

INCIDENCE OF HOOF LAMENESS AND ASSOCIATED STAPHYLOCOCCAL WOUND CONTAMINANTS AMONG HORSES AT OBOLLO-AFOR MARKET IN ENUGU STATE, NIGERIA

¹EZE, Chinedu Athanasius, ¹OGBANYA, Kenneth Chiedozi, ²EZE, Didacus Chukwuemeka and ¹OKONKWO, Noble

¹Department of Veterinary Surgery and Radiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

²Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Corresponding Author: Ogbanya, K. C. Department of Veterinary Surgery and Radiology, University of Nigeria, Nsukka, Enugu State, Nigeria. **Email:** Kenneth.ogbanya@unn.edu.ng **Phone:** +234 8036027640

ABSTRACT

Hoof is the commonest site of lameness in horses; the condition is exacerbated by infection resulting from contaminants which are very common in their environment. This study determined the incidence of hoof lameness of horses in Obollo-Afor market. Relationship between lameness and sex, type of lameness, level of different limb involvements among the affected horses, presence of Staphylococcal contaminant and its phenotype identification as well as the antibiogram of the hoof wound isolates were determined. One hundred and seventy horses were presented for sale during the study period (May – July, 2012). The ratio of male to female was 7:3. Approximately, thirty-four percent (34.1%) of both sexes have one type of lameness or the other. In male and female population, 38.4% and 22.2% were affected by lameness, respectively. The major types of lameness shown by the affected horses were either fore (male – 12.8%, female – 8.9%) or hind limbs (male – 22.4%, female – 11.1%) as well as wobbling (5.4% of both sexes). Staphylococcus species were isolated in 76% of the samples collected and of this number 22% was haemolytic while the rest were non-haemolytic isolates. Both haemolytic and non-haemolytic isolates were susceptible to Gentamicin, Tigecycline in that order.

Keywords: Horses, Hoof lameness, Staphylococcal infection, Haemolytic and non-haemolytic isolates, Antibiotic susceptibility

INTRODUCTION

Lameness is an abnormality of gait that is caused by pain or restriction of movement. Most of the injuries are muscular or skeletal lameness (Churchill, 1979; La Pierre, 2001). Lameness can be as a result of chipped bones, bowed tendons and other soft tissue injuries. Foot injuries can be caused by trauma such as sharp objects like nails, penetrating the sole of the hoof, damage to the corium from decreased blood flow, bacteria migrating into the defects,

fissures and cracks in the white line or as a result of degenerative process. Hoof is the commonest site of lameness in horses; the condition is exacerbated by infection resulting from contaminants which are very common in their environment. Factors that predispose horses to lameness and hoof infection include; improper hoof trimming, insufficient natural movement, unhygienic living condition and weakened immune system (Khanna *et al.*, 2007).

The presenting clinical signs may be in form of abnormal stance such as pointing the toe, resting one leg more than another, abnormal movement like head nod (forelimb lameness), hip hike (hindlimb lameness), reduced arc of foot flight-often seen as stiffness or reluctance to flex the limb normally, shortened stride length (shortened swing phase of the stride) and abnormal foot placement such as landing toe first to spare the heel (La Pierre, 2001). In practice, many hoof injuries and lameness end up as surgical cases, depending on the type of condition, its origin and advancement or severity. For effective management of these conditions, one's expertise in hoof anatomy, and surgery and good knowledge of the common microbial contaminants as well as its susceptibility to commonly available antimicrobial drugs should not be in doubt.

Both coagulase-negative and coagulase-positive Staphylococci cause diseases (Bergdoll, 1989), the former being increasingly recognised as opportunistic pathogens. Staphylococcal infection occurs on the hoof areas because the organisms can be found on the soil, air and water where they are easily picked by the hoof (Duguid *et al.*, 1975). It can enter the hoof through penetrating open wound. Staphylococcus is one of the major micro-organisms that cause hoof wound infection thereby setting up more perfect environment for other types of micro-organisms to invade. Hoof infection can come in form of hoof abscess (infected wound), ringbone, navicular disease, laminitis, thrush and osteomyelitis (Littlewood *et al.*, 1998).

