Infection, Genetics and Evolution 70 (2019) 53–60

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Prevalence and multilocus genotypes of *Giardia duodenalis* infecting pigs in Ogun state, Nigeria

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ARTICLE INFO

Keywords:
ELISA
Giardia
Nigeria
Pig
Prevalence
Zoonosis

ABSTRACT

Giardia duodenalis is an intestinal flagellated protozoan parasite that is infectious to humans and a wide range of animals worldwide. While varying prevalence rates have been reported in pigs worldwide, there are currently no published reports on the genotypes of *Giardia* infecting pigs in any African country. The present study is on the prevalence and genotypes of *G. duodenalis* in 209 pigs raised on four farms in Ogun State Nigeria. Using an enzyme-linked immunosorbent assay (ELISA) kit, *Giardia duodenalis* coproantigens were detected on all farms and in 25.4% (53/209) of pigs sampled. However, there was no significant influence ($p > 0.05$) of age, sex and stool consistencies of the pigs on the distribution of the infection. Genotyping of *Giardia duodenalis* in all ELISA-positive samples, achieved by the amplification of the small subunit ribosomal RNA (*ssu rRNA*), glutamate dehydrogenase (*gdh*), triosephosphate isomerase (*tpi*) and beta giardin (*bg*) genes, identified 14 and 37 assemblage B and E isolates respectively while mixed infection by both assemblages was recorded in two isolates. Novel nucleotide substitutions were identified in four assemblage B isolates at the *ssu rRNA* locus. Genetic diversity was observed among the assemblage B isolates after multiple alignment analyses of the *gdh*, *tpi* and *bg* sequences whereby sub-assemblages BII ($n = 2$), BIII ($n = 9$) and BIV ($n = 3$) were identified. The assemblage B isolates from pigs in this study were phylogenetically related to isolates from humans, marmoset and cattle while the assemblage E isolates were related to isolates from sheep, goats and cattle. These findings suggest that pigs in southwest Nigeria predominantly harbour *G. duodenalis* isolates that could be infectious to other animal species and to a lesser extent, isolates that may be of zoonotic importance.

1. Introduction

Giardia is a gastrointestinal protozoan parasite that infects humans and a wide variety of domestic and wild animals (Akinkuotu et al., 2018; Koehler et al., 2014; Squire and Ryan, 2017; Zhang et al., 2012). Infected hosts are often asymptomatic or present with diarrhoea whose severity depends on host immune status and concurrent infection with other pathogens (Robertson et al., 2010).

The disease is transmitted faeco-orally via consumption of food and water contaminated with *Giardia* cysts (Karanis et al., 2007; Smith et al., 2007). Additionally, the age, immune status, proximity to animals, potability of drinking water, personal and environmental hygiene are some factors reported to predispose humans to the infection (Budu-Amoako et al., 2012; Squire and Ryan, 2017), while age, stocking density, floor type of pens, system of management, presence and abundance of flies, potability of drinking water and method of waste disposal are factors that reportedly predispose animals to the infection (Maddox-Hyttel et al., 2006; Xiao et al., 1994; Yui et al., 2014).

To date, eight *G. duodenalis* assemblages (A-H), which are different in their host specificities, have been identified by Polymerase Chain Reaction (PCR), either in combination with Restriction Fragment Length Polymorphism (RFLP) or sequencing of the PCR amplicons (Thompson and Ash, 2016; Wegayehu et al., 2016; Zahedi et al., 2017). Assemblages A and B have a wide host range, being capable of infecting humans and various mammalian species while assemblages C–H are more host-specific and predominantly infect non-human mammalian hosts (Zahedi et al., 2017; Zhang et al., 2012). However, there have been reports of sporadic human infections by assemblages C, D, E and F (Fantinatti et al., 2016; Zahedi et al., 2017). Sub-assemblages have only been identified within assemblage A (AI-AIII) and B (BI-BIV) with all,

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https://doi.org/10.1016/j.meegid.2019.02.017
Received 31 July 2018; Received in revised form 22 January 2019; Accepted 16 February 2019
Available online 21 February 2019

except sub-assemblage AIII, assumed to be of zoonotic importance (Thompson and Ash, 2016).

Giardiasis in pigs has been reported worldwide with varying prevalence rates (Agumah et al., 2015; Brhanie et al., 2014; Farzan et al., 2011; Fava et al., 2013; Li et al., 2017; Minetti et al., 2014; Petersen et al., 2015; Siwila and Mwape, 2012; Stojecki et al., 2015; Syakalima et al., 2015; Yui et al., 2014). The variations in prevalence rates have been attributed to differences in the age categories of pigs studied, ecological characteristics of the study area, study designs and diagnostic tools employed (Feng and Xiao, 2011; Lim et al., 2013; Thompson and Ash, 2016). Furthermore, various genotyping studies have reported a predominant infection of pigs by assemblage E and much lesser by assemblages A and B (Armson et al., 2009; Hamnes et al., 2007; Li et al., 2017; Petersen et al., 2015). To the best of our knowledge, there are currently no available reports on the genotypes of *G. duodenalis* infecting pigs in Africa.

