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Ovine footrot: Bacterial isolates and their sensitivity pattern to antimicrobial agents in Oyo State, Nigeria

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ABSTRACT

Aim: Purpose of the study was to determine the incidence of foot rot concerning breeds, sex, and ages, isolates and characterises the bacterial organisms, and establishes their sensitivity pattern to commonly used antimicrobial agents in Nigerian breeds of sheep in Oyo State, Nigeria.

Method and materials: Total 66-foot rot swab samples from sheep with clinical symptoms of foot rot infection were screened for the bacterial organisms between January and June 2023. Standard microbiological methods were adopted for microbiological analyses and antimicrobial susceptibility tests.

Results: Findings revealed that 36 out of 66 screened sheep were positive for either one or both commonest bacterial aetiological organisms (*Dichelobacter nodosus* and *Fusobacterium necrophorum)*. Overall positivity of foot rot etiological organisms of 54.55% was observed. The study revealed that the Yankasa breed was the most susceptible breed, with 63.64%, while the Balami breed of sheep was the least susceptible, with 09.09%. The severity of foot rot showed that sheep having severe infection had the highest positivity (61.10%), while sheep with mild infection had the lowest percentage positivity (25%). The two isolated bacteria (*Dichelobacter nodosus* and *Fusobacterium necrophorum*) were highly resistant to oxytetracycline and tylosin and moderately resistant to sulphadimidine and amoxicillin. But they showed high sensitivity to clindamycin and moderate sensitivity to ofloxacin and enrofloxacin.

Conclusion: It was concluded that foot rot was more common in Yankasa, Ewe, and adult sheep than in other breeds, rams, and young animals. The most common organisms in foot rot were *Dichelobacter nodosus* and *Fusobacterium necrophorum,* which were highly sensitive to clindamycin.

Keywords: Antimicrobial, bacterial, foot rot, occurrence, sheep

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Introduction

In Nigeria, small ruminants play an essential socio-economic role in people's lives: they are slaughtered during ceremonies and festivals and serve as a source of ready cash to smallholder farmers (Olaogun and Oyetoyinbo, 2020). Nigeria has an estimated sheep population of 47,926,860 head with livestock alone contributing about 6% of Nigeria's Gross Domestic Product (GDP) and about 60% of rural dwellers making their livelihoods through livestock production (Aminu et al., 2021). Despite this seemingly huge population, livestock product consumption levels are approximately one-seventh and one-quarter of those in the developed world. This has been linked to suboptimal livestock productivity that does not match human population growth, resulting in declining per capita production of animal products (Aminu *et al*., 2021). One of the limiting factors to optimum livestock production in Nigeria is disease as previously described by (Olaogun *et al*., 2025). One important clinical disease of livestock in Nigeria is foot rot.

Foot rot is an infectious, clinically well-defined

contagious disease that results in severe lameness and financial losses due to decreased flock productivity, particularly in sheep, cattle, and goats. Because the lesions are so painful and frequently cause lameness, the disease affects sheep globally and poses a serious threat to their wellbeing. Foot rot has a significant financial impact on the sheep business since output losses in terms of meat and wool are massive (Marshall *et al.,* 1991). In addition to those losses, the price of curative, preventative, and control methods can be high as well (Winter, 2008). Reduced ewe reproduction (Wassink *et al.,* 2010b), longer lamb fattening times, decreased milk yield, and lower-quality wool are the main causes of direct costs (Gelasakis *et al.,* 2010; Härdi-Landerer *et al.,* 2017).
Foot rot is caused by a complex bacterial combination, of which *Dichelobacter nodosus* is a crucial component, invading the epidermal tissue of the hooves (Allworth, 2014). Unlike other bacteria, *D. nodosus* is rarely discovered in soil or sheep faeces. The bacterium releases toxins that result in the superficial layer of the interdigital skin dying and allowing the growth of additional germs like spirochetes (Green and George, 2008). *Staphylococcus* and *Streptococcus* bacteria, which are opportunistic in nature and commonly present at the nail area and on the skin surface, have a secondary impact on the progression of the disease (Bath *et al.,* 2011).

