EPIDEMIOLOGICAL INVESTIGATION OF PESTE DES PETITS RUMINANTS ON RURAL GOAT BASED ON TEACHING VETERINARY HOSPITAL, CHITTAGONG VETERINARY AND ANIMAL SCIENCES UNIVERSITY, BANGLADESH

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Roll No.: 0214/03
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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Epidemiology

Department of Medicine and Surgery
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DECEMBER 2016

Authorization

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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Dedication

I dedicate this MS research work
to my beloved parents

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List of Abbreviations

Abbreviation	Elaboration
%	Percentage
µl	Micro liter
BCS	Body Condition Score
BDSL	Biological Diagnostic Supplies Ltd
BLRI	Bangladesh Livestock Research Institute
bp	Base pair
CELISA	Competitive Enzyme-Linked Immunosorbent Assay
CI	Confidence Interval
CVASU	Chittagong Veterinary and Animal Sciences University
DNA	Deoxy Ribo Nucleic Acid
EDTA	Ethylene Diamine Tetra Acitic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
Fc-r RIIB	Fc-Gamma-RIIB Signaling in B cells
gm	Gram
IFN	Interferon
ILRI	International Livestock Research Institute
MAb	Monoclonal Antibody
ml	Milliliter
Nm	Nanometer
OD	Optimal Density
OIE	World Organization for Animal Health
OIE	Office International des Epizooties
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PI	Percent Inhibition
PPR	Peste des Petits Ruminants
PPRV	Peste des Petits Ruminants Virus
PRTC	Poultry Research and Training Centre
QA	Quality Assurance
RLDL	Regional Leading Diagnostic Laboratory
RNA	Ribo nucleic acid
RNAse	Ribonuclease
rpm	Rotation Per Minute
RSU	Regional Support Unit
RTPCR	Reverse Transcription Polymerase Chain Reaction
SAARC	South Asian Association for Regional Cooperation
SELISA	Sandwich Enzyme-Linked Immunosorbent Assay
TVH	Teaching Veterinary Hospital
UV	Ultra violet
VTM	Viral Transport Media
WAHID	World Animal Health Information Database

Abstract

Bangladesh is an agro based country where livestock is a cardinal component of agriculture. Goat rearing has gained the popularity as a subsidiary and sometimes as a main occupation among rural families, marginal farmers, children, landless laborers and distressed women for its low investment and high profit. Infectious diseases are important hindrance for goat rearing, whereas Peste des Petits Ruminants (PPR) is the most devastating viral disease in goats.

A descriptive study 1 followed by case-control study was conducted on clinical PPR cases in goats at Teaching Veterinary Hospital (TVH) of Chittagong Veterinary and Animal Sciences University (CVASU) during 15 August 2015 to 30 December 2016. The aim of this study was to estimate proportionate prevalence of PPR, measure the frequency of clinical signs, drugs prescribed against PPR and determine the potential risk factors associated with the occurrence of PPR. Another descriptive study 2 also carried out to estimate proportionate PPR prevalence on the basis of clinical signs and symptoms and molecular testing, PPR sero-prevalence and assess the effect of PPR on lymphocyte and neutrophil counts in goats at TVH of CVASU and other selected sites of Chittagong Metropolitan City.

A total of 922 clinically diagnosed PPR goat cases and 2766 non PPR goat cases as control with epidemiological data were extracted from the TVH recording system for the case-control study. For another descriptive study 2 a total of 138 PPR suspected goats were studied in the selected study sites (50 suspected goats at TVH during November 15, 2015 to January 30, 2016 and 88 suspected goats at other sites during August15, 2015 to October 15, 2016).

Blood samples and nasal swabs were collected from all goats. Blood samples were used for PPR serology and hematological evaluation whereas swab samples were used for PPR molecular diagnosis.

Most frequent group of clinical signs of PPR in goats was nasal discharge, stomatitis and diarrhoea (17.5%), followed by coughing, stomatitis and diarrhoea (15.1%) and nasal discharge with coughing and diarrhoea (14.7%).

Most frequent drugs prescribed against PPR in goats were sulphonamides (70.2%) followed by aminoglycosides (15.4%).

Regardless of study type the proportionate prevalence of PPR in goats was varied from 21.3% to 28.7%. Whereas the proportionate PPR sero prevalence was 50%. The PPR cases in vaccinated goats were nil in the descriptive study 2. PPR infection significantly decreased lymphocyte counts but significantly increased neutrophil counts in studied goats.

Rainy season had 2.4 times higher odds of PPR in goats than in combined summer and winter seasons ($p<0.001$), combined intensive and semi intensive systems of rearing had 4.3 times higher odds of PPR in goats than in combined free range and tethering ($p<0.001$), Black Bengal Goat had 1.2 times higher odds than in Jamunapari and Cross ($p=0.015$) and cachectic body condition had 5.7 times higher odds than in fair and good conditions ($p<0.001$).

This study may explore frequent clinical signs and symptoms in PPR infection, diagnose clinical PPR through molecular testing, assess relationship between PPR and checking blood parameters. This study also determine potential risk factors measuring strength of association, and undertake the level of drug usage, proportionate prevalence and sero prevalence in the descriptive study 1 and 2.

In conclusion, diagnosis of clinical PPR should be based on clinical signs with blood profiling and molecular testing in some cases, extra care should be taken during rainy season turned out as PPR season. The goats should be vaccinated against PPR before starting of the rainy season, hygiene should be improved in intensive and semi intensive rearing system, nutritional management should be considered in cachectic body condition.

Key words: Peste des Petits Ruminants, Prevalence, Risk factors, Haematology

Chapter I: Introduction

Bangladesh is an agro based country. In this country the total livestock population is about 196.03 millions representing 14.8 millions of goat population (Anon, 2017d). Goats are reared by rural families, marginal farmers, children, landless laborers and distressed women who cannot afford to rear cattle. Goat is also known as " poor man's cow"(Islam et al., 2013). It contributes to the national economy of Bangladesh through providing high quality meat, milk and skin. It acts as a support for poverty alleviation, income generation, creation of employment opportunity and food production (Debnath, 1995).

Infectious diseases are most significant constraints to the goat rearing in Bangladesh. Among infectious diseases PPR, pneumonia, goat pox, enterotoxaemia, mastitis, myiasis, tetanus, and ringworm etc are predominant in goats in this country (Sharifuzzaman, 2015).

Peste des Petits Ruminants caused by Peste des Petits Ruminants Virus (PPRV) is an acute, highly contagious, and febrile transboundary viral disease of small ruminants as well as wild animals. It has a widespread distribution throughout southern Asia, Arabia, the Middle East, West and Central Africa (Banyard et al., 2010). PPR is endemic in different countries such as Bangladesh, India, Pakistan, Nepal, Bhutan, Afghanistan and China (Muthuchelvan et al., 2014).

Peste des Petits Ruminants Virus is an envelope, single stranded, non segmented, negative sense RNA virus (Bailey et al., 2005) which belongs to the genus Morbillivirus, under the family Paramyxoviridae (Chauhan et al., 2009) of order Mononegavirales (Mehmood et al., 2009). PPRV consists of six structural protein as Nucleocapsid protein (N), Phosphoprotein (P), Matrix protein (M), Fusion protein (F), Hemagglutinin protein (H), Polymerase protein (L) and two non-structural proteins, C and V (Banyard et al., 2010). It has four distinct lineages as 1, 2, 3 and 4 and one single serotype (Dhar et al., 2002).

This disease in goat is characterized by sudden high fever (Islam et al., 2014), oculonasal discharge (Munir, 2010), pneumonia (Rahman et al., 2011b), stomatitis and profuse diarrhoea (Rahman et al., 2011c) leading to severe dehydration followed by death. High

morbidity and mortality up to 100% occurs due to PPR which results in heavy economic losses. So, the current study was conducted to explore the most frequent clinical signs and symptoms associated with PPR in goats in field conditions seems to aid in tentative diagnosis.

Different approaches are practiced to prevent and control PPR in goats. Vaccination is one of the approaches to control it. In addition various antimicrobial drugs with other symptomatic and supportive drugs are used to treat PPR in goats. However, the level of drug usage against PPR has not been assessed yet. The present study was therefore aimed to measure the level of drug uses against clinical PPR in goats.

The prevalence of PPR in goats was reported to be variable across the world such as 32.8% in India (Balamurugan et al., 2012), 45% in The Republic Niger (Farougou et al., 2013), up to 83% in Turkey (Özkul et al., 2002), 6.8% in Ghana (Folitse et al., 2016) and 10.1-50.3% in Bangladesh (Sarker and Islam, 2011; Islam et al., 2013; Parvez et al., 2014; Bupasha et al., 2015; Sharifuzzaman, 2015; Rahman et al., 2016). Most of the cited studies used either small sample size or clinical signs based tentative diagnosis to estimate PPR prevalence. Therefore, a study with statistically sound sample size and better diagnostic technique were warranted to determine PPR prevalence.

The reported sero prevalence of PPR in goats ranging 9-63.4% in different countries of the world (Singh et al., 2004b; Abraham et al., 2005; Khan et al., 2007; Raghavendra et al., 2008; Janus et al., 2009; Zahur et al., 2011). However, in Bangladesh 25% to 49.2% sero prevalence was estimated in goats (Razzaque et al., 2004; Banik et al., 2008). Hence, the study was undertaken to explore the seroprevalence of PPR in goats.

Many earlier studies determined crucial risk factors associated with the occurrence of PPR in goats. They were as housing ($P=0.008$) (Islam et al., 2016), breed ($p<0.01$) (Sarker and Islam, 2011; Parvez et al., 2014; Salih et al., 2014), age ($p<0.001$) (Sarker and Islam, 2011; Mahajan et al., 2012; Parvez et al., 2014), seasonal influence ($p<0.001$) (Sarker and Islam, 2011; Parvez et al., 2014), rearing system ($p<0.05$) (Salih et al., 2014), sex ($p<0.05$) (Sarker and Islam, 2011; Salih et al., 2014). However, most of these studies did not account proper statistical analysis to determine risk factors associated with the occurrence of clinical PPR cases in goats. Therefore, the current case-control study was

targeted to determine potential risk factors associated with the occurrence of clinical PPR cases in goats.

Lymphocyte count declines quickly due to the destruction of lymphoid organs (Sharifuzzaman, 2015) which leads to immunosuppression (Abraham et al., 2005) through reducing antibody production in body. Neutrophil count during PPR infection increases to prevent secondary bacterial infection (Sharifuzzaman, 2015). Accounting blood parameters in refining PPR diagnosis could increase the efficiency of diagnosis of PPR for the places where modern and sensitive diagnostic techniques are scared. The present study was therefore aimed to assess the effect of PPR on blood parameter.

The specific objectives of the present study were as follows:

1. To measure the frequency of clinical signs and symptoms associated with clinical PPR cases in goats at Teaching Veterinary Hospital (TVH) of Chittagong Veterinary and Animal Sciences University (CVASU).
2. To determine the frequency of drugs prescribed against clinical PPR cases in goats at TVH.
3. To estimate proportionate prevalence and sero prevalence of PPR in goats at TVH and other respective sites.
4. To explore the potential risk factors having association with clinical PPR occurrence in goats at TVH.
5. To assess the effect of PPR on blood parameters in goats including both TVH and other study sites.

Chapter II: Review of Literature

Relevant literatures on PPR, pathogenesis, clinical signs along with post mortem lesions, prevalence, seroprevalence of PPR, effect on white blood cell, associated risk factors, surveillance and epidemiological studies, consequence, diagnostic approach along with prevention and control have thoroughly been reviewed in this chapter. The basic conception of this section is to provide up to date information with previous studies as well as to determine the gap and justify the current Master's research on epidemiological investigation of PPR in Chittagong metropolitan areas. The review findings of crucial published and unpublished articles have been presented under the headings as below:

2.1 Peste des Petits Ruminants

Peste des Petits Ruminants are an acute, highly contagious viral disease of sheep and goats caused by PPR virus. This virus is the fourth member of Morbillivirus genus (Kul et al., 2007) under the family Paramyxoviridae, sub family Paramyxovirinae (Gibbs et al., 1979a) and order Mononegavirales. The virus has a close relationship with other member of Morbillivirus genus such as Human Measles virus, Canine distemper virus, Porcine distemper virus, Dolphin Morbillivirus and Porpoise Morbillivirus (Barrett, 1994).

