ANTIBIOGRAM OF BACTERIAL ISOLATES ASSOCIATED REPRODUCTIVE ABNORMALITIES IN SHEEP IN GWAGWALADA–FCT, NIGERIA

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ABSTRACT

A survey of bacterial isolates associated with reproductive anomalies was conducted in slaughtered ewes in Gwagwalada abattoir in order to establish infection occurrence and design adequate antibiotic intervention strategy. A multistage convenient random sampling was employed over a three months study period (March – May, 2011). Out of one hundred and nine (109) ewes examined during meat inspection forty-one (41) showed various forms of reproductive defeats. Collected swab cultures indicated twenty-two (22) had microbial growths which on identification and biochemical characterization revealed *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis,* and *Pseudomonas aeroginosa.* Antibiotic sensitivity testing using standard disc diffusion technique against Amoxicillin, Ampicillin, Erythromycin, Ciprofloxacin, Gentamycin and Tetracycline showed that Ciproflaxin, Erythromycin and Gentamycin were the most sensitive while the isolates were resistant to Tetracycline. Amoxicillin and Ampicillin showed variable intermediate effects on the isolate. The overall susceptibility showed 68.2% isolates were sensitive and 31.8 % resistance to the antibiotics, which was statistical significance analysis ($P<0.05$) by chi square analysis. The age and breed of ewe also showed significant association with the occurrence of reproductive disorders, as yankasa breed (70.7%) within the age 13-36 months were most commonly affected. In conclusion, this study provides preliminary information on the bacterial agents associated with reproductive disorders in ewes with corresponding susceptibility pattern. In addition, Nutrient agar is a potential substitute to Mueller Hinton agar. It is suggested that routine bacteriological examination and antibiotic susceptibility testing, in conjunction with mycological investigation is necessary in order to guide against misuse of unwarranted drugs.

Key words: Antibiogram, bacterial isolates, reproductive abnormalities, ewe, Gwagwalada, Nigeria

INTRODUCTION

Small ruminants are ubiquitous with an estimated total population of 56.6 million head throughout the country. The estimated sheep population is about 22.1 million as goats' outnumbered sheep by a ratio of three to two (RIM, 1992). Sheep are the second most numerous pastoral species with small flocks accompanying many cattle herds in the north and Middle Belt regions. There are four main types of native sheep: the Balami, Uda, Yankasa and West African Dwarf. Balami and Uda are kept in the semi-arid regions, West African Dwarf sheep in the south and Yankasa are well spread throughout the country (FAO. 1983). All Nigerian sheep are eaten daily as source of protein supply and used for wool, but

are rarely milked, but there is also a marked variation in demand coinciding with religious festivals. As a result, there are dramatic seasonal price fluctuations and in some areas the household fattening of sheep for sale is a major economic activity (Bourn et al., 1994).In spite this purposeful use of the sheep, their productivity is hindered by management and disease (Kudi et al., 1997) especially those causing reproductive anomalies with resultant economic burden on the livelihood of owners (Lamorde, 1997). Therefore information on microorganisms associated with reproductive disorders is essential for diagnosis, treatment and control of low productivity in livestock especially sheep. The need for update information on the antibiotic susceptibility of possible bacterial organisms associated with reproductive disorders necessitated this investigation in slaughtered sheep consumed in the study area.

MATERIAL AND METHODS

Study area: Gwagwalada is one of the five Local Government Area Councils of the Federal Capital Territory of Nigeria, together with Abaji, kuje, Bwari, Kwali and Abuja City. Gwagwalada is also the name of the main town in the Local Government Area, which has an area of 1,043 km² and a population of 157,770 at the 2006 census (Anon, 2011). The postal code of this area is 902 (Anon, 2009), a town where the University of Abuja is located (Awowole-Browne, 2007). Satellite Images of Gwagwalada indicates its geographical coordinates as 8° 56' 29" North, 7° 5' 31" East (3D Google Earth). The abattoir is located mid-way between the popular 'kasuwan dere' and the Federal Radio Coporation of Nigeria (FRCN) along old kutunku road. The abattoir has being the main source of wholesome meat for the culturally diverse inhabitants of Gwagwalada metropolis and its environs.

