www.ajbrui.net

Afr. J. Biomed. Res. 13 (January 2010) 33 - 37

Research article

Bovine Streptococcal Mastitis in Southwest and Northern States of Nigeria

***Amosun E. A, Ajuwape A.T.P. and Adetosoye A. I.**

Department of Veterinary Microbiology and Parasitology,
Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT: An investigation was carried out to identify the streptococci species isolated from clinical cases of bovine mastitis in Kwara, Kaduna and Oyo States of Nigeria. Milk samples from 200 clinically mastitic udders were bacteriological studied. A total of 130 streptococci isolate belonging to six species of streptococci, namely S. uberis, *S. agalactiae, S. dysgalactiae, S. epidemicus, S. bovis, S. equinus* were recovered from the milk examined. Streptococcus uberis was the most frequently encountered species with an incidence of (55.4%) followed by *Streptococcus agalactiae (24.6%), Streptococcus dysgalactiae (12.3%) Streptococcus zoopidemicus (3.9%) Streptococcus bovis (2.3%) and Streptococcus equinus* (1.5%). These species of streptococcus are of great public importance.

Key words: Streptococcus, Mastitis, Bovine.

INTRODUCTION

Milk is mostly produced by smallholders and semi-nomadic livestock owners in all tropical countries like Nigeria. Hence, the milk produced is mostly consumed by producers themselves, with little or nothing sold for cash, except occasionally and in seasons of surpluses (Chamberlain, 1989). Cows, water buffalo, sheep, goats and camels have been found to provide milk in different developed and developing countries, however cows have been the main suppliers of milk (Chamberlain, 1989). Mastitis is the inflammation of mammary gland or udder. It is characterised by palpable changes in the consistency of the mammary tissue and changes in the appearance of the milk (Radostits *et al*, 1994; Oliveira *et al*, 2000; Viera-da-Motta *et al*, 2001; Menzies and Ramanoon, 2001). Mastitis leads to economic losses in terms of reduced milk yield or milk quality, early culling of severely affected animals. It results to expensive antibiotic treatment, veterinary services and losses of the young ones (MacDonald and Low, 1985; Buriel, 1997; Sordiell *et al*, 2000; Leitner *et al*, 2001). Streptococcus is isolated frequently from bovine mammary glands (Freney et al. 1992; Baron *et al*, 1994; Facklam. 2002; Fortin et al, 2003). *Streptococcus agalactiae, S. dysgalatiae* and *S uberis* have been reported as the three most common aetiological agents of mastitis (Calvinho and Oliver, 1998; Leigh, 1999; Khan *et. al*., 2003). Other Streptococcal species such as *S. uberis, S. agalactiae, S. dysgalactiae, S. epidemicus, S. bovis, S. equinus* have been implicated in bovine mastitis, although they are relatively infrequent (Lammler. 1991; Leigh. 1999; Khan *et. al.*, 2003). *Streptococcus agalactiae* has been widely reported as an important pathogen of both animals and man (Schuctat and Wenger, 1994; Keefe, 1997; Mosabi *et al*, 1997; Ko, *et. al.,* 2001). This organism primarily infects the cisterns and the ductal system of the mammary gland. An irritant is produced, causing inflammation of the gland which is mostly subclinical with occasional clinical symptoms (Myllys *et. al.*, 1995). Accumulation of bacteria waste products intensifies the inflammatory response resulting in destruction of milk producing tissues and reduced milk yield or produce agalactia. It has been reported that *Streptococcus agalactiae* rarely cause severe illness, however, extensive scarring of a quarter may render it

Manuscript received: February 2009; Accepted: July 2009
Author for correspondance :
E-mail: elizabethamosun@yahoo.com; Tel. +234 805 5123 892

unproductive in subsequent lactation. (Myllys *et al*, 1995). Other species of streptococcus including *S. equinus, S. dysgalactiae, S. equisimilis, S. zooepidemicus* have been isolated from bovine intramammary infections (Watts, 1989; Calivinho and Oliver 1998).