The etiological agent is an envelope, non segmented, single stranded, negative sense RNA virus (Bailey et al., 2005). The envelope is 8 to 15 nm in thickness, with spikes of 8.5 to 14.5nm length. The spikes formed by the two viral glycoproteins, the Hemagglutinin (H) and the fusion (F) proteins which are essential in the first steps of the host cell infection by the virus (Diallo et al., 1989).Genome size of PPR virus is 15-16 kb with negative polarity (Bhuiyan et al., 2013). PPRV consists of 15948 nucleotides which encodes six structural protein like Nucleocapsid protein (N), Phosphoprotein (P), Matrix protein (M), Fusion protein (F), the Hemagglutinin protein (H), Polymerase protein (L) and two non-structural proteins, C and V (Banyard et al., 2010).

Peste Des Petits Ruminants was first identified in Cote d'Ivore (the Ivory Coast) in West Africa in 1940 (Taylor, 1984). In 1956 the causative agent of PPR and Rinderpest were thought closely related to each other (Mornet et al., 1956). Due to the strong clinical

resemblance between Rinderpest and PPR, adaptation of a variant of Rinderpest virus to small ruminants was thought to be responsible for PPR.

Small ruminants, especially sheep and goats are mainly affected by PPR (Anderson and McKay, 1994). Different levels of virulence in sheep and goats are exhibited due to the virus. Clinically it is a severe fast spreading disease in sheep and goats but goats are severely affected than sheep (Nkangaga, 2014).Guinean breeds of goats such as West African dwarf, Logoon, Kindi and Djallonke are highly susceptible to PPR (Couacy-Hymann et al., 2007a).There are differences on susceptibility between breeds e.g., the dwarf breeds of goats are more susceptible to PPR than Sahelian breeds (Diop et al., 2005). Cattle and pigs develop antibody after experimental PPR virus infection but clinical signs are not manifested (Anderson and McKay, 1994). Buffaloes and camels are also susceptible to infection but do not overt clinical signs and unable to transmit the disease to other animals. There is only one serotype of PPR virus, whereas on the basis of partial sequencing of F gene at least four distinct genetically grouped lineages (1, 2, 3 and 4) are present (Dhar et al., 2002).

After first identification of the PPRV the spread of it was confined to sub Saharan Africa, the Middle East and Indian subcontinent. According to OIE World Animal Health Information Database (WAHID), PPR is currently found in the Middle East, North Africa, West Africa, South Africa, East Africa, and parts of Asia (Banyard et al., 2010). According to OIE PPR is currently reported as an endemic disease in Bangladesh, India, Bhutan, Iran, Iraq, Israel, Kuwait, Nepal, Afghanistan, Bahrain, Benin, Turkey, Yemen, Tajikistan, Saudi Arabia, Angola, Algeria, Comoros, Uganda, Kenya, Egypt, Tunisia, Cameroon, Eritrea, Ethiopia, Ghana, Guinea, Buinea Bissau, Nigeria, Sudan, Tanzania, The Republic of Congo, and Mali (Abubakar and Munir, 2014).

Among many factors close contact between animals plays a vital role in the transmission of PPR. Inhalation acts as an important path of spreading infective fine droplets into the air forming aerosols during coughing and sneezing as expired air contains a high level of virus excretion (Mulindwa et al., 2011).

The incubation period of PPR is 4 to 10 days (Islam et al., 2001). During incubation period it can spread through movement of infected animals. PPR infected animals shed

virus in exhaled air, in secretions and excretions just 10 days after the onset of clinical signs. Virus shedding occurs from infected goats before the appearance of clinical signs (Couacy-Hymann et al., 2007b). The infected animals excrete large quantities of virus through secretion or excretion such as nasal fluids, tears, saliva, urine, faeces and semen (Misinzo et al., 2015). Virus can also be transmitted by infected dam through feeding milk to the offspring (Lefevre and Diallo, 1990). Virus can be shed out from the infected one after post recovery which can also be found in faeces after 11 weeks of recovery (Ezeibe et al., 2008). Contaminated bedding, water troughs, mangers and areas also act as sources of transmission of this disease (Sil et al., 1995).

2.2 Pathogenesis, Clinical Signs and Post-mortem Lesions of Peste des Petits Ruminants

2.2.1 Pathogenesis:

PPRV has an affinity to lymphoid and epithelial tissues for which respiratory route serves as a portal

↓

At the time of infection the virus is received by antigen presenting cell which is present in intraepithelial space and lamina propria of the respiratory mucosa (Yanagi et al., 2006)

↓

It is then transported to the place of viral replication, located in the regional lymphoid tissue of pharyngeal, mandibular lymph node and tonsil

↓

Infected lymphocyte helps in spreading the infection by both lymphatic and vascular systems (Osunkoya et al., 1989)

↓

Cytopathic effect is observed in different organs due to infection

↓

During infection the fusion occurs between viral envelope and cell membrane of the extracellular space

↓

Viral nucleocapsid is carried to the cell surface, whereas the later forms modified segments to the plasma membrane containing the viral envelope proteins

↓

Budding process helps in maturation of virus from the plasma membrane in a heteromorphic fashion

↓

Two or three days after infection and about 1 to 2 days prior to appearance of clinical sign viremia can develop which helps the virus disseminate to spleen, bone marrow, mucosa of both gastrointestinal and respiratory systems (Gibbs, 1981)

.

2.2.2 Clinical Signs

2.2.2.1 Acute Form

The following clinical signs were reported to be produced under acute form of PPR in goats in different countries (Sil et al., 1995b; Chauhan et al., 2009; El-Rahim et al., 2010a; Sarker and Islam, 2011; Fentahun and Woldie, 2012; Gurcay et al., 2013; Islam et al., 2013; Islam et al., 2014; Sharifuzzaman, 2015; Sharma et al., 2015; Rahman et al., 2016). They included high temperature (104-106°F), watery oculo-nasal discharge which becomes mucopurulent in nature, obstruction of nostrils leading to dyspnea, coughing followed by bronchopneumonia, adhesion of eyelids, conjunctivitis, stomatitis followed by salivation leading to anorexia, watery diarrhoea with foul odour causing dehydration, emaciation, dullness, abortion in pregnant animals.

2.2.2.2 Per-acute Form

Per-acute stage is seen in young goats. Appeared clinical signs are same as acute form but occur more rapidly leading death of 100% infected animals after 5 to 6 days (Charbonnier and Laveissiere, 2015).

2.2.2.3 Sub Acute Form

Sub acute form continues not more than two weeks (Fentahun and Woldie, 2012). This stage is least severe than other stages. After 5 days of incubation period a moderate fever is appeared which lasts only for 1 to 2 days (Charbonnier and Laveissiere, 2015).

2.2.2.4 Sub Clinical Form

In this stage no clinical signs are appeared which can be revealed through serological investigations (Charbonnier and Laveissiere, 2015).

2.2.3 Post-mortem Lesions

Many earlier studies documented the following post-mortem lesions in goats affected by PPR (Kul et al., 2007; Chauhan et al., 2009; El-Yuguda et al., 2009; Munir, 2010; Rahman et al., 2011c; Sahinduran et al., 2012).

Lesions were emaciation, faeces soiling in hindquarters, necrotic lesion with congestion on the oral cavity, tongue, gums and palate including the whole gastrointestinal tract.

Erosions with characteristic haemorrhagic ulcerative lesion in the mouth, pharynx including upper esophagus were found. Along with congestion there was also haemorrhagic lesion in the intestinal mucous membrane of abomasum, rumen, reticulum, omasum, colon and rectum. Haemorrhagic plug was observed in the ileocecal valve, at the cecocolic junction and in the rectum. Haemorrhagic Zebra or tiger stripes was also identified in the caeco-colic junction. Reddened lining with erosive creamy exudates in nasal cavities were found. Signs of bronchopneumonia with consolidation and atelectasis on the trachea were identified containing foamy liquid. Congestion of the lung was seen with dark red or purple color, firm in touch located in the anterior and cardiac lobes. Erosive lesions were present in larynx and trachea. Shunken eyeballs, congested, enlarged, edematous, soft and swollen lymph node were found. Necrotic lesion was present in intestinal lymph node. Congested, necrotic and bloated spleen was identified. Extensive necrotic lesions were detected in the Peyer's patches, spleen, thymus, pulmonary lymph node causing ulceration. Ulcer lesion was also found in the mucosae of urinary tract, pin point hemorrhage was seen in the liver and erosive lesions were observed in genital mucosa of females.

2.3 Occurrence of Peste des Petits Ruminants Outbreak

Outbreak of PPR may take place in a restricted geographical area, or may extend over several countries which lasts for a few days or weeks, or for several years (Anon, 2017c). In many developing countries especially in Asia, Europe and Africa PPR is now enlisted as one transboundary disease for small ruminants and also endemic in most of the developing countries of the world (Banyard et al., 2010).

Outbreaks of PPR have been recorded in Jordan (Rweyemamu et al., 2000), India (Shaila et al., 1989; Balamurugan et al., 2010), Pakistan (Ahmad et al., 2005; Zahur et al., 2014), Nigeria (El-Yuguda et al., 2009), Arabian peninsula (Abu-Elzein et al., 1990), Ethiopia (Roeder et al., 1994), Turkey (Yener et al., 2004), Iran (Abdollahpour et al., 2006), Egypt (El-Rahim et al., 2010b),Tibet and China (ZhiLiang et al., 2009), Afghanistan, (Wang et al., 2015), Morocco (Sanz-Alvarez et al., 2008),Tajikistan (Kwiatek et al., 2007) and Sudan (Khalafalla et al., 2010).

In Bangladesh PPR had been identified in Black Bengal goats during a severe outbreak in 1993 which was confirmed by British reference laboratory (Islam et al., 2001). The virus

was first detected in Meherpur district (Sil et al., 1995c).Till now about 2307 outbreaks have been reported among which 611 in Dhaka, 396 in Chittagong, 360 in Khulna, 289 in Rajshahi, 291in Rangpur and 144 in Sylhet (FAO, 2017).

2.4 Prevalence and Incidence of Peste des Petits Ruminants

The overall prevalence of PPR in goats based on clinical signs has been reported in India (32.8%) (Balamurugan et al., 2012), Pakistan (31.3%) (Aslam et al., 2009), The Republic of Niger (45.0%) (Farougou et al., 2013), Turkey (0.9 % to 82.6%) (Özkul et al., 2002) and Ghana (6.8%) (Folitse et al., 2016).

The prevalence of PPR in goats based on clinical signs and lesion appeared to be variable across Bangladesh as 50.3% in Potuakhali (Islam et al., 2013), 12.5% in Kushtia (Sharifuzzaman et al., 2015), 18.8% in Rangpur (Sil et al., 1995b), 20.6% in Rajshahi (Sarker and Islam, 2011), 39% in Thakurgoan (Bupasha et al., 2015) and 10.1% in Chittagong (Parvez et al., 2014).

Only few published studies on PPR in goats are available in Chittagong. Therefore comprehensive future studies were required.

2.5 Sero-prevalence of Peste des Petits Ruminants

The seroprevalence of PPR in goats varied regionally such as 9% to 63.4% (Singh et al., 2004b; Abraham et al., 2005; Khan et al., 2007; Raghavendra et al., 2008; Janus et al., 2009; Zahur et al., 2011), 0.6% to 176% (Adel et al., 2004; Osman et al., 2009; Salih et al., 2014).

However, the recorded sero-prevalence of PPR in goats in Bangladesh was 25% to 49.2% (Razzaque et al., 2004; Banik et al., 2008)

2.6 Effect of Peste des Petits Ruminants on Differential Leukocyte Counts

Peste Des Petits Ruminants virus has a significant effect on white blood cells which can be observed during the acute phase of viral infection (Rajak et al., 2005). Among different kinds of white blood cells lymphocyte, neutrophil are affected most due to PPRV causing lymphopenia and neutrophilia.

Lymphocyte declines quickly in PPRV infection as this virus directly affects lymphoid organs (Sharifuzzaman et al., 2015). As a result virus can replicate resulting induction of immune response (Abraham et al., 2005) reducing level of antibody production in body.

To protect the body from secondary bacterial infection due to immunosuppression neutrophil percentage starts to increase gradually (Sharifuzzaman, 2015). According to previous studies it is confirmed that due to PPRV infection percentage of lymphocyte declines up to 21%-40%, whereas neutrophil percentage increases up to 58%-72% (Sil et al., 1995a; Islam et al., 2014).

