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First report of *Echinococcus granulosus sensu stricto* (G1) in Nigeria, West Africa

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Short Communication

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Abstract

Echinococcus granulosus sensu stricto is regarded to have the highest zoonotic potential of all *Echinococcus* taxa. Globally, human infection due to this species constitutes over 88.44% of the total cystic echinococcosis (CE) burden. Here, we report a CE infection in a Nigerian camel caused by *E. granulosus* G1 genotype. To the best of our knowledge, this report is the first encounter of the G1 genotype in the West Africa sub-region where the G6 genotype is reportedly prevalent, suggesting that the epidemiology of this highly zoonotic group could have a wider host range and distribution in the sub-region, and emphasizes the need for further investigation into the genetic diversity of *Echinococcus* spp. in Nigeria and across the sub-region.

Introduction

Cystic echinococcosis (CE) is a parasitic zoonosis caused by cestodes. Regardless of the current knowledge on the genetic diversity, host range and the taxonomic challenges among certain members of the *Echinococcus* complex, it remains a neglected zoonotic disease across the world, and more so in Nigeria. Infection with this cestode results in serious veterinary and public health concerns (Deplazes *et al*., 2017) as economic losses are estimated to reach billions of US dollars annually (WHO, 2013). *Echinococcus granulosus sensu stricto* (*s.s.*) (G1, G3), is regarded to have the highest zoonotic potential, while other species in the complex include *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus canadensis* (G6–10) and *Echinococcus felidis*, with preference to different intermediate hosts (Lymbery, 2017). Therefore, exploring the genetic variation within/between species and the species diversity in an endemic area is important to the control and management of CE. However, there are still contentious issues regarding the taxonomic status of the *E. canadensis* group (G6/7 G8/10) (Thompson, 2008; Lymbery *et al*., 2015; Nakao *et al*., 2015; Laurimäe *et al*., 2018).

In Nigeria, investigations have shown that the northern part of the country is endemic of CE. Yet, only recently was the G6/7 genotype reported to be majorly responsible for CE in animals in endemic communities (Ohiolei *et al*., 2019a). In humans, CE is poorly investigated, although in recent times two unusual presentations have been reported: one is a case of musculoskeletal involvement with HIV coinfection in a patient admitted to The University of Jos Teaching Hospital in north-central Nigeria (Ozoilo *et al*., 2007), and an orbital cystic echinococcosis manifested in protrusion of the eye and poor vision in the University College Hospital, Ibadan south-western Nigeria (Fasina & Ogun, 2017). Nonetheless, it is believed that CE in humans is often misdiagnosed (Fasina & Ogun, 2017), and the species/genotypes responsible for infection remain unknown. Here, we encountered and molecularly identified what we believed is the first record of the G1 genotype in a camel host from a northern Nigerian state, and highlight the potential public health importance.

Material and methods

Sample collection, polymerase chain reaction (PCR) amplification and sequencing

A fertile hydatid cyst of camel origin was collected in December 2018 from Sokoto modern abattoir, Sokoto state, Nigeria (13.0059°N, 5.2476°E) at postmortem during a routine inspection. Genomic DNA extraction was performed on a cut piece of the germinal layer using Qiagen DNeasy Blood and Tissue DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Amplification of the complete mitochondrial *cox*1 (1674 bp) (Kinkar *et al*., 2019) and *nad*1 (894 bp) gene was achieved using previously described primer pairs (Wu *et al*., 2018) in addition to a *nad*5 gene fragment (about 680 bp) (Kinkar *et al*., 2018a). PCR was conducted in 25 µl final volume using 2 mM $MgCl_2$, 0.2 mM dNTPs, 5 µl 5× Taq buffer, 10 pmol of each primer, 0.5 µl Ex Taq DNA polymerase (5 U/µl, Takara), 0.5 µl of genomic

