MICROBIAL ASSOCIATION WITH SUSPECTED CUTANEOUS LEISHMANIASIS (CL) LESIONS ON DOGS IN JOS-SOUTH PLATEAU STATE NORTH-CENTRAL, NIGERIA

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Abstract

Cutaneous Leishmaniasis is a zoonotic, neglected tropical disease of public health importance in some parts of the world. Dogs are reported as reservoir hosts of this disease. Secondary microbial infections cause complications of increasing tissue destruction and septicaemia. This study determined the association of microorganisms with suspected CL lesions on dogs and their susceptibility to drugs using Micro-bacteriological and Mycological diagnostic techniques. 72 swab samples and lesion scrapings were collected from active cutaneous wounds using sterile swab sticks and surgical blades on Mongrels with suspected CL lesions. The samples were diagnosed for *Leishmania* amastigotes by microscopy using Giemsa stained technique. Swabs were inoculated into Blood agar (BA), MacConkey's agar (MCA), Desoxycholate-citrate agar (DCA) and Triple sugar iron agar (TSIA) and incubated at 37 ºC for 24hrs for bacterial isolation. Suspected CL lesion scrapings were also inoculated into Sabouruad dextrose agar (SDA), sealed with masking tape and incubated at room temperature and observed for fungal growth after 7 days. There was zero (0.0%) occurrence of *Leishmania* amastigotes. 39 (54.175) *Staphylococcus* species was isolated from MCA, 9(12.50%) from DCA and 54(75.0%) from BA. 9(12.50%) *Streptococcus* spp. was isolated from MCA, 6(8.33) from DCA and 3(4.17) from BA. 14(19.44) *Bacillus* spp. was isolated from MCA, 21(29.17) from DCA and 13(18.06) from BA. 3(4.17) *Enterobacter* spp., *Serratia* spp. and *Alkaligenes* spp. were isolated from TSIA. 24(33.33) *Aspergillus* spp., *Trychophyton* spp. and *Mucor* spp. were isolated, 18(25.00) *Microsporum* spp., 12(16.67) *Penicillium* spp. and 9(12.50) *Candida* spp. Bacteria and fungi are associated with suspected CL lesions. Secondary microbial infected CL lesions could be treated with Ofloxacin, Gentamicin and Tetracycline.

Keywords: Microbial Association; Suspected Cutaneous Leishmaniasis Lesions; Dogs; *In Vitro* Antimicrobial Screening

Introduction

Leishmaniasis is one of the diverse and complex and important vector borne diseases of human caused by several species of *Leishmania* with a complex ecology and epidemiology, (Manu *et al*., 2006; Sharma and Singh, 2008, Daniel *et al.,* 2017). Leishmaniasis manifests in multifaceted forms as: Cutaneous Leishmaniasis (CL), Mucosal Cutaneous Leishmaniasis (MCL), Diffuse Cutaneous Leishmaniasis (DCL) and Visceral Leishmaniasis (VL) (Manu *et al*., 2006, Daniel *et al.,* 2017). *Leishmania* species are transmitted exclusively via the bite of female sandflies belonging to two genera, *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Grimaldi and Tesh, 1993; Moncaz *et al*., 2012). Worldwide human prevalence of the disease is reported to exceed 12million cases with an estimated annual incidence of 400,000 cases, occurring in over 88 countries among a population at risk of about 400 million (Alvar *et al.,* 2008; Mayrink *et al*., 2010; Durrani *et al.,* 2011).

Cutaneous Leishmaniasis (CL) is a worldwide public health and social problem in many developing countries. Old World cutaneous leishmaniasis (OWCL) is present in many endemic areas in North Africa, the Mediterranean, the Middle East, the Indian subcontinent and Central Asia. The species responsible for OWCL are mainly *L. major* and *L. tropica* (Alraijhi, 2003). *Leishmania infantum* and *L. donovani* can also cause localised CL but are observed less frequently in the Mediterranean areas (Alraijhi, 2003, Reithinger *et al*., 2007). *Leishmania*, a genus of flagellate protozoa (suborder: Trypanosomatidae, order: Kinetoplastida) has worldwide distribution humans (Barral and Barral-Netto, 1994).