In Nigeria, giardiasis is under-reported with up to 41.5% prevalence in humans (Biu et al., 2009; Efunshile et al., 2015; Inabo et al., 2011; Maikai et al., 2012; Nwanguma and Alumanah, 2008; Obiukwu et al., 2008). Furthermore, the only genotyping study in humans identified only sub-assemblage AII (Maikai et al., 2012). In animals, the prevalence ranges between 5.6% and 72.3%, being reported in cattle (Magaji et al., 2013), dogs (Abubakar et al., 2015), goats (Akinkuotu et al., 2016), pigs (Agumah et al., 2015) and rabbits (Akinkuotu et al., 2018). At the time of writing this report, only one study in rabbits by Akinkuotu et al. (2018) characterized the *Giardia* genotypes in any animal species in the country.

This study was therefore conducted to determine the prevalence and genotypes of *G. duodenalis* infecting pigs in a southwestern state of Nigeria using an enzyme-linked immunosorbent assay (ELISA) and multilocus genotyping (MLG) of four genes respectively.

2. Materials and methods

2.1. Study area, animals and farms

This cross-sectional study was conducted in Ogun State, southwestern Nigeria which is located between latitude 6.2°N and 7.8°N and longitude 3.0°E and 5.0°E. Four swine farms, each located at Odeda, Ilaro, Sagamu and Ayetoro (Fig. 1), were selected for this study based on the owner's willingness to participate in the study and availability of pigs of different age categories. The pigs on these farms were intensively managed in demarcated pens with concrete floors while the source of drinking water was a dug well located within the vicinity of each farm. On all the farms, recently weaned piglets from different sows were usually grouped and reared together.

2.2. Sample collection

A total of 209 faecal samples were individually collected from pigs on all farms in this study, between October 2015 and April 2016. These were collected from 64 adults (> 6 months of age), 74 post-weaned pigs (above 6 weeks to 6 months of age) and 71 pre-weaned piglets (up to 6 weeks of age) either per rectum from the adult and pre-weaned pigs or after being freshly voided by the pre-weaned piglets. Samples were then transported, in cold packs, to the Veterinary Parasitology laboratory, College of Veterinary Medicine (COLVET), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria where they were immediately prepared and screened for *G. duodenalis* coproantigens by ELISA. The ELISA tests were run within four hours of collecting samples. The ELISA-positive samples were then stored at -20 °C for up to three days pending DNA extraction.

2.3. Stool sample preparation

The ethyl-acetate sedimentation technique was performed on each faecal sample using the procedure described by Erdman (1981). Briefly, 2 g of each sample was emulsified with 10 ml of distilled water and the mixture was filtered with a sieve and 9 ml of the filtrate was transferred to a 15 ml test tube into which 3 ml of ethyl-acetate was added. The test tube was sealed with a rubber stopper and vigorously agitated. The stopper was removed and the test tube was centrifuged at 600 x g for seven minutes. An applicator stick was used to detach the fat plug and the supernatant was decanted. The remaining sediment was used for ELISA and DNA extraction.

2.4. Detection of Giardia duodenalis coproantigens by ELISA

Coproantigens were detected using a *G. duodenalis*-specific ELISA stool kit (RIDASCREEN® *Giardia*; R-Biopharm, AG, Germany) as per the manufacturer's instructions while the optical densities (OD) of each reaction were measured at 450 nm in a 96-well ELISA plate reader (BIOTEX; ELx800, USA).

2.5. DNA extraction

Genomic DNA was extracted from *Giardia* coproantigens-positive samples by use of the AccuPrep™ stool genomic DNA extraction kit (Bioneer, Korea) according to the manufacturer's instructions. All DNA was eluted in 100 µl of 10 mM Tris buffer, packed in dry ice and transported to the Atlantic Veterinary College, University of Prince Edward Island, Canada where the molecular analyses were performed.

2.6. PCR amplification of Giardia duodenalis

Nested PCR protocols previously described by Appelbee et al. (2003), Caccio et al. (2008) and Sulaiman et al. (2003) were used to amplify *G. duodenalis* target fragments of the *ssu rRNA, gdh* and *tpi* genes respectively while a semi-nested PCR protocol was used to amplify the *bg* gene (Mahbubani et al., 1992). A hot-start step of 95 °C for five minutes was included in each round of PCR. The properties of all primers used (Table 1) have been described in our earlier report (Akinkuotu et al., 2018).