Several factors may be responsible for the occurrence of foot rot in a flock. These include mechanical injury or puncture wound resulting in weakening and thinning of the inner digital skin, especially between the toes. Prolonged wetness or contact with a moist environment. Overstocking or overcrowding that creates an extremely contaminated environment. Sheep grazing in a damp terrain with rough or sharp pasture. Clinical signs of foot rot, includes distinctive discharge that smells bad, in severe cases, entire hoof capsule may shed, and chronic infection causes hooves to drastically deform and enlarge. Interdigital space swelling, fever, anorexia and decreased milk production are associated with disease. In general, disease causes decreased productivity because of reduced growth rate, infertility-related issues, and higher morbidity and mortality (Dhungyel *et al.,* 2013; Wassink *et al.,* 2010).

According to Green *et al*. (2007), antibiotics can be applied to treat and prevent transmission of pathogens causing foot rot either on an individual basis or a whole-flock basis according to the national regulation of different countries. Antibiotics such as oxytetracycline are recommended to be used either as a topical spray at an early stage of foot rot or as an injectable form of long acting oxytetracycline in severe forms of foot rot (Wassink *et al*., 2010; Strobel *et al*., 2014; Duncan *et al*., 2014). Several authors have recommended other antibiotics that can be used in the treatment of foot rot in sheep, and they are as follows: Penicillin/Streptomycin (Winter, 2008), Tylosin (Moore *et al*., 2005), Lincomycin/Spectinomycin combination (Venning *et al*., 1990), Tilmicosin (Winter, 2008), Erythromycin (Ware *et al*., 1994), and Gamithromycin (Strobel *et al*., 2014).

Despitehuge economic losses associated with foot rot that may include poor performance (feed to meat conversion ratio), fertility problems, increasing culling rates, loss of weight and growth retardation, premature culling, and increased cost of veterinary care and treatment. Its epidemiological data in Nigerian breeds of sheep remain scarce. Also, the global issue of antimicrobial resistance vis-à-vis inappropriate use, abuse of antimicrobial agents, and generalized lack of antimicrobial stewardship in food animals. That are more prevalent in Nigeria with its serious deleterious public health consequences, necessitate the needs for this study. Furthermore, available data revealed a lack of information on foot rot in sheep vis-à-vis its incidence among breeds, ages, and sexes of Nigerian sheep. There have not been any previous data on common bacterial organisms in foot rot and their pattern of sensitivity to commonly used veterinary antimicrobial agents in Nigerian sheep.

The study therefore, enumerated the incidence of foot rot regarding breeds, sexes and ages; isolate the most common bacterial organisms; and establish their sensitivity pattern to commonly used antimicrobial agents in Nigerian breeds of sheep. This will allow for improvement in treatment, prevention, and control measures of foot rot cases and thus help in alleviating productivity losses, animal welfare situations, and livestock production in general.

Materials and Methods

The study was conducted based on the recommendation of the ethical standard of the University of Ibadan's Research Ethics Committee (UI-ACUREC). The protocol and procedures

employed were reviewed and approved by the Ethical Standard of Research Committee.

The study was carried out at some farms and Akinyele livestock market in Oyo State, Nigeria. Oyo State is an inland state situated in the southwest geopolitical zone of Nigeria. It lies entirely in the tropics between latitude 7.1°N and 9.2°N and longitude 2.7°E and 4.56°E of the Greenwich Meridian and covers a land area of about 28,454 square kilometres. The state shares contiguous internal boundaries with Ogun State to the south, Kwara State to the north, and Osun State to the east, while westwards, it presents a common international border with the Republic of Benin and partly with Ogun State. Oyo State has been in the front race as a livestock-producing community with sizeable commercial livestock farming, involving huge enterprises and many households. (Olusoji and Sunday, 2016)

Studied animals and samples collection: The breeds of sheep that were sampled include West African Dwarf, Yankasa, Balami, and Uda. Each of these breeds was morphologically examined, identified, and classified according to body conformation, height, and other distinguishable characteristics. Sex was established based on their reproductive organ's appearance. The ages of the animals were classified based on the record of birth and rostral dentition, while the degree of severity of foot rot was classified based on the extent of the lesion and gait of the animal, as previously described by (Olaogun and Jeremiah, 2018;Olaogun and Esan, 2024).

