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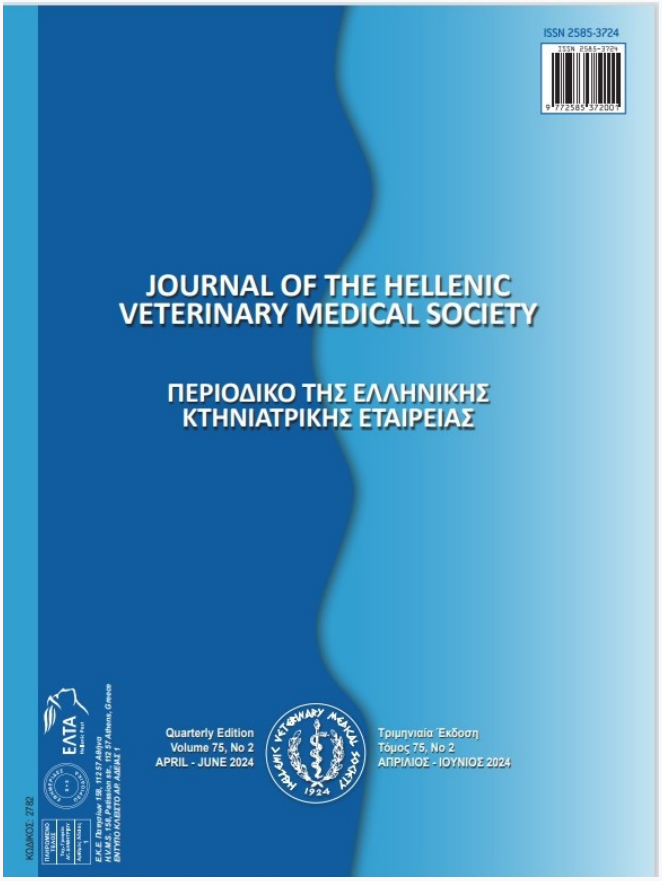

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Research article
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Postexposure *Pestes-des-petits-ruminants* vaccination with oxytetracycline therapy mitigates severe disease and increases survivability in natural infections

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ABSTRACT: The potentials of post-exposure intranasal (IN) and subcutaneous (SC) Peste des petits ruminants vaccination (PPRVc) with concurrent oxytetracycline (oxytet.) treatment in the management of PPR outbreaks in goats were investigated. Twenty-eight (28) West African dwarf goats, were randomly assigned into 7 groups: A (Oxytet.), B (IN - PPRVc), C (IN-PPRVc + Oxytet.), D (SC-PPRVc), E (SC-PPRVc + Oxytet.), F (Infected, untreated control), and G (Normal control). Natural PPRV infection was induced by the co-habitation method and following the onset of pyrexia in 75% of the goats, the interventions were administered accordingly. The leukocytic profiles were evaluated on days 1, 3, 5, 7 and 14 post-intervention (p.in). The clinical scores (CS), mortality pattern, lung lesion scores (LLS) and pathology were also evaluated post-intervention. A survivability score of 50% was observed in groups C and E with clinical scores of 2.06 and 3.90, while 100% mortality was recorded in the other groups with clinical scores of 4.38, 5.89, 7.05 and 6.92, for groups A, B, D and F, respectively. A mild decrease in the total leukocyte counts and absolute lymphocytes counts was observed in all the PPRV exposed groups by day 3 through 5 p.in but increased significantly ($p<0.05$) in groups C and E by day 14 p.in. Also, groups C and E showed the least mean (s.e.m) lung lesion scores of 0.61(0.12) and 0.64(0.15), respectively and also showed a relatively normal pulmonary, illeal, splenic and lymph node histomorphology, compared to the other groups. Postexposure PPR vaccination with concurrent oxytetracycline therapy significantly altered the known clinicopathology of PPR in goats, mitigated the disease severity and resulted in higher survivability in natural PPR infection.

Keywords: *Peste-des-petit ruminant*; Postexposure vaccination; Clinical score; Intranasal vaccination; Lung Lesion Score

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INTRODUCTION

The endemic status of *Peste des petits ruminants* (PPR) in most of Africa, South-east Asia and Arabian Penisula gives rise to frequent outbreaks in susceptible animals with ravaging effects on goat and sheep production (Kumar et al., 2014; Woma et al., 2016; Balogun et al., 2017; Ahaduzzaman, 2021). During PPR outbreaks, symptomatic treatment with antibacterials, antidiarrheals, antiprotozoals, and intravenous fluid therapy are common (Abubakar and Irfan, 2014); a practice which is expensive, requires the services of trained personnel, yet resulting in ambivalent disease outcomes. The use of post-exposure vaccination in the management of clinically sick animals is not a common practice. However, some recent studies have proposed the potentials of post-exposure vaccination effectiveness in the management of viral diseases in both experimental and field cases (Yu et al., 2004; Rupprecht et al., 2009; Keckler et al., 2013; Okwor et al., 2012). Post-exposure vaccination has been shown to either modify or alter the clinical course of the disease among those who are already infected (Lauterbach et al., 2010; Gallagher and Lipsitch, 2019), a feat which has pitched it as a viable emergency management approach during disease outbreaks. The use of Post-exposure PPR vaccination in the management of PPR outbreak has been previously reported (Abubakar et al., 2015; Ezeasor et al., 2021). Ezeasor et al., (2021), reported that early post-exposure PPR vaccination alone, in an exposed goat herd with less than 20% PPR morbidity resulted in 60% survivability. Relatedly, some post-exposure vaccination studies on influenza and small pox have postulated that percentage survivability increases, the earlier the vaccine is administered post-exposure (Barefoot et al., 2009; Lauterbach et al., 2010; Keckler et al., 2013). However, the disease outcomes in morbidly advanced cases may differ. In this report, we describe the clinicopathological and pathomorphological observations in PPR post-exposure vaccinated goats at 75% morbidity. Abubakar et al., (2015) reported higher survivability following post-exposure PPR vaccination with concurrent penicillin-streptomycin, trimethoprim or sulfadiazine treatments. The morbidity and mortality patterns associated with PPR infection in small ruminants are exacerbated by secondary bacterial infections in advanced morbidity scenarios (Emikpe et al., 2010; Kumar et al., 2014). Oxytetracyclines, a broad-spectrum antibacterial is currently reported to possess strong antiviral properties (Rothan *et al.,* 2014; Liao *et al.,* 2019). Considering the effects of secondary bacterial infections in clinical course of PPR, it is possible that post-exposure PPR vaccination with concurrent oxytetracycline treatment may potentially improve survivability. This study reports the effects of post-exposure vaccination with or without oxytetracycline therapy in the management of advanced PPR outbreaks in West African dwarf goats.