On the other hand, *Streptococcus uberis* is known worldwide as an environmental pathogen responsible for a high proportion of cases of clinical and subclinical mastitis in lactating cows and is also the predominant organism isolated from mammary gland during the non lactating period (Bradley, 2002; Khan *et. al.,* 2003).

The main source of infection is the udder of infected cows, although when hygiene is poor, contamination of the environment may provide an additional source of infection. The teat of the udder and skin of cattle, milkers hand, floors, utensils and cloths are often heavily contaminated when good hygiene is not maintained. Sores on the teat are the commonest sites outside the udder for the persistence of the organism (Radostits *et. al.*, 2000). The purpose of this study was to investigate the incidence of streptococci bovine mastitis in Nigeria dairy farms in southwestern and northern Nigeria.

MATERIALS AND METHODS

Samples collection

The milk samples were collected from White Fulani, Bunaji, Red Bororo, Kuri, Friesian crossed with White Fulani and Bunaji which were managed under semi-intensive system. Two-hundred milk samples were collected from cows with clinical mastitis from farms in three different states namely Oyo State. Kwara and Kaduna State of Nigeria. Milk samples were collected from June 2005 to March, 2006. The milk samples were obtained asceptically from the affected udders and the initial stream of fore milk was discarded. About 5ml of milk from each cow was collected in sterile labeled bottle. Samples were transported on ice (Coleman® flask) to the our laboratory for analysis.

Bacteriology

The milk samples were inoculated on to 7% sheep blood agar (oxoid Columbia blood agar®) MacConkey agar No. 2 (Oxoid CM 109®) plates and incubated aerobically at 37°C for 24-72hr. Haemolysis and pigmentation were scored after 24h. Colonies yielding Gram-positive cocci with catalase-negative and oxidase negative reaction were subjected to CAMP test and Esculin hydrolysis as described by (Cruikshank *et. al.,* 1975, Barrow and Feltham, 1993). Carbohydrates utilization was conducted in peptone water as described by (Cruickshank *et. al*, 1975) containing lactose, maltose, mannitol, raffinose glycerol, salicin, sorbitol, sucrose and trehalose respectively. All carbohydrates were inoculated with O.lml of the bacteria suspension and incubated at 37^0C for 24hr. Positive reactions were indicated by a change from straw colour to pink colour using Andrade's indicator (Cruickshank et al, 1975).

Serotyping

Serological grouping of isolates were performed with a commercial latex agglutination kit for the identification of streptococcal groups A, B, C, D, F and G. Streptococci were tested using the broth method described by the manufacturer (Oxoid). A drop was dispensed from each latex reagent into the six circular rings on the reaction card. Pasteur pipette was used to add 1 drop of extract to each of the six rings. Mixing sticks provided was used to spread the mixture over the entire area of the ring using a separate stick for each ring. Visible agglutination within 1min was considered positive.

RESULTS

Out of the 200 milk samples studied for streptococci infection, streptococcus species were isolated from 130 (65%) of the milk samples. The serological and biochemical characteristics of the 130 streptococci isolated are as shown in Table 1. The isolates were found to belong to 6 distinct species namely *Streptococcus uberis* was the most common streptococci with an incidence of 55.38% followed by *S. agalactiae* 24.62% while *Streptococcus dysgalactiae; S. zooepidemicus; S. bovis* and *S. equinus* had an incidence of 12.31%, 3.85%, 2.3%, 1.54%, respectively (Table 2).

Seventy-two isolates were identified as non-groupable streptococci *(Streptococcus uberis).* Thirty-two isolates were identified as group B streptococci *(S. agalctctiae).* They were all CAMP test positive, exhibited (β-haemolysis on sheep blood agar and none hydrolyse esculin (Table 2).

