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Haematological, Serum Biochemical and Acute Phase Protein Profiles of Sheep with Footrot Infection caused by *Dichelobacter nodosus*

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ABSTRACT

Foot-related diseases are among the most common herd health issues worldwide, leading to heavy burden on the sheep and their owner including heavy socio-economic losses. This study investigated the haematological, serum biochemical and acute phase protein profiles of sheep with footrot infection caused by *Dichelobacter nodosus*. Blood samples from 15 sheep with footrot and 10 healthy controls were collected and analyzed for possible alterations in haematological, serum biochemical parameters and acute phase protein (APPs). Sheep with footrot showed significant changes in haematological parameters including left-shift, neutrophilic leukocytosis, lymphopenia and monocytosis. Serum biochemical assay revealed significant ($P<0.05$) increase in the concentration of total protein, cortisol, glucose, haptoglobin and fibrinogen while the concentration of albumin was significantly decreased. The finding of this study emphasizes the importance of haematological, serum biochemical and APP analytes in the diagnosis and monitoring of footrot in sheep. The finding of this current study could be used for the development of point-of-care diagnostic markers that would aid prompt diagnosis and treatment of footrot in sheep.

Keywords: Albumin; Bacterial infections; *Dichelobacter nodosus*; Fibrinogen; Haptoglobin.

INTRODUCTION

Footrot is a bacterial infection of the interdigital skin and a well-defined clinical disease that primarily affects sheep's foot resulting in lameness. This condition is of great welfare and economic concern to sheep farmers worldwide (Adama and Yahya, 2008; Zanolari *et al.,* 2021). The pathogenesis of Footrot is often believed to be complex and multifactorial with the primary causal pathogen been the gram-negative anaerobic bacterium *Dichelobacter nodosus* (Blanchard *et al*., 2021). The organism thrives well in moist environment with improper hoof trimming, poor hoof hygiene, poor nutrition, stress and concurrent diseases being among the numerous predisposing factors that could lead to physical damage to the interdigital skin thereby setting the stage for bacterial replication in the damaged skin leading to interdigital dermatitis (ID) which is characterized by inflammation and sloughing off of the superficial epidermal layer of the foot (Maboni, 2017; Ardüser *et al*., 2019) As the condition progresses, it results into footrot with resultant separation of the hoof horn capsule from the underlying tissues (Davenport *et al*., 2014; Smith *et al*., 2014; Kuhnert *et al*., 2019). An opportunistic bacterium, *Fusobacterium necrophorum* has been incriminated to play a vital role in the disease progression, promoting interdigital dermatitis and permitting further replication of *D. nodosus* (Gelasakis *et al.,* 2019; Basa *et al*., 2020; Zanolari *et al.,* 2021). In Nigeria, seasonal dependency occurs for the prevalence of *Dichelobacter nodosus*, with higher prevalence recorded during the rainy season (Adama and Yahya, 2008).

Acute phase response (APR) is a broad term used to describe a range of systemic and metabolic changes in an animal that act together to neutralize the negative effects of inflammation, infection, trauma, ischemic necrosis, or malignant growth (Kushner and Mackiewicz, 2020; Ehlting *et al*., 2021). An increase in the levels of acute phase proteins (APP) triggered by proinflammatory cytokines such as interleukin 1β, interleukin 6 and tumour necrosis factor-α is an integral part of acute phase response (APR) which occur solely to protect the host and restore the body condition back to its normal function (de Carvalho Filho *et al*., 2020). APPs are generally classified as positive (increase) and negative (decrease) with the positive APPs been further described as minor, moderate and major according to their magnitude of increase (Ceciliani *et al*., 2002; Leclere *et al*., 2015).

Few researchers have reported alterations in haematological and some serum biochemical parameters

associated with footrot caused by *Dichelobacter nodosus* infection in ruminants (Saleh *et al*., 2019, Kontturi *et al*., 2020; Szponder *et al*., 2021). However, studies on APR in footrot infection are scant (El-Deeb *et al*., 2022). There is paucity of information about the clinico-pathological changes associated with footrot infection in Nigeria. Hence, we undertook this study to determine the haematological, serum biochemical and acute phase protein responses observed in footrot infection in sheep caused by *Dichelobacter nodosus*. This study will highlight the role of these indices on the pathophysiology of the disease to enhance effective diagnosis and prompt treatment.