MATERIALS AND METHODS

Criteria for Identifying Hoof Lameness:

The horses were observed in walking and on standing positions. In a normal horse, the forelimbs are positioned at the same level with one another, so that the weight is shared equally between them. A lame horse may try to avoid bearing weight on the affected leg by either pointing or carrying the limb (Figure 1). In walking position, a horse with forelimb lameness will raise its head (nod) when the

lame leg hits the ground to reduce the weight on the affected limb. When the lame hind limb hits the ground, the head is dropped, while the hip on the affected side is hiked.



Figure 1: Plate showing antimicrobial susceptibility profile on nutrient agar

Furthermore, palpation and hoof tester methods were employed in identifying parts of limbs that are lame (Gyang, 1986). Based on the above criteria, each horse was identified either as having fore limb, hindlimb or shifting limb lameness.

Sample Collection: Effective restraint was achieved by the use of halter method with the help of assistants. The dung in the hoof was removed using hoof knife. The hoof surface was copiously washed with non-contaminated clean water. Following the examination of the hoof, hoof knife was used to remove dead and necrotic tissues. The content of fresh exposed hoof lesions or infected wounds was expressed and sterile swab sticks were used to collect the samples. Collected samples were labelled and placed on ice pack for transportation to the Microbiology Laboratory of the Faculty for immediate processing.

Culture, Isolation and Identification of Micro-Organism:

The samples were cultured on nutrient agar with 7% salt solution (appropriately labelled for each sample). The inoculated plates were then incubated at 37 °C for 24 hours. Growths obtained after culture isolation were purified in another prepared nutrient agar with salt. Growths obtained after

purification were macroscopically characterized using standard method (Cheesbrough, 1991).

Phenotypic Identification

Catalase and mannitol tests: Hydrogen peroxide was added directly to 24-hour growth on a nutrient agar slant. A positive result was indicated by bubbling. For mannitol salt agar (MSA), the acidity of the media caused the pH indicator (phenol red) to turn yellow indicating presence of *Staphylococcus* species (Kateete *et al.*, 2010). Haemolytic activity test (Figure 2) and haemolytic characterization were carried out as described by Boyce (1985) and Chow *et al.* (1983), respectively.

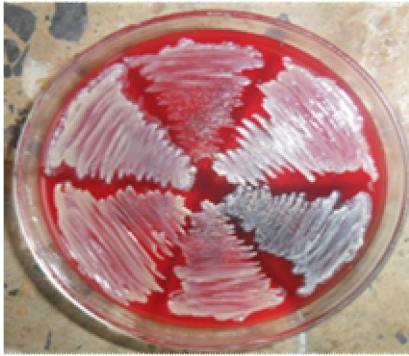


Figure 2: Plate showing haemolysis on blood agar

Antibiotic Sensitivity Test: Antibiotic susceptibility test was carried out on the various isolates to establish antibiotic profiles for each of them. This was done using the disc diffusion method as described by Wayne (2011).

RESULTS

Out of 170 horses encountered during the study period, 125 were males, while 45 were females. Percentage of total number affected was $58/170 \times 100 = 34.2\%$, the ratio of male to female was 7:3 (Table 1).

Incidence Rate (IR): I.R per 100 population per month was $58/170 \times 100 = 34.11$ new cases per 100 populations per month. Percentage of hoof lameness associated with the forelimb was $26/58 \times 100 = 44\%$.

Percentage of hoof lameness associated with the hindlimb was $33/58 \times 100 = 56\%$. Out of the 58 samples collected, the number of *Staphylococcus* isolated was 44 (Table 3). The result of the haemolytic activity showed that 8 and 4 isolates were β -haemolytic at 37°C and 4°C respectively, whereas 32 isolates were non haemolytic. The antimicrobial susceptibility test (Table 3) revealed that Gentamicin (aminoglycoside) consistently exhibited high efficacy against the Staphylococcal isolates. Tigecycline were also effective followed by Ciprofloxacin.

