UNIVERSITY OF NAIROBI

MOLECULAR EPIDEMIOLOGY OF *ECHINOCOCCUS* AND *TAENIA* SPECIES IN DOGS FROM CYSTIC ECHINOCOCCOSIS ENDEMIC AREAS IN KENYA

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A thesis submitted in Fulfilment of the Requirements for Award of the Degree of Doctor of Philosophy in Applied Parasitology of the University of Nairobi

School of Biological Sciences

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of a degree. Where other people's work has been used, this has been properly acknowledged and referenced in accordance with the University of Nairobi's requirements

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ABSTRACT

Dogs are reservoirs and hosts for several parasites which cause zoonoses of public health significance worldwide. All four hookworm species of canines are zoonotic and cause infections ranging from transient skin irritations to prolonged 'creeping eruptions', eosinophilic enteritis and patent intestinal infections. Cestodes belonging to the Taeniidae family are of medical and veterinary importance in resource poor communities and cause cystic echinococcosis (CE), cysticercosis and coenurosis in livestock and humans. There is scanty data on the prevalence of intestinal parasites of dogs and their impact on public health in Kenya. Furthermore, the role of domestic dogs in maintaining the transmission cycles and environmental contamination with infective stages of these parasites remains unknown in Kenya. This study aimed at establishing the prevalence and distribution of *Echinococcus granulosus* sensu lato (s. l.), *Taenia* spp. and other zoonotic intestinal parasites in dogs from CE endemic areas of Kenya.

Dog faecal samples were collected from the environment in Turkana, Meru, Isiolo and Narok (Maasai Mara) counties and microscopically examined for the detection of intestinal parasites using McMaster and zinc chloride flotation-sieving technique. Genotyping of hookworms, *Echinococcus* and *Taenia* spp. was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and or DNA sequencing. *Echinococcus* and *Taenia* spp. haplotypes were determined by sequencing and analysis of cytochrome C oxidase subunit 1 gene (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) genes respectively.

Of the 1,621 faecal samples, 701 (43.24%) contained at least one parasite. Eleven parasites genera/families were identified namely hookworms, Taeniidae, *Spirometra* spp., Coccidia, *Toxocara* spp., *Trichuris* spp., *Toxascaris leonina*, *Dipylidium caninum*, *Anoplocephala* spp., *Mesostephanus* spp. and *Uncinaria stenocephala* (in order of frequency). Hookworms were the most common parasites detected followed by taeniids. Microscopy detected hookworm eggs in 490/1621 (30.23%, 95% CI 27.99–32.53) faecal samples. The prevalence of hookworms was high in counties receiving higher rainfall (Narok 46.80%, Meru 44.88%) and low in those with a more arid climate (Isiolo 19.73%, Turkana 11.83%). In a subset of 70 hookworm eggs positive faecal samples *Ancylostoma caninum* (n = 59), *A. braziliense* (n = 10) and *A.* cf. *duodenale* (n = 1) were identified. Eleven percent (178/1,621) of faecal samples had taeniid eggs, among them 4.4% (71/1,621) were *Echinococcus* spp. eggs. Area-wise, the faecal prevalence of *Echinococcus* spp. was 9.2% (48/524) in Turkana, 4.0% (20/500) in Maasai Mara, 0.7% (2/294) in Isiolo and 0.3%

(1/303) in Meru. Four *Echinococcus* spp. were identified with *E. granulosus* sensu stricto (s. s.) being the dominant *Echinococcus* taxon, followed by *E. canadensis* (G6/7) that was detected in 51 and 23 faecal samples, respectively. *E. ortleppi* and *E. felidis* were rare and only detected in 5 and 2 faecal samples respectively. Overall 79/1621 (4.9%) faecal samples contained eggs of *Taenia* or *Hydatigera* (8.0% in Turkana, 4.8% in Isiolo, 3.8% in Maasai Mara and 1.3% in Meru). *Taenia hydatigena* and *T. multiceps* were the most frequent, found in 36 and 15 samples, respectively. Other eggs detected in the faecal samples belonged to *T. serialis* (sensu lato), *T. madoquae* (the first record in domestic dogs), *T. ovis*, *T. saginata* and *Hydatigera taeniaeformis*. Polymorphism of *nad1* sequences revealed 22 and 8 haplotypes of *T. hydatigena* and *T. multiceps*, respectively, but only a few haplotypes showed wide geographical distribution for both species.

This study identified zoonotic parasites in dogs that pose potential public health risk to humans. Canine hookworm species and genetic variability of *T. hydatigena* and *T. multiceps* are reported for the first time in Kenya. The data confirms differences in diversity and abundance of *Echinococcus* and *Taenia spp*. between regions of Kenya and demonstrates that domestic dogs play a role in linking the domestic and sylvatic cycles of *Echinococcus* and *Taenia* spp. The unusual detection of *A.* cf. *duodenale*, *T. saginata* and *E. felidis* in dog faeces indicates coprophagy as a common behaviour of dogs. These findings emphasize the need for control measures such as enforcing laws for restraining stray dogs, dog population management, regular deworming of dogs, proper disposal of slaughter offal and dog faeces and public health educational awareness programmes.

DEDICATION

I wish to dedicate this thesis to my late father Rev. Canon Julius Kakundi Kiilu, who has been my inspiration through-out all my education levels.

