%), ocular discharge (8%) and abortion (1.66%);
- PPR outbreak investigation covering 23 districts of Bangladesh, using molecular detection technique of RT-PCR, the highest percentage (78.84%) of PPR virus was detected in the samples collected from infected goats and sheep at Savar region, Dhaka. Whereas, in Mymensingh the prevalence was lowest (20%). The overall prevalence of PPR was found to be 54.59% (out of the total of 403 PPR samples tested 220 were positive).
- Vaccination against PPR using the locally produced vaccine (of DLS) was able to reduce the outbreak (determined using RT-PCR test) of PPR from 21-42% (in unvaccinated flocks) to 3% (in the flocks one year after vaccination).
- In an effort to develop PPR free zone three unions Nowapara from Debhata, Satkhira; Raypura from Gangni, Meherpur and Damodarpur from Badarganj, Rangpur were selected where 80% of the population of goats and sheep were vaccinated using the PPR vaccine of DLS. The study revealed that herd immunity could be developed using the locally produced PPR vaccine. The sero-positivity was found to be maintained at 86.89%; 93.06%; and 88.11% when tested after one year of vaccination of the animals in Debhata (Satkhira), Gangni (Meherpur) and Badarganj (Rangpur) respectively.

- Impact on income from goat farming was found to be increased at one year post-vaccination (due to reduced sheep and goat mortality), that was evidenced by the reduction in the number of low income group of farmers from 89.33% to 42.66% and the increasing in the number of medium income group of farmers from 9.67% to 52.67% along with the increase in high income group of farmers from 1% to 4.67%.
- This study developed polyclonal antibodies (antisera) against PPR virus in rabbit and goats.
- This study also identified and isolated PPR viruses in Vero cells.
- The technique of growing Vero cells on coverslips and staining with H&E for the ease of detection of CPE in PPRV infected cells has been adopted for the first time in Bangladesh.
- This study introduced successfully RT-PCR and PCR protocols to detect the co-infecting viruses like FMD, pox and orf with PPR viruses in the field samples. Confirming that the PPRV isolated from a field sample is free from other commonly found co-infecting viruses will help trouble free propagation of PPRV in Vero cells for attenuation/isolation process of the virus.
- The locally developed primary rabbit and goat anti PPRV antibodies and the isolated PPRV in Vero cell culture was successfully used in sandwich ELISA to detected antibodies against field infectivity of goats due to PPRV.
- The isolated PPR virus was also used in indirect ELISA to detect anti PPRV antibodies. This test results correlate with the findings of sandwich ELISA.
- Until now (during submission of PCR) 32^{nd} passages in Vero cells have been completed. Following each passage the infectivity of cells due to PPRV were confirmed by using RT-PCR. It is hoped that serial passaging into Vero cells will be continued in the laboratory, beyond the project period, till proper attenuation of PPR virus is achieved and vaccine seed is developed.
- Sequencencing of the partial N gene of the isolated PPR viral RNAs from the 1^{st}, 5^{th}, 10^{th}, 15^{th} and 20^{th} passages in Vero cells showed nucleotide replacements (point mutations) at nucleotide level 23 (G to A), 159 (T to C) and 169 (A to T) indicating that the attenuation is in progress.
- Phylogenetic analysis of partial N gene revealed these isolates to be very closely relate to the China, Tibet and Indian isolates with nucleotide percent identity of 96.73% to 98.19%.

- This study was unable to generate mouse monoclonal anmtibodies agaist PPR viral epitopes due to time constraints. However, the work is going on.

Key words

PPR, RT-PCR, ELISA, PPR free zone, Vero cell culture, Phylogenetic analysis of partial N protein gene of PPR, PPR vaccine seed, Passaging PPRV in Vero cells, Detection of CPE in PPRV infected Vero cells on coverslips by staining with H&E.

B. Implementation Status

1. Procurement (Component wise):

Coordination Component (BARC)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment	Steel Almira (1) Scanner (1)	(24415+10000) =34415	100%	100%	
(b) Lab &field equipment	-	-	-	-	-
(c) Other capital items	-	-	-	-	-

Component -1 (BLRI)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment	a)Computer table (01) b)Computer chair (01) c)Visitor chair (04) d)Steel Almira (01) e)File Cabinet (01) f) Refrigerator (02) g)Laptop (01) h)Scanner (01) i)UPS (offline) (01) j)Laser Printer (01)	5,000 3,500 16,000 24,000 15,000 60,000 60,000 10,000 10,000 20,000	a)Computer table (01) b)Computer chair (01) c)Visitor chair (04) d)Steel Almira (01) e)File Cabinet (01) f) Refrigerator (02) g)Laptop (01) h)Scanner (01) i)UPS (offline) (01) j)Laser Printer (01)	6,000 3,000 14,000 25,000 15,000 64,000 54,000 11,000 10,500 22,000	

(b) Lab &field equipment	Distilled water plant (Double)- 01	250,000	Distilled water plant (Double)- 01	245,000	
(c) Other capital items					