A total of 66 sheep showing clinical symptoms of foot rot were sampled across the locations using a sterile swab stick to take swab samples from the infected wound on each of the sheep. The breed, sex, and age of sheep, and level of severity of foot rot infection were recorded appropriately (Olaogun and Adedokun, 2020a, Olaogun and Adedokun, 2020b). Foot rot swab samples were sampled anaerobically from indigenous breeds of sheep, and clinical manifestations of foot rot were observed with the use of swab sticks. The samples were transferred in an anaerobic jar from the site of collection to the bacteriological laboratory of the Department of Veterinary Medicine, University of Ibadan.

Bacterial Isolation and Identification: Before culturing, the media used were kept in jars along with a commercial kit (AnaeroGenTM, Oxoid Ltd, Basingstoke, Hampshire, England) to provide anaerobiosis. Samples were grown directly on blood and MacConkey agar culture in a Co_2jar at 37°C for 48 hours based on standard procedures as described by (Cowan, 1993; Olaogun *et al*.,2016; Olaogun *et al*., 2018). The bacterial organisms were initially established based on the morphological characteristics of the culturing colonies and the organism. Samples were subsequently cultured on Eugon agar enriched for isolation of D. nodosus and Fusobacterium selective agar (FSA) with 5% defibrinated sheep blood and other components (i.e., haemin, tween 80, and dithioeritrol), but without antibiotic supplements (i.e., neomycin and vancomycin) for isolation of *D. nodosus* and *F. necrophorum*. Colonies were evaluated under a microscope (BX43, Olympus, Tokyo, Japan) after incubation at 37 °C for 3 d in terms of morphology, haemolytic properties, incubation needs, and Gram staining (Ozgen *et al*., 20`5). To confirm the identification of *F. necrophorum*, a marked β-haemolysis on blood agar enriched with 5% sheep blood in conjugation with resistance to vancomycin and susceptibility to polymyxin and the specific biochemical tests were applied as described by (Quinn *et al*., 2011). Positivity of foot rot was determined based on isolation of either *D. nodosus*, *F. necrophorum* or both organisms, while samples that were negative for any of the two organisms were classified as foot rot negative sheep.

Antibiotic susceptibility tests: The test was carried out using the Kirby-Bauer agar diffusion method. The foot rot swab samples that tested positive for either *Dichelobacter nodosus*, *Fusobacterium necrophorum* or both were streaked onto Tryptic soy agar (TSA) and incubated for 24 hours. An antibiotic disc containing eleven antibiotics that includes amoxicillin, gentamicin, oxytetracycline, sulphadimidine, enrofloxacin, ciprofloxacin, erythromycin, tylosin, penicillin-streptomycin, ofloxacin, and clindamycin was prepared using 1 ml of the drugs to 1 ml of distilled water. Mueller-Hinton agar (MHA) was prepared and used as a growth medium for the test. The colony of bacteria was put inside a new swab stick with normal saline and mixed until it was free from cloudiness and spread throughout the surface of the Mueller-Hinton agar plate prepared. The antibiotic sensitivity discs were impregnated on the surface of the Mueller-Hinton agar and were incubated for 24 hours. A ruler with the nearest millimetre was used to measure the inhibition zone diameters. Isolates were classified as susceptible, intermediate and resistant, as described by (Wayne, 2017).

Statistical analysis: All data were analysed using JMP® 11, NC: SAS Institute Inc. software version. Descriptive statistics were used to establish the distribution and number/percentage of occurrence of foot rot in relation to foot rot based on location, breed, sex and age of sheep.

Results and Discussion

It was revealed the distribution of clinical foot rot infection in sheep in Oyo State (Table 1). Out of a total of 66sheep showing clinical symptoms of foot rot sampled, 66.67% were found in Akinyele Sheep and Goats Market, 30.30% were found in Olarenle Oloko Farm, Ogbomosho, while the least, 3.03%, were found in the University of Ibadan Teaching and Research Farm. Breeds distribution shows as follows: The highest is 63.64% in the Yankasa breed, followed by 15.15% in the WAD, 12.12% in the Uda, and the least being 09.09% in the Balami breed of sheep. Sex distribution shows 72.73% in females and 27.27% in males, while age distribution indicates 63.64% in adults and 36.36% in young animals, respectively.