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1. **Mulinge, E.,** Magambo, J., Odongo, D., Njenga, S., Zeyhle, E., Mbae, C., Kagendo, D., Wassermann, M., Kern, P., Romig, T., *Echinococcus* species in dogs in different regions of Kenya. 12th European Multicolloquium of Parasitology, 20 – 24th July, 2016, Turku, Finland

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LIST OF ABBREVIATIONS AND ACRONYMS

CE – Cystic echinococcosis

AE – Alveolar echinococcosis

DALY – Disability adjusted life year

SCT – Sedimentation and counting technique

US – Ultrasonography

CT – Computed tomography

MRI – Magnetic resonance imaging

IHAT – Indirect hemagglutination antibody test

LAT – Latex agglutination test

PAIR – Puncture aspiration injection respiration

CLM – Cutaneous larva migrans

EPG – Eggs per gram

CPG – Cysts per gram

FEC – Faecal egg counts

SG – Specific gravity

BLAST – Basic Local Alignment Search Tool

RFLP – Restriction fragment length polymorphism

LAMP – Loop mediated isothermal amplification

ELISA – Enzyme-linked immunosorbent assay

DTT – Dithiothreitol

dNTPs – deoxyribonucleotide triphosphates

EDTA – Ethylenediaminetetraacetic acid

KOH – Potassium hydroxide

KCl – Potassium chloride

NaOH – Sodium hydroxide

$MgCl_2$ – Magnesium chloride

NaCl – Sodium hydroxide

Tris – (hydroxymethyl) aminomethane

HCl – Hydrochloric acid

NADH – Nicotinamide adenine dinucleotide

rRNA – Ribosomal ribonucleic Acid

ITS – Internal transcribed spacer

Cox1 – Cytochrome C oxidase subunit 1

DNA – Deoxyribonucleic acid

PCR – Polymerase chain reaction

WAAVP – World Association for the Advancement of Veterinary Parasitology

ACUC – Animal Care and Use Committee

CESSARi – Cystic echinococcosis sub-Saharan Africa Research initiative

AMREF – African Medical and Research Foundation

WHO-IGWE – World health organisation-Informal Working Group on Echinococcosis

TroCCAP – Tropical Council for Companion Animal Parasite

SERU – Scientific Ethics Review Unit

CMR – Centre for Microbiology Research

KEMRI – Kenya Medical Research Institute

DVS – Department of Veterinary Services

MoH – Ministry of Health, Kenya

UoN – University of Nairobi

WHO – World health organisation

NEB – New England Biolabs

SPSS – Statistical Package for the Social Sciences

CHAPTER ONE: INTRODUCTION

1.1 *Echinococcus*

Echinococcosis is an important zoonosis caused by adult or larval stages of dog tapeworms of the genus *Echinococcus* and the family Taeniidae. The genus *Echinococcus* has 9 valid species based on their different morphological characteristics, host-specificity and molecular phylogeny. These include: *E. granulosus* sensu stricto, *E. equinus, E. ortleppi, E. canadensis* (G6 – G10), *E. felidis, E. multilocularis, E. vogeli, E. oligarthra and E. shiquicus* (Nakao *et al*., 2013b; Nakao *et al*., 2013c; Lymbery, 2017). *E. granulosus* and *E. multilocularis* are the causative agents of cystic echinococcosis (CE) and alveolar echinococcosis (AE) respectively, and are of medical and public health importance globally (Moro *et al*., 2009; Deplazes *et al*., 2017). *Echinococcus vogeli* and *E. oligarthra* cause polycystic and unicystic echinococcosis respectively, conditions of less medical and public health importance in Neotropics where they occur (Romig *et al*., 2017). *E. shiquicus* is transmitted in a predator–prey relationship involving Tibetan fox and plateau pika as the definitive and intermediate host respectively and is restricted to the Qinghai-Tibet plateau region of China (Xiao *et al*., 2005). *E. felidis* has been detected in African wild carnivores, warthogs and hippopotamus in Africa (Halajian *et al*., 2017; Romig *et al*., 2017). The zoonotic potential of *E. shiquicus* and *E. felidis* is unknown (Boufana *et al*., 2013a; Romig *et al*., 2017). Classification of *E. granulosus* s. l. was established previously based on genetic variation (partial sequencing of mitochondrial DNA), intermediate host affinities, geographical distribution, morphological, biological and biochemical differences. *E. granulosus* s. l. is made up of at least five species namely; *E. granulosus* s. s., *E. equinus, E. ortleppi, E. canadensis* (G6–G10) and *E. felidis* (Nakao *et al*., 2013b; Nakao *et al*., 2013c; Romig *et al*., 2017).

1.2 Cystic echinococcosis and *Echinococcus* infection in dogs

Cystic echinococcosis (CE) is an important zoonosis with a global distribution and is caused by the larval stage of the tapeworm *E. granulosus* s. l. The parasite life-cycle involves two mammalian hosts; a carnivorous definitive host which harbours the adult worm in the small intestine and intermediate host animals mainly of herbivorous and omnivorous, which contain the larval stages (metacestodes) within various organs (Thompson, 2017). In dogs, *Echinococcus* spp. infection remains asymptomatic regardless of the parasite burden. However studies involving definitive hosts are necessary to estimate prevalence, worm burden and *Echinococcus* spp. Data from these studies are used in accessing distribution, transmission dynamics, risks of infection for humans and other intermediate hosts (Carmena and Cardona, 2013) and the role played by dogs in environmental contamination with zoonotic taeniids. Diagnosis of *Echinococcus* infection in dogs has several challenges because tapeworm eggs are shed erratically and are indistinguishable from those of *Taenia* spp. (Craig *et al*., 2015).

1.3 *Taenia* species

The genus *Taenia* consists of nearly 50 valid species most of which remain genetically uncharacterized (Hoberg, 2006; Lavikainen *et al*., 2008). *Taenia* spp. complete their life cycles in two mammalian hosts; a carnivorous or omnivorous definitive host which harbours the adult tapeworm in the small intestines. The herbivorous/omnivorous intermediate host acquire infection by ingesting eggs passed through faeces to form larval stages of the parasite called cysticerci or coenuri which can be localized in different organs depending on the *Taenia* species. Although several *Taenia* spp. utilize dogs as the definitive host they can develop in different mammalian intermediate host species to complete their life cycles (Loos-Frank, 2000).

