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Antimicrobial Susceptibility of Aerobic Bacteria and Fungi Isolated from Cases of Equine Ulcerative Lymphangitis in Kano Metropolis, Nigeria

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ABSTRACT

This study was carried out to isolate possible aerobic bacteria and fungi that may be associated with cases of ulcerative lymphangitis in horses in Kano metropolis and also carry out an antimicrobial susceptibility tests on the bacteria isolated. In this study, 36 wound swabs were collected from horses with clinical cases of ulcerative lymphangitis in Kano metropolis, Nigeria for purposes of culture, isolation and identification of bacterial and fungal organisms associated with the disease. *Corynebacterium pseudotuberculosis, Staphylococcus* spp. and *Streptococcus* spp. were isolated with frequencies of 22.22, 30.56 and 11.11%, respectively; 36.11% of the samples yielded no bacterial isolates. 38.46% of the fungal organisms isolated were *Aspergillus fumigatus*, 26.92% were *Aspergillus niger*, 23.08% were *Mucor* spp. and 11.54% were *Trichophyton* spp. The antibiotic sensitivity carried out on the bacterial isolates showed fluroquinolones (ciprofloxacin and perfloxacin) and amoxicillin to be the most effective against most of the organisms isolated but at varying degrees. In this study, both bacteria and fungi were implicated in the disease process of ulcerative lymphangitis in Kano, Nigeria and the breeds most affected were: the 'Arewa', 'Sudan' and 'Talon' breeds (all Arabian cross in nature). In the treatment of ulcerative lymphangitis, an antimicrobial sensitivity should be carried out in order to ascertain the most sensitive drug(s) to be used and where this is not possible, the fluoroquinolones or the penicillins could be used alongside anti-mycotic agents.

Key words: Ulcerative lymphangitis, bacteria, fungi, fluoroquinolones, penicillins, Kano

INTRODUCTION

Corynebacterium spp. is gram-positive pleomorphic rods that are associated with two clinical syndromes in horses: abscesses and ulcerative lymphangitis (Miers and Ley, 1980). Infections are most common in the pectoral muscles, but other locations are possible. Affected muscles are swollen and edematous and contain variably sized zones of localized suppurative inflammation. Fever is common. The causative agent is readily isolated from the affected tissues and can be seen in aspirates from intramuscular abscesses (Valentine and McGavin, 2007). Intramuscular abscess due to *Corynebacterium pseudotuberculosis* occurs almost exclusively in horses in arid regions of Western United States and Brazil. Bacterial exotoxins such as phospholipase D are normally

released which contribute to vascular damage and inhibition of neutrophil function. *C. pseudotuberculosis* is a gram positive pleomorphic facultatively anaerobic bacillus present within the soil. It can enter the muscle by penetrating open wounds (Valentine and McGavin, 2007). While a number of microorganisms have been associated with ulcerative lymphangitis, *Corynebacterium pseudotuberculosis* is the most common cause (Pratt, 2009). However, in some other cases a bacterial culture may be negative. This negative result may be recorded because the microorganism responsible is difficult to culture (e.g. many *Mycoplasma* species) or the organism has been effectively eliminated by the immune system and the pathology is due to an excessive immune response after the organism has been cleared. The organism may even be a fungus. Other implicated organisms include *Streptococcus* spp. *Staphylococcus* spp. *Rhodococcus equi* (Bain, 1963) and *Pseudomonas aeruginosa* (Azizuddin and Chandrasekharan, 1954; Littlewood *et al.,* 1998). In Nigeria, ulcerative lymphangitis is found in all regions especially in the Northern part (Useh *et al.*, 2005; Mshelia *et al.*, 2010) where horse population is higher and where there is regular concentration of horses for polo and racing tournaments and during celebrations like the Durbar.

The disease is sporadic occurring as scattered outbreaks in horse populations. When it occurs it can be devastating causing extensive lymphangitis and skin ulcerations which usually result in lameness. The disease can be severely debilitating in some cases and can even result in deaths. Various treatment protocols are being used in the management of ulcerative lymphangitis but the disease seems to almost always recur in an animal that has once had the clinical disease. Many have attributed this to the location of the causative organism in the lymphatic system, thus due to the selective permeability of the lymphatics it is believed that very low concentrations of the drugs get to the organism causing the disease. Also, *Corynebacterium pseudotuberculosis* is an intracellular organism as the high lipid content of the cell wall contributes to its survival within macrophages (Valentine and McGavin, 2007) therefore an antibiotic could be effective *in-vitro* that may not be effective for the organism *in-vivo* (Orsini *et al.*, 2005).

