PATHO-BIOLOGICAL STUDIES OF PPR VIRUS IN EXPERIMENTALLY INFECTED GOATS WITH REFERENCE TO IMMUNOMODULATORY ACTIVITY OF *NIGELLA. SATIVA* SEEDS

K. Aqil[1], Muti-ur-Rehman Khan[2], A. Aslam[2], A. Javeed[2], R. Qayyum[1], S. Masood[2,] A. W. Manzoor[1], F.[1], F. Yasmeen[1], M. L. Sohail[3] and S. Umar[4]

[1]Veterinary Research Institute (VRI) Lahore, Pakistan; [2]University of Veterinary and Animal Sciences Lahore, Pakistan
[3]University College of Veterinary and Animal Sciences, The Islamia University of Bhawalpur Pakistan
[4]Faculty of Veterinary & Animal Sciences, PirMehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan
Corresponding Author E-mail: drniazi@uvas.edu.pk

ABSTRACT

Peste des petits ruminants (PPR) is a transboundary viral disease of small ruminants that causes huge economic losses in developing world. The present project was aimed to report the gross and microscopic alterations and to evaluate the immunomodulatory and therapeutic response of *N. Sativa* in goats experimentally infected with peste des petits ruminants virus (PPR). For this purpose, twenty goats were divided into four groups (A, B, C & D). Goats of group A were fed *N. Sativa* seeds seven days prior to the inoculation of infection, while group C was given *N. Sative* seeds on the very day of infection inoculation. Group B and D were kept as positive and negative control, respectively. Clinical signs score, histopathological changes, haematology, liver function parameters, renal function parameters and serum biochemical parameters were recorded after every seven days, until 21st day post infection. Results revealed statistically significant changes between treatment and control groups. Haematological parameters (TLC, TEC, Hb, PCV) were significantly higher ($P<0.05$) in *N. Sativa* treated group. Liver enzymes (ALP and AST) decreased significantly ($P<0.05$) in group A throughout the course of the study. Creatinine was also significantly lower ($P<0.05$) in *N. Sativa* fed goats as compared to control group on day 21. Serum protein, albumin, calcium, potassium and magnesium was significantly higher ($P<0.05$) in group fed with *N. Sativa* seven days prior to infection. Likewise, clinical signs, histopathological scoring was significantly lower ($P<0.05$) in treated group A. All these changes highlight the immunomodulatory action of dietary supplementation of *N. Sativa* and its role in suppressing the pathogenicity of PPR virus in goats.

Key words: goats, histopathology, immunomodulation, *Nigella Sativa*, PPR.

INTRODUCTION

Peste des petits ruminants (PPR), is an acute, highly contagious and economically important transboundary viral disease of sheep and goats associated with high morbidity and mortality (Balamurugan *et al*., 2014). It is caused by PPR virus, a morbillivirus of the *Paramyxoviridae* family. Disease severity depends on species infected, breed or virus strain (Wernike *et al*., 2014). PPR is prevalent in developing countries of Africa and Asia, where small ruminants play a vital role in sustainable agriculture employment (Abubakar and Munir, 2014)

In acute cases, pyrexia (41°C), depression, anorexia, dryness of the muzzle along with nasal and lachrymal discharge and excessive salivation are evident (Parida *et al*., 2015). In later stages, diarrhea, labored coughing, abdominal breathing leading to dyspnea, weight loss and emaciation may cause death. The morbidity rate can reach up to 100% with a high case fatality rate in the acute infection (Pope *et al*., 2013). The main histopathological findings of PPR are seen in the digestive, respiratory and lymphoid systems. The digestive system lesions involve erosive, ulcerative stomatitis and fibrinohemorrhagic enteritis and hemorrhages in the mucosa of abomasum (Al-Dubaib, 2009). The respiratory lesions involve bronchitis, bronchiolitis, interstitial pneumonia, syncytial cells and presence of intracytoplasmic and intranuclear inclusion bodies in bronchiolar and ~~alveoler~~alveolar epithelium (Aytiken *et al*., 2011). Initially virus replicates in the tonsillar tissue and lymph nodes at the inoculation site. It is hypothesized that virus infected immune cells in the respiratory mucosa migrate to the local lymphoid tissue, where it replicates and enters the general circulation (Herbert *et al*., 2014). PPR virus is diagnosed by various laboratory diagnostic tests which are aimed at detection of antigen (virus isolation, antigen capture ELISA) or antigenic genetic material (RT-PCR, real time PCR) and antibodies against the virus (virus neutralization, competitive ELISA). However, the efficiency of laboratory diagnosis can be greatly influenced by the integrity of the sample received, often affected by the conditions of its by collection and transportation (Parida *et al*., 2015).

Antimicrobial agents of plant origin such as essential oils and plants extracts have gathered significant importance as alternatives to the antibiotics to avoid secondary infection, which may help in reducing the mortality. *Nigella Sativa* (*N. Sativa*) is one of such alternatives that could be used as feed additives and its antimicrobial activity has proved to be a substitute for conventional antimicrobial drugs (Azeem *et al*., 2014). The wide ranges of benefits of *N. Sativa* include its use as immunomodulator, renal protective, analgesic, hepato-protective, antioxidant and gastroprotective (Ahmad *et al*., 2013).

Pakistan is home to 70.3 million goats (Pakistan Economic Survey 2015-2016) with 37 well recognized breeds (Abubakar and Munir, 2014). Every year, PPR be the reason of loss of thousands of small ruminants causing substantial deterioration in small ruminants production and economics of farmer (Munir *et al*., 2013). For better understanding of disease, a comprehensive analysis of clinical, biochemical and haematological parameters is mandatory (Aytiken et al., 2011). Keeping this in mind, the present study was designed to detect the hematological, serum biochemical, and histopathological findings in goats, experimentally infected with PPR and to evaluate the immunomodulatory effects of *N. Sativa* as an economical therapeutic agent to enhance cellular and humoral immunity against PPR.

MATERIALS AND METHODS

Twenty healthy animals were procured from market and kept in isolated sheds at the Veterinary Research Institute (VRI), Lahore, Pakistan. PPR virus was acquired from National Agriculture Research Council, Islamabad, Pakistan. PPR virus was passaged on Vero-cell lines at VRI and inoculums was optimized in tissue culture with lethal dose ($TCLD_{50}$) 10^3/ml were prepared.

The goat feed was arranged comprises of metabolizable energy 3000 Kcal/kg and total protein 22%, having no antibiotic and toxin binder. ~~The~~ ~~every~~Every batch of the basal feed was analyzed for zearalenone, aflatoxin and ochratoxin before feeding. *N. Sativa* seeds (NSS) were obtained from a local market of district Lahore, Pakistan. The taxonomic identification of the plant seeds ~~were~~was done at University of Agriculture, Faisalabad, Pakistan. The plant seeds were grounded into a coarse powder and used in the diets @ 5 gram per kg feed.

