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# *Echinococcus ortleppi* and *Echinococcus canadensis* G6/7 affect domestic animals in western Zambia

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ABSTRACT

Cystic echinococcosis (CE) is endemic in many parts of sub-Saharan Africa. In contrast to the eastern part of the continent, very little data exists on the current disease situation in southern Africa including Zambia. This study determined frequency and species identity of *Echinococcus* spp. circulating in livestock and dogs in the Western Province of Zambia. Cysts were collected in slaughterhouses at meat inspection (cattle) and during examination of home slaughtered pigs, while dog faecal samples were collected per-rectum and examined microscopically for the presence of taeniid eggs. Individual taeniid eggs from faecal samples and individual protoscoleces from cysts were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and/or sequencing of the NADH-dehydrogenase subunit 1 (*nad1*) and cytochrome C oxidase 1 (*cox1*) gene. Fifty-four of 2000 cattle (2.7%) were found infected with a total of 65 cysts, predominantly fertile lungs cysts; all cysts were identified as *Echinococcus ortleppi*. Two out of 52 home-slaughtered pigs (3.8%) were infected with a fertile lung cyst each; both cysts were also identified as *E. ortleppi*. Microscopic examination revealed 10/289 dog faecal samples to contain taeniid eggs, of which four samples (two each) contained *Echinococcus canadensis* (G6/7) or *Taenia hydatigena*, respectively. This is the first insight in the *Echinococcus* species circulating in Zambia providing premises for further studies into transmission dynamics of CE in the southern African region.

## 1. Introduction

Cystic echinococcosis (CE) is a zoonotic disease listed by WHO as a neglected tropical disease (NTD) (WHO 2013). It is caused by the metacestodes (cysts) of *Echinococcus granulosus* sensu lato (s.l.). In the domestic cycle, the principal intermediate hosts are livestock species such as sheep, cattle, pigs, camels, horses and donkeys, while the main definitive host is the domestic dog. Within the *E. granulosus* s. l. cluster, five distinct species are currently recognized: *E. granulosus* sensu stricto (s.s.) (G1/G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6-G10), and *E. felidis* (Nakao et al., 2007; Hüttner et al., 2008; Thompson 2008; Maillard et al., 2009; McManus 2013; Romig et al., 2017; Vuitton et al., 2020). Although human and animal CE is known to be widespread in Africa both north and south of the Sahara (Deplazes et al., 2017), the frequency and epidemiology of the various causative species are still poorly known. Molecular data are available from 12 countries in sub-Saharan Africa, but often consist of single records and case descriptions (reviewed in Deplazes et al., 2017). *Echinococcus granulosus* s.s. and *E. canadensis* G6/7, which are responsible for most human cases of CE worldwide (88.44% and 11.07%, respectively - Alvarez Rojas et al. 2014), are also by far the most frequent causes of human CE in Africa (Cardona and Carmena 2013), exceptions being one *E. ortleppi* infection in the Republic of South Africa (RSA) (Mogoye et al., 2013) and the case of an aberrant genotype related to *E. granulosus* s. s. recently discovered in Ethiopia (Wassermann et al., 2016).

A detailed description of the epidemiological situation in Africa is difficult to achieve, since the availability and quality of data on CE amongst the various countries are as diverse as the environmental conditions (Macpherson and Wachira 1997). While eastern Africa,

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particularly Sudan and Kenya, are comparatively well researched, only isolated records, often old and/or with dubious validity, are available from western, central and southern Africa (Romig et al., 2011). From the limited data, however, it is already clear that more CE intermediate hosts and more *E. granulosus* s. l. species have been reported in sub-Saharan Africa than from any other continent, owing to the existence of large numbers of wild ungulate species (Macpherson and Wachira 1997; Hüttner and Romig 2009). This poses a very complex epidemiological situation, because the various species of *E. granulosus* s. l. are known or suspected to differ in host specificity, including the potential to cause disease in humans. As *E. granulosus* s. s. is the most important agent of human CE (Alvarez Rojas et al. 2014), the apparently uneven geographical distribution of human CE in Africa may therefore be influenced by the presence or absence of certain *Echinococcus* species.

