HUMAN CUTANEOUS LEISHMANIASIS (CL) IN PLATEAU AND NASARAWA STATES, NORTH-CENTRAL NIGERIA

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Abstract

Studies on human cutaneous leishmaniasis (CL) were carried out using Clinical, Parasitological and Molecular (PCR) diagnostic techniques in Plateau and Nasarawa States, North-Central Nigeria. The population size was determined by Sample Size Calculator (survey software)-The Survey System (Creative Research Systems, 2012). The study population was 469 individuals comprising of 255 and 214 were examined from Plateau and Nasarawa States, (240 male, 229 females) aged 3 to 65 years. 469 humans comprising of 255(54.37%) from Plateau State and 214(45.63%) from Nasarawa State were examined. Scars 353/469(75.27%) were more prevalent, lesions (3.84%) and rashes/nodules (2.56%), more prevalent on the legs 327/469(69.72%). Male to female prevalence ratio of clinical diseases was 1:1. Scars 76(21.53%) and 73(20.68%) were more prevalent in 20-29 years and 10-19 years. Lesions (44.44% and 50%) and rashes/nodules (27.78% and 33.33%) were more prevalent in <10 years and 10-19 years. Scars (29.40%), lesions (61.11%) and rashes/nodules (58.33%) were more prevalent among pupils/students. Individuals who domiciled 10-19 and 20-29 years in the localities had high prevalence of scars (21.81% and 21.53%), those who domiciled <10 years had more lesions (38.89%), and rashes/nodules (50%). Individuals with national and local travel histories had high prevalence of scars (50.42% and 47.88%), lesions and rashes/nodules more prevalent in individuals with local travel histories (100%). Significant difference (P<0.05) existed between the prevalence of clinical disease and anatomical sites of infection, age, occupation, period of domiciliation and travel history. Results present 5/18(27.78%) prevalence of CL by parasitological techniques, 4/5(80%) in male, pupils/students, age groups <10 years, with local travel histories and 1/469(0.21%) accounting for 0.28% prevalence of CL by PCR in male, pupil, 14 years with local travel history and scars in Kanana, Plateau State. The study established CL endemicity in Kanana, Plateau State and first reported the occurrence of human CL in Gitata and Kagbu, Nasarawa State.

Key Words: Human, Cutaneous Leishmaniasis, Plateau, Nasarawa, North-Central, Nigeria

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). Leishmaniasis manifests in multifaceted forms as: Cutaneous Leishmaniasis (CL), Mucosal Cutaneous Leishmaniasis (MCL), Diffuse Cutaneous Leishmaniasis (DCL) and Visceral Leishmaniasis (VL) (Lainson and Corbett, 2004; Manu *et al*., 2006). *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 have exceeded 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).

CL of the Old World (oriental sore) is caused by *Leishmania tropica, L. major* and *L. aethiopica* and is distributed throughout the Mediterranean and non- Mediterranean areas including West Africa (Ashford and Bettini, 1987). CL involving only the skin may be characterized by one to dozens of lesions (Centre for Food Security and Public Health, 2009; Dermnet, 2016). Inoculation occurs after a sand fly bites on exposed part of the body (usually the legs, arms, neck, or face) (Stark, 2015a, b). CL is zoonotic, hence, considered as an important public health problem leading to severe stigmatization of affected individuals when lesions occur on exposed extremities (Reithinger *et al.,* 2010). Clinical features of CL, depending on the species of *Leishmania*, include smooth nodules (nodular form), flat plaques or hyperkeratotic wart-like lesions, "wet" or "dry" ulcers, papular lesions, oriental sores, ulcers (infiltrating ulcerating) and erythematous types (Centre for Food Security and Public Health, 2009; Dermnet, 2016).