Ulcerative lymphangitis has been reported in Nigeria since the 1980s and has been reported to be a devastating disease of horses causing reduced performance of recovered sporting horses (Addo, 1980, 1983; Useh *et al.*, 2005). The purpose of this study was to ascertain the exact micro organisms involved in this disease process and their response to various antibiotics so as to effectively control the disease entity which has over the years been refractory to treatment with the common antibiotics as reported in other part of the world (Singh, 2010).

METERIALS AND METHODS

Study area and sample collection: Clinical cases of ulcerative lymphangitis in horses were identified at various locations in the city of Kano in Northern Nigeria. The work was carried out between May to September, 2009. Locations covered were the Polo Club, Race course and the Kano City, all are within Kano metropolis. Kano is located in the North-Western part of Nigeria falling within latitude 8°30' N and longitude 12°30' E. The horses were restrained (using halters and hobbles) and the lesions were then properly disinfected using cotton wool soaked with methyl spirit to avoid any extraneous contamination of the samples to be collected. The pus was expressed by pressing the lesions with a gloved hand. Uncontaminated swabs of the pus were then taken, labeled and placed on ice packs. In the case of those horses that did not have the skin ulcers but had developing abscesses, the skin over the abscess was properly disinfected and aspirates of the abscesses were taken using 5 mL syringes and 18 G needles. These were also labeled and immediately put on ice packs for transportation to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for immediate processing.

Cultural isolation and macroscopic identification: The samples once at the laboratory were cultured on sheep blood agar (appropriately labeled for each sample) by smearing the surface of the media with the sample swab sticks and primary, secondary and tertiary streaks were made using improvised Pasteur loops. The growth media were then incubated at 37°C for 24 h. The sample swabs were also smeared on the surface of Sabouraud's Dextrose Agar (SDA) contained in sample bottles for fungal isolation. Incubation was done for 3-14 days at room temperature. Growths obtained after cultural isolation were described macroscopically, i.e., cultural characteristics, stating their color, size, shape, hemolytic effect on blood agar, smell, texture and consistency according to standard methods by Cheesbrough (1991).

Microscopic identification: The bacterial growths were Gram-stained and viewed with the light microscope under oil emersion objective (X100), while the fungal growths were stained with lactophenol cotton blue stain and viewed under low objective (X10 or X40).

Biochemical tests: The isolates were subjected to biochemical characterization using catalase test, oxidase test, coagulase test, urease test and nitrate reduction test.

Antibiotics sensitivity test: Antibiotics susceptibility test was carried out on the various isolated bacterial organisms to establish an antibiotics profile for each of them. This was done using the disc diffusion method which was introduced in the 1940s by Heatley (1944). Pure cultures of the various bacterial organisms were smeared evenly on the surfaces of freshly prepared nutrient agar plates. The antibiotic discs (from Abtek biologicals Ltd.) were strategically placed on the smeared surfaces and incubated at 37°C for 24 h after which the zones of inhibition were measured in millimeters and recorded.

RESULTS

Table 1 showed that *Staphylococcus* spp. was the most frequently isolated organism from the cases of ulcerative lymphangitis examined with a frequency of 30.56%. *Corynebacterium* spp. was next with 22.22% and the least frequently isolated was *Streptococcus* spp. having a frequency of isolation of 11.11%. The remaining samples yielded no growths (36.11%).

Table 2 showed that *A. fumigatus* was the most frequently isolated fungus with a frequency of 38.46%, followed by *A. niger* with a frequency of 26.9%, then *Mucor* spp. (23.08%) and lastly *Trichophyton* spp. having a frequency of 11.54%.

Table 1: Frequency of isolation of bacterial organisms associated with ulcerative lymphangitis in Kano, Nigeria

Bacteria types	No. of isolates	Total No. of samples collected	Frequency of isolation (%)
Corynebacterium spp.	8	36	22.22
Staphylococcus spp.	11	36	30.56
Streptococcus spp.	4	36	11.11

Table 2: Frequency of isolation of fungal organisms associated with ulcerative lymphangitis in Kano, Nigeria

Fungal isolates	Frequency of isolation (%)
Aspergillus fumigatus	38.46
Aspergillus niger	26.92
Trichophyton spp.	11.54
Mucor spp.	23.08

Table 3: Antibiotics sensitivity of *Corynebacterium* spp. associated with ulcerative lymphangitis in Kano, Nigeria

Antibiotics	Concentration (mcg)	Zone of inhibition (mm)	Interpretation
Pefloxacin	10	10	++
Gentamicin	10	11	++
Zinnacef	20	9	+
Amoxycillin	30	6	+
Rocephin	30	8	+
Ciprofloxacin	10	>15	+++
Streptomycin	30	8	+
Septrin	30	13	++
Erythromycin	10	13	++