MATERIALS AND METHODS

Vaccine and vaccine preparation

Pest des petit ruminants (PPR) vaccine derived from the attenuated Nigeria 75/1 strain at a concentration of 3 log10 /ml TCID50 was used. A vaccine vial of 50 doses was reconstituted as recommended by the manufacturer. For vaccination via the intranasal route, the reconstituted vaccine was admixed with rehydrated *Irvingia gabonensis* seed gum extract as described by Ezeasor et al., (2020). The gum extract was admixed with the PPR vaccine at a vaccine-to-gum ratio of 1:1 using a mechanical stirrer. For the parenteral vaccine administration, 1ml of reconstituted vaccine was injected subcutaneously, using 1ml disposable syringes.

Antibacterial agent

Oxytetracycline (20% LA), produced by Kepro (Deventer, Netherlands) for deep intramuscular injection at 1ml/10kg b.w. was used for this study.

Animal Experiment

Twenty-eight (28) male West African dwarf goats randomly assigned into seven groups (n=4), designated A, B, C, D, E, F and G were used for the study. The animals were kept for a period of two weeks prior to the study, to allow for acclimatization of the animals to the new husbandry regime. Blood was collected via jugular venipuncture at the end of the acclimatization period to confirm PPR sero-negative status of the experimental animals, using H-based PPR blocking ELISA kit.

To mimic natural infection, direct-contact was chosen as challenge protocol as described by Jarikre et al., (2016). Here, PPR virus (PPRV) sick goats confirmed by Agar gel immunodiffusion (AGID) were introduced into the experimental groups at a ratio of 1:4 and allowed to co-habit for at least, three days. Following the onset of clinical signs of pyrexia in at least 75% of the goats in each group and confirmation of infection by AGID test, the following treatments/ interventions was carried out: Group A goats

were treated with oxytetracycline only. Group B goats received PPR vaccine via the intranasal route using IG seed gum as adjuvant. The vaccine-gum mixture (1:1) was administered using a calibrated dropper into both nostrils (1 ml each) while restraining the head of the animals for at least 1 minute, to prevent the goats from shaking off the mixture. Group C goats received PPR vaccine via the intranasal route and treated with oxytetracycline. Group D goats received PPR vaccine via the subcutaneous injection only, while group E goats received PPR vaccine subcutaneously and treated with oxytetracycline. Group F goats served as the PPRV control (not vaccinated, not treated), while group G served as the normal control (not vaccinated, not treated, not exposed to PPRV).

The animals were examined twice a day (morning and evening) for a period of 14 days and all relevant clinical signs were recorded. Blood work was done to determine baseline values and subsequently on days 1, 3, 5, 7 and 14 post-interventions (p-in) and detailed necropsy was carried out on all mortalities and euthanized goats. All gross lesions were duly noted and sections of the lungs, mesenteric lymph nodes, spleen and gastrointestinal tracts was collected for histopathological examination.

Determination of Clinical Score

After establishment of PPRV infection and commencement of designated vaccine/oxytetracycline interventions, the experimental groups were carefully observed daily for clinical signs and evaluation of disease severity using a predetermined scoring rubric as described by Pope et al., (2013) with some modifications (Table 1). The determination of the clinical score (CS) which was initially based on graded severity scores of the demeanor, pyretic response, ocular/nasal discharges, oral lesions, diarrhoea, and respiratory symptoms, was modified to include the time (days) taken for death to occur post-intervention

Table 1. Severity scoring guide for assessment of animals infected with PPRV.

Parameter	Score	Description
Demeanour	0	Normal
	1	Mildly inactive. The animal is slow to rise and move
	2	Mildly inactive and depressed. Mild inappetence
	3	Inactive, apathetic, and anorexic.
	4	Severe depression, unable to stand, extreme lethargy, dehydration
Fever	0	RT <39.5°C
	1	RT >39.5°C but <40°C
	2	RT >40.0°C but <41°C
	3	RT > 41°C or >39.5°C > 5 days
	4	RT > 41°C or >39.5°C for >5 days followed by rapid fall <38.8°C
Ocular/nasal discharges	0	None
	1	Watery Ocular discharge
	2	Watery to mucoid oculo-nasal discharge; mild conjunctivitis
	3	Mucopurulent oculo-nasal discharges
	4	Severe mucopurulent oculo-nasal discharges
Oral lesions	0	None
	1	Congested oro-nasal mucosa and buccal papillae
	2	Pin-prick lesions within the mucosa of the buccal cavity
	3	Clear erosive lesions on oro-nasal mucosae
	4	Severe erosive lesions in the mucosa of the buccal cavity or lip.
Respiratory Signs	0	Normal
	1	Slight tachypnoea
	2	Tachypnoea/ mild cough
	3	Tachypnoea/dyspnoea with persistent coughing
	4	Marked tachypnoea/dyspnoea with persistent coughing
Feces	0	Normal
	1	Soft and pasty
	2	Runny
	3	Frank diarrhea
	4	Muco-hemorrhagic diarrhea

Table 2. Scoring guide for the lung lesion score.

Score	Description
0	Normal
1	Small lesion < 5% of the lobe affected
2	Approximately 25% of the lobe affected
3	Approximately 50% of the lobe affected
4	Approximately 75% of the lobe affected
5	Diffuse lesion. 100% of the lobe affected.

(p.in) in the individual goats. Animals presenting with a severity score of 4 in "demeanor" for more than 3 hours were euthanized on ethical grounds. The Clinical score (CS) for each group was determined using the formula:

Clinical Score (CS) = Group Severity Score (SS) ÷ Total Death Time (days) in group.

Haematological examination

The total leukocyte counts were determined by the haemocytometer method (Thrall and Weiser, 2002). The differential leukocyte counts were done on Leishman stained thin blood smears, and the percentage cell count were converted to absolute counts (Thrall and Weiser, 2002).

Pathological examination

Comprehensive necropsy was conducted and the extent of the gross lesions in the lungs was determined and scored as described by Baird et al., (2012) with modifications. The scoring system, initially based on the occurrence of pneumonic consolidation alone, was adjusted to include the presence of passive hyperemia. The extent of lung lesion was determined by visually assessing all the lung lobes and giving them a score of 0 to 5 based on the percentage of surface area that was affected (Table 2). The sum of the individual lobe scores was determined in each group and the group means were computed as the lung lesion score (LLS). Samples from the lungs, mesenteric lymph nodes, spleen, and gastrointestinal tract were collected and fixed in 10% phosphate-buffered formalin for histopathological examination. Tissue sections collected for histopathological examination were prepared using standard techniques (Bancroft and Layton, 2013). The hematoxylin and eosin (H&E) stained sections were examined with a Motic™ compound light microscope. The photomicrographs were taken using C-Mount™ microscope camera.

Statistical analysis

With the exception of the clinical scores, all data are presented in mean ± standard error. One-way ANOVA was used to compare the difference between the group means of the lung lesion scores using GraphPad prism 6, statistical software. Inferential statistics for the hematology was done using repeated measures ANOVA on SPSS version 27 for Microsoft Windows 10. Significance was accepted at $p<0.05$.

