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Original Article

PCR-based detection of *Schistosoma bovis* in cattle in Maiduguri Metropolis and Jere Local Government Areas in Borno State, Nigeria

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ABSTRACT

Objective: To determine the prevalence of *Schistosoma* infection in cattle in Maiduguri Metropolis (MMC) and Jere Local Government Areas (LGAs) of Borno State, Nigeria.

Materials and Methods: Blood samples (n=200) were collected from cattle consisting of one hundred (100) each from five (5) ward levels each of MMC and Jere LGAs. DNA samples were extracted from the serum samples, analysed and quantified using a Nano-drop machine. The extracted DNA were subjected to polymerase chain reaction (PCR).

Results: The overall prevalence of *Schistosoma* infection was 2% (n=200). Jere LGA had 3% (n=100) while MMC had 1% (n=100). There was no statistical significant association in prevalence rate in the two LGAs studied (*P*=0.621) (*P*>0.05). At the ward levels, Custom Area in Jere LGA had 15%, Jiddari ward in MMC had 5%, and the remaining ward levels had no cases. Of the 103 female and 97 male cattle screened, the prevalence in female was 1(0.97%) and 3(3.09%) in the male. Of the 177 serum samples from above 1year (adult) examined, 4 (2.26%) were positive and none in the young. There was no statistical significant association in prevalence rate among ward levels, sex groups and age groups in the study areas (*P*=0.621) (*P*>0.05), (*P*=0.356) (*P*>0.05) and (*P*=1.000) (*P*>0.05) respectively. Of the eight (8) breeds screened, Kuri had 2.7%, Sokoto Gudali (1.82%), Abore (2%), Red Bororo (2.63%), White Fulani, Porland, Mbala and Wafara recorded no cases. The difference in prevalence rates among the breeds based on the trend of occurrence of *Schistosoma* infection were not significantly associated statistically (*P*=1.000) (*P*>0.05).

Conclusion: There is a prevalence of *Schistosoma* infection in cattle in the two LGAs of Borno state. It is recommended that a system be established to maintain preventive and control programs.

KEYWORDS

Cattle, Cattle breeds, ELISA, PCR, Prevalence

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INTRODUCTION

Schistosomiasis is a disease caused by trematode; it is widely distributed in different parts of the world (Kassaw, 2007). The most obvious sign of Urinary Schistosome is blood in urine (Belding, 1965). *Schistosoma haematobium* was the first to be described during an autopsy in an Egyptian patient and this gave the popular name for all forms of schistosomiasis as 'bilharziasis' in honour of his discovery during the Eighteen century. *Schistosomes* dwell in the blood of animals and humans causing disease. It belongs to the genus *Schistosoma*; family, *Schistosomatidae*; order, *Digenea*; class, *Trematoda*; phylum, *Plathylelminths* and kingdom, *Animelia* (Webster and Southgate, 2006).

About 20 species are currently recognized. Among those recognized include: *S. bovis*, *S. mansoni, S. intercalatum, S. haematobium, S. guineensis, S. rodhaini, S. curassoni* and *S. hippopotami* (Hanlet et al., 2009). *Schistosomes* are not hermaphrodites, the male's body forms a gynaecophoric channel in which it holds the female sex; they feed squarely on host blood (Rumnajek, 1987). The distribution of *Schistosomes* in man is governed by the existence of suitable intermediate molluscan hosts (Belding, 1965). Mott et al. (1993) gave an estimate of 90 million people infected in Africa alone and about 100 million others at risk of infection. *S. haematobium* co-exists with *S. mansoni* and the distribution of the two species is somehow confusing.