Study population/animals: All the sheep that were brought for slaughter at Gwagwalada abattoir during the study period were considered and used for this investigation. These animals were brought in the mornings before slaughter from the livestock market behind the Area Council veterinary clinic attached to the Gwagwalada central market, within a distance of about two-four kilometers to the abattoir. Others brought from distant neighboring states are kept in the lairage on arrival to rest for a minimum of 24-48hrs depending on the travel distance, traumatic injuries and the consent of owner before slaughter. The livestock superintendent is usually notified prior to slaughter of these sheep for documentation purpose.

Sampling technique and sample size: Convenient random sampling method was adopted where all the sheep slaughtered on the floor between March-May 2011 was sampled based on detection of visible gross abnormality in the anatomical size of the reproductive organs of ewes post slaughter. Forty-two (42) cases were observed and considered for this investigation. The age of ewes was noted based on dentition method and breed as described by Adu and Ngere (1979).

Sample collection: suspicious or defective genital tracts of ewes during meat inspection were excised, examined and swabs collected with sterile stick and placed in plastic container bottle containing transport medium (Nutrient broth). These samples were transported to the

Bacteriology Laboratory of the Federal College of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute, Vom on cold ice packs and stored at 4^{o}C until.

Bacteriological investigation

Isolation of Pathogens: Collected sample were inoculation onto Blood Agar (BA) and MacConkey Agar (MCA) plates as described by Cheesbrough, (2002). MCA and BA plates were incubated aerobically at 37 ^{o}C for 24 hour. Post incubation, colonial and cultural morphology were read in accordance to conventional methods (Cowan, ST and Steel, KJ 1990) and documented. Pure isolates were obtained by repeated sub culturing of these organisms onto Nutrient Agar (NA) plates, and then maintaining on NA agar slants at 4 ^{o}C until required.

Identification and biochemical characterization of Bacterial Isolates: The cultural and physiological characteristic of isolates was observed and noted 24 hours post incubation. Colonies were gram stain and observed under x 100 magnificant. Gram negative were sub cultured on Eosin methylene blue agar plates and incubated at 37^{o}C. Motility test and other biochemical reactions such as Catalase, Coagulase, Indole, Urease, Oxidase and Citrate test were further conducted described by Cheesbrough, (2002). Results obtained were compared with the Identification and Biochemical reaction tables illustrated by Baker and Silverton, (1998).

Sensitivity susceptibility testing: sensitivity test was conducted using swabs of each isolates inoculated into different test tubes containing Nutrient broth and incubated for 24hours at 37^{o}C. The swab of each isolates from the Nutrient broth was smeared out on Nutrient agar. A multi-antibiotic sensitivity disc was placed on smeared surface of NA using a sterile thumb forceps. All the plates were incubated at 37^{o}C for 24hrs after which a millimeter rule was used to measure the diameter of zone of inhibition. Based on the diameter sizes, the zone of inhibition was divided into sensitive and resistant. This test was repeated on Mueller Hinton agar to ensure consistency in results obtained. Isolates to be inoculated on the surface of Mueller-Hinton agar were incubated overnight until turbidity is equivalent to 0.5 Mcfaland standards, allowed for few minutes at room temperature. Then antimicrobial susceptibility was performed on Mueller –Hinton agar by the standard disk diffusion method recommended by the National committee for clinical laboratory standards (NCCLS, 2002). This was done by dipping a sterile swab stick in to overnight broth and carefully swabbing the entire surface of Mueller –Hinton agar plates. The antibiotics tested against the test the test organism were amoxicillin (30ug), ampicillin (30ug), erythromycin (10ug), ciprofloxacin (30ug), gentamycin (10ug), and tetracycline (30ug). The antibiotic multi disc (Oxoid) was then placed on the surface of the inoculated plates and gentlely pressed. The plates were incubated at 37^{o}C for 18-24hrs. The diameters of the zone of inhibition were scored as sensitive or resistant by comparing with recommended values on standard charts (NCCLS, 2002).