Twenty goats were divided into (A, B, C, & D) four groups, each having 5 goats. All experimental animals were de-wormed with oxfandazole and ivermectin on day 1st of their arrival at experimental site. All the experimental animals were kept in insect proof separate pens and were given 14 days for acclimatization. During these 14 days, all the animals were regularly monitored for the vital parameters (temperature, pulse and respiration) and presence of any clinical sign or disease. All the animals were non-vaccinated for PPR virus. Feed and fresh water were provided *ad-libitum*. Proper and regular cleaning of experimental pens were also assured by trained animal handlers.

Animals of group A were given feed with *N. Sativa* seeds, seven days prior to inoculation of infection. Group C were fed *N. Sativa* seeds from the very day of inoculation of infection, where as group B (positive control) and D (negative control) were given routine feed without any addition of *N. Sativa* seeds. All the groups were inoculated with 5ml of PPR virus intra-tracheally (10^3 TCID 50/ml), except group D which was kept as control and was given distill water. Animals were monitored for clinical signs for 21 days post infection.

Competitive enzyme linked immunosorbent assay kit were used for analysis of serum sample, manufactured by World Rinderpest Reference Laboratories, Pirbright, UK (Abubakar and Munir, 2014). Samples were tested for anti-PPRV antibodies in terms of percentage inhibition (PI). The values of the optical density (OD) were changed to PI. Using Immunoskan reader manufactured by Flow Laboratories, UK, we read the ELISA plates at 492 nm wavelength. Result calculation was done automatically by software produced by FAO/IAEA, Vienna, Austria. The said program converted the optical density (OD) values to percentage inhibition (PI) using the following formula (Mahmoud and Galbat, 2017):

$$PI\% = 100 - (\text{mean OD of test wells} / \text{Mean OD of cma wells}) \times 100$$

Where, OD represents the optical density value and cma points to the MAb control. Inhibition values (more than 50% were considered positive.)

2 ml of blood was collected on days 7th, 14th and 21st of experimental trial after disinfecting the collection site with pre-pared alcohol swab, and collected in each of plain red top and anticoagulant coated purple top ~~vacutainers,~~.vacutainers. Blood collected in anticoagulant coated vacutainer was stored for hematological analysis and in plain vacutainers for serum biochemical analysis. Hematological parameters studied included; total erythrocyte count (TEC), total leukocyte count (TLC), hemaglobin (Hb) and packed cell volume (PCV) were analyzed by automated hematological analyzer (Abacus Junior Vet 5, Diatron, Hungary).

Biochemical parameters including total serum protein (TSP), albumin, cholesterol and glucose were determined by Technicon RA-XT auto-analyzer (biousa, CA, USA), while calcium, magnesium and potassium were analyzed by automated blood chemistry analyzer Hitachi 705 (Hitachi, Japan), using Thermo Fisher Scientific Inc. (USA) reagents.

Liver function test including alkaline phosphatase (ALP), bilirubin, aspartate transaminase

(AST) and alanine aminotransferase (ALT) and kidney function test including creatinine and blood urea nitrogen (BUN) were analyzed using Pictus 700 chemistry analyzer (Diatron, Hungary).

Animals from each group were slaughtered on 7th, 14th and 21st days post-infection to study the gross and histopathological lesions. Liver, spleen, intestine, lung and lymph nodes were stored in liquid nitrogen. The tissues were fixed in 10% formalin, embedded in paraffin, and sections of 4 mm were prepared and stained by eosin and haematoxylin (Bancroft and Gamble, 2007).

Kidney, lungs, liver, spleen and mesenteric lymph nodes of animals from group B were processed through RT-PCR for the confirmation of PPRV on 7th, 14th and 21st days post inoculation. RNA was extracted using RNA extraction method (Luka *et al.,* 2012). The PPRV-specific set of primers was used for the reaction (PPRVF1b: 5′-AGT ACA AAA GAT TGC TGA TCA CAG T-3′ and PPRVF2d: 5′-GGG TCT CGA AGG CTA GGC CCG AAT A-3′) to amplify a 448-bp cDNA. Live lypholized PPR vaccine, made by the (VRI) Veterinary Research Institute, Lahore, Pakistan was used as positive control. From the mixture of tissue and vaccine, RNA was procured from the group B animals using RNA easy Mini Kit (Qiagen, Germany), according to manufacturer's protocol. The RT-PCR was accomplished with Qiagen One- Step RT-PCR kit (Qiagen, Germany). Thermocycling protocol followed was: reverse transcription at 50ºC for 30 min, initial de-naturation and activation of polymerase at 94ºC for 15 min, followed by 35 cycles of de-naturation, annealing and extension at 94ºC for 1 min, 50ºC for 1 min and 72ºC for 2 min, respectively, and final elongation at 72ºC for 7 min (Rahman *et al.,* 2011~~).The~~). The RT-PCR products were analyzed by electrophoresis on 2% agarose in TBE buffer gel stained with ethidium bromide. Products were visualized on an UV trans illuminator. Vaccinal strain of PPRV was used as positive control.

Statistical analysis: Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software Inc. La Jolla, CA, USA). Data were expressed as the mean ± standard deviation of the mean (SDM). Data regarding hematology, serum biochemistry, clinical sign scoring, gross lesions and ~~histopatholgy~~histopathology were analyzed using analysis of variance (ANOVA). The statistical differences were considered significant at $P < 0.05$ unless otherwise stated.

RESULTS

Animals of Group A treated with *N. Sativa* showed mild clinical signs during the course of experiment while the animals of Group B showed severe clinical signs after inoculation and mortality (20%) was also noted. Group "C" had moderate clinical signs and symptoms (Table 1). It was apparent that animals which were fed NSS showed no sign of disease. Table 2 shows mean antibody titer in experimental groups. Results showed significant difference (P<0.05) in mean antibody titer between group A and C, with higher in group A (49.75) as compared to group C (31.80).

Hematological alterations in experimental animals during the course of the disease are shown in table 3. Results revealed a significant decrease (P<0.05) in leukocyte count in group B, while there was no significant difference (P>0.05) notable in group A and C. Likewise, no significant changes (P>0.05) were observed in TEC, Hb and PCV. Serum biochemical parameters i.e. Bilirubin, ALP, AST, ALT, BUN and creatinine showed significant elevation (P<0.05) in group B, whereas no such significant changes (P>0.05) were seen in group A and C (Table 4, 5).