MATERIALS AND METHODS

Experimental sheep

The study was conducted on fifteen (15) adult female Yankassa sheep presenting with clinical signs of ovine footrot. The experimental animals were weighing approximately 30 - 45kg and aged between 1 and 2 years. They were housed in a well-maintained private sheep farm along Mandate Area, Ilorin. The control group consisted of ten (10) healthy female sheep of the same breed, similar age and weight. The climatic condition of Ilorin is characterized by both rainy and dry seasons. This study was conducted at the peak of rainfall in August with the average temperature around 28^{o}C and relative humidity of 65%. All the animals were well fed, watered and cared for in accordance with Directive 2010/63/EU on animal care. The clinical observation and vital parameters including rectal temperature, pulse and respiratory rate were recorded at the time of sampling.

Interdigital sample collection and microbiological analysis

The lesions were thoroughly cleaned with cotton wool soaked in sterile (distilled) water and a sterile cotton swab moistened with distilled water was used to obtain sample from lesion between the interdigital space of the hoof of sheep with clinical footrot. The swab was immediately streak on 4% hoof agar plate at site of sampling and the plate are placed on anaerobic jar and incubated for 4 days. Positive growth was subculture severally until they are free of contaminating bacteria and were then subculture in 2% hoof agar for further biochemical characterization (Stewart and Claxton, 1993; Buller and Eamens, 2014).

Blood Sampling and Laboratory Analysis

Blood samples (5 ml) was collected into a plain tube to obtain serum for biochemical analysis and a tube containing ethylenediaminetetraacetic acid (EDTA) for determination of full blood count using an automated haematology analyzer (Mindray Biomedical, China). To obtain serum, 5ml of the blood sample was centrifuged at 6,000rpm for 10 minutes and the sera was carefully harvested and stored at -20^{o}C until required. The sera were used to assay for biochemical and acute phase protein profile. Glucose, total protein, albumin and the biochemical indicators of organic damage, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), total bilirubin, cholesterol, urea and creatinine were determined using commercial reagent kits (Randox, UK) and automated biochemistry analyser (Roche, India) according to the recommendations of the manufacturer. Cortisol, fibrinogen and haptoglobin were assayed by enzyme-linked immunosorbent-based kits (MyBioSource, USA).

Statistical analysis

Data was prepared using Excel (Microsoft Office for Mac, Version 2022) and presented as mean ± standard error of mean (SEM) for continuous variables. Student's *t*-test was used to compare the means using Statistica software version 13.1 (TIBCO Software Inc., USA). Differences were considered statistically significant at $P < 0.05$.

Ethical statement

All applicable international, national, and or institutional guideline for the care and use of animals was strictly followed. The approval for this study was obtained from the Ethical committee on Animal use, University of Ilorin, Ilorin with approval number UREC/FVM/15/32TA017. Owner's consent was sought prior to the commencement of the study.

RESULTS

Clinical Examination Finding

Clinical observation and vital parameters of sheep with footrot was as presented (Tables 1- 2). Findings revealed decreased weight loss due to anorexia and an acute onset of lameness affecting one/both toe(s) of the forelimb. Some of the sheep were seen stamping their toe on the ground due to severe pain. Interdigital dermatitis characterized by loss of hair, reddening and swollen interdigital space tissues was seen (Figure 1). in severe cases, separation of the part of the hoof horn from skin beneath were observed (figure 2). Some of the lesion had a characteristic foul smell. Rectal temperature, respiratory and heart rate was significantly increased.

Table 1: Clinical examination finding in acute footrot infection in sheep (N = 15).