Data analysis: All data was expressed as frequency and percentage, Chi square was used to establish the goodness of fit as described (Mahajan, 1997).

RESULTS

Out of the one hundred and thirty five ewes genital organs examined forty one (41) showed different gross pathology which include, Fetal (death) waste (46.3%), Mucometra (7.3%), Fetal maceration (4.9%), Pyometra (17.1%), and Hydrometra (24.3%), (Table 1). The distribution of abnormalities by breed showed that Yankasa, 29 (70.7%), were more predisposed than Balami, 4 (9.8%) and Uda, 8 (19.5%), (Table 2). Ewes within 13-36 months were most susceptible to reproductive problems (Table 3). Out of the Forty-one (41) swabs collected and screened, Twenty seven specimens (27) yielded microbial growth (65.9%), 22 (81.5%) of these were bacteria isolates, 5 (18.5%) were fungi-like (yeast) on BA while 14 (34.1%) yielded no microbial growth (Table 4). Antibiogram susceptibility of isolates indicated, *Escherichia coli* were sensitive to amoxicillin, ampicillin, erythromycin, ciprofloxacin, gentamycin, and resistant to tetracycline. *Staphylococcus aureus* isolates were sensitive to amoxicillin, erythromycin, ciprofloxacin, gentamycin, and resistant to ampicillin, and tetracycline. *Streptococcus pyogenes* were most sensitive to ampicillin, erythromycin, ciprofloxacin, and resistant to amoxicillin, gentamycin and tetracycline. *Proteus mirabilis* were most susceptible to erythromycin, ciprofloxacin, gentamycin, and resistant to tetracycline. *Pseudomonas aeroginosa* was seen to be most ciprofloxacin, gentamycin and resistant to amoxicillin, ampicillin and tetracycline (Table 5). The sensitivity distribution pattern of the six (6) antibiotics routinely used, showed that 15 (68.2%) isolates out of the 22 bacterial isolates were sensitive to Ciprofloxacin, Erythromycin, and gentamycin antibiotics while 7 (31.8%) were resistant which was statistically significant by Chi square ($P<0.05$) analysis. Ciprofloxacin (CIP) was the most sensitive of these antibiotics, followed by Erythromycin (ERY) and Gentamycin (GEN) while Tetracycline (TET) was the least susceptible. Similar antibiotic susceptible pattern was observed for Nutrient agar.

Table 1: Types of reproductive abnormalities in sheep

Type of Disorder	Number affected	Percentage (%)
Fetal death/waste	19	46.3
Mucometra	3	7.3
Fetal maceration	2	4.9
Pyometra	7	17.1
Hydrometra	10	24.3
Total	**41**	**100**

Prevalence rate 20.9%

Table 2: Reproductive abnormalities amongst different breeds of ewes in Jos abattoir

Breed	Number examined	Number affected	Percentage (%)
Yankasa	101	29	70.7
Balami	65	4	9.8
Uda	30	8	19.5
Total	**196**	**41**	**100**

Table 3: Reproductive abnormalities amongst different age groups of ewe in Jos abattoir

Age (Months)	Number examined	Number affected	Percentage (%)
Less than 12	4	-	-
13-24	10	21	51.2
25-36	167	15	36.6
36 and above	15	5	12.2
Total	**196**	**41**	**100**

Table 4: Microbial growth distribution from specimen collected

Growth	Frequency	Percentage (%)
Bacterial isolates	22	53.7
Fungal isolates	5	12.2
No growth	14	34.1
Total	**41**	**100**

Table 5: Sensitivity pattern of isolates to selected antibiotics on Mueller Hinton agar

