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Serological screening of Peste des petits ruminants, comparative haemodynamic changes and serum biochemical profiles in symptomatic and asymptomatic sheep and goats in Enugu State

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Target Audience: *Veterinarians, Para-veterinarians, Livestock breeders, Animal scientists, Animal handlers, Animal health administrators, Researchers and students*

Abstract

Haemo-biochemical profile of small ruminants (SR) within age range 12-18 months exhibiting clinical signs suggestive of Peste des petits ruminants (PPR) (n=80) and apparently healthy ones (n=20) was studied. Blood samples (5 ml) were collected from the SR displaying a combination of the following clinical signs: nasal and ocular discharges, cough, diarrhoea, respiratory distress, weakness and emaciation, and from the apparently healthy ones. Sera and whole blood were analysed for the presence of PPRV antibodies using competitive ELISA kit as a screening test and haemo-biochemical profile using standard techniques respectively. Out of the 80 SR exhibiting clinical signs suggestive of PPR, sera from 51 (63.8%) were positive for the PPRV antibody and all of the apparently healthy ones were PPRV seronegative. There was a significant association ($p<0.05$) between those displaying clinical signs and seropositivity. The mean white blood cell of PPRV positive sheep and goats were significantly lower ($p<0.05$) than apparently healthy sheep and goats. Significant differences ($p<0.05$) in the packed cell volume (PCV), red blood cell (RBC) and platelet counts were observed in PPRV positive goats when compared with apparently healthy goats. The aspartate transaminase, alanine aminotransferase, total protein, albumin, urea, creatinine and potassium of PPRV positive sheep and goats differ significantly ($p<0.05$) from the apparently healthy sheep and goats. PPRV positive goats had significant higher ($p<0.05$) total bilirubin and significant lower ($p<0.05$) sodium level than the apparently healthy goats. Antidiarrheal agents with aqua solution together with immuno-modulators could help improve the management of PPR infection in SR.

***Keyword**: Biochemical profile; Clinical sign; Goats; Haematological profile; Peste des petits ruminants infection; Sheep*

Description of Problem

Peste des petits ruminants (PPR) is a viral disease of small ruminants (1). It tops the agenda as an important trans-boundary animal disease (TAD) with significant economic consequences in especially developing countries, thus, has been targeted for eradication by Food and Agricultural

Organisation (FAO) and World Health Organisation (WHO) (2). The disease is caused by *Peste des petits ruminants virus* (PPRV) recently renamed *Small Ruminant Morbillivirus* (SRM) (3). It is a single-stranded ribonucleic acid (RNA) virus belonging to the genus *Morbillivirus*, family *Paramyxoviridae* (4). PPR is an acute, febrile and highly contagious disease affecting domestic and wild small ruminants. This disease is characterised by pyrexia, serous to mucopurulent nasal and ocular discharges, coughing, respiratory difficulty, diarrhoea, pneumonia and necrotic ulcers on the oral commissures, tongue, lips and on dental pad (5). This is usually followed by death or recovery from the disease (5,6). It causes high morbidity (100%) and mortality rate that can reach up to 90% especially in naive animals (7). The disease has been reported to be more severe in goats than sheep based on epidemiological studies (8,9). Different methods are used to diagnose the disease: the presenting clinical signs as a tentative diagnosis (10,11); serology as a screening test (12,13); and molecular (Polymerase Chain Reaction (PCR)) approach (5,14,15) as a confirmatory diagnoses. The use of clinical signs alone is not sufficient in the diagnosis of PPR as many respiratory (contagious caprine pleuro-pneumonia, pneumonic pasteurellosis) and gastro-intestinal diseases (salmonellosis and helminthosis) present similar clinical signs and thus are considered as differential diagnoses (16). Information from the profiling will guide in the management of PPR. The disease is progressive and causes diarrhoea leading to dehydration and loss of electrolytes which in turn affect the haemo-biochemical parameters (17,18). There are many conflicting reports of haematology and biochemical profile of PPR infected small ruminants (6,17,19,20,21,22). Per-acute form of the disease is usually observed in kids of four months old which has been known to occur immediately after the depletion of maternal immunity, thus is highly fatal in young animals (23). Hence the knowledge of the haemogram and biochemical profiles of sheep and goats suffering from PPR infection in Enugu State, Nigeria can be helpful in the proper clinico-therapeutic management of the disease. Thus, the purpose of this study was to compare the alterations in the haemo-biochemical profile of sheep and goats with Peste des petits ruminants infection in Enugu State Nigeria.