The PCRs were performed in a 50 µl reaction volume comprising 1 X Q-solution (Qiagen), 1 X CoralLoad® PCR buffer containing 1.5 mM $MgCl_2$ (Qiagen), 200 mM of each dNTP, 0.5 µM of each primer, 2.5 units of HotStar® Taq polymerase (Qiagen) and 2 µl of the tested DNA. The primary and secondary PCRs were similar except that 2 µl of the primary PCR products was used as the DNA template in the secondary PCR and the annealing temperatures of the *ssu rRNA* and *bg* genes were changed (Table 1). Positive and negative controls were also included at each PCR step.

Electrophoreses of all secondary PCR amplicons were performed on 1% agarose gel stained with SYBR™ Safe DNA gel stain (Invitrogen) while visualization and image capture were achieved using the VersaDoc™ 5000MP imaging system (BioRad).

2.7. Sequencing of amplified genes

Secondary PCR products from all four genetic loci were sequenced in both directions using secondary PCR primers at the Macrogen USA Corp., MD, USA. The forward and reverse sequences and their chromatograms were visually inspected for each isolate to confirm single nucleotide polymorphisms and mixed infections after which the consensus sequences were constructed. Each consensus sequence was compared to similar published sequences available at the GenBank™ database by Basic Local Alignment Search Tool (BLAST) analysis (http://www.ncbi.nlm.nih.gov/blast). Consensus sequences were then aligned with reference GenBank™ sequences using the ClustalW multiple alignment function in BioEdit version 6 (Hall, 1999).