Except for large-scale livestock surveys in RSA in the 1960s that indicated CE to be widespread, little data exist on CE from the southern part of Africa. However, recent studies, mainly based on opportunistic sampling, showed the presence of *E. granulosus* s. s., *E. equinus, E. ortleppi, E. canadensis* and *E. felidis,* indicating that these regions may have the highest genetic diversity of CE in Africa (Deplazes et al., 2017). Here, we present a molecular survey of livestock and dogs for frequency and identity of *Echinococcus* spp. in western Zambia, being the first study of this kind in southern Africa

## 2. Materials and methods

### 2.1. Study area

*Echinococcus* cysts from livestock and dog faecal samples were collected in Western Province of Zambia from March 2014 to November 2017. Western Province lies between longitudes 22° and 25° East and latitude 13° 30` and 17° 45` South. The province covers an area of 126,386 km$^2$, which represents about 17% of the total land surface of Zambia (Fig. 1). About 10% (12,950 km$^2$) of the total land area of the province consists of a vast sandy upland. The province has a dry and cold winter (April to July), hot and dry season (August to October), and hot and wet summer (November to March). The annual flooding of the Zambezi plains controls the pattern of life for the people and livestock in Western Province with people practising transhumant subsistence livelihood. According to the 2017/2018 Livestock and Aquaculture Census, Western Province of Zambia was host to 450,949 cattle, 69,062 goats, 310 sheep, 92,635 pigs, and 116,469 dogs. The animal husbandry practices are centred on traditional livestock rearing involving free range and communal grazing for all livestock including pigs. Dogs are often used in herding of livestock and guarding them at night.

Cattle are rarely slaughtered at home with the preferred mode of slaughter at abattoirs in Mongu and Senanga towns for commercial purposes. However it is general practice that carcasses of cattle and other livestock that die from various causes are consumed by local people, and the preferred method of offal disposal is feeding of dogs. Pigs and goats are mostly slaughtered unsupervised at home for consumption. There is no formal organised slaughter for goats and pigs in Western Province. Some traders buy pigs and goats and transport them to the capital city Lusaka and other towns in the Copper-belt Province.

Mongu is the capital of the Western Province of Zambia where most of the cattle from the other districts in the province are slaughtered in two major abattoirs. Nkeyema district is immediately adjacent to the expansive Kafue National Park, which is home to a large variety of African wildlife. Dog faecal samples were collected from Mongu, Limulunga, Nkeyema and Kaoma to enable comparison between the wildlife interface and the purely domestic cycle areas in Kaoma-Nkeyema and Mongu-Limulunga areas, respectively (Fig. 1).

### 2.2. Cyst sampling

Prior to commencement of the study, meat inspectors at the two slaughterhouses in Mongu underwent an in-house refresher training in recognition of hydatid cysts in various organs of carcasses according to the procedures recommended by FAO/UNEP/WHO (1994). The slaughtered cattle were subjected to thorough post-mortem inspection and all organs with lesions were identified and samples were collected.

Two thousand cattle were examined in the two slaughterhouses in Mongu. For practical reasons, sampling was not truly at random: all cattle were examined that were slaughtered at randomly selected days/times. Due to the absence of an organised pig slaughter in Western Province, sample collection in pigs was opportunistic during home slaughter in various informal slaughtering sites in Mongu and Limulunga. Cyst contents were inspected macro- and microscopically for the presence of protoscoleces. Cysts with protoscoleces were considered fertile while cysts without protoscoleces and calcified cysts were considered non-fertile. Protoscoleces and pieces of cyst wall (germinal layer) of each cyst were stored in 70% ethanol at room temperature for molecular characterisation. Each cyst was fixed and stored as an isolate. Viability of the protoscoleces was examined under the microscope using the dye exclusion principle after staining with 0.1% eosin stain for 15 min.