Humans are the major reservoirs of human *Schistosomes*, apart from the role of humans as the major reservoirs of infection, other mammals have been incriminated and as such not less than 38 mammals have been found to be naturally infected by *S. mansoni* (Rumnajek, 1987). Akande and Odetola (2013) reported that water habitats influence the incidence of schistosomiasis. The water habitats can be shallow pools, ponds, small earth dams, rivers and marshes, irrigation channels, lakes and reservoirs. The two main instances of hybridization that involved the human (*S. haematobium*) with bovine *Schistosoma* (*S. bovis*) and then between *S. mansoni* with *S. rodhaini* is actually a puzzle and of public health significance. In both instances, the danger is that changes to the genome could result to enhanced pathology or virulence, either in humans or animals which could actually exacerbate the public health, veterinary and conservation burden of the new hybrid infection. More recently, Gomes et al. (2006), Hung et al. (2008) and Lier et al. (2009) established and developed SYBR Green (a fluorescent based technology) and *Taq* Man probe real time PCR assays for the detection and quantification of *Schistosoma* l DNA. They reported that they are potentially useful for quantification of parasite burden in *Schistosoma* infection. Therefore, PCR allows the use of PCR-based DNA/serum diagnosis for routine purposes in laboratories in less developed countries with fewer resources. Thus, this study was designed to determine the prevalence of *Schistosoma* infection in Cattle in Maiduguri Metropolis and Jere Local Government Areas of Borno State, Nigeria.

MATERIALS AND METHODS

Study area: The study was conducted in Maiduguri Metropolis and Jere Local Government Areas of Borno State. The boundaries of the state are the Republic of Niger to the North, Cameroon Republic to the east and to the Northeast by Chad Republic (**Figure 1**). Within the country, its neighbouring states are Adamawa to the South, Yobe to the West and Gombe to the Southwest.

Study design: A cross- sectional study was conducted in accordance with the method of Thrusfield (2002). Blood samples were aseptically collected from cattle on herd basis using a multistage sampling technique. Ten (10) out of 27 wards initially selected by simple random sampling from both LGAs were subjected to the next stage of survey. At each of the 10 selected wards and average of 3 cattle were selected from an earlier 5 cattle herds randomly selected for blood collection.

Sample size: Sample size for the cattle was calculated using the formula Z^2pq/L^2 where Z=1.96, p=prevalence, q=1-p, L=level of significant (5%) as described by Thrusfield (2002). By using known prevalence rate of 12.5% for Schistosomiasis in cattle (Mersha and Belay (2012)), a total of 168 samples was the true representative of the sample population. However, a total number of 200 blood samples were collected in order to increase precision (20 samples coming from each of the ten wards).

Blood sample collection from cattle: Blood samples were collected from cattle after proper restrain. Sex, age, breed, source of water, date and location of the animals were determined and recorded at the time of sample collection. 10 mL of blood was aseptically collected from the jugular veins using 10 mL, 18Gx1. 5 inches syringes and needles. The blood samples collected were poured into non-heparinized universal bottle for serum to be separated and collected. In the laboratory, the blood samples were further centrifuged at 3,000 g for 15 min. Thereafter clarified sera was decanted into clean labelled serum vials and stored at –20ºC until analysed.

Extraction of DNA from Serum Samples: Samples of DNA were extracted from the serum samples using a

Quick -gDNA kit. The kit contained Genomic lysis Buffer, DNA Pre-wash Buffer, g-DNA wash Buffer,