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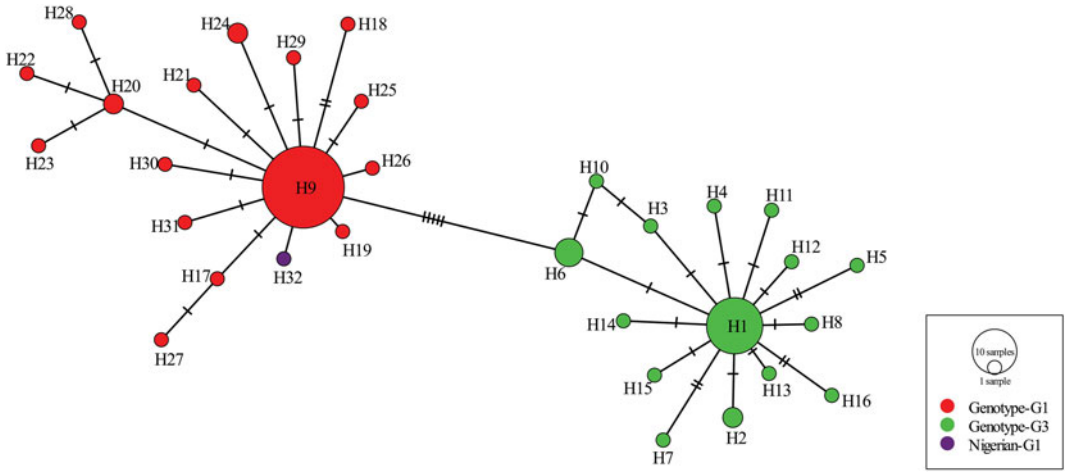

Fig. 1. Median-joining network of the *nad*5 mitochondrial genes of a panel of 86 sequences consisting of *Echinococcus granulosus sensu stricto* representatives isolates/haplotypes from across the world, including our Nigerian isolate (haplotype H32). Reference genotype G1 sequences (51) = GenBank: MG672170–MG672220; genotype G3 sequences (34) = GenBank: MG682511–MG682544.

DNA extract (~50 ng) and RNAse free water up to the final volume of 25 µl. The reaction cycled 35 times under the following conditions: denaturation at 95°C for 30 s, annealing at 55°C for 40 s and elongation at 72°C for 60–90 s after an initial denaturation at 95°C for 5 min, followed by a final extension at 72°C for 10 min.

Amplicons were visualized by electrophoresis in 1.5% (w/v) agarose gels in 1 × TAE (40 mM Tris-acetate, 2 mM EDTA, pH 8.5), stained with GelRed™, and viewed under UV light. The PCR products were purified using an Agarose Gel DNA Purification Kit (Axygen Biosciences, CA, USA), and then cloned to a pMD19 T-vector (Takara Bio, Japan) prior to sequencing (Beijing Tsingke Biotechnology Co., Ltd., China).

Identification and phylogenetic network analysis

Nucleotide sequences where viewed manually for correction of any nucleotide misread followed by alignment with retrieved sequences from Genbank in BioEdit v7.2.6 (Hall, 1999). The identity of the isolate was confirmed via a nucleotide sequence BLAST search using the NCBI BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with G1 nucleotide sequences from other parts of the world. The median-joining network was inferred based on the sequences of the mitochondrial *nad*5 gene using PopART (http://popart.otago.ac.nz). Reference genotype G1 sequences for the network analysis were from Kinkar *et al.* (2018b) (GenBank: MG672170–MG672220), while genotype G3 sequences were from Kinkar *et al.* (2018c) (GenBank: MG682511–MG682544).

Results and discussion

The BLAST result of the mitochondrial sequences confirmed the identity of the isolate as G1 genotype with a 99–100% similarity with other G1 sequences deposited in GenBank. The median-joining network (fig. 1) based on the *nad*5 gene(680 bp) further distinguished the G1 genotype from G3 genotype. Meanwhile, A few nucleotide polymorphisms were observed between the Nigerian G1 isolate and a reference G1 sequence (GenBank accession: AB786664; host: human; origin: China) as follows: *cox*1 267A–267G; *nad*1 117T–117C; and *nad*5 355C–355T and 578T–578C. The G1 sequences from this study have been deposited in GenBank under the following accession numbers: MN199126 (*cox*1), MN199127 (*nad*5) and MN199128 (*nad*1).