RESULTS

Clinical Observations

Clinical signs and lesions consistent with PPR in goats were observed in all the PPR exposed groups. Typical PPR clinical signs like mucopurulent oculonasal discharges, swelling of the lips as well as pasting of the perineal areas with diarrhoeic faeces were observed in all the PPR exposed groups (Figure 1). However, these symptoms varied in severity between the groups and resulted in 50% survival in Groups C and E while all the animals in groups A, B, D, and F died before the end of the study period. The severity of the clinical symptoms were quantified and the summary of the severity scores and the computed clinical scores for each of the experimental groups is presented in Table 3. The computed clinical scores were highest in group D (7.05), followed in decreasing order by groups F (6.92), B (5.89) and A (4.38), all with 100% mortality on days 11, 7, 8 and 11 post-intervention, respectively. Groups C (2.06) and E (3.90) had the lowest clinical scores with 50% mortality on days 13 and 10 post-intervention, respectively. Similarly, the post-intervention survival time was highest in groups C and E with 49 and 48 days, respectively, while groups A, B, D and F had a post-intervention survival times of 21, 27, 20 and 12 days, respectively.

Haematology

The summary results of the total WBC counts and absolute lymphocyte counts (ALC) are presented in Figure 2. No significant difference between the experimental groups was observed in the total WBC counts on day 1 p-in. However, by day 3p-in, there was an obvious decrease in group B and Group F, which was

Figure 1: PPR infected West African Dwarf goat showing severe mucopurulent oculonasal discharges and swelling of the lips (A); and perineal area pasted with diarrhoiec feces (B).

Table 3. Computed clinical scores for West African dwarf goats exposed to PPRV and subsequently vaccinated and/or treated with oxytetracycline (LA).

Group and Serial number		Days Post-vaccination/Treatment														T	SS	PiST	CS	M (%)
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**					
A	**A1**	1	1	0	2	3	0	0	8	8	8	**12***	-	-	-	43	92	21	4.38	100
	A2	0	4	4	4	4	8	-	-	-	-	-	-	-	-	24				
	A3	1	5	8	**11***	-	-	-	-	-	-	-	-	-	-	25				
	A4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
B	**B1**	9	-	-	-	-	-	-	-	-	--	-	-	-	-	9	159	27	5.89	100
	B2	2	2	6	6	10	9	13	7	9	-	-	-	-	-	64				
	B3	2	1	1	3	6	6	10	7	5	-	-	-	-	-	41				
	B4	1	0	3	5	9	7	10	10	-	-	-	-	-	-	45				
C	**C1**	1	1	1	1	0	0	0	0	0	0	0	0	0	0	4	101	49	2.06	50
	C2	2	2	3	1	6	7	6	9	-	-	-	-	-	-	36				
	C3	0	0	0	0	3	0	0	3	3	3	5	5	5	3	30				
	C4	0	2	0	0	2	0	3	1	0	5	6	5	7	-	31				
D	**D1**	3	7	8	12	12	12	14	16	-	-	-	-	-	-	84	141	20	7.05	100
	D2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2				
	D3	2	1	2	3	6	8	9	8	8	4	4	-	-	-	55				
	D4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
E	**E1**	1	0	0	2	3	4	9	8	6	8	3	3	3	3	53	187	48	3.90	50
	E2	1	0	3	3	5	5	6	6	6	6	-	-	-	-	41				
	E3	0	0	0	3	3	3	4	4	3	3	3	3	3	3	35				
	E4	2	0	0	1	3	7	11	14	9	11	-	-	-	-	58				
F	**F1**	4	5	8	9	4	9	12	-	-	-	-	-	-	-	51	83	12	6.92	100
	F2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	4				
	F3	5	4	8	11	-	-	-	-	-	-	-	-	-	-	28				
	F4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				

Dashed (-) boxes represent dead animals.
Days with an Asterix (*) depict euthanasia on ethical grounds for animals that scored 4 on "general signs"
T: Total severity score per animal
SS: Group Severity score
PiST: Post-intervention Survival time for the group (days)
CS: Clinical Scores
M: Percentage mortality

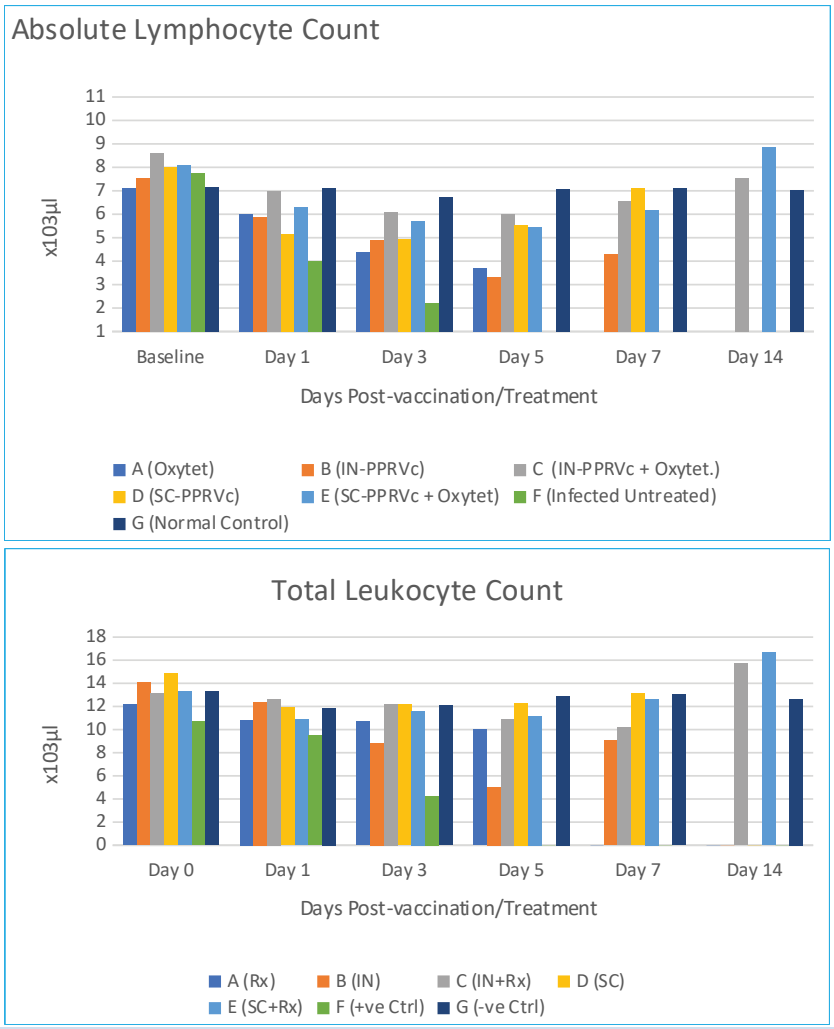

Figure 2. The total leukocyte counts and absolute lymphocyte counts of PPRV exposed and vaccinated/treated West African dwarf goats (Mean ± SEM).