Humans are the definitive hosts of "human *Taenia*", *Taenia solium* (Linnaeus, 1758), *Taenia saginata* (Goeze, 1782) and *Taenia asiatica* (Eom & Rim, 1993). In humans *T. solium,* causes cysticercosis (neurocysticercosis) and taeniasis, as humans serve both as intermediate and definitive hosts respectively. Both *T. saginata* and *T. asiatica* cause taeniasis in humans who are the final host of these species. Additionally, humans can also be infected with zoonotic taeniid species such as *Hydatigera taeniaeformis* (Batsch, 1786), *T. crassiceps* (Zeder, 1800), *T. multiceps* (Leske, 1780) and *T. serialis* (Gervais, 1847). *Taenia multiceps* and *T. serialis* cause cerebral and subcutaneous coenurosis respectively in humans and in small ruminants. *Taenia crassiceps* cause proliferative cysticercosis, a fatal condition in immunocompromised individuals. Both adult worms and larval stages of *H. taeniaeformis* are rare infections in humans. Other *Taenia* spp. of veterinary importance include *T. hydatigena* (Pallas, 1766) and *T. ovis* (Cobbold, 1869), both taxa utilizing dogs as definitive hosts and causing cysticercosis in livestock (Hoberg, 2002, 2006).

1.4 Intestinal parasites of dogs

Dogs are reservoirs of several zoonotic parasites. The role of dogs in transmitting human infections has been recognized worldwide (Deplazes *et al*., 2011). The transmission of these zoonoses is influenced by poor hygiene and overcrowding, poor veterinary extension services and limited knowledge of zoonoses (Traub *et al*., 2005). The canine hookworms *Ancylostoma caninum, Ancylostoma braziliense, Ancylostoma ceylanicum* and *Uncinaria stenocephala* are well known for their detrimental impacts on canine health (Miller, 1968; Georgi *et al*., 1969). However, in addition to their veterinary significance, all four canine hookworm species have zoonotic potential. In humans, these species produce cutaneous larva migrans (CLM) or 'ground itch', which is usually self-limiting (Bowman *et al*., 2010).

1.5 Problem statement

Cystic echinococcosis is a zoonotic disease of public health and socio-economic significance. The World health organisation (WHO) has categorized CE among priority neglected tropical diseases whose further research input is needed to reduce the global burden. Over 3 billion US$ per year is lost to treatment of human cases and losses in livestock production (WHO, 2013, 2015). CE is an emerging or re-emerging disease in many parts of the world with a particular impact in developing countries where extensive livestock farming is practiced (Jenkins *et al*., 2005). In terms of global disease burden i.e. disability-adjusted life years (DALYs) loses due to CE amounts to 1.6 to > 3 M. At least 2 – 3 million people worldwide are affected by CE, where it causes significant morbidity (Budke *et al*., 2006). One of the highest annual incidences of human CE ever reported (176–220 cases/100,000) was in Turkana, Kenya (French and Nelson, 1982; French *et al*., 1982). Previous studies have identified domestic dogs as the main definitive hosts for *E. granulosus* in Kenya, with high prevalence reported in pastoral communities (Eugester, 1978; Macpherson *et al*., 1985; Wachira *et al*., 1994; Buishi *et al*., 2006).

1.6 Justification

Despite the availability of data on CE in livestock and humans and for *Echinococcus* infections in wildlife (Addy *et al*., 2012; Mutwiri *et al*., 2013; Kagendo *et al*., 2014; Mbaya *et al*., 2014; Odongo *et al*., 2018), recent insights into the situation and role of dogs in maintaining the transmission is still lacking in endemic areas of Kenya. Data on *Echinococcus* infection in dogs is useful in estimating the relative infection pressure to humans and intermediate hosts, in determining the role dogs play in environmental contamination and in maintaining the transmission cycles. Besides species identification, understanding the genetic variability of *Echinococcus* spp. is important; for example within the *E. granulosus* s. s. cluster, different microvariants may exhibit differences in

host-specificity, pathogenicity, rate of development, transmission dynamics and drug sensitivity. These important parameters may influence diagnosis, treatment, prevention and control of CE. Presently in Kenya, very little is known of *Taenia* spp. of dogs and the diseases they cause in humans and livestock. There is limited data on the prevalence of intestinal parasites of dogs and their significance on public health in Kenya. The available data is either three decade old, or the information is based on data from a limited sample size or from single locations (Wachira *et al*., 1993b; Buishi *et al*., 2006).

1.7 Research questions

1. Are dogs from Turkana, Meru, Isiolo and Maasai Mara (CE endemic areas) infected with *Echinococcus* and *Taenia* species and/or other zoonotic intestinal parasites?

2. Which *Echinococcus* and *Taenia* species are common in dogs in CE endemic areas?

3. What *Echinococcus* and *Taenia* haplotypes (variants) are common in dogs in CE endemic areas?

1. 8 Objective

1.8.1 Overall objective

The main objective of the study was to establish the prevalence and distribution of species of *E. granulosus* s. l., and *Taenia* in dogs from CE endemic areas in Kenya.

1.8.2 Specific objectives

1. To assess the prevalence and distribution of *E. granulosus* s. l., *Taenia* spp. and other zoonotic intestinal parasites in dogs from CE endemic areas.

2. To identify the species of *E. granulosus* s. l. and *Taenia* in dogs from CE endemic areas.

3. To determine the genetic variability (haplotypes) of major *Echinococcus* and *Taenia* species from CE endemic areas.

CHAPTER TWO: LITERATURE REVIEW

2.1 Life cycle of *Echinococcus granulosus* s. l.

The life cycle of *E. granulosus* s. l. is illustrated in Fig. 2.1. *E. granulosus* s. l. has an indirect life cycle that involves two mammalian hosts. Carnivores serve as the definitive and harbour the adult tapeworm in the small intestine, while herbivores and omnivores act as the intermediate hosts where the larval metacestode stage proliferates asexually in visceral organs. Humans act as the dead-end host. Members of the Canidae family are the main definitive hosts while those of Felidae and Hyaenidae families play minor roles in transmission. The definitive host are infected upon ingesting raw offal containing viable protoscoleces. Ingested protoscoleces evaginate, attaching to the canine intestinal mucosa and develop into adult tapeworms. Within the small intestines the adult tapeworm releases fertilized eggs or gravid proglottids in faeces to the environment and these are accidentally ingested by the intermediate host. The eggs are activated in the small intestines and the released oncosphere larvae penetrate the intestinal mucosa, enter the blood stream and are transported to various organs where the cysts develop. The metacestode multiply asexually to produce viable protoscoleces which develop into adult worms upon consumption by definitive hosts (Thompson, 2017).