Table 6 shows the data regarding serum biochemical profile in both treated and untreated groups. Non-significant (P>0.05) higher total serum protein was observed in groups A (5.11) when compared to group C (5.09). Whereas, significant difference (P<0.05) was recorded when compared to groups B (4.88) and D (4.63). Mean serum albumin was significantly different (P<0.05) in group A (2.38) and group C (3.39). Highest calcium level was detected in group D (9.08) with significant difference (P<0.05) as compared to group B (6.56) and C (6.40). ~~Similarly~~Similarly, serum potassium was significantly higher in group D (6.48) as compared to group A (4.08). Significant difference was observed in serum cholesterol level among treatment and control groups, with highest in group C (54.75), followed by group B (43.08), group A (39.92), and least in group D (23.67). Table 7 shows significant difference (P<0.05) in clinical signs scoring among experimental group with highest scoring in group B (7.43) followed by group C (5.00), A (2.00) and no clinical sign was observed in negative control group D (0.00). Gross pathological lesions scoring revealed that group B animals were severely affected by PPRV at any stage of infection as compared to other experimental groups. Groups C showed mild to moderate lesions, while group A was safer from the infection (table 8). Results showed significant higher (P<0.05) mean for post mortem lesion scoring in group B (14.75) followed by group C (7.57), group A (4.28) and no lesions were seen in control group D (0.00) (Table 8).

The pathognomonic lesions observed in lungs were broncho-interstitial pneumonia, intracytoplasmic inclusion bodies and syncitial cell formation. ~~Infilteration~~Infiltration of pneumocytes was also observed in lungs in infected animals along with heart failure cells (Fig., 1 & Plate 1). The severity of lesions was much more pronounced in infected non treated group (B) than infected treated groups (A & C). ~~Hyperaemic~~Hyperemic ~~oedematous~~edematous mucosa

was observed in intestines with ulcerated focal areas (Fig., 2). Sinusoids narrowing resulted due to hepatocytomegally, while eosinophilic intranuclear inclusion bodies were observed in hepatocytes and pyknotic nuclei with degenerating hepatic cells (Plate 2). Acute spleenitis with mononuclear cells infiltration, ~~trabaeculae~~trabeculae and capsule were obvious (Fig., 3). Prominent microscopic lesions in lymphoid organs included intra cytoplasmic inclusion bodies, syncytial cell formation, depletion and necrosis of lymphoid tissues with numerous lymphocyte infiltration with pyknotic nuclei, seen from day 14 to 21 of infection in untreated group B (Plate 3).

Highest mean score for histopathological changes was observed in group A (7.50) on day 14 post infection. While, in untreated group B, highest score was recorded on day 7 of experiment (7.67). In treated group C, highest mean accumulative score was recorded on day 21 post infection (7.33). Statistical analysis showed significant difference of scoring on day 7 of experiment while on day 14 only group C showed significant difference ($P<0.05$) while on day 21 ($P>0.05$) no significant change of scoring was recorded (Table 10). A RT-PCR product of 372BP was amplified from liver, kidney, lungs, spleen and mesenteric lymph nodes collected from the goats of group B (Plate 4) confirming PPRV presence in these organs (Figure 4).

Table 1. Clinical signs and post-mortem changes in control and treatment groups of goats experimentally infected with PPR virus.

Clinical parameter	Group A			Group B			Group C			Group D		
	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day
Rectal temp. (°F)	105	102	102	106	104	105	104	102	103	102	103	102
Pulse (bpm)	117	107	111	114	120	108	112	111	112	109	102	109
Respiration (bpm)	41	38	40	42	39	39	39	35	37	35	40	37
Nasal discharge	+	+	-	+++	+++	++	+++	++	++	-	-	-
Ocular discharge	+	+	-	+++	++	+++	++	++	++	-	-	-
Coughing	+	+	-	++	+++	+	+	+	+	-	-	-
Respiratory distress	+	+	-	+++	++	++	+++	++	+	-	-	-
Stomatitis	+	++	+	++	++	+++	++	+	++	-	-	-
Ulcerative erosions in Bucal mucosa	+	+	+	++	+	++	++	++	++	-	-	-
Diarrhea	-	++	-	+++	+++	+++	+++	++	+	-	-	-
Mortality	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

- No lesion, + mild, ++ moderate, +++ severe

Table 2. Mean anti-body titer of goats in experimental groups during the course of the study

Group	Days			Mean
	7th	14th	21st	
A	50.32 ± 0.20^{a}	50.43 ± 0.21^{a}	47.90 ± 1.64^{ab}	49.75 ± 0.49^{C}
B	24.38 ± 2.12^{de}	22.81 ± 3.35^{de}	17.33 ± 0.88^{de}	22.10 ± 1.57^{C}
C	21.99 ± 2.63^{de}	27.40 ± 8.15^{cd}	54.00 ± 0.58^{a}	31.80 ± 4.75^{B}
D	16.85 ± 2.26^{e}	20.09 ± 4.75^{de}	37.33 ± 1.20^{bc}	23.05 ± 3.04^{C}

Means sharing similar letter in a row or in a column are statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 3. Hematological changes in treatment and control groups of experimental goats infected by PPR virus.

Parameters	Group	Days			Mean
		7th	14th	21st	
TLC µg	**A**	8.48 ± 0.12^{de}	7.21 ± 0.04^{g}	8.92 ± 0.05^{cd}	8.17 ± 0.22^{B}
	B	4.71 ± 0.11^{i}	5.35 ± 0.12^{h}	4.18 ± 0.09^{i}	4.79 ± 0.15^{D}
	C	7.70 ± 0.11^{fg}	7.98 ± 0.53^{ef}	7.86 ± 0.02^{f}	7.83 ± 0.17^{C}
	D	10.71 ± 0.22^{a}	9.65 ± 0.09^{b}	9.40 ± 0.15^{bc}	10.03 ± 0.20^{A}
Hb	**A**	9.46 ± 0.16^{ab}	9.07 ± 0.01^{b}	9.70 ± 0.06^{a}	9.39 ± 0.10^{AB}

	B	9.14±0.08^{b}	8.48±0.21^{c}	6.07±0.09^{d}	8.15±0.38^{C}
	C	9.23±0.06^{ab}	9.15±0.33^{b}	9.03±0.50^{b}	9.16±0.15^{B}
	D	9.50±0.12^{ab}	9.70±0.07^{a}	9.73±0.12^{a}	9.63±0.07^{A}
TEC µg	**A**	13.19±0.25^{a}	9.50±0.26^{cd}	9.03±0.01^{cd}	10.92±0.59^{A}
	B	12.60±0.23^{ab}	9.25±0.09^{cd}	8.76±0.13^{de}	10.52±0.54^{AB}
	C	11.79±0.64^{b}	10.03±0.43^{c}	7.57±0.29^{e}	10.15±0.58^{B}
	D	11.76±0.42^{b}	8.53±0.15^{de}	12.53±0.32^{ab}	10.88±0.54^{A}
PCV	**A**	27.60±0.75	26.75±0.85	27.34±0.87	27.25±0.45^{A}
	B	27.79±0.58	26.73±0.83	29.63±0.68	27.90±0.50^{A}
	C	25.56±1.00	32.20±4.85	32.63±2.36	29.54±1.89^{A}
	D	29.96±1.62	34.57±1.79	28.30±0.35	31.08±1.13^{A}

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 4. Liver function parameters in goats of experimental groups infected with PPR virus.