Table 1: Distribution of clinical foot rot in sheep based on location, breed, sex and age in Oyo state

Location	**Frequency (n=66)**	**Percentage (%)**
OlarenleOloko farm (A1)	20	30.30
University of Ibadan Teaching and Research Farm (A2)	2	3.03
Akinyele Sheep and Goat market (A3)	44	66.67
Breed	Frequency (n=66)	Percentage (%)
West African Dwarfs (WAD)	10	15.15
Yankasa	42	63.64
Balami	6	09.09
Uda	8	12.12
Sex	Frequency (n=66)	Percentage (%)
Male	18	27.27
Female	48	72.73
Age	Frequency (n=66)	Percentage (%)
Young	24	36.36
Adult	42	63.64

It was represented positivity for *F. necrophorum* and B. *nodosus* among foot rot-infected sheep in Oyo State (Table 2). The highest positivity of organisms was 60% at Olarenle Oloko farm, Ogbomosho, followed by 54.55% at Akinyele Sheep and Goat Market and 0% at University of Ibadan Teaching and Research Farm. Overall positivity of foot rot etiological organisms of 54.55% was observed in Oyo State.

Table 2: Number and percentage positivity for F. necrophorum and B.nodosus among foot rot infected sheep based on locations in Oyo State

Locations	**Total sampled N=66**	**Number positive N=36**	**% Positive %=54.55%**
Olarenle Oloko farm (A1)	20	12	60%
University of Ibadan Teaching and Research Farm (A2)	2	0	0%
Akinyele Sheep and Goat market (A3)	44	24	54.55%

It was showed the number and percentage of samples positive for *F. necrophorum* and B. *nodosus* in different breeds of foot rot-infected sheep in Oyo State (Table 3). The highest positivity of 100% was observed in the Balami breed, followed by 66.67% in Yankasai, 25% in Uda and the least positivity of 0% in West African Dwarf sheep.

Table 3: Number and percentage positivity for *F. Necrophorum* and *B. Nodosus* based on breeds of foot rot infected sheep in Oyo State.

Breeds	**Total sampled N=66**	**Positive N=36**	**Negative N=30**	**% Positive %=54.55%**
West African Dwarfs (WAD)	10	0	10	0%
Yankasa	42	28	14	66.67%
Balami	6	6	0	100%
Uda	8	2	6	25%

It was presented the number and percentage of samples positive for *F. necrophorum* and B. *nodosus* in different age groups of foot rot-infected sheep in Oyo State (Table 4). The highest positivity of 75% of foot rot etiological bacterial organisms was detected among 3-year age category, followed by 66.70% observed among 5-year category, 50% in 4-year category, 40% positivity among 2-year category and 0% in 1-year age group of foot rot-infected sheep.

It was recorded the number and percentage of samples positive for *F. necrophorum* and B. *nodosus* in different sexes of foot rot-infected sheep in Oyo State (Table 5). The highest positivity of 62.50% of foot rot etiological bacteria was observed in female sheep compared to the lowest positivity of 22.20% detected in male sheep.

Table 4: Number and percentage positivity for *F. Necrophorum* and *B. Nodosus* based on age groups of foot rot infected sheep in Oyo State**.**

Ages	Total sampled N=66	Positive N=36	Negative N=30	% Positive %=54.55%
1year	4	0	4	0%
2 years	20	8	12	40%
3 years	24	18	6	75%
4 years	12	6	6	50%
5 years	6	4	2	66.70%

Table 5: Number and percentage positivity for *F. Necrophorum* and *B. Nodosus* based on sexes of foot rot infected Sheep in Oyo State.

Sex	Total sampled N=66	Positive N=36	Negative N=30	% Positive %=54.55%
Male	18	6	12	33.33%
Female	48	30	18	62.50%

It was recorded the number and percentage of samples positive for *F. necrophorum* and B. *nodosus* based on different levels of severity of infection of foot rot in sheep in Oyo State (Table 6). The highest positivity of 61.10% was observed among severely affected foot rot-infected sheep, followed by 54.55% in the moderate infection category, and the least positivity of 25% was observed in animals categorised as mildly infected.

Table 6: Number and percentage positivity for *F. Necrophorum* and *B.nodosus* based on severity of foot rot in infected sheep in Oyo State.