only significant ($p<0.05$) in group F. By day 5p-in, the total WBC counts of group B was significantly lower ($p<0.05$) compared the other groups. This however, reversed by day 7 p-in, during which no significant difference ($p>0.05$) was observed between the experimental groups. By day 14 p-in, an obvious increase was observed in groups C and E, which were the only groups with surviving goats in the PPRV-exposed groups. The absolute lymphocyte counts (ALC) showed lower ALC from day 1 to 5 p-in in all the PPRV-exposed experimental groups when compared with their baseline values and the unexposed control group. At days 1 and 3 p-in, the absolute lymphocyte values of group F were significantly ($p<0.05$) lower than those of the other PPRV-exposed experimental groups. Also, at days 3 and 5p-in, a significantly ($p<0.05$) lower ALC was observed in groups A and B, compared to normal control. By day 7p-in, the ALC values showed a slight increase in all the PPRV-exposed experimental groups, however, group B still had a significantly ($p<0.05$) lower ALC, compared to the normal control. By day 14, an obvious increase was observed in groups C and E, however they did not differ significantly ($p>0.05$) compared to the normal control.

Pathological Observations

Lesions consistent with PPR infection in goats were observed in the PPRV infected groups, irrespective of group differences. Blunting of the oral papillae and mild to severe, multifocal erosions and ulcers on the lips, buccal mucosa and tongue were mostly observed. The oral lesion were most severe in group F goats. In the lungs, variable pneumonic lesions ranging from passive hyperaemia to mild, random, patchy or severe, widespread areas of lung consolidation were observed (Figure 3). These pulmonary lesions served as the basis for evaluation of the lung lesion

score (LLS). The mediastinal lymph nodes were mostly swollen and oedematous and there was mild to moderate hyperaemia of the small intestinal serosa with congestion of the mucosal folds (Figure 4). All the animals that survived in the PPR vaccinated and oxytetracycline treated groups (Groups C and E) did not show gross lesions except for the labial swelling and crusty lesions at the commissures of the lips.

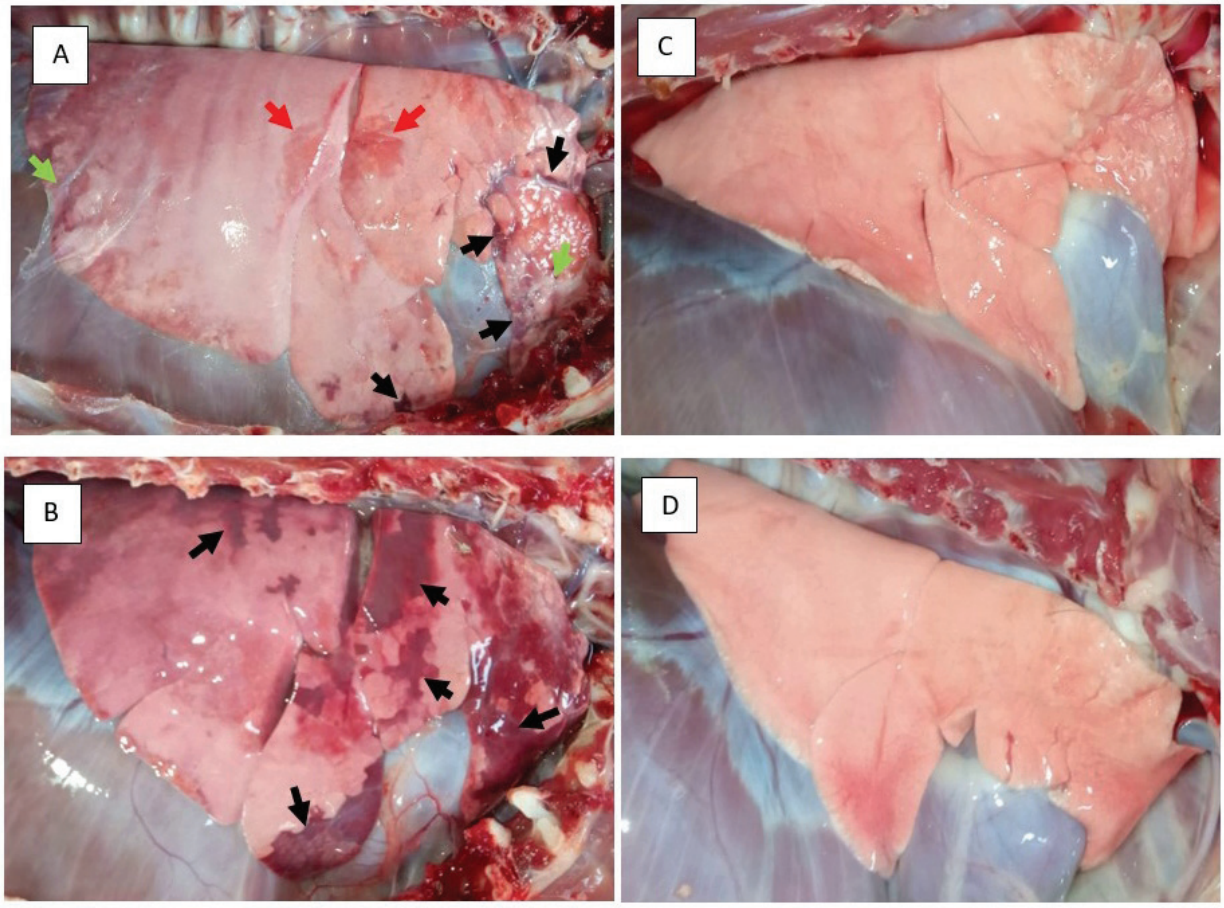

Figure 3: Photographs of the lungs from PPRV exposed and vaccinated/treated WAD goats. A: Widespread patchy areas of hyperemia (red arrow) and consolidation (Black arrow) with fibrin mat on the serosa of the cranial and caudal lobe (green arrow) was observed in group B (IN only). B: Multifocal widespread areas of lung consolidation (black arrow) was the predominant pneumo-pathology observed in group D (SC only). The goats the survived in Group C (IN+Rx) and Group E (SC+Rx) did not show any pulmonary lesion (C and D).

