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ORIGINAL ARTICLE

Prevalence and molecular characterization of cystic hydatidosis in livestock slaughtered in southern Mozambique

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Abstract Hydatid disease is a parasitic zoonosis caused by genotypes of the genus *Echinococcus*. This disease inflicts economic loses in livestock and cause public health burden in resource poor mostly in developing countries. The aim of this study was to determine the prevalence and identity of the genotypes responsible for hydatid cysts in cattle, goats and pigs slaughtered at selected abattoirs of southern provinces of Mozambique. Cysts were collected from liver and lungs and hydatid confirmation was made by cystic membrane observation and visualization of protoscoleces by light microscope. Thirty-two hydatid cysts from 817 cattle and two from 68 pigs were collected from local slaughterhouses and slabs. DNA was extracted from protoscoleces of each cyst together with the cystic membrane and amplified based on the mitochondrial subunit 1 of the *cox1* and *nad1* gene. The overall prevalence of hydatid cysts was 3.9% in cattle, 2.9% in pigs and none of the goats were found with cysts. All cysts collected from cattle and pigs were identified as *Echinococcus ortleppi* (G5) with a minimum homology of 99% on BLAST analysis. Our results confirm the presence of *E. ortleppi* in cattle and pigs in southern Mozambique at a low prevalence and further studies are recommended to determine the risk factors favoring the transmission of this zoonotic parasite in the resource-poor livestock farming communities of this region.

Keywords *Echinococcus ortleppi* (G5) · Hydatid cyst · Cattle · Pigs · *cox1* · *nad1* · Mozambique

Introduction

Species of *Echinococcus granulosus* sensu lato are causative agents of cystic echinococcosis in herbivores, omnivores and humans (Ammann and Eckert 1996). The parasite is prevalent in poor rural livestock farming areas where livestock husbandry is traditional and commonly associated with uncontrolled movement of stray, shepherd and hunting dogs, which contributes to dispersion and contamination of pasture with taeniid eggs (Eckert and Deplazes 2004; Possenti et al. 2016). The disease is maintained by feeding dogs with uncooked infected offals in areas where home slaughter is practiced without veterinary inspection combined with lack of knowledge on the parasite life cycle, which consequently leads to incorrect disposal of infected organs (Bourée 2001). Infections in human may lead to severe or fatal disease course due to the rupture of the cyst or organ impairment due to cyst size (McManus et al. 2003).

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There are to date, nine genotypes and one additional strain reported from *E. granulosus* sensu lato (Nakao et al. 2013) and they were separated into five species namely *E. granulosus* sensu stricto (G1 + 3), *E. equinus*, *E. ortleppi*, *E. canadensis* (G6/7, 8, 10) (Romig et al. 2017; Deplazes et al. 2017; Macin et al. 2021) and *E. felidis* (Hüttner et al. 2008), all with zoonotic potential reported except *E. felidis* (Thompson 2020). Through phylogenetic analysis, the genotypes G6/G7 and G8,G10 were observed to group into two different clades suggesting distinct species (Laurimäe et al. 2018). Besides genetic variations, there has been reports of variations in the pathogenicity, antigenicity and drug response which may dictate in the development of region-specific control measures (Manterola and Otzen 2016).

In the African region, most cases of hydatid disease are confined in Eastern and Northern countries where the disease is highly endemic (Eckert and Deplazes 2004). In Southern Africa, recent data on echinococcosis were from slaughtered ruminants of Zambia (Nonaka et al. 2011; Banda et al. 2013, 2020) and Namibia (Krecek et al. 1990) although the parasite has been well described before with greater contributions from South Africa (Ortlepp 1934; Verster 1961, 1965).

In Mozambique, livestock, especially ruminants are concentrated in the southern region because of less challenge with trypanosomiasis compared to the northern area of the country and cattle is the preferred specie due to high commercial value compared to other species (Timberlake and Reddy 1986; Rocha et al. 1991). On the other hand, pig farming in Mozambique is mainly compromised by African swine fever (Penrith et al. 2007; Quembo et al. 2018), however, in relation to parasitic diseases in pigs most studies conducted have been in *Taenia solium* and cysticercosis (Afonso et al. 2011; Pondja et al. 2015; Chilundo et al 2017, 2018; Nhancupe et al 2019). Beside the reports on echinococcosis in livestock and dogs in Mozambique by de Castro-Amaro (1960) and Ferreira (1980), to our knowledge the only report available on *Echinococcus* in humans in Mozambique is from Noormahomed et al. (2014) who conducted a serological study in HIV + patients. Hence, there is paucity of information on the *Echinococcus* genotypes circulating in Mozambique, and the role of domestic animals in the transmission cycles of the parasite in humans. In southern Africa, hydatid cysts isolated from wildlife were characterized using molecular techniques in Namibia by Wassermann et al. (2015) and in South Africa by Halajian et al. (2017).

The present study reports on the prevalence and molecular characterization of hydatid cysts collected from cattle and pigs slaughtered in slaughterhouses and slabs in southern of Mozambique.

Materials and methods

Study area

The study was conducted between August 2017 and July 2019 in selected districts of Maputo, Gaza and Inhambane in southern Mozambique. Livestock production in these districts follows a traditional system and plays an important role in the livelihood of communities (Hendrickx et al. 2015). Cattle and goats are the main source of wealth and livelihood for the communities.