So, the current study was aimed to test the effect of PPR on lymphocyte and neutrophil in goats.

2.7 Risk Factors Associated with Peste des Petits Ruminants in Goat

A risk factor may be any kind of attribute or exposure which increases the likelihood of developing the disease. Various risk factors were reported to be responsible for PPRV infection in goat, such as housing (OR=0.29; P=0.008; matcha housing versus mud housing) (Islam et al., 2016), having history of single vaccination within 1 year (OR=0.21, p=0.02; twice vaccination within 1 year versus single vaccination within 1 year) (Islam et al., 2016), breed ($p<0.01$) (Sarker and Islam, 2011; Parvez et al., 2014; Salih et al., 2014), age ($p<0.001$) (Sarker and Islam, 2011; Mahajan et al., 2012; Parvez et al., 2014), seasonal influence ($p<0.001$) (Sarker and Islam, 2011; Parvez et al., 2014), rearing system ($p<0.05$) (Salih et al., 2014), housing ($p<0.05$) (Salih et al., 2014), sex ($p<0.05$) (Sarker and Islam, 2011; Salih et al., 2014), immune status ($p=0.005$) (Bupasha et al., 2015), large flock size (Al-Majali et al., 2008), introduction of a new stock (Fentahun and Woldie, 2012), visiting live animals market (Al-Majali et al., 2008) and inadequate veterinary services (Al-Majali et al., 2008).

In the above mentioned most of the risk factors were determined through univariate analysis on the basis of chi square value estimation in the earlier studies but strength of association was not measured through multivariate factor analysis.

So, the current study was aimed to determine potential risk factors responsible for PPR in goats through measuring strength of association in multivariate factor analysis.

2.8 Drug Related Review

According to previous studies in the world there is no antiviral medication against PPRV infection in goat has been approved yet (Kumar et al., 2014). However, on the effect of potential antiviral against PPRV infection very few studies were undertaken (Nizamani et al., 2011; Raheel et al., 2013). For mouth sore antiseptic ointment and to check concurrent bacterial infection antibiotics can be applied (Kumar et al., 2014).

In many Bangladeshi studies therapy of antibiotics like sulphonamide, oxytetracycline, metronidazole to check secondary bacterial infection and as other supportive drugs antihistamine, oral saline and hyper immune serum for recovery in goats were evidenced (Sil et al., 1995b; Islam et al., 2013; Islam et al., 2014; Sharifuzzaman, 2015; Yousuf et al., 2015).

2.9 Surveillance and Epidemiological Studies

Peste Des Petits Ruminants is a transboundary disease that is of significant economic, trade importance for a considerable number of countries which can easily spread to other countries, reach epidemic proportions requires management and co-operation between several countries. In Afghanistan a piloting system for epidemio-surveillance network was launched in 14 provinces in 2010, whereas in Nepal an epidemiological surveillance with disease reporting system was established in 1996 which became an independent organization in 2004 (Kalam, 2013).

In Bangladesh active surveillance is carried out for Avian Influenza with the support of world donors. Poor passive surveillance system under DLS is being operated for other diseases. Passive disease surveillance of TVH of CVASU is far better because of accurate data recording system. However, data analysis, reporting and feedback systems are yet to be functioning. So, this epidemiological study highlighted the way of functioning all components of surveillance cycle at TVH of CVASU.

2.10 Consequences of Peste des Petits Ruminants

PPRV causes immunosuppression due to the damage of lymphoid organs resulting viremia which predispose the host to secondary bacterial infection associated with high mortalities (Beckford et al., 1985). There is strong evidence of a virulent strain Izatnagar/94 causing acute leucopenia, lymphopaenia and reduction of level of antibody

(Rajak et al., 2005). Viral infection inhibits interferon (IFNs) production, alters cytokine response, suppresses the inflammatory response, destroys leukocytes and inhibits immunoglobulin synthesis which results in acute immunosuppression (Schneider-Schaulies et al., 2001). The duration of immunosuppression may last for weeks which is closely associated with the severity of pathological lesions where peripheral blood cells are also infected (Jagtap et al., 2012). Antibody production is inhibited due to the binding of nucleoprotein of virus and Fc-γ RII B cells (Kerdiles et al., 2006).

In susceptible goat populations the range of morbidity is about 80 to 90% (Nkangaga, 2014). Severe outbreak occurs due to the movement of animals into an endemic area (Kusiluka and Kambarage, 1996). At the period of outbreak infection are severely occurred in enzootic area above 50%, ranges up to high 90% (Radostits et al., 2007). In a separate study conducted by (Nwoha et al., 2013) the rate of mortality of PPR was found of 20 to 90%, in dwarf goat. Case fatality may become higher in arid or semi arid region (Abu-Elzein et al., 1990). During the outbreak of PPR in Bangladesh morbidity and mortality were 74.1% and 54.8% respectively (Islam et al., 2001).

Peste Des Petits Ruminants is an OIE (Office International des Epizooties) enlisted disease (Kumar et al., 2014). In the countries where PPR generally occurs, it is considered as a most important disease. In most of the countries animals are regarded as a important source of protein (Fentahun and Woldie, 2012). Goat Contributes in nutrition and in cash income of poor farmer as they are considered as a poor man's cow in Africa and South Asia (Staal et al., 2009). According to the International Livestock Research Institute (ILRI), PPR has been identified as one of the animal diseases which should be controlled for removing poverty in countries named West Africa and South Asia indicating the disease as an economically important one (Perry, 2002). The infection leads to the severe poverty in farmers which also make the poor and very poor people destitute.

The annual losses in goat's mortality due to PPR were estimated to be US$ 1.5 million in Nigeria (Hamdy et al., 1976), US$ 39 million in India (Bandyopadhyay, 2002), US$ 1.5 million in Iran and US$ 15 million in Kenya (Thombare and Sinha, 2009).

A family's income is greatly affected by this disease. After the global eradication program of Rinderpest in cattle important measures are taken by national and international organizations. In south Asia the first control program for PPR was taken by

India in 2010. In 2013 for controlling PPR two pilot projects were run in Africa with financial support of European commission (Kumar et al., 2015). The preparedness plans should be reviewed, border control should be strengthen and surveillance should be developed (Fentahun and Woldie, 2012) in order to control PPR.

2.11 Diagnosis

On the basis of epidemiological investigation as well as clinical findings a tentative diagnosis of PPR can be made. Some cardinal clinical signs like oculonasal discharge, stomatitis, bronchopneumonia and diarrhoea with severe dehydration help to make a tentative diagnosis. Diagnosis can be made also after postmortem examination by observing congestive, erosive lesion of different mucosal tissues and the bronchopneumonic lesion in the lung (Roeder et al., 1999a).

Various molecular techniques are developed for PPRV diagnosis such as PCR (Abubakar et al., 2012), RT-PCR (sensitivity-12.5% and specificity-100%) (Abubakar et al., 2012), Nucleic acid hybridization (Abubakar et al., 2012).

Many tests have been also developed for detection of PPRV antibody in serum such as C-ELISA (sensitivity-99.4% and specificity-94.5%) (Zohari et al., 2012), S-ELISA (sensitivity-88.9% and specificity-92.8%) (Singh et al., 2004a), Immunocapture ELISA (Roeder et al., 1999b), Indirect ELISA (Balamurugan et al., 2007), Blocking ELISA (Saliki et al., 1993). Besides, other techniques like Virus Neutralization Test (VNT) (Wohlsein and Singh, 2015), Hemagglutination (HA) (Wosu, 1991) and Hemagglutination Inhibition (HI) (Manoharan et al., 2005) can also be used.

This present study relied on clinical signs for descriptive study 1 and clinical signs along with molecular and serological test for descriptive study 2 to explore the possibility of refining clinical based PPR diagnosis.

2.12 Prevention and Control

In order to eradicate PPR, veterinary capacities should be reinforced (Grant, 2013), the surveillance system should be strengthen (Grant, 2013), hygiene should be improved up to the mark and targeted vaccination should be allowed. Outbreak of PPR can be regulated by implementing quarantine, vaccination, movement control, disease monitoring, sanitary slaughter, cleaning and disinfection. In the SAARC countries an

animal health regional support unit (RSU) was established in 2010 through FAO based in Kathmandu and Nepal on highly pathogenic, emerging diseases, control approaches and monitoring (Kumar et al., 2014).

Nowadays many researches are going on to obtain an attenuated DIVA (Differentiation of Infected and Vaccinated Animals) vaccine in the world for saving both time and money. These vaccines can be recombinant vector vaccines obtained by deletion, substitution and insertion of gene in order to express foreign genes (Charbonnier and Laveissiere, 2015)

Due to restriction of lineage 4 in Asia, a lineage specific PPR vaccine has been improved in India through continuous passage which gained great popularity (Singh, 2011). Using bivalent vaccine was evidenced in India safe for inducing immune response in goats (Hosamani et al., 2006).

In Bangladesh for controlling PPR two types of vaccine are being practiced, as conventional live attenuated vaccine developed by Bangladesh Livestock Research Institute (BLRI) but produced by Livestock Research Institute (LRI) (Rahman et al., 2011a). But this vaccine has poor thermo stability and required cold chain system which cannot be maintained properly in field level leading deterioration of vaccine efficacy. So to overcome the vaccine failure another thermo stable vaccine is experimentally developed by BLRI (Chowdhury et al., 2004; Rahman et al., 2011a). It can be stored at room temperature (Siddique et al., 2006). As the temperature of Bangladesh fluctuates with variation of season so testing of immune efficacy and shelf life of vaccine is needed.

2.13 Conclusion

In conclusion conception of PPR, prevalence, associated risk factors of PPR, consequences, drugs against PPR, diagnostic approach along with prevention and control strategy have been discussed and assessed the justifications to perform the current study.

Chapter III: Materials and Methods

3.1 Chittagong Metropolitan City

Chittagong Metropolitan city, the second largest city of Bangladesh is located at 22°22' N and 91°48' E (Anon, 2017b). Around 6.5 million people live in this city. The city is in tropical monsoon climate area where annual temperature varies from 13°C to 32°C with 5.6 mm to 727.0 mm rainfall and 70 to 85% humidity (Anon, 2016).

Chittagong is well recognized both as a major coastal seaport and a financial centre on the bank of Bay of Bengal. It straddles the coastal foot hills of the Chittagong Hill tracts in southeastern Bangladesh. The southern bank of the city is surrounded by Karnaphuli river which enters into the Bay of Bengal in an estuary located 12 km west of downtown Chittagong (Anon, 2017a). Different ethnic groups of people live in harmony in this Metro City. They include Muslim(dominant), Hindu, Buddhist and Christan, Chakma, Marma, Tripura, Tanchangya, Lushai, Pankho, Bawm, Mro, Khyang, Khumi and Chak (Anon, 2017e).

3.2 Livestock Population in Chittagong Metropolitan City

As a subsidiary or major economic source livestock is reared by the people of Chittagong in small scale household farm or medium to large scale commercial farm. In metropolitan city the total population of Cattle, Baffalo, Sheep and Goat are 51290, 578, 2438, and 30320, respectively (Personal communication, District Livestock Officer, Department of Livestock Services, Chittagong, Bangladesh, 2017). Goats are reared in intensive, semi intensive, free range and tethering systems in this country. Goat houses are generally kachha (Bengali word) (Islam et al., 2016), muddy and brick walled types. Floor is earthen kachha and brick finished or cemented. As roofing materials paddy straw or other materials are generally used. Farmers usually keep their goats within their own houses, but some farmers keep goat in a separate goat houses.

The main purpose of goat rearing is to earn money by selling them at market which is an alternate source of income. Goat rearing acts as a great support for poor and marginal

people and helps in poverty alleviation. Some people in the metro city also rear goat as hobby.

3.3 Teaching Veterinary Hospital and its Passive Disease Surveillance System

Teaching Veterinary Hospital (TVH) of Chittagong Veterinary and Animal Sciences University (CVASU) is one of the reputed veterinary hospitals in Bangladesh because of its teaching and learning facilities as well as public and referral services. Teaching Veterinary Hospital of CVASU is well equipped hospital for animal disease diagnosis. This hospital was established in 2002. This hospital receives different clinical cases from areas in and around the Metro City along with various referral cases from neighboring upozilla (sub-districts). Along with pet animals (dog, cat, rabbit and pet birds) on an average 30 goats, 2-3 cattle and 1-2 sheep are daily brought to TVH for diagnosis, treatment and advisory services (Personal communication, Director of TVH). Goats affected with different diseases like PPR, mastitis, pneumonia, nutritional deficiency as well as different surgical affections are daily being received by TVH.