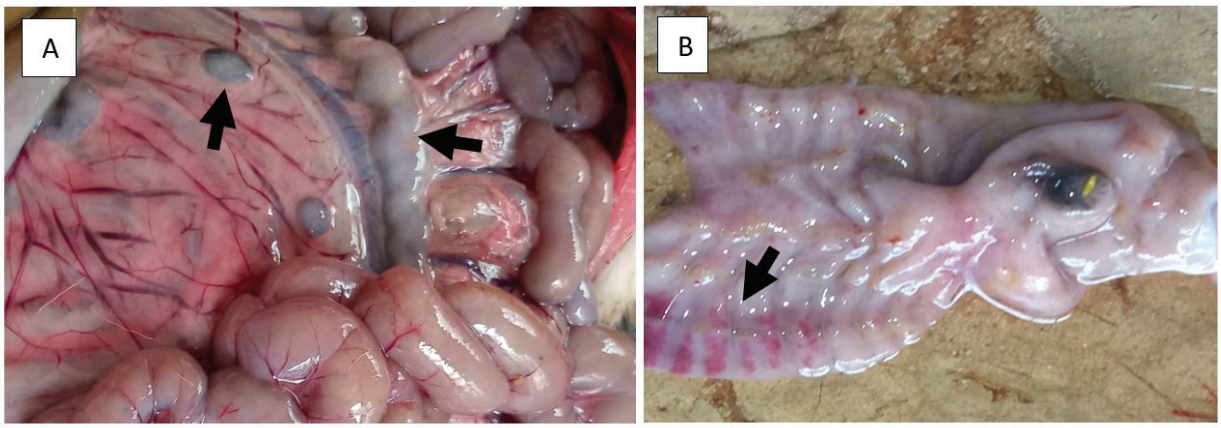

Figure 4: PPR infected West African Dwarf goat showing: (A) enlarged and oedematous mesenteric lymph nodes (arrow) and hyperaemic intestinal serosa; and (B) linear congestion (zebra markings) on the mucosa of the colon (arrow).

Lung lesion scores

Groups B, C, and E goats showed pneumo-pathological lesions in 50% of the animals from each group whilst in groups A, D and F goats, pneumopathological lesions were present in all the goat carcasses. The mean (S.E.M) values of the LLS are presented in Table 4. Group F goats had the highest mean (S.E.M) LLS of 2.5 (0.26), which differed significantly ($p<0.05$) from those of groups A, C and E, with mean (SEM) LLS of 0.90 (0.22); 0.61 (0.12) and 0.64 (0.15), respectively. Group D goats had the second-highest mean LLS of 1.93 (0.37) which also differed significantly ($p<0.05$) from those of groups A, C and E. Group B goats had a mean LLS of 1.57 (0.07) and did not differ significantly ($p>0.05$) from groups A, C and E. Group G goats (Normal Control) had no visible lung lesion and thus presented with a score of 0.

Histopathological findings.

In the lungs, the histopathological findings were mainly hyperemia of the interalveolar septae, proliferation of the alveolar pneumocytes, presence mixed leukocytic exudates with multinucleated giant cells in the alveoli, bronchi and bronchioles. The severity pattern varied in the lungs with group F goats showing the most severe lesion (Figure 5). Similar but less severe histopathological changes were observed in group A, B and D goats and the two goats that died in groups C and E. In these groups, the most consistent pulmonary lesion was mild to severe hyperemia of the interalveolar capillaries. Proliferation of the alveolar pneumocytes with inflammatory cellular exudates in alveoli, bronchial/bronchiolar inflammation, and the presence of giant cells in alveolar lumen was present, but inconsistent, being most severe in group D followed by groups B and E and then least severe in groups A and C. The lung samples collected from the goats that survived until the end of the study period in groups C and E showed the normal pulmonary histomorphology. Gastrointestinal lesions were present in all the PPR virus exposed groups irrespective of the vaccination/treatment differences and were most severe in the goats that died at the peak of diarrhea. The lesions observed in the duodenum, jejunum, ile-

Table 4. Lung lesion scores (LLS) of experimental goats determined at necropsy (Mean ± SEM).

GROUPS	SN	Right Lung				Left lung			LLS
		Right Cranial lobe	**Right middle lobe**	**Right caudal lobe**	**Accessory lobe**	**Left cranial lobe**	**Left middle lobe**	**Left caudal lobe**	
Group A	**A1**	1	1	0	2	1	0	0	**0.90±0.22^{a}**
	A2	3	0	3	2	0	0	0	
	A3	2	1	0	1	2	0	0	
	A4	0	0	0	0	0	0	0	
Group B	B1	5	5	5	5	5	5	5	**1.57±0.07^{ab}**
	B2	2	1	1	2	1	2	0	
	B3	0	0	0	0	0	0	0	
	B4	0	0	0	0	0	0	0	
Group C	**C1**	0	0	0	0	0	0	0	**0.61±0.12^{a}**
	C2	3	3	1	0	1	0	2	
	C3	0	0	0	0	0	0	0	
	C4	1	1	1	1	1	1	1	
Group D	**D1**	1	1	0	3	0	2	0	**1.93±0.37^{b}**
	D2	3	1	0	2	1	2	3	
	D3	5	1	0	2	0	0	4	
	D4	3	4	1	5	4	4	2	
Group E	**E1**	0	0	0	0	0	0	0	**0.64±0.15^{a}**
	E2	1	2	1	2	1	1	1	
	E3	0	0	0	0	0	0	0	
	E4	1	1	1	3	1	1	1	
Group F	**F1**	4	3	2	4	4	3	4	**2.5±0.26^{b}**
	F2	5	4	3	3	2	3	1	
	F3	2	3	2	3	1	1	3	
	F4	3	1	0	3	0	2	1	

*Different Superscripts indicate significant difference ($p<0.05$).

um, and colon were villous atrophy, necrosis of the enterocytes lining the villi, necrosis of the mucosal glands and crypt cells, mild to moderate infiltration of inflammatory cells into the lamina propria of the mucosa, and depletion of lymphocytes in the Peyer's patches. In the groups that survived till the end of the study period (Group C and E goats), the duodenum and jejunum showed the normal histomorphology while the ileum showed mild depletion of the lymphocytes in the Peyer's patches only (Figure 6). Group G goats showed normal gastrointestinal histomorphology. In the lymphoid organs, moderate to severe depletion of the lymphocytes was observed in all the PPRV exposed groups that succumbed to the infection. However, in groups C and E, the animals that survived until the end of the study period showed an increased cellularity in the B-cell areas of the splenic white pulp (Figure 7) and cortex of the lymph nodes (Figure 8).