Cattle, goats and pigs carcasses were inspected for hydatid cysts at selected slaughterhouses with special attention to internal organs such as the lungs, liver, spleen, heart and kidney. Cattle from districts Chockwé, Chibuto, Mapai and Guijá of Gaza province and from districts of Mabote and Homuíne of Inhambane province were slaughtered in the Municipal slaughterhouse of Maputo and cattle from district of Magude of Maputo province in two slaughterhouses located in Magude district. Sampling was conducted according to the information on the availability of animals for slaughter provided by the local inspector and all carcasses of slaughtered animals on day of visit were inspected. Pigs inspected for the presence of cysts in this study originated from slabs in market village in district of Mabote, in localities of Ramiro and Chicorroa in Zavala district in the province of Inhambane; from market village of Mapai district, from Samora Machel market and two residences in the district of Chibuto in the Gaza province. Goats inspected at the municipal slaughterhouse of Maputo were from Mapai and Magude districts, and cattle from Chockwé, Guijá, Mapai, Mabote, Homuine and Zavala districts (Fig. 1).

In positive cases, cysts were carefully dissected from the affected organ, placed into plastic containers and labelled with the following information; name of slaughterhouse and animal origin, date of collection, animal species, identification number and location of cyst(s). Samples were immediately transported to the Parasitology laboratory at the Faculty of Veterinary Medicine, Eduardo Mondlane University for further processing.

Microscopic analysis

In the laboratory, a small amount of the cystic fluid was aspirated using a sterile syringe, placed in a microscope slide and observed under light microscope for the presence of protoscoleces. An incision was made on the cyst wall and isolated the cyst membrane using a scalpel blade (Fig. 2a, b). The remainder of the cyst fluid was transferred to a Falcon tube (15 ml) and centrifuged at 180 g for 10 min. The pellet was preserved together with the cyst

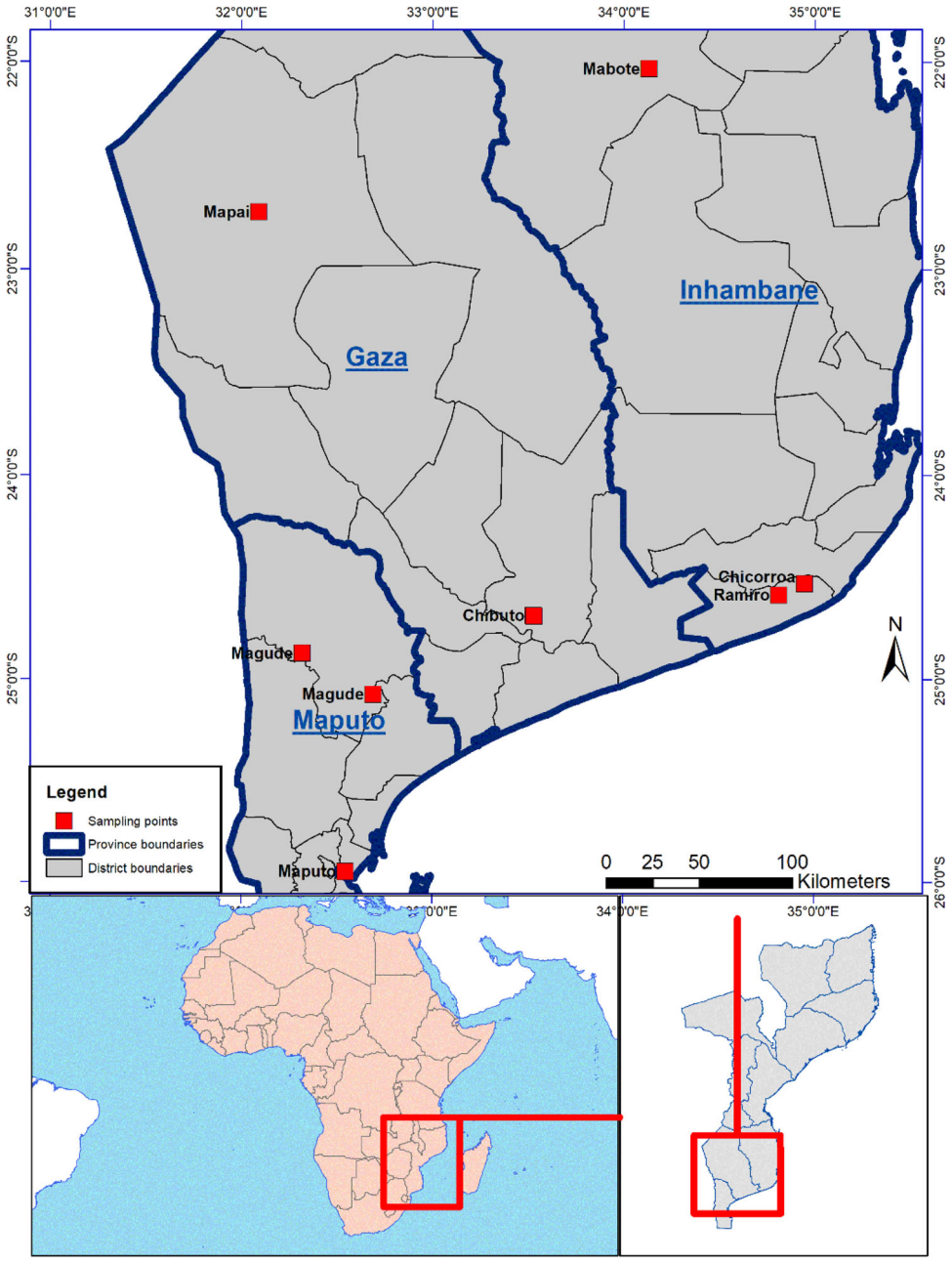

Fig. 1 Location of slaughter houses/slabs in Provinces of Maputo, Gaza and Inhambane in southern Mozambique

membrane in absolute ethanol for molecular analysis. Cyst viability was verified by observation of flame cells activity, and by eosin (0.1%) staining of protoscoleces as described by Smyth and Barrett (1980). Stained protoscoleces and calcified cysts were considered as non-viable and unstained as fertile or viable (Fig. 2c).