Teaching Veterinary Hospital has an excellent paper based disease surveillance data recording system for individual clinical case. In the hospital all types of diseases and disease condition along with basic epidemiologic information for individual case are being accurately recorded in the registered case sheet. The case sheet includes a case registration number, date of registration, name and address of the owner, owners complain, category of case based on owners complain, rearing system, breed, age, sex, body condition score (BCS), number of parity and body weight, clinical signs and system involved, temperature, tentative diagnosis followed by confirmatory diagnosis in some cases, drugs prescribed, and case follow up. Passive disease surveillance system at TVH of CVASU has been presented in Figure 3.1 and 3.2.

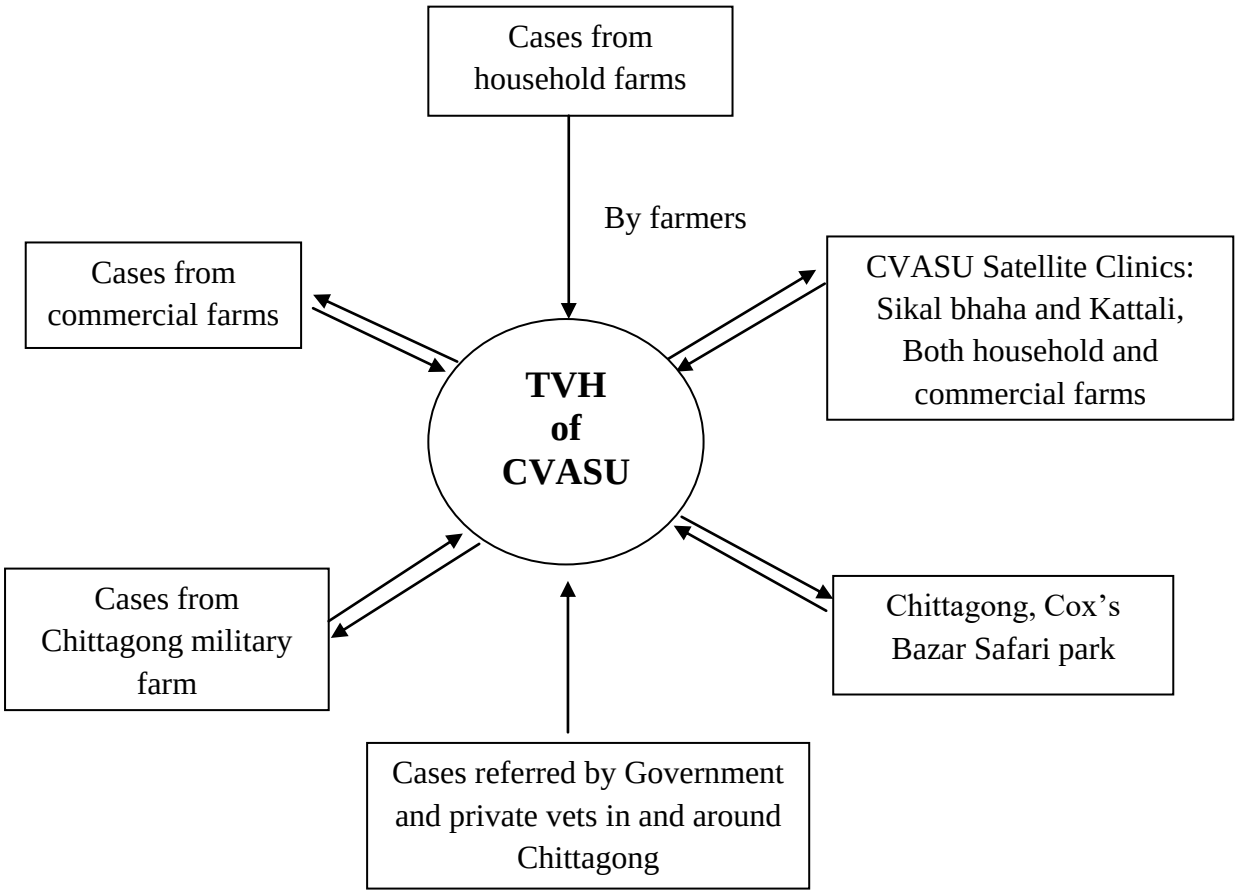

Figure 3.1: Passive surveillance network of Teaching Veterinary Hospital at Chittagong Veterinary and Animal Sciences University (Sources of disease data)

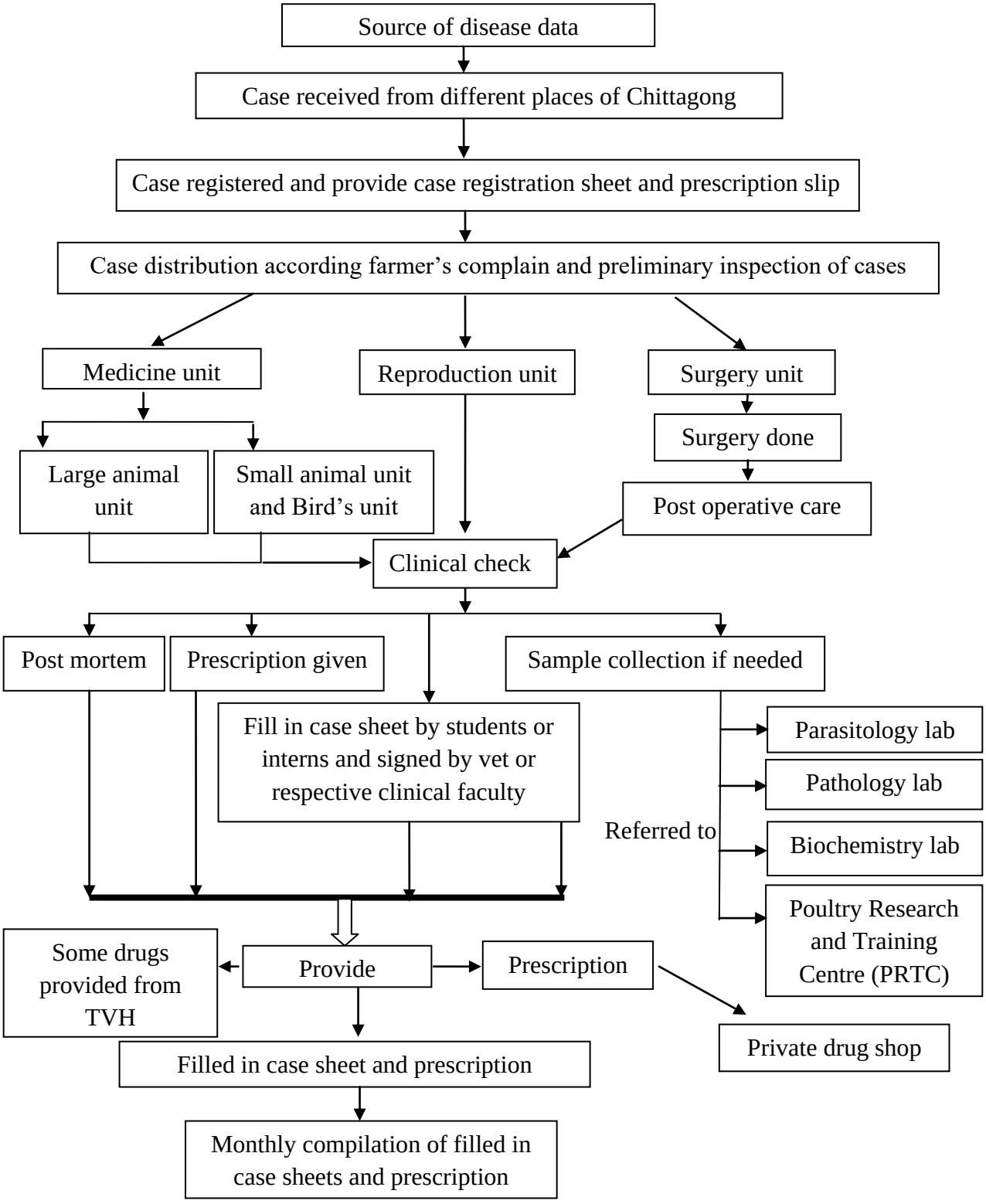

Figure 3.2: Activities flow chart of passive disease surveillance at Teaching Veterinary Hospital of Chittagong Veterinary and Animal Sciences University

3.4 Study Design, Sample Size, Sampling and Data Recording

Teaching Veterinary Hospital of CVASU based passive surveillance data of PPR in goats were used for the present study. All clinically diagnosed PPR goat cases recorded (922 during January 2011 to December 2015) were included for a descriptive study 1 followed by a case-control study. A PPR case was clinically characterized or described by coughing, nasal discharge, pneumonia, lacrimation, conjunctivitis, diarrhea, stomatitis, frothy salivation and fever. A goat that had at least three clinical signs of the aforementioned list was considered as a PPR suspected case. Clinical faculty of Chittagong Veterinary and Animal Sciences University and TVH veterinarian diagnosed all Peste des Petits Ruminants cases. Average 3 non-PPR clinical goat cases per PPR case were taken as control (N=2766). Non-PPR goat cases recorded during the same time period as PPR goat cases.

Detail case and control distribution in different areas in and around Chittagong during 2011-2015 has been given in Appendix-I and presented in Figure 3.3:

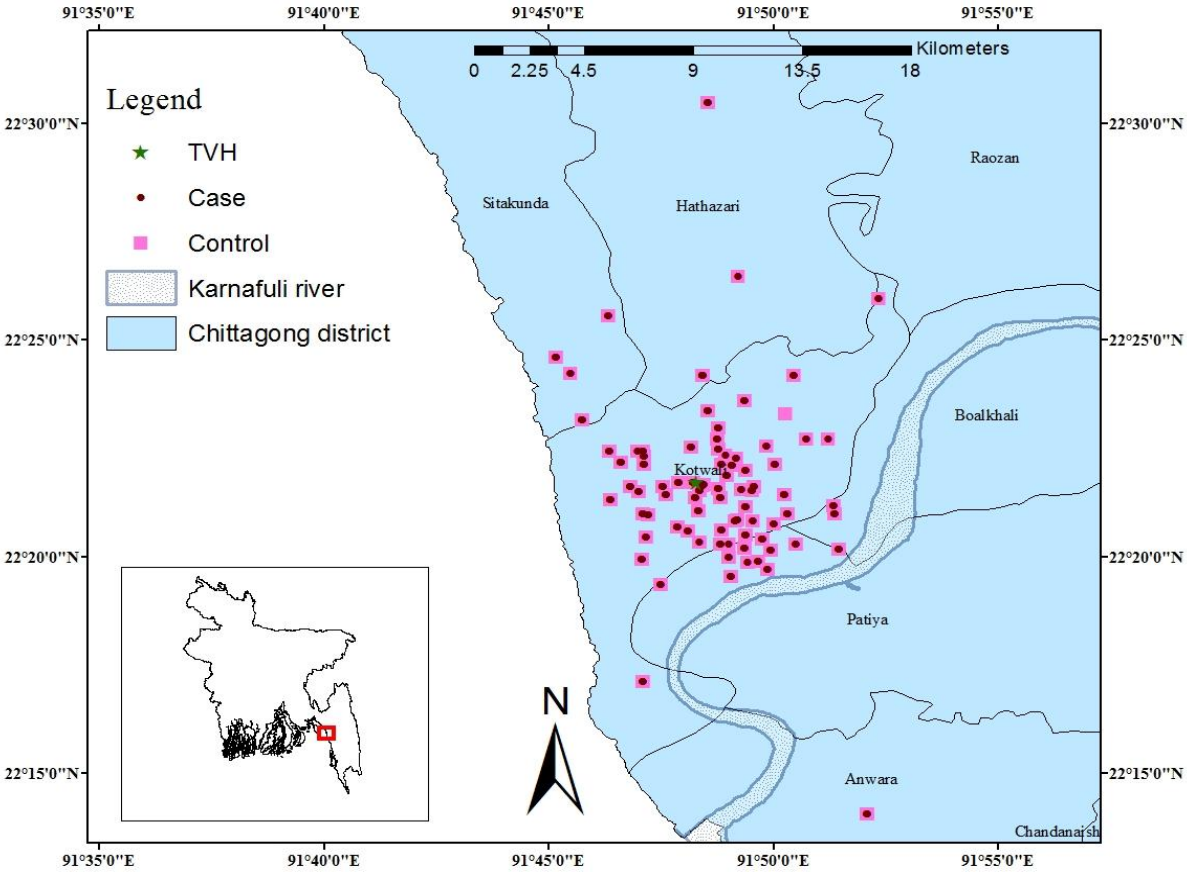

Figure 3.3: Spatial distribution of Peste des Petits Ruminants and non- Peste des Petits Ruminants goat cases in the study

Google map (https://www.google.com.bd/maps) was used to get geocoordinates of the location of the cases and controls based on the address recorded in the hospital registration sheet. ArcGIS-ArcMap version 10.2 (ESRI, USA) was used to produce map and locate cases and control individuals.