Severity of infection	Total sampled N=66	Positive N=36	Negative N=30	% Positive %=54.55%
Severe	36	22	14	61.11%
Moderate	22	12	10	54.55%
Mild	8	2	6	25.00%

It was revealed the susceptibility pattern of *Fusobacterium necrophorum* to commonly used veterinary antimicrobial agents as follows: findings indicate 100% resistance to erythromycin (R), tylosin (T), oxytetracycline (O) and amoxicillin (A); and 80% resistance to sulphadimidine (S) and 40% resistance to both clindamycin (L) and ofloxacin (F), respectively (Fig. 1). 20% resistance was observed with Enrofloxacin (E), Penicillin-Streptomycin (P), Ciprofloxacin (C), and Gentamicin (G), respectively. The organism was 20 % sensitive and 60% intermediately susceptible to enrofloxacin (E), penicillin-streptomycin (P), and ciprofloxacin (C), respectively. While the organism was 20% sensitive and 40% intermediately susceptible to Clindamycin (L) and Ofloxacin (F), respectively.

It was revealed the sensitivity pattern of *Dichelobacter nodosus* to commonly used veterinary antimicrobialagents as follows: findings indicate the five isolates were 100% resistant to Tylosin (T), Oxytetracycline (O), and Sulphadimidine (S). 80% and 60% resistance to gentamicin (G) and amoxicillin (A), respectively (Fig. 2). 40% resistance to erythromycin (R), while they were 20% resistant to enrofloxacin (E), penicillin-streptomycin (P), ciprofloxacin (C) and ofloxacin (F), respectively. All five isolates were 40% sensitive to Clindamycin (L) and 20% sensitive to Enrofloxacin (E), Erythromycin (R), and Ofloxacin (F), respectively. The isolates were 80% intermediately sensitive to penicillin-streptomycin (P) and ciprofloxacin (C), respectively. They were 60% intermediately sensitive to Enrofloxacin (E), Clindamycin (L) and Ofloxacin (F), respectively. While they were 40% intermediately sensitive to erythromycin (R) and amoxicillin (A), respectively.

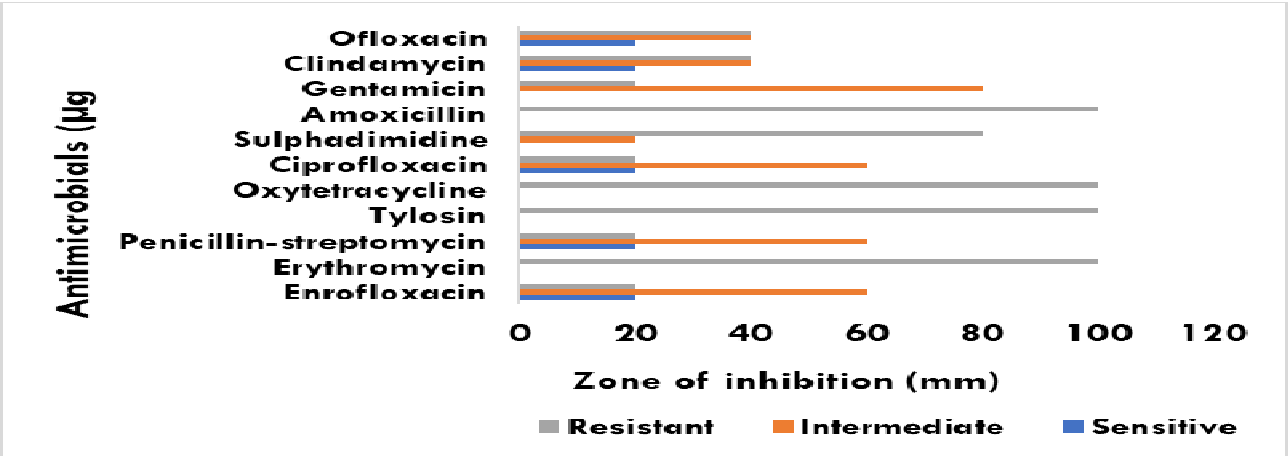

Fig. 1: Graphical representation of the summary of the antibiotics sensitivity result for *Fusobacterium necrophorum.* Number of isolates n = 5

