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Vaginal Carriage of Antimicrobial Resistant Lancefield Group G Streptococci among Dogs in Lagos Metropolis, Nigeria

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ABSTRACT

Streptococcus species are common colonisers of urogenital tracts in dogs. While their involvement is becoming evident in clinical infections like foetal abortion and pathology of multiple organs in dogs, there is a paucity of information on the pathogenicity of the organism in apparently healthy dogs in Nigeria. In this study, 154 samples were obtained from 154 apparently healthy fertile and infertile bitches in Lagos State, Nigeria. Using the Lancefield classifications, 14 Streptococcal isolates were found to belong to the pathogenic Group G. Biochemical characterization was studied, Haemolysis on blood agar showed all isolates to be haemolytic *Streptococcal canis* and antimicrobial susceptibility testing revealed their susceptibility pattern. Eight (57.14%) of the isolates were from infertile bitches compared with 6 (42.86%) from fertile bitches. Regular contact of the dog owners with their vaginal discharge may constitute a potential source of higher risk of disease of streptococcal origin of public health importance.

1. INTRODUCTION

Many infectious agents have been suspected to induce infertility in bitches. Canine Distemper and Canine Herpes viruses are well known to cause infertility and parasite like *Neospora caninum* has also been documented as a cause of canine infertility (Robbe *et al*., 2016). *Streptococcus* species have usually been seen as commensals of the urogenital tract. More recently, however, Givens and Morley (2008), Pinho *et al.,* (2013) identified *streptococcus* species as one of the important causes of foetal and embryonic mortality and infertility in bitches and can be frequently isolated from the vagina, cervix and uterus (Watts *et al* 1996).

In dogs, pathogenic species of streptococcus infections typified by *Strep. canis* belong to Lancefield group G, (DeWinter and Prescott 1999; Lamm *et al*, 2010). Zoonotic infections of *Strep. canis*, especially of dog and owners have been documented by several authors (Lam *et al*, 2007; Pinho, *et al* 2013., Fulde and Valentin-Weigand 2013., Lacave *et al* 2016). Taniyama *et al* 2017, recently, reported a human case of uncomplicated bacteremia following a dog bite, caused by *Strep. canis* from the Lancefield Group G. In Nigeria, not much of Streptococcal Lancefield classification has been done on the dogs from most of the communities, neither has there been any link between human infections caused by *Strep. canis* and dog bite. Also not much attention been paid to the Public Health importance of *Strep. canis* infection of dogs in most of the communities in the country.

In this study, an attempt was made to characterise vaginal Streptococcal isolates from fertile and infertile bitches using haemolysis, Lancefield grouping and antibiotic resistance studies.

2. MATERIALS AND METHODS

Sample collection and isolation: 154 vaginal swabs (one sterile swab per bitch) were randomly collected under aseptic condition from 154 fertile and infertile dogs in Lagos metropolis using sterile cotton - tipped wooden swab sticks and kept in a cold box with frozen ice packs till analysis. Infertility status of bitches was based on history provided by the dog owners. A bitch was considered infertile if it was unable to produce any puppy or insufficient number of puppies after two successive mating by a fertile stud (Allain, 2011). Each vaginal swab was plated onto Columbia blood agar (CBA), MacConkey agar (MAC) plates for the isolation of aerobic bacteria, and incubated for 48 hours at 37℃. All the isolates obtained were separated for pure culture on nutrient agar with 7% sheep blood agar. Suspected streptococci colonies were phenotypically characterized according to their colonial morphologies, Gram staining and biochemical activities such as catalase and oxidase activities, Indole and Voges-Proskauer reactions, and production of acid from arabinose, sorbitol and mannose (Lysková *et al*, 2007a, Lysková *et al*, 2007b, Lam *et al* 2007).

2.1.0. **Haemolytic reaction**: Biotyped streptococci isolates were typed for hemolytic reaction according to Cheesbrough, (2000). Briefly, typed streptococci were cultured on Columbia agar base medium (Oxoid U.K.) supplemented with 7% sheep blood. The plates were then incubated at 35-37℃ for 48hours and typical hemolytic pattern were identified as complete (β-hemolytic), partial (α-hemolytic) and non-hemolytic (Gamma, γ-haemolysis) (Perez-Trallero *et al* 2007).

2.1.1. Biotyping: Each identified streptococcus isolates was further biotyped into Lancefield groups according to protocol described by Perez-Trallero *et al* (2007).