2.2 Taxonomy of *Echinococcus* and *Taenia* species

2.2.1 *E. oligarthra*

E. oligarthra causes human unicystic echinococcosis and is widely distributed in the Neotropics (South, Central America and northern Mexico). Transmission of *E. oligarthra* is via sylvatic cycle involving wild felids as definitive host and a wide range of wild rodents as intermediate hosts. Natural infections have been reported in wild felids in South and Central America including puma (*Puma concolor*), jaguar (*Panthera onca*), ocelot (*Leopardus pardalis*), pampas cat (*Leopardus*

colocola), jaguarundi (*Herpailurus yagouaroundi*) and Geoffroy's cat (*Leopardus geoffroyi*) (D'Alessandro and Rausch, 2008). The natural intermediate hosts for *E. oligarthra* are wild rodents including agoutis (*Dasyprocta punctata*), spiny rat (*Proechimys* sp.) and pacas (*Cuniculus paca*) (D'Alessandro and Rausch, 2008; Romig *et al*., 2017). *E. oligarthra* is of less public health significance with only few human infections attributable to this species in Venezuela, Brazil and Surinam (D'Alessandro, 1997; Soares Mdo *et al*., 2013).

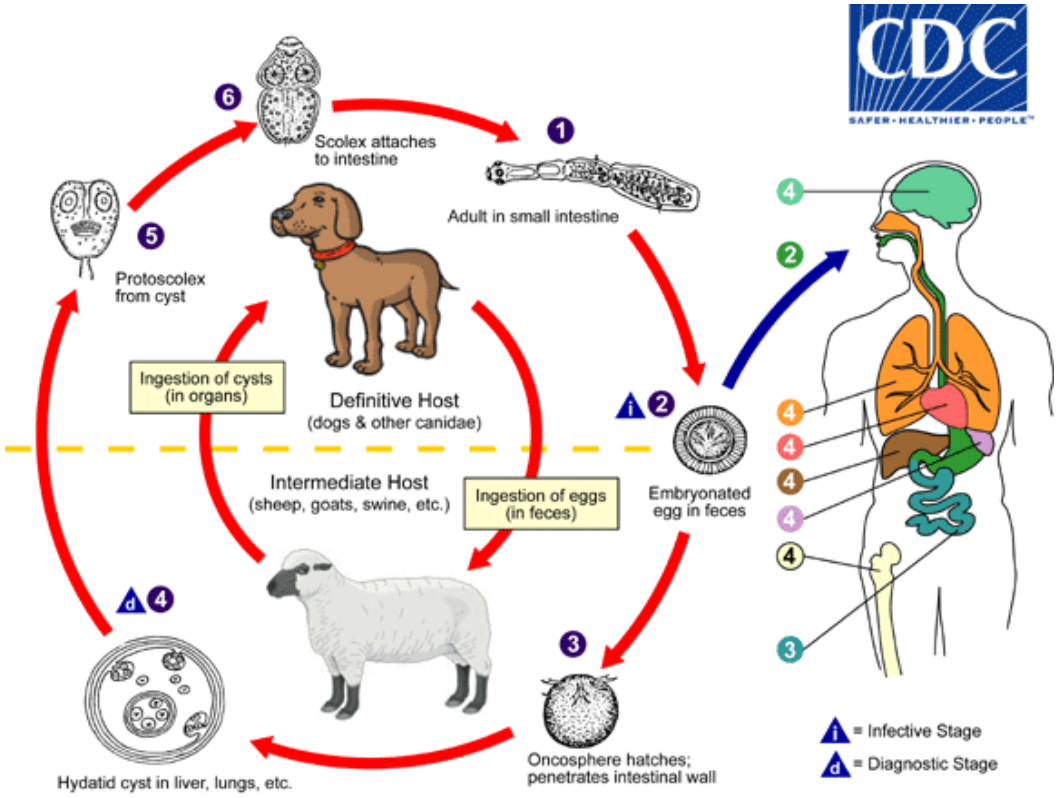

Fig. 2. 1 Life cycle of *Echinococcus granulosus*. Source: http://www.dpd.cdc.gov/dpdx accessed on 13/07/2018.

2.2.2 *E. vogeli*

E. vogeli is the causative agent of polycystic echinococcosis in humans mainly in the Neotropical region largely due to limited geographical habitat of the only known definite host bush dog (*Speothos venaticus*). The intermediate host of *E. vogeli* are pacas (*C. paca*), agoutis (*Dasyprocta* sp.) and nine-banded armadillos (*Dasypus novemcinctus*). Transmission is primarily sylvatic however the habit of hunters feeding infected viscera of pacas to domestic dogs risks human infection with *E. vogeli* (D'Alessandro and Rausch, 2008; Romig *et al*., 2017). Human infections with *E. vogeli* have been reported in Brazil, Argentina, Chile, Peru, Colombia, Venezuela, Uruguay and Surinam (Carmena and Cardona, 2014).

2.2.3 *E. shiquicus*

E. shiquicus is transmitted in predator – prey relationship involving Tibetan fox (*Vulpes ferrilata*) and plateau pika (*Ochotona curzoniae*) as definitive and intermediate host respectively. Its geographical distribution is restricted to the Qinghai-Tibet plateau region of China, a factor solely determined by the existences of these hosts (Romig *et al*., 2017). The adult tapeworm has been detected by PCR in domestic dogs, however it not clear whether this species can utilize dogs as definitive hosts (Boufana *et al*., 2013a). No human infection with *E. shiquicus* has been reported (Xiao *et al*., 2006b).