Dogs are reported to play vital role as reservoir hosts of this disease (Seixas *et al*., 1994; Andrade-Narvaes *et al*., 2003; Silveira *et al*., 2004; Boakye *et al*., 2005; Yoshie do Rosario *et al*., 2005; Kimutai *et al*., 2009; Shaw *et al*., 2009; Reithinger *et al*., 2010). A statement from Center for Disease Control and Prevention (2016), says that leishmaniasis has been found in a few dogs imported to the United States from areas of the world where the disease is prevalent. According to Velez *et al*., (2012) species of *Leishmania* such as *Leishmania braziliensis* and *Leishmania panamensis*, among others, can infect dogs and produce symptoms and signs similar to CL in humans. Although dogs may be considered as potential reservoir for CL (Seixas *et al*., 1994; Reithinger *et al*., 2010) there are few records of studies on dog CL in Nigeria (Daniel *et al.,* 2017, Daniel, 2018) and in a statement made by Velez *et al*., (2012) there are very few studies describing canine CL in South America. Dogs can be infected when the sand flies bite them (Center for Disease Control and Prevention, 2014; WHO, 2014 and 2016).

Clinical signs of CL in dogs are variable and can mimic other infections and skin lesions in dogs (Centre for Food Security and Public Health 2009). Common cutaneous syndrome is a non-pruritic exfoliative dermatitis, nodules, ulcers or scabs, atypical skin lesions including postular rashes, panniculitis, depigmentation, erythema multiforme, digital and nasal hyperkeratosis, and cases that resemble alopcea areata or pemphigus foliaceus found especially around the nose, eyes (the face), ears, feet, and perinea regions where dogs have less hair (Centre for Food Security and Public Health 2009; Bako, 2012; Kent *et al*., 2013).

Cutaneous leishmaniasis lesions have been reported to associate with bacteria and fungi infections (Ikeh, 1996, Centre for Food Security and Public Health 2009; Darogha, 2009, Garg *et al.,* 2009, Idowu *et al*., 2011, Ahmed, 2012, Daman, (2012), and Pyendang, (2012), Daniel *et al.,* (2017). These researchers observed the growth of bacteria isolates such as *Staphylcoccus aureus*, *Escherichia coli* and *Proteus vulgaris* and fungi such as *Aspergillus flavus,Rhizopus*, and P*enicillium* in surface open wounds of human. According to Hengameh and Sadeghian (2008), secondary bacterial infection of the CL will increase the tissue destruction and the resulting scar. The study determined the association of microorganisms with suspected CL lesions on dogs using Micro-bacteriological and Mycological diagnostic techniques and *In Vitro* Antimicrobial Screening in Jos-South Plateau State North-Central, Nigeria.

MATERIALS AND METHODS

Description of study area and sample collections

The study was carried out in the 4 districts (Du, Gyel, Kuru and Vwang) of Jos-South Local Government Area (LGA) of Plateau State, Nigeria. Plateau State lies in the Niger – Benue trough (Nigerian Middle- belt). Plateau State is a mountainous area in the north-central of the country with captivating rock formations. Its bearing is 9º10'N 9º45'E. It has an area of about 30,913 square

kilometres. The population of the state is place at 4, 178, 712 million during the 2006 census. Jos South, which capital is Bukuru with bearings 9º40'N 9º52' E has an area of about 5,104 square kilometres, an estimated total population of around 306,716 people. It is bounded by Jos- North and Jos- East LGAs - to the northeast, Bassa LGA- to the northwest, Riyom LGA - to the southwest and Barkin Ladi LGA - to the southeast (NIPOST, 2009; Plateau State Government, 2015).

Ethical clearance was obtained from Plateau State Ministry of Health. Dog owner's informed consents were sought prior to commencement of clinical examination, lesion snip swab sample collections. Familiarization and advocacy visits were made accompanied by oral enlightenment campaigns about the disease leishmaniasis and possible association of microorganisms with the surface CL lesions. House-to-house screening of 72 dogs with characteristic active CL lesions was made. Samples were collected by trained veterinary health personnel. The wounds were first cleaned using sterile cotton swabs and aseptically placed in peptone water. The specimens were collected by gently rotating sterile swabs in the wounds. The areas affected were cleaned with 70% alcohol, lesion scrapings were taken by making small incisions at the swollen edges of the affected areas to depths of 2mm with needles, raised and cut with surgical blades. The cut tissues were shared into two, one part teased and fixed on grease-free glass slides for *Leishmania* amastigote diagnosis, and one part wrapped in foil papers for fungal isolation. The teased and fixed tissues were examined in the Parasitology laboratory and the swab sticks and wrapped lesion snips were surveyed for bacteria and fungi in the Bacteriology Laboratory of the Federal College of Veterinary and Medical Laboratory Technology, Vom respectively.