DISCUSSION

It was observed from this study that the prevalence rate of hoof lameness was high (34%) within the period of study. Though, the study was not an all year round research, but the findings agree with the work of Khan *et al.* (2005) who attributed the high prevalence rate of lameness in horses to favourable environmental conditions (high moisture and humidity) which enhances bacterial proliferation. Hoof lameness was observed more in males than females, probably due to strenuous exercises that the males are usually engaged on. In Northern Nigeria where these animals were coming from, horses are kept for Polo tournament and males appear more promising in this event. In the present study the hind limb lameness was observed more than the fore limb probably due to more injuries observed in the hind limb of the studied animals. Wobbling as observed in this study was as a result of weakness.

This particular type of lameness (wobbling movement) was also characterized by concurrent injuries on both fore and hind limbs and usually the affected horses were observed to be emaciated. In the study, for the fact that 76% of the equine hooves screened incriminated *Staphylococcus* was an indication of high rate of prevalence of *Staphylococcus* in the study area. This rate is higher than 43% reported by Weese *et al.* (2005). This high prevalence poses danger to both human and animal population since *Staphylococcus* is a zoonotic bacterium that spread fast among

Table 1: Types of lameness exhibited among the examined horses at Obollo market, Enugu State, Nigeria

Type of lameness	Male (N =125)	Percentage (%)	Female (N =45)	Percentage (%)	Total percentage (%)
Wobbling	4	3.2	1	2.2	5.4
Pointing fore limb	16	12.8	4	8.9	21.7
Pointing hind limb	28	22.4	5	11.1	33.5
Hanging limb	0	0	0	0	0
Limping	0	0	0	0	0
Dragging foot	0	0	0	0	0
Total (%)	48	38.4	10	22.2	60.6

Table 2: Frequency of occurrence of hoof wound infection in horses sold at Obollo market, Enugu State, Nigeria

Month	Number of Stallion	Number of mares	Number of new cases of hoof infection	Number of hoof from fore limb	Number of hoof from hind limb
2/May/2012	59	13	5	7	11
6/June/2012	55	23	1	10	14
25/July/2012	56	12	4	9	7
Total	170	48	10	26	33

Table 3: Antimicrobial susceptibility of *Staphylococcus* associated with hoof infection in horses sold at Obollo market, Enugu State, Nigeria

Antimicrobial agents	Zone Diameter of Breakpoints	Number of isolates Tested	Number Resistance	Percentage Resistance (%)	Number Susceptible	Percentage Susceptible (%)
<i>CIP</i> (5µg)	≤ 15- ≥ 21	44	22	50	22	50
<i>S</i> (10µg)	≤14 – ≥22	44	44	100	0	0
<i>GEN</i> (120µg)	≤ 6 - ≥10	44	0	0	44	100
<i>SXT</i> (25µg)	≤10 – ≥16	44	44	100	0	0
<i>TIG</i> (15µg)	≤23 – ≥29	44	17	39	27	61

Legends: *CIP* = Ciprofloxacin, *S* = Streptomycin, *SXT* = Cotrimaxole, *TIG* = Tigecycline, *GENT* = Gentamicin

animals and between animals and human populations. In the study also, 8% of the isolates were haemolytic at 37°C. The result of the haemolytic activity showed that 8 and 4 isolates were β-haemolytic at 37°C and 4°C respectively whereas 32 isolates were non-haemolytic. The health implication on the affected animals is very obvious, because haemolytic *Staphylococcus* cause both shock and death in the affected animals (Boyce, 1985). This study showed that *Staphylococcus* infection is emerging as important hoof pathogens that can predispose horses to lameness.

The antimicrobial susceptibility test revealed that Gentamicin consistently exhibited high efficacy against the *Staphylococcal* isolates. Tigecycline were also effective probably because they have high specificity for gram-positive bacteria. Next in efficacy was Ciprofloxacin.

This susceptibility to the above antibiotics is clinically relevant since in the field situation knowing that the chances of the *staphylococcus* as a contaminant may be high in hoof injuries and surgeries, the use of these drugs in their order of efficacy can be used to manage the condition both in curative and prophylactic situations.