Molecular analysis

DNA was extracted from 5 µl of protoscoleces together with a small piece of cyst membrane tissue or from the membrane tissue only in case of absence of fluid using a commercial kit (Genomic DNA™ Tissue MiniPrep Kit, ZYMO Research Corporation USA) following the manufacturer instructions with a modification of the initial incubation time to 6 h. The subunit 1 of the cytochrome c oxidase (*cox1*) and the subunit 1 of NADH dehydrogenase (*nad1*) genes were amplified using the primers JB3: 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′, JB4: 5′-TAAA-GAAAGAACATAATGAAAATG-3′ for *cox1* and JB11: 5′-AGATTCGTAAGGGGCCTAATA-3′ and JB12: 5′-ACCACTAACTAATTCACTTTC-3′ for *nad1* (Bowles et al. 1992; Bowles and McManus 1993), following cycling conditions described by Gasser et al. (1999) with an

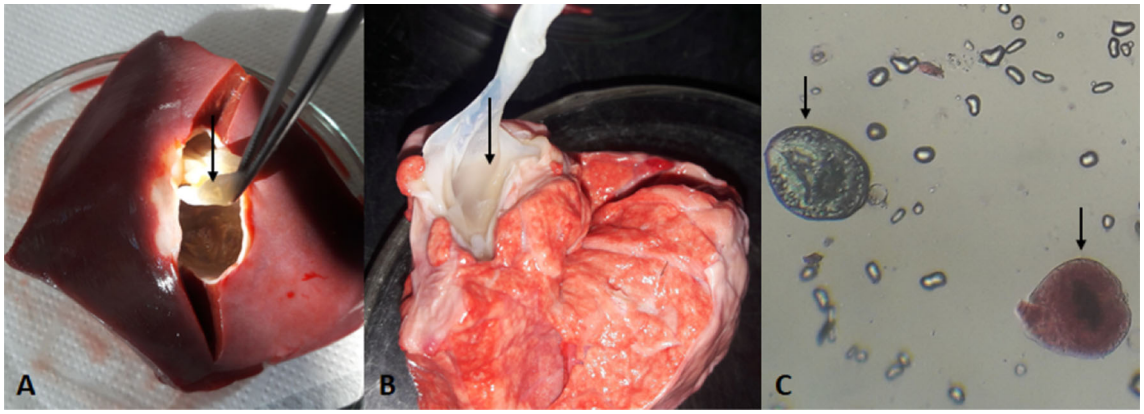

Fig. 2 Hydatid cyst as pointed by arrow in liver (**a**), lung (**b**) and viable and non-viable protoscolices (**c**) (stained in red-non viable and unstained-viable) (X10)

adjustment of the initial elongation time: initial denaturation of 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 1 min and finally an elongation at 72 °C for 5 min. The amplification was performed in 25 µl reaction volume composed by 1 µl of forward and reverse primers (10 µM), 7.5 µl of dH_2O, 12.5 µl of Taq master mix (Thermo Fisher Scientific, USA) and 3 µl of DNA. Visualization of PCR products were made in agarose gel 1.5% stained with ethidium bromide.

Amplicons of fifteen samples for *cox1* and thirteen for *nad1* were sent to Inqaba Biotechnical Industries Ltd. in Pretoria, South Africa, for sequencing in one direction using the forward primer. The obtained sequences were assembled, manually edited and aligned with homologue sequences from the GenBank database with Clustal W (Thompson et al. 1997) using the BioEdit program (Hall 1999). Sequences were trimmed to a common length of 212 nucleotides for *cox1* and 480 nucleotides for *nad1* regions. jModeltest (Posada 2008) was used to select the best nucleotide substitution model for neighbor-joining (NJ), Maximum likelihood (ML) and Bayesian Inference analyses. The GTR + I and HKY + 1models were selected for *cox1* and *nad1* under the AIC information criterion, respectively (Hasegawa et al. 1985). The Neighbour-joining and maximum likelihood trees were generated using PAUP* 4.0 (Swofford 2002) and the nodal support for both methods were estimated using the 1000 booststrap pseudo-replicates. The Bayesian analysis was executed in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The four Markov chains were run for 5 million generations to that the standard deviation of the split frequencies was less than 0.01. The first 500,000 trees were discarded as burnin. The phylograms were generated using the 50% majority-rule consensus trees, with the nodal support values indicated as posterior probabilities.

Results

Prevalence of hydatid cyst in livestock

From 817 and 68 carcasses of cattle and pig inspected for hydatid cysts, 3.9% (32/817) and 2.9% (2/68) respectively, were found positive for hydatid cyst and none of the goat carcasses (233) was positive (Table 1). In cattle high prevalence value compared to other districts (4.2%) was observed in districts of Chibuto and Magude. In pigs cysts were only from the Chibuto district of Gaza province, and all were collected from lungs and classified as fertile. In cattle the lung (29/32) was the most affected organ (90.6%) compared to 9.4% in liver (3/32) and 93.1% (27/29) of cysts collected in lungs were classified as fertile and two (2/29) as calcified (6.9%) as shown in Tables 2 and 3.