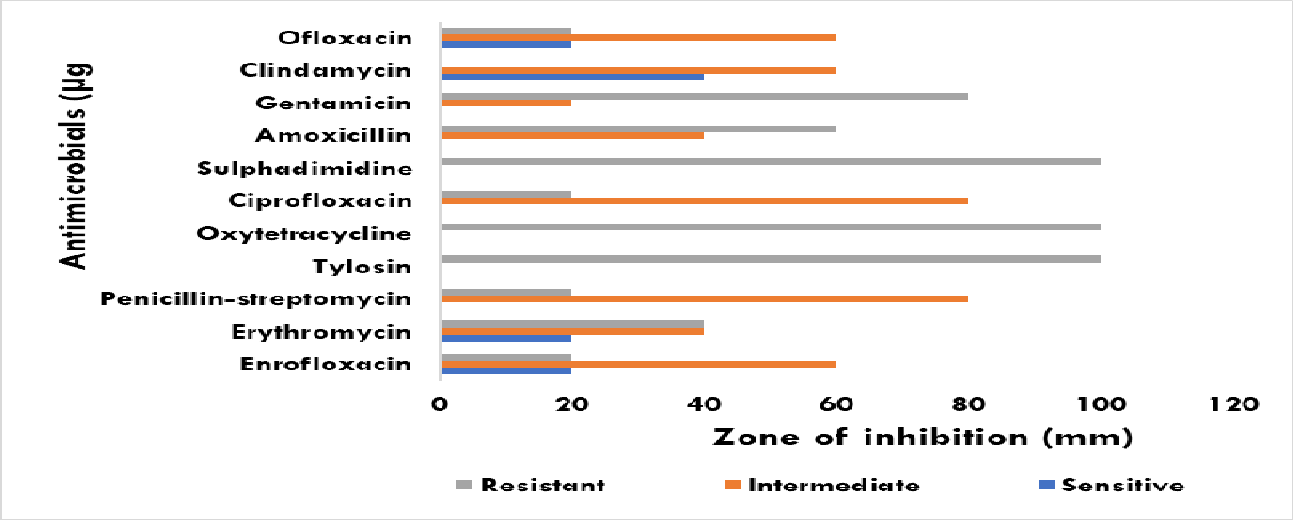

Fig. 2. Graphical representation of the summary of the antibiotics sensitivity result for *Dichelobacter nodosus*. Number of isolates n = 5

Most cases of foot rot encountered during this study were accompanied by various degrees of lameness. This observation is in tandem with the earlier report of (Knappe-Poindecker *et al*., 2014), who stated that the presence of severe hoof lesions is always manifested in lameness in most of the affected sheep. Foot lesions, especially deep-rooted lesions, increase the chance of contamination and infection with pyogenic bacteria, which can cause foul abscesses due to pyogenic bacteria behaviour and odour. These findings also agree with the observation of (Aguiar *et al*., 2011; Bitrus *et al*., 2017) in terms of infection and clinical presentation. Observation from this present study reveals lesion severity ranges from mild to severe and purportedly relates to the nature of the proteolytic enzymes produced by *Dichelobacternodosus*, although contributions from other bacteria are also likely. *Dichelobacter nodosus* produces several extracellular proteases, including gelatinase, elastase, fibrinogenase, collagenase and caseinase, which agrees with the report of (Green, 1985), who also isolated *Dichelobacternodosu s*from cases of foot rot. Breeds' susceptibility to foot rot among Nigerian indigenous sheep indicates that all breeds are susceptible, but some are more susceptible, as there was more incidence of foot rot in the Yankasa breed compared to other breeds.

This observation conforms with the earlier findings of (Emery *et al*., 1984; Greber and Steiner, 2013), who in their various works reported that there was evidence that some breeds, such as Merinos (a popular breed in Australia and New Zealand for wool production) or brown-headed meat sheep (a Swiss multi-purpose breed), are more susceptible than others; however, such findings could not be confirmed by other studies. The general observation of more cases of foot rot in adult animals compared to young animals may be due to low immune status associated with old age and the presence of concurrent infection in adult animals because of immunosuppression. This is in conformity with the earlier observation of (Ardüser *et al*., 2020), who also reported a higher incidence of foot rot in adult animals compared to young ones. This finding, however, contradicts the findings of (Angell *et al*., 2018), who reported that yearling sheep (adults) are far less likely to have foot rot than lambs. More incidences of foot rot cases observed in female sheep compared to male sheep may be associated with the generally larger population of female sheep (ewe) compared to male sheep (ram) and may also be due to the higher fragility of females and the more resilient potential of rams compared to ewes. This agrees with the observation of (Raadsma and Egerton, 2013; Storms *et al*., 2022), who observed in their various studies more cases of foot rot in female compared to male animals.