### 2.3. Dog faecal sampling

Dog faecal sampling was done in two areas, Kaoma-Nkeyema and Mongu-Limulunga, with a targeted sample of 150 dogs per area. Samples were obtained per rectum during dog mass rabies vaccination campaigns organised by the District Veterinary Office with a contribution of materials by the research team. Permission from dog owners was obtained before sampling was undertaken. Faecal samples were individually labelled and preserved in 70% ethanol until use. Approximately 10 to 20 g faecal material was collected from dogs of different age and sex.

### 2.4. Cyst DNA extraction

DNA was extracted from a single protoscolex or piece of germinal layer by lysing in 0.02 M NaOH at 95 °C for 10 min as previously described by Nakao et al. (2003). In few instances where the above process failed to yield adequate DNA, genomic DNA was extracted as described by Dinkel et al. (2004): about 0.5 g cyst wall (germinal layer) was cut into small pieces and digested in the presence of 2 mg/ml proteinase K in 500 µl of 10 mM Tris–HCl (pH 7.5), 10 mM EDTA, 50 mM NaCl, 2% sodium dodecyl sulphate and 20 mM dithiothreitol. DNA was extracted using phenol–chloroform–isoamyl alcohol (25:24:1) with subsequent ethanol precipitation. After drying, the DNA was dissolved in 100 µl nuclease-free water.

### 2.5. Parasitological examination of dog faecal samples for taeniid eggs

About 2–3 g faecal material was processed using a zinc chloride flotation and sieving method (Mathis et al., 1996) and examined microscopically for the presence of taeniid eggs. In brief, the faecal samples were drained of ethanol, transferred to 15 ml falcon tubes and rinsed with 8–12 ml of distilled water. Four parts of zinc chloride (specific gravity = 1.45) were mixed with one part of the faecal pellet, and, after flotation, the top layer containing taeniid eggs was filtered through two sequential sieves of 50 µm and 22 µm (Franz Eckert GmbH, Germany) respectively, using distilled water. The second sieve (22 µm) was inverted to wash off retained taeniid eggs with distilled water via a funnel into a 15 ml falcon tube. After centrifugation, excess distilled water was removed and the pellet containing taeniid eggs was stored in 2 ml micro centrifuge tubes containing 70% ethanol. Individual taeniid eggs were isolated using a micro-pipette under the microscope (4x or 10x) and transferred into 0.2 ml thin wall PCR tubes containing 10 µl of 0.02 M NaOH, lysed at 99 °C for 10 min and used directly in the PCR (Nakao et al. 2003).