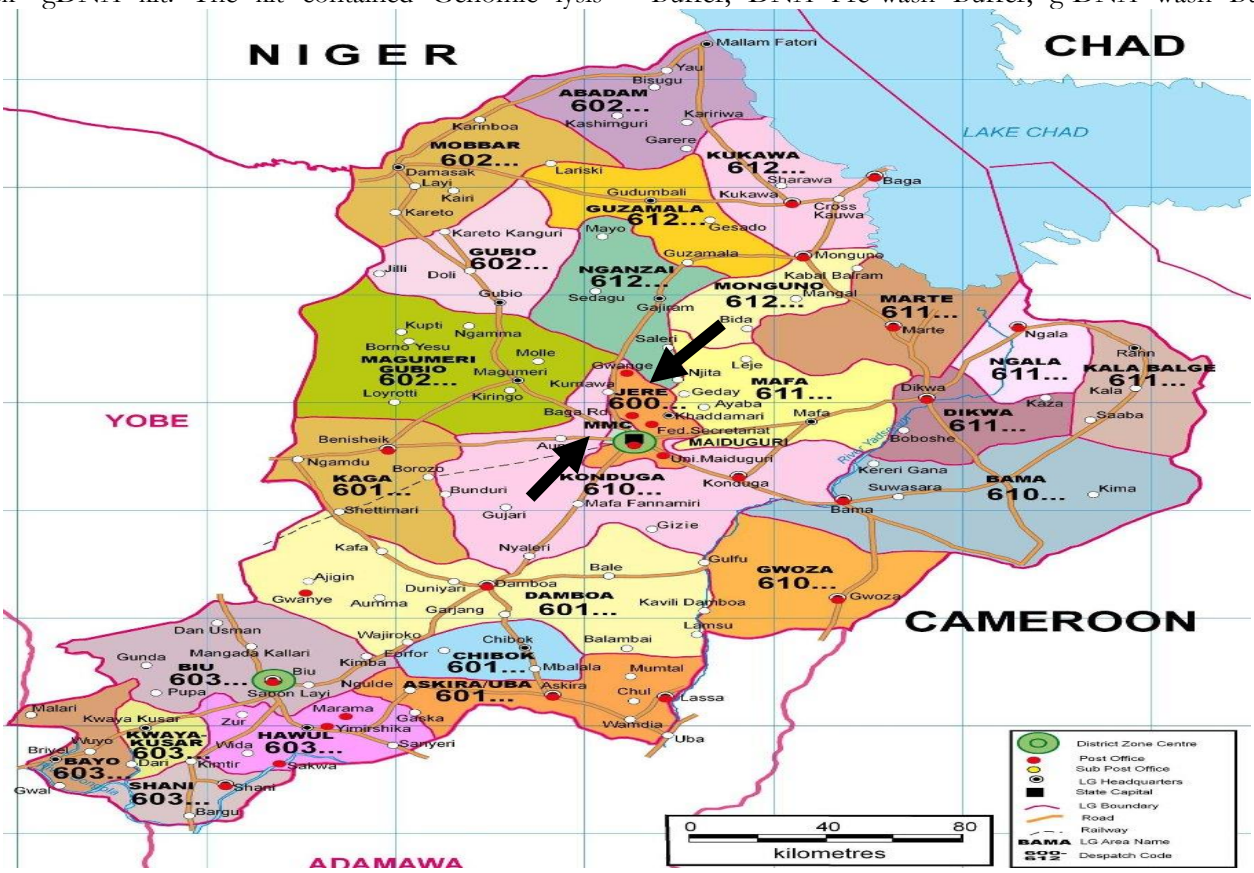

Figure 1. Map showing the study areas. The black arrows indicate the study areas. (source: Google map)

DNA elution Buffer, Zymo-Spin Columns and collection tubes.

Polymerase chain reaction: Genomic lysis Buffer (400 µL) was added to 100 µL of serum and vortexed 4-6 seconds then left 5-10 seconds at room temperature. Mixture was then transferred into in collection tubes, centrifuged at 10,000xg for one min. The Zymo-Spin Column was transferred to a new tube and 200 µL of DNA Pre-wash was added, centrifuged at 10,000 g for 1 min. 500 µL of g-DNA was added to the spin and centrifuged at 10,000 g for one minute. The spin was transferred to a clean micro centrifuge tube and mixed with 50 µL DNA Elution Buffer, then incubated at room temperature, centrifuged at top speed for 30 Sec to elute the DNA. The eluted DNA was stored at about -20ºC for further use in PCR. Polymerase Chain Reaction was carried out by first preparing a PCR premix which consisted of: dNTPS (10 mM, lot: 00159538), primers (forward: AAA-AAA-GAA-TGA-ACG-AAA-TCG-GTG, reverse: ACC-TCT-TCG-AAA-TCC-TTC-CAG-CCT), buffer, *Taq* polymerase (11146173001, version 23), double-diluted water and template (DNA and Serum). The final reaction volume was 50 µL each for DNA and Serum Templates. The reaction set-up was made up of 5 steps in the PCR Thermocycler: Initial Denaturation at 94ºC for 2 min, Denaturation at 94ºC for 30 Sec, Annealing at 61ºC at 30 Sec, Extension at 72ºC for 30 Sec and Holding at 4ºC.