For the current descriptive study 1 followed by case-control study, the following epidemiological data were extracted per individual from TVH paper based record keeping system. The data were rearing system, breed, age, sex, BCS, parity number, body weight, clinical signs and symptoms, diagnosis and drugs prescribed.

In order to substantiate the descriptive study 1 another descriptive study 2 was also conducted on goats brought to TVH of CVASU and goats in other sites of Chittagong Metropolitan City.

A total 138 suspected goats for PPR were considered for a descriptive study 2. Within the study period in TVH total 150 goats were brought for treatment in which 50 goats were suspected for PPR by observing clinical signs and symptoms. Similarly, in Firingibazar slaughter house 30 goats were suspected within 80 goats, in Dewanhat slaughter house 15 goats were suspected within 95goats, in Bibirhat Live Goat Market 10 goats were suspected within 55 goats, in Sagorika Live Goat Market 8 goats were suspected within 50 goats, in AK Khan Household Goat Farm 10 goats were suspected within 30 goats, in Pahartali Household Goat Farm 10 goats were suspected within 25 goats and in Segunbagan Household Goat Farm 5 goats were suspected within 15 goats.

Table 3.1: Distribution of Peste des Petits Ruminants suspected goats in different study sites

Sites	Total number of goats	Number of clinically PPR suspected goats
Teaching Veterinary Hospital	150	50
Firingibazar Slaughter House	80	30
Dewanhat Slaughter House	95	15
Bibirhat Live Goat Market	55	10
Sagorika Live Goat Market	50	8
A K Khan Household Goat Farm	30	10
Pahartali Household Goat Farm	25	10
Segunbagan Household Goat Farm	15	5

Different epidemiological data for the suspected goats from different study sites were collected for the descriptive study 2. Peste des Petits Ruminants vaccination data were additionally recorded. Clinical signs of PPR were noted through observations and direct physical examination. Case definition of PPR was followed as the definition being made for the case-control study. The record keeping sheet has been given in Appendix-II.

3.5 Sample Collection under Descriptive Study 2

Both nasal swab and blood samples were collected from each individual animal. Nasal sample was used for PPR molecular diagnosis, whereas blood sample was used for PPR antibody evaluation and differential leukocyte counts.

Proper personnel protective measures were ensured before collecting samples. Individual nasal swab sample was collected using sterile cotton swab stick and then placed in sterile cryo-vial containing 5ml viral transport media (VTM) and marked with unique identification number. The VTM was prepared as instructed by Healing and Organization, (2006). Immediately after collection the samples in cryo-vials were placed in an insulated ice box containing ice gel and immediately transferred to Poultry Research and Training Centre (PRTC) laboratory, CVASU. In the PRTC laboratory the samples were temporarily stored at -80^0C. Then the samples were shifted to SAARC PPR RLDL (South Asian Association for Regional Cooperation Regional Leading Diagnostic Laboratory) in

Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka. In BLRI the samples were kept at -80^{0}C for further laboratory evaluation.

A 4 ml of blood sample was collected aseptically from jugular vein of each selected animal using sterile disposable syringe. Single drop of blood sample was immediately placed on a sterile slide for producing a direct thin smear for hematological examination and the rest of the blood sample in the syringe was poured into sterile falcon tube without adding any kind of anticoagulants. Individual falcon tube and subsequent slide were given identical and unique identification number. The falcon tubes containing blood samples were placed as 45^0 inclined position and transferred to the PRTC laboratory using insulated ice box. In the laboratory the falcon tube containing blood samples were kept in refrigerator overnight. Then the serum was separated by centrifugation at 5000 rpm for 15 minutes. After centrifugation serum samples were transferred to eppendorf tube with identical identification number of the falcon tube and stored at -20 ℃ until being transported to the SAARC PPR RLDL, BLRI Savar, Dhaka. In BLRI the serum samples were stored at -20°C until further evaluation.

3.6 Blood Smear Preparation

The blood smear was prepared as the process described by Alim et al. (2012). In brief a drop of blood was placed at right end of a clean grease free smooth unbroken ended slide. Then the slide was taken on a piece of paper on the table holding firmly by the force or thumb finger on left hand. The even edge of the second slide was placed near the drop of blood towards the middle of the slide. The second slide was drawn towards the drop of the blood at an angle of 45^0 and the blood was spread along the edge. Keeping the second slide in the same angle a quick even push was given with a uniform force towards the other end of slide. Then the slide was allowed to be dried. The dried slides with unique identification numbers were taken to the Physiology Laboratory of CVASU for staining and hematological examination.

3.7 Laboratory Evaluation

3.7.1 Molecular Testing of Peste des Petits Ruminants

3.7.1.1 Ribo Nucleic Acid (RNA) Extraction

Nasal samples were evaluated as pool sample, each pool consisting of 5 nasal swabs. When one pool sample was positive all 5 samples of pool was regarded as positive. The pool samples were extracted according to the manufacturer's protocol (RNeasy® Mini Handbook, 4th edition, April 2006 by Qiagen). In brief 250 µl pooled swab sample suspension was taken in an eppendorf tube followed by adding 450 µl of RLT (working buffer). After then the mixture was vortexed and allowed for 3 minutes incubation at room temperature. Then 700 µl of 70% ethanol was added in the tube for making the volume of mixture up to 1400 µl following gently mixing and vortexing. After then 700 µl was transferred from the eppendorf tube containing mixture to the RNeasy spin column with a 2 ml collection tube and centrifuged @ 10,000 rpm for 30 seconds. Then the flow through was discarded immediately and remaining 700 µl mixed solution was again transferred into the respective spin column. Further, it was again centrifuged for 30 seconds at 10,000 rpm. Then the residue was discarded again followed by adding 700 µl of RW1 buffer to the spin column and again immediately centrifuged @ 10,000 rpm for 30 seconds. Then the flow through was poured off immediately followed by adding 500 µl of RPE in the respective spin and subjected to centrifugation @ 10,000 rpm for 30 seconds. Immediately the residue was discarded again following repeatedly adding of 500 µl of RPE in the respective spin column which was subjected to centrifugation @ 10,000 rpm for 1 minute. Finally, the spin column was placed into 1.5 ml eppendorf tube. After then a volume of 50 µl of RNAase free water was added into the centre of the spin column, and again centrifuged @10,000 rpm for 1 minute. At last the respective spin column was discarded immediately followed by labeling of eppendorf tube containing extracted RNA with specific identification which was stored at -20 ^{0}C for short term and long term period until the test being performed.

3.7.1.2 Conventional Reverse Transcriptase Polymerase Chain Reaction

Reverse Transcription Polymerase Chain Reaction (RT-PCR) protocol followed by agarose gel electrophoresis technique was adopted for the detection of PPR viral RNA from nasal swab extracts as described by Yousuf et al. (2015). One step RT-PCR kit (Qiagen Street 1, 40724 Hilden, Germany) was used for the RT-PCR.

3.7.1.3 Analyses of Reverse Transcription-Polymerase Chain Reaction Products by Agarose Gel Electrophoresis

Agarose gel electrophoresis technique was performed for analyzing the RT-PCR products described by Gurcay et al. (2013). In brief, Agarose gel was prepared by dissolving agarose powder in 1x TAE Buffer containing the mixture of Tris base, Boric acid and EDTA to a final concentration of 1.5 % (w/v). After then, the slurry was heated in a microwave oven until the Agarose was fully dissolved. Then immediately ethidium bromide was added to the agarose solution @ 0.05 µl/ml. The agarose solution containing ethidium bromide was poured into the gel casting tray to which the comb was properly positioned. After the complete setting of gel the comb was removed following the transfer of gel into the electrophoresis tank which was covered with 1x TAE buffer. PCR products were mixed with DNA loading buffer (5 vol. DNA solutions with 1 vol. of Bromophenol blue as loading buffer) followed by loading to individual slots of the gel. As a size standard, a DNA size marker was also loaded to one slot for better understanding. Then electrophoresis process was run at 100V for 30 minute. After completing the electrophoresis the gel was immediately washed with running water followed by placing on the UV trans-illuminator in the dark chamber of the image viewing and documentation system. Finally, the image was visualized on the monitor following the image was printed as well as saved electronically.

Composition of master's mix, thermal profile and the details of primers were given in Tables 3.2 and 3.3 according to instruction of Qiagen Street 1, 40724 Hilden, Germany.

Table 3.2: The composition of master mixture for Polymerase Chain Reaction, thermal profiles and composition of agarose gel used for electrophoresis

Category	Item	Volume/Quantity/ Duration
Master mixture	Nuclease free water	12 µl
	5X Qiagen one-step RT-PCR buffer	5 µl
	dNTP Mix (10 mM of each dNTP)	1 µl
	Primer forward (100 pmol/µl)	0.5 µl
	Primer reverse (100 pmol/µl)	0.5 µl
	Qiagen one step RT-PCR enzyme mix	1.0 µl
Agarose gel	100 bp DNA ladder	3 µl
	TAE buffer (1X)	50 ml
	Electrophoresis grade agarose	0.75 gm
	Loading dye	2 µl
	Ethidium bromide	2.5 µl
Thermal profile	Reverse transcription step	50^0 C for 30 minutes
	Initial denaturation	95^0 C for 15 minutes
	Denaturation	94^0 C for 30 seconds
	Annealing	55^0 C for 30 seconds
	Elongation	72^0 C for 30 seconds
	No. of cycles	35
	Final elongation	72^0 C for 10 minutes

Table 3.3: Primers used for amplification of N gene of Peste des Petits Ruminants Virus

Primer	Sequence	Position	Size	Ref.
NP3: Forward	5´-TCTCGGAAATCGCCTCACAGACTG-3´	1232-1255	351 bp	(Couacy-Hymann et al., 1995)
NP4: Backward	5´-CCTCCTCCTGGTCCTCCAGAATCT-3´	1583-1560		

3.7.2 Serological Testing of Peste des Petits Ruminants

Only 48 serum samples (N=138) were considered for serological analysis by using CELISA technique because of financial constraint. These limited number of serum samples were randomly selected from the list of samples that had low lymphocyte and high neutrophil counts due to lymphopenia and neutrophilia during PPR infection (Maina et al., 2015). Among all serum samples at least four samples and two samples were selected randomly from clinically PPR affected goats and non-PPR goat, respectively for each study site maintaining 2:1 ratio for CELISA testing. The CELISA test was performed as described by Banik et al. (2008).

Preparation of stock and working solution has been given in the Appendix III.

3.7.2.1 Description of Competitive Enzyme Linked Immunosorbent Assay Protocol

A commercial PPR CELISA kit produced by BDSL (Biological Diagnostic Supplies Ltd 25 Main Street, Irvine, KA11 4AQ, Scotland) and The Pirbright Institute for Animal Health was used for ELISA test to detect the anti-PPR antibody of the serum samples using standard process described by Banik et al. (2008). The test protocol was based on the basis of competition between the monoclonal antibodies against Hemagglutinin (H) protein of PPR virus and test serum samples to bind with PPR antigen (Couacy-Hymann et al., 2007c; Banik et al., 2008).The procedure is described below.