Lancefield group C contains two species, *S. dysgalactiae* and *S. zooepidemicus. S. zooepidemicus* exhibit beta-haemolysis on blood agar which distinguishes these organisms from the alpha-haemolytic *S. dysgalacticie, S. zooepidemicus* hydrolysed esculin while *S. dysgalactiae* didn't. *S. dysgalactiue* is differentiated from *S. zooepidemicus* on basis of trehalose utilization. *S. dysgalactiae* utilized trehalose while *S. zooepidemiciis* did not (Table 2).

Lancefield group D contains two species *S. bovis* and *S. equinus. S. bovis* fermented lactose, mannitol and raffinose while *Streptococcus equinus* did not fermented sugars (Table 2).

Table 1:
Summary of Streptococci isolated from clinical mastitic Cows in Ibadan, Ilorin and Kaduna

Isolates	Ibadan (Oyo State n = 66)		Ilorin (Kwara State n = 36		Kaduna (Kaduna State n = 28		Total = 130	
	No Positive	Incidence (%)	No Positive	Incidence (%)	No Positive	Incidence (%)	No Positive	Incidence (%)
S. uberis	47	71.21%	16	44.44%	9	32.14%	72	55.38%
S. agalactiae	19	28.79%	8	22.22%	5	17.86%	32	24.62%,
S. dysgalactiae	-	-	8	22.22%	8	28.57%	16	12.31%
S. zooepidemicus	-	-	2	5.56%	3	10.71%	5	3.85%
S. bovis	-	-	2	5.56%	1	3.57%	3	2.32%
S. equinus	-	-	-	-	2	7.14%	2	1.54%
Total	66	100%	36	100%	28	100%	130	100%

n - no of isolates

Table 2:
Biochemical and Serological Identification of Streptococci Isolated from Mastitic Cows

Organisms	Haemolysis	CAMP test	Esculin hydrolysis	Lactose	Mannitol	Raffinose	Sorbitol	Sucrose	Trealose	Salicin	Lancefield
Groupable *Streptococcus agalactiae*	β	100%	0	85.5%	0	0	0	100%	100%	100%	B
S. dysgalactiae	α	0	0	63.8%	0	0	73.9%	100%	100%	52.5%	C
S. zooepidemicus	β	0	100	100	0	0	100%	100%	0	100%	C
S. bovis	α	0	100	100	95.1%	100%	0	100%	60.5%	100%	D
S. equinus	α	0	57.8%	0	0	0	0	100	0	100%	D
Non-groupable *S. uberis*	α	0	100%	100%	100%	0	100%	100%	100%	100%	ng s

ng s = Non-groupable

DISCUSSION

In this study, 72 of 130 isolates (55.38%) were identified as *S. uberis.* None of the organisms could be grouped using A, B, C, D, F and G coagulation reagents. Typically, the organisms are esculin positive and are lactose, mannitol, sorbitol, sucrose, trehalose and salicin fermenters. *Streptococcus uberis* is the most frequently isolated streptococcal species from bovine mammary gland. (McDonald and McDonald, 1976) reported that *S. uberis* accounted for 56.5% of 455 streptococcal isolates from 72 dairy herds. This finding is similar to our findings in this study. (Bramley and Dodd, 1984) reported that 73% of British herds harbored at least one cow infected with *S. uberis* and that this organism was responsible for 14% of clinical mastitis cases. (Watts, 1988) reported that 98 of 317 isolates (30.9%) were identified as *S. uberis.*

Our findings support the observation of (Keefe, 1997) that the prevalence of infection with group B streptococci can reach 44% in infected herds. Recently, (Ekin and Gurturk, 2006) also recorded 44.7% group B streptococci from bovine mammary glands. The *S. agalactiae* encountered in this study was 100% CAMP

test and 0% esculin positive, respectively. This finding conforms to that earlier reported by (Watts, 1988).