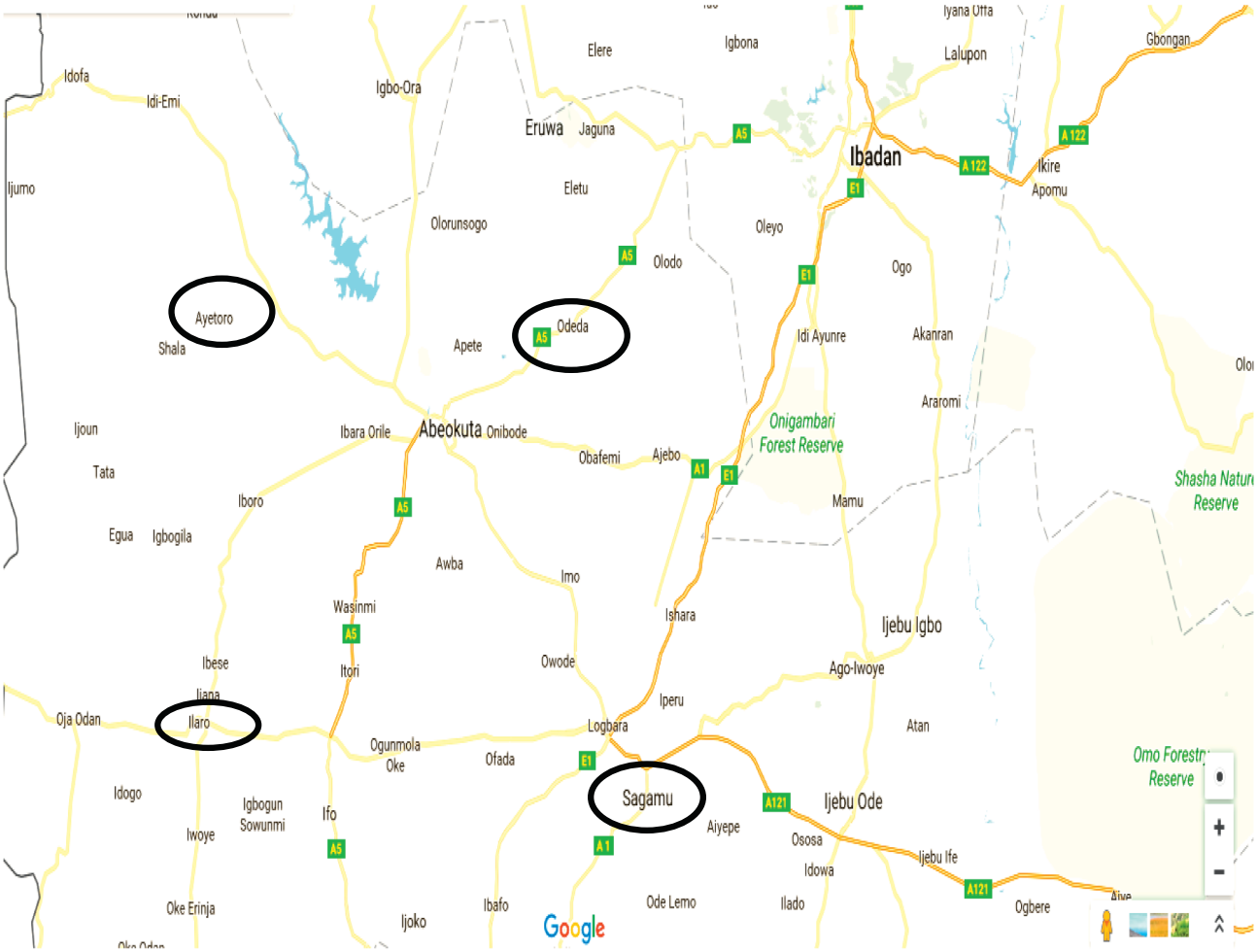

Fig. 1. Map of the study area with sampling sites being annotated (reprinted from Google, 2018).

2.8. Phylogenetic analyses

Separate phylogenetic trees were constructed using three and four representative *ssu rRNA* and *gdh* isolates respectively from pigs in this study with six and nine reference GenBank™ *ssu rRNA* and *gdh* isolates respectively. *Giardia microti* (AF006676) and *G. ardeae* (AF069060) sequences were used to root the *ssu rRNA* and *gdh* trees respectively, these trees were constructed using maximum likelihood (ML) with the Tamura-Nei model of the Molecular Evolutionary Genetic Analysis (MEGA) 7.0 software (Kumar et al., 2016). Statistical support for the ML tree topology was assessed by 1000 bootstrap replicates (Felsenstein, 1985).

2.9. Statistical analyses

The prevalence of the infection was obtained from the results of the ELISA and was computed using descriptive statistics while Chi-square test was used to compare the prevalence rates among the categories of pigs considered in this study at 5% level of precision. All analyses were performed using the IBM Statistical Package for Social Sciences (SPSS) software version 24 (IBM Analytics, NY, USA).

3. Results

3.1. Prevalence of Giardia duodenalis coproantigens

Giardia duodenalis coproantigens were detected on all four farms and in 25.4% (53/209) of all pig samples in this study. The highest 24 (33.8%), intermediate 14 (21.9%) and lowest 15 (20.3%) prevalence rates were recorded in the pre-weaned piglets, adult and post-weaned pigs respectively but there was no significant ($p > 0.05$) association between *Giardia* infection and age. Furthermore, the infection was slightly ($p > 0.05$) higher in females (25.7%) than in males (25.0%) and in diarrhoeic pigs (28.6%) than those that passed formed stools (22.5%). The prevalence rates of the infection on the farms studied ranged from 21.2% to 31.4% but were not statistically ($p > 0.05$) significant (Table 2).

3.2. Giardia duodenalis genotypes found in pigs

Amplification was achieved in the 53 ELISA-positive samples at the *G. duodenalis ssu rRNA* and *gdh* loci. Genotyping at these genetic loci revealed that all categories of pigs considered harboured assemblages B and E with infection by assemblage E (73.6%) being significantly ($p < 0.05$) higher than those by assemblage B (30.2%) on all farms. Mixed infection with both assemblages was observed in two isolates (Table 2).

Ten non-clustered assemblage B isolates at the *ssu rRNA* locus had 100% sequence identity with one another and the *G. duodenalis* assemblage B isolated from rabbits in Nigeria (MG018739) and humans in Uganda (KY658187). Furthermore, four assemblage B isolates at this locus had four single nucleotide polymorphisms (SNPs) and were 99% identical to the reference GenBank™ assemblage B isolate from humans (KY658187) (Table 3). Additionally, all non-clustered assemblage E isolates ($n = 37$) at this genetic locus were identical to one another and the *G. duodenalis* assemblage E isolate from cattle in USA (AY655701).

Table 1
Primers used for PCR amplification at *ssu rRNA*, *gdh*, *tpi* and *bg* genes of *Giardia duodenalis* in pigs.

Genetic loci	Primer name	Primer sequence (5′–3′)	Annealing temperature [a]	Fragment size (bp)	References
ssu rRNA	Gia2029[b] Gia2150c[b]	AAGTGTGGTGCAGACGGACTC CTGCTGCCGTCCTTGGATGT	55	497	Appelbee et al., 2003
	RH11 RH4	CATCCGGTCGATCCTGCC AGTCGAACCCTGATTCTCCGCCAGG	59	292	Hopkins et al., 1997
bg	G7[b] G759[b]	AAGCCCGACGACCTCACCCGCAGTGC GAGGCCGCCCTGGATCTTCGAGACGAC	65	753	Mahbubani et al., 1992
	G376 G759	CATAACGACGCCATCGCGGCTCTCAGGAA GAGGCCGCCCTGGATCTTCGAGACGAC	64	384	
tpi	AL3543[b] AL3546[b]	AAATIATGCCTGCTCGTCG CAAACCTTTCCGCAAACC	50	605	Sulaiman et al., 2003
	AL3544 AL3545	CCCTTCATCGGIGTAACTT GTGGCCACCACICCCGTGCC	50	530	
gdh	Gdh1[b] Gdh2[b]	TTCCGTRTYCAGTACAACTC ACCTCGTTCTGRGTGGGCCA	50	754	Caccio et al., 2008
	Gdh3 Gdh4	ATGACYGAGCTYCAGAGGCACGT GTGGCGCARGGCATGATGCA	50	520	

[a] Temperature in degrees Celsius (°C).
[b] Primers used in the primary PCR step.