2.2.4 *E. multilocularis*

E. multilocularis causes alveolar echinococcosis (AE) and is widespread in the northern hemisphere. The parasite is transmitted primarily in a sylvatic cycle involving the red fox (*Vulpes vulpes*) as the main definitive host and wild rodents of the family Arvicolinae (voles) as the most important intermediate host. Other wild canids that act as definitive hosts of this species include: arctic fox (*V. lagopus*), coyote (*Canis latrans*), wolf (*C. lupus*), golden jackal (*C. aureus*), raccoon-

dog (*Nyctereutes procyonoides*), sand fox (*V. corsac*), and the Tibetan fox (*V. ferrilata*). Domestic dogs may also serve as definitive host of *E. multilocularis* and are usually linked with human infections. Both domestic and wild cats (*Felis silvestris*) are also known definitive hosts but have a lesser role in the transmission of this tapeworm. *E. multilocularis* has a wide range small mammals as intermediate hosts depending on the endemic areas, and they include many voles species, Muskrats (*Ondatra zibethicus*), some members of Murinae subfamily, coypus (*Myocastor coypus*), European beavers (*Castor fiber*), several members of Lagomorphs, hamsters, some shrews and lemming species (Romig *et al*., 2017). Human infections with *E. multilocularis* are common in Eurasia, sporadic in North America and Russia (Deplazes *et al*., 2017).

2.2.5 *E. granulosus* sensu lato

E. granulosus s. l., consists of five species that cause CE in intermediate hosts and include: *E. granulosus s. s., E. equinus, E. ortleppi, E. canadensis* (G6 – G10), *E. felidis* (Nakao *et al*., 2013b; Nakao *et al*., 2013c; Lymbery, 2017).

2.2.5.1 *E. granulosus sensu stricto*

Earlier, the term *E. granulosus* was used to represent all agents of CE but was later categorized into three strains (sheep strain, Tasmanian strain and buffalo strain) which assumed genotypes G1 – G3 respectively (Bowles *et al*., 1992). This cluster now known as *E. granulosus* s. s. has been shown to constitute more haplotypes other than previously known (G1 – 3) after analysis of longer sequences of *cox1* gene and other target genes (Nakao *et al*., 2013b; Romig *et al*., 2015). *E. granulosus* s. s. is predominantly transmitted in domestic cycles involving domestic dogs and livestock, but sheep seems to be the most significant intermediate host as they are frequently infected with highly fertile cysts compared to other domestic intermediate hosts (goats, cattle, yak, camels, alpacas, pigs, donkeys) (Romig *et al*., 2017). Kinkar *et al*. (2017) proposed the exclusion

of genotype (G2) from *E. granulosus* s. s. and that genotypes G1 and G3 be distinct genotypes based on mitochondrial genome but should be considered as one species on basis of nuclear gene sequences. Further, the recent discovery of *E. granulosus* omo genotype from an Ethiopian man and cattle in Kenya adds to the growing complexity of *E. granulosus* s. s., this genotype was placed phylogenetically in between *E. granulosus* s. s. and *E. felidis* clades (Wassermann *et al*., 2016).

The role of wild carnivores in the transmission of *E. granulosus* s. s. is illustrated by the emergence of sylvatic cycles or spill-over events from domestic cycles such as the presence of adult worms in wolves, jackals (Europe & Iran), wild dogs (Namibia), red foxes and dingoes (Australia). The presence of fertile or infertile cysts of *E. granulosus* s. s. in wide range of wild herbivores has been demonstrated in different ecological areas. Some of these wild herbivores include: wild boars, red deer, wild sheep, squirrels, gazelles, antelopes, feral pig, warthog and macropods, some of these hosts may be suitable intermediate hosts while others are dead-end hosts (Romig *et al*., 2017).

E. granulosus s. s. is the predominant taxon identified in all characterised livestock CE studies in Kenya (Wachira *et al*., 1993a; Dinkel *et al*., 2004; Hüttner *et al*., 2009; Romig *et al*., 2011; Addy *et al*., 2012; Mbaya *et al*., 2014; Odongo *et al*., 2018), and reaches fertility in all livestock ruminants, particularly in sheep (Njoroge *et al*., 2002; Addy *et al*., 2012; Mbaya *et al*., 2014). Sheep are commonly slaughtered at home and are often the preferred source of meat in Maasailand (Macpherson *et al*., 1985; Addy *et al*., 2012). This coupled with high cyst fertility, lack of stringent meat inspection, poor abattoir conditions and the high numbers of roaming dogs may explain the abundance of *E. granulosus* s. s. in Kenya. *E. granulosus* s. s. has also been described in wildlife from lions, hyenas and wildebeests in Kenya, suggesting a spill-over of domestic cycle because of human encroachment to conservation areas (Romig *et al*., 2017).

A significant proportion (88%) of global cases of human CE is due to *E. granulosus* s. s. (Alvarez Rojas *et al*., 2014), while accounting for (83 – 100%) in Kenya (Wachira *et al*., 1993a; Dinkel *et al*., 2004; Hüttner *et al*., 2009; Casulli *et al*., 2010; Romig *et al*., 2011; Mutwiri *et al*., 2013). The high prevalence in humans' correlates well with the wide spread and abundance of *E. granulosus* s. s. in definitive and intermediate hosts. The fact that this species has very low specificity in intermediate hosts may contribute to increased pathogenicity to humans compared to other agents of CE (Romig *et al*., 2017).

2.2.5.2 *E. equinus*

E. equinus previously known as the *E. granulosus* "horse strain" or G4 genotype (Bowles *et al*., 1992; Bowles and McManus, 1993; Thompson and McManus, 2002), is principally transmitted between domestic dogs and members of Equidae family. Worldwide, confirmed cases of this species are few owing to the fact that cyst materials from earlier studies were not genetically characterized but were assumed to be *E. equinus* (Boufana *et al*., 2014; Romig *et al*., 2017). *E. equinus* has been reported in horses and mules in Europe, from donkeys in Turkey, Egypt and Tunisia (Aboelhadid *et al*., 2013; Boufana *et al*., 2014; Simsek and Cevik, 2014; Romig *et al*., 2015; Simsek *et al*., 2015). In Etosha National Park of Namibia, a sylvatic cycle between lions and black-backed jackals as definitive hosts, and plains zebras as intermediate hosts was established (Wassermann *et al*., 2016). In an ongoing CE study in donkeys in Kenya, *E. equinus* has been identified in only one cyst among approximately 100 cysts that have been characterized (Mulinge *et al*., unpublished data). Besides equids, this species was characterized in red ruffed lemur (*Varecia rubra*) from a zoo in UK (Boufana *et al*., 2012). No human infections associated with this species have been reported.