Parameters	Group	Days			Mean
		7th	14th	21st	
Alkaline Phosphatase (µ/l)	**A**	242.20±2.71^{d}	257.00±0.91^{bc}	240.33±0.88^{d}	246.67±2.48^{B}
	B	263.60±2.25^{ab}	255.75±0.85^{c}	270.67±4.26^{a}	262.75±2.14^{A}
	C	217.60±3.93^{e}	212.25±0.85^{e}	216.00±0.58^{e}	215.42±1.71^{C}
	D	202.00±0.32^{fg}	204.75±3.66^{f}	197.00±1.53^{g}	201.67±1.46^{D}
SGPT (ALT) µ/l	**A**	44.60±1.21^{a}	42.50±0.65^{ab}	45.00±2.65^{a}	44.00±0.83^{A}
	B	40.00±1.30^{abc}	39.75±0.85^{abc}	34.33±2.91^{cde}	38.50±1.11^{B}
	C	30.20±1.71^{e}	28.50±1.55^{ef}	36.67±1.20^{bcd}	31.25±1.29^{C}
	D	32.40±2.20^{de}	29.75±3.54^{ef}	24.00±2.08^{f}	29.42±1.76^{C}
SGOT (AST) µ/l	**A**	27.80±0.86^{c}	25.25±0.63^{cd}	21.33±0.67^{de}	25.33±0.87^{C}
	B	34.00±1.67^{b}	38.50±0.65^{a}	38.67±1.45^{a}	36.67±1.01^{A}
	C	38.40±0.93^{a}	19.50±0.65^{ef}	25.67±2.40^{cd}	28.92±2.60^{B}
	D	25.00±1.52^{cd}	17.00±2.04^{f}	32.67±1.33^{b}	24.25±2.01^{C}
Bilirubin	**A**	0.71±0.01^{bc}	1.28±0.05^{a}	0.33±0.09^{d}	0.81±0.11^{A}
	B	0.74±0.08^{bc}	0.30±0.02^{d}	0.80±0.06^{b}	0.61±0.07^{B}
	C	0.27±0.01^{d}	0.65±0.01^{c}	0.65±0.03^{c}	0.49±0.06^{C}
	D	0.16±0.01^{e}	0.15±0.01^{e}	0.29±0.01^{d}	0.19±0.02^{D}

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5. Kidney function parameters in goats of experimental groups infected with PPR virus.

Parameters	Group	Days			Mean
		7th	14th	21st	
Blood Urea Nitrogen (BUN) mg/dl	**A**	38.00±1.00^{ab}	37.75±1.38^{abc}	38.67±1.45^{ab}	38.08±0.66^{A}
	B	28.00±1.00^{ef}	26.75±0.85^{f}	29.00±2.31^{ef}	27.83±0.73^{C}
	C	31.00±0.71^{de}	33.50±1.19^{d}	41.67±1.76^{a}	34.50±1.42^{B}
	D	33.80±1.07^{cd}	33.75±2.95^{cd}	34.67±1.45^{bcd}	34.00±1.04^{B}
Creatinine (mg/dl)	**A**	0.700±0.07	0.775±0.09	0.533±0.09	0.683±0.05^{A}
	B	0.560±0.09	0.725±0.10	0.967±0.12	0.717±0.07^{A}
	C	0.580±0.10	0.625±0.17	0.700±0.11	0.625±0.07^{A}
	D	0.840±0.04	0.725±0.08	0.867±0.03	0.808±0.04^{A}

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 6. Means score of serum biochemical values in goats of experimental groups infected with PPR virus.

Parameters	Group	Days			Mean
		7th	14th	21st	
Serum Protein (g/dl)	A	5.20±0.03^{b}	4.63±0.05^{cde}	5.60±0.06^{a}	5.11±0.12^{A}
	B	4.72±0.06^{cd}	4.75±0.06^{cd}	5.30±0.15^{b}	4.88±0.09^{B}
	C	5.54±0.10^{a}	4.73±0.06^{cd}	4.83±0.07^{c}	5.09±0.12^{A}
	D	4.60±0.03^{de}	4.83±0.08^{c}	4.40±0.11^{e}	4.63±0.06^{C}
Albumin (g/dl)	A	2.60±0.08^{de}	2.13±0.08^{f}	2.37±0.03^{ef}	2.38±0.07^{B}
	B	2.10±0.04^{f}	2.33±0.06^{ef}	2.23±0.09^{f}	2.21±0.04^{B}
	C	3.60±0.11^{ab}	3.13±0.16^{c}	3.40±0.06^{bc}	3.39±0.09^{A}
	D	3.46±0.02^{ab}	3.75±0.12^{a}	2.77±0.33^{d}	3.38±0.14^{A}
Calcium (g/dl)	A	7.22±0.07^{c}	7.28±0.08^{c}	8.00±0.58^{b}	7.43±0.16^{B}
	B	6.32±0.05^{d}	6.45±0.13^{d}	7.10±0.06^{c}	6.56±0.11^{C}
	C	6.40±0.13^{d}	6.48±0.12^{d}	6.30±0.10^{d}	6.40±0.07^{C}
	D	9.64±0.07^{a}	9.18±0.12^{a}	8.00±0.58^{b}	9.08±0.23^{A}
Potassium (meq/l)	A	4.54±0.07^{e}	3.83±0.07^{f}	3.67±0.33^{f}	4.08±0.14^{B}
	B	2.58±0.04^{g}	2.55±0.06^{g}	2.20±0.06^{d}	3.23±0.34^{C}
	C	3.74±0.08^{f}	3.98±0.08^{f}	4.67±0.09^{e}	4.05±0.12^{B}
	D	5.64±0.08^{c}	7.50±0.14^{a}	6.53±0.09^{b}	6.48±0.25^{A}
Cholesterol (mg/dl)	A	37.60±0.60^{d}	45.00±1.87^{c}	37.00±0.58^{d}	39.92±1.25^{C}
	B	55.60±0.87^{b}	36.25±1.11^{d}	31.33±0.33^{e}	43.08±3.27^{B}
	C	46.20±0.58^{c}	73.00±3.46^{a}	44.67±0.33^{c}	54.75±4.04^{A}
	D	25.40±0.51^{f}	23.50±1.19^{fg}	21.00±0.58^{g}	23.67±0.68^{D}
Glucose (mg/dl)	A	21.16±0.09^{d}	14.30±0.11^{g}	18.67±0.33^{e}	18.25±0.90^{C}
	B	15.62±0.09^{f}	18.10±0.23^{e}	20.60±0.35^{d}	17.69±0.61^{C}
	C	26.74±0.45^{b}	15.63±0.56^{f}	24.83±0.12^{c}	22.56±1.52^{B}
	D	20.94±0.25^{g}	26.85±0.47^{b}	29.33±1.20^{a}	25.01±1.12^{A}
Magnesium (mg/dl)	A	1.48±0.04^{e}	1.68±0.09^{de}	2.17±0.03^{bc}	1.72±0.09^{B}
	B	1.68±0.07^{de}	1.60±0.04^{de}	1.43±0.03^{e}	1.59±0.04^{B}
	C	2.52±0.06^{ab}	2.75±0.48^{a}	2.07±0.03^{cd}	2.48±0.17^{A}
	D	2.60±0.04^{ab}	2.80±0.12^{a}	2.60±0.06^{ab}	2.67±0.05^{A}