Agarose gel electrophoresis: Agarose gel of 1.2% was prepared by weighing 1.2 gm of the Agarose-LE-Analytic grade V3121 gel on an analytic balance and dissolving it in 100 µL of TAE using a magnetic stirrer/heater and allowed to boil and settle. Ethidium bromide (0.5 µL) was then added and mixed. TrayThe Agrose was poured into the casting tray and a gel casting comb was inserted in the tray and allowed to settle, after settling, the comb was removed and the casted gel was then transferred into the gel tank and flooded with TAE to the maximum limit of the tank. The template samples were loaded into the wells using a Template Loading Dye (TLD). The DNA Ladder was also loaded and gel tank covered. Actually the gel tank has a cathode and anode electrode which was then connected to the Electrophoresis Power tank (Cleaver 3000). Electrophoresis was run at 150-V for 15 min.

Afterward, the gel was removed and blotted to dry with a laboratory towel. The dried electrophoresed gel was later transferred into a UV Trans-illuminator (Clearer, Scientific) where the amplicons were illuminated. A gel documenting system with a photographic imager was used to snap shot the image.

Statistical analysis: Data generated were analysed using a computer software Graphpad instat 3. Tables, Percentages, Chi-square were analysed from the data generated in this study.

RESULTS

The overall prevalence of *Schistosoma* infection in cattle in Maiduguri Metropolis and Jere LGAs of Borno State is presented in **Table 1**. Out of the 200 cattle tested in both LGAs, 2% (n=4/200) were positive by PCR assay. Jere LGA had the highest number of cattle positive by ELISA (3%; n=100) than MMC (1%; n=100). At the ward levels, Custom Area in Jere LGA had the highest number of *Schistosoma* positive (15%) followed by Jiddari ward in MMC with 5%. All the remaining ward levels spread

Table 1: Prevalence of *Schistosoma* infection in cattle in Maiduguri and Jere Local Government Areas of Borno State based on PCR assay.

LGA	WARDS	No. tested	PCR + (%)	*P*-value	OR	95% C.I Lower-Upper
MMC						
	Gwange	20	0(0.0)			
	Shuwari	20	0(0.0)			
	Bolori	20	0(0.0)			
	Jiddari	20	1(5.0)			
	B/bulin	20	0(0.0)			
Sub-total		100	01(1.0)	0.62	0.32	0.03-3.19
Jere						
	Dusuman	20	0			
	K/mari	20	0			
	G/long	20	0			
	M/sari	20	0			
	C/area	20	3(15.0)			
Sub-total		100	03(3.0)		3.06	0.31-29.9
Total for both LGAs		200	4(2.0)			

B/bulin=Bullabulin, K/mari=khaddamari, G/long=Gongulong, M/sari=Maimusari and C/area=Custom area.

Table 2: Prevalence of *Schistosoma* infection in cattle in Maiduguri and Jere Local Government Areas of Borno State based on Age.

Sex	No. Tested	PCR + (%)	*P*-value	OR	95% C.I (Lower-Upper)
Female	103	1(0.97)	0.35	0.307	0.313-3.00
Male	97	3(3.09)		3.255	0.332-31.85
Total	200	4(2.0)			

Table 3: Prevalence of *Schistosoma* infection in cattle in Maiduguri and Jere Local Government Areas of Borno State based on Age.

Age (Years)	No. Tested	PCR + (%)	*P*-value	OR	95% C.I (Lower-Upper)
Adult(>1)	177	4(2.26)	1.000	1.219	0.635-23.38
Young(<1)	23	0(0.0)		0.820	0.042-15.73
Total	200	4(2.00)			

Table 4: Prevalence of *Schistosoma* infection in cattle in Maiduguri and Jere Local Government Areas of Borno State based on the trend of occurrence in breeds.

Group Trend	N0. Tested	PCR + (%)	*P*-value	OR	95% C.I (Lower-Upper)
A	180	4(2.00)	1.000	1.045	0.054-20.13
B	20	0(0.0)		0.956	0.049-18.42
Total	200	4(2.0)			

Group A=kuri, Red Bororo,Sokoto Gudali and Abore, Group B=White Fulani, Porland, Mbala and Wafara.