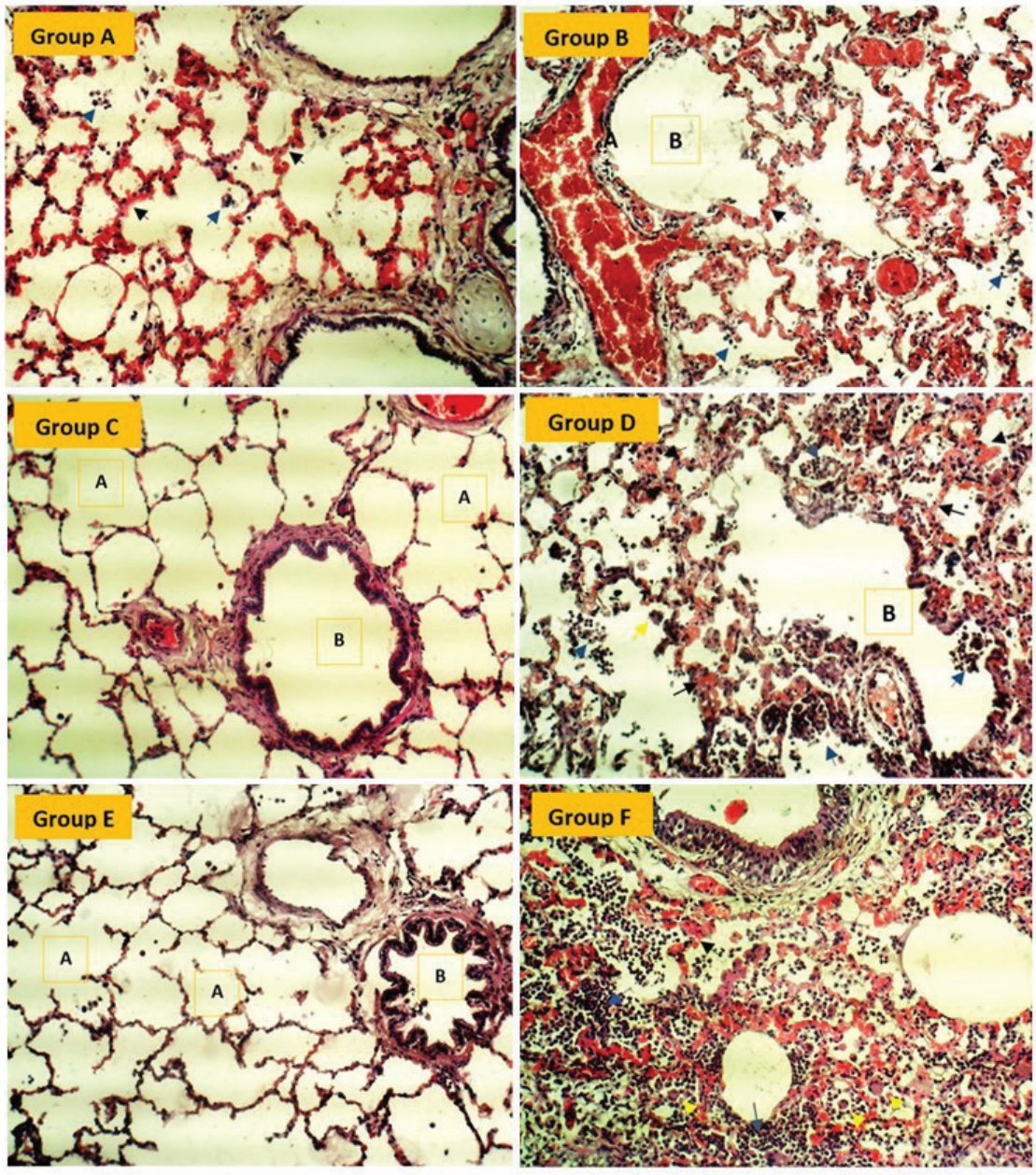

Figure 5. Histopathology of the lungs from the PPRV exposed and vaccinated and/or treated goats. Group A and B goats showed marked hyperemia of the interalveolar septae (black arrow) with the presence of mild scant cellular exudates in the alveoli (blue arrow). In group D, hyperemia of the interalveolar septae (black arrow) and the presence of mild to moderate cellular exudates in the alveoli (blue arrow) can be observed. The goats that survived in groups C and E showed the normal pulmonary histomorphology. In group F, thickening of the interalveolar septae (black arrow) with marked cellular exudates (blue arrow) with multinucleated giant cells (yellow arrow) in the alveolar lumen (blue arrow) were observed. Bronchiole (B); Alveoli (A). H&E Mag. x160.