2.2.5.3 *E. ortleppi*

The first description of this species was in dogs that had been fed with hydatid cysts from cattle in South Africa, where the adult worm was detected. It was previously known as a cattle strain of *E. granulosus* or G5 genotype and later renamed *E. ortleppi* (Bowles *et al*., 1992; Thompson and McManus, 2002). *E. ortleppi* is well adapted to cattle where majority of the cysts reach fertility and are mainly found in the lungs. *E. ortleppi* is globally distributed but rare even in cattle keeping areas. This species has also been detected in other domestic intermediate hosts including sheep, goats, and pigs. Metacestodes of *E. ortleppi* have been found in a zebra and oryx antelopes (*Oryx gazella*), in Buffalo, captive deer and monkeys (Obwaller *et al*., 2004; Pednekar *et al*., 2009; Boufana *et al*., 2012; Romig *et al*., 2017).

In Kenya, *E. ortleppi* was first detected in a pig isolate (Dinkel *et al*., 2004) and later in cattle from Isiolo (Mulinge *et al*., unpublished data) and Maasailand (Addy *et al*., 2012). The study by Mbaya *et al*. (2014) identified Meru and Isiolo as major foci of *E. ortleppi* where it was isolated from cattle, goats and sheep in a total of 51/258 (19.8%) cysts examined. Human infections with *E. ortleppi* have been reported although rarely, possibly due to the infrequency of this species in other hosts or resistance to infection in humans (Alvarez Rojas *et al*., 2014).

2.2.5.4 *E. canadensis* (G6 – G10)

E. canadensis (G6 – 10) is a complex species consisting of four genotypes: the camel strain (G6), pig strain (G7), G8 (American' cervid strain) and G10 (Fennoscandian'cervid strain). The G6/7 cluster is transmitted primarily in a domestic cycles involving (camels – dogs) and (pigs – dogs) and occurs globally. *E. canadensis* (G6/7) cysts attain high fertility rates in camels and pigs. The (G8/10) cluster is mainly transmitted in sylvatic cycles involving wolves and cervids particularly in the north of Eurasia and America (Nakao *et al*., 2013c; Romig *et al*., 2017). However, the cervid strains (G8 and G10) can also be transmitted in domestic or semi-synanthropic life cycles involving

dogs as the definitive host, domesticated reindeer and wild cervids respectively (Oksanen and Lavikainen, 2015). Lymbery *et al*. (2015) has suggested allocation of genotypes *E. canadensis* (G6 – G10) into three distinct species *E. intermedius* for G6/7, *E. borealis* for G8 and *E. canadensis* for G10 according to molecular phylogeny of mitochondrial genes. Recently, two distinct species were proposed for G6/7 and G8/10 clusters based on sequencing of mitochondrial genes (Addy *et al*., 2017b) and nuclear genes (Laurimae *et al*., 2018), but opposed by Yanagida *et al*. (2017) after finding shared alleles within these two clusters in regions where different mitochondrial DNA linkages occur sympatrically.

E. canadensis (G6/7) cluster transmission may also involve other intermediate hosts besides camel or pigs. Goats can be suitable intermediate hosts of *E. canadensis* (G6/7) in the absence of camels or pigs (Soriano *et al*., 2010a; Addy *et al*., 2012). The involvement of sylvatic cycles between wolves or red foxes and wild boars in Europe is not clear and it remains to be determined whether infections with *E. canadensis* (G6/7) are not the result of spill-over of domestic cycles of dogs and pigs. The infection of oryx antelopes and lions with *E. canadensis* (G6/7) in Namibia points to a possible sylvatic transmission without the involvement of domestic dogs (Romig *et al*., 2017).

E. canadensis (G6/7) causes considerable morbidity in humans and is associated with 11% of human CE cases characterized worldwide. Human infections with G8 and G10 are rare and cause benign form of CE (Alvarez Rojas *et al*., 2014). *E. canadensis* is the main cause of human CE in Sudan, Egypt, Austria and Poland, however it could be argued that *E. granulosus* s. s. is rare or absent in animals from these countries (Romig *et al*., 2015). In Kenya, *E. canadensis* (G6/7) accounts for between 15-17% of human CE characterised (Casulli *et al*., 2010; Mutwiri *et al*., 2013). *E. canadensis* (G6/7) was isolated in dogs in Turkana accounting for 38% *Echinococcus*

adult worms (Wachira *et al*., 1993a), where the CE prevalence is high in camels (61.4%) (Njoroge *et al*., 2002) and goats (30% to 92%) (Wachira *et al*., 1993a; Dinkel *et al*., 2004; Hüttner *et al*., 2009). *E. canadensis* (G6/7) was rare in sheep and cattle in Maasailand, but was shown to be common in goats where it attained high fertility rate (Addy *et al*., 2012). In Meru and Isiolo it occurs in abundance in camels and goats, but is rare in sheep and cattle (Mbaya *et al*., 2014).

2.2.5.5 *E. felidis*

Initial records of *E. felidis* emanated from lions in South Africa through morphological description and was originally known as *E. granulosus felidis* and later as "lion strain" of *E. granulosus*. Over the last decade this species has been detected in wild carnivores including lions and hyenas (Hüttner *et al*., 2009; Kagendo *et al*., 2014; Romig *et al*., 2017). Metacestodes of this species have been recovered in warthogs (*Phacochoerus africanus*) (Hüttner *et al*., 2009; Romig *et al*., 2017) and recently in hippopotamus (*Hippopotamus amphibius*) (Halajian *et al*., 2017).

Several studies previously conducted in Kenya (Maasailand) failed to detect *E. felidis* in livestock (Wachira *et al*., 1993a; Hüttner *et al*., 2009; Addy *et al*., 2012; Odongo *et al*., 2018). This species was also absent in about 500 wildebeest sampled from Maasai Mara (Kagendo *et al*., 2014). There are no records of human infection with *E. felidis*, although it is worth noting that this species belongs to the same clade as *E. granulosus* s. s. therefore warranting further investigations (Hüttner *et al*., 2008; Romig *et al*., 2017).