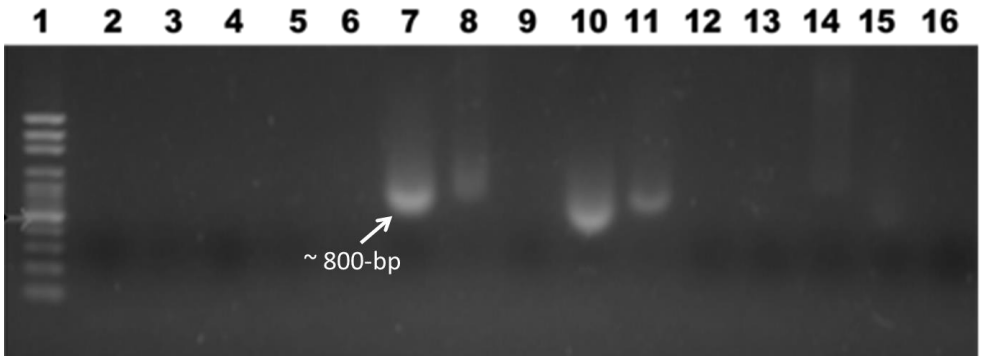

Figure 2: Amplicon bands of *Schistosoma bovis* on a casted gel after gel electrophoresis. Lane 1=Ladder (100-bp), Lane 2-6=Negative, Lane 7-8=Positive, Lane 9=Negative, Lane 10-11=Positive, Lane 12-15=Negative and Lane 16=Negative control.

among the two LGAs recorded negative cases of *Schistosoma* infection when the cattle were screened. There was no statistical significant association between the LGA and positive serological reaction to *Schistosoma* infection (*P*>0.05).

The prevalence of *Schistosoma* infection by PCR in MMC and Jere LGAs of Borno State based on Sex are shown in **Table 2**. Out of the 103 serum samples from female cattle tested, 1 (0.97%) was positive. Among the Males, 3 (3.09%) were positive. There was no statistical significant association in prevalence rate among the sex group in the study area (*P*>0.05.

The prevalence of *Schistosoma* infection by PCR in cattle in MMC and Jere LGAs of Borno State based on Age are shown on **Table 3**. Out of the 177 serum samples from cattle aged above 1year (adult) examined, 4 (2.26%) were positive , while out of 23 serum samples from cattle less than one year(young) none was positive by PCR . There was no statistical significant association in prevalence rate among age groups and the positive serological reaction to *Schistosoma* infection in the study areas (*P*>0.05).

The prevalence based on the trend of occurrence of *Schistosoma* infection among the eight (8) breeds is presented in **Table 4**. Out of the eight (8) breeds screened the highest number of cases recorded was in Kuri breed with 2.70%. This was followed by Sokoto Gudali with 1.82% positive cases. The prevalence of other breeds recorded is as follows: Abore- 2%, Red Bororo-2.36%, White Fulani, Porland, Mbala and Wafara recorded no cases of *Schistosoma* infection. However, the difference in prevalence rates among the breeds were not statistically significantly associated (*P*>0.05).

Out of the 200 serum samples examined, 3 (3%) and 1 (1%) were positive for serum-PCR amplification in Custom Area and Jiddari ward levels of Jere and MMC LGAs of Borno State respectively. The casted gel is having 16 wells. The first (1) and the last (16) lanes are the ladder (100-bp) and negative control respectively. Lanes 7, 8, 10 and 11 are positive lanes with amplicon bands. While lanes 2, 3, 4, 5, 6, 9, 12, 13, 14 and 15 showed no amplicon bands (Plate 1).

DISCUSSION

The current study provides information on the prevalence of *Schistosoma* infection among cattle in MMC and Jere LGAs of Borno state, Nigeria. The overall prevalence of *Schistosoma* infection among cattle in the study area was 2% (n=200). This value is of public health importance because it has been established that *S. bovis* can cause infection in children (Huyse et al., 2009). In a seroprevalence study in the same areas among cattle, we found an overall 10% prevalence, which was higher than our present study based on PCR (Hambali et al., 2016). This variation might be due to less sensivity of seroprevalence study as compared to PCR-based study. Because of the close interaction between the cattle and herdsmen with their families in the study area, the cattle can be a potential reservoir of the infection. The result of the present study is lower than 12.3 and 12.5% reported by Almage (2007) and Zelalem (2010). The differences could be attributed to different diagnostic technique used in this study. The study area also have less water bodies compared to areas with high prevalence and the difference in management of unwanted offals and other waste materials in the herds and environs. Pontes et al. (2003) also reported that a lower PCR results might be due to inhibition of the amplification reaction or DNA degradation during transportation or uneven distribution of constituents. The prevalence was higher in Jere LGA than MMC. The association between the animals in the two LGAs and the occurrence of the infection was not statistically significant (*P*>0.05). This means that the location of the animal does not affect the occurrence of