Component 2 (BAU)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment	LS	83000	LS	83000	
(b) Lab &field equipment	LS	2955962	LS	2954510	
(c) Other capital items	01	699000	01	699000	

2. **Establishment/renovation facilities:** (None for any of the components of BARC, BLRI and BAU)

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized:

Coordination Component (BARC)

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training: Control of PPR in Bangladesh	102	58	160	6 days	
(b) Workshop: Annual workshop	125	39	164	3 days	
(c) Others (if any)	-	-	-	-	-

Component-1 (BLRI)

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	14	06	20	02 Days	
(b) Workshop	-	-	-	-	
(c) Others (if any)	-	-	-	-	

Component-2 (BAU) None

C. Financial and Physical Progress (Combined & Component wise)

Financial and Physical Progress (Combined)

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	8946707	18025082	7629475	950520	71.24	
b. Field research/lab expenses and supplies	6889734		6867445	2122	49.68	
c. Operating expenses	1074973		933219	58623	59.53	
d. Vehicle hire and fuel, oil & maintenance	137711		136576	-9961	66.39	
e.Training/workshop/seminar etc.	875600		695600	169780	63.33	
f. Publications and printing	200000		0	200000	0	
g. Miscellaneous	433802		325994	122338	56.04	
h. Capital expenses	1214615		1214615	0	70.58	
Total	19773142	18025082	**17802925**	**1493422**	90.03	

Financial and Physical Progress (Component wise)

Coordination Component (BARC)

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	33,85,375	39,10,245	23,60,032	5,93,309	69.71	
b. Field research/lab expenses and supplies	-		-		0	
c. Operating expenses	6,03,977		2,16,498		35.84	
d. Vehicle hire and fuel, oil & maintenance	1,34,996		19,996		14.81	
e. Training/workshop/seminar etc.	7,54,000		6,05,600		80.32	
f. Publications and printing	3,15,000		-		0	
g. Miscellaneous	3,53,747		80,395		22.72	
h. Capital expenses	34,415		34,415		100	
Total	**55,81,510**	**39,10,245**	**33,16,936**	**5,93,309**	**84.43**	

Component-1 (BLRI)

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	29,72,119	26,06,927	23,76,884	230,043	79.97	
b. Field research/lab expenses and supplies	39,33,772	39,15,057	39,12,935	2,122	99.47	
c. Operating expenses	5,20,316	500,110	497,570	2,540	95.66	
d. Vehicle hire and fuel, oil & maintenance	97,715	86,619	96,580	-9,961	98.84	
e.Training/workshop/seminar etc.	90,000	79,780	90,000	-10,220.00	100	
f. Publications and printing	0	0	0	0	0	
g. Miscellaneous	99,240	87,970	95,670	-7,700	96.40	
h. Capital expenses	481,000	481,000	481,000	0	100	
Total	**81,94,162**	**77,57,463**	**75,50,639**	**206,824**	**97.33**	

Component-2 (BAU)

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	2012379	5957374	2010859	Nil	33.75	
b. Field research/lab expenses and supplies	2955962		2954510		49.59	
c. Operating expenses	238000		175075		2.94	
d. Vehicle hire and fuel, oil & maintenance	20000		20000.		0.34	
e. Training/workshop/seminar etc.	-		-		-	
f. Publications and printing	-		-		-	
g. Miscellaneous	71929		97729		1.64	
h. Capital expenses	699200		699200		11.74	
Total	**5997470**	**5957374**	**5957374**	**Nil**	**100**	

D. Achievement of Sub-project by Objectives (Tangible form): Technology generated/developed:

Coordination Component (BARC): Not applicable

Component-1 (BLRI): No technology was generated

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
-	-	-	-

Component-2 (BAU)

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
(a) To develop biologics for the diagnosis of PPR	Development of polyclonal antibodies against PPRV: i) Vero cell adapted PPR virus was inactivated using BEI ii) The inactivated viral suspension was admixed with Freund's complete adjuvant iii) Immunization of rabbit and goat with the inactivated PPR virus mixed with Freund's adjuvant applying booster doses iv) Serum samples were collected from the vaccinated animals following 30 days of immunization.	Anti PPRV antibodies in rabbit and goats developed; those were used in "Indirect ELISA" to detect antibodies against PPRV and in "Sandwich ELISA" to detect both the anti PPRV antibodies and PPRV from the field cases.	Laboratories could adopt the technology of the development of polyclonal antibodies against PPRV for using in ELISA to detect PPRV and anti PPRV antibodies.
(b) To develop vaccine seed to control PPR	i) Isolation and molecular identification of PPR virus from field cases ii) Exclusion of samples co-infecting with viruses like goat Pox, Orf and FMD. iii) Propagation of PPRV in Vero cell culture and serial passaging of the virus into the cell culture after confirming the infectivity using RT-PCR iv) Rapid detection of CPE in coverslip culture of Vero cells infected with PPRV. v) Monitoring the progress of attenuation by sequencing of partial N gene of PPRV at the different stages of passage in Vero cells.	i) Presence of PPRV in the lymphnode sample was confirmed using RT-PCR. ii) Samples co-infected with FMD, Pox and Orf virus as detected by RT-PCR (PPR and FMD viruses) and PCR (Pox and Orf viruses), were discarded. iii) So far, 32nd passages of PPR Virus in Vero cells have been completed. iv) Detection of PPRV induced CPE in coverslip culture of Vero cells stained with H&E has been adopted successfully. v) Point mutations were observed at nucleotide levels 23 (G to A), 159 (T to C) and 169 (A to T) indicating that the attenuation is in progress following serial passages in Vero cells.	The protocol adopted for the development of PPR vaccine seed under this sub-project could be used for vaccine seed development from other viruses like FMD, Pox, Orf, etc. It is hoped that serial passaging into Vero cells will be continued in the BAU laboratory beyond the project period, till proper attenuation of PPR virus is achieved and vaccine seed developed or if it is not possible for BAU to continue the work then the half processed Vero cell adapted PPRV may be transferred to BLRI or DLS for further processing towards the development of a vaccine seed.