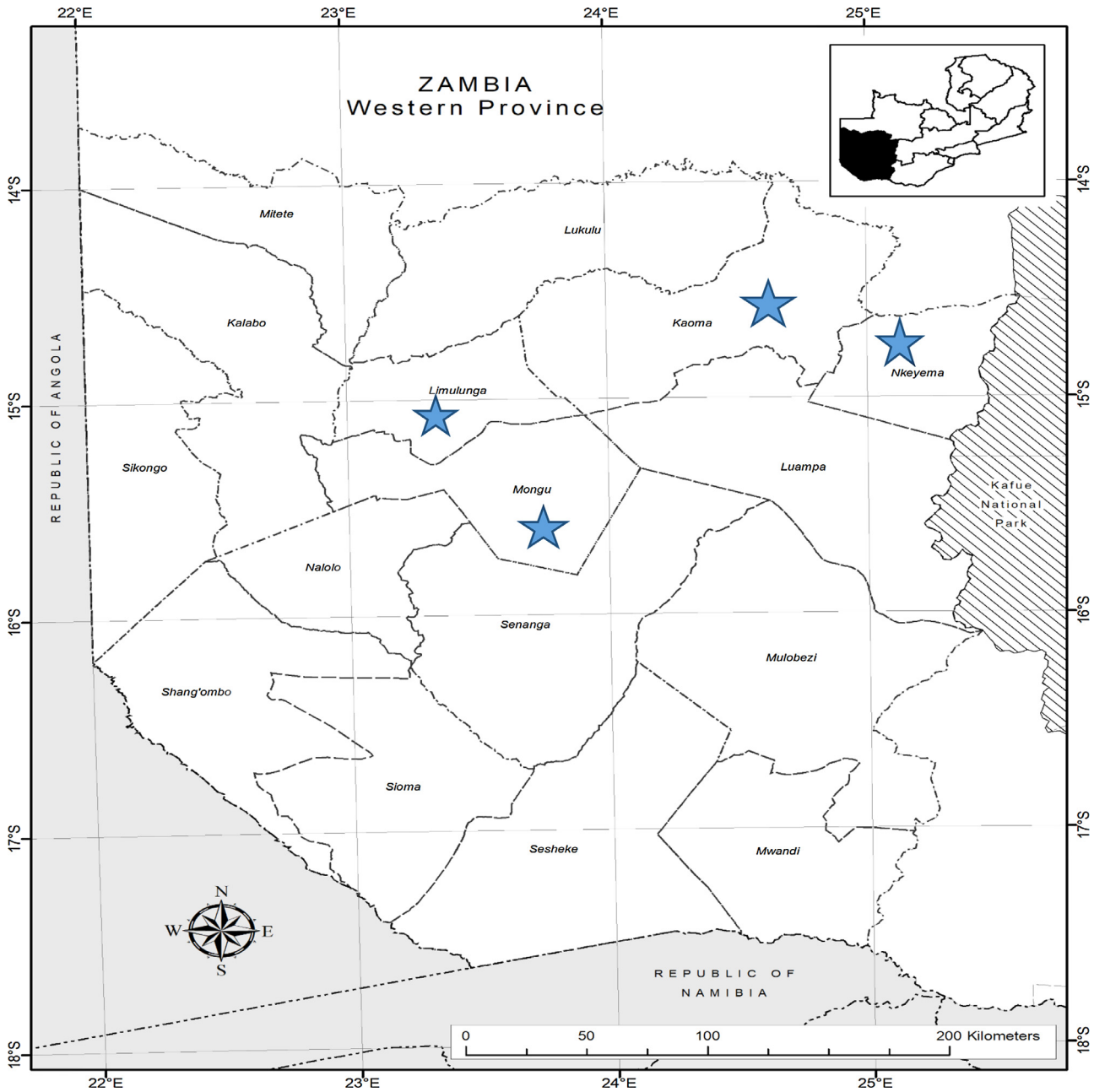


**Fig. 1.** Map of Western Province of Zambia (insert map of Zambia showing the location of Western Province). Study areas are marked by star symbols.