Cases of human CL have been reported in West Africa since the mid-19th century to the 21st century (Jellife, 1955; Mc Millan, 1957). The lack of data on parasitological tests is partly due to difficulty in growing the parasites *in vitro* (Manu *et al*., 2006). Parasitological techniques are tedious, inconveniencing, does not detect current and previous infections (Monroy-Ostria *et al*., 1997; Hepburn 2003). According to Abdallah *et al*., (2014), Center for Disease Control and Prevention (2014b), WHO (2014) and Leishrisk, (2015), every year 500,000 people contract VL and 1,500,000-2,000,000 people develop CL, representing only 30% of the cases and the other 70% goes unreported, mostly in tropical countries. Cases of human CL are on the increase in Nigeria (Agwale *et al*., 1993; Ikeh *et al*., 1993; Agwale *et al*., 1998; Igbe *et al*., 2009). There is need therefore, for more epidemiological studies of CL employing different techniques to adopt an easy, rapid, noninvasive, specific and sensitive method for mass screening and detection of current and previous infections, achieved through immunological and cellular techniques which encompasses molecular and cellular biology of antigen recognition and specific immune reactions and non-specific (Grimaldi and Tesh, 1993; Manu *et al*., 2006).

Clinical, parasitological and Polymerase chain reaction (PCR) techniques have been used by several researchers (Bensoussan *et al*., 2006; Daboul, 2011; Weina and Elston, 2012). Parasitological techniques are reported to be quick, fast and low cost but have limited sensitivity (Hepburn, 2003; Venazzi *et al*., 2006) and most reliable methods to confirm diagnosis of leishmaniasis from superficial scrapings of the ulcers and isolation of the parasite by inoculation of culture or in susceptible experimental animals which can provide material for further investigations (e.g., isoenzyme analysis) (Monroy-Ostria *et al*., 1997). According to Felipe *et al*., (2011) whenever there is suspicion of leishmaniasis, only the laboratory diagnosis can give final answer.

The PCR can be reliably used for the diagnosis of CL when rapid species identification is needed (Bensoussan *et al*.,

2006). The techniques based on PCR are highly sensitive and specific but need to be adopted for field use (Kimutai *et al.,* 2009; Fagundes *et al*., 2010). Venazzi *et al*., (2006) and Durrani *et al*., (2011) reported PCR to be extremely sensitive, can accurately detect *Leishmania* parasites and can be used to detect extremely small amounts, so little quantities as 1 pentagram (10^{-15}g) of *Leishmania* kDNA equivalent to $^{1}/10$ of parasite thereby offering an alternative way for the demonstration of parasites in clinical samples.

William *et al*., (2004); Leder and Peter (2011) and WHO (2014), reported that occupational exposure such as military and other related occupation involving travels, widespread deforestation are important factors in the epidemiology of CL. According to Vilela *et al*., (2013) and WHO (2014), agricultural projects and irrigation schemes, environmental and climatic changes, poor housing and domestic sanitary conditions may increase sand fly breeding and resting sites, as well as their access to humans; diets lacking protein-energy, iron, vitamin A and zinc may increase risk of CL that can progress to kala-azar.

Materials and Methods

Study Area

The study was carried out in 9 and 8 local government areas (LGAs) of Plateau and Nasarawa States, North-Central Nigeria, including Barkin Ladi, Bassa, Bokkos, Jos East, Jos South, Langtang North, Langtang South, Pankshin and Qua'an Pan LGAs of Plateau State. Others are Akwanga, Awe, Karu, Keana, Kokona, Lafia, Nassarawa and Nassarawa Eggon LGAs of Nasarawa State. The two states both lay in the Niger – Benue trough (Nigerian Middle- belt) and their vegetations are generally Guinea Savannah (Plateau State Government, 2015). The criteria of selection of the LGAs from the two states were based on random selection by balloting, adopted from the Carter Centre Jos, Standardized Integrated Epidemiological Mapping/Baseline Survey of Schistosomiasis. Plateau and Nasarawa States are mountainous areas with captivating rock formations. Plateau State lies between latitudes $9^{o}10^{'}$N and longitudes $9^{o}45^{'}$E, an altitude of about 1,200 meters, an area of about 30,913 square kilometers and an estimated 4, 178, 712 million people during the 2006 population census. Nasarawa State lies between latitudes 8^{o}32'N and longitudes 8^{o}18'E with an area of about 27,117 square kilometers and estimated 1.5 million people (during the 2006 population census). The major occupations of the people in Plateau and Nasarawa States include agriculture and mining, farming, fishing, dyeing, weaving, carving and blacksmith (Encyclopedia, 2016; North Central Region Guide, 2016).