E: Information/Knowledge generated/Policy generated

Coordination Component (BARC): Not applicable

Component 1 (BLRI)

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
a) Sero-epidemioly study of PPR in small ruminants. b) Outbreak investigation of PPR using RT-PCR. c) Development of PPR free zone.	a) A total of 3933 blood samples (27 selected districts) were collected from unvaccinated goats and sheep and the sera were tested using c-ELISA for the presence of PPRV antibody. b) A total of 403 PPR suspected samples were collected from outbreak investigation areas (23 selected districts). c) Three unions, one each, from Debhata,Gangni and Badarganj upazilas were selected for developing PPR free zone. More than 80% of the sheep and goat population in the areas were vaccinated against PPR	a) The overall sero-prevalence was 44.95% with the lowest (29.36%) being found in Rangpur and highest in Sirajganj (65.60%). b) PPR outbreak pattern before vaccination was around 21%-25%. But, one year after vaccination the outbreak of PPR came down to 3% in the vaccinated villages that was 19% in unvaccinated villages. c) The sero-positivity was found to increase steadily to 85.72%, 90.82% and 81.89%; 90.76%, 83.54% and 95.45%; 86.14%, 93.06% and 88.11% at 2 months, 6 months and 1 year of post vaccination in Debhata, Gangni and Badarganj respectively.	a) The study revealed that around 55% of the goat and sheep population in Bangladesh are at risk of contracting PPR. b) This study revealed that vaccination of small ruminants with PPR vaccine was able to reduce the outbreak of PPR. c) The present study revealed that herd immunity could be developed using the locally produced PPR vaccine. However, for declaring the areas as PPR free zone the present high level of sero-positivity has to be maintained at least for 3 years and beyond through vaccination of new generation of animal population and stringent sero-surveillance study.

Component-2 (BAU)

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
(a) To develop biologics for the detection of PPR.	Used PPRV in immunizing goats and rabbits for the development of anti PPRV antibodies.	Developed polyclonal antibodies against PPRV in goats and rabbit	Help in developing sandwich and indirect ELISA to detect anti PPRV antibodies and PPR virus.
(b) To develop vaccine seed against PPR	Identification of PPR virus in the suspected samples using RT-PCR Exclusion of samples co-infecting with viruses like goat Pox, Orf and FMD. Propagation of PPR virus in Vero cells and attenuation of the virus through serial passaging into the cells. Sequencing and Phylogenetic analysis of partial N gene of the virus.	Obtained PPR virus, free from contaminating viruses (Goat pox, Orf and FMD). Successfully adapted PPRV in Vero cells. So far, 32nd passages in the Vero cells have been completed. Phylogenetic analysis of partial N gene of the virus revealed that these isolates are very closely relate to the China, Tibet and Indian isolates with nucleotide percent identity of 96.73% to 98.19%.	The knowledge generated on the development PPR vaccine seed from the local isolates of PPR viruses will be helpful in vaccine seed formulation for other viruses.

F. Materials Development/Publication made under the Sub-project

Coordination Component (BARC)

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.		01	Training Manual on Control of PPR in Bangladesh
Journal publication			
Video clip/TV program			
News Paper/Popular Article			
Other publications, if any			

Component-1 (BLRI): No such publication made

Component-2 (BAU)

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication	01		Molecular detection of co-infecting viruses from suspected Peste des Petits Ruminants (PPR) outbreaks with special emphasis on isolation and partial characterization of PPR viruses in Bangladesh.
Video clip/TV program			
News Paper/Popular Article			
Other publications, if any **(Poster Presentation**)		01	Designing Polymerase Chain Reaction Techniques for the detection of PPR, Orf, Goat pox and FMD in clinically infected Black Bengal goats. This poster awarded at 26th Bangladesh Society for Veterinary, Education and Research (BSVER) ASCON 2020.