In brief 50 µl of N-PPR antigen solution was added to all wells of the ELISA micro-titre plate, tapping the side of the microtitre plate to ensure that the antigen is evenly

distributed over the bottom of each well. Then the whole micro-titre plate was kept overnight at 4^0C. At the following day washing of the whole micro-tire plate for 5 minutes using washing buffer was done for 3 times followed by adding 40 µl blocking buffer in each well. Then 10 µl conjugate control (Cc), 10 µl of strong positive control (C^+), 10 µl negative control (C^-) and 10 µl monoclonal antibody (MAb) were added in the well number A1, A2 and B1, B2; C1, C2 and D1, D2; E1, E2 and F1, F2 and G1, G2 and H1, H2, respectively. Again 10 µl of test serum was added in test wells followed by 50 µl MAb to all wells except the conjugate control wells (A1, A2 and B1, B2). Then the micro-titre plate was incubated at 37°C for an hour in an incubator with shaking (150-160 rpm) facilities. After incubation the plate was washed for three times in washing buffer for 5 minutes. Then 50 µl of rabbit anti mouse conjugate solution was added in each well. After incubation at 37°C for an hour in an incubator with shaking (150-160 rpm) the micro-titre plate was washed for 5 minutes with washing buffer for 3 times. Again 50 µl of substrate/Chromogen mixture was added in each well and placed in the dark for 10 minutes by wrapping the micro-tire plate with aluminium foil. Then 50 µl of stopping solution was added to each well. Finally, the micro-titre plate was read for OD (Optimal Density) values with BioTek multichannel spectrophotometric ELISA plate reader with interference filters of 492 nm. The reader was run as the manufacturer protocol. The reading data was placed into data sheet of Microsoft® Excel programme and saved in the computer hard disc with specific identification. The final reading was calculated manually.

3.7.2.1.1 Data Expression for Competitive Enzyme Linked Immunosorbent Assay

The reading of micro-titre plate was used in two types of data analysis described as follows.

A) For the Quality Assurance (QA) acceptance Percent Inhibition (PI) was used. These PI values were calculated as follows:

PI = 100 - [(Replicate OD of each Control/ Median OD of MAb control Cm) x 100]

B) For the acceptance of replicate values for test sera and diagnostic interpretation the Percent Inhibition (PI) was calculated as follow:

PI = 100 - [(Replicate OD of each test serum/ Median OD of MAb control Cm) x 100]

The test was accepted as OD values of MAb control (Cm) and PI values of different controls lied within the accepted limits written in QA Fact sheet with the supplied CELISA kit. Sera having PI greater than 50% were considered to be PPR positive.

3.7.3 Hematological Evaluation

3.7.3.1 Differential Leukocyte Counting

A total of 138 blood films were smeared using Wright's staining technique as described by Al-Badrani (2013) placing in a fixed place under light microscope by using immersion oil. Then the cells were identified by using high power objective 100x following parallel stripe method. The counting was continued till 100 cells. Different types of cells were counted by tally method. Then percentage of Lymphocyte, Neutrophil, Eosinophil, Monocyte and Basophil were recorded.

3. 8 Statistical Evaluation

Field and laboratory data were entered into Microsoft Excel 2007 spread sheet. Data were cleaned, sorted, coded and checked for integrity in MS Excel 2007. Afterwards, data were exported to STATA-IC-13 *(StataCrop, 4905, Lakeway Drive, College station, Texas 77845, USA)* for conducting epidemiological analysis.

3.8.1 Descriptive Analysis

Descriptive statistics (frequency number and percentages) were calculated to express the clinical cases of goats diagnosed at TVH of CVASU (2011-2015) along with temporal distribution of PPR and frequency distribution of symptoms of clinical PPR, frequency distribution of drugs prescribed against PPR in which major drugs were grouped according to mode of action, generic composition and use followed by other supportive drugs. Proportionate prevalence was calculated for PPR based on clinical signs and symptoms as well as molecular test results, whereas relationship between PPR cases and non-PPR cases in contrast to lymphocyte and neutrophil counts were expressed in mean and median number with minimum and maximum.

3.8.2 Risk Factor Analysis

3.8.2.1 Univariate Analysis

Season of the year was categorized into summer (March-June), winter (November-February) and rainy (July-October). Again Body Condition Score (BCS) was determined through numeric scoring on the basis of eye estimation. Body scores 1 and 2 presents cachectic followed by 3 for fair and 4 for good. Body weight and age were categorized on the basis of percentile as they were not normally distributed.

Disease diagnosis was recorded for all cases and controls as binary outcome variable to obtain an estimate of the fraction of cases brought in with PPR relative to other diagnoses. Factors of year, season, rearing system, breed, BCS, sex, body weight and age were initially assessed by Fisher's exact test to identify univariate association with the prevalence of PPR. The level of significance was determined as $p \leq 0.05$.

3.8.2.2 Multivariate Analysis

Fisher Exact's significant ($p \leq 0.05$) factors were forwarded to multivariate analysis. Before fitting the logistic regression model the season was regrouped as summer and winter together and rainy. Breed was regrouped as Jamunapari and Cross together and Black Bengal Goat. The logistic regression model was fitted by backward-elimination procedure of the variables one by one considering Wald test significant p-value. The presence of confounding factors, interaction factors and presence of collinearity were investigated. A change of the coefficient of more than 10% for individual factor between full model and reduced model was considered to indicate the presence of confounding, whereas the likelihood ratio test's significant p-value of ≤ 0.05 was considered to identify interaction factors. Chi-square test between the independent variables was used to check for the presence of collinearity. The sensitivity of the final model was determined using Receiver Operating Curve and Hosmer-Lemeshow goodness-of-fit test. Finally, the results were presented for each predictor variable as an odds ratio (OR) and 95% CI.

Chapter IV: Results

A) Descriptive Study 1 followed by Case-control Study

4.1 Descriptive Analysis

4.1.1 Clinical Cases of Goats at Teaching Veterinary Hospital of Chittagong Veterinary and Animal Sciences University from 2011-2015

Annual distribution of different clinical cases in goats is presented in Table 4.1.The frequency percentage of myiasis, mastitis and PPR in goats ranged 2.9-7.6%, 2.6-5.6% and 21.3-28.7%, respectively. The proportionate prevalence of parasitic infestation, reproductive cases, acidosis and anorexia was 9.4-11.2%, 2.4-4.9%, 8.7-10.5% and 3.7-4.8%respectively

Table 4.1: Overview of diseases/ disease conditions diagnosed in goats at Teaching Veterinary Hospital of Chittagong Veterinary and Animal Sciences University (2011-2015)

Year		PPR	Mastitis	Myiasis	Parasitic infestation	Reproductive cases	Acidosis	Anorexia	Others*	All
2011	N	151	40	31	67	17	63	34	307	710
	%	21.3	5.6	4.4	9.4	2.4	8.9	4.8	43.2	19.4
2012	N	177	31	58	76	23	66	28	302	761
	%	23.3	4.1	7.6	9.9	3.0	8.7	3.7	39.7	20.8
2013	N	188	19	35	79	21	75	27	283	727
	%	25.9	2.6	4.8	10.9	2.9	10.3	3.7	38.9	19.8
2014	N	196	23	21	82	20	77	34	282	735
	%	26.7	3.1	2.9	11.2	2.7	10.5	4.6	38.4	20.1
2015	N	210	20	31	69	36	73	27	265	731
	%	28.7	2.7	4.2	9.4	4.9	9.9	3.7	36.3	19.9

***Others: Vitamin and mineral deficiency, Respiratory cases (Sneezing and coughing) and surgical cases**

4.2 Temporal Distribution of Peste des Petits Ruminants in Goats

Seasonal distribution of PPR at TVH has been presented in Table 4.2.The estimated proportionate prevalence of PPR in rainy season was 65.6% followed by18% in winter season and 16.4% in summer season.

Table 4.2: Seasonal distribution of Peste des Petits Ruminants in goats at Teaching Veterinary Hospital of Chittagong Veterinary and Animal Sciences University (N=922)

Season	Month	N	%
Winter	November-February	166	18.0
Summer	March-June	151	16.4
Rainy	July-October	605	65.6

4.3 Recorded Clinical Signs and Symptoms of Peste des Petits Ruminants in Goats

Diarrhoea, stomatitis and nasal discharge together were recorded as the most frequent clinical signs of PPR (17.5%) followed by diarrhea, stomatitis and coughing together (15.1%), diarrhea, nasal discharge and coughing combined (14.7%) and diarrhea (4.1%) only. The detail results of clinical signs are presented in Table 4.3.

Table 4.3: Frequency distribution of symptoms of clinical Peste des Petits Ruminants in goats at Teaching Veterinary Hospital of Chittagong Veterinary and Animal Sciences University (N=922)

Clinical signs and symptoms	N	%
Diarrhea, stomatitis and nasal discharge	162	17.5
Diarrhea, stomatitis and coughing	139	15.1
Diarrhea, nasal discharge and coughing	136	14.7
Diarrhea and coughing	76	8.2
Diarrhea and nasal discharge	64	6.9

Diarrhea and stomatitis	63	6.8
Diarrhea, stomatitis, nasal discharge and lacrimation	55	6.1
Diarrhea, stomatitis and salivation	48	5.2
Diarrhea	38	4.1
Diarrhea, anorexia, pneumonia, nasal discharge and conjunctivitis	32	3.4
Diarrhea, pneumonia and coughing	25	2.9
Diarrhea and pneumonia	19	2.1
Diarrhoea and dehydration	18	1.9
Coughing, lacrimation and diarrhoea	16	1.8
Diarrhea, dyspnea and stomatitis	15	1.6
Diarrhoea, pneumonia and skin lesion	7	0.8
Diarrhea, nasal discharge and depression	5	0.5
Diarrhea, stomatitis, salivation and skin lesion	4	0.4

4.4 Frequency of Using Different Drugs Prescribed Against Peste des Petits Ruminants in Goat at Teaching Veterinary Hospital

Ranges of drugs prescribed in treating PPR have been enlisted in Table 4.4. Sulphadimidine sodium was the most frequently prescribed antibacterials against PPR (70.2%) followed by Gentamicin (15.4%) and Ceftriaxone (4.6%). Among the combined preparations of aminoglycosides with sulphonamide were used frequently (1.8%) than aminoglycosides with penicillin (0.8%). The details of drugs prescribed against PPR are given in Table 4.4.

Table 4.4: Frequency distribution of prescribed different drugs against Peste des Petits Ruminants in Goat at Teaching Veterinary Hospital during 2011-2015

Classes of drugs	Category	n	%
Sulphonamides	Sulphadimidine	647	70.2
Aminoglycosides	Gentamicin	142	15.4
Cephalosporin	Ceftriaxone	42	4.6
Betalactum	Amoxicillin	35	3.7
Aminoglycosides and Betalactum	Sulphadiazine, Sulphadimidine, Sulphapyrimidine and Streptomycin	18	1.8
Fluroquinolone	Ciprofloxacin	18	1.9
Tetracycline	Oxytetracycline	13	1.4
Supportive drugs*	Antihistamine and Saline	436	47.3
	Vitamin and Saline	195	21.2
	Saline	146	15.8
	Antihistamine and Antipyretic	53	5.7
	Antihistamine and Vitamin	51	5.5
	Antipyretic and Saline	41	4.50

4.5 Risk Factor Analysis

4.5.1 Univariate Analysis

The prevalence of PPR in goats significantly varied by year, season, rearing system, breed, BCS and weight ($p \leq 0.05$) (Table 4.5)

Table 4.5: Univariate association between risk factors and the binary response variable of Peste des Petits Ruminants in goats

Factor	Category	N	PPR		p-value
			Positive (%)	Negative	
Year	2011	713	151 (21.2)	562	0.012
	2012	763	177 (23.2)	586	
	2013	728	188 (25.8)	540	
	2014	752	196 (26.1)	556	
	2015	732	210 (28.7)	522	
Season	Winter (November-February)	646	166 (25.7)	480	<0.001
	Summer (March-June)	1296	151 (11.7)	1145	
	Rainy (July-October)	1746	605 (34.7)	1141	
Rearing system	Intensive and semi-intensive	2424	793 (32.7)	1,631	<0.001
	Free range and tethering	1229	120 (9.8)	1109	
Breed	Black Bengal	1059	294 (27.8)	765	0.015
	Jamunapari and cross	2629	628 (23.9)	2001	

Body Condition Score	Cachectic	178	233 (56.7)	411	<0.001
	Fair to good	2126	446 (17.3)	2572	
Sex	Male	1732	433 (25.0)	1299	1.000
	Female	1,956	489 (25.0)	1,467	
Body weight (Kg)	≤ 9.5	941	195 (20.7)	746	0.002
	>9.5 to ≤20	1927	511 (26.5)	1416	
	>20 to ≤87	820	216 (26.3)	604	
Age (Month)	≤5 month	935	216 (23.1)	719	0.28
	>5 to ≤18	2000	517 (25.9)	1483	
	>18 to ≤60	753	189 (25.1)	564	

4.5.2 Multivariate Analysis

After adjusting the factors each other the final logistic model was developed (Table 4.6) PPR was 2.4 times more during rainy season in comparison to summer and winter together (95% CI: 1.9-2.9; p<0.001). The prevalence of PPR was 4.3 times higher among those goat population reared in intensive and semi-intensive system than those goat population reared in free range and tethering system (95% CI: 3.3-5.5; p<0.001). Goats with cachectic BCS had 5.7 times more likely affected by PPR than goat with fair to good BCS (95% CI: 4.5-7.1; p<0.001). However, Black Bengal goat had 1.2 times more likely to be affected by PPR than Jamunapari and other cross breed (95% CI: 1.0-1.5; p=0.04).

