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Prevalence of mastitis and drug resistance of bacterial isolates from milk of slaughter goats in South East, Nigeria

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ABSTRACT

Aim: The study was aimed to determine the prevalence of mastitis and antibiograme of bacterial isolates from affected slaughter goats' milk in Ikpa, Nsukka.
Method and materials: The study population comprised of lactating slaughter goats at Ikpa slaughterhouse which was the converging point of goats sourced locally and from other states. Total sample of 120 does were collected and transported in flasks containing ice to the laboratory of the Veterinary Public Health University of Nigeria, Nsukka for further analysis.
Results: Overall, mastitis prevalence of 60.8% was recorded. Also, prevalence of 55.3 and 94.1% were observed in milk from clinically normal and abnormal udders, respectively. Mastitis was significantly associated (OR = 12.7, 95%CI = 2.16-278.9, P = 0.002) with clinical state of udder. Bacteria isolated were *Staphyloccocus aureus* (50.0%), *Streptococus* spp (33.0%), *E. coli* (10.0%) and *Proteus* spp (7.0%). The isolates were resistant to commonly used antimicrobials.
Conclusion: It was concluded that isolates from the does' milk were resistant to common antimicrobials. Need is felt to organise awareness campaigns for goat farmers to employ hygienic methods of goat management and milking to reduce mastitis and imprudent antimicrobial usage. Government should also enact and enforce laws on compulsory pasteurization of milk to protect consumers in Nigeria.

Keywords: Antimicrobials, Mastitis, Resistance, Slaughter Goats.

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Introduction

Milk is known to be one of the complete foods. Goat milk is noted for its high quality and similarity to human milk in digestibility (Almaas et al., 2006). In developing countries, goat milk is very important serving as a source of nutrition and income to the rural and peri-urban populations. However, one major problem of milk production is mastitis which is the most expensive disease of the dairy industry (Ruegg and Reinemann, 2002). Mastitis can broadly be classified as being clinical or subclinical. Largely, the difference between the two is the presence or absence of external inflammation (Dimitrov and Stoimenov, 2018). The subclinical mastitis however, is much more common than clinical mastitis in dairies (Khan and Khan, 2006).

Clinical mastitis is noted for its sudden onset, redness of udder, pain and reduced milk of the quarters affected. Milk from such udder appears abnormal having flakes, clots, strings or watery. Severe mastitis cases show apparent systemic signs and animal may exhibit fever, go off feed or into shock (Mcfadden, 2011). On another hand, both udder and milk in sub-clinical mastitis appear normal but detectable only by measuring the somatic cell count (SCC) content. Usually, high SCC indicates mastitis (Mcfadden, 2011). One method of measuring SCC is the use of the California Mastitis Test (CMT). The test is one of the most reliable, accurate animal-side method of screening for subclinical mastitis (Ruegg and Reinemann, 2002). High CMT scores are commensurate with increased probability of having the disease in a quarter (Mcfadden, 2011). However, the SCC range in goats is yet to be standardized at one reference range (Dimitrov and Stoimenov, 2018) and cut-off points can differ from one operator to another (McDougall et al., 2001). Also, SCC is affected by a number of other factors including viral infection, age, stage of lactation, level of production and fraction of milk

being sampled (McDougall et al., 2001). The CMT result therefore, is not confirmatory but used for testing and selecting samples for microbiological assessment (Dimitrov and Stoimenov, 2018).

In Nigeria, milk production does not satisfy the quantity required and one of the causes of this inadequacy is mastitis. The disease leads to heavy losses due to reduction in milk production, culling and replacement of infected animals, cost of veterinary care, reduction in milk quality, shortened lactation period, discard of milk as well as risk of antibacterial residues in milk (Gelasakis et al., 2018; Khan and Khan, 2006; Mcfadden, 2011). Antibacterial residues in foods of animal origin have been blamed as one of the causes of antibiotic resistance (Abakpa et al., 2015). In Nigeria where antibiotic use is not controlled and self medication of livestock is common among farmers (Njoga et al., 2018), there are widespread reports of antibiotic treatment failures in food animals due to antibacterial resistance (Animale and Épidémiologie, 2016; Hambali and Mustapha, 2015; Oluwasile et al., 2014). Thus, diseases due to drug resistant etiologic agents are difficult to manage clinically in animals and man (Obaro et al., 2018).In addition, presence of resistant bacterial strains in food animals spells danger to public health due to possible horizontal resistance gene transfer to the normal intestinal flora and potential pathogenic organisms in the intestine of the consumers (Hambali and Mustapha, 2015). Considering the paucity of information on mastitis and drug sensitivity status of the aetiologic agents in slaughter goats in Nigeria, this study was conducted to screen and determine the antibiogram of isolates from slaughter goats' milk at Ikpa Slaughterhouse, Nsukka Local Government Area (LGA), Enugu State, Southeast, Nigeria.