G. Description of generated Technology/Knowledge/Policy:

i. Technology Factsheet (title of the technology, introduction, description, suitable location/ecosystem, benefits, name and contact address of author)

Title of the technology: PPR vaccine seed developed from the local isolates of PPR viruses

Introduction

Peste des Petits Ruminants (PPR) is globally known as goat/ sheep plague, a vital OIE listed transboundary animal disease (TAD) of small ruminants. The disease is endemic Bangladesh. PPR is much devastating in goats in rural areas due to high morbidity (10-100%), high mortality (up to 100%) and finally heavy production losses (due to death of infected goats). It needs regular sero-monitoring and immunization with an effective viral vaccine. Commercially available diagnostics are expensive, and there are controversies about the effectiveness of the vaccine used in Bangladesh. This study is designed to developed diagnostics for PPR virus (antigen and antibody based) and development of an effective PPR viral vaccine to combat field infectivity.

Steps followed to develop PPR vaccine seed:

a. Propagation of PPR virus in Vero cells for vaccine seed formulation
b. Preparation of PPR viral antigen
c. Development of polyclonal antibodies against PPRV in rabbit and goats
d. Demonstration of cytopathic effect of PPRV in Vero cells on cover slip culture.

Description

Propagation of PPR virus in Vero cells for vaccine seed formulation: A total of 36 clinically infected goats were collected from various locations of Bangladesh. The bronchial, pharyngeal and mesenteric lymph nodes were collected aseptically in cryotubes, snap frozen and preserved at -20˚C. The viral RNA was extracted using SV Total RNA Isolation System (Promega, USA). The purity and concentration of extracted RNA was measured at 260nm/ 280nm in a Nanodrop^{TM} spectrophotometer (IAEA, Scibersd off, Vienna). A 260nm/ 280nm ratio of~2.0 was generally considered as "pure" and used for reverse transcriptase polymerase chain reaction (RT-PCR) detection of N gene of PPRV. The RT-PCR was carried out using the primer sequences (Forward: gctctgtgattgcggctgagc and Reverse: cctggtcctccagaatcttggcc) obtained from a commercial source (AIT Biotech, Singapore). An RT-PCR amplicon of 402bp was considered as positive and 30 samples yielded positive response. Differential diagnosis of pox virus, Orf virus and FMD virus were made by using PCR and RT-PCR tests for the identification of co-infection along with the PPR virus positive samples. Among the PPR positive samples two samples showed pox virus co-infectivity, two samples showed FMD virus co-infectivity and one sample showed Orf virus co-infectivity. Co-infected samples (n=05) were discarded. From the PPR viral positive samples RT-PCR amplification of N protein gene of PPRV was carried out and sequenced (carried out in Apical Scientific laboratory, Malaysia). Designed primers for the detection of PPRV (Table 1) were used in sequencing of N protein gene. A total of 25 truly PPR viral infected samples were processed for further study. However, for the convenient of study, six samples were adapted in culture and the phylogenic analysis of partial N gene of selected PPR samples showed that the isolated virus was 95-98% homology with viruses circulating in Indian and China but it made a separate sub-cluster indicating evolution at small extend.

PPR virus from the prescapular, pharyngeal or bronchial lymph nodes of affected goats (n=05) were propagated in Vero cell line. The lymph nodes were macerated using sterile mortar and pestle in the presence of liquid nitrogen, to prepare a 20% (w/v) tissue homogenate in Dulbecco's phosphate buffered saline (PBS). The suspension was centrifuged (800g) for 10mins to remove debris. The supernatant was collected in a fresh Falcon tube; part of the suspension was used to identify PPR viruses by RT-PCR. PCR and RT-PCR tests were also carried out to

identify any contaminating viruses like goat pox, foot and mouth disease (FMD), and contagious ecthyma (Orf) present in the samples or not; PPR viruses, free from other contaminating viruses, were grown in Vero cell line. Part of the suspension was treated with gentamicin at the rate of 500µg/ml for 30mins at room temperature. The treated suspension was stored in aliquots (100µl/ tube) at -80°C until used. Prior to use, the suspension was passed through a syringe filter (0.2µm pore size) and the filtrate was used as the inoculums.