Table 4.6: Outputs of the final logistic regression model

Factors	Category	OR	95% CI	p-value
Season	Summer and Winter	1		
	Rainy	2.4	1.9-2.9	<0.001
Rearing system	Free range and tethering	1		
	Intensive and Semi-intensive	4.3	3.3-5.5	<0.001
Body Condition Score	Fair to good	1		
	Cachectic	5.7	4.5-7.1	<0.001
Breed	Jamunapari and cross	1		
	Black Bengal Goat	1.2	1.0-1.5	0.04

B) Descriptive Study 2

4.6 Proportionate Prevalence of Peste des Petits Ruminants on the basis of Clinical Signs and Symptoms

The overall proportionate prevalence of PPR in Chittagong Metropolitan area on the basis of clinical signs and symptoms was 27.6% (N=500). The proportionate prevalence of PPR was 33.3% (N=150) in TVH followed by 37.5% (N=80) in Firingibazar, 15.8% (N=95) in Dewanhat, 18.2% (55) in Bibirhat, 40% (N=25) in Pahartali, 33.3% (N=30) in AK Khan, 16% (N=50) in Sagorika and 33.3% (N=15) in Segunbagan (Table 4.7).

Table 4.7: Proportionate prevalence of Peste des Petits Ruminants in goats based on clinical signs and symptoms in Chittagong Metropolitan City

Site	Total goats	Clinically suspected goats (%)	95% CI
*TVH	150	50 (33.3)	26-41
Firingibazar	80	30 (37.5)	27-49
Dewanhat	95	15 (15.8)	9-25
Bibirhat	55	10 (18.2)	9-31
Pahartali	25	10 (40)	21-61
AK Khan	30	10 (33.3)	17-53
Sagorika	50	8 (16)	7-29
Segunbagan	15	5 (33.3)	11.8- 61.6
Total	**500**	**138 (27.6)**	**24-32**

*TVH: Teaching Veterinary Hospital

4.7 Proportionate Prevalence of Peste des Petits Ruminants on the basis of Molecular Testing

The overall proportionate prevalence of PPR based on molecular testing of nasal samples in Chittagong Metropolitan area was 21.7% (N=138). According to the study sites the proportionate prevalence of PPR was 20% (N=50) in TVH of CVASU followed by 16.7% (N=30) in Firingibazar, 50% (N=10) in Bibirhat, 33.3% (N=15) in Dewanhat and 50% (N=10) in Pahartali (Table 4.8).

Table 4.8: Proportionate prevalence of Peste des Petits Ruminants in goats based on molecular testing on nasal swabs in Chittagong Metropolitan City

Site	No of sample	No of PPR positive (%)	95% CI
*TVH	50	10 (20.0)	10.0-33.7
Firingibazar	30	5 (16.7)	5.6-34.7
Dewanhat	15	5 (33.3)	11.8-61.6
Bibirhat	10	5 (50.0)	18.7-81.2
Pahartali	10	5 (50.0)	18.7-81.3
AK Khan	10	0	0-30.9
Sagorika	8	0	0-36.9
Segunbagan	5	0	0-52.2
Total	**138**	**30 (21.7)**	**15.2-52.2**

*TVH: Teaching Veterinary Hospital

4.8 Association between Proportionate Prevalence of Peste des Petits Ruminants and Vaccination Status of Goat

The proportionate prevalence of PPR was 0% (95% CI: 0-8.4%) in vaccinated goats and 33.3% in non vaccinated goats (95% CI: 22.2-41.5%).

4.9 Sero- Prevalence of Peste des Petits Ruminants in Goats

Half of the samples had PPRV antibody in this study and no site specific variation was evidenced (Table 4.9).

Table 4.9: Proportionate Sero-prevalence of Peste des Petits Ruminants in goats of Chittagong Metropolitan City based on Competitive Enzyme-Linked Immunosorbent Assay

Site	No of serum samples from PPR suspected goats (PPR Ab +)	No of serum samples from apparently healthy goats (PPR Ab+)
*TVH	4 (2)	2 (1)
Firingibazar	4 (2)	2 (0)
AK Khan	4 (2)	2 (0)
Sagorika	4 (2)	2 (0)
Bibirhat	4 (2)	2 (0)
Segunbagan	4 (2)	2 (2)
Dewanhat	4 (2)	2 (0)
Pahartali	4 (2)	2 (0)
Total	**32 (16)**	**16 (3)**

*TVH: Teaching Veterinary Hospital

4.10 Distribution of Lymphocytes and Neutrophils in Peste des Petits Ruminants

Overall lymphocyte counts were comparatively lower in PPR affected goats (37.1% in TVH, 41.8% in Firingibazar, 42.4% in Bibirhat, 32.2% in Dewanhat and 38.6% in Pahartali) than in non PPR affected goats (40.2% in TVH, 52.7% in Firingibazar, 49.2% in Bibirhat, 64.5% in Dewanhat and 41.2% in Pahartali).

In contrast overall neutrophil counts were higher in PPR affected goats (64.3% in TVH, 60.8% Firingibazar, 60.4% in Bibirhat, 67% in Dewanhat and 60.8% in Pahartali) than in non PPR affected goats (57.2% in TVH, 52.6% in Firingibazar, 54.8% in Bibirhat, 55.5% in Dewanhat and 54.4% in Pahartali) (Table 4.10).

Table 4.10: Distribution of Lymphocyte and Neutrophil in Peste des Petits Ruminants positive goats/non-Peste des Petits Ruminants goats

Site	PPR (+ve)	Mean		Median		Min-Max		PPR (-ve)	Mean		Median		Min-Max	
		L	N	L	N	L	N		L	N	L	N	L	N
*TVH	10	37.1	64.3	37	65	30-45	60-68	40	40.2	57.2	32.5	58	30-75	39-64
Firingibazar	5	41.8	60.8	43	61	34-45	59-63	25	52.7	52.6	45	55	30-78	37-65
Bibirhat	5	42.4	60.4	43	59	37-45	57-65	5	49.2	54.8	44	57.5	30-78	39-65
Dewanhat	5	32.2	67	30	67	27-40	65-68	10	64.5	55.5	71	55	38-78	51-61
Pahartali	5	38.6	60.8	38	61	28-47	56-65	5	41.2	54.4	52	55	30-78	49-57

*TVH: Teaching Veterinary Hospital, Min- Minimum and Max-Maximum

Chapter V: Discussion

Peste des Petits Ruminants is most devastating and economic important disease of goats in Bangladesh. The present epidemiological study was conducted in TVH of CVASU and other sites of Chittagong Metro City in order to explore proportionate prevalence, associated risk factors, clinical signs and symptoms and to get an insight of drugs used for treatment of this disease. In this section all important findings in the current study with implication, limitations, conclusions and recommendations have been discussed.

5.1 Prevalence of Peste des Petits Ruminants in Goats

In the current study the overall proportionate prevalence of PPR in goats in Chittagong Metropolitan area were 27.6% and 21.7% in descriptive study 2 and (21.3-28.7%) in descriptive study 1. Similar observation was reported in Turkey (Özkul et al., 2002) and Bangladesh (Rajshahi) (20.6%) (Sarker and Islam, 2011). Different Bangladeshi studies reported the PPR prevalence as 8.9% in Chittagong (Parvez et al., 2014), 18.8% in Rangpur, 12.5% in Kushtia (Sharifuzzaman et al., 2015), 50.3% in Potuakhali (Islam et al., 2013) and 39% in Thakurgoan (39%) (Bupasha et al., 2015).

In some overseas studies researchers also reported variable results such as 6.8% in Ghana (Folitse et al., 2016), 47.9% in Niger (Farougou et al., 2013) and 32.8% in India (Balamurugan et al., 2012).

5.2 Sero-prevalence of Peste des Petits Ruminants in Goats

In the current study half of the serum samples was sero positive, (31.2% in PPR affected goats and 18.8% in healthy goats) which could be due to wild PPR virus exposure or previous PPR vaccine. The present finding is supported by the studies conducted in Bangladesh(49.2%) (Razzaque et al., 2004) and in Pakistan(52.9%) (Zahur et al., 2011). This finding can be compared with the findings of previous studies indicating either higher or lower seroprevalence as 43.3% in Punjab (Khan et al., 2007), 33-35% in India (Singh et al., 2004b; Raghavendra et al., 2008), 63.4% in Kerala (El-Rahim et al., 2010a), 17.6% in Tibet and China (ZhiLiang et al., 2009), 9% in Afghanistan (Abraham et al., 2005), 15.1% in Saudiarabia (Janus et al., 2009), 50.7% in Sudan (Osman et al., 2009) 45% in Egypt (Salih et al., 2014) and 0.6% in Ethiopia (Adel et al., 2004).

5.3 Risk Factors Associated with Peste des Petits Ruminants

The current study identified season as a significant risk factor (OR=2.4; Rainy season versus combined season of Summer and Winter) which is consistent with the many studies in Bangladesh (Mondal and Yamage, 2014; Parvez et al., 2014) and another study in the world (Martrenchar et al., 1997). PPR occurs more in rainy season due to heavy rainfall as this season is favorable for the survival and spread of virus (Abubakar et al., 2009).However few studies determined winter season as risk factor for the occurrence of PPR in goats (Gupta et al., 2007; Sarker and Islam, 2011). Because in winter season dusty and dry winds initiates the spread of PPR (Sarker and Islam, 2011). Saprophytic organism in the air passage initiate pneumonia (Sarker and Islam, 2011).

Rearing system (OR=4.3; Intensive and Semi-intensive versus Free range and Tethering) was identified another significant risk factor. This result however differs from an Indian study where free ranging or tethering systems had potential influence on the occurrence of PPR in goats (Balamurugan et al., 2012). As in free range or tethering animals get poor veterinary care leading to susceptibility to PPR infection. During free ranging movement of animals occur from one place to another are responsible for spread of the virus (Singh et al., 2004a)

The present study determined breed as a potential risk factor for PPR (OR=1.2, Black Bengal versus Jamunapari and Cross) which is agreed with number of Bangladeshi studies in Bangladesh (Sarker and Islam, 2011; Islam et al., 2013; Parvez et al., 2014; Sharifuzzaman et al., 2015) and in Sudan (Salih et al., 2014). Black Bengal Goats are highly susceptible to PPR (Sarker and Islam, 2011).

Body Condition Score was also identified as a potential risk factor for PPR in goat (OR=5.7; Cachectic versus Fair to Good) which is a new finding in the current study. However the finding makes biologically sense because cachectic animals become immunologically compromised which could lead to different infections in goats (Yeh and Schuster, 1999).

5.4 Clinical Signs Associated with Peste des Petits Ruminants

Most frequent clinical signs in PPR affected goats in this study were high fever, nasal discharge, lacrimation, coughing, stomatitis and diarrhoea which are in-lined with the

previous studies conducted in different countries in the world such as in Turkey (Ozkul et al., 2002; Gurcay et al., 2013), in Egypt (Muse et al., 2012), in Tanzania (Kulkarni et al., 1996) and in Pakistan (Abubakar et al., 2009). These studies additionally reported the following clinical signs: high fever, oculonasal discharge, lacrimation, stomatitis, anorexia, coughing, diarrhoea and depression. The noted clinical signs in this study could help diagnose PPR in goats in the field condition.