Diagnosis of Leishmania amastigotes from Suspected CL Lesions on Dogs

On reaching the laboratory, the fixed tissues were stained with Giemsa, kept for 1hour, washed with distilled and allowed to dry. The smears were examined microscopically using Immersion oil (X100) objectives as described by Bryceson (1976), adopted by Agwale (1996), Manu (1998), Daniel *et al*., (2017) and Daniel (2018).

Isolation of Bacteria from Suspected CL Lesions on Dogs

Bacteria isolation was carried out adapting the methods of Cowan and Steel, (1974); Finegold and Martin (1982); Esekwe (1993) and Norrell *et al*., (1997). The 72 swab sticks for bacteria isolation inoculated into BA, MCA, DCA and TSIA were incubated at 37ºC for 24hrs. Inoculates were spread on the different agar media using the streak method and incubated at 37 ºC for 24hrs. The smears from each of the morphologically different colonies observed on the agar were, heat-fixed with Gram stain, and examined microscopically. Bacteria were identified from their form, size, reaction to Gram stain, and colony characteristics on the culture media. The smears were microscopically examined for bacteria according to the method described by Bauer *et al.,* (1966).

Isolation of Fungi from Suspected CL Lesions on Dogs

The 72 lesion scrapings were inoculated into SDA, incubated at room temperature and observed for fungal growth after 7 days. Fungi were macroscopically identified in accordance with Cheesbrough, (2000) techniques.

RESULTS

Results showed that surface CL lesions were more prevalent on the ears than on the head and zero percent (0.0%) occurrence of Leishmania amastigotes in the suspected CL lesions examined (Table 1). Plate 1: Representatives of CL Lesions observed: on

the ears and on the head. Results revealed 54(75%) of *Staphylococcus* spp. on BA, 39(54.17%) on MCA and 9(12.50%) on DCA; 9(12.50%), 6(8.33) and 3(4.17%) was isolated on MCA, DCA and BA respectively. *Bacillus* spp. was isolated on 21(29.17%) DCA plates, 14(19.44%) MCA plates and 13(18.06%) BA plates respectively. *Enterobacter* spp., *Serratia* spp. and *Alkalignes* spp. were isolated each on 3(4.17%) plates of TSIA, (Table 2). Plate 2 showed bacterial growth on some agar and zone of inhibition of antimicrobial discs on test bacteria isolates. Table 3 and Plate 3 showed isolates of fungi on SDA. Of the 72 plates, 24/72 (33.33%) showed the incidence of *Aspergillus* spp., *Trychophyton* spp. and *Mucor* spp., followed by *Microsporum* spp. 18 (25%), *Penicillium* spp. 12(16.67) and *Candida* spp. 9(12.50). The results showed multiple growths of the microorganisms on the different agar.

Table 1: Site of Infection Related Prevalence of *Leishmania* amastigotes from Suspected CL Lesions on 72 Dogs

Site of Infection	No. (%) Examined with Lesions	No. (%) Examined with *Leishmania* amastigotes
EAR	**63 (87.50)**	**0 (0.0)**
HEAD	**9(12.50)**	**0 (0.0)**
TOTAL	**72 (100)**	**0 (0.0)**

Table 2: Bacteria isolate from Suspected CL Lesions of 72 dogs

Bacteria Isolates	Agar/Frequency of Occurrence of Isolates			
	MCA (%)	DCA (%)	BA (%)	TSIA (%)
Staphylococcus sp	39(54.17)	9(12.50)	54(75)	NIL (0.00)
Streptococcus sp	9(12.50)	6(8.33)	3(4.17)	NIL (0.00)
Bacillus spp	14(19.44)	21(29.17)	13(18.06)	NIL (0.00)
Enterobacter spp	NIL(0.00)	NIL (0.00)	NIL(0.00)	3(4.17)
Serratia spp	NIL(0.00)	NIL (0.00)	NIL(0.00)	3(4.17)
Alkalignes spp	NIL(0.00)	NIL (0.00)	NIL(0.00)	3(4.17)