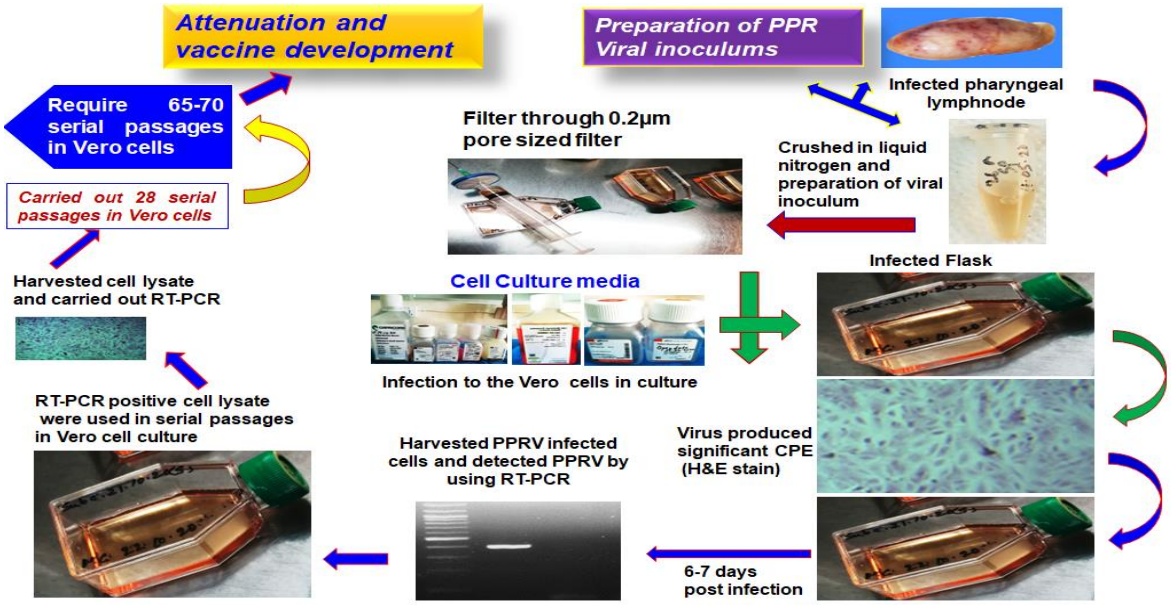

Figure28: Isolation, adaptation and attenuation of PPRV in culture.

For isolation of PPR virus, pharyngeal lymph nodes from the infected goats were chopped finely with scissors, crushed using sterile pestle and mortar in liquid nitrogen, made fine suspension in 10ml PBS. The suspension was centrifuged at 4000g for 5 minutes and the supernatant was inoculated in monolayer of goat kidney cells. After 5 passages in goat kidney cell culture the viral inoculum was then used to inoculate 75-80% confluent monolayer of Vero cells in flasks. The cytopathic effect was examined under microscope following 5-7 days of infection. Infected Vero cells on the cover slips were also examined under microscope after staining with H&E to visualize the cytoplasmic and nucleus specific CPE. The cell culture flask infected with PPRV was used in viral RNA extraction and detection of N protein gene of the viruses by using RT-PCR. Until now 32nd passages of PPRV in Vero cells has been completed and the process of serial passaging is being continued.

Preparation of PPR viral antigen: To achieve this PPR virus was initially propagated in goat kidney cells. After five blind passages in goat kidney cells the cell lysate was tested for the presence of PPRV by using N gene specific RT-PCR. The cell lysate containing PPRV was then used to inoculate monolayer of Vero cells. Confluent monolayer of Vero cells was prepared in 25cm^2 flask following standard subculture procedure using Eagle's medium (MEM) enriched

with 5 to10% fetal calf serum. For isolation of the virus 200µl of each sample was inoculated in duplicate flasks. The cells were examined daily for cytopathic effects (CPE). Each sample was subjected to three blind passages irrespective of the appearance of CPE. After each passage the cell culture supernatant was tested by RT-PCR for the detection of PPR virus as described. Until now 32nd passages of PPR virus in Vero cell culture have been carried out and each successful passage was confirmed by determining the PPR viral RNA in the inoculum by using RT-PCR. Attenuation of PPR virus in Vero cell line is still going on. Viral RNA from 1st, 5th, 10th, 15th and 20th passages was amplified using RT-PCR and the cDNAs were subjected to sequencing, sequence analysis and determination of clade position of the virus. Complete attenuation require about 70 passages in Vero cells.

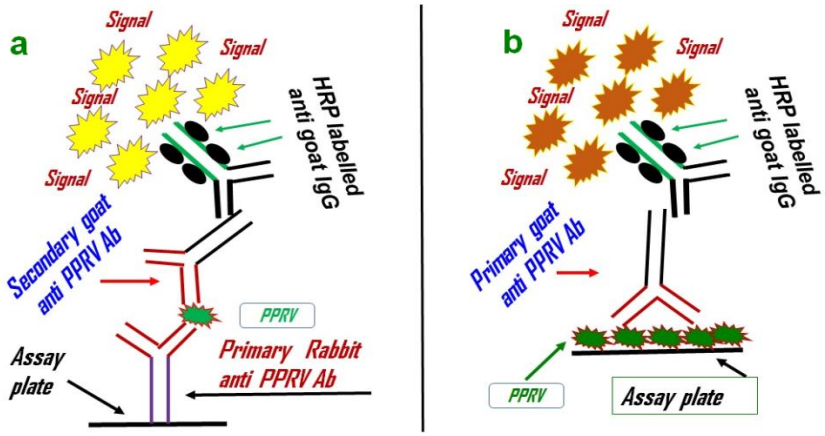

Figure 29: Schematic diagram for the detection of anti PPRV antibodies and PPR viruses from infected goats by using sandwich ELISA (a). The project has generated primary rabbit anti PPRV antibodies, primary goat anti PPRV antibodies and PPR viruses. This sandwich ELISA can be used to detect both the anti PPRV antibodies and PPR viruses from the field cases in Lab condition. The PPRV as isolated in this study was also used in an indirect ELISA to detect polyclonal (anti sera) antibodies against PPRV (b).

