Hematological and serum biochemical profiles of a natural African swine fever virus infection in pigs

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Summary
African swine fever (ASF) is a contagious viral disease that affects pigs of all ages, inducing hemorrhagic fever with high mortality and severe threat to pig production. This study investigated the hematological and serum biochemical abnormalities associated with a natural ASF virus (ASFV) infection in pigs. A total of 100 serum samples of pigs from piggery suspected of ASFV infection were screened for antibodies by ELISA. Thirty-two blood samples from serologically positive pigs and 32 negative pigs were undergo to hematological and serum biochemical analyses following standard procedures. The results showed that the mean values of the red blood cell (RBC) count, total white blood cell (TWBC) count, absolute lymphocyte count, absolute monocyte count, serum total protein (TP) and globulin were significantly ($p < 0.05$) lower while the mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), absolute neutrophil count and serum gamma glutamyl transferase (GGT) were significantly ($p < 0.05$) higher in the infected than the healthy pigs. There were no significant differences ($p > 0.05$) in the mean values of the packed cell volume (PCV), hemoglobin concentration, absolute eosinophil count, cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities between the infected and healthy pigs. Hence, natural ASFV infection may have caused alterations in the hematological and serum biochemical parameters in the infected pigs. The generated data could complement the existing laboratory diagnostic techniques such as polymerase chain reaction, direct fluorescence antibody test, indirect fluorescent antibody test and ELISA in the diagnosis of ASF in pigs.

Introduction

African swine fever (ASF) is a highly contagious hemorrhagic, often fatal viral disease of pigs caused by African swine fever virus (ASFV) of the genus *Asfivirus*, in the family *Asfarviridae*. It is a large, enveloped, double-stranded DNA virus (Alonso *et al.* 2018) and the only known vector-borne DNA virus (Dixon *et al.* 2011).

African swine fever is maintained in Africa by a natural cycle of transmission between wart hogs and the soft tick vector *Ornithodorus moubata*. A cycle of maintenance between domestic pigs and *Ornithodoros* has been identified in Kenya (Gallardo *et al.* 2011). Contaminated feed as a transmission vehicle for introducing trans-boundary animal diseases onto high-biosecurity swine operations has been recognized as a major risk factor since the introduction of porcine epidemic diarrhea virus into the United States in 2013 (Dee *et al.* 2018). The virus is transmitted oro-nasally in the domestic pigs followed by penetration into the pharyngeal mucosa and through the lymphatics, the virus enters the mandibular or retropharyngeal lymph nodes, where viral replication occurs. The virus then spreads through circulatory system to other organs and tissues of the body (Sanders *et al.* 2012).

The disease is characterized by pyrexia (40-42 °C),

anorexia, severe disseminated hemorrhage, ataxia, and depression in domestic pigs with case-fatality rates approaching 100% (Blome *et al.* 2013). The clinical forms of the disease include per-acute, acute, sub-acute or chronic and even asymptomatic, with incubation period in natural infections varying from 4 to 19 days and clinical course ranging from less than seven days post-infection in acute form, to several weeks, or even months, in chronic form (Beltran-Alcrudo *et al.* 2017). The major lesions due to ASF occur in the circulatory system, lymphoid tissues, and renal system and include hemorrhages under the skin; enlarged, edematous, and completely hemorrhagic lymph nodes with extensive necrosis of the lymphoid follicles; epicardial and endocardial hemorrhages; enlarged, friable, markedly congested and dark-red to black spleen with rounded edges; and petechiae on the capsule of the kidneys with pneumonia, fibrinous pericarditis, and edematous lymph nodes in the chronic form of the disease (Sanchez-Vizcaino *et al.* 2015; Beltran-Alcrudo *et al.* 2017). The infected animals have marked leucopenia and severe impairment of lymphoid organs, characterized by lymphocytic and significant cellular depletion in spleen, lymph nodes and other lymphoid tissues (Ramino-Ibanez *et al.* 1995).

Pork is the most consumed meat from terrestrial animals, accounting for over 37 percent of global meat intake, followed closely by chicken (35.2%) and beef (21.6%) (FAO 2013). However, in a recent report by the Food and Agricultural Organization, pork dropped to second position after poultry as the most consumed meat in the world because of the ravaging effect of African swine fever in China which is the world leading producer of pork (FAO 2019). According to Beltran-Alcrudo and colleagues (Beltran-Alcrudo *et al.* 2017), African swine fever is a severe threat to pig production systems, in that it not only threatens food security and challenges the livelihoods of pig producers and other actors in the supply chain but may also have major consequences on international trade as a result of trade restrictions. The disease, however, is not zoonotic.