Materials and Methods

Study Area

The study was conducted in the Ikpa slaughter house, Nsukka LGA which was chosen because it has the highest rate of slaughter in Enugu North senatorial zone. Nsukka LGA, lies between latitudes $60^{o}\ 18^{1}$ and $70^{o}\ 06^{1}$ North, and longitudes $60^{o}\ 52^{1}$ and $70^{o}\ 54^{1}$ East. It covers a land area of approximately 3,961 square kilometers. The LGA is bounded in the North by Igbo-Eze South LGA; on the South-East by Udenu LGA, South West by Igbo-Etiti LGA and North West by Kogi State (Onunkwo et al., 2011).

Study Design and Population

The work adopted the cross-sectional study method. The study population comprised of lactating slaughter goats at Ikpa slaughterhouse which is the converging point of goats sourced locally and from other states.

Sample Size Determination

With the statistical formula for cross-sectional studies $N = 1.96 * P(1 - P)/d^2$ as cited (Agada et al., 2017), the minimum sample size of 103 does was calculated using the prevalence of 15.5% earlier recorded from goats screened for mastitis in Northern Nigeria (Tanimomo et al., 2015). However, a total of 120 does were sampled.

Sample collection and handling

For each goat, the udder was first examined for abnormalities (dermatitis, inflammation, laceration etc) and ecto-parasites. It was washed with clean water, allowed to dry and cleaned with cotton wool soaked in 70% ethyl alcohol. Few streams of milk were discarded then 5mls collected into sterile universal sample bottles. Each bottle was labeled and the goat's breed, udder appearance and milk characteristics noted. All samples collected were then transported in flasks containing ice to the laboratory of the Veterinary Public Health University of Nigeria, Nsukka for further analysis.

California Mastitis Test (CMT)

The CMT kit (V. Kruse Company, Denmark) contains the CMT paddle and reagent. The paddle has four wells corresponding to each teat of the cattle udder. The test was conducted using the procedure earlier described in goats (McDougall et al., 2001). The results were interpreted depending on the type of gel formed (Anon, 2010 accessed 14/3/2019 from http://www.infovets.com/demo/demo/smrm/D100.HTM; Shearer and Harris, 1992): as mild gel formation which disappears after 3mins (+); moderate gel formation (++) and Heavy gel formation (+++)

Bacteriological Isolation

This was done following the protocol described by the International Commission on Microbiological Specifications for Foods (ICMSF, 1996)

Identification of isolates

Identification of the isolates was carried out as described (Quinn et al., 2003).

Total Aerobic (Viable) and Coliform Counts

This was done as described (Cappuciono and Sherma, 2011).

Antibiotic sensitivity test

Antimicrobial susceptibility of the bacteria isolates was determined by the disc diffusion method (Clinicaland Laboratory Standards Institute [CLSI, 2010]).

Data analysis

Data generated were analysed using chi-square with the STATA Version12 and OpenEpi and probability values less than the critical value (α = 0.05) considered significant.

Results and Discussion

The CMT result showed 60.8% prevalence of mastitis among goats slaughtered in Ikpa slaughterhouse. The breed specific prevalence was 57.0% in Kano brown, 86.0% in Red Soko to and 60.0% in West African Dwarf goats. Prevalence of mastitis in udders with obvious clinical signs was 94.1% and 55.3% among those showing no clinical signs. Mastitis was significantly associated with udder appearance (OR=12.73, 95%CI = 2.162-278.9, P = 0.002), with those showing clinical signs being 12.7 times more likely to have mastitis than the ones without obvious clinical signs (Table 1). The culture result showed that *Staphylococcus* spp was isolated from 50.0% of the samples, Streptococcus spp from 33.0%, E. coli from 10.0% and Proteus spp from 6.7% (Table 2). The mean cfu for McConkey agar was 2.06 x 10^{10} (SE 1.51 x 10^{10} and 95%CI=1.67 x 10^{10} – 7.85 x10^{10}). The mean cfu for nutrient agar was 4.76 x 10^{9} (SE 5.81 x 10^{8} and 95%CI=8.67 x 10^{8} – 3.24 x10^{9}) as shown in Table 3. The *Staphylococcus* spp,*Streptococcus* spp, *E. coli* and *Proteus* spp were found to be resistant to common antibacterial drugs (Table 4).