In West Africa, CE is largely under-investigated, unlike the northern and eastern sub-regions where data on CE epidemiology and the population genetic structure of the causative agents are available (Deplazes *et al.*, 2017; Lymbery, 2017). However, the few available data for the West Africa sub-region showed that *E. canadensis* G6/7 is basically responsible for CE in both humans and livestock (Mauti *et al.*, 2016; Deplazes *et al.*, 2017; Ohiolei *et al.*, 2019a). Meanwhile, the role of camels as potential intermediate hosts for the G1 genotype has been documented in most African and Asian countries (Deplazes *et al.*, 2017). Also, *E. granulosus s.s.*, which consists of genotypes G1 and G3, is responsible for over 88.44% of global human CE burden (Alvarez Rojas *et al.*, 2014). In Africa, *E. granulosus s.s.* and *E. canadensis* G6/7 are both responsible for the majority of CE infection in livestock and humans (Addy *et al.*, 2012; Boufana *et al.*, 2014; Deplazes *et al.*, 2017), with cattle, sheep, goats and camels being massively involved in the transmission and maintenance of the infection (Ernest *et al.*, 2009; Boufana *et al.*, 2014; Deplazes *et al.*, 2017; Ohiolei *et al.*, 2019a), especially in poor pastoral communities with high stray dog populations and poor waste disposal systems in slaughterhouses (Ernest *et al.*, 2009; Omadang *et al.*, 2018).

In Nigeria, although the high prevalence of CE in livestock attracted a lot of concern between 1970 and 1990, not much has been done lately (see the review by Ohiolei *et al.*, 2019b). In humans, the seemingly low CE prevalence has been attributed to poor surveillance, lack of differential diagnosis and poor knowledge by medical personnel (Fasina & Ogun, 2017; Ohiolei *et al.*, 2019b). In our previous study, where we reported the G6/7 genotype as being responsible for CE infection in livestock, the absence of the G1 genotype could have been a result of sample size limitation, as emphasized (Ohiolei *et al.*, 2019a). Nonetheless, the G1 genotype in the current study further suggests that other species/genotypes could potentially be present in the country and even within states. Furthermore, while transboundary animal movement through animal trade or smuggling could play a potential role in the distribution and prevalence of CE, the extent to which it influences the epidemiology in Nigeria remains unknown

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as most West African countries are largely uninvestigated (Deplazes *et al.*, 2017; Ohiolei *et al.*, 2019a). However, infection with the G6 genotype in a Nigerien migrant was recently detected at a referral centre in Northern Italy. Based on the data, the authors suggested that the individual is potentially from an active transmission zone (Angheben *et al.*, 2017). Interestingly, Sokoto, a state in the north-west zone of Nigeria where G6/7 genotype was previously reported (Ohiolei *et al.*, 2019a) and also the G1 genotype in the present study, borders Niger Republic to the north, indicating the possibility of transboundary CE transmission. Besides being a border state where unchecked animal movement could contribute to CE transmission, the state also shows other high-risk factors propagating CE transmission such as high frequency of stray dogs within the city metropolis and dog access to slaughterhouses. The city also hosts a significant number of households within the metropolis that keep dogs as pets that are most times allowed to roam freely, thus increasing the risk of human infection.

In conclusion, to the best of our knowledge, this study represents the first report of *E. granulosus s.s.* G1 genotype in Nigeria and the West Africa sub-region. With the previous report of the G6/7 genotype and the current detection of the genotype with the highest zoonotic potential, there is thus a need for heightened surveillance considering the public health significance of both genotypes. Furthermore, we recommend a massive screening of animal hosts alongside people living in endemic zones and human patients presenting symptoms suspected of CE in view of evaluating the burden of CE due to this genotype across the country and sub-region, as the previous absence of this genotype could be largely due to poor surveillance or a lack of molecular studies in the past. We also suggest future studies to consider the potential impact of animal movement across borders on the overall CE prevalence in the country.

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Conflicts of interest. None.

Ethical standards. Not applicable.

References

Addy F, Alakonya A, Wamae N *et al.* (2012) Prevalence and diversity of cystic echinococcosis in livestock in Maasailand, Kenya. *Parasitology Research* **111**, 2289–2294.

Alvarez Rojas CA, Romig T and Lightowlers MW (2014) *Echinococcus granulosus sensu lato* genotypes infecting humans—review of current knowledge. *International Journal for Parasitology* **44**, 9–18.

Angheben A, Mariconti M, Degani M, Gobbo M, Palvarini L, Gobbi F, Brunetti E and Tamarozzi F (2017) Is there echinococcosis in West Africa? A refugee from Niger with a liver cyst. *Parasites & Vectors* **10**, 232.

Boufana B, Lahmar S, Rebai W, Ben Safta Z, Jebabli L, Ammar A, Kachti M, Aouadi S and Craig PS (2014) Genetic variability and haplotypes of *Echinococcus* isolates from Tunisia. *Transaction of the Royal Society of Tropical Medicine and Hygiene* **108**, 706–714.