Table 4: Antibiotics sensitivity of *Staphylococcus* spp. associated with ulcerative lymphangitis in Kano, Nigeria

Antibiotics	Concentration (mcg)	Zone of inhibition (mm)	Interpretation
Pefloxacin	10	8	+
Gentamicin	10	7	++
Zinnacef	20	7	+
Amoxycillin	30	11	++
Rocephin	30	0	Resistant
Ciprofloxacin	10	11	++
Streptomycin	30	7	+
Septrin	30	7	+
Erythromycin	10	0	Resistant

Table 5: Antibiotics sensitivity of *Streptococcus* spp. associated with ulcerative lymphangitis in Kano, Nigeria

Antibiotics	Concentration (mcg)	Zone of inhibition (mm)	Interpretation
Pefloxacin	10	8	+
Gentamicin	10	7	+
Zinnacef	20	8	+
Amoxycillin	30	>15	+++
Rocephin	30	0	Resistant
Ciprofloxacin	10	14	++
Streptomycin	30	11	++
Septrin	30	0	Resistant
Erythromycin	10	13	++

Table 3 showed the inhibitory effects of commonly used antibiotics on *Corynebacterium* spp. Ciprofloxacin had complete inhibitory effect indicating highest sensitivity. This was followed by pefloxacin, gentamicin, septrin and erythromycin. The least sensitivity was exhibited by zinnacef, amoxicillin, rocephin and streptomycin.

Table 4 showed the inhibitory effects of commonly used antibiotics on *Staphylococcus* spp. It showed that gentamicin, amoxycillin and ciprofloxacin had a high inhibitory effect, while pefloxacin, zinnacef, streptomycin and septrin had a lower inhibitory effect. *Staphylococcus* spp. showed resistance to rocephin and erythromycin.

From Table 5, it could be seen that amoxycillin had a complete inhibitory effect on *Streptococcus* spp. Ciprofloxacin and erythromycin also had high inhibitory effects. Pefloxacin, gentamycin and zinnacef exhibited low sensitivity. *Streptococcus* spp. was resistant to rocephin and septrin.

DISCUSSION

Breed predisposition has not been a topic of discussion with regards susceptibility to ulcerative lymphangitis. But in this research, the most frequently affected horses belonged to either of the 'Arewa', 'Sudan' or 'Talon' breeds (all Arabian cross in nature). This may be due to management practices. The expensive and Argentine horses used for polo are usually managed under strict hygienic conditions because they are highly valuable, thus they are less likely to pick up this infection as it is related to poor hygiene. It was also observed from this study that the most affected horses were between the ages of 3 and 7 years. Conceivably, this could be attributed to a high level of activity seen at this age range which normally results in injuries that could get contaminated with the micro-organisms that cause ulcerative lymphangitis. This finding agreed with the report by Pratt (2009) which states that 'horses of 1- 5 years of age are more susceptible to infection by *Corynebacterium pseudotuberculosis*, but adult horses of any age could be affected'. It was also observed from this research, relying on available records, that ulcerative lymphangitis was more prevalent during the rainy season. This is in agreement with reports by Khan *et al.* (2005) who stated that *Corynebacterium pseudotuberculosis* infection in horses is seasonal, with a peak incidence in late summer and fall. This can be attributed to the favorable environment for bacterial proliferation available during the rainy season due to high moisture and humidity. Some of the cases of ulcerative lymphangitis from which samples were collected had lesions on the pectoral muscles (Fig. 1) which showed that they had "pigeon breast" form of the disease. Some other ones showed typical nodular and ulcerative lesions affecting the entire limbs (Fig. 2) which agreed with descriptions given by Khan *et al.* (2005). The disease caused by *Corynebacterium pseudotuberculosis* is said to manifest in three different forms which are: ulcerative lymphangitis, external and internal abscesses (Aleman and Spier, 2002).

The pattern of organisms isolated from the cases of ulcerative lymphangitis from Kano metropolis is in agreement with the findings of Azizuddin and Chandrasekharam (1954) and Bain (1963), who in their various studies, reported possible aetiologies of lymphangitis to include *Corynebacterium pseudotuberculosis*, *Staphylococcus* spp. and *Streptococcus* spp., etc. Table 1 showed the presence of other bacteria such as *Staphylococcus* and *Streptococcus* aside the main causative agent, *Corynebacterium pseudotuberculosis*. This is because ulcerative lymphangitis is commonly

Fig. 1: Lesion on the breast muscle of a horse in Kano, Nigeria caused by *Corynebacterium pseudotuberculosis* infection showing typical 'Pigeon breast' form of the disease