### *2.6. Polymerase chain reaction (PCR) for cyst and egg isolates*

DNA was amplified by nested PCR. The target of the PCR was a 1073–1078 bp long fragment including the complete *nad1* (NADH dehydrogenase subunit 1) gene. The primers used for the first amplification were forward 5′-TGG AAC TCA GTT TGA GCT TTA CTA-3′, reverse 5′-ATA TCA AAG TAA CCT GCT ATG CAG-3′ and for the second forward PCR 5′-TAT TAA AAA TAT TGA GTT TGC GTC-3′ and reverse 5′-TCT TGA AGT TAA CAG CAT CAC GAT-3′ as described previously by Hüttner et al. (2008). For the egg lysates another nested PCR targeting part of the *nad1* gene was performed using primers 5′ – TGT TTT TGA GAT CAG TTC GGT GTG – 3′ and 5′ – ATA TCA AAG TAA CCT GCT ATG CAG – 3′ in the primary PCR and 5′ – CAG TTC GGT GTG CTT TTG GGT CTG – 3′ and 5′ – TCT TGA AGT TAA CAG CAT CAC GAT – 3′ in the secondary resulting in a product of 545 – 552 bp (Hüttner et al., 2008). Briefly, in the first PCR, a 50 µl reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM of each dNTP, 12.5 pmol of each external primer, 1.25 U Ampli-Taq polymerase (Applied Biosystems) and 1 µl of the egg or larval lysate was constituted. Subsequent to the first PCR, a nested PCR was performed using 1 µl of the first amplification product as template. The second PCR reaction mixture was identical to the first, except for the primers. All thermal reactions were performed for 35 cycles of denaturation (94 °C for 30 s), annealing (55 °C for 30 s) and elongation (72 °C for 60 s) and post cycling extension at 72 °C for 5 min. Amplification results were detected on 1.5% (w/v) agarose gel stained with Gel Red® (Biotium, Inc.). All egg lysates with a negative PCR result were further investigated with a second PCR, targeting a 1019 bp long fragment of the cytochrome c oxidase 1 (*cox1*) gene. In contrast to the *nad1* primers, which are derived from the sequences of *Echinococcus*, the primers used for the *cox1* amplification are more specific for *Taenia* species. Primers for the first PCR were: forward 5′- ACT AAT ATA TTT TCT CGT ACT TC - 3′, reverse 5′- CAT GAT GCA AAA GGC AAA TAA ACC - 3′ and for the nested PCR, forward 5′- TTG ATC GTA AAT TTA GTT CTG - 3′ and

reverse 5′- CAT AGA TTT ACA AAA TC - 3′. The reaction and cycling conditions were identical to those used for *nad1* PCRs.

### 2.7. Restriction fragment length polymorphism (RFLP) of nad1 of cyst and egg isolates

The *nad1* amplicons obtained from cysts were digested as described previously (Hüttner et al. 2009) with the restriction enzyme *Hph*I (Fermentas GmbH, Germany). A total reaction mixture of 30.5 µl, constituted 15 µl PCR amplicons, 2 µl 10x buffer B (supplied with enzyme), 13.1 µl DNase/RNase-free water and 0.5 µl (5 U) enzyme. Reaction mixture was incubated at 37 °C for 4 h, followed by deactivation of the enzyme at 65 °C for 20 min. Banding patterns of the digest were visualised on 3% (w/v) agarose gel primed with HD Green™ DNA stain. PCR amplicons from the egg lysate were digested with *Hph*I enzyme according to Mulinge et al. (2018). Briefly, 10 µl of the nested PCR product, 0.5 µl (5 U) *Hph*I endonuclease (Thermo Scientific), 2 µl 10x buffer B and 7.5 µl of nuclease free water were added in a 20 µl final volume. Genotypes of samples were determined by comparing their banding patterns to defined patterns of known positive samples of *E. granulosus* s. s., *E. ortleppi, E. canadensis* G6/7 and *E. felidis*. PCR products that presented unclear banding patterns including one pig cyst isolate, and those of the *cox1* amplification, were purified using the High Pure PCR Product Purification Kit (Roche, Germany) following the manufacturer's protocol and sequenced at GATC Biotech AG, (Germany) using the nested reverse primer.

### 2.8. Data analysis

DNA sequences for cyst and taeniid eggs isolates were viewed and manually edited using GENtle v. 1.9.4 (http://gentle.magnusmanske.de). The sequences were compared with those on NCBI database using nucleotide BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/) to determine the species.

## 3. Results

### 3.1. Cysts in cattle and pigs

Out of 2000 examinded cattle, 54 animals contained a total of 65 cysts (prevalence estimate 2.7%). All cysts were collected and examined. A total of 64 out of 65 cattle cysts were fertile and contained viable protoscoleces; all except four cyst were located in the lungs. All the cattle cysts were identified by the *nad1* PCR-RFLP as *E. ortleppi* (Table 1). Two of 52 examined pigs contained one fertile lung cyst each (prevalence estimate 3.8%); both cysts also belonged to *E. ortleppi*. The *nad1* sequence of one cyst showed 99.8% identity with GenBank deposit AB235846 (Table 1 and Fig. 2) and is deposited under accession number KU842046 (Addy et al., 2017).