Table 2
Prevalence and genotypes of *Giardia duodenalis* infecting pigs in Ogun state.

		ELISA			Genotyping at *ssu rRNA* and *gdh* loci	
Parameters	Categories	All	No. of pigs sampled	*p*-value	No. of assemblages (%)	
			Positive (%)		B	E
Age	Pre-weaned piglets	71	24 (33.8)		8 (33.3)	17 (70.8)*
	Post-weaned pigs	74	15 (20.3)	0.129	5 (33.3)	11 (73.3)*
	Adult	64	14 (21.9)		3 (21.4)	11 (78.6)*
Sex	Males	108	27 (25.0)	0.902	7 (25.9)	22 (81.5)*
	Females	101	26 (25.7)		9 (34.6)	17 (65.4)*
Stool consistency	Diarrhoeic	98	28 (28.6)	0.316	11 (39.3)	23 (82.1)*
	Non-diarrhoeic	111	25 (22.5)		5 (20.0)	16 (64.0)*
Farms	Odeda	48	13 (27.1)		3 (23.1)	11 (84.6)*
	Ilaro	51	16 (31.4)	0.615	4 (25.0)	12 (75.0)*
	Sagamu	58	13 (22.4)		5 (38.5)	9 (69.2)
	Ayetoro	52	11 (21.2)		4 (36.4)	7 (63.6)
All pigs		209	53 (25.4%)		16 (30.2%)	39 (73.6%)

* Significantly ($p < .05$) higher values in each category.

Table 3
Genetic variants of assemblage B isolates identified in pigs at the *ssu rRNA* genetic loci.

Variant	Accession No.	Number	Nucleotide positions			
			214	240	246	247
Reference	KY658187		G	C	C	G
Variant 1	MH620357	10	G	C	C	G
Variant 2	MH620358	4	T	T	A	A

The nucleotide sequence information for the two non-clustered assemblage B variants and assemblage E isolates at the *ssu rRNA* gene were deposited in GenBank™ under the accession numbers MH620357, MH620358 and MH620359 respectively.

The non-clustered assemblage E isolates (n = 37) at the *gdh* locus had 100% sequence identity with one another and the GenBank™ assemblage E isolates from sheep in China (KC960648) and cattle in Ghana (KY711410). The sequence data for this isolate were deposited in GenBank™ under the accession number MH644769.

Multiple alignment and BLAST analyses of the 14 non-clustered assemblage B sequences at the *gdh*, *tpi* and *bg* loci characterized these isolates as sub-assemblages BII, BIII and BIV with the highest occurrence of sub-assemblage BIII. However, at the *bg* locus, sub-assemblage BI was assigned to two isolates which were identified as sub-assemblage BIII at the *gdh* and *tpi* loci while poor amplification results were observed in two isolates (Table 4). The sequence data of the sub-assemblages BII, BIII and BIV isolates at the *gdh*, *tpi* and *bg* loci were deposited in GenBank™ under the accession numbers: MH644766-MH644768 for the *gdh* gene, MH644770-MH644772 for the *tpi* gene, and MH644774-MH644776 for the *bg* gene.

Designation of an isolate as assemblage E by MLG was made at the *ssu rRNA* and *gdh* loci while assemblage B genotypes were identified using the four genetic loci. Using MLG, 37 and 14 assemblage E and B isolates respectively were identified in pigs while two isolates with mixed assemblage infections were observed. Furthermore, non-concordant (sub)-assemblage designation at the genetic loci was observed in two isolates (Table 4).

Table 4
Multilocus genotypes of *Giardia duodenalis* isolates in pigs at the four genetic loci.

Genotypes identified at each genetic locus						MLG
Assemblage	Number	*ssu rRNA*	*gdh*	*tpi*	*bg*	
E	37	E	E	[a]	[a]	E
E and B	2	E and B	E and B	[a]	[a]	E and B (Mixed)
B	2	B	BII	BII	BII	BII
B	5	B	BIII	BIII	BIII	BIII
B	2	B	BIII	BIII	–	BIII
B	2	B	BIII	BIII	BI	BIII
B	3	B	BIV	BIV	BIV	BIV

Poor amplification was observed.
[a] PCR amplification was not performed.