There are no vaccines available for ASF and the supportive therapy usually employed in the management of the disease to reduce morbidity and mortality can be improved with the knowledge of hematological and biochemical profiles of ASF infected pigs. In addition, data on the hemato-biochemical profiles of a natural ASF infection in pigs are scarce.

The present study, therefore, investigated a natural ASF infection in a piggery in Nnewi, Anambra State, Nigeria, with special emphasis on the hematological and serum biochemical findings associated with the disease, with the aim of generating data that could aid supportive therapy and complement the existing laboratory diagnostic techniques in the diagnosis of ASF.

Materials and methods

Animals and flock history

One hundred pigs from a piggery located in Nnewi, Anambra State, Nigeria, with a natural ASF infection, were sampled. The pigs sampled were between 6-7 months of age. The farm with a population of 767 pigs had lost 415 pigs as at the time of sample collection. Breed of pigs stocked include Large white, Landrace, Duroc and Cambrough. The records at the farm showed the breakdown of the 767 pigs to comprise 346 males and 421 females. Drugs used for therapy on the farm include long acting oxytetracycline, penicillin/streptomycin and diminazene aceturate.

The study was performed in accordance with the ethical standards and the use of animals complied with local animal laws, guidelines and policies of Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

Blood sample collection

Blood samples were aseptically collected via the jugular venipuncture, after the animals were properly restrained using nose snares. Blood samples were collected between 9:00 and 11:00 am into two sets of sample bottles (one containing anticoagulant-ethylene diamine tetra-acetic acid-EDTA for hematology and the other devoid of EDTA for serum biochemical analysis) and submitted to the laboratory, in cold packs.

Serological test

Assay for antibody detection was carried out using ASFV-Ab Rapid ELIZA Test Kit (Ring Biotechnology Co Ltd, Beijing 100176, China) following method described by WOAH (OIE 2016) and the results were interpreted according to the instructions of the manufacturers.

Hematological analyses

The packed cell volume (PCV) was determined by the microhematocrit method, red blood cell (RBC) counts, and total white blood cell counts (TWBC) by hemocytometer method while the differential white blood cell counts were determined by Leishman technique (Thrall and Weiser 2002). The hemoglobin concentration was determined by

cyanomethemoglobin method (Higgins *et al.* 2008) while the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae.

Serum biochemical analyses

Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum gamma glutamyl transferase (GGT) and cholesterol were determined by colorimetric method using Quimica Clinica Aplicada test kits. Total protein was determined by direct Biuret method and serum albumin by bromocresol green method using commercial kits. All analyses were read using Diatek blood biochemistry analyzer (Wuxi Hiwell Diatek Instrument co. Ltd, China). Serum globulin was calculated by subtracting albumin from total protein.

Statistical analyses

The data generated from the laboratory procedures were statistically analyzed by independent t-test using statistical software program (SPSS for Windows version 20) and the level of significance was considered at $p < 0.05$.

Results

Clinical findings

Clinical signs such as fever, loss of appetite, depression, recumbency, hyperemia of the skin of the ear, abdomen and legs, respiratory distress, vomiting, emaciation, swollen joints, and lameness, typical of ASF were manifested by the pigs sampled in this study.

Erythrocytic alterations

The mean value of the RBC count was significantly ($p < 0.05$) lower while the mean values of the MCV and MCHC were significantly ($p < 0.05$) higher in the infected than in the healthy pigs. However, there were no significant differences ($p > 0.05$) in the mean values of the PCV and hemoglobin concentrations between the infected and healthy pigs (Table I).

Leukocytic changes

The mean values of TWBC count, absolute lymphocyte count and absolute monocyte count in the infected pigs were significantly ($p < 0.05$) lower than those of the healthy pigs, while the mean value of the absolute neutrophil count in the infected pigs was significantly ($p < 0.05$) higher than the healthy pigs. There was no significant difference ($p > 0.05$) in the mean value of the absolute eosinophil count between the infected and healthy pigs (Table II).