Molecular characterization of *Echinococcus granulosus* sensu lato

Sequence analysis showed that the sequences of the isolates from cattle and pigs were identical. BLAST analysis of 15 samples (two from pigs and 13 from cattle) identified the cysts isolates as *E. ortleppi* based on the *cox1* gene. These isolates showed a homology of 99% to previously published *E. ortleppi* sequences from Zambia (KU743915.1), Namibia (KX138068.1), Brazil (KT337323.1) and Austria (MG976769.1). These sequences were deposited in GenBank under the accession numbers MZ220587-MZ220601. Phylogenetic analysis showed that the in-group formed a monophyletic clade (A) with the outgroup (Fig. 3). This clade showed the relationship between *E. granulosus* s.s., *E. ortleppi* and *E. canadensis*. Analyses showed that *E. granulosus* s.s. is more distantly related to other *Echinococcus* species. Although this species formed a strong supported clade (clade C) by neighbor-joining and Bayesian inference with *E. canadensis* and

Table 1 Prevalence of *Echinococcus* metacestodes in carcasses of slaughtered cattle and pigs in selected districts of southern Mozambique

Province	District	Cattle		Pigs	
		N	P (%)	N	P (%)
Gaza	Chockwé	60	1 (1.6)	–	–
	Chibuto	189	8 (4.2)	15	2 (13.3)
	Mapai	369	13 (3.5)	9	0
	Guijá	24	1 (4.1)	–	–
	Subtotal	642	23 (3.6)	24	2(8.3)
Inhambane	Mabote	150	5 (3.3)	6	0
	Homuíne	25	1 (4)	–	–
	Zavala	–	–	38	0
	Subtotal	175	6 (3.4)	44	0
Maputo	Magude	72	3 (4.2)	–	–
	Subtotal	72	3 (4.2)	–	–
	Total	817	32 (3.9)	68	2 (2.9)

N number of inspected animals, *P* Number positive

Table 2 Distribution of *Echinococcus* metacestodes by organ in slaughtered cattle and pigs in southern Mozambique

Province	District	Animal species			
		Cattle		Pig	
		Lungs (NI = 29)	Liver (NI = 3)	Lungs (NI = 2)	Liver
Gaza	Chockwé	1	0	–	–
	Chibuto	8	0	2	0
	Mapai	11	2	0	0
	Guijá	1	0	–	–
Inhambane	Mabote	5	0	0	0
	Homuíne	1		–	–
	Zavala	–	–	0	0
Maputo	Magude	2	1	–	–
Total		29	3	2	0

NI number of animals with infected organ

Table 3 Classification of hydatid cysts collected from cattle and pigs in southern Mozambique

Animals	Infected organs examined	Cysts examined	Non-viable N	Calcified N	Fertile N
Cattle	Lungs	29	0	2 (6.9)	27 (93.1)
	Liver	3	1 (33.3)	0	2 (66.6)
Pigs	Lungs	2	0	0	2 (100)
	Liver	0	0	0	0

N number of examined cysts

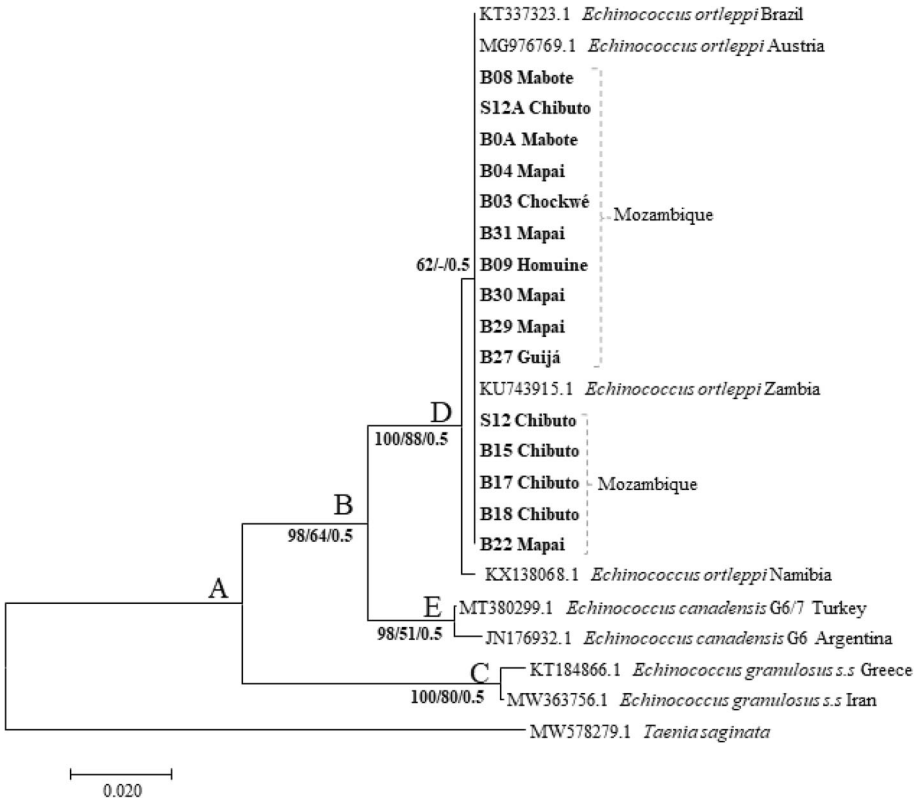

Fig. 3 Neighbor-joining tree based on 220 nucleotide sequences of the *cox1* region, illustrating the relationship between the samples collected from different districts of southern Mozambique, and the GenBank derived sequences. The nodal support values shown in the order of Neighbor-joining, Maximum likelihood and Bayesian inference

E. ortleppi, *E. granulosus* s.s. showed a genetic distance of 9% from *E. canadensis*, and 10% from *E. ortleppi*. The results showed that isolates from this study formed a well-supported clade with *E. ortleppi* sequences from GenBank database, which formed a monophyletic sister clade (clade B) to the *E. canadensis* isolates from GenBank. This close relationship was further confirmed by a 4% genetic distance between the two species.