### 3.2. Taeniid eggs in dog faecal samples

Out of 289 dog faecal samples that were microscopically screened, ten samples were positive for taeniid eggs. Of these, only four contained eggs that gave amplification products which could be analysed by RFLP or sequencing, respectively. Twenty-five taeniid eggs from two samples were identified as *T. hydatigena*. The obtained sequences of the mitochondrial *cox1* gene showed 99% identity to previously published sequence of *T. hydatigena* (accession number FJ518620 (Liu et al., 2011)). The sequences are deposited in GenBank under accession numbers MN216148 - MN216153. The RFLP banding pattern of *E. canadensis* (G6/7) was found in six eggs from the other two samples. This result was confirmed by sequencing of the *nad1* gene. The sequences showed 99% identity with GenBank deposit AB208063 (Nakao et al., 2007), and are deposited under accession number MN 216,154 (Tables 2 and 3).

## 4. Discussion

The rather low prevalence of CE in cattle confirms the results of an earlier survey in the same study area, where 2.1% of 4061 cattle were found infected with unspecified *Echinococcus* cysts (Banda et al., 2013). The presence of *E. ortleppi* in western Zambian cattle is in accordance with recent records of this parasite in cattle from neighbouring Namibia (Aschenborn, cit. in Deplazes et al., 2017) and Gauteng Province in South Africa (Mogoye et al., 2013), and cattle infection exclusively with this species may be typical for larger parts of southern Africa. Older large-scale prevalence surveys from the Republic of South Africa and the Kingdom of Eswatini (formerly Swaziland) also reported prevalence levels of <10% in cattle with a predominance of lung location and high fertility of the cysts, which are both typical for *E. ortleppi* in cattle; morphological data from the time are also suggestive of this parasite (Verster 1962; Verster and Collins 1966; Mitchell 1977). *Echinococcus ortleppi* is particularly well adapted to cattle as intermediate hosts. This is indicated by high fertility of the cysts - 64 out of 65 in our study -, while the low prevalence, also observed in other world regions, has been explained by the fact that cattle slaughter is often restricted to larger facilities with effective offal disposal (in contrast to frequent home slaughter e.g. of sheep, where dogs have ready access to cysts) (Addy et al., 2012; Romig et al., 2017). In regions where CE prevalence in cattle is higher, e.g. parts of eastern Africa, the majority of cysts belong to *E. granulosus* s. s.. These cysts are often sterile or calcified, and cattle infection with *E. granulosus* s. s. is believed to be the result of spill-over from the concurrent sheep-dog lifecycle. Therefore, the absence of *E. granulosus* s. s. cysts from cattle in our study indicates absence or extreme rarity of *E. granulosus* s. s. transmission in western Zambia (where few sheep are kept), or even in larger parts of southern Africa.

The examination of 52 home-slaughtered pigs in our survey is the first study from Africa, where both prevalence and genetic identity of the cysts were established. Previous molecular records of cysts from pigs are restricted to four cysts in Kenya (two *E. granulosus* s. s., one *E. ortleppi,* one *E. canadensis* G6/7) (Dinkel et al., 2004) and two cysts in Ethiopia (one *E. granulosus* s. s., one *E. ortleppi*) (Tigre et al., 2016). Prevalence surveys for unspecified CE in African pigs are limited to few countries, as pigs were rather recently introduced as important meat sources in most of Africa: Ethiopia (no infection amongst 150 pigs) (Fromsa and Jobre 2011), Tanzania (4.3% amongst 70 pigs) (Ngowi et al., 2004), Nigeria (several studies with prevalences up to 55.9% amongst 320 pigs) (lit. in Deplazes et al., 2017) and South Africa (0.4% to 3.5% amongst 67,720 pigs from various parts of the country) (Verster and Collins 1966). Our findings of fertile *E. ortleppi* cysts in