In this study Lancefield group C contains two species, *S. dysgalacliae* and *S. zooepidemicus. S. zooepidemicus* exhibited β-haemolysis on sheep blood agar, which distinguishes these organisms from alpha-haemolysis *of S. dysgalacliae.* All sixteen isolates (12.31%) identified as *S. dysgalacliae* in this study exhibited serological and biochemical characteristic similar to those described previously for bovine *S. dysgalactiae* by (McDonald and McDonald, 1976). *S. zooepidemicus* is differentiated from *S. dysgalactiae* on the basis of trehalose utilization. *S. zooepidemicus* utilizes lactose, sucrose and sorbitol but not trehalose (Farrow and Collins, 1984). In this study, two species reacted with group D antisera. Of these, 3(2.31%) produced 100% esculin hydrolysis, and 100%, 95.1%. 100% and 60.5% of lactose, mannitol, raffmose and trehalose fermentation, respectively. The strains were identified as *S. bovis* (Barrow and Felthman, 1993). The second strain under group D Lancefield group of streptococci have an incidence of 1.54%. This strain produced 57.8% esculin hydrolysis and 100% for sucrose and salicin, but lactose, mannitol, raffmose. sorbitol and trehalose were not fermented, hence, identified as *S. equinus* (Barrow and Feltham, 1993). In this study a total of six Lancefield groups were determined for 130 gram-positive, catalase-negative cocci. In conclusion, the results of the present study indicate the accurate identification of streptococcal species. Work on the distribution of these organisms and define their role in bovine mastitis is in progress.

REFERENCES

Baron, E.J., L.R. Peterson and S.M. Fine-gold (1994); Streptococci and related genera. Pages 336-337 in Bailey and Scott's Diagnostic Microbiology. 9th ed. Mosbv, St. Louis. MO.

Barrow, G.T., Feltham, R.K.A. (1993); Cowan and steel's manual for the identification of medical bacteria, third edition Cambridge University Press. Cambridge. 331pp.

Bradley, A.J. (2002); Bovine mastitis: An evolving disease. *Vet. J.* 164: 116-128.

Bramley, A.J. and Dodd, F.H. (1984); Reviews of the progress of dairy science: mastitis control progress and prospects. J. Dairy Res. 51:481-521.

Buriel, A.R. (1997); Dynamics of intramammary infection in the sheep caused by coagulase-negative staphylococci and its influence on udder tissue and milk composition. *Vet. Rec.* 140, 419-423.

Calvinho, L.F. and Oliver, S.P. (1998); Factor influencing adherence of streptococcus dysgalactiae to bovine mammary epithelial cell monolayers. J. Vet. Med. B. 45, 161-170.

Chamberlain. A. (1989); Milk production in the tropic. Intermediate Tropical Agriculture Series. First edition.

Cruickshank, R., Duguid, J.P., Marmion, B.P., Swan, R.H.A. (1975); Medical microbiology. 12th ed. Churchill Livinstone. Edinburgh London. 587pp.

Ekin, I.H. and K. Gurturk (2006); Characterisation of bovine and human group B streptococci isolated in Turkey. Journal of Medical Microbiology 2006, 55, 517-521.

Facklam, R. (2002); What happened to the streptococci: Overview of taxonomic and nomenclature changes. Clin. Microbiol. Rev. 15:613-630.

Farrow, J.A.E. and Collins, M.D. (1984); Taxonomic studies on streptococci of serological groups C.G.. and L. and Possibly related taxa. Syst. Appl. Microbiol., 5:483-493.

Fortin, M.. S. Messier, J. Pare, and R. Higgins (2003) Identification of catalase-negative, non-β-haemolytic, gram-positive cocci isolated from milk sample. Clin. Microbiol. 41:106-109.

Freney, J., S. Bland. J. Etienne, M. Desmonceaux, J.M. Boeufgras. and J. F. Seurette. (1992); Description and evaluation of the semiautomated 4-hour rapid ID 32 strep method for identification of streptococci and members of related genera. J. Clin. Microbiol. 30:2657-2661.

Keefe, G.P. (1997); *Streptococcus agalactiae mastitis: a review.* Can Vet. .1. 38, 429-437.