Development of polyclonal antibodies against PPRV in rabbit and goats: As the PPR virus got adapted in cell culture and 4th passage showed stable replication in Vero cells the bulk culture of the viruses (about 500ml volume) was achieved by replicating virus in Vero cells in larger flasks. Following inoculation of the virus in Vero cells (5-7 days of culture), the cell culture flasks were subjected to 4 freezing and thawing cycles. The cell culture fluid was then collected in Falcon tubes. Following centrifugation at 3000rpm for 15 minutes, the cell culture supernatant was collected in fresh Falcon tubes. The Falcon tubes containing the virus infected supernatant were then subjected to low speed centrifugation (5000 rpm for 10 minutes) to remove cell debris. The supernatant was collected aseptically and subjected to precipitation using 8% (w/v) polyethylene glycol (PEG) along with 2.3% (w/v) sodium chloride. This

mixture was incubated overnight at +4°C. Then the cultured fluid was centrifuged at 32000rpm for 2hrs. The pellet was collected and added 10ml PBS. A 70% sucrose solution was prepared and took into the bottom of the ultracentrifuge tube then a 30% sucrose solution was overlaid on it, on top of which viral pellet suspension was added. This mixture was centrifuged for 50000rpm for 1hr. The white band above the 70% sucrose solution was collected by aspiration in an eppendorf tube, centrifuged at 15000 rpm for 5 minutes using a microcentrifuge machine. The supernatant was discarded and the pellet was carefully aspirated and collected in a fresh eppendorf tube that was then tested for the presence of PPRV by RT-PCR. The pellet from the PPR viral culture supernatant containing infective viral particles was inactivated by using binary ethylenimine (BEI), at a concentration of 1mMand 2mM. This concentration of BEI was generated by treating 0.1M 2-bromoethylamine hydrobromide with 0.2N NaOH for 1 hour at 37°C. This solution was then be added to the virus suspension to give the desired concentration of BEI, i.e. 1% v/v for 1mM BEI and 2 % v/v for 2mM BEI. Periodically, the virus-containing bottle was inverted in order to inactivate virus in the air space above the liquid level. Excess BEI was neutralized at the end of the inactivation period with 0.1 % w/v sodium thiosulphate. The extent of viral inactivation was determined by growing the inactivated samples in Vero cells, studied the CFE and detected the viral component by using RT-PCR. The flask containing Vero cells and inoculated with BEI inactivated viruses was not infected and the cell lysate did not show any amplicon in RT-PCR indicating complete inactivation of the viruses. The inactivated viral suspension (1ml) was admixed with (1:1, v/v) 1ml Freund's complete adjuvant. Primary immunization was carried out with the Freund's complete adjuvant and goats (n=03, 0.5ml, Figure 30a) and rabbit (n=05, 0.1 ml, Figure 30b) were immunized subcutaneously with the antigen. Subsequent boost was carried out using Freund's incomplete adjuvant (1:1, v/v) at an interval of 7 and 15 days. Serum samples from the vaccinated rabbit and goats were collected by cardiac puncture following 30 days of immunization. This antibody was used in sandwich ELISA and indirect ELISA to detect anti PPRV antibodies.

Figure 30: Raising hyper immune serum in goat (a) and rabbit (b) with the PPR virus propagated in Vero cells. About 0.25ml of PPR viral suspension was admixed with 0.25ml of Freund's complete adjuvant and injected subcutaneously (s.c) in goats (0.5ml) and rabbit (0.1 ml). Following 7 and 15 days of priming, the goats and rabbits were boosted with 0.25 ml and 0.1 ml of PPR viral suspension respectively admixed with 0.25ml of Freund's incomplete adjuvant through s.c route. Following 15 days of boosting 10ml of venous blood from the immunised goats and 7-8ml of cardiac blood from the rabbit were collected in Falcon tubes from where antisera were collected (c). Both of the antisera were used in sandwich ELISA.

Demonstration of cytopathic effect of PPRV in Vero cells on cover slip culture: Cytopathic effect or cytopathogenic effect (CPE) refers to structural changes in host cells that are caused by viral invasion. The infecting virus causes a number of changes that include lysis of the host cell, cell aggregation, cell sheet separation, rounding of cells, syncytia formation, cytoplasmic vacuolation, nuclear aggregation, appearance of nuclear or cytoplasmic inclusion bodies, absence of cell death without lysis, etc. All of these morphological changes in the host cells caused by viral invasion is said to be the CPE. The CPE of any virus in culture can be studied by growing infected cells in flasks and examining the flasks under microscope. However, unstained cells seldom showed distinct and specific changes in cells under microscopic examination. The routinely used staining method is H&E staining (Luna, 1968) but never practiced for the PPRV specific CPE. Moreover, H&E staining cannot be carried out in tissue culture flasks. This study adapted Vero cell culture on cover slips and stained the infected and un-infected Vero cells on cover slips by using H&E staining.