Materials and Methods

Ethical statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, with approval number (UNN/eTC/16/82217) obtained from the Animal Ethics Committee of the University of Nigeria.

Study Animals

Small ruminants (fifty-one ovine and twenty-nine caprine) showing clinical signs suggestive of PPR (e.g. ocular and nasal discharges, encrustation in the medial canthus, ulcerative stomatitis, coughing, difficulty/labored breathing, diarrhoea) that were encountered between December 2017 and June 2018, and apparently healthy small ruminants (ten ovine and ten caprine) were purposively sampled in Enugu State Nigeria.

Sample Frame

A multistage sampling was performed with two hierarchical stages. First, three Local Government Areas (L.G.A) were selected by simple random selection method. Secondly, from each L.G.A selected, live sheep and goat markets, households that keep sheep and goats and veterinary clinics were purposively selected based on market

management and traders/owner's consent, respectively.

Blood sample collection

Blood sample (5 ml) was aseptically collected via the jugular vein from 100 small ruminants (SR) in Enugu State, Nigeria. Of these, 80 SR [sheep (n= 51) and goats (n= 29)] were showing clinical signs suggestive of PPR (pyrexia, mucopurulent nasal and ocular discharges, coughing, respiratory difficulty, diarrhoea) while 20 SR [sheep (n=10) and goats (n=10)] were apparently healthy (not showing any clinical signs of disease). A unit volume of each blood sample was put into a tube containing ethylenediamine tetra acetic acid (EDTA) and was used for haematology, while 4ml were put into a plain tube and was allowed to clot, serum was harvested by centrifugation at 1500 x g for 10 minutes, and stored at -20°C until needed for serology and serum biochemistry.

Table 1: Scoring of Clinical signs of suspected sheep and goats included in the study

Clinical score	Clinical description
0	Rectal temperature of less than 39.5°C, normal respiratory rate (sheep 15-40; goats 10-30 cpm) and normal faeces.
1	Watery ocular discharge, slight tachypnoea, soft faeces and temperature of greater than 39.5°C but less than 40°C
2	Watery to mucoid oculonasal discharge, reddened eyes and mild conjunctivitis, diarrhoea, depression, mild inappetance, tachypnoea, mild cough and rectal temperature above 40°C but less than 41°C
3	Mucopurulent nasal and ocular discharges with severe conjunctivitis, erosive stomatitis, frank diarrhoea, tachypnoea, dyspnoea, coughing and rectal temperature above 41°C or above 39.5°C for more than five days
4	Severely depressed, unable to stand, extremely lethargic, dehydrated, with mucopurlent nasal and ocular discharges, severe conjunctivitis and stomatitis, mucohaemorrhagic diarrhoea, marked tachypnoea/dyspnoea, cough and rectal temperature above 41°C or above 39.5°C for more than 5 days followed by a rapid fall in temperature (less than 38°C)

Classification of sheep and goats into clinical categories

The clinical score for assessment of animals infected with PPRV as described by Pope et al (24) was used to segregate sheep and goats into clinical categories as shown in Table 1. However, for the purpose of this study, only those with clinical scores "2" and "3" were classified as "Showing clinical signs" and thus used for this study.