5.5 Effects of Peste des Petits Ruminants on Lymphocyte and Neutrophil

In the current study the lymphocyte count was found lower in PPR affected goat in comparison to healthy animal which may be due to destruction of lymphoid tissues as the PPR virus has strong affinity to lymphoid organs (Khan et al., 2012; Pope et al., 2013) Contrarily, neutrophil count was higher in PPR affected goats than healthy goats. This could be due to having concurrent bacterial infection which is supported by earlier studies in Kenya (Sil et al., 1995a; Maina et al., 2015) and in Bangladesh (Islam et al., 2014; Sharifuzzaman, 2015; Naznin et al., 2016).

5.6 Drugs Prescribed Against Peste des Petits Ruminants in Goats

Sulphonamides, Aminoglycosides were used most frequently against PPR affected goats at Teaching Veterinary Hospital (TVH) to check secondary bacterial infection. Other supportive drugs as Antihistamine and Saline were combindly used for recovery. Successful PPR treatment with sulphur drug has also been reported by Gupta et al. (2007); Islam et al. (2013); Sharifuzzaman (2015) in Bangladesh and also in India (Sahu et al., 2014). This drug is also comparatively cheaper, available and sensitive against bacterial infections. Frequent use of other supportive drugs like antihistamine, vitamin and saline for rapid recovery was also found in the previous studies in Bangladesh (Islam et al., 2013; Islam et al., 2014).

5.7 Vaccine Efficacy

The current study was conducted to ascertain the proportionate prevalence of PPR in vaccinated and non vaccinated goats. The proportionate prevalence of PPR was 0% in vaccinated goats and 33.3% in non vaccinated goats which is indicator of vaccine success and the finding is supported by Gibbs et al. (1979b); Islam et al. (2013); Sharifuzzaman

(2015). However, the finding should be considered cautiously as the current case-control study did not contain bigger sample size.

5.8 Limitations of the Study

5.8.1 Descriptive study 1 followed by Case-control Study

i) Proportionate prevalence of PPR was estimated due to lack of denominator data.

ii) Many missing data were noticed in hospital record keeping sheet.

iii) As clinical and epi data were recorded by non epi faculty, information bias might have occurred.

iv) Diagnosis was based on clinical signs and symptoms. So, diagnostic error might happen with similar diseases.

5.8.2 Descriptive Study 2

i) Sample size was small.

ii) Only 30.7% of serum samples obtained was evaluated for PPR antibody determination because of financial constraints.

iii) Nasal samples were evaluated as pool samples for molecular testing because of budget constraint.

Chapter VI: Conclusions, Recommendations and Future Directions

Conclusions

The overall proportionate PPR prevalence in goats were 27.6% (N=500), 21.7% (N=138) and 21.3-28.7% (N= 922) in descriptive study 2 and descriptive study 1.The overall sero-prevalence of PPR was 50%. Rainy season, intensive and semi intensive systems of rearing, Black Bengal Goat and lower BCS had significantly higher contribution to the occurrence of PPR in goats.

Frequent group of clinical signs of PPR encountered was nasal discharge, stomatitis and diarrhoea (17.5%).

Average lymphocyte count declined in PPR affected goats, which might be due to destruction of lymphoid organs. However, average neutrophil count increased which could be due to secondary bacterial infection.

Vaccinated goats had no PPR cases in the descriptive study 2 which indicates success of vaccination, however, this finding should be considered cautiously as the sample size was small.

For recovery sulphur drugs, aminoglycosides, antihistamine, vitamin and saline were frequently used in goats against PPR.

Recommendations

1. The current study proposes the following signs to diagnose clinical PPR along with low lymphocytic count. They include nasal discharge, coughing, stomatitis and diarrhoea. This recommendation is based on the current descriptive study along with blood and molecular testing.
2. Extra care should be taken during rainy season, vaccination against PPR should be completed before starting the rainy season.
3. Hygiene status should be improved to reduce the occurrence of PPR in goats under intensive and semi intensive rearing systems.
4. Improvement of goat nutritional management should be considered as goats with lower BCS had higher PPR cases.

5. Sulphonamide, aminoglycosides, antihistamine and saline were more frequently prescribed. So sulphur and aminoglyside drugs can be administered to check secondary bacterial infection and antihistamine, vitamin and saline for recovery.

Future directions

1. A comprehensive prospective cohort study based on molecular and serological test can be conducted.
2. A vaccine trial against PPR in goats can be assessed rigorously in the field condition.
3. Drug trials against PPR can be conducted.

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Appendix I: Case and control distribution in different area during 2011-2015

Sources	No of PPR cases	%	No of non PPR cases	%
AK Khan	7	28.0	18	72.0
Agrabad	7	24.1	22	75.9
Akbar shah	19	17.8	88	82.2
Alangkar	5	25.0	15	75.0
Ambagan	25	26.6	69	73.4
Amin colony	1	25.0	3	75.0
Anowara	7	21.9	25	78.1
Bakalia	5	29.4	12	10.6
Bandartilla	4	25.0	12	75.0
Barapul	8	40.0	12	60.0
Bibirhat	1	25.0	3	75.0
Bisha colony	11	15.3	61	84.7
CDA Market	7	20.6	27	79.4
CRB	9	24.3	28	75.7
Chaktai	6	28.6	15	71.4
Chalk bazar	15	23.4	49	76.6
Chandan nagar	10	22.2	35	77.8
Chowdhury nagar	16	32.0	34	68.0
City gate	6	28.6	15	71.4
Colonel hat	30	23.8	96	76.2
Dampara	13	30.2	30	69.8
Dewanhat	7	25.0	21	75.0
Eid gah	7	28.0	18	72.0
Enayet bazar	10	25.0	30	75.0
Fakirhat	14	21.9	50	78.1
Fateyabad	1	25.0	3	75.0
Faujdarhat	7	35.0	13	65.0
Ferojshah	8	25.0	24	75.0

Sources	No of PPR cases	%	No of non PPR cases	%
Foys lake	17	26.2	48	73.8
GEC	3	20.0	12	80.0
Goal pahar	9	23.1	30	76.9
Hazi para	0	0	6	100
Kalamia bazar	7	24.1	22	75.9
Kathal bagan	3	18.8	13	81.2
Kattali	22	26.5	61	73.5
Khulshi	50	25.3	148	74.7
Lalkhan bazar	23	25.3	68	74.7
Madarbari	1	7.1	13	92.9
Malipara	1	25.0	3	75.0
Master lane	9	25.0	27	75.0
Monsurabad	1	7.7	12	92.3
Muradpur	8	28.6	20	71.4
Nalapara	4	30.8	9	69.2
Nasirabad	29	24.8	88	75.2
Nayabazar	12	25.0	36	75.0
Oxyzen	6	28.6	15	71.4
Pahartali	53	26.5	147	73.5
Panchlaish	4	30.8	9	69.2
Panir tanky	5	26.3	14	73.7
Panjabi lane	1	14.3	6	85.7
Pathantuli	3	20.0	12	80.0
Poly technical	1	25.0	3	75.0
Port colony	4	21.1	15	78.9
Rahman nagar	3	25.0	9	75.0
Rail gate	3	25.0	0	75.0
Sabujbag	4	25.0	12	75.0
Sadarghat	4	19.1	17	80.9

Sources	No of PPR cases	%	No of non PPR cases	%
Sagorika	12	26.1	34	73.9
Salimpur	2	40.0	3	60.0
Santinagar	2	25.0	6	75.0
Saraipara	13	26.5	36	73.5
Shamsherpara	2	25.0	6	75.0
Shegunbagan	23	25.8	66	74.2
Shersha	12	24.5	37	75.5
Sholoshahar	5	31.3	11	68.7
Technical	6	15.8	32	84.2
Textile	7	28.0	18	72.0
Tiger pass	10	27.0	27	73.0
Tulatali	8	26.7	22	73.3
Vatiyary	4	30.8	9	69.2
WASA	2	25.0	6	75.0
Wireless	63	27.0	170	73.0

Appendix II: A Questionnaire for epidemiological investigation of PPR on rural goat based on TVH, CVASU, Bangladesh

General information:

- Name of the owner…….
- Occupation of owner……..
- Education of owner……...
- Date of data and sample collection……..
- Types of farm: Backyard/ Family farm/ Commercial Farm
- Total number of goats….Male….Female……..Kid……..
- Location…… Upazilla…….
- Latitude……. Longitude……
- Rearing system: Intensive / Semi intensive /Tethering /Free ranging
- Environment of rearing area : Hot / Cold / Moderate / Moisty
- Season …….
- Goats are mixed with neighbor goats: Yes/No
- Last date of PPR identified……..
- Any recent outbreak of PPR? Yes/No
- Any death history due to PPR: Yes/ No (if yes how many…….)

Individual level information:

- Breed of goat: Local / Cross /Jamnapari / Black Bengal /Other…… (specify)
- Age of goat……
- Sex of goat: Male/Female
- If female number of parity…….
- Physiological status : Pregnant/ Not-Pregnant
    - If pregnant please specify duration of pregnancy……
- Lactation status: Lactating/ Non-lactating (if lactating please specify how much milk is produced per day)……
- Body weight……
- BCS: Cachectic / Fair / Poor /Good/ Obese
- Temperature……..

- Nasal discharge : Yes /No
- Sneezing :Yes / No
- Coughing : Yes /No
- Abnormal respiratory sound: Present/ Absent
- Lacrimation: Yes/ No
- Stomatitis : Yes/ No
- Faeces : Normal /Watery /Pasty /Other
- Dehydration : Yes / No
- Duration of illness…….
- The goat is vaccinated : Yes/ No
- If vaccinated time of vaccination……..
- Which vaccine was used……..
- From which vaccination was done……

Appendix III: Preparation of stock and working solution

Stock preparation

Stock	Preparation
Antigen stock	Freeze dried contents of a vial was mixed with 1.0 ml of the sterile distilled water until completely dissolved. Then the antigen stock was stored at -20^{0}C
Anti-PPRV Monoclonal Antibody stock	Anti-PPR Monoclonal Antibody stock was made by mixing the freeze dried contents of a vial supplied with the kit with 1.0 ml sterile distilled water and was mixed gently until completely dissolved. The stock was stored at -20^{0}C
Conjugate stock	The freeze dried contents of a vial supplied with the kit was mixed with 1.0 ml of sterile water until completely dissolved. After preparing the stock was preserved at -20^{0}C
Positive Control Serum	Supplied positive control with the kit was dissolved with 1.5 ml distilled water. Finally the stock was stored at -20^{0}C
Negative Control Serum	Supplied negative control with the kit was dissolved with 1.0 ml distilled water. Finally the stock was stored at -20^0 C
Chromogen stock solution	One O-phenylenediamine dihydrochloride (OPD) tablet of 3.7mM supplied with the kit was dissolved in 75 ml distilled water in a falcon tube. Then the stock was kept at -20^0 C
Substrate stock solution	Supplied One Hydrogen peroxide tablet in the empty Azlon bottle was dissolved in 10 ml deionized water (5% solution, 882Mm) in a falcon tube. It was stored at 4^{0}C until used the test start
Antigen working solution (Working dilution: 1:100)	Antigen Stock solution 40 µl PBS stock 4000 µl Above materials mixed just immediate before the test start.
Monoclonal antibody (MAb) working solution (Working dilution: 1:100)	Monoclonal antibody stock solution 40 µl Blocking buffer 4000µl Above materials mixed just immediate before the test start

Stock	Preparation
Conjugate working solution (Working dilution: 1:100)	Conjugate stock solution 40 µl Blocking buffer 4000 µl Above materials mixed just immediate before the test start
Chromogen-substrate working solution (Working dilution: 1:250)	Substrate stock 16 µl Chromogen stock (OPD solution) 4000 µl Above materials mixed just immediate before the test start
Washing buffer	Distilled water 800 ml PBS stock 20 ml Above materials mixed just immediate before the test start

Brief Biography

Brishti Barua passed Secondary School Certificate Examination (SSC) with GPA 5.00 in 2005 and Higher Secondary Certificate Examination (HSC) with GPA 4.80 in 2007. DR. Brishti achieved her Doctor of Veterinary Medicine Degree with GPA 3.62 in 2012 (held in 2014) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, she is a candidate for the degree of MS in Epidemiology under the Department of Medicine and Surgery, Faculty of Veterinary Medicine, CVASU. She has boundless interest in exploration on the epidemiology of Peste des Petits Ruminants in goats.