Isolates	Antibiotics						RES	SEN	Total
	AMO	AMP	CIP	ERY	GEN	TET			
E. coli	S	S	S	S	S	R	1	5	6 (27.3%)
S. aureus	S	R	S	S	S	R	3	5	8 (36.4%)
Streptococcus	R	S	S	S	R	S/R	1	2	3 (13.6%)
P.mirabilis	S/R	S/R	S	S	S	R	1	2	3 (13.6%)
Ps.aeroginosa	R	R	S	R	S	R	1	1	2 (9.1%)
Total isolates	**2S,2R**	**2S,2R**	**5S**	**4S**	**4S**	**4R**	**7**	**15**	**22**
Frequency (%)							**31.8**	**68.2**	**100**

10mm or less-Resistant= (R) and 11mm or more-sensitive=(S)

Key:

AMO- Amoxicillin AMP-Ampiclox
CIP-Ciprofloxacin ERY-Erythromycin
GEN-Gentamycin TET-Tetracycline

DISCUSSION

In this survey, the prevalence of reproductive problems amongst ewes represents 20.9% involving mostly the yankasa breeds (70.7%) within the age range of 13-24 and 25-36 months. The age and breed showed significant association by chi square analysis ($P<0.05$).This high record could be associated with the sexually active periods and the ubiquitous nature of the yankasa breed in the study area. Microbiological analysis of the samples collected shows a total of twenty-two (22) isolates belonging to five Genera were isolated from the forty-one (41) ewe specimen examined. The bacterial isolates were the highest (53.7%), with occurrence of *Escherichia coli* (27.3%), *Staphylococcus aureus* (36.4%), *Streptococcus pyogenes* (13.6%), *Proteus mirabilis* (13.6%) and *Pseudomonas aeroginosa* (9.1%). This was statistically significant by chi square ($P<0.05$). The bacterial isolates in this study connotes reports of similar studies conducted by Weka et al., 2010 in some parts of the country which reported *Escherichia coli* and *Actinomyces pyogenes* as important bacterial agents that cause reproduction problems in ewes which results in

abortion and or fetal and neonatal deaths as earlier associated with congenital infections (Otesile et al., 1982; Ojo, 2006).

The highest representation of *Staphylococcus aureus* isolates maybe unconnected to its ubiquitous nature and affinity for residency in the reproductive tracts, which was associated with lamb mortality (Ahmed et al., 2010), causing purulent infection in surviving lamb (Haughey, 1980). *Escherichia coli* isolated in these samples could be associated to its ubiquitous and opportunist nature as earlier reported by Haughey, (1980). *Streptococcus pyogenes, Proteus mirabilis,* and *Pseudomonas aeroginosa* were also isolated in low numbers, which indicates that they are not uncommon associates of reproductive problems in ewes. It is also important to note that these visually identified abnormalities may also have being associated with some other infectious agents particularly fungi as indicated in some of the initial culture growths which would have required further clarification. The observed resistance of all isolates to tetracycline is not unconnected to its misuse by livestock farmers and practitioners. Nutrient agar holds forth a promising alternative for Mueller Hinton agar in a situation or countries where Mueller Hinton agar is not available and urgent presumptive laboratory diagnosis is required. In conclusion, bacterial pathogens are responsible for high rate of reproductive problems commonly encountered in ewe in the study area. These associated bacterial isolates were most susceptible to Ciproflaxin, Erythromycin and Gentamycin. Therefore, it is recommended that quality samples should be collected and handled properly for bacteriologic and mycological investigations as well as susceptible testing so as to enhance the quality of treatment and preventive intervention strategies which would guide against antibiotic misuse.

ACKNOWLEDGEMENT

Authors wishes to acknowledge the efforts of Dr. Dandam and Mr. Isaac of the Gwagwalada Area Council Veterinary Clinic and the Abattoir respectively for their efforts during the sample collection.

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