**Table 1**
Prevalence, fertility and organ location of *Echinococcus* spp. cysts in cattle and pigs.

| Animal species (number) | Prevalence | Mean number of cysts per animal | Cyst fertility (fertile / sterile) | Cyst location (lung / liver) | Species/ genotype (n samples) RFLP | *nad1* sequencing |
|---|---|---|---|---|---|---|
| Cattle (*n* = 2000) | 54/2000 (2.7%) | 1.2 | 64 / 1 | 61 / 4 | EO (65) | – |
| Pigs (*n* = 52) | 2/52 (3.8%) | 1.0 | 2 / 0 | 2 / 0 | EO (2) | EO (1) |

EO = *Echinococcus ortleppi* (G 5).

**Fig. 2.** Free-roaming domestic pig in the Zambesi floodplains near Mongu (photo T. Romig).

**Table 2**
Prevalence and species identification of taeniid eggs in dogs.

| Area | No. faecal samples | No. taeniid positive samples on microscopy/PCR | Identified taeniid species on RFLP and sequencing (n samples) | |
|---|---|---|---|---|
| Kaoma / Nkeyema | 137 | 9/4 | EC (2) | TH (2) |
| Mongu / Limulunga | 152 | 1/0 | – | – |

EC = *Echinococcus canadensis* (G6/7), TH = *Taenia hydatigena*.

**Table 3**
Distribution and identity of taeniid eggs in individual dog faecal samples.

| Sample Number | Origin (district) | Number of taeniid eggs on microscopy | Number of PCR amplifiable eggs | Species identified |
|---|---|---|---|---|
| 1 | Kaoma | 32 | 24 | *T. hydatigena* |
| 2 | Kaoma | 1 | 1 | *T. hydatigena* |
| 3 | Kaoma | 1 | 0 | – |
| 4 | Nkeyema | 1 | 0 | – |
| 5 | Nkeyema | 1 | 0 | – |
| 6 | Mongu | 1 | 0 | – |
| 7 | Nkeyema | 8 | 1 | *E. canadensis* (G6/7) |
| 8 | Nkeyema | 4 | 0 | – |
| 9 | Kaoma | 2 | 0 | – |
| 10 | Nkeyema | 6 | 5 | *E. canadensis* (G6/7) |

Zambian pigs show that these are competent intermediate hosts for this parasite and may, in addition to cattle, play a hitherto unrecognised role in the perpetuation of the *E. ortleppi* lifecycle. This should be further explored given the fact that, in Zambia as well as in many other parts of Africa, pigs are often allowed to roam free and have access to dog faeces (Fig. 2), and are eventually slaughtered at home without supervision. Previously, the lifecycle of *E. ortleppi* was thought to be almost exclusively based on cattle as intermediate hosts (lit. in Romig et al., 2017). Apart from pigs, there are vey few records of infections of other mammals: buffaloes in India (Zhang et al., 1999), goats and sheep in Kenya (Mbaya et al., 2014), a camel in Sudan (Ahmed et al. 2013), and captive monkeys, deer and a porcupine (Plesker et al., 2009; Boufana et al., 2012; Hodzic et al., 2018).

Human infections with *E. ortleppi* are known, but occur far less frequently than with other species such as *E. granulosus* s.s. and *E. canadensis* (G6/7) (Alvarez Rojas et al. 2014). Only twelve human cases of *E. ortleppi* infection are known from various parts of the world (Argentina, Brazil, Mexico, India, China, Vietnam, Netherlands, France and South Africa) (Alvarez Rojas et al. 2014; Shi et al., 2019). The presence of *E. ortleppi* in Zambian cattle and pigs therefore poses only a mild threat to human beings in the province. This could explain why a recent ultrasound survey carried out in humans in the province gave a very low prevalence estimate of CE (Banda, unpublished).