METHODOLOGY

Ethical clearance was obtained from Plateau and Nasarawa States Ministries of Health. Participants' informed consents were sought prior to commencement of clinical examination, lesion snip and blood sample collections. Familiarization and advocacy visits were made accompanied by oral enlightenment campaigns about the disease leishmaniasis. The Community Directed Distributors (CDDs) of the Primary Health Care (PHC) centres, Social Mobilization Officers (SMOs) and the Monitoring and Evaluation Officers (M & Es) from the local government headquarters served as facilitators and links to the community leaders during the campaigns.

Study Population

The human population size was determined by Sample Size Calculator (survey soft ware)-The Survey System (Creative Research Systems, 2012). The study population was 469 individuals comprising

of 255 and 214 were examined from Plateau and Nasarawa States, (240 male, 229 females) aged 3 to 65 years.

Clinical Diagnosis

Clinical assessment of participants was with respect to the presence of lesions, scars, rashes/nodules by the collaborating PHC centers. Demographic information (gender, age, marital status, occupation, travel history and period of domiciliation in the study area) was obtained from each participant by interview as described by Yoshie do Rosario *et al.*, (2005), Dermnet (2015) and Stark (2015a, b).

Parasitological Diagnosis

Sample collections were assisted by trained health and veterinary workers in line with ethical standard. Lesion snips were collected from 18 individuals (10 from Plateau State and 8 from Nasarawa State) with suspected active cases of CL lesions. Areas affected were aseptically cleaned using packed and sterilized cotton wool. Incisions were made at the edge of the affected areas to depths of 2mm with needles, raised and about 2g of the tissues cut with surgical blades. The cut tissues were divided into two parts, one part teased on clean glass slides, fixed in 70% alcohol. The other part was wrapped in foil paper and kept for PCR assay. On reaching the Parasitology Laboratory of the Federal college of Veterinary and Medical Laboratory Technology, Vom, the fixed tissues were stained with Giemsa stain and kept for 1 hour for amastigotes to absorb the stain. They were washed with buffers solution and allowed to air dry. The smears were searched microscopically for amastigotes using Immersion oil (100X) Objectives as described by Bryceson (1976); Agwale (1996), Manu (1998) and Manu *et al*., (2006).

PCR Assay

Blood Sample Collections

Five milliliters (5ml) sterile syringes and needles were used to collect 3ml of blood from cubital veins of subjects as described by Yoshie do Rosario *et al.*, (2005). The 3ml of blood was put into EDTA bottles, packed in plastic containers containing frozen ice packs at 2^{o} to 8^{o}C as described by Agwale (1996) and Manu (1998) and transported to Parasitology laboratory of the Federal college of Veterinary and Medical Laboratory Technology, Vom. The plasma was harvested and stored at -20^{0}C. Samples were taken to Biotechnology Laboratory of the National Veterinary Research Institute, Vom and assayed by PCR for *Leishmania* species DNA.

DNA Extraction from Lesion Snips and Blood

QIAamp® DNA Blood Mini kit (QIAGEN®, www.qiagen.com) was used. DNA isolation was carried following manufacturer's instructions.

Leishmania Oligochromatograpic-Test (Leishmania OligoC-Test) kit manufactured by Coris BioConcept Science Park CREALYS B- 5032 GEMBLOUX, BELGIUM. (Tel: +32(0)81.719.917; Fax: +32(0)81.719.919: E-mail: info@corisbio.com; Website: www.corisbio.com; IFU-5305/TB/04) was used and adhering strictly to Manufacturer's instruction. Post- PCR room temperature was raised to 55^{o}C and the *Leishmania* Oligo-Strip running buffer and assay tubes were pre-heated. 40µl of heated *Leishmania* Oligo-Strip running buffer was added in the bottom of heated assay tube and replaced in the heater. 40µl of PCR products (kept on ice) was added and mixed thoroughly by pipetting, and the assay tube replaced in the heater. The *Leishmania* Oligo-Strip was immediately dipped in the assay tube and closed with stopper. The assay was

incubated in the heater at 55^{o}C for 10min. The results obtained were read and recorded.