Fig. 2: Lesions of ulcerative lymphangitis in a horse in Kano, Nigeria, showing typical pattern of attack on the lymphatic vessels which may affect the entire limb

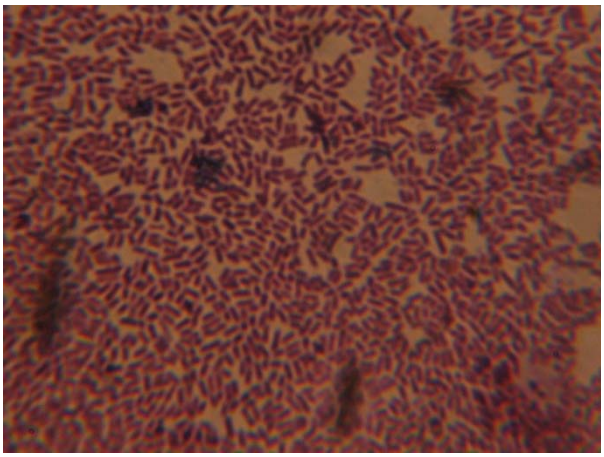

Fig. 3: Microscopic appearance of *Corynebacterium pseudotuberculosis* isolated from a horse with ulcerative lymphangitis in Kano, Nigeria showing typical gram positive rods at X 1000 magnification

associated with a wound which serves as a likely portal of entry for other bacteria. The specific species of *Corynebacterium* isolated in this study is *Corynebacterium pseudotuberculosis*. This is because the bacteria isolated were gram positive rods with typical Chinese letter arrangements (Fig. 3). They exhibited a narrow zone of **β**-hemolysis when cultured on sheep blood agar, they were catalase positive, fermenters on oxidative fermentative medium, urease positive and nitrate negative being distinguishing features for *Corynebacterium pseudotuberculosis* as reported by Efstratiou and George (1999). Though fungal agents have been incriminated in the disease process of ulcerative lymphangitis aside bacteria, no specific species have been implicated to the best of our knowledge. In this study however, fungal agents such as *Aspergillus fumigatus, A. niger, Trycophyton* spp. and *Mucor* spp. were isolated (Table 2). The cases could not have been said to be epizootic lymphangitis because the dimorphic fungus, *Histoplasma capsulatum var farciminosum* was not isolated which is reported as the causative agent of epizootic lymphangitis (OIE, 2008). The

findings in this work with regards the fungal isolates, may explain why ulcerative lymphangitis has been refractory to treatment with antibiotics in Kano.

The antimicrobial susceptibility tests carried out on the three bacterial isolates in this study generally revealed that the fluoroquinolones (especially ciprofloxacin) and the penicillins (especially amoxicillin) consistently exhibited high efficacy against all the bacteria. This was so probably because of the fact that the fluoroquinolones, being a third generation group of antibiotics, are bactericidal and have a broad spectrum of activity; therefore they are very potent and resistance to them by bacteria is minimal. The penicillins on the other hand were also very effective probably because they have high specificity for gram-positive bacteria. Next in efficacy were the aminoglycosides (especially streptomycin and gentamycin) and then the macrolide antibiotics especially erythromycin. The other antibiotics were zinnacef, rocephin and septrin which exhibited very low sensitivity and even complete resistance in some instances.

CONCLUSION AND RECOMMENDATIONS

From the results of this research, it can be said that *Corynebacterium pseudotuberculosis*, *Staphylococcus* spp., *Streptococcus* spp. and fungi are involved in the disease process of ulcerative lymphangitis. Ciprofloxacin and amoxicillin are the most effective antibiotics against the bacterial agents responsible for ulcerative lymphangitis in Kano, Nigeria.

It is therefore recommended that in the management of horses, a high level of hygiene should be observed. All skin wounds and bruises should be promptly washed, disinfected and dressed to prevent contamination with bacteria and fungi which could progress to ulcerative lymphangitis.

In the treatment of cases of ulcerative lymphangitis, a drug sensitivity test should be carried out on organisms encountered. Where this is not possible, the fluoroquinolones or penicillins can be used with a degree of certainty to produce favorable results. The first choice is ciprofloxacin because it is bactericidal, broad spectrum, distributes well in tissues and more importantly for our case, it is known to accumulate in the macrophages and polymorphonuclear leucocytes thus effective against intracellular organisms (Aliu, 2007; Dowling *et al*., 2008) and the main causative agent here is *Corynebacterium pseudotuberculosis* which is said to be intracellular. The use of antifungal agents both topically and systemically is also recommended.

Finally, it is worth mentioning that this disease has threatened the entire equine population in Nigeria and the developing world alike raising a fundamental issue about equine welfare (Fazili and Kirmani, 2011).

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