3.3. Phylogenetic analyses

Evaluation of the phylogenetic relationship of the three representative *G. duodenalis* isolates at the *ssu rRNA* locus revealed their distribution into assemblages B and E clades. The two assemblage B isolates (MH620357 and MH620358) clustered with assemblage B isolates from human (AF199477) and cattle (KJ888984) while the assemblage E isolate (MH620359) clustered with assemblage E isolates from sheep (KT922264) and goats (AF199488) (Fig. 2).

Similarly, the four representative isolates at the *gdh* locus were distributed into assemblage B and E clades. The sub-assemblages BIII and BIV isolates from pigs in this study clustered near their respective isolates from humans (AF069059 and KX228245) while the sub-assemblage BII isolates from the studied pigs clustered with an isolate from a marmoset (AY178752). Furthermore, the assemblage E isolates from pigs in this study sub-clustered near isolates from cattle (KF843923.1) and sheep (KP635110.1) (Fig. 3).

4. Discussion

Giardiasis is a common gastrointestinal illness reported worldwide

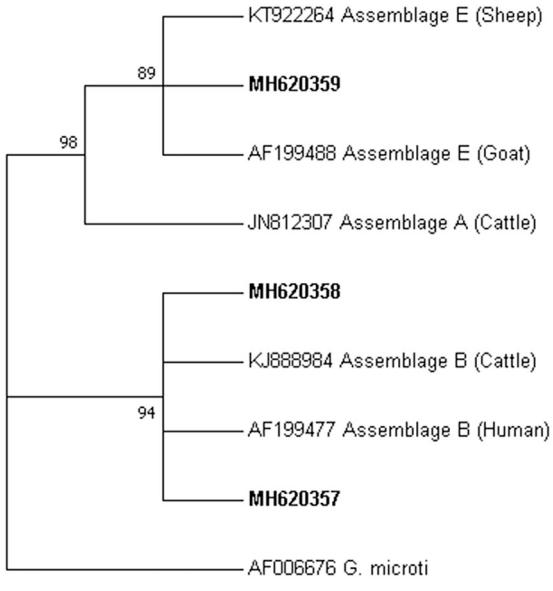

Fig. 2. Phylogenetic tree of representative *G. duodenalis* assemblages B and E isolates of pigs at the *ssu rRNA* gene constructed using the Tamura-Nei model of the maximum likelihood method. Bootstrap values (> 80%) from 1000 replicates are shown at each node. The tree is rooted by *G. microti* (AF006676). Accession numbers of sequences from this study are in bold while reference sequences from GenBank™ have accession number, assemblage designation and host origin.

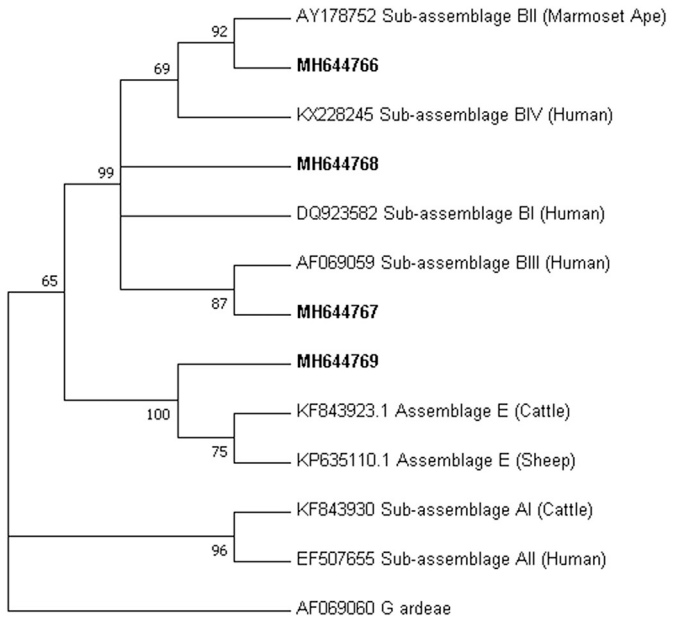

Fig. 3. Phylogenetic tree of representative *G. duodenalis* assemblages B and E isolates of pigs at the *gdh* gene constructed using the Tamura-Nei model of the maximum likelihood method. Bootstrap values (> 60%) from 1000 replicates are shown at each node. The tree is rooted by *G. ardeae* (AF069060). Accession numbers of sequences from this study are in bold while reference sequences from GenBank™ have accession number, assemblage designation and host origin.