Results

Of the 469 studied 353(75.27%) had scars, 18(3.84%) had characteristics active CL lesions and 12(2.56%) had rashes/nodules. Apparently healthy individuals who had no noticeable scars, lesions and rashes/nodules were 86(18.34%). The clinical manifestations of the disease were more prevalent on the legs 327(69.72%), followed by the hands 38(8.10%) and least on the head 18(3.84%), (Table 1). Male and female occurrence of scars was 50.42% and 49.58%, lesions 50% and rashes/nodules 58.33% and 41.67% (Table 2). Highest prevalence of scars was found among ages 20-29 years and 10-19 years (21.53% and20.68%), whereas lesions and rashes/nodules were more prevalent in ages <10 years (44.44% and 27.78%) and 10-19 years (50.0% and 33.33%), (Table 3). Pupils/students and farmers showed the highest prevalence of scars (29.40% and 28.90%), lesions (61.11% and 6.33%), and rashes/nodules (58.33% and 25.0%), (Table 4). The highest prevalence of scars (21.81% and 21.53%) was found among individuals who had lived 10-19 years and 20-29 years, lesions and rashes/nodules were more prevalent (38.89% and 50.0%, and 50.0% and 33.33%) in individuals who had lived <10 years and 10-19 years in the localities (Table 5). The clinical manifestations of the disease were more prevalent among the married and unmarried individuals: scars 55.52% and 41.08%, lesions 50% and 33.33% and rashes/nodules 41.67% and 33.33% (Table 6). Scars were more prevalent (50.42% and 47. 88%) among individuals with national and local travel histories while lesions and rashes/nodules were high in individuals with local travel histories (100% each) (Table 7). Results showed an overall prevalence of 5/18(27.78%) by parasitological technique with 80% in the males and 20% in the females. The results revealed also, high prevalence of the disease in individuals ages <10 years (80%) and 40-49 years (20%), (Tables 8 and 9). High prevalence of the disease was revealed also among the pupils/students (80%) and farmers (20%) with local and national travel histories (Tables 10 and 11). PCR results showed an overall 1/469(0.21) prevalence of CL representing 1/353(0.28%) male, pupil, 14 years old with scars and local travel history (Table 12).

Table 1: Clinical and Anatomical Site of Infection-Related Prevalence of Human CL

Clinical signs	No. (%) examined	Anatomical Site of Infection		
		No. (%) Head	No. (%) Hands	No. (%) Legs
App. Healthy	86(18.34)	0(0.0)	0(0.0)	0(0.0)
Scars	353(75.27)	8(44.44)	29(76.32)	316(96.64)
Lesions	18(3.84)	2(11.11)	4(10.53)	10(3.06)
Rashes	12(2.56)	8(44.44)	5(13.16)	1(0.31)
Total	**469(100)**	**18(3.84)**	**38(8.10)**	**327(69.72)**

χ^2 =114.263, df=4 P<0.001 Number of valid cases 383

Table 2: Gender-Related Prevalence of Human Clinical CL

Gender	**No. (%) Examined**	**Clinical Signs**			
		No. (%) App. Healthy	**No. (%)Scars**	**No. (%)Lesions**	**No. (%) Rashes**
Male	240(51.17)	46(53.49)	178(50.42)	9(50.50)	7(58.33)

Female	229(48.83)	40(46.51)	175(49.58)	9(50.50)	5(41.67)
Total	**469(100)**	**86(18.34)**	**353(75.27)**	**18(3.84)**	**12(2.56)**

χ^2 =.520, df=3, *P*=.915 Number of valid cases 469

Table 3: Age-Related Prevalence of Human Clinical CL

Age (Year)	No. (%) Examined	Clinical Signs			
		No. (%) App. Hlthy Exam.	No. (%) Scars Exam.	No. (%) Lesions Exam.	No. (%) Rashes Exam.
<10	45(5.59)	5(5.81)	26(7.37)	8(44.44)	6(50.0)
10-19	91(19.40)	9(10.47)	73(20.68)	5(27.78)	4(33.33)
20-29	93(19.83)	14(16.28)	76(21.53)	2(11.11)	1(8.33)
30-39	87(18.55)	24(27.91)	62(17.56)	1(5.56)	0(0.0)
40-49	69(14.71)	17(19.77)	50(14.16)	2(11.11)	0(0.0)
≥50	84(17.91)	17(19.77)	66(18.70)	0(0.0)	1(8.33)
Total	**469(100)**	**86(18.34)**	**353(75.27)**	**18(3.84)**	**12(2.56)**