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.
Significant difference was calculated by DMRT.

Table 7. Accumulative scoring of clinical signs in goats of experimental groups infected with PPR virus.

Parameters	Group	Accumulating scoring of Clinical Signs			Mean
		7thDPI	14th DPI	21stDPI	
Nasal discharge	A	1	1	0	9
	B	3	3	3	7
	C	3	3	1	0
	D	0	0	0	9
Ocular discharge	A	1	1	0	2
	B	4	3	1	8
	C	2	3	1	6
	D	0	0	0	0
Coughing	A	1	1	0	2
	B	2	3	2	7
	C	1	2	2	5
	D	0	0	0	0
Respiratory distress	A	1	1	0	2
	B	3	3	3	9
	C	2	1	1	4

	D	0	1	0	1
Stomatitis	**A**	1	1	1	3
	B	3	1	2	6
	C	1	1	2	4
	D	0	0	0	0
Ulcerative erosions in buccal mucosa	**A**	0	1	1	2
	B	1	1	2	4
	C	1	2	2	5
	D	0	0	0	0
Diarrhoea	**A**	0	1	0	1
	B	3	2	5	10
	C	1	1	2	4
	D	0	0	0	0

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 8. Accumulating Scoring of Post Mortem values in goats of experimental groups infected with PPR virus.

Parameters	**Group**	**Accumulating scoring of Clinical Signs**			**Mean**
		7thDPI	**14th DPI**	**21stDPI**	
Haemorrhagic trachea	**A**	0	1	0	1
	B	2	3	9	14
	C	1	2	5	8
	D	0	0	0	0
Lung consolidation	**A**	0	0	0	0
	B	2	3	8	13
	C	1	2	5	8
	D	0	0	0	0
Liver congestion	**A**	1	0	0	1
	B	2	3	16	21
	C	2	2	10	14
	D	0	0	0	0
Enteritis	**A**	0	0	3	3
	B	3	3	8	14
	C	2	2	3	7
	D	0	0	0	0
Splenomegaly	**A**	0	1	0	1
	B	2	3	8	13
	C	2	3	5	10
	D	0	0	0	0
MLN enlargement	**A**	1	0	0	1
	B	3	3	9	15
	C	3	2	6	11
	D	0	0	0	0
BLN enlargement	**A**	1	0	0	1
	B	3	3	9	15
	C	3	1	4	8
	D	0	0	0	0

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 9. Accumulative ~~histropathological~~histopathological lesions score in in goats of experimental groups infected with PPR virus.

Organs	Liver			Lungs			Spleen				Kidneys			MLN		Intestine					Accumulative Score
	S	D	I	B	P	I	S	N	D	M	T	N	G_H	I	S	D_N	B_S	D_P	H	Z	
Days Post Infections(DPI)																					
07	2	2	1	2	3	1	2	3	2	0	0	1	2	3	2	1	1	0	1	2	31
14	3	3	2	3	3	2	1	3	0	2	3	3	2	2	3	3	2	1	2	2	45
21	3	3	1	3	1	2	3	0	2	3	1	3	2	2	2	3	3	3	2	1	43
Days Post Infections(DPI)																					
07	3	2	2	3	3	2	3	3	3	2	3	3	2	3	3	1	1	0	3	2	47
14	3	3	3	3	3	1	2	3	3	1	3	1	2	2	2	3	2	2	3	1	46
21	3	3	2	2	1	3	2	3	3	2	1	2	2	2	3	2	3	3	1	2	45
Days Post Infections(DPI)																					
07	2	1	2	3	3	2	0	0	2	3	1	0	3	3	3	3	2	0	1	2	36
14	3	1	2	3	3	2	0	0	1	2	3	1	3	2	3	1	2	1	1	2	36
21	3	3	1	3	1	3	1	1	3	3	2	2	3	3	1	3	3	2	1	2	44

Where:

S → ~~Syncitial~~Syncytial Cell Formation, D → Disruption of Hepatic Cord, I→ Inclusion Bodies, B→ Broncho Interstitial Pneumonia, P → Proliferative changes in Alveoli D → Degenerative Changes, M → Macrophage ~~Infilteration~~Infiltration, T → Tubular Degeneration , Gh → ~~Glomarular~~Glomerular ~~haemorrhages~~hemorrhages, D_n → Depletion and Necrosis of Lymphoid, B_s→ Blunting and Stunting of Villie, D_P → Depletion of Lymphocytes in Payers' Patches, H → Hyperplasia of epithelial mucosa , Z→ Zebra Marking