the infection. The higher prevalence recorded in Jere LGA was from Custom Area, this could be associated with presence of more water bodies like River Ngadda, River Yedzaram and River Gombole on the Jere axis as compared to MMC axis.

In the current study, the prevalence rate of *S. bovis* was higher in males as compared to females. There was no statistical significant association between sex and occurrence of the infection (P>0.05). The prevalence rate was higher in the young than adult. The higher prevalence in male in this study is lower than the 15.4% as reported by Mersha and Belay (2012). This could be attributed to the fact that more of the male are practically grazed around for nutrient than the female within the study area. The prevalence rate was higher in the young than adult. The prevalence was higher in the adult than in the young in this study and is lower than 12.5% as reported by Mersha and Belay (2012). There was no statistical significant association among age groups and occurrence of the infection (P>0.05). The higher prevalence in adult could be due to increase in level of contamination of grazing area with the Cercariae and the exploring nature of the adult animal. From this study, the prevalence by breed was higher in Kuri than other breeds. There was no statistical significant association among the breeds and the occurrence of the infection (P>0.05). The higher prevalence in Kuri breed might be due to exposure to marshy areas invaded by cercariae or due to lower numbers of other breeds sampled. The serum samples were subjected to PCR and after gel electrophoresis, amplicon bands were vivid, the bands lied at 800-bp which is in harmony with the report of the PCR based approach for the amplification of *Schistosoma bovis* by Sandoval and Siles-Luca (2006). Furthermore, DNA samples were extracted from the serum samples and subjected to Nano-technology measurement where the Nucleic Acid concentration ranged from 0.1-15.4 ng/µL. Extracted DNA samples that were subjected to PCR amplification failed to show vivid amplicon bands. The failure of the extracted DNA samples from serum to amplify sequelled to a trouble shooting. The forward and reverse primers were later subjected to oligonucleotide concentration analysis where the absorbance was maximum at 260 nm wavelength which is in line with the standard absorbance rate of ideal quality primers. The sequence also tallied, thereby ruling out the primer effect. *Taq*-Polymerase potency was also put into consideration even though it was the same *Taq*-polymerase that was used in serum amplification which gave amplicon bands. This also rulled out *Taq*-polymerase hypo-potency. PCR amplification conditions were set for the Ideal amplification and conditions were adhered to during the PCR reaction (94ºC: 2 min [94ºC; 30 Sec - 61ºC; 30 Sec - 72ºC; 30 Sec] x35 - 4ºC). The same conditions were used for serum PCR and amplicon bands were seen. This suggests that the amplification conditions could be ideal. The Template samples were also reviewed from preparation to storage. Serum and DNA samples were stored at -20ºC as required by the report of Sandoval and Siles-Luca (2006) and kept away from other factors that could denature them. The absence of amplicon bands could be attributed to 'host specific' DNA inhibitors encountered during preparation, transportation and or manipulation of the samples. Our tropical nature might have affected the keeping quality of the DNA sample, a temperature lower than -20ºC (-60ºC to -80ºC) might have prevented the occurrence putting in mind that room temperature in Spain and a temperature of -20ºC might have aided the amplification reported by Sandoval and Siles-Luca (2006).

CONCLUSION

This study provided the base line data on the prevalence and distribution of *Schistosoma* infection in cattle in MMC and Jere LGAs of Borno state, Nigeria. Because of the public health importance of *Schistosoma* infection, there should be an officially coordinated system to aid in instituting and maintaining preventive and control programmes. There is need for regular provision of safe and wholesome water to both animals and humans.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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Nothing to declare.

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