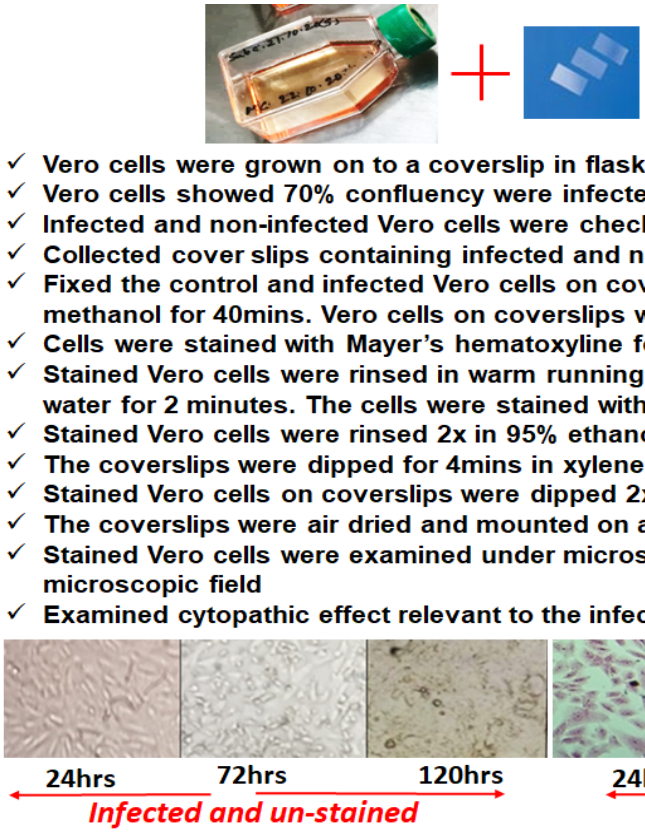

Figure 31: The cytopathic effect (CPE) of PPR virus in Vero cells on cover slip culture. Vero cells infected with PPRV; un-stained (left) and stained (right) with H&E at 24hrs, 03 days (72hrs) and 05 days (120hrs) of infection. The CPE as seen in Vero cells comprises of round up, cell aggregation, syncytia formation and detachment from the surface. Such changes are distinctly visible on coverslips stained with H&E (right) which however are indistinct in unstained cells (left).

Suitable area/Location: Not applicable.

Benefit: This is a lab based technology that could be used for the diagnosis of PPR infection in sheep and goats as well as for the development of PPR vaccine using local virus isolates. Hence it is hoped that the technology would be useful for the scientists and vaccine production/disease diagnostic institutes both from the public and private sectors.

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ii. Effectiveness in policy support (if applicable)

Coordination Component (BARC)
Not applicable.

Component-1 (BLRI)
The knowledge gained from the trial of the development of PPR free zone and the information generated on the efficacy of locally produced PPR vaccine would help to develop appropriate policy support in relation to planning for PPR eradication program as per OIE.

Component-2 (BAU)
Not applicable

H. Technology/Knowledge generation/Policy Support (as applied):

i. Immediate impact on generated technology (commodity & non-commodity):

Coordination Component (BARC)
Not applicable

Component-1 (BLRI)
Primary knowledge in relation to the development of PPR free zone and the information generated on the efficacy of locally produced PPR vaccine would help develop appropriate policy support in relation to planning for PPR eradication program as per OIE.

Component-2 (BAU)
a.This study developed rabbit and goat anti PPRV antibodies (antisera). These antibodies can be used in sandwich ELISA and indirect ELISA to detect subclinical infectivity of goats due to PPR virus or detection of immunity against PPR viral vaccine

b.The PPR viral vaccine seed formulation is in progress. Until now 32^{nd} passages in vero cells was carriedout. Further attenuation of PPR virus in Vero cells is going on. There is a hope to geenrate vaccine seed against PPR virus.

ii. Generation of new knowledge that help in developing more technology in future:

Coordination Component (BARC)
Not applicable

Component-1 (BLRI)
Not applicable

Component-2 (BAU)

-The polyclonal antibodies generation technologies adapted in this study can be used in other infectious diseases and development of detection protocols at local settings

-PPR virus isolation, adaption and attenuation protocols in Vero cells can also be used in other infectious diseases like FMD, Lumpy skin diseases, goat pox, Orf viruses etc.