in different age categories of humans and various domestic and wild animals (Faria et al., 2016; Hogan et al., 2014; Petersen et al., 2015; Stojecki et al., 2015; Wegayehu et al., 2016; Wegayehu et al., 2017; Zhang et al., 2016). In pigs, various studies that utilized microscopy (Brhanie et al., 2014; Fava et al., 2013; Matos et al., 2016; Yui et al., 2014), immunology (Budu-Amoako et al., 2012; Farzan et al., 2011; Petersen et al., 2015; Siwila and Mwape, 2012; Stojecki et al., 2015; Syakalima et al., 2015) and PCR (Armson et al., 2009; Beck et al., 2010; Farzan et al., 2011; Fava et al., 2013; Li et al., 2017; Minetti et al., 2014) in diagnosis of the infection have been reported worldwide with varying prevalence rates.

In Nigeria, *Giardia* infection has been reported in humans and domestic animals (Abubakar et al., 2015; Agumah et al., 2015; Akinkuotu et al., 2016; Akinkuotu et al., 2018; Biu et al., 2009; Efunshile et al., 2015; Inabo et al., 2011; Magaji et al., 2013; Maikai et al., 2012; Nwanguma and Alumanah, 2008; Obiukwu et al., 2008) and majority of these studies employed microscopic diagnostic techniques. To the best of our knowledge, this may be the pioneer study in Nigeria that employed immunological techniques to detect the infection in pigs.

The 25.4% prevalence rate of *Giardia* coproantigens in pigs in this study was higher than the rates recorded by Agumah et al. (2015) and Gagman et al. (2015) in pigs reared in Jos, Nigeria. It was also higher than the rates reported in Japan (Yui et al., 2014), Ethiopia (Brhanie et al., 2014), Turkey (Kirkoyun Uysal et al., 2009) and Brazil (Fava et al., 2013; Matos et al., 2016). The higher sensitivity of the ELISA used in this study than that of light microscopy utilized in these reports may account for the higher prevalence rate observed.

The prevalence rate in this study was also higher than those reported in several molecular studies in pigs worldwide (Armson et al., 2009; Beck et al., 2010; Farzan et al., 2011; Fava et al., 2013; Hamnes et al., 2007; Li et al., 2017; Minetti et al., 2014). However, apart from the reports of Olson et al. (1997) and Budu-Amoako et al. (2012), higher prevalence rates were reported in porcine studies that employed immunologic diagnostic techniques (Farzan et al., 2011; Petersen et al., 2015; Siwila and Mwape, 2012; Stojecki et al., 2015; Syakalima et al.,

2015). The differences in these reported prevalence rates may be associated with variations in study designs, age of pigs studied and system of management in sampled piggeries. Furthermore, the high prevalence rate recorded in the present study may be due to factors such as the wet climatic conditions of the study area, abundance of mechanical vectors such as flies and rodents in and around the sampled piggeries and the concrete floor of the pens which have all been suggested to facilitate the survival and transmission of *Giardia* cysts in herds (Maddox-Hyttel et al., 2006; Xiao et al., 1994; Yui et al., 2014). The presence of these factors on all studied farms may also be responsible for the similar prevalence rates observed among them.

Giardia infection was recorded in all age categories of pigs in this study although no significant influence of age on the prevalence of the infection was observed. This observation corroborates the submissions of Hamnes et al. (2007) and Armson et al. (2009) and suggests that *Giardia* infection in the studied pig herds was likely influenced more by non-age related factors such as husbandry practices, lack of potable drinking water and the designs of the pens. Furthermore, the high infection rate recorded in suckling piglets in this study supports the reports of Xiao et al. (1994) but contrasts with the widely reported predominance of *Giardia* infection in post-weaned pigs (Armson et al., 2009; Budu-Amoako et al., 2012; Kirkoyun Uysal et al., 2009; Maddox-Hyttel et al., 2006; Petersen et al., 2015; Siwila and Mwape, 2012; Yui et al., 2014). The high rate of infection of post-weaned pigs have been attributed to the long pre-patent period of *Giardia* and changes (social, environmental and nutritional) associated with weaning (Montagne et al., 2007).

The similar infection rates recorded in both sexes of pigs in this study conforms with the reports of Siwila and Mwape (2012), Brhanie et al. (2014) and Gagman et al. (2015) and suggests that the various breeding practices in the studied piggeries do not influence the transmission of the infection within each herd.

Although diarrhoea has been significantly associated with giardiasis in pigs (Armson et al., 2009; Hamnes et al., 2007; Maddox-Hyttel et al., 2006; Matos et al., 2016), high infection rates have also been recorded in asymptomatic pigs (Armson et al., 2009; Petersen et al., 2015; Yui et al., 2014). This therefore implies that diarrhoea is not a reliable clinical feature of giardiasis since co-infection with other pathogens commonly occurs (Budu-Amoako et al., 2012; Kirkoyun Uysal et al., 2009). This may be responsible for the similar infection rates observed between pigs with diarrhoea and pigs with formed stools in this study.