Serum biochemical findings

The mean values of the serum total protein and globulin were significantly ($p < 0.05$) lower while the mean value of serum GGT was significantly ($p < 0.05$) higher in the infected than the healthy

Table I. *The erythrocytic profile of pigs infected with African swine fever virus.*

Parameters	Groups of pigs		p-values
	Infected pigs (n = 32)	Healthy pigs (n = 32)	
PCV (%)	28.56 ± 1.05	30.00 ± 0.09	0.178
Hb conc (g/dl)	10.03 ± 0.34	9.72 ± 0.06	0.364
RBC count ($x10^6$)	8.16 ± 0.25[a]	9.09 ± 0.01[b]	0.001
MCV (fl)	35.01 ± 0.47[a]	33.09 ± 0.05[b]	0.000
MCHC (g/dl)	35.23 ± 0.46[a]	32.38 ± 0.10[b]	0.000

All data are expressed as mean ± standard error mean (SEM);
n = Number of animals sampled; [a, b]Different alphabetical superscripts on mean in a row indicate significant difference ($p < 0.05$) between the groups.

Table II. *The total white blood cell counts (TWBC) and differential white blood cell counts of pigs infected with African swine fever virus.*

Parameters	Groups of pigs		p-values
	Infected pigs (n = 32)	Healthy pigs (n = 32)	
TWBC count ($x10^3$)	17.75 ± 0.67[a]	29.65 ± 0.13[b]	0.000
Lymphocyte count ($x10^3$)	6.28 ± 0.31[a]	19.93 ± 0.05[b]	0.000
Neutrophil count ($x10^3$)	9.99 ± 0.29[a]	6.60 ± 0.05[b]	0.000
Monocyte count ($x10^3$)	0.35 ± 0.03[a]	2.01 ± 0.05[b]	0.000
Eosinophil count ($x10^3$)	1.11 ± 0.08	1.11 ± 0.02	0.942

All data are expressed as mean ± standard error mean (SEM);
n = Number of animals sampled; [a, b]Different alphabetical superscripts on mean in a row indicate significant difference ($p < 0.05$) between the groups.

Table III. *Some biochemical changes in pigs infected with African swine fever virus.*

Parameters	Groups of pigs		p-values
	Infected pigs (n = 32)	Healthy pigs (n = 32)	
Total Protein (g/dl)	7.45 ± 0.06[a]	8.14 ± 0.09[b]	0.000
Albumin (g/dl)	4.92 ± 0.12	5.04 ± 0.06	0.376
Globulin (g/dl)	2.53 ± 0.07[a]	2.99 ± 0.00[b]	0.000
GGT (IU/L)	8.26 ± 2.23[a]	0.93 ± 0.03[b]	0.002
ALT (IU/L)	75.23 ± 5.43	64.89 ± 0.66	0.063
AST (IU/L)	120.43 ± 7.73	124.70 ± 1.07	0.586

All data are expressed as mean ± standard error mean (SEM);
n = Number of animals sampled; [a, b]Different alphabetical superscripts on mean in a row indicate significant difference ($p < 0.05$) between the groups.

pigs. However, there were no significant differences ($p > 0.05$) in the mean values of serum cholesterol, ALT and AST between the infected and healthy pigs (Table III).

Discussion

African swine fever is a highly contagious disease of wild and domestic pigs with high rates of morbidity and case fatality and constitutes a severe threat to pig production. It is listed by the World Organization for Animal Health (WOAH) as a notifiable disease. However, the disease has no known therapy and vaccines are not readily available. This underscores the need for more research to elucidate the pathophysiology of ASF as well as generate data that could complement the existing laboratory techniques in the diagnosis of ASF in pigs. The present study, therefore, investigated the hemato-biochemical alterations associated with a natural ASF infection in pigs.

The mean value of the red blood cell count was significantly lower ($p = 0.001$) in the infected pigs. This could be attributed to hemorrhagic blood loss. The ASF is a hemorrhagic disease and the significant drop in the RBC count was expected. The significantly higher MCV ($p = 0.000$) and MCHC ($p = 0.000$) in the infected pigs recorded in the present study are indicative for the presence of reticulocytes produced by increased erythropoiesis in response to the hemorrhagic blood loss.