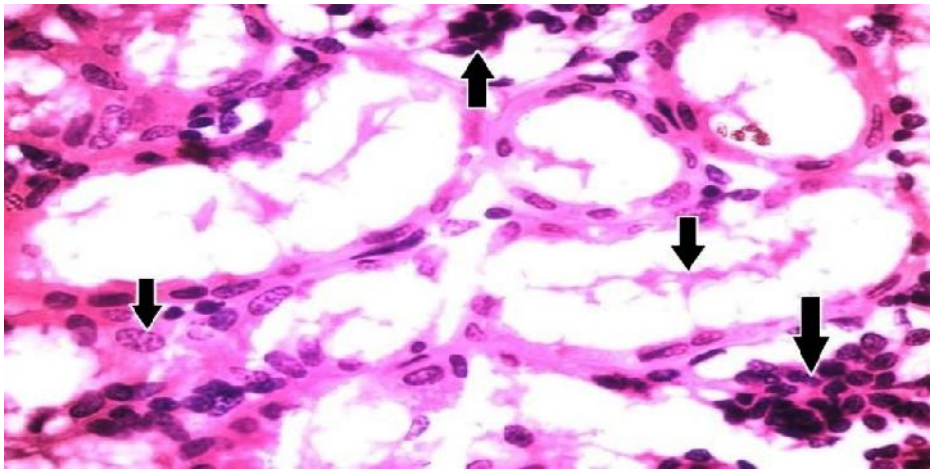
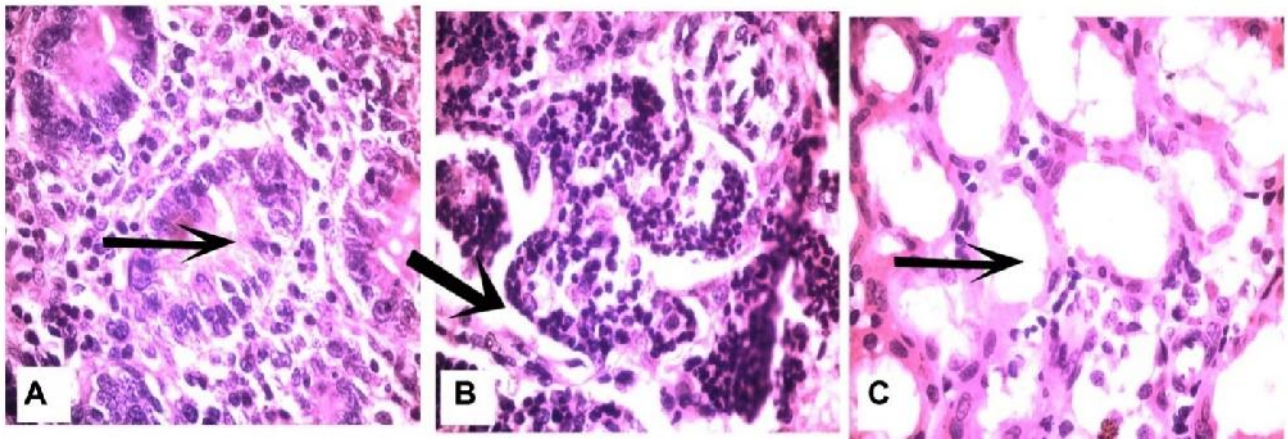


Figure 1. Lung section showing multinucleated syncytial and intracytoplasmic inclusion bodies in alveolar epithelium.



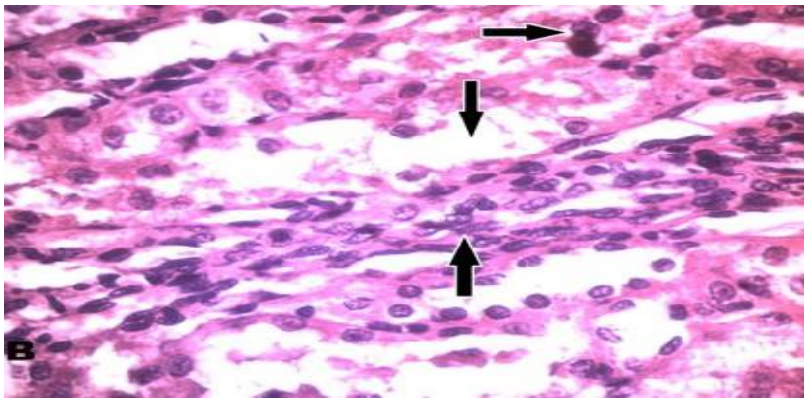


Plate 1. Lungs of goats showing broncho interstitial pneumonia (A), histiocyte proliferation (B), oedema (C).
Fig 3. Section of Spleen showing depletion of lymphoid ~~cells(~~cells (↓) and infiltration of neutrophils(↑& →).

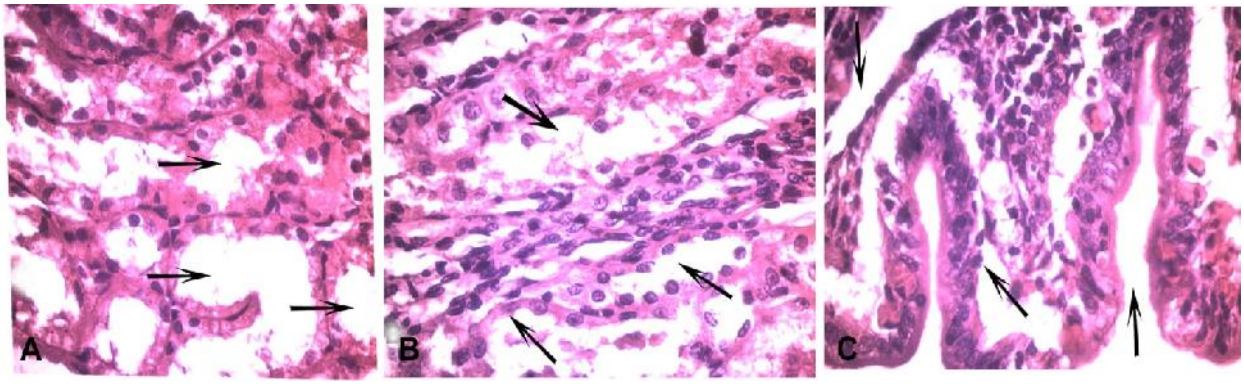


Plate ~~3:MLN~~3: MLN showing lymphoid cells depletion (A), infiltration of neutrophils (B), multinucleated synctial cell formation (C) in goats inoculated with PPR virus.