-Co-infectivity of goats with FMD, Orf and pox was found to be a common occurrence. It needs to be tested all the samples of caprine origin against PPR, Pox, Orf and FMD before selecting and isolating targeted viruses in culture.

iii. Technology transferred that help increased agricultural productivity and farmers' income: None yet

iv. Policy support: None yet

I. Information regarding Desk and Field Monitoring

i. Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):

Coordination Component (BARC):

Description	Output
Coordination meeting	Coordination meeting was arranged at three months interval
Workshop	Arranged annual review workshop
Training	Arranged officer's training
Research activities	Research activities of the sub-project was monitored at one month interval

ii. Field Monitoring (date & no. of visit, name and addresses of team visit and output): Coordination Component (BARC):

Date	No. of visit	Name of team members	Addresses of team visit	Output
12.02.2020	01	Dr. Mohammad Rafiqul Islam Dr. Mohammad Showkat Mahmud Dr. Md. Ismail Hossain	Bangladesh Agricultiral University	Monitoring team visited the experimental fields and laboratories activities along with the PI of this project.
29.07.2020	01	Dr. Mohammad Rafiqul Islam Dr. Kazi M. Kamaruddin Dr. Mohammad Showkat Mahmud Dr. Md. Nure Alam Siddiky	Bangladesh Livestock Research Institute	Monitoring team visited on-going activities of the project regarding samples collection, test and analysis.
10.01.2021	01	Dr. Mohammad Rafiqul Islam Dr. Kazi M. Kamaruddin Dr. Md. Nure Alam Siddiky	Bangladesh Agricultiral University	Monitoring team visited the experimental fields and laboratories activities along with the PI of this project.

Component-1 (BLRI):

Sl. No.	Date	Name of team members	Address	Output
01	14.05.2019	Dr. Miah Sayed Hasan, Dr. Suraiya Parvin	BARC	Satisfactory
02	29.07.2020	Dr. Mohammad Rafiqul Islam Dr. Mohammad Showkat Mahmud Dr. Kazi M. Kamaruddin Dr. Md. Nure Alam Siddiky	BARC	a. Seroprevalence (41.78%) of PPRV was found in goats and 59.93% positive for clinical samples. b. For PPR free zone, OIE-FAO guideline should be followed. BLRI components are advised to include stage wise activities in methodology. c. Data should be presented as pre-vaccination and post vaccination.

Component-2 (BAU)

Date	No. of Visit	Name and addresses of team members	Output
21.3.2019	01	Dr. Md. Mosharrof Uddin Molla, Chief Scientific Officer, BARC, Dhaka.	Activities are going on. Steps to be taken after approval of procurement plan.
12.02.2020	01	Dr. Md. Rafiqul Islam, Chief Scientific Officer, Livestock Division, BARC, Dhaka	Research progress should be smoothly run.
11.01.2021	01	Dr. Kazi M. Kamaruddin, Consultant, NATP, BARC, Dhaka	Development of Monoclonal antibody should be start.
06.03.2021	01	Dr. Md. Harunur Rashid, Director, PIU, BARC, Dhaka	Development of PPR vaccine works should ne continue.

iii. Weather data, flood/salinity/drought level (if applicable) and natural calamities:

Not applicable

J. Sub-project Auditing (covers all types of audit performed)

Coordination Component (BARC):

Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Internal	No objections raised	5,33,648	Satisfactory	
Internal	No objections raised	5,59,247	Satisfactory	

Component-1 (BLRI):

Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
FAPAD	No	29,65,601	Satisfactory	
FAPAD	No	31,12,886	Satisfactory	

Component-2 (BAU)

Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial audit	No objection	2873380	Satisfactory	**Completed**
Financial audit	No objection	1376951	Satisfactory	**Completed**

K. Lessons Learned:

- The study revealed that around 55% of the goat and sheep population in Bangladesh are at risk of contracting PPR through which outbreaks of the disease are being continued and the PPR virus is being circulated in the field. The study further revealed that outbreak of PPR could be blocked through mass vaccination using either imported vaccine or locally produced PPR vaccine however, for using local vaccines, capacity of the PPR vaccine production lab has to be increased.
- It was also revealed through this study that about 17% of the PPR infected goats were co-infected with goat pox, Orf and foot and mouth diseases viruses. For PPR vaccine seed production, it is important that the sample is free from any contaminating viruses and to get rid of the co-infecting viruses the RT-PCR and PCR designed under this study could be used successfully.

L. Challenges (if any):

- Farmers were reluctant to get their goats vaccinated against PPR.
- There was lack of skilled manpower for proper vaccination

- Maintaining cool-chain for vaccine transportation and delivery was a problem
- New entry of goats (purchase of new goats or distribution of goats by NGO) in the household or village was the most important risk factor for PPR virus circulation
- Due to covid-19 outbreak lab work was affected seriously as Bangladesh Agricultural University was under movement restriction for a period of one year.
- Lab works sometimes interrupted due to shortage of chemical which was imported from abroad.

M. Suggestions for Future Planning (if any):

- This study provides preliminary information on PPR sero-prevalence, socioeconomic condition and possible associated risk factors. Therefore, we recommend a more systematic intensive and active serological and virological surveillance programs in the areas in addition to implementing intensive vaccination campaigns. More time was required to establish a completely PPR free zone in the selected areas.
- For PPR vaccine seed production and then formulation of PPR vaccine along with the development of monoclonal antibodies required more time with more funding.

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