Clinical Observation	Score	Percentage occurrence (%)
Hoof lesion	15/15	100
Interdigital dermatitis (Benign footrot)	2/15	13.3
Severe footrot with hoof separation	13/15	87.7
Lameness	15/15	100

Table 2: Vital Parameters (Mean ± SEM) of infected and control sheep

Group	Temperature (^{o}C)	Respiratory rate (cycles/minute)	Heart rate (beats/minute)
Sheep with footrot (N =15)	39.3 ± 0.15^{b}	35.68 ± 4.20^{b}	71.34 ± 2.32^{b}
Control Sheep (N = 10)	37.5 ± 0.08^{a}	22.8 ± 3.51^{a}	86.58± 3.74^{a}

Means with different superscripts ^{a,b} indicates statistically significant differences at P<0.05

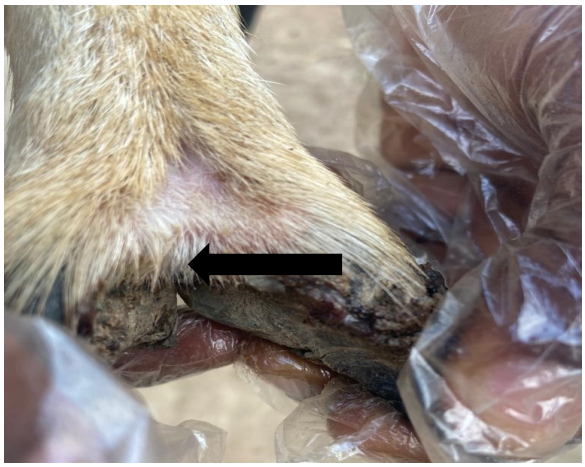

Figure 1: Benign footrot: Infected hoof showing interdigital dermatitis characterized by loss of hair, reddening and swollen interdigital space with the hoof still intact (Arrowed).

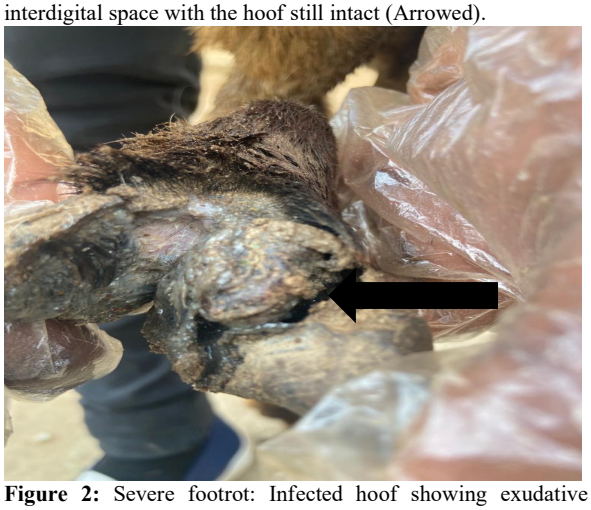

Figure 2: Severe footrot: Infected hoof showing exudative inflammation characterized by necrosis of the epidermal tissue of the interdigital skin and hoof matrix, resulting in separation of the hoof from the underlying soft tissue (Arrowed).

Microbial Isolation and Identification of *D. nodosus*

Out of the fifteen interdigital swabs taken from sheep with acute footrot, only thirteen (86.7%) were found to be positive according to the morphology of the colonies, the remaining two (13.3%) showed no growth. The colonies were grayish white transparent with elevated center, irregular edges and has a distinct butyric acid odor. Microscopic examination of stained smear revealed Gram negative rods with terminal enlargement characteristic of *D. nodosus*. The result of the biochemical tests was negative for catalase, indole production, nitrate hydrolysis, starch hydrolysis, glucose fermentation but positive for H_2S production.

Haematological parameters

The mean (± SEM) hematological parameters of infected and control sheep was as presented. Hemogram of sheep with footrot were normal and comparable to those of normal sheep. However, the leucogram revealed significant changes in total white blood cell, neutrophils, bands, lymphocytes, eosinophils and monocytes counts (Table 3).