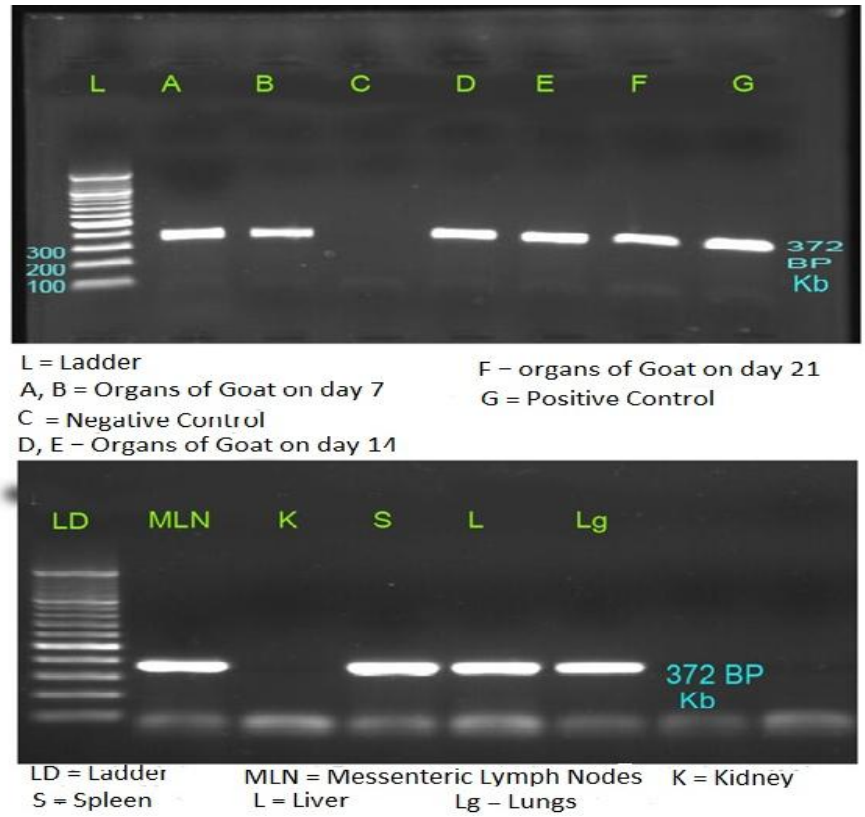


Figure 4. RT-PCR product of 372 BP from the organs of group B goats on 7, 14 & 21 days.

DISCUSSION

In this study fever, nasal and ocular discharge, stomatitis, ulceration in buccal cavity and diarrhea were reported as the main clinical signs during the course of the disease. Presence of diarrhea was also reported by Nwoha *et al*., (2013), which depicts the localization of viral agent in the digestive tract of infected animals, and is the main cause of mortality because of dehydration. Similar findings were reported by Parida *et al*., (2015) who reports all the mentioned clinical signs as the key signs of the disease, with possible variation which may be attributed to differences in virulence of viral strain and the immunological status of the study subject. Troung *et al*., (2014) also reported similar clinical signs specifically rise in body temperature and nasal and ocular discharge from 4th day of inoculation of infection, while ocular ulcerations were reported at 6th day post infection. Pope *et al*., (2013) reported a peak in appearance of clinical signs from 6th to 8th day post infection. Rahman *et al*., (2011) also reported similar findings during an outbreak of PPRV in goats of Bangladesh. During an experimental infection study, Osman *et al*., (2009) reported all the clinical signs in milder form during the course of the disease which is not in line with the findings of this study. This difference can be explained on the basis of individual variability among animals to cope up with the infection, which causes variation in severity of disease.

Present study reports hemorrhagic trachea, enteritis, spleenomegaly and lymph node enlargement as the characteristic postmortem findings from 7th day onward after inoculation of infection. Similar findings were reported by Chauhan *et al*., (2011) who reported characteristic pathology of PPR virus in respiratory and digestive tract further confirming these systems as the localization sites of PPRV. Likewise, histopathology studies revealed syncytial cell formation, broncho-interstitial pneumonia, proliferative changes in alveoli and disruption of hepatic cord. All these findings confirm the presence of virus in organs of respiratory and digestive systems as previously reported (Aytiken *et al*., 2011, Osman *et al*., 2009). Similar to this study, Troung *et al*., (2014) also reported syncytia in goats as the characteristic feature of morbilli virus infections.

Present study reports a detectable increase in antibody titer from day 7 of infection. Similar results were reported by Osman *et al*., (2009) who reported significant increase at 7th and following days after inoculation of infection because of humoral immune response caused by exposure of animals to the virus (Maina *et al*., 2015).

Hematology of infected goats in present study revealed marked decrease in leukocyte, which can be explained because of ~~immunosupression~~immunosuppression activity of morbillivirus, causing secondary bacterial infections

(Aytiken *et al*., 2011). Parida *et al*., (2015) also reported leucopenia because of profound immunosuppression caused by PPR virus. Similar findings were reported by Naznin *et al*., (2014).

As PPR is a viral disease, there exists no particular treatment for the disease and post-exposure therapeutic approaches for infection are not described much in the literature (Balamurugan et al., 2014). Numerous studies ~~reports~~report the use of *N. Sativa* as liver tonics, anti-diarrheal, analgesics, and anti-bacterial. Extensive studies on the herb have explored a wide spectrum of its pharmacological actions including immunomodulatory, antimicrobial and anti-inflammatory, properties, etc. Because of its miraculous power of healing, *N. sativa* has got the place among the top ranked evidence based herbal medicines (Ahmad *et al*., 2013). Current story describes the immunomodulatory and therapeutic effect of this herb against PPR virus in experimentally infected goats. *N. Sativa* prevented the occurrence of clinical signs and significant decrease in clinical signs, gross and histopathological lesions were observed in *N. Sativa* treated group A.

During this study, *N. Sativa* treated group experienced lower liver enzyme levels after treatment. Similar hepatoprotective activity of the herb was also reported by Krishnan and Muthukrishnan (2012), who reported decrease in liver enzymes level after treatment with *N. Sativa* seeds. *N. Sativa* is hypothesized to keep intact the integrity of hepatocellular membrane, which when disrupts release liver enzymes in cytoplasm. This study showed nephroprotective activity of *N. Sativa* during the course of the disease. Similar findings were reported by Ahmad *et al*., (2013) who reported decreased values of serum BUN and creatinine when fed in diet. All these pharmacological functions of *N Sativa* is because of thymoquinone, the active ingredient in the herb, owing ~~hepato~~hepatic and nephroprotective properties.

N. Sativa showed comprehensive therapeutic potential in current study, as reported by Salem and Hossain, (2000) who described immunomodulation due to increase in CD4 and CD8 cells which ultimately increase the IFN-g production. *N. Sativa* encouraged T cell stimulation and proliferation causing viral clearance and decrease in severity of lesions and clinical signs among *N. Sativa* fed goats, highlighting the anti-viral ad immunomodulatory activity of the herb.

Conclusion: This study reports the immune responses against PPR virus in the presence of *N. Sativa*. *N.Sativa* was found to show immunomodulatory effects and has the ability to induce cellular immune responses inhibiting the replication of virus. Enhanced cell-mediated, humoral immune responses help in early viral clearance, leading reduced pathogenicity of PPR virus in goats. This communication highlights the importance of N. Sativa feeding in goats for protection against PPR virus

Acknowledgements: We thank to the laboratory staff of Veterinary Research Institute (VRI) Lahore for their help in this research project.