Serological detection of antibody against PPRV

The sera were evaluated for the presence of PPRV antibodies using commercial competitive enzyme-linked immunosorbent assay (c-ELISA) kit (ID-VET® Grabel, France) as described by Libeau et al (12). This diagnostic kit was developed by FAO reference laboratory (CIRAD-EMVT, Montpellier, France) and it detects PPRV nucleoprotein (N) antibody. The optical density (OD) of the positive and negative controls met the validation criteria as indicated by the manufacturer (12). The competition percentage (CP %) for each sample was calculated adopting the formulae:

$$CP\ \% = \frac{OD\ samples}{OD\ Negative\ Control}\ X\ \frac{100}{1}$$

Sera samples with CP % less than 50 were considered PPRV positive according to the manufacturer's instructions.

Determination of haemo-biochemical profiles

Packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell count (RBC), white blood cell count (WBC) and platelet counts were determined as described by Coles (25) and Schalm et al (26). Serum alanine aminotransferase (ALT) and aspartate transaminase (AST) were determined by the Reitman-Frankel colorimetric method (27,28); total protein (TP) by the Biuret method, and albumin by bromocresol green method (29); total bilirubin (TB) and direct bilirubin (DB) by the modified Jendrassik-Grof method (30); urea by the Berthelot-Searcy method (31); creatinine by the modified Jaffe method (32); potassium (K) by Hillman and Beyer (33); and sodium (Na) by Henry et al (34), all conforming to manufacturers' instructions.

Table 2: Haematological parameters of PPRV infected and apparently healthy sheep and goat in South East Nigeria

	Sheep			Goat		
Parameters	**PPRV infected**	**Apparently health**	**p value**	**PPRV infected**	**Apparently health**	**p value**
Packed cell volume (%)	31.1 ± 3.57	25.3 ± 1.64	0.16	30.8 ± 0.71	21.8 ± 1.54	**0.00**
Haemoglobin concentration (g/dl)	9.25 ± 0.46	8.94 ± 0.59	0.68	8.33 ± 0.22	8.31 ± 0.38	0.97
Red blood cell count (10^6 /µl)	7.61 ± 0.42	6.29 ± 0.71	0.13	13.38 ± 0.32	8.27 ± 0.36	**0.00**
White blood cell count (10^4 /µl)	7.04 ± 0.60	11.9 ± 0.77	**0.00**	5.2 ± 0.64	10.71 ± 1.21	**0.00**
Platelet count (10^6 /µl)	9420 ± 1648	12880 ± 1460	0.13	4440 ± 747.6	11480 ± 967.9	**0.00**

The results were expressed as mean ± standard error of mean

Table 3: Biochemical parameters of PPRV infected and apparently healthy sheep and goats in South East Nigeria

	Sheep			Goats		
Parameters	**PPRV infected**	**Apparently health**	**p value**	**PPRV infected**	**Apparently health**	**p value**
Aspartate transaminase (AST) (µ/l)	74.1 ± 3.3	60.4 ± 5.0	**0.036**	88.7 ± 2.22	76.4 ± 4.46	**0.026**
Alanine aminotransferase (ALT) (µ/l)	15.4 ± 1.07	10.9 ± 1.59	**0.031**	19.1 ± 0.55	16.4 ± 1.07	**0.041**
Total protein (TP) (g/dl)	68.7 ± 3.31	78.6 ± 2.19	**0.022**	63.8 ± 2.39	69.98 ± 1.01	**0.033**
Albumin (g/dl)	28.9 ± 1.07	32.4 ± 1.05	**0.032**	31.9 ± 1.95	37.1 ± 0.62	**0.025**
Total bilirubin (TB) (mg/dl)	2.51 ± 0.56	2.38 ± 0.34	0.848	2.22 ± 0.07	1.66 ± 0.18	**0.013**
Direct bilirubin (DB) (mg/dl)	1.93 ± 0.35	1.92 ± 0.38	0.982	1.44 ± 0.09	1.63 ± 0.16	0.33
Urea (µmol/l)	8.27 ± 0.43	6.13 ± 0.78	**0.028**	7.69 ± 0.23	6.15 ± 0.60	**0.03**
Creatinine (µmol/l)	168.8 ± 6.61	142.16 ± 10.45	**0.046**	196.31 ± 3.46	167.56 ± 12.8	**0.025**
Sodium (mEg/l)	126.97 ± 3.31	122.59 ± 6.26	0.545	121.34 ± 5.36	135.45 ± 2.67	**0.032**
Potassium (mEg/l)	4.01 ± 0.37	5.08 ± 0.32	**0.041**	2.98 ± 0.33	4.04 ± 0.07	**0.008**