2.1.2. Antimicrobial susceptibility testing: In this study, commonly used antibiotic agents were selected for disc diffusion testing. These included amoxicillin (10µg), ciprofloxacin (5µg), cefuroxime (30µg), ceftazidime (30µg), ceftriazone (30µg), chloramphenicol (10µg), gentamycin (10µg) and streptomycin (10µg). The strains were grown overnight on 7% sheep blood agar plates at 37^0C. A bacterial suspension of 0.5McFarland standard was plated on 7% sheep blood agar and antibiotics discs were placed. The inhibitory zone diameters obtained around the antibiotic disks were measured after incubation for 24 h at 37 ℃ and interpreted according to CLSI (2014) guidelines.

2.1.3. Minimum Inhibitory Concentration Determination (MIC): Standard broth micro-dilution method was used to determine the MIC of each antibiotic against identified isolate in the following dilution range of 0.5–64 µg/mL. MIC of each antibiotic was interpreted according to CLSI guidelines (2014).

3.0 Statistical Analysis

Prevalence of beta-hemolytic group G *Streptococcus* among fertile and infertile bitches was analyzed using statistical package for Social Sciences (SPSS.16) and presented as percentages in tables. Antimicrobial susceptibility pattern data was analyzed using descriptive statistics such as percentages.

4. RESULTS

Among the 154 bitches sampled, frequency of streptococcus were more in 82 bitches that are infertile (53.2%) than in 72 fertile bitches (46.80%).

4.1. Biochemical characteristics: Fourteen (14) of 154 samples were streptococcal isolates and were Catalase, Oxidase and Coagulase negative but Indole and Vogues-Prokauer positive. Acid was also produced from arabinose, sorbitol and mannose. Nine out of the 14 isolates (5.8%) showed α-haemolytic activities, 4 (2.6%) showed β-haemolytic activities, but only 1 (0.7%) showed γ-haemolytic activities.

The 14 Streptococcus species were found to belong to Lancefield G sero-group. Six were isolated from the fertile bitches compared to 8 that were from the infertile group. One bitch out of 72 (1.3%) among the fertile bitches possess streptococci Dual antigen, but 2 of 82 bitches (2.4%) had Dual antigen and were detected among the infertile bitches Table 1.

4.1.2. Antimicrobial Susceptibility Profile: The Antimicrobial susceptibility of 14 streptococci isolates obtained from this study showed high rate of resistance to cefuroxime (85.7%), amoxicillin (78.6%), chloramphenicol, (71.4%) and ceftazidime (64.3%). The isolates were also resistant to Ceftriaxone (50%), gentamycin (50%) and streptomycin (21.4%), Table 2.

4.1.3. Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration of various tested antibiotic suggest high rate of resistance to amoxicillin, cefuroxime, ceftazidime, and ceftriaxone chloramphenicol with MIC level greater than 16µg/mL.

Table 1. Lancefield sero-grouping of Streptococcus species found among the bitches

	A	B	C	D	F	G	Dual Antigen
	n (%)						
Fertile bitches (72)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(3.9)	1(1.3)
Infertile bitches (82)	0(0.0)	1(0.7)	2(1.3)	1(0.7)	0(0.0)	8(5.2)	2(2.4)

Bitches examined (N) Lancefield grouping of *Streptococcus species* found in bitches vaginal isol**ates** (N=154)

N=total number of bitches examined; n=number of bitches with *Streptococcus species*;
% = percentage rate of *Streptococcus species*)

Table 2. Antimicrobial susceptibility pattern (N=14)

Antibiotic	**Susceptibility rate**			***MIC (0.5–64 µg/mL)**
	S	I	R	**n(%)**
Amoxicillin	0(0.0)	3(21.4)	11(78.6)	14(100)
Ciprofloxin	11(78.6)	3(21.4)	0(0.0)	0(0.0)
Cefuroxime	2(14.3)	2(14.3)	12(85.7)	12(85.7)
Ceftazidime	3(21.4)	3(21.4)	9(64.3)	11(78.6)
Ceftriaxone	4(28.6)	3(21.4)	7(50.0)	10(71.4)
Chloraphenicol	4(28.6)	4(28.6)	10(71.4)	10(71.4)
Gentamycin	5(35.7)	2(14.3)	7(50.0)	9(64.3)
Streptomycin	6(42.9)	5(35.7)	3(21.4)	3(21.4)

5. DISCUSSION

Streptococcus species in Group G have been found to be associated colonizers of the urogenital system in dogs and cats and have been implicated in fetal or neonatal septicemia, leading to abortion or neonatal death, respectively (Lamm *et al* 2010).