χ^2 =70.276, df=15, *P*<0.001 Number of valid cases 469

Table 4: Occupation-Related Prevalence of Human Clinical CL

Occupation	No. (%) Examined	Clinical Signs			
		No. (%) App. Healthy Exam.	No. (%) Scars Exam.	No. (%) Lesions Exam.	No. (%) Rashes Exam.
Pupil/Student	133(28.36)	11(12.79)	104(29.40)	11(61.11)	7(58.33)
Farming	121(25.80)	10(11.63)	102(28.90)	6(33.33)	3(25.00
Civil Servant	93(19.83)	46(53.49)	47(13.31)	0(0.0)	0(0.0)
Business	62(13.22)	12(13.95)	50(14.16)	0(0.0)	0(0.0)
Artisan	25(5.33)	3(3.49)	21(5.95)	1(5.56)	0(0.0)
Dependant	35(7.46)	4(4.65)	29(8.22)	0(0.0)	2(16.67)
Total	**469(100)**	**86(18.34)**	**353(75.27)**	**18(3.84)**	**12(2.56)**

χ^2 =98.723, df=15, *P*<0.001 Number of valid cases 469

Table 5: Period of Domiciliation-Related Prevalence of Human Clinical CL

Period of Domiciliation (Year)	No. (%) Examined	Clinical Signs			
		No. (%) App. Healthy Exam.	No. (%) Scars Exam.	No. (%) Lesions Exam.	No. (%) Rashes Exam.
<10	88(18.87)	22(25.58)	53(15.01)	7(38.89)	6(50.0)
10-19	109(23.24)	19(22.09)	77(21.81)	9(50.0)	4(33.33)
20-29	88(18.76)	12(13.95)	76(21.53)	0(0.0)	0(0.0)
30-39	77(16.42)	8(9.30)	67(18.98)	0(0.0)	2(16.67)
40-49	52(11.09)	17(19.77)	35(9.92)	0(0.0)	0(0.0)
≥50	55(11.73)	8(9.30)	45(12.75)	2(11.11)	0(0.0)
Total	**469(100)**	**86(18.34)**	**353(75.27)**	**18(3.84)**	**12(2.56)**

χ^2 =48.245, df=15, *P*<0.001 Number of valid cases 469

Table 6: Marital Status-Related Prevalence of Human Clinical CL

Marital Status	No. (%) Examined	Clinical Signs			
		No. (%) App. Healthy Exam.	No. (%) Scars Exam.	No. (%) Lesions Exam.	No. (%) Rashes Exam.
Married	268(57.14)	58(67.44)	196(55.52)	9(50.0)	5(41.67)
Unmarried	178(37.95)	23(26.74)	145(41.08)	6(33.33)	4(33.33)
Widow	20(4.26)	3(3.49)	11(3.12)	3(16.67)	3(25.0)
Divorced	3(0.64)	2(2.33)	1(0.28)	0(0.0)	0(0.0)
Total	**469(100)**	**86(18.34)**	**353(75.27)**	**18(3.84)**	**12(2.56)**

χ^2 =187.764, df=9, *P*<0.001 Number of valid cases 469

Table 7: Travel History-Related Prevalence of Human Clinical CL

Travel History	No. (%) Examined	Clinical Signs			
		No. (%) App. Healthy Exam.	No. (%) Scars Exam.	No. (%) Lesions Exam.	No. (%) Rashes Exam.
Local	224(47.76)	25(29.07)	169(47.88)	18(100)	12(100)
National	224(47.76)	46(53.49)	178(50.42)	0(0.0)	0(0.0)
International	21(4.48)	15(17.44)	6(1.70)	0(0.0)	0(0.0)
Total	**469(100)**	**86(18.34)**	**353(75.27)**	**18(3.84)**	**12(2.56)**