The mean value of the TWBC count was significantly lower ($p = 0.000$) in the infected pigs than the healthy ones. This finding agrees with the report of Afoyoa and colleagues (Afoyoa *et al.* 2014). Gomez-Villamandos and colleagues (Gomez-Villamandos *et al.* 1995) attributed the leucopenia that occurs in ASF to extensive necrosis of lymphocytes in the lymphoid tissues, as lymphocytes represent the largest proportion of leukocytes (Territo 2019). In addition, the lower TWBC count recorded in the present study was contributed by significantly lower absolute monocyte count in the infected pigs.

The mean value of the absolute lymphocyte count was significantly lower ($p = 0.000$) in the infected pigs. This finding agrees with the reports of Gomez-Villamondos and colleagues (Gomez-Villamondos *et al.* 2003) and Afayoa and colleagues (Afayoa *et al.* 2014). This could be attributed to apoptosis of lymphocytes induced by cytokines produced by ASFV infected monocytes and macrophages (Gomez-Villamondos *et al.* 2003). Lymphopenia is mostly associated with conditions that affect the bone marrow including viral infections that temporarily disrupt bone marrow functions (Kliegman and St. Geme 2019). The lower absolute lymphocyte count in the infected pigs recorded in the present study may be ascribed to a combination of temporary disruption of bone marrow function and apoptosis of lymphocytes.

The significantly higher ($p = 0.000$) absolute neutrophil count recorded in the present study may be attributed to a compensatory response of bone marrow stem cells to the depletion of lymphocytes in the lymphoid tissues following ASFV induced apoptosis and necrosis.

The mean absolute monocyte count was significantly lower ($p = 0.000$) in the infected pigs than the healthy ones in the current study. Afayoa and colleagues (Afoyoa *et al.* 2014) in contrast to the present finding reported a significantly increased monocyte count in the infected pigs in an experimental study. The difference between the two studies could be attributed to the phase of the disease when sample collection was done as the case in the current study may be ascribed to the destruction and depletion of the virus-infected monocytes.

The mean value of serum total protein in the infected pigs was significantly lower ($p = 0.000$) than the healthy ones. This could be attributed to low dietary protein intake following anorexia (Awekew *et al.* 2017). Protein-losing enteropathy and decreased hepatic biosynthesis of albumin could also be implicated in the hypoproteinemia observed in the present study (Nwoha *et al.* 2013). In addition, significantly lower globulin (immunoglobulins) level observed in the present study may have contributed to the significantly lower serum protein.

The mean value of serum globulin in the infected pigs was significantly lower ($p = 0.000$). The lower serum globulin correlates with lower absolute lymphocyte count recorded also in this study which was because of ASF induced apoptosis of lymphocytes.

The mean value of serum GGT in the infected pigs was significantly higher ($p = 0.002$) than that of the healthy pigs. This finding concurs with the report of Afayoa and colleagues (Afoyoa *et al.* 2014). Serum gamma-glutamyl transferase (GGT) has been shown to be a sensitive marker of cholestasis and has been found to be a valuable tool in the diagnosis of hepatobiliary disorders (Kasper *et al.* 2018). According to Mason and colleagues (Mason *et al.* 2010), GGT evaluation is an enzymatic liver function test that has been available for several decades and has been initially used as a sensitive indicator of fatty liver disease and hepatitis. The significantly higher serum GGT level recorded in the current study could be attributed to multiple organ damage caused by ASF which includes the liver and hepatobiliary system.

Conclusions

The results of the present study support that the disease causes alterations in the hematological and serum biochemical parameters in the infected pigs. The disease is associated with significantly lower RBC count, TWBC count, absolute lymphocyte count, absolute monocyte count, serum total protein and globulin levels, and significantly higher MCV, MCHC, absolute neutrophil count and serum GGT level. Evaluation of hematological and serum biochemical parameters in pigs could therefore be used to complement the existing laboratory diagnostic techniques such as polymerase chain reaction, direct fluorescence antibody test, indirect fluorescent antibody test and ELISA in the diagnosis of ASF in the case of suspected outbreak of the disease. Knowledges of the hemato-biochemical changes may be useful to predict the prognosis of the disease. Veterinarians should consider the hematological and biochemical profiles of ASF to formulate the effective supportive therapy so as to ameliorate the morbidity and high case fatality caused by the disease.

Ethical standards

The research was carried out in accordance with the Ethics and Regulations guiding the use of animals, and handling of animals during sample collection complied with institutional animal welfare laws, guidelines, and policies and with ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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