Although *Strep. canis* isolates present considerable variability in biochemical profiles, the biochemical results obtained in this study is consistent with the study of Whatmore *et al* (2001) on *Strep. canis*. Also the MIC and basic antibiotic patterns of the streptococcus isolates are consistent with the Antibiotic resistant patterns of pyogenic Lancefield Group G of the *Strep. canis* of Pinho *et al* (2013).

Dewinter *et al* (1999) reported *Strep. canis* to be the most common streptococcal species found in dog infections. In this study, the Haemolysis analysis and the Lancefield Streptococcal species characterization results are consistent with the results of Bert and Lambert-Zechovsky, 1997 and Lamn *et al* (2010). It is therefore strongly suggestive that the streptococcal isolates in this study are most likely *S. canis.*

The studies of Clark *et al* (1984), Skyes *et al* (2005), Lyskova *et al* 2007b and Lamm *et al* (2010), isolated *Strep. canis* as the most common Streptococcal isolates found in dogs in clinical infections such as dermatitis, otitis externa, pneumonia, infective endocarditis, abortion and neonatal death.

In contrast however, the isolates from this study were from apparently healthy bitches with no obvious lesions or observed clinical signs. The main observable sign was infertility. This shows that dogs infected with the pathogenic *Strep. canis* may or may not present with visible clinical signs on dogs.

While the *Strep. canis* from other studies, referred to above, were recovered from infected lungs, hearts, aborted fetuses of bitch, similar *Strep. canis* isolates have been obtained from the milk of cows and cats with mastitis (Pasevantyo *et al* 2007., Hassan *et al* 2007). The Streptococcal isolates from this study were from the vagina swabs.

Strep. canis transmission among domestic animals living in close proximity has been documented by Tikofsky and Zadoks (2005). *Strep. canis* have also been found to be responsible for outbreaks of clinical and subclinical mastitis with bacterial shedding in milk in cattle herds (Chaffer *et al* 2005).

It is therefore suggested that bitch should be considered as potential source of septicemias, dermatitis and pneumonia that are of Streptococcal origin in other livestock within the locality and such bitch should be considered in the management of such infections.

Although this study has documented the isolation and characterization of *Strep.* canis from vaginal swabs, it is not impossible for the *Strep. canis* to be found in vaginal discharges of the bitches. It is also not impossible for such discharges to be of high risk of human contamination of dog owners and children that have close contact with apparently healthy dogs or dogs with subclinical infections.

Although *Strep. canis* infection was previously thought to be rare in humans, it has been associated with leg ulcers in dog owner (Bert and Lambert-Zechovsky 1997, Lam *et al* 2007; meningitis in man (Jacobs *et al* 1993) and severe bacteremia with cellulitis in immuno-compromised patient (Taniyama *et al*., 2017). An expanding human population concomitant with an increasing number of dogs owners favour the conditions for the transmission of *Strep. canis* streptococci to man. This study may be suggesting that *Strep. canis* is becoming a Public Health risk even in apparently healthy dogs. Therefore the search for the pathogen is recommended whenever patients that have history of contacts with dogs present with systemic symptoms.

6. REFERENCES

Abdulwahed, A. H., Ömer, A., Usleber, E. 2005. Identification of *Streptococcus canis* isolated from milk of dairy cows with subclinical mastitis. J. Clin. Microbiol. 43 (3): 1234-1238.

Allain, F**.** 2011. Infertility in bitches and queens: Recent advances. Rev Bras Reprod Anim. Belo Horizonte. 35(2):202-209.

Bert, F., Lambert-Zechovsky, N. 1997. Septicemia caused by *Streptococcus canis* in a human. J Clin Microbiol. 35:777–779.

Chaffer, M., Friedman, S., Saran, A., Younis, A. 2005. An outbreak of *Streptococcus canis* mastitis in a dairy herd in Israel. N Z Vet J. 53: 261–264.

Cheesbrough, M. 2000. Microbiology. In Medical Laboratory Manual for Tropical Countries. ELBS Edition. Cambridge UK Cambridge University Press. pp 26–58.

Clark, R.B., Berrafati, J.F., Janda, J.M., Bottone, E.J. 1984. Biotyping and exoenzyme profiling as an aid in the differentiation of human from bovine group G streptococci. J Clin Microbiol. 20:706–710.

Clinical and Laboratory Standard Institute. 2014. Twenty-First Informational Supplement, CLSI Document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing.

DeWinter, L. M., Prescott, J. F. 1999. Relatedness of *Streptococcus canis* from canine streptococcal toxic shock syndrome and necrotizing fasciitis. Can. J. Vet. Res. 63: 90–95.