2.2.6 *Echinococcus* spp. haplotypes

An understanding of the genetic variability of *Echinococcus* spp. is important because even within a species different microvariants may exhibit differences in host-specificity, pathogenicity, rate of development, transmission dynamics, and drug sensitivity. These important parameters may influence diagnosis, treatment, prevention and control of CE. *E. granulosus* s. s. has been shown

to constitute more haplotypes other than previously known (G1 – 3) after analysis of longer sequences of *cox1* gene and other targets (Nakao *et al*., 2013b; Romig *et al*., 2015). The *E. canadensis* (G6 – 10) cluster still remains unresolved (Nakao *et al*., 2013c; Lymbery *et al*., 2015; Romig *et al*., 2015; Yanagida *et al*., 2017; Addy *et al*., 2017b; Laurimae *et al*., 2018). Genotypes G6 and G7 are of great significance in the African continent. However it is not clear whether the internal structure of *E. canadensis* (G6/7) is influenced by different geographical dispersal or host preference (G6 for camel, G7 for pigs) (Addy *et al*., 2017b).

2.2.7 *Taenia* species in dogs

Although several *Taenia* spp. utilize dogs as the definitive host, they can develop in different mammalian intermediate host species to complete their life cycles (Loos-Frank, 2000). Fig. 2.2 shows the life cycle of coenurosis caused by *T. multiceps* and *T. serialis* in small ruminants and lagomorphs respectively. Other *Taenia* species which utilize domestic dogs as their definitive host have similar life cycles but may develop in different intermediate hosts and or organs (Table 2.1).

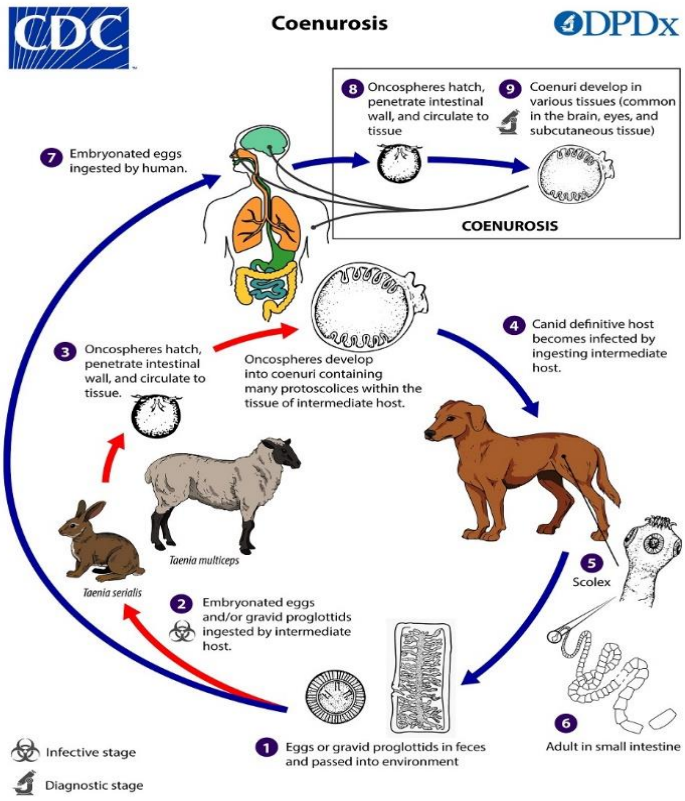

Fig. 2. 2 The life cycle of Coenurosis. Source: https://www.cdc.gov/dpdx/coenurosis accessed on 04/04/2020.

Table 2.1 *Taenia* species and their intermediate host and organ or tissue of development

Taenia species	Intermediate hosts	Organ or tissue of development
T. hydatigena	Sheep, goats, pigs	Omentum, mesentery and liver surface
T. multiceps	Sheep, goats, humans	Brain, spinal cord, subcutaneous fascia, intramuscular and peritoneal areas
T. ovis	Sheep, goats	Cardiac, diaphragmatic and skeletal muscle
T. serialis	Hares, rabbits, rodents, humans	Brain, subcutaneous, intermuscular tissue.
T. crassiceps	Rodents, hares, rabbits, humans	Subcutaneous tissue, peritoneal and pleural cavities
T. pisiformis	Hares, rabbits	Liver capsule, omentum and mesentery

Taenia spp. that utilize dogs as definitive host are described in the subsequent sections.

2.2.7.1 *Taenia hydatigena*

The larval stage of *T. hydatigena*, Cysticercus tenuicollis causes cysticercosis in ruminants, a condition that is widespread globally. The definitive hosts of this tapeworm include; the dog, fox, wolf and coyotes while several species of domestic and wild herbivores and pigs serve as the intermediate hosts. Majority of the metacestodes are usually attached to the omentum and mesentery, but can be found occasionally on the liver surface. There are however descriptions of metacestodes in unusual locations such as lungs, kidneys, brain and in reproductive organs (Hoberg, 2002). Majority of metacestodes infection are chronic and asymptomatic, thus rarely detected until slaughter (Christodoulopoulos *et al*., 2008). The acute form of infection is rare in sheep and reports of outbreaks of severe visceral cysticercosis in lambs are rare (Livesey *et al*., 1981; Koutsoumpas *et al*., 2013; Scala *et al*., 2016). Infection with metacestodes of *C. tenuicollis* is of economic importance due the condemnation of infected organs such as liver and death of young animals due to traumatic hepatitis (Radfar *et al*., 2005; Nourani *et al*., 2010; Scala *et al*., 2016). Only the sporadic death of single lambs has been described in various Asian countries (Radfar *et al*., 2005; Nourani *et al*., 2010). In Kenya, two previous separate studies conducted in Turkana examined dogs after necropsy and revealed high prevalence between 66.7 and 89.5% of *T. hydatigena* adult worms (Jenkins *et al*., 1991; Buishi *et al*., 2006).