Analysis of the *nad1* region corresponded with that of the *cox1* region. For this gene, PCR failed on two samples and they were excluded from the analysis. BLAST analysis also confirmed the identification of the 13 isolates from this study as *E. ortleppi*, with a homology of 99% to sequences from China (MN058592.1), Sudan (KU842045.1), Kenya (KX010904.1) and Ethiopia (KU842044.1) and are deposited into the GenBank database under the accession numbers MZ254630–MZ254642. Phylogenetic analysis of the *nad1* sequences produced a phylogenetic tree which was congruent to that of the *cox1*. The tree confirmed the identification of our isolates as *E. ortleppi*, and further showed similar relationship between the *Echinococcus* species as observed with the *cox1* region. Each *Echinococcus* species formed its own strongly supported clade, clade C representing *E. granulosus* s.s. whilst clades D and E represents *E. ortleppi* and *E. canadensis* respectively. Within the *E. ortleppi* clade, the phylogenetic tree showed that isolates from this study more related to the sequence from China (MN058592.1), and these isolates formed a strongly supported monophyletic sister clade to isolates from Sudan (KU842045.1), Kenya (KX010904.1) and Ethiopia (KU842044.1) (Fig. 4).

Discussion

Results from this study confirmed that 15 out of 34 hydatid cysts investigated in cattle and pigs in southern Mozambique were *E. ortleppi* (G5). The prevalence of 3.9% in cattle was low, but within the range of 2.1% and 4.2% reported in Zambia and Tanzania (Banda et al. 2013; Nonga and Karimuribo 2009; Komba et al. 2012 and Banda et al. 2020). High prevalence rates between 13.2 and 48.7% have also been reported in Tanzania (Ernest et al. 2009; Mellau et al. 2010). In cattle, the parasite was reported in all provinces of the southern region of Mozambique with values ranging from 1.6 to 4.2%; unlike in pigs, where the parasite seems to be confined only in the district of Chibuto in Gaza province. Dogs are the main hosts for the parasite and according to a study of Miambo et al. (2016) in Magude district, dogs are mainly kept by local farmers for hunting and shepherding of livestock which results in contamination of pasture areas with parasite eggs.

There is paucity of reports on the prevalence of hydatid cysts in pigs in Southern African countries and the

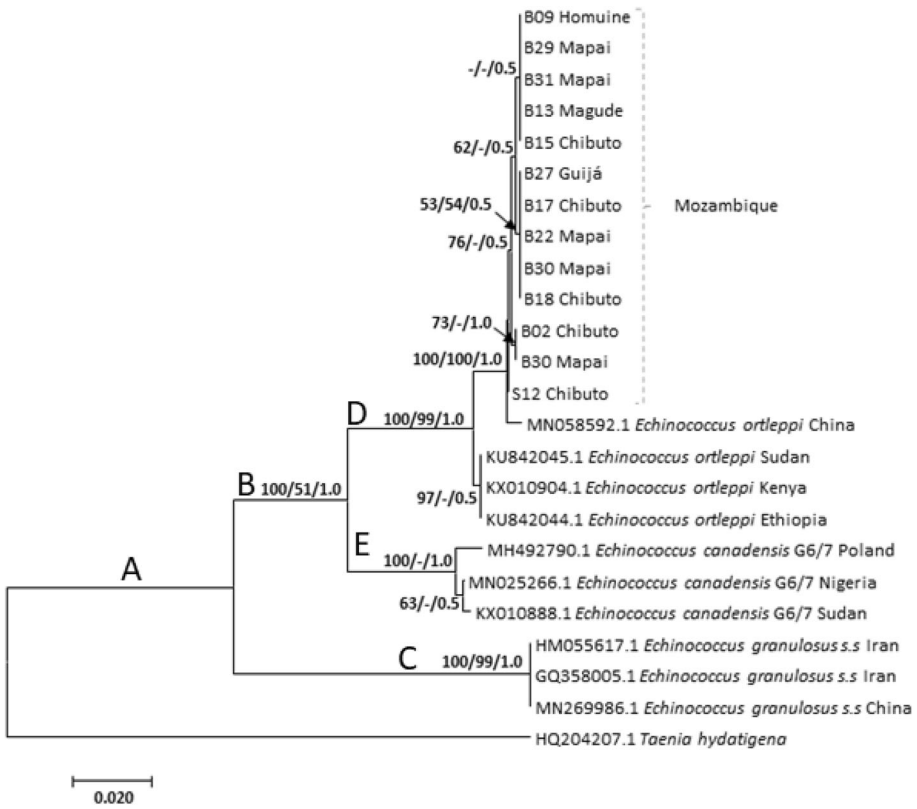

Fig. 4 Neighbor-joining tree based on 485 nucleotide sequences of the *nad1* region, showing the relationship between the samples collected from different districts of southern Mozambique, and the GenBank derived sequences. The nodal support values shown in the order of Neighbor-joining, Maximum likelihood and Bayesian inference

prevalence of 2.9% found in pigs in this study is relatively high compared to the percentage of 0.36% previously reported in Mozambique (de Castro-Amaro 1960), 0.4% in Tanzania (Mkupasi et al. 2011) and lower than the value of 4.3% reported in Tanzania (Ngowi et al. 2004). The number of districts in southern Mozambique, including Chibuto and Zavala are known to regularly consume pork meat produced by local small-scale farmers, however, African swine fever in the country still remain a major constraint on production (Penrith et al. 2007). Nonetheless, research emphasis has been given to cysticercosis caused by *Taenia solium* which is endemic in Tete province (Pondja et al. 2010, 2015; Chilundo et al. 2017, 2018) and incriminated as the main cause of economic loss in small-scale pig farming (Trevisan et al. 2018) and seizures due to neurocysticercosis in the affected population (Afonso et al. 2011; Assane et al. 2017; Saldanha et al. 2018). The asymptomatic course of echinococcosis in dogs and livestock and the chronic nature and slow progression of disease in humans may be some of the reasons of limited studies and lack of prioritization. Lungs were the most affected organs compared to liver in all districts and similar findings were reported by Ernest et al. (2009), Banda et al. (2012) and Banda et al. (2020). Fertility levels of cysts in cattle (93.1%) and pigs (100%) may be an indicator of the risk and importance of this animal species in the transmission of the parasite to dogs. In the municipal slaughterhouse of Maputo, according to the local inspector, lungs were sold to feed dogs and this can be an indicator of what is occurring in other slaughterhouses or slabs.