The results were expressed as mean ± standard error of mean

Data analysis

Chi-square statistical test was done using Graph Pad Prism statistical package version 5.2 for Windows (GraphPad Software, La Jolla, California, USA, http://www.graph pad.com) to evaluate the

association between the presence of PPRV antibody and display of clinical signs. Student's t-test was used for the comparison of mean values between PPRV-infected SR and apparently healthy SR. The analyses were accepted at $p<0.05$.

Results

Antibody analysis

Serum samples from 51 (63.8%; 32 sheep and 19 goats) of the 80 SR with clinical signs suggestive of PPR were positive to the PPRV antibodies, while 29 (19 sheep and 10 Goats) samples were seronegative to PPRV antibodies. There was a significant association ($p<0.05$**)** between the display of clinical signs and seropositivity.

Haematology

There was significant decrease ($p<0.05$) in the mean WBC of PPRV positive sheep when compared to apparently healthy sheep (Table 2). There were significant decreases ($p<0.05$) in the mean WBC and platelets counts, and significant increases ($p<0.05$) in the mean PCV and RBC count of PPRV positive goats when compared to apparently healthy goats.

Serum biochemistry

There were significant decreases ($p<0.05$) in the mean TP, ALB and potassium ion concentrations, and significant increases ($p<0.05$) in the mean AST, ALT, creatinine and urea level of PPRV positive sheep when compared to apparently healthy sheep (Table 3). There were significant decreases ($p<0.05$) in the mean TP, ALB, Sodium ion concentration and potassium ion concentration, and significant increases ($p<0.05$) in the mean AST, ALT, creatinine, urea and TB level of PPRV positive goats when compared to apparently healthy goats.

Discussion

In the present study, the finding revealed an association between the displayed clinical signs and seropositivity to the disease. This concurs with previous reports (35), as presence of these clinical signs could be used to make a tentative diagnosis of PPR. Thus, the exhibition of clinical sign suggestive of PPR and presence of PPRV antibody could be said to be an active infection.

The haematological and serum biochemical profile were significantly altered in PPRV positive SR. The significant increases in PCV and RBC observed in PPRV positive goats agree with the findings of Kataria et al (19) and Islam et al (22). The increased PCV and RBC values observed may be attributed to the diarrhoea resulting in dehydration, and consequently haemoconcentration in the PPRV affected goats. The finding is however, at variance with the reports of some previous authors (6,36,37,38) who found a significant decrease in the PCV and RBC of PPR positive sheep and goats, which they attributed to massive haemorrhages in different visceral organs (6,36,38) observed during postmortem examination (36). Aikhuomobhogbe and Orheruata (39) reported a non-significant difference in the PCV of PPR infected goats. These variations could also be attributed to the different stages of the disease as it is a progressive one having the following stages: incubation, prodromal, pneumonic and diarrhoeal stages.

The significant decrease in the WBC (leucopenia) observed in the PPRV positive sheep and goats agrees with the reports of previous authors (19,20,22,37,38). But it contrasted with Das et al (6) and Balogun et al (40). Leucopenia observed in the present study could be attributed to the lymphotropic activity of the PPR virus as the virus has an affinity for the lymphoid organ. The stage of

the infection and the immune status of the host can also influence the leucocyte status of the animal. Decreased WBC leads to marked immunosuppression of the host immunity which may promote the establishment of and aggravates the course or severity of secondary bacterial infection or other underlying diseases. Thus, this complicates the management of the disease.