Serum Biochemical Parameters

The mean (± SD) serum biochemical parameters of infected and control sheep was as presented. Significant increase in total protein, cortisol and glucose concentration was observed in sheep. While the concentration of urea, creatinine, total bilirubin, cholesterol, and the activities of AST, ALT and ALP were comparable between infected and control sheep (Table 4).

Acute phase response

The mean (± SEM) acute phase protein (APP) parameters of infected and control sheep was as presented. Significant increase in positive acute phase protein: fibrinogen, haptoglobin and a decrease in negative acute phase protein: albumin was evident in the sheep with footrot (Table 5).

Table 3: Haematological Parameters (Mean ± SEM) of *D. nodosus*-infected and control sheep

Parameters	Sheep with footrot (N = 15)	Control sheep (N = 10)
Haematocrit index (%)	28.33±1.06^{a}	27.88±0.74^{a}
Haemoglobin (g/L)	9.83±0.72^{a}	9.06±0.98^{a}
RBC (x 10^{12}/L)	8.97±0.42^{a}	9.13±0.29^{a}
WBC (x10^{9}/L)	15.31±1.22^{b}	9.24±1.95^{a}
Neutrophil (x10^{9}/L)	8.56±0.89^{b}	4.79±1.49^{a}
Bands (x10^{9}/L)	2.49±0.18^{b}	0.00±0.00^{a}
Lymphocyte (x10^{9}/L)	3.42±1.33^{b}	5.67±0.72^{a}
Monocyte (x10^{9}/L)	0.63±0.07^{b}	0.31±0.03^{a}
Eosinophil (x10^{9}/L)	0.29±0.30^{a}	0.25±0.40^{a}

Key: The same alphabet indicates that the means were comparable (P>0.05); Different alphabet represent statistically significant difference from control at P<0.05

Table 4: Serum biochemical Parameters (Mean ± SEM) of *D. nodosus*-infected and control sheep

Parameters	**Sheep with footrot (N = 15)**	**Control sheep (N = 10)**
Total protein (g/dL)	95.46±13.1[a]	96.02±5.75[a]
Urea (mg/dL)	55.03±4.34[a]	53.03±9.67[a]
Creatinine (mg/dL)	38.34±15.77[a]	40.29±11.05[a]
Total bilirubin (mg/dL)	30.56±2.70[a]	33.73±1.45[a]
Cholesterol (mg/dL)	158.45±210.3 [a]	157.76±132.14[a]
AST (U/L)	86.05±11.04[a]	88.17±7.23[a]
ALT (U/L)	30.15±2.43[a]	31.18±1.85[a]
ALP (U/L)	19.52±2.59[a]	21.75±1.51[a]
Glucose (mg/dL)	97.45±3.46[b]	82.39±1.01[a]
Cortisol (mg/dL)	6.49±0.59[b]	4.95±0.27[a]

Key: AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline Phosphatase, The same alphabet indicates that the means were comparable (P>0.05); Different alphabet represent statistically significant difference from control at P<0.05

Table 5: Acute Phase Protein Parameters (Mean ± SEM) of *D. nodosus*-infected and control sheep

Group/Parameters	**Negative APPs**	**Positive APPs**	
	Albumin (g/dL)	**Fibrinogen (g/dL)**	**Haptoglobin (g/dL)**
Sheep with footrot (N =15)	35.72 ± 0.15[b]	35.81 ± 4.20[b]	84.34 ± 2.32[b]
Control Sheep (N = 10)	39.39 ± 0.08[a]	22.60 ± 3.51[a]	71.58± 3.74[a]

Key: The same alphabet indicates that the means were comparable (P>0.05); Different alphabet represent statistically significant difference from control at P<0.05

DISCUSSION

Footrot is a herd health problem leading to lameness and reduced in productivity of sheep in footrot endemic countries including Nigeria. This study report for the first time the isolation and identification of *D. nodosus* from the interdigital swap sample collected from Yankassa sheep with lesions suggestive of acute footrot in Ilorin, Kwara State. The observation that 86.7% of the samples collected were positive for *D. nodosus* reiterate the importance of this organism in the establishment and pathogenesis of footrot infection in Yankassa sheep and this current finding agrees with those of previous researchers (Hoby *et al*., 2020; Kraft *et al*., 2020; Storms *et al*., 2021; Szponder *et al*., 2021; Storms *et al*., 2022; Loosli *et al*., 2023). In this study, all the examined sheep were lame and had severe hoof lesion which could be due to toxaemia induced by the causative bacteria, *D. nodosus*.