χ^2 =78.584, df=6, *P*<0.001 Number of valid cases 469

Table 8: Gender-Related Parasitological Prevalence of Human CL by Microscopy of Lesions

Gender	No. (%) Examined	No. (%) Positive
Male	9(50.0)	4(80.0)
Female	9(50.0)	1(20.0)
Total	**18(100)**	**5(27.78)**

χ^2 =2.492, df=1, *P*=114 Number of valid cases 18

Table 9: Age-Related Parasitological Prevalence of Human CL by Microscopy of Lesions

Age (Year)	No. (%) Examined	No. (%) Positive
<10	8(44.44)	4(80.0)
10-19	6(33.33)	0(0.0)
20-29	1(5.56)	0(0.0)
30-39	1(5.56)	0(0.0)
40-49	2(11.11)	1(20.0)
≥50	0(0.0)	0(0.0)
Total	**18(100)**	**5(27.78)**

χ^2 =5.538, df=4, *P*=.236 Number of valid cases 18

Table 10: Occupation-Related Parasitological Prevalence of Human CL by Microscopy of Lesions

Occupation	No. (%) Examined	No. (%) Positive
Pupil/Student	12(66.67)	4(80.0)
Farming	5(27.78)	1(20.0)
Civil Servant	0(0.0)	0(0.0)
Business	0(0.0)	0(0.0)
Artisan	1(5.56)	0(0.0)
Dependant	0(0.0)	0(0.0)
Total	**18(100)**	**5(27.78)**

χ^2 =.720, df=2, *P*=.698 Number of valid cases 18

Table 11: **Travel History-Related Parasitological Prevalence of Human CL by Microscopy of Lesions**

Travel History	No. (%) Examined	No. (%) Positive
Local	17(94.44)	4(80.0)
National	1(5.56)	1(20.0)
International	0(0.0)	0(0.0)
Total	**18(100)**	**5(27.78)**

χ^2 =18.000, df=1, *P*<0.001 Number of valid cases 18

Table 12: Clinical Signs-Related Prevalence of Human CL by PCR

Clinical Signs	No. (%) Examined	No. (%) Positive
App. Healthy	86(18.34)	0(0.0)
Scars	353(75.27)	1(0.28)*
Lesions	18(3.84)	0(0.0)
Rashes	12(2.56)	0(0.0)
Total	**469(100)**	**1(0.21)**

*Male, Pupil, 14 years old with local travel history

$\chi 2$ = No statistics computed because data are incomparable

Discussion

The observation of scars, characteristic active lesions, rashes/nodules and their multiple occurrence on legs, hands and head regions of individuals agrees with the reports of Bañuls *et al.,* (2011), Leder and Peter (2011) and Bsrat *et al.*,(2015) who attributed this to possibly the exposed nature of these parts of the body. Bsrat *et al.,* (2015) reported also, the occurrence of clinical diseases on unusual locations such as genital organs, buttocks and palms. The 69.72% prevalence of the clinical signs on the legs and least on the head agrees with the works of Manu (1998) and Leder and Peter (2011) possibly due to over protection of the head region which carries the sense organs and contrasts the works of Nawaz *et a*l., (2010) and Bsrat *et al.,* (2015) who observed high infection on the face (head region) than the hands and legs. No significant difference (*P*>0.05) existed in clinical manifestations of the disease between the males and females. This agrees with the results of Manu (1998) and Leder and Peter (2011) who observed no significant difference in infection rates between the genders in the prevalence of active lesions or scars. However, it contradicts the observations of Bsrat *et al.,*