Two out of 289 dog faecal samples contained eggs of *E. canadensis* (G6/7). This does not constitute a prevalence, as taeniid egg detection in faeces of carnivores has a very low diagnostic sensitivity (uneven shedding of eggs, no detection of prepatent infections), which was further reduced in our study by the high proportion of eggs whose DNA could not be amplified. However, this is the only currently feasible approach for molecular characterisation of the *Echinococcus* species in living dogs. Given the presence of *E. ortleppi* in cattle and pigs, the absence of this species in the dogs is somewhat unexpected. However, prevalence of livestock CE was low, and transmission may be focal

under high-risk conditions (e.g. in the vicinity of slaughter facilities). Also, the low number of sampled dogs may contribute to this lack of *E. ortleppi* detection. As *Echinococcus* tapeworms are usually found in large numbers in the intestines of definitive hosts, the presence of one highly infected dog may suffice to contaminate large areas of pasture with tapeworm eggs.

This is the first detection of *E. canadensis* G6/7 in Zambia. This genotypic cluster contains two microvariants, G6 (or 'camel strain') and G7 (or 'pig strain'), whose minor genetic differences are of unclear relevance for host predilection and infectivity (Addy et al., 2017; Laurimae et al., 2018). *Echinococcus canadensis* G6/7 is distributed worldwide, in Africa it is present in a dog-camel lifecycle throughout the camel-raising areas of northern, western and eastern Africa (lit. in Deplazes et al., 2017). Further south, where camels are absent, goats and possibly pigs seem to be most important intermediate hosts (Dinkel et al., 2004; Addy et al., 2012). In southern Africa, it was so far only detected in five human patients from various parts of the Republic of South Africa (Mogoye et al., 2013), and in several wild definitive and intermediate hosts in Namibia (Aschenborn, cit. in Romig et al., 2017). Globally, G6/7 is regarded as the second most common cause of human CE, only preceded by *E. granulosus* s. s. (Alvarez Rojas et al. 2014). Its detection in Zambia raises public health concerns and the elucidation of the lifecycle is a priority. Cattle can be ruled out as important infection sources for dogs, as none of 2000 cattle in our study contained a cyst of this parasite, and cattle infections with *E. canadensis* in other parts of the world are also rare (Deplazes et al., 2017; Romig et al., 2017). The most likely intermediate hosts are pigs (despite the absence of cysts in our small sample of 52 animals) and goats, which could not be examined during this study. Both species are known to be competent hosts for this parasite (Wachira et al., 1993; Dinkel et al., 2004; Varcasia et al., 2007). It will therefore be of interest to examine *Echinococcus* cyst isolates from goats in the study area, although this poses logistic challenges as goats are only slaughtered at home. In addition, scavenging of dogs on carcasses of wild ungulates cannot be ruled out. Both positive samples originated from dogs in Nkeyema district, which borders the extensive Kafue National Park, and wild mammals are known to be suitable hosts for *E. canadensis* G6/7 in neighbouring Namibia (Aschenborn, cit. in Romig et al., 2017). The involvement of wildlife in the transmission of *Echinococcus* spp. in Zambia is therefore in need of study.

## 5. Conclusion

The present study provides baseline data on the infection of livestock and dogs with *Echinococcus* spp. in Western Province of Zambia. This is the first molecular survey done in the region so far. The results obtained have shown that the predominant *Echinococcus* species in the province in cattle and pigs is *E. ortleppi*, and pigs may be more important for the lifecycle of this parasite than previously assumed. The presence of *E. canadensis* (G6/7) in dogs calls for additional studies including the examination of goats and wild ungulates. *Echinococcus granulosus* s.s. is either absent or very rare in western Zambia, possibly caused by the small number of sheep kept, as sheep are the major domestic animals that harbour fertile *E. granulosus* s. s. cysts.

## Declaration of competing interest

The authors declare that there is no conflict of interest.

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