Deplazes P, Rinaldi L, Alvarez Rojas CA *et al.* (2017) Global distribution of alveolar and cystic echinococcosis. *Advances in Parasitology* **95**, 315–493.

Ernest E, Nonga HE, Kassuku AA and Kazwala RR (2009) Hydatidosis of slaughtered animals in Ngorongoro district of Arusha Hydatidosis of slaughtered animals in Ngorongoro district of Arusha region, Tanzania. *Tropical Animal Health and Production* **41**, 1179–1185.

Fasina O and Ogun OG (2017) Hydatid cyst of the orbit in a young Nigerian female: a case report. *Ghana Medical Journal* **51**, 204–206.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposiums Series* **41**, 95–98.

Kinkar L, Laurimäe T, Acosta-Jamett G *et al.* (2018*a*) Distinguishing *Echinococcus granulosus sensu stricto* genotypes G1 and G3 with confidence: a practical guide. *Infection, Genetics and Evolution* **64**, 178–184.

Kinkar L, Laurimäe T, Acosta-Jamett G *et al.* (2018*b*) Global phylogeography and genetic diversity of the zoonotic tapeworm *Echinococcus granulosus sensu stricto* genotype G1. *International Journal for Parasitology* **48**, 729–742.

Kinkar L, Laurimäe T, Balkaya I *et al.* (2018*c*) Genetic diversity and phylogeography of the elusive, but epidemiologically important *Echinococcus granulosus sensu stricto* genotype G3. *Parasitology* **145**, 1613–1622.

Kinkar L, Korhonen PK, Cai H *et al.* (2019) Long-read sequencing reveals a 4.4 kb tandem repeat region in the mitogenome of *Echinococcus granulosus* (*sensu stricto*) genotype G1. *Parasites & Vectors* **12**, 238.

Laurimäe T, Kinkar L, Moks E *et al.* (2018) Molecular phylogeny based on six nuclear genes suggests that *Echinococcus granulosus sensu lato* genotypes G6/G7 and G8/G10 can be regarded as two distinct species. *Parasitology* **145**, 1929–1937.

Lymbery AJ (2017) Phylogenetic pattern, evolutionary processes and species delimitation in the genus *Echinococcus*. *Advances in Parasitology* **95**, 111–145.

Lymbery AJ, Jenkins EJ, Schurer JM and Thompson RC (2015) *Echinococcus canadensis*, *E. borealis*, and *E. intermedius*. What's in a name? *Trends in Parasitology* **31**, 23–29.

Mauti S, Traoré A, Crump L, Zinsstag J and Grimm F (2016) First report of *Echinococcus granulosus* (genotype G6) in a dog in Bamako, Mali. *Veterinary Parasitology* **217**, 61–63.

Nakao M, Lavikainen A and Hoberg E (2015) Is *Echinococcus intermedius* a valid species? *Trends in Parasitology* **31**, 342–343.

Ohiolei JA, Yan HB, Li L *et al.* (2019*a*) Cystic echinococcosis in Nigeria: first insight into the genotypes of *Echinococcus granulosus* in animals. *Parasites & Vectors* **12**, 392.

Ohiolei JA, Yan H-B, Li L, Zhu G-Q, Muku RJ, Wu Y-T and Jia W-Z (2019*b*) Review of cystic echinococcosis in Nigeria: a story of neglect. *Acta Parasitologica*10.2478/s11686-019-00124-x

Omadang L, Chamai M, Othieno E, Okwi A, Inangolet FO, Ejobi F, Oba P and Ocaido M (2018) Knowledge, attitudes and practices towards cystic echinococcosis in livestock among selected pastoral and agro-pastoral communities in Uganda. *Tropical Animal Health and Production* **50**, 11–17.

Ozoilo KN, Iya D, Kidmas AT, Uwumarogie O and Hassan S (2007) Anterior abdominal wall hydatid cyst; an unusual presentation. *Nigerian Journal of Medicine* **16**, 181–182.

Thompson R (2008) The taxonomy, phylogeny and transmission of *Echinococcus*. *Experimental Parasitology* **119**, 439–446.

WHO (2013) *Sustaining the drive to overcome the global impact of neglected tropical diseases: Second WHO report on neglected diseases*. Geneva, World Health Organization.

Wu Y, Li L, Zhu G *et al.* (2018) Mitochondrial genome data confirm that yaks can serve as the intermediate host of *Echinococcus canadensis* (G10) on the Tibetan Plateau. *Parasites & Vectors* **11**, 166.