Cystic echinococcosis is regarded endemic in sub-Saharan Africa; however, most countries have limited to no information on this parasitic disease (Wahlers et al. 2012). The lack of taxonomically useful morphological characters of the adult worm and the great plasticity of the cystic larvae in the intermediate and aberrant hosts has resulted in the taxonomy of *Echinococcus* controversial (Thompson and McManus 2002; Knapp et al. 2011). The members of this genus exhibit similar phenotypic traits, which supports the idea that *Echinococcus* is a monophyletic entity (Knapp et al. 2011). Available evidence based on the DNA analysis of the mitochondrial genes showed that *E. granulosus* is a complex of species and genotypes exhibiting a marked genetic variability (Cardona and Carmon 2013). According to Wahlers et al. (2012), this complex of species is prevalent in sub-Saharan Africa and the genotypes may be associated with varying virulence and host preference.

Analysis of our sequences identified fifteen isolates for *cox1* and thirteen for *nad1* as *E. ortleppi*, also known as genotype G5 affecting both pigs and cattle from southern Mozambique. This species has been previously reported in neighboring countries such as South Africa Mogoye et al. 2013).

The phylogenetic analysis of our isolates showed the species (*E. ortleppi* along with our isolates, *E. canadensis* and *E. granulosus*) as a paraphyletic entity, based on the *cox1* gene. This observation corresponded to that reported by Knapp et al. (2011), however, differs from the monophyletic entity reported by Saarma et al. (2009). The analysis also showed that *E. granulosus* isolates formed a monophyletic sister clade to *E. ortleppi* and *E. canadensis*. This is not surprising since the basic taxonomy of *Echinococcus* genus based on the mitochondrial gene reported *E. ortleppi* (G5), *E. granulosus* sensu stricto (G1 + G3) and *E. canadensis* (G6/G7, G8, G10) as part of the nine genotypes of *E. granulosus* s.l. and that the genotypes G6–G7 and G8–G10, which represents *E. canadensis* form their own clade, representing a distinct species (Wahlers et al. 2012).

This is the first study reporting on the presence of *E. ortleppi* in cattle and pigs from southern Mozambique confirmed through genetic studies of the cysts. This is significant taking into account that *E. ortleppi* has been reported to infect humans (Romig et al. 2015; Dybicz et al. 2019). From our study, we conclude that resource-poor rural farming communities of southern Mozambique are at risk of being exposed to *E. ortleppi* (G5) infection and we recommend future studies aimed to investigate the potential reservoirs of the parasite and the risk factors related to the transmission in order to raise awareness to communities at risk.

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Authors' contribution RDM, SMSA and SM conceived and designed the experiments. RDM collected the samples from the field. RDM, SMSA, MPM and SM performed the experiments. RDM and MPM analysed the data. RDM wrote the article. RDM, SMSA, EVN, MPM and SM read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no competing interest.

Ethics statement This research has been approved by the Research Council of the Veterinary Faculty, Eduardo Mondlane University, Maputo, Mozambique and have therefore been performed in accordance with the ethical standards.

References

Afonso SMS, Vaz Y, Neves L, Pondja A, Dias G, Willingham AL III, Vilhena M, Duarte PC, Jost CC, Noormahomed EV (2011) Human and porcine *Taenia solium* infections in Mozambique: identifying research priorities. Anim Health Res Rev 12:123

Ammann RW, Eckert J (1996) Cestodes: *Echinococcus*. Gastroenterol Clin 25:655–689

Assane YA, Trevisan C, Schutte CM, Noormahomed EV, Johansen MV, Magnussen P (2017) Neurocysticercosis in a rural population with extensive pig production in Angónia district, Tete Province, Mozambique. Acta Trop 165:155–160

Banda F, Nalubamba KS, Muma JB, Munyeme M, Munang'andu HM (2012) A cross-sectional study investigating cystic hydatidosis in slaughtered Cattle of Western Province in Zambia. ISRN Parasit 2013:468163

Banda F, Nalubamba KS, Muma JB, Munyeme M, Munang'andu HM (2013) A cross-sectional study investigating cystic hydatidosis in slaughtered Cattle of Western Province in Zambia. Int Sch Res Notices 2013:1–9

Banda F, Sikasunge C, Francis A, Wassermann M, Mackenstedt U, Peter K, Daugschies A, Mulinge E, Romig T (2020) *Echinococcus ortleppi* and *Echinococcus canadensis* G6/7 affect domestic animals in western Zambia. Acta Trop 211:105648

Bourée P (2001) Hydatidosis: dynamics of transmission. World J Surg 25:4–9

Bowles J, McManus DP (1993) NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. Int J Parasitol 23:969–972

Bowles J, Blair D, McManus DP (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol Biochem Parasitol 54:165–173

Cardona GA, Carmena D (2013) A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. Vet Parasitol 192:10–32