Fulde, M., Valentin-Weigand, P. 2013. Epidemiology and pathogenicity of zoonotic streptococci. Curr. Top. Microbiol. Immunol. 368: 49-81

Givens, M.D., Marley, M. S. 2008. Infectious causes of embryonic and fetal mortality. Theriogeneology. 70: 270 -85

Hassan, A.A., Akineden, O., Usleber, E. 2007. Identification of *Streptococcus canis* isolated from milk of dairy cows with subclinical mastitis. J. Clin Microbiol. 43:1234–1238.

Jacobs, J. A., de Kron, M. C. T., Kellens, J. T. C., Stobberingh, E. E. 1993. Meningitis and sepsis due to the Group G Streptococcus. Eur. J. Clin. Microbiol. Infect. Dis. 12:224–225.

Lacave, G., Coutard, A., Troché, G., Augusto, S., Pons, S., Zuber, B., Laurent, V., Amara, M., Couzon. B., Bédos, J.P., Pangon, B., Grimaldi, D. 2016. Endocarditis caused by *Streptococcus canis*: an emerging zoonosis? Infection. 44(1):111-4.

Lam, M.M., Clarridge, J.E., Young, E.J., Mizuki, S. 2007. The other group G Streptococcus: increased detection of *Streptococcus canis* ulcer infections in dog owners. J Clin. Microbiol. 45:2327–2329.

Lamm, C.G., Ferguson, A.C., Lehenbauer, T.W., Love, B.C. 2010. Streptococcal infection in dogs: a retrospective study of 393 cases. Vet Pathol. 47:387–395

Lysková, P., Vydržalová, M., Královcová, D., Mazurová, J. 2007(a). Prevalence and Characteristics of *Streptococcus canis* strains isolated from Dogs and Cats Acta Vet. Brno. 76: 619-625.

Lyskova, P., Vydrzalova, M.,Mazurova, J. 2007(b).

Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. J. Vet. Med. A. Physiol. Pathol. Clin. Med. 54(10):559-63.

Perez-Trallero, E., Montes, M., Orden, B., Tamayo, E., Garcia-Arenzana, J.M., Marimon, J. M. 2007. Phenotypic and genotypic characterization of *Streptococcus pyogenes* isolates displaying the MLSB phenotype of microlide resistance in Spain 1999 to 2005. Antimicrob. Agents. Chemother. 51(4):1228-33.

Pesavento, P.A., Bannasch, M.J., Bachmann, R., Byrne, B.A., Hurley, K.F. 2007. Fatal *Streptococcus canis* infections in intensively housed shelter cats. Vet. Pathol. 44:218–22

Pinho, M.D.S., Matos, C., Pomba, A. Lübke-Becker, L.H., Wieler, S., Preziuso, J. Melo-ristino M. Ramirez. 2013. Multilocus Sequence Analysis of *Streptococcus canis* confirms the zoonotic origin of human infections and reveals genetic exchange with *Streptococcus dysgalactiae* subsp *equisimilis* J. Clin. Microbiol. 51. 4. 1099-1109

Robbe, D., Passarelli, A., Gloria, A., Di Cesare, A., Capelli, G., Iorio, R., Traversa, D. 2016. *Neospora caninum* seropositivity and reproductive risk factors in dogs. Exp. Parasitol. 164: 31-5

Sykes, J.E., Kittleson, M.D.,Pesavento, P.A., Byrne, B.A., MacDonald, K.A., Chomel, B.B. 2005. Evaluation of the relationship between causative organisms and clinical characteristics of infective endocarditis in dogs: 71 cases (1992-2005). J. Am. Vet. Med. Assoc. 228: 1723–1734.

Taniyama, D., Abe, Y., Sakai, T., Kikuchi, T., Takahashi, T. 2017. Human case of bacteremia caused by Streptococcus canis sequence type 9 harboring the *scm* gene. IDCases. 7: 48–52. doi: 10.1016/j.idcr.2017.01.002 Published online. Jan 20.

Tikofsky, L.L., Zadoks, R.N. 2005. Cross-infection between cats and cows: origin and control of

Streptococcus canis mastitis in a dairy herd. J. Dairy Sci. 88:2707–2

Watts, J.R., Wright, P.J., Weather, K.C. 1996. Uterine, Cervical and Vaginal microflora of the normal bitch throughout the reproductive cycle. J. Small Animal Practice. 37: 54-60

Whatmore, A. M., Engler, K. H., Gudmundsdottir, G., Efstratiou, A. 2001. Identification of isolates of *Streptococcus canis* infecting humans. J. Clin Microbiol. 39: 4196-4199.