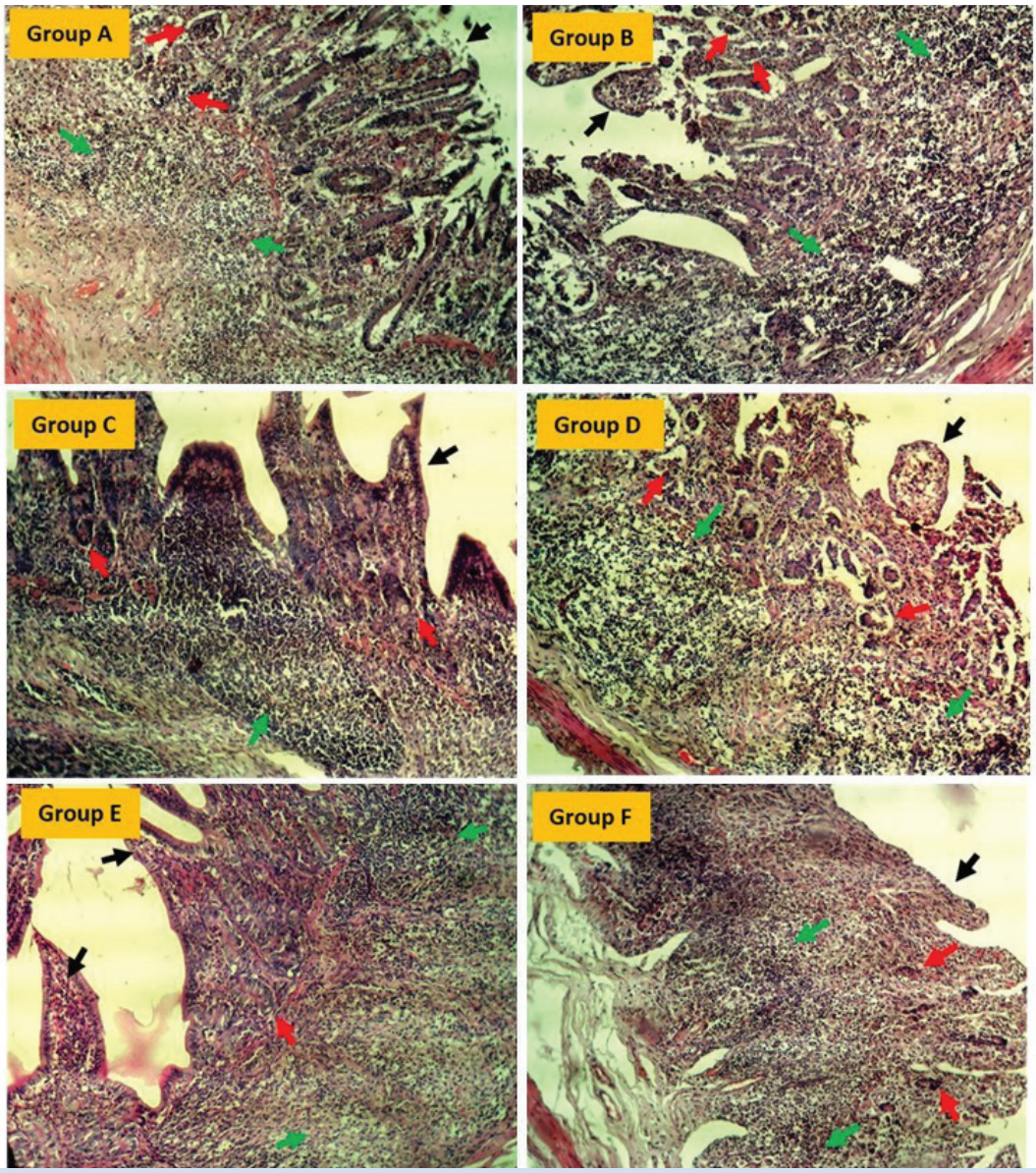

Figure 6. Histopathology of a section of the intestine (ileum) from the PPRV exposed and vaccinated and/or treated WAD goats. Sections of the ileum from Groups A; B; D and F goats showing the necrosis of lining cells of the mucosal glands (red arrow), sloughing of the enterocytes (black arrow), and severe lymphocyte depletion in the Peyer's patches (green arrow). In Group C and E goats, the animals that survived showed normal mucosal histomorphology and a mild depletion of the lymphocytes in the Peyer's patches (green arrow). Villi (black arrow); crypts (red arrow).H&E Mag. x160

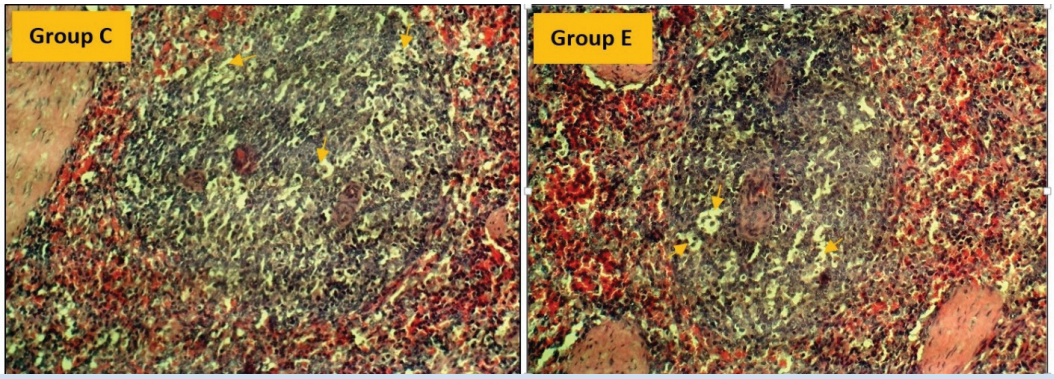

Figure 7: Photomicrographs of the spleen collected from PPRV-exposed, vaccinated, and treated WAD goats showing increased cellularity, as well as 'moth-eaten' appearance of the lymphoid follicles due to a moderate degree of scattered lymphocytolysis (arrow). H&E x200

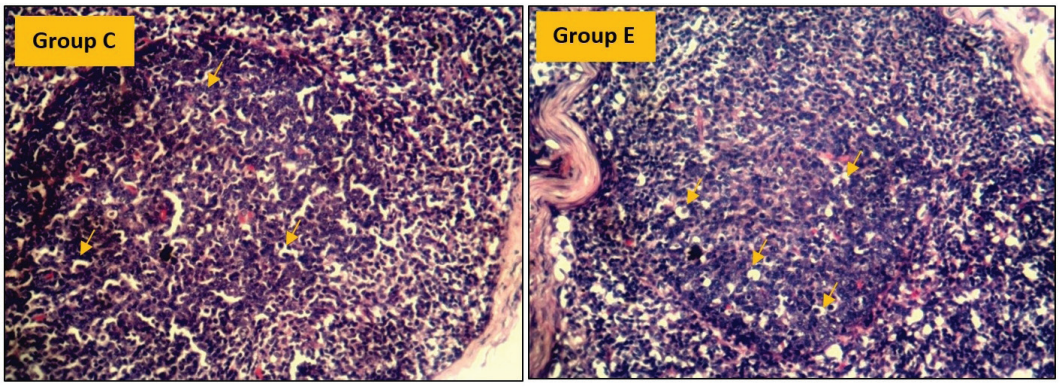

Figure 8: Photomicrographs of the mediastinal lymph node collected from PPRV exposed, vaccinated and treated goats that survived until the end of the study in groups C and E , showing a comparable increase in cellularity, as well as "moth-eaten" appearance of the lymphoid follicles due to moderate degree of scattered lymphocytolysis (arrow). H&E x200

DISCUSSION

In this study, we report the management of PPR outbreak using post-exposure PPR vaccination and oxytetracycline. In the PPR vaccinated and oxytetracycline treated groups, the disease generally ran an acute course resulting in 50% mortality and computed clinical scores far lesser than what was observed in the other groups. The application of clinical scoring systems in veterinary medicine have been used by several authors to evaluate the relationship between treatments and outcomes (Pope et al., 2013; Oma et al., 2016; Buczinski et al., 2021). The clinical scoring system is an important research tool in determining the most efficacious treatment or to form an accurate prognosis (Hayes et al., 2010). The computed clinical scores, as observed in this study suggest that concurrent PPR vaccination (intranasal or subcutaneous) and oxytetracycline administration is a better approach for managing PPR outbreaks than either vaccination (intranasal and subcutaneous) or oxytetracycline therapy, alone. Published information on the survivability of PPR clinically sick goats following post-exposure vaccination and/or treatment is sparse in the assessable literature. However, the available report is quite similar to the findings of this study. Abubakar *et al.,* (2015) reported higher survivability in post-exposure PPR vaccinated (SC) and antibacterial-treated goats. In the present study, post-exposure PPR vaccination of PPR clinically sick goats either via the subcutaneous route or the intranasal route (using a mucoadhesive polymer as delivery agent) with concurrent oxytetracycline treatment altered the course of the disease and resulted in higher survivability compared to vaccination alone or treatment alone.

A marked decrease in the total leukocyte counts and absolute lymphocyte count was observed in group F (PPRV control) by day 3 p-in. Leukopenia due to lymphopenia is consistent with PPRV infection, and it is associated with extensive necrosis in the lymphoid organs (Peyer's patches, spleen, lymph nodes), hence reducing the circulating peripheral blood leukocytes population by not less than 25% (Kumar *et al.,* 2004; Kul *et al.,* 2007; Pope *et al.,* 2013; Kumar *et al.,* 2014).