Khan, I.U., A.A. Hassan, A. Abdulmawjood. C. Lanimler, W. Wolter, and M. Zschock. (2003); Identification and epidemiological characterization of *Streptococcus uberis* isolated from bovine mastitis using conventional methods. J. Vet. Sci. 4:213-223.

Ko, W.C., Lee, H.C., Wang, L.R., Lee, C.T., Liu, A.J. and Wu, J.J. (2001); Serotyping and antimicrobial susceptibility of group B streptococcus over an eight-year period in southern Taiwan. *Eur J. Clin. Microbiology Infect Dis* 20, 334-339.

Lammler, C. (1991); Biochemical and serological properties of *Streptococcus uberis.* J. Vet. Med. Ser. B. 38:737-742. Leigh. J.A. 1999. *Streptococcus uberis:* A permanent barrier to the control of bovine mastitis. Vet. J. 157:225-238 McDonald, J.S. 1984. Streptococcal and staphylococcal mastitis. Vet. Clin. North Am. 6:269-285.

Leigh, J.A. (1999); Streptococcus uberis: A permanent barrier to the control of bovine mastitis. *Vet. J.* 157: 225-238.

Leitner. G., Chaffer, M., Zamirs, Mor, T., Glickman, A., Winkler, M. Weisblit, L. and Saran. A. (2001); Udder disease etiology, milk somatic cell

and NAGase activity in Israel Assaf Sheep throughout lactation Small Ruminant Research 39:107-112.

MacDonald, I. and Low, J. (1985); Livestock Rearing in the tropics. Macmillan Publishers Ltd.. London.

McDonald, T.J. and McDonald, J.S. (1976); Streptococci isolated from bovine intramammary infections. Am. J. Vet. Res. 37:377-381.

Menzies, P.I., Ramanoon, S.Z. (2001); Mastitis of sheep and goats. *Vet. Clin. North Am. Food Anim. Practical* 17, 333-358.

Mosabi, J.M., Arimi, S.M. and Kangethe, E.K. (1997) Isolation and characterization of group B streptococci from human and bovine sources within and around Nairobi. *Epidemiol Infect* 118, 215-220.

Myllys, V. and Rautala, H. (1995); Characterization of clinical mastitis in primiparous heifers. *J. dairy Sci.* 78: 538-545.

Oliveira, P. Watts. J., Salmon, S. and Aarestrup, M. (2000); Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in the Europe and United States. Dairy Science Journal, 83:855-862.

Radostits, O.M., Gray, C.C., Blood, D.C., Hinchcliff, K.W. (2000); Veterinary medicine. A textbook of the disease of cattle, sheep, pigs, goats and horses. 9th edition W.B. Saunders Company, London P. 996-1008.

Radostits. O.M., Blood. D.C., Gray, C.C. (1994); Veterinary medicine. A textbook of diseases of the cattle, sheep, pigs and horses. 8th edition. Bailliere Tindall. London. *P.* 403-417.

Schuctat, A. and Wenger, J.D. (1994); Epidemiology of group B streptococci disease. *Epidemiol Rev.* 16, 374-402.

Sordielli, D.O., Buzzola, F.R., Gomez, M.I., Steele-Moore, L., Berg. D. Gentilini, E., Catalanno, M, Reitz. A.J., Toller, Srud. T., Denamiel, G., Jeric, P. and Lee. J.C. (2000) Capsule expression by bovine isolates *of Staphylococcus aureus* from Argentina: Generic and Epidemiologic analyses. Journal of Clinical Microbiology. 38:846-850.

Vieira-da-Motta, O., Folly, M.M., Sakyiama, C.C.H. (2001); Detection of different staphylococcus aureus strains in bovine milk from subclinical mastitis using PCR and routine techniques. *Braz. J. Microbiol*. 32, 27-31.

Watts, J. L. (1988); Characterization and identification of streptococci isolated from mammary gland. J. Dairy Sci. 71:1616-1624.

Watts, J.L. (1989); Evaluation of the minitek gram positive set for identification of streptococci isolated from bovine mammary gland. J. Clin. MicrobioL 27:1008-1010.