REFERENCES

Abubakar, M. and M. Munir (2014). Peste des petits ruminants virus: an emerging threat to goat farming in Pakistan. Transboundary. Emerg. Dis. 61(s1): 7-10.

Ahmad, A., A. Husain, M. Mujeeb, S.A. Khan, A.K. Najmi, N.A. Siddique, Z.A. Damanhouriand, and F. Anwar (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. Asian. Pac. J. Trop. Biomed. 3: 337-352.

Ahmad, A., A. Husain, M. Mujeeb, S.A. Khan, A.K. Najmi, N.A. Siddique, Z.A. Damanhouri, and F. Anwar (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. Asian. Pacific. J. Trop. Biomed. 3(5): 337-352.

Al-Dubaib, M. A (2009). Peste des petitis ruminants morbillivirus infection in lambs and young goats at Qassim region, Saudi Arabia. Trop. Anim. Health. Prod. 41: 217-220.

Aytekin, I., N. Mamak, A. Ulucan and A. Kalinbacak (2011). Clinical, haematological, biochemical and pathological findings in lambs with Peste des Petits Ruminants. Kafkas. Uni. Vet. Fak. Derg. 17: 349-355.

Azeem, T., R. Zaib, S. Umar, M. Asif, M. Arif, and A. Rahman (2014). Effect of Nigella Sativa on poultry health and production: a review. Science Letters. 2: 76-82.

Balamurugan, V., D. Hemadri, D., M.R. Gajendragad, R.K. Singh, and H, Rahman (2014). Diagnosis and control of peste des petits ruminants: a comprehensive review. Virus. Dis. 25(1): 39-56.

Bancroft, J.D., M. Gamble (2007). Theory and Practice of Histological Techniques. 6th. Stockholm. 56-59.

Chauhan, H.C., P.S. Lambade, A. Sen, A.I. Dadawala, P.B. Ranaware, B. Chandel, D.V. Joshi, S.S. Patel, K. Pankaj, N.M. Shah, and H.N. Kher (2011). The use of pathological and histopathological techniques in the diagnosis of peste des petits ruminants in India. Vet. Italia. 47(1): 41-47.

Herbert, R., J. Baron, C. Batten, M. Baron, G. Taylor (2014). Recombinant adenovirus expressing the haemagglutinin of peste des petits ruminants virus (PPRV) protects goats against challenge with pathogenic virus; a DIVA vaccine for PPR. Vet. Res. 45: 24.

Krishnan, N. and S. Muthukrishnan (2012). Effect of Nigella sativa seed extract on carbon tetrachloride-induced hepatotoxicity in rats. J. Acute. Med. 2(4): 107-113.

Luka, P.D., C. Ayebazibwe, D. Shamaki, F.N. Mwiine and J. Erume (2012). Sample type is vital for diagnosing infection with peste des petits ruminants virus by reverse transcription PCR. J. Vet. Sci. 13: 323-325.

Maina, S.M., C.G. Gitao, and P.K. Gathumbi (2015). Hematological, serological and virological findings in sheep and goats experimentally infected with lineage III peste des petits ruminants virus isolates in Kenya. J. Exp. Biol. 3: 81-88.

Mahmoud M.A, and S.A. Galbat (2017) Outbreak of foot and mouth disease and peste des petits ruminants in sheep flock imported for immediate slaughter in Riyadh, Veterinary World, 10(2): 238-243.

Munir, M., A. Saeed, M. Abubakar, S. Kanwal, and M. Berg (2013). Molecular characterization of Peste des Petits Ruminants viruses from outbreaks caused by unrestricted movements of small ruminants in Pakistan. Transbound. Emerg. Dis. doi: 10.1111/tbed.12089.

Naznin, M., M. Ahaduzzaman, S. Chowdhury, and P.K. Biswas (2016). Prevalence and clinico-pathological parameters of PPR infected goats and their response to antibiotic treatment at Panchlaish, Chittagong, Bangladesh. Int. J. Natural. Sci. 4(2): 1-7.

Nwoha, R.I., J.O. Omamegbe, and N.O. Nwakundu (2013). Clinico-pathological findings in west African dwarf goats with Peste Des Petits Ruminants Infection. Philippine. J. Vet. Anim. Sci. 39(1): 125-132.

Osman, N.A., A.S. Ali, and M.A. Fadol (2009). Pathological, serological and virological findings in goats experimentally infected with Sudanese Peste des Petits Ruminants (PPR) virus isolates. J. Gen. Mol. Virol. 1(1): 1-6.

Parida, S., M. Muniraju, M. Mahapatra, D. Muthuchelvan, H. Buczkowski, and A.C. Banyard (2015). Peste des petits ruminants. Vet. Microbiol. 181(1): 90-106.

Pope, R.A., S. Parida, D. Bailey, J. Brownlie, T. Barrett, and A.C. Banyard (2013). Early events following experimental infection with peste-des-petits ruminants virus suggest immune cell targeting. PLoS. One. 8(2): e55830.

Rahman, M.A., I. Shadmin, M. Noor, R. Parvin, E.H. Chowdhury and M.R. Islam (2011). Peste des petits ruminants virus infection of goats in Bangladesh: Pathological investigation, molecular detection and isolation of the virus. The Bangladesh Veterinarian, 28: 1-7.

Rahman, M.A., I. Shadmin, M. Noor, R. Parvin, E.H. Chowdhury, and M.R. Islam (2011). Peste des petits ruminants virus infection of goats in Bangladesh: Pathological investigation, molecular detection and isolation of the virus. Bangla. Vet. 28(1): 1-7.

Salem, M.L. and M.S. Hossain (2000). In vivo acute depletion of CD8 (+) T cells before murine cytomegalovirus infection upregulated innate antiviral activity of natural killer cells. Int. J. Immunopharmacol. 22: 707-18.

Truong, T., H. Boshra, C. Embury-Hyatt, C. Nfon, V. Gerdts, S. Tikoo, L.A. Babiuk, P. Kara, T. Chetty, A. Mather, and D.B. Wallace (2014). Peste des petits ruminants virus tissue tropism and pathogenesis in sheep and goats following experimental infection. PLoS. One. 9(1): e87145.

Wernike, K., M. Eschbaumer, A. Breithaupt, J. Maltzan, H. Wiesner, M. Beer, and B. Hoffmann (2014). Experimental infection of sheep and goats with a recent isolate of peste des petits ruminants virus from Kurdistan. Vet. Microbiol. 172(1): 140-145.