In the goats treated with oxytetracycline alone, a slightly similar trend to that of PPRV control was observed. The total leukocyte counts did not decrease; however, the absolute lymphocyte counts showed a moderate decrease on days 3 and 5 p-in though, within the normal reference range. A similar pattern was also observed in the vaccinated and oxytetracycline treated goats. This observation may suggest that the oxytetracycline may have influenced the effects of PPRV on the leukocytic profiles. Oxytetracycline, a broad-spectrum antibacterial agent, was used at the recommended doses. Some authors have described tetracyclines and their derivatives to have antiviral effects against several RNA viruses by inhibiting RNA replication (Yang *et al.,* 2007; Ng *et al.,* 2012; Rothan *et al.,* 2014; Liao *et al.,* 2019). It has also been reported that the antiviral effects of tetracyclines may also be indirect, involving several mechanisms through which the virus enters and replicate in the cells (Mosquera-Sulbaran and Hernandez-Fonseca, 2020). According to this report, tetracyclines may block protein synthesis in a host cell, hence inhibiting virion formation. It has also been reported that tetracyclines may have an anti-apoptotic effect on host cells and this may cause a decrease in the spread of the virus (Wang *et al.,* 2003; Griffin *et al.,* 2010). PPRV is an

RNA virus, hence it is possible that administered oxytetracycline may have exerted an antiviral effect on the PPRV, hence mitigating its effect on the host's leukocytic profile.

In the goats vaccinated via the intranasal route (only), a gradual decrease in the total leukocyte counts and absolute lymphocyte counts was observed on days 3 and 5 p-in, which differed significantly from the normal control. However, these patterns were reversed by day 7 p-in. This observation may be indicative of a virus-induced lymphocytolytic activity with a counter-effort by the immune system to surmount the immunosuppressive advances of the PPRV. In the goats vaccinated via the subcutaneous routes (only), the TLC did not differ significantly from the normal control. However, 100% mortality was recorded in group D despite showing a relatively normal leukocytic profile. The outcome of PPR infection is known to depend on the ability of the animals to mount a specific immune response to PPRV (Olaleye *et al.,* 1989; Kumar *et al.,* 2014) and inhibit PPR-induced immunosuppression which manifests as leukopenia due to lymphopenia (Munir, 2013; Kumar *et al.,* 2014). Hence, the fairly good leukocytic profile observed in groups B (Intranasal vaccine only) and D (Subcutaneous vaccine only) implies that upregulation of the cellular immune response alone, may not be sufficient to influence a favorable disease outcome following post-exposure vaccination. On the other hand, the disease outcomes were different in groups where post exposure vaccination was combined with oxytetracycline treatment. With regards to the high survival rates and lesser clinical scores observed in these groups, it is likely that a combined effect of immune system upregulation and the antiviral effect of tetracycline could have been responsible for the favorable disease outcomes in these groups. Oxytetracycline is a very potent broad-spectrum antibacterial agent and in consonance with its antiviral activities, its usage may have decreased the viral load, minimized secondary bacterial complications in the PPR virus-exposed animals, thus enabling the viral-suppressed immune system to bounce back.

The gross and histopathological lesions observed in all the PPR virus exposed groups in this study are consistent with documented reports of PPR virus in goats (Pope et al., 2013; Kumar et al., 2014; Truong et al., 2016). In the lungs, a lesion pattern suggestive of vaccinal and/or antibacterial treatment influence was observed. Based on the lung lesion scoring technique employed in this study, the lung lesion scores were highest in PPRV control goats, and least in goats that received oxytetracycline alone, and those that received post-exposure vaccinated and oxytetracycline, concurrently. Antibiotics are key components in the therapeutic protocol used for the management of PPR in goats, with the primary purpose of inhibiting or mitigating secondary bacterial complications due to the PPR virus induced immunosuppression (Abubakar and Irfan, 2014; Abubakar et al., 2015). Oxytetracycline is a common and cost effective broad-spectrum antimicrobial drug effective against a wide range of bacterial organisms (Ghanem et al., 2015; Sukanata et al., 2018). Hence, its use may have mitigated or inhibited the development of secondary bacterial pulmonary infections and their associated pulmonic lesions. Among the goats with relatively lower LLS, the goats vaccinated via the intranasal route showed the least scores. The possibility of a synergistic effect mediated by the vaccination route and antibacterial treatment is also possible. Vaccination via the intranasal route has been reported to rapidly induce a local mucosal immune response in the lower respiratory tract (Davis, 2001; Gerdts et al., 2006). An earlier study on intranasal PPR vaccination of goats showed better pulmonary protection compared to the subcutaneous or intramuscular routes of vaccination following experimental PPR virus infection (Emikpe et al., 2013). Hence, coupled with the antibacterial and antiviral effects of oxytetracycline, the post-exposure PPR vaccination via the intranasal route may have induced some level of mucosal immune response which mitigated the pulmonic effects of the virus.

In the intestines, the observed lesions were similar in all the groups, as most of the animals died following exacerbated diarrhea. However, the goats that survived until the end of the study period showed a relatively normal intestinal histomorphology, except for a mild depletion in the lymphoid cell populations of the Peyer's patches of the ileum. Diarrhea is one of the hallmarks of the acute forms of PPR and is usually followed by death due to severe dehydration (Munir, 2013; Pope *et al.,* 2013). Therefore, it is likely that a greater percentage survivability would have been attained in the PPRV-infected and vaccinated and/or treated goats if therapeutic protocols for the control of caprine diarrhea were considered in this study. In the spleen and mesenteric lymph nodes, lymphocytolysis with moderate-to-marked depletion of the lymphocytes in the lymphoid follicles were the major finding in all the PPRV-exposed goats that died before

the end of the study. This is in agreement with the reports of Munir (2013), Pope *et al.,* (2013), Balamurugan *et al.,* (2014), and Kumar *et al.,* (2014) which also attributed these findings to the lymphocytolytic effects of the PPR virus. The histopathological lesions in the spleen and mesenteric lymph nodes were similar in all the PPRV-exposed groups, except in the goats that survived until the end of the study in groups C and D, which showed scanty lymphocytolysis and increased cellularity in the B-cell areas of the splenic white pulp and cortex of the mesenteric lymph nodes. Though without obvious germinal centers, increased B cell cellularity in the spleen and lymph nodes are known to be associated with acute immune responses (Elmore 2006a; Elmore 2006b). This observation also suggests that a possible mechanism following PPR post-exposure vaccination is the development of humoral immune response which in synergy with the antiviral effects of oxytetracycline, may have reduced the viral load and accelerated the recovery.

CONCLUSION

This study has shown that concurrent PPR post-exposure vaccination and oxytetracycline treatment have the potential to modify the disease course and improve survivability in advance morbidity cases better than vaccination alone or oxytetracycline treatment alone. This is evidenced by the observed mortality pattern, clinical scores, clinicopathological and pathomorphological observations. Based on the findings of this study, PPR vaccination in combination with oxytetracycline treatment in the management protocol of PPR outbreaks should be encouraged and done as early as possible after evidence of infection is observed, so as to minimize fatal losses associated with the disease.

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CONFLICT OF INTEREST

None declared

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