Table3: Fungi Isolates from Cutaneous Leishmaniasis Lesions of 72 Dogs on SDA Medium

Fungal isolates	***Aspergillus* sp**	***Microsporum* sp**	***Trychophyton* sp**	***Penicillium* sp**	***Candida* sp**	***Mucor* sp**
Frequency of occurrence (%)	**24(33.33)**	**18(25.00)**	**24(33.33)**	**12(16.67)**	**9(12.50)**	**24(33.33)**

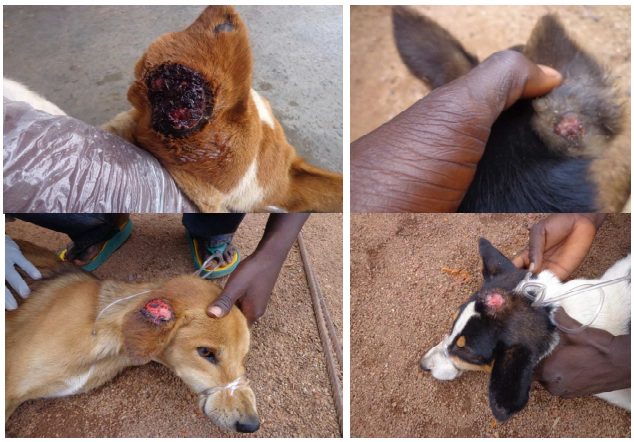

Plate 1: Representatives of CL Lesions observed: on the ears and on the head

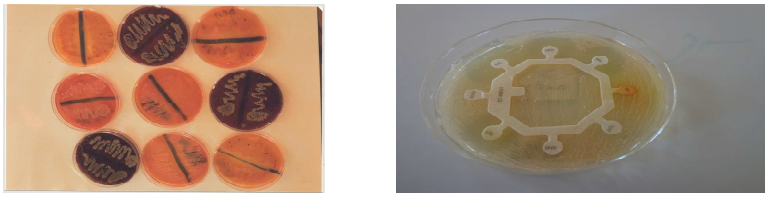

Plate 2 From left, Plates of bacterial growth on some agar and right Plate of zone of inhibition of antimicrobial discs on test bacteria isolates

Plate 3: Plates of fungal isolates: From top left *Aspergillus* sp., *Trichophyton* sp. and *Penicillium*, *Candida* sp., *Trichophyton* sp. and *Mucor* sp.,; from bottom left *Aspergillus* sp. and *Mucor* spp., *Microsporum* spp. and *Mucor* spp

DISCUSSION

The high incidence of the lesions on the ear of the dogs conforms to the works of Centre for Food Security and Public Health (2009), Bako (2012) and Kent *et al*., (2013) who observed highest prevalence of the clinical signs on the ears of dogs than the other parts of the body. This part of the dog body may be suggestive of preferred site for sand fly bites. However, non-isolation of *Leishmania* amastigotes from the lesions may imply that the lesions observed were not due to sand fly but due to other insects' bites. It may also imply mimicry of other infections and visceral diseases. This agrees with the report of Centre for Food Security and Public Health (2009), which stated that clinical signs of CL in dogs are variable and can mimic other infections and skin lesions in dogs with visceral disease. The isolation of *Staphylcoccus* spp., *Streptococcus* spp and *Bacillus* spp. in clinically active CL lesions in the study area conforms to the works of Darogha, (2009), Garg *et al*, (2009), Idowu *et al*, (2011), Ahmed (2012) and Bako (2012) who isolated similar organisms in cutaneous lesions on both humans and dogs. The incidences of *Enterobacter* spp., *Serratia* spp and *Alkaligenes* spp further confirms the association of bacteria with surface cutaneous lesions.

The isolation of *Aspergillus* spp*, Microsporum* spp*, Trichophyton* spp. *Candida* spp*, Mucor* spp and P*enicillium* spp in wounds on dogs in this study, agrees with the works of Ikeh, (1996), Ahmed, (2012), Bako (2012), Daman (2012), and Pyendang, (2012) who isolated similar fungi in wound on human. The isolation the same species of bacteria and fungi could suggest microbial zoonosis.

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