(2015) and Patient (2015) who reported high infection rate in the males (7.6%) rather than females (5.9%) and about 2:1 male to female ratio prevalence of CL. The high prevalence of lesions and rashes/nodules in ages <10 years and 10-19 years partly agrees with the reports of Manu (1998), Nawaz *et al.*, (2010) and Bsrat *et al*., (2015), and partly disagrees with the reports of Manu (1998), Nawaz *et al*., (2010) and Bsrat *et al*., (2015) who observed low prevalence of infection in age groups<10 years and ≥11 years old. Attributed to probably due to poverty in rural areas that might lead to lack of proper care by parents/guardians of children <10 years, or due to the active nature of individuals between 10 years and 19 years (teen age). This concurs with the earlier works of William *et al., (*2004) and Leder and Peter, (2011) who stated that leishmaniasis is associated with rural areas and poverty. High prevalence of clinical diseases among pupils/students and farmers, could possibly be associated with age, active behaviours and farming occupation which has to do with entering bushes, forested areas, hills and even sandy places, irrigational and other agricultural practices which pre-expose them to greater risk of sand fly bites. This concurs with the reports of Vilela *et al.*, (2013) and WHO (2014), who respectively reported that agricultural projects, irrigation schemes, environmental and climatic changes, settlement of people in forest areas can increase the prevalence of CL in rural and periurban areas. The high prevalence of lesions and rashes/nodules among individuals who have domiciled stayed <10 years and 10-19 years may suggest that the disease is endemic in the study areas, current infection and with continued transmission of the disease in the area as stated by Lysenko & Beljaeve (1987) The study observed high prevalence of clinical disease in individuals who are married. This could be attributed to poverty and rural behavior where early marriages are encouraged for parents to get relieve from their responsibilities while some continue their schooling in their matrimonial homes. The study observed high prevalence of clinical disease in individuals who have not traveled wide. This contradicts the report of William *et al*., (2004) and Leder and Peter (2011) who stated that the disease is associated with travelers, such as immigrants, and military personnel who might import the disease to a non endemic CL area as they travel.

The 27.78% prevalence of CL by Giemsa's stained preparation is a confirmation of the occurrence of CL in these study foci. According to Daboul (2011); Weina & Elston (2012) and Henry *et al.*, (2015) parasitological diagnosis is still considered the gold standard and reliable method to confirm diagnosis of leishmaniasis and the identification of the parasites in thin smears made from superficial scrapings of the ulcers. The result indicates that the disease is current in the study foci as stated by Monroy-Ostria *et al*., (1997) and Hepburn (2003) that the detection of parasites in lesion scrapings of patients with active lesions is an indication of current infection and that it is difficult to detect infection by parasitological methods in patients with more than one year evolution. Center for Food Security and Public Health, (2009) reported that amastigotes are easiest to find in recent or active lesions. The 80% and 20% prevalence of the disease in male and female human, ages <10 years and 40-49 years, pupil/students and farmer with local and national travel histories is a pointer that the disease is endemic in the study area and agrees with the works of Lysenko and Beljaeve (1987), Monroy-Ostria *et al*., (1997) and Hepburn (2003) who stated that infection in ages between 11 and 20 years

and below is an indication that infection is endemic in the area under consideration.

The 0.21% prevalence representing 0.28% of infection by PCR in a male, pupil, 14 years old with scars (not active lesion) and with local travel history from Kanana village in Langtang South LGA of Plateau State is an indication of the existence of CL in the area. This concurred with the work of Lima *et al.,* (1997), who reported that the presence of infection in individuals with healed lesions (scars) is an indication that the individual still contains the parasites and that although, lesions can resolve leaving scars, the healed lesion can still contain the parasites. This conforms also with the reports of Belli *et al.,* (1998), Kimutai *et al.,* (2009), Fagundes *et al.,* (2010) and Felipe *et al.,* (2011) who stated that detection of *Leishmania* DNA by PCR suggests active infection and is epidemiologically relevant for identifying foci of active transmission and designing control strategies. Similarly, the occurrence of the infection in individual between the ages 10-19 years conforms to the works of Manu (1998) and Sharifi *et al.*, (1998) who speculated that this category of persons are more prone to risk of infection due to their nature of being active and, in rural areas, this category of individuals mostly fend for themselves and some engaged in agricultural and hunting activities.

Limitations

The unavailability of serological and molecular diagnostic test kits for the diagnosis of CL in Nigeria was a setback to this study. The PCR test kit used for this research had to be imported from the United States of America.

Recommendation

Recommendation is made for public health organization bodies to make available and affordable serological and molecular test kits for the diagnosis of leishmaniasis in Nigeria.

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