Chilundo AG, Mukaratirwa S, Pondja A, Afonso S, Miambo R, Johansen MV (2017) Prevalence and risk factors of endo-and ectoparasitic infections in smallholder pigs in Angónia district, Mozambique. Vet Parasitol Reg Stud Rep 7:1–8

Chilundo AG, Johansen MV, Pondja A, Miambo R, Afonso S, Mukaratirwa S (2018) Piloting the effectiveness of pig health education in combination with oxfendazole treatment on prevention and/or control of porcine cysticercosis, gastrointestinal parasites, African swine fever and ectoparasites in Angónia District, Mozambique. Trop Anim Health Prod 50:589–601

de Castro-Amaro E (1960) Contribution à l'etude de l'hydatidose au Mozambique. Bull off Int Epizoot 54:488–491

Deplazes P, Rinaldi L, Rojas CA, Torgerson PR, Harandi MF, Romig T, Antolova D, Schurer JM, Lahmar S, Cringoli G, Magambo J (2017) Global distribution of alveolar and cystic echinococcosis. Adv Parasitol 95:315–493

Dybicz M, Borkowski PK, Jonas M, Wasiak D, Małkowski P (2019) First report of *Echinococcus ortleppi* in human cases of cystic echinococcosis in Poland. BioMed Res Int 2019:1–15

Eckert J, Deplazes P (2004) Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev 17:107–135

Ernest E, Nonga HE, Kassuku AA, Kazwala RR (2009) Hydatidosis of slaughtered animals in Ngorongoro district of Arusha region, Tanzania. Trop Anim Health Prod 41:1179

Ferreira MLEP (1980) Occurrence of the multivesicular form of hydatidosis in cattle in mozambique. Anim Health Prod 28:254–258

Gasser RB, Zhu X, McManus DP (1999) NADH dehydrogenase subunit 1 and cytochrome c oxidase subunit I sequences

compared for members of the genus *Taenia* (Cestoda). Int J Parasitol 29:1965–1970

Halajian A, Luus-Powell WJ, Roux F, Nakao M, Sasaki M, Lavikainen A (2017) *Echinococcus felidis* in hippopotamus, South Africa. Vet Parasitol 243:24–28

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98

Hasegawa M, Kishino H, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174

Hendrickx SCJ, Maute FR, Cunhete D (2015) Caracterização dos sistemas de produção e comercialização das carnes vermelhas no sector familiar nos corredores de Maputo e Limpopo em Moçambique: Resultados do Estudo de Base. ILRI Nota Informative De Investigação 42:1–5

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. J Bioinform 17:754–755

Hüttner M, Nakao M, WassermannT SL, Boomker JDF, Dinkel A, Sako Y, Mackenstedt U, Romig T, Ito A (2008) Genetic characterization and phylogenetic position of *Echinococcus felidis* (Cestoda: Taeniidae) from the African lion. Int J Parasitol 38:861–868

Knapp J, Nakao M, Yanagida T, Okamoto M, Saarma U, Lavikainen A, Ito A (2011) Phylogenetic relationships within *Echinococcus* and *Taenia* tapeworms (Cestoda: Taeniidae): an inference from nuclear protein coding genes. Mol Phylogenet Evol 61:628–638

Komba EVG, Komba EV, Mkupasi EM, Mbyuzi AO, Mshamu S, Mzula A and Luwumba D (2012) Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: implications for public health. Tanzan J Health Res 14(2):131–138

Krecek RC, Boomker J, Penzhorn BL, Scheepers L (1990) Internal parasites of giraffes (*Giraffa camelopardalis angolensis*) from Etosha National Park, Namibia. J Wildl Dis 26:395–397

Laurimäe T, Kinkar L, Moks E, Romig T, Omer RA, Casulli A, Umhang G, Bagrade G, Irshadullah M, Sharbatkhori M, Mirhendi H (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. J Parasitol 145:1929–1937

Macin S, Orsten S, Samadzade R, Colak B, Cebeci H, Fındık D (2021) Human and animal cystic echinococcosis in Konya, Turkey: molecular identification and the first report of E. equinus from human host in Turkey. Parasitol Res 120:563–568

Manterola C, Otzen T (2016) Epidemiología Molecular de la Equinococosis Quística: Caracterización Genotípica en Humanos y Diferentes Animales. Int J Morphol 34:1472–1481

McManus DP, Zhang W, Li J, Bartley PB (2003) Echinococcosis. The Lancet 362:1295–1304

Mellau LSB, Nonga HE, Karimuribo ED (2010) A slaughterhouse survey of liver lesions in slaughtered cattle, sheep and goats at Arusha, Tanzania. Res J Vet Sci 3:179–188

Miambo RD (2016) Prevalence of gastro-intestinal parasites of livestock and dogs and risk factors for transmission with emphasis on giardia and cryptosporidium in Magude district, Maputo province, Mozambique. M. Sc. dissertation, University of Kwazulu-Natal, Durban

Mkupasi EM, Ngowi HA, Nonga HE (2011) Prevalence of extra-intestinal porcine helminth infections and assessment of sanitary conditions of pig slaughter slabs in Dares Salaam city, Tanzania. Trop Anim Health Prod 43:417–423

Mogoye BK, Menezes CN, Wong ML, Stacey S, Delft DV, Wahlers K, Wassermann M, Romig T, Kern P, Grobusch MP (2013) First insights into species and genotypes of *Echinococcus* in South Africa. Vet Parasitol 196:427–432

Nakao M, Lavikainen A, Yanagida T, Ito A (2013) Phylogenetic systematics of the genus *Echinococcus* (Cestoda: Taeniidae). Int J Parasitol 43:1017–1029