Several reports are available on the genotypes of *G. duodenalis* in pigs worldwide (Armson et al., 2009; Beck et al., 2010; Farzan et al., 2011; Fava et al., 2013; Li et al., 2017; Minetti et al., 2014). However, available published reports and one review (Akinkuotu et al., 2018; Berrilli et al., 2012; Helmy et al., 2014; Hogan et al., 2014; Squire and Ryan, 2017; Squire et al., 2017; Wegayehu et al., 2017) indicate that *G. duodenalis* genotypes have been identified in cattle, goats, sheep, rabbits and wildlife in Africa while there is no report on the genotypes of *Giardia* in pigs. This may therefore be the first report on the genotypes of *Giardia* species infecting pigs in Africa.

The *ssu rRNA*, *gdh*, *tpi* and *bg* genetic loci used for genotyping in this study are commonly employed in several epidemiological studies (Macedo de Godoy et al., 2013; Maikai et al., 2012; Wegayehu et al., 2016; Wegayehu et al., 2017). Furthermore, the similarly high diagnostic performance of the *ssu rRNA* and *gdh* genes reported in previous studies (Akinkuotu et al., 2018; Wegayehu et al., 2016; Wegayehu et al., 2017) informed our initial amplification of the DNA samples using these genes.

The MLG of *Giardia* isolates employed in this study has been reported to be more reliable in assemblage designation than single locus genotyping (Akinkuotu et al., 2018; Liu et al., 2014; Pantchev et al., 2014). This observation relates to the inconsistent assemblage designation of an isolate arising from the different genetic loci (Liu et al., 2014; Ryan and Cacciò, 2013). This is evident in this study as sub-assemblage BI was assigned to an isolate at the *bg* locus which was genotyped as sub-assemblage BIII at the *gdh* and *tpi* loci.

Pigs in this study were infected with *G. duodenalis* assemblages B and E with the latter being predominant. These observations, similarly reported by Farzan et al. (2011) and Stojecki et al. (2015), suggest that most pigs in the study area harbour the livestock-adapted assemblage E genotype. Additionally, the assemblage E isolates in this study, being identical to an isolate derived from cattle and phylogenetically related to isolates from sheep and goats at the *ssu rRNA* locus and cattle and sheep at the *gdh* locus, are therefore assumed to have been acquired from or be capable of infecting other animal species in the study area.

Contrary to several reports in which assemblage A was the zoonotic genotype identified in pigs (Armson et al., 2009; Caccio et al., 2008; Li et al., 2017; Maddox-Hyttel et al., 2006; Petersen et al., 2015), assemblage B may be the incriminated zoonotic genotype of pigs in Nigeria. Although there is limited information on the distribution of assemblage B subtypes in previous genotyping studies on pigs, MLG of the *G. duodenalis* isolates in the studied pigs revealed a predominance of sub-assemblage BIII. It is however noteworthy that the sub-assemblages BII, BIII and BIV isolates identified in these pigs have also been reported in humans worldwide (Anim-Baidoo et al., 2016; Di Cristanziano et al., 2014; El Fatni et al., 2014; Mbae et al., 2016), thereby implying that humans in Nigeria may also harbour these sub-assemblages even though only sub-assemblage AII has so far been reported (Maikai et al., 2012). This suggests that these pigs may have acquired the infection from workers on the farms and may also be involved in the possible zoonotic transmission cycle of the infection. It is therefore necessary to determine the prevalence of *Giardia* by the detection of coproantigens and/or serology and also identify the genotypes of *Giardia* species infecting humans and various animal species in various parts of the country which will elucidate the epidemiological scenario of the infection.

5. Conclusions

Pigs reared in Ogun State, southwest Nigeria are predominantly infected with the livestock-adapted assemblage E and lesser by the potentially zoonotic assemblage B. Genetic diversity was recorded among the assemblage B isolates whereby two variants were observed at the *ssu rRNA* locus and three sub-assemblages were identified at the *gdh*, *tpi* and *bg* loci. Being the pioneer genotyping study on *Giardia* of pigs in Africa, the results from this study provides a foundation on which further genotyping studies on *Giardia* species infecting humans and various animal species can be conducted in Nigeria and other African countries.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgement

We would like to thank Haili Wang for technical assistance.Financial support.This study was supported by the Tertiary Education Trust Fund (TETFund) Academic Staff Training and Development Intervention grant (grant number: 2012/2013) of the Federal Government of Nigeria. The funder was not involved in study design, data collection, analysis and interpretation or manuscript preparation and submission.

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