The observed decrease (although not significant) in the platelet count (thrombocytopenia) in PPRV positive goats is similar to the finding of Sahinduran et al (20). Platelets are important components of blood that are responsible for haemostasis. Studies have shown that PPR virus causes thrombocytopenia (41) which may result from decreased production of platelets in the bone marrow or from increased peripheral destruction or from increased consumption which occurs with severe trauma and disseminated intravascular coagulation or a combination of these three factors (42). PPRV is known to cause disseminated intravascular coagulopathy (DIC) (20).

The significant increases in AST and ALT activities observed in PPRV positive sheep and goats is similar to the findings of Aytekin et al (17) and Sahinduran et al (20). The increased serum levels of both enzymes indicate affection and infection in liver and various organs where they are found. PPR infection has been reported and confirmed to be associated with liver damage (40). Multifocal areas of coagulative necrosis and vacuolation of hepatocytes have also been reported (43) in sheep and goat naturally infected with PPRV showing significant increases in mean serum AST and ALT concentrations.

The significant lower total protein and albumin observed in PPRV positive sheep and goats is similar to the findings of Das et al (6), Kataria et al (19) and Islam et al (22). This may be attributed to nephrotic damage associated with PPR infection, thus, resulting in kidney damage/dysfunction (40).

The significant increases in urea and creatinine levels recorded in PPRV positive sheep and goats agrees with the reports of previous authors (18,20,40). Urea remains a by-product in liver and creatinine is a by-product of creatinine phosphate in muscles. Both are normally removed from the blood via urine. Thus, their presence in the blood in high level is considered to be indicative of renal dysfunction.

The significant decrease in potassium ions observed in PPRV positive sheep and goats agrees with the report of Balogun et al (40). But it contrasted with findings of Sahinduran et al (20) and Islam et al (22). This low level of potassium ions could result from diarrhoea which is a symptom of PPR infection.

The significant increase in total bilirubin concentration observed in PPRV positive goats agrees with the findings of previous authors (6,18,41). Damaged liver makes the removal of bilirubin impossible as the unconjugated bilirubin which is toxic cannot be expelled from the body leading to hyperbilirubineamia. Also, the significant decrease in sodium ion concentration observed in PPRV positive goats agrees with the findings of Balogun et al (40), but contrast with the findings of Das et al (6) and Kataria et al (19). This decreased level of sodium ion concentration could also result from gastrointestinal and renal losses of fluid (i.e. diarrhoea and kidney impairment respectively) which is a feature of PPR infection.

In the present study, the non-significant differences observed in most of the haematological parameters of PPRV positive sheep agrees with the findings of Truong et al (44). It has been reported that goats are more susceptible and severely affected by PPRV than sheep which was based on

epidemiologic studies (8). Truong et al (44) reported the absence of viral RNA load in the whole blood of PPRV infected sheep while detectable levels were observed in PPRV infected goats at day 8 post infection. He also reported a significant wide spread PPRV induced pathology, severe oral lesions, haemorrhagic and necrotic ceacal and colonic tissue in goats which were absent in sheep.

The knowledge of the haemogram and biochemical profiles of SR suffering from PPR infection as revealed in this study will be helpful in the proper clinical and therapeutic management of the disease. It is therefore, pertinent for clinicians to incorporate fluid therapy, hepatonics, anti diarrhoeic and immune-modulating agents into the management therapy of PPR infection to enhance recovery rate.

Conclusion and Applications

1. The haemo-biochemical analysis of PPRV positive SR showed evidence of damage to the liver and kidney with marked immunosuppression and altered serum biochemical profile.
2. This haemo-biochemical data obtained provides valuable information that can be used for diagnosis and management of PPRV infection by livestock clinicians.
3. The study also revealed that goats are more severely affected by PPRV as reflected on the significant alterations observed in their haemo-biochemical profile.
4. Monitoring of haemo-biochemical parameters of PPRV-infected SR could serve as a prognostic indicator to access the level of pathology of the progressive disease.

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