Ngowi HA, Kassuku AA, Maeda GEM, Boa ME, Willingham AL (2004) A slaughter slab survey for extra-intestinal porcine helminth infections in northern, Tanzania. Trop Anim Health Prod 36:335–340

Nhancupe N, Noormahomed EV, Afonso S, Svard S, Lindh J (2019) Further evaluation of recombinant Tsol-p27 by enzyme-linked immunoelectrotransfer blot for the serodiagnosis of cysticercosis in pigs from Mozambique. Parasit Vectors 12(1):1–4

Nonaka N, Nakamura S, Inoue T, Oku Y, Katakura K, Matsumoto J, Mathis A, Chembesofu M, Phiri IGK (2011) Coprological survey of alimentary tract parasites in dogs from Zambia and evaluation of a copro-antigen assay for canine echinococcosis. Ann Trop Med Parasit 105:521–531

Nonga HE, Karimuribo ED (2009) A retrospective survey of hydatidosis in livestock in Arusha, Tanzania, based on abattoir data during 2005–2007. Trop Anim Health Prod 41:1253

Noormahomed EV, Nhacupe N, Mascaro-Lazcano C, Mauaie MN, Buene T, Funzamo CA, Benson CA (2014) A cross-sectional serological study of cysticercosis, schistosomiasis, toxocariasis and echinococcosis in HIV-1 infected people in Beira, Mozambique. PLoS Negl Trop Dis 8:e3121

Ortlepp RJ (1934) *Echinococcus* in dogs from Pretoria and vicinity. Onderstepoort J Vet Sci Anim Ind 3:97–108

Penrith ML, Pereira CL, Da Silva ML, Quembo C, Nhamusso A, Banze J (2007) African swine fever in Mozambique: review, risk factors and considerations for control. Onderstepoort J Vet Res 74:149–160

Pondja A, Neves L, Mlangwa J, Afonso S, Fafetine J, Willingham AL III, Thamsborg SM, Johansen MV (2010) Prevalence and risk factors of porcine cysticercosis in Angonia District, Mozambique. PLoS Negl Trop Dis 4:e594

Pondja A, Neves L, Mlangwa J, Afonso S, Fafetine J, Willingham AL III, Thamsborg SM, Johansen MV (2015) Incidence of porcine cysticercosis in Angónia District, Mozambique. Prev Vet Med 118:493–497

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Possenti A, Manzano-Román R, Sánchez-Ovejero C, Boufana B, La Torre G, Siles-Lucas M, Casulli A (2016) Potential risk factors associated with human cystic echinococcosis: systematic review and meta-analysis. PLoS Negl Trop Dis 10:e0005114

Quembo CJ, Jori F, Vosloo W, Heath L (2018) Genetic characterization of African swine fever virus isolates from soft ticks at the wildlife/domestic interface in Mozambique and identification of a novel genotype. Transbound Emerg Dis 65:420–431

Rocha A, Starkey P, Dionisio AC (1991) Cattle production and utilization in smallholder farming systems in southern Mozambique. Agric Syst 37:55–75

Romig T, Ebi D, Wassermann M (2015) Taxonomy and molecular epidemiology of *Echinococcus granulosus* sensu lato. Vet Parasitol 213:76–84

Romig T, Deplazes P, Jenkins D, Giraudoux P, Massolo A, Craig PS, Wassermann M, Takahashi K, Rue MDL (2017) Ecology and life cycle patterns of *Echinococcus* species. Adv Parasitol 95:213–314

Saarma U, Jõgisalu I, Moks E, Varcasia A, Lavikainen A, Oksanen A, Simsek S, Andresiuk V, Denegri G, González LM, Ferrer E (2009) A novel phylogeny for the genus *Echinococcus*, based on nuclear data, challenges relationships based on mitochondrial evidence. J Parasitol 136:317

Saldanha V, Saldanha G, Reys RP, Benson CA, Noormahomed EV (2018) Neurocysticercosis in child bearing women: an overlooked condition in Mozambique and a potentially missed

diagnosis in women presenting with Eclampsia. EC Microbiol 14:736
Smyth JD, Barrett NJ (1980) Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. Trans R Soc Trop Med Hyg 74:649–652
Swofford DL (2002) Paup*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sunderland, MA: Sinauer Associates
Thompson RC (2020) The molecular epidemiology of Echinococcus infections. Pathogens 9:453
Thompson RA, McManus DP (2002) Towards a taxonomic revision of the genus *Echinococcus*. Trends Parasitol 18:452–457
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 25:4876–4882
Timberlake JR, Reddy SJ (1986) Potential pasture productivity and livestock carrying capacity over Mozambique. Serie Terra e Água. Instituto Nacional de Investigação Agronomica, Maputo
Trevisan C, Devleesschauwer B, Praet N, Pondja A, Assane YA, Dorny P, Thamsborg SM, Magnussen P, Johansen MV (2018) Assessment of the societal cost of *Taenia solium* in Angónia district, Mozambique. BMC Infect Dis 18:127
Verster A (1961) Helminth research in South Africa V. *Echinococcus* in South Africa. J S Afr Vet Assoc 32:181–186
Verster AJM (1965) Review of *Echinococcus* species in South Africa. Onderstepoort J Vet Res 32:7–118
Wahlers K, Menezes C, Wong ML, Zeyhle E, Ahmed ME, Ocaido M, Stijnis C, Romig T, Kern P, Grobusch MP (2012) Cystic echinococcosis in Sub-Saharan Africa. Lancet Infect Dis 12:871–880
Wassermann M, Aschenborn O, Aschenborn J, Mackenstedt U, Romig T (2015) A sylvatic lifecycle of *Echinococcus equinus* in the Etosha National Park, Namibia. Int J Parasitol Parasites Wildl 4:97–103