The variation in severity of foot rot as observed in this study is in conformity with (Abbott and Egerton, 2003), who also described that the severity is a function of the virulence factors of the *D. nodosus* strain. Mild strains of *D. nodosus* will result in interdigital dermatitis with no corneal tissue separation; this made it difficult to distinguish clinically interdigital skin inflammation majorly associated with *F. necrophorum*.

Green and George (2008) had previously reported that mild foot rot regresses spontaneously in most

infected animals as the environment becomes dry.

The primary detection and classification of samples with either or both of *Fusobacterium necrophorum* and *Dichelobacter nodosus* as foot rot-infected sheep in this study is in tandem with the findings of (Winter, 2008; Bennett *et al*., 2009), who also reported that foot rot is caused by the synergistic action of two anaerobic Gram (-ve) bacteria, namely *F. necrophorum* and *D. nodosus.* Incidentally, more sheep were positive with *F. necrophorum* compared to *D. nodosus.* This may be because *F. necrophorum* are commensals in the rumen and can be seldomly detected in the faeces, and environmental contamination may be inevitable, especially in moist or unhygienic environments in general. This agrees with the earlier report of (Kontturi *et al*., 2019), who also detected that *F. necrophorum* was the main aetiology of interdigital phlegmon in cattle. But this is in contrast with (Wani and Samanta, 2006), who reported *D. nodosus* as the main aetiological agent and that *F. necrophorum*played a synergistic effect in the pathogenesis of foot rot, as it causes interdigital dermatitis that enables invasion by *D. nodosus.*

F. necrophorum was observed to be moderately susceptible to enrofloxacin, penicillin-streptomycin, and ciprofloxacin. While being resistant to erythromycin, tylosin, oxytetracycline and amoxicillin. This pattern of susceptibility differs from the earlier report of (Bergsten et al., 2017), who observed that all isolates of *F. necrophorum* were all susceptible to all tested antimicrobial agents, which were penicillin, tetracycline, cefuroxime and cefotaxime. This may be due to different test methods adopted in the two studies; the earlier test was determined by E-test, while our study adopted the Kirby-Bauer agar diffusion method. This also contradicts the earlier report of antimicrobial resistance not being characteristic of *F. necrophorum* in interdigital phlegmon in Norwegian dairy herds (Knappe-Poindecker *et al*., 2013). This different observation may be associated with the appropriate regulations and enforcement necessitating the judicious/prudent use of antimicrobial agents in dairy operations in Norway.

D. nodosus was observed to be moderately susceptible to clindamycin, enrofloxacin, erythromycin, and ofloxacin, while being completely resistant to tylosin, oxytetracycline, and sulphadimidine and being moderately resistant to gentamicin and amoxicillin. This was in agreement with the observation of (Frosth *et al.,* 2013), who observed that all *D. nodosus* isolates from sheep herds with clinical signs of foot rot were susceptible to enrofloxacin, erythromycin, penicillin and tetracycline.

Conclusion

It was concluded that higher occurrence of foot rot was in Yankasa breed, Ewe and adult sheep compared to other breeds, Ram and young animals. *Fusobacterium necrophorum* and *Bacteroides nodosus* were the most common organisms isolated from foot rot cases in Nigerian breeds of sheep. The organisms were susceptible to Clindamycin and moderately sensitive to Ofloxacin and Enrofloxacin. *Recommendation:* Clindamycin is recommended as the most effective antimicrobial agent, followed by Ofloxacin and Enrofloxacin. Most importantly, attention should be given to the management of sheep by ensuring that the environment where sheep are kept is properly cleaned and dry. Appropriate policies guiding the prudent/judicious use of antimicrobial agents in food animals should be formulated and strictly enforced.

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