The elevated body temperature, respiratory rate and heart rate in sheep with footrot are key findings suggesting a systemic inflammatory response to infectious condition (Seixas *et al*., 2021). Hyperthermia, a classical sign of acute inflammation indicates that the sheep's immune response to the presence of D. *nodosus*, the increased respiratory rate could be due to compensatory mechanism by the body to meet up with the increased oxygen demand during inflammation, while the elevated heart rate is likely due to the sympathetic nervous system response to inflammation and/or stress by increasing blood and oxygen flow to affected tissues. This observation is consistent with previous studies reporting similar changes in sheep with footrot caused by *D. nodosus* (Szponder *et al.,* 2017).

The knowledge of haematological and serum biochemical parameters in ruminants are necessary to improve therapy and institute proper disease prevention measures (Delano *et al*., 2002). The observation that the haemogram of sheep with acute footrot infection were normal and comparable with those of control sheep is suggestive of the fact that *D. nodosus* had no severe effect on these parameters during the acute phase of the infection. Conversely, the leukogram revealed a classical acute inflammatory response to systemic bacterial infection which is characterized by leukocytosis, neutrophilia, lymphopenia and mild monocytosis. This current report agrees with previous finding in cattle (Shibahara *et al*., 2002).

In this study, the elevated levels of glucose and cortisol in sheep with footrot could be attributed to stress response to infection. Cortisol induced hyperglycaemia is a frequent finding in stress and inflammation while the increased cortisol level could be due to stress (pain) induced activation of the hypothalamic-pituitary-adrenal axis (Mifsud *et al*., 2018). The finding of this study is consistent with previous research that reported increased cortisol levels in sheep with footrot. The comparable total protein levels observed in control sheep and sheep with footrot is an indication to the fact that the overall protein production is not affected by the infection while the hypoalbuminaemia, however indicates protein loss due to acute phase protein response since albumin is a negative acute phase protein. The discrepancy observed in total protein and albumin concentration suggests hepatic compensatory mechanism to maintain protein production by prioritizing globulin over albumin. Interestingly, the unchanged levels of urea, creatinine, AST, ALT and ALP total bilirubin, cholesterol, in sheep with footrot are notable findings which suggests that renal and hepatic functions as well as lipid metabolism and were not significantly altered by *D. nodosus* infection in sheep.

Acute phase proteins are produced by the liver in response to inflammation and tissue damage (Gruys *et al*., 2005; Bode *et al*., 2012). The concentrations of the two positive acute-phase proteins (APP), namely fibrinogen and haptoglobin assayed in this study were significant

increased in sheep with footrot caused by *D. nodosus*. According to Carvalho *et al*. (2012), the fibrinogen, haptoglobin and α1acid glycoprotein APP concentrations did not increase in the course of foot rot in sheep. Breed variation could be responsible for this observable differences.

Conclusion

This study reports the haematological, serum biochemical and acute phase protein profiles in sheep with footrot caused by *D. nodosus* in Yankassa sheep flock in Ilorin, Kwara State. *Dichelobacter nodosus* was isolated from the interdigital lesion of all lame sheep studied. This study has provided useful insights into the pathophysiology and clinicopathological features of footrot caused by D. nodosus in sheep. Further study should be done to investigate the role of APPs in the pathogenesis of footrot and their potential as diagnostic markers and therapeutic strategies for footrot in sheep.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

JAA was a supervisor, that was involved in conceptualization, experimental design, and critical review of the manuscript. OAA, AAA, and AA applied the experiment and were involved in drafting the work. HA and ICI made substantial contribution to the research concept. All authors reviewed and approved the final version of this manuscript for publication.

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