Conclusion: It is recommended that in the horse management, a high level of hygiene should be observed. Hoof injuries, wound and bruises should be promptly washed, disinfected and dressed to prevent contamination with micro-organisms which could progress to lameness. In the treatment of cases of hoof infections, a drug sensitivity test should be carried out before instituting therapy, in order to avoid development of antibiotic resistant strains (Ettema *et al.*, 2010).

REFERENCE

- BERGDOLL, M. S. (1989). *The Microbiological Safety of Foods in Health Care Settings*. Academic Press, New York.
- BOYCE, J. M. (1985). Detection of hemolytic activity of *Staphylococcus aureus* with cathra replication. *Journal of Clinical Microbiology*, 21(5): 835 – 837.
- CHEESBROUGH, M. (1991). *Medical Laboratory Manual for Tropical Countries*. Volume II, Butterworth-Heinemann Limited, Oxford.
- CHOW, A. W., GRIBBLE, M. J. and BARTLETT, K. H. (1983). Characterization of the hemolytic activity of *Staphylococcus aureus* associated with toxic shock syndrome. *Journal of Clinical Microbiology*, 17(3): 524 – 528.
- CHURCHILL, E. A. (1979). The methodology of diagnosis of hind leg lameness. *Proceedings of the American Association of Equine Practitioners*, 25: 297 – 304.
- DUGUID, J. P., ANDERSON, E. S., ALFREDSON, D. A., BARKA, R. and OLD, D. C. (1975). A new biotyping scheme for *Salmonella typhimurium* and its phylogenetic significance. *Journal of Medical Microbiology*, 8: 149 – 166.
- ETTEMA, J., OSTERGAARD, S. R. and KRISTENSEN, A. R. (2010). Modelling the economic impact of three lameness causing diseases using herd and cow level evidence. *Preventive Veterinary Medicine*, 95: 64 – 73.
- GYANG, E. O. (1986). *Introduction to Large Animal Surgery*. Agitab Publications Limited, Zaria, Nigeria.
- KATEETE, P. P., KIMANI, C. N., KATABAZI, F. A., OKENG, A., OKEE, M. S., NANTEZA, A. JOLOBA, M. N. and NAJJUKA, F. C. (2010). Identification of *Staphylococcus aureus*: DNase and mannitol salt agar improved the efficiency of tube coagulase test. *Annals of Clinical Microbiology and Antimicrobials*, 9: 23 – 29.
- KHAN, C. M., LINE, S. E. and ANDERSON, R. C. (2005). *The Merck Veterinary Manual*, Ninth Edition, Merck and Company Incorporated, Whitehouse Station, New Jersey, USA.
- KHANNA, T., FRIENDSHIP, R., DEWEY, C. and WEESE, J. S. (2007). Methicillin resistance *Staphylococcus aureus* colonization in pigs and pig farmers. *Veterinary Microbiology*, 128(3-4): 298 – 303.
- LA PIERRE, K. C. (2001). *Applied Equine Podiatry*. http://www.appliedequinepodiatry.org/Text_Files/Applied_Equine_Podiatry_Article.pdf. Accessed June 13, 2014.
- LITTLEWOOD, J. D., BARBET, J. L. BARLEY, M. J. CRAIG, J. M. THOMSETT, L. R. and WALTON, G. S. (1998). The Skin. Pages 270 – 324. In: HIGGINS, A. and WRIGHT I. M. (Editors), *The Equine Manual*, W.B. Saunders, Philippines.
- WAYNE, D. U. (2011). *Collage of American Pathologist on Laboratory Accreditation Programme*, Clinical and Laboratory Standards Institute, ASM Press, Washington DC, USA.
- WEESE J. S., ROUSEAN, J., TRAUB-DARGATZ, J. I., WILLEY, B. M., MCGEER, A. J. and LOW, D. E. (2005). Community-associated methicillin resistance *Staphylococcus aureus* in horse and humans who work with horses. *Journal of American Veterinary Medical Association*, 226(4): 580 – 583.