The study found mastitis to be prevalent in goats slaughtered in Nsukka, Nigeria. The prevalence recorded (60.8%) was higher than 15.5% (Tanimomo et al., 2015) and 10% (Ameh et al., 1993) observed in Northern Nigeria, 50.9% in Kenya (Mahlangu et al., 2018) and 38.75% in Bangladesh (Ferdous et al., 2018). The difference in prevalence between the studies could be as a result of variation in CMT scores between operators since the SCC range in goats is yet to be standardized (Dimitrov and Stoimenov, 2018) and cut-off points therefore could differ from one operator to another (McDougall et al., 2001). According to McDougall et al. (2001), non standardisation affects the specificity and sensitivity of CMT. The high prevalence recorded in this work could be attributed to the fact that the sample was drawn from slaughter animal population since farmers in Nigeria are known to sell their female animals for slaughter not for profits but in cases of disease or to solve pressing problems (Ogugua et al., 2018). It is possible that the owners sold the goats due to enlarged or gangrenous udder, unproductivity in the form of high doe and kid mortality, as well as lowered milk production which are common in mastitis (Ferdous et al., 2018). Mastitis has been noted as one of the most important reasons for disposing female animals (Bradley, 2014). The high prevalence also might possibly be attributed to teat dipping therapy during dry period which helps to reduce cases of mastitis (Koop et al., 2010) not being a common practice among goat farmers in Nigeria. Such situations, where farmers do not make use of required routine preventive and control measures rather than treatment of clinical cases, increases the occurrence of the disease (Abebe et al., 2016). The prevalence of mastitis in the study area is important economically due to lowering of milk yield (Gelasakis et al., 2018). Also, subsequent episodes of clinical mastitis result in further reduction in quantity of milk produced and animals that experience clinical mastitis are known to never reach their full potential subsequently, in levels of milk production (Bar et al., 2007).

The high SCC recorded in this study is an indication of high level of intra-mammary (IM) infection (Green et al., 2004). Also, this study found clinical mastitis (CM) to be associated with SCC which also increased with bacterial count. This is in agreement with other studies that showed SCC to increase with bacterial infection (Green et al., 2004; Moroni et al., 2005). The SCC has been shown as the best predictor of mastitis in sheep and goats (McDougall et al., 2001). However, SCC is affected by the type of organism responsible for the mastitis. While Moroni et al. (2005) found SCC to increase with *S. aureus* infection; Green et al. (2004) found it to decrease; although the former worked with goats and the latter with cattle. The isolation rate of bacteria was found to have increased with CMT results in agreement with McDougall et al. (2001). The organisms incriminated for mastitis in the Ikpa slaughterhouse were *Staphylococcus* spp, *Streptococcus* spp, *Proteus* spp and *E. coli*. The isolation of *Staphylococcus aureus* and Streptococcus spp in this work is in agreement with what was recorded elsewhere in Maiduguri (James et al., 1999), North West Nigeria (Ameh et al., 1993), Bangladesh (Ferdous et al., 2018) and Kenya

(Mahlangu et al., 2018). These two are known to be among the bacteria usually incriminated in mastitis cases, although the isolation of E. coli have been commonly reported (Abdalhamed et al., 2018; Ameh et al., 1993; Ameh et al., 1999; Ferdous et al., 2018; Mahlangu et al., 2018). The isolation of these organisms is of public health importance because they can cause food poisoning when ingested in milk or its products without pasteurization (Gelasakis et al., 2018) especially enterotoxin producing strains of *Staphylococcus* (Omar and Mat-Kamir, 2018). Being that Staphylococcus invasiveness is known to depend on the human host factors (Van et al., 2009), exposure to it is a cause for public health concern given the high HIV burden in Nigeria (Habib et al., 2010). Isolation of *Staphylococcus aureus* which is mainly an environmental bacteria (Green et al., 2004) indicates unhygienic nature of the goat environment that possibly led to intramammary (IM) infection in the affected goats. This is reflected in the heavy bacterial counts recorded in the study. Invasion of the mammary gland could have occurred due to poor hygiene level during production and milking in the farms of origin (Ameh et al., 1999) or from the heavily contaminated Ikpa slaughter environment (Nwanta et al., 2010).

The isolates were found to be resistant to all the antimicrobials tested. The organisms could have become drug resistant due to sub-therapeutic treatment by farmers(Chen et al., 2013). This is worrisome since antimicrobials are the only means of treating cases of mastitis due to bacterial infections. Therefore, mastitis cases due to these organisms could result to mortality in herds and increased cost to the farmers. It has been reported that many farmers do not observe withdrawal periods before the sale of animal products to the public (Njoga et al., 2018). This could have clinical and economic effects due to stimulation of drug resistance and reduction of milk quality, respectively. Drug residue in milk is of public health importance given that continued exposure of intestinal microflora to low doses of the drugs exerts enough selection pressure for resistance to develop among the exposed organisms (Nasir et al., 2015). Also, resistant organisms in milk transmit resistance genes to the normal intestinal microflora of the consumers of such milk. Finally, the risk of developing diseases from these drug resistant bacteria is quite high given that consumption of milk without pasteurization is common in Nigera (Ofukwu et al., 2008).

Finally, the major limitation of the work is that it was conducted on slaughter goats. Slaughterhouse surveys do not represent the true field situation but they signify the existence of the disease in the area where the animals were sourced from.

Conclusion

It was concluded that mastitis was prevalent among slaughter goats in Ikpa Slaughter house. The isolates from the does' milk were resistant to common antimicrobials. Need is felt to organise awareness campaigns for goat farmers to employ hygienic methods of goat management and milking to reduce mastitis and imprudent antimicrobial usage. Government should also enact and enforce laws on compulsory pasteurization of milk to protect consumers in Nigeria.

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