2.2.7.2 *Taenia multiceps*

Dogs, jackals, foxes and coyotes are the definitive hosts of *T. multiceps*, while the intermediate hosts include sheep, goats, horses, cattle, camels, deer and pigs. The metacestode stage, Coenurus cerebralis, is a cerebral or non-cerebral parasite, that cause coenurosis in small ruminants (Scala *et al*., 2007). The development of the metacestode in the brain or the spinal cord is associated with

severe clinical symptoms that include; depression, cycling, blindness, head deviation and ataxia. Infected ruminants scam to death leading to huge economic losses (Avcioglu *et al*., 2011). Transmission of this parasite is facilitated by feeding dogs with coenuri cysts of sheep or goats after opening the skull or leaving infected carcasses unattended (Scala and Varcasia, 2006).

The presence of *T. multiceps* in small ruminants has been recorded from neighbouring countries of Tanzania (Miran *et al*., 2015), Ethiopia (Asmare *et al*., 2016), and Egypt (Desouky *et al*., 2011; Amer *et al*., 2017). Coenurosis occurs globally apart from Australia although it is more prevalent in the Mediterranean areas (Scala and Varcasia, 2006). The status of coenurosis in livestock and possibly in humans is generally undocumented in Kenya. Nevertheless, as known in other endemic areas such as in Italy, Egypt, North America, the United States, South Africa, Europe, India, Brazil, and Israel (Scala and Varcasia, 2006; Lescano and Zunt, 2013), presence of *T. multiceps* in the population could pose serious health risk and disease burden to humans where coenurosis may manifest in several neurological disorders.

Variant forms of coenurosis occur due to Coenurus gaigeri occurring subcutaneously, in skeletal muscles and other muscles of goats. Non-cerebral coenurosis although rare, and has been reported in goats in the Middle East (Oryan *et al*., 2014). However, it has been shown that Coenurus cerebralis and Coenurus gaigeri are caused by the same parasite, with the former causing cerebral coenurosis in sheep while the latter causing non-cerebral form in goats, with therefore suggesting the name Coenurus multiceps for both forms (Akbari *et al*., 2015).

2.2.7.3 *Taenia ovis*

In addition to the domestic dog, *T. ovis* uses other canids including foxes and wolves as definitive host and small ruminants, sheep and goats, as intermediate hosts where it develops as cysticerci in the cardiac, diaphragmatic and skeletal muscle. Disease burden and economic losses in livestock

mainly stems from condemnation of infested carcasses (Eichenberger *et al*., 2011; De Wolf *et al*., 2014). Dogs infected with *T. ovis* have been associated with outbreaks of cysticercosis in sheep flocks in Canada (Soehl, 1984), England (Eichenberger *et al*., 2011) and China (Shi *et al*., 2016). In China and mainland Australia *T. ovis* infection is a threat to the sheep industry (Jenkins *et al*., 2014; Zheng, 2016). In Australia, red foxes were identified as the main definitive host of this species (Jenkins *et al*., 2014). A related species, *T. krabbei*, is morphologically similar to *T. ovis* and is transmitted in a sylvatic cycle, using cervids as the intermediate host and carnivores such as dogs, red foxes and wolves as definitive hosts (Al-Sabi *et al*., 2013).

2.2.7.4 *Taenia serialis*

T. serialis uses domestic dogs as definitive hosts and lagomorphs as intermediate hosts and is rarely found in squirrels, other rodents, cats and humans. Cerebral coenurosis due to *T. serialis* infection are rare in humans globally but coenuri can also be found in other tissues (Segovia *et al*., 2001; Lescano and Zunt, 2013). The adult tapeworms of *T. serialis* in *C. anthus* (golden wolf) have been characterized in Kenya (Allsopp *et al*., 1987; Zhang *et al*., 2007).

2.2.7.5 *Taenia crassiceps*

Domestic dogs and felids are the definitive hosts for *T. crassiceps* while small mammals including mice, rabbits, and other rodents are intermediate hosts. Cysticerci of *T. crassiceps* can develop in the muscle and subcutaneous tissues, but may also be found within the peritoneal and pleural cavities. *T. crassiceps* has a proliferative cysticercus that develops asexually by budding. Consequently, ingestion of only one or a few eggs can result in massive infections (Hoberg, 2002; Lescano and Zunt, 2013). Human infection with *T. crassiceps* are rare and have been reported mainly from immunocompromised individuals, where the parasite can cause proliferative

cysticercosis (Francois *et al*., 1998; Wunschmann *et al*., 2003). *T. crassiceps* is common in Europe, North America, and Asia (Lescano and Zunt, 2013).

2.2.7.6 *Taenia pisiformis*

The adult worm of *T. pisiformis* develops in the small intestine of members of Canidae and Felidae (Saeed *et al*., 2006; Lahmar *et al*., 2008; Bagrade *et al*., 2009) while using lagomorphs as the main intermediate host. Cysticercus pisiformis in the intermediate host is usually located in the liver capsule, omentum and mesentery and causes autologous poisoning, emaciation, immunosuppression and death (Owiny, 2001; Zhou *et al*., 2008). *T. pisiformis* is widely distributed globally, corresponding with the occurrence of the intermediate and definitive hosts (Yang *et al*., 2012). *T. pisiformis* is a non-zoonotic cestode, however infection in rabbits results in economic losses in rabbit rearing countries (Yang *et al*., 2014).

2.3 Geographical distribution of cystic echinococcosis (*E. granulosus* s. l.)

Cystic echinococcosis occurs worldwide that is in South and North America, Asia, Europe, Africa and Australia (Deplazes *et al*., 2017). A summary of the epidemiology of CE in north, west, central and southern Africa is described here. A detailed description of CE epidemiology is discussed for East Africa. The geographical distribution of CE in intermediate hosts and incidences in humans in Africa are shown in Fig. 2.3 and Fig. 2.4 respectively.

2.3.1 Africa (North, west, central and southern)

CE is endemic in North African countries of Tunisia, Morocco, Libya, Algeria and Egypt and is transmitted through domestic cycles involving dogs and livestock. Human CE is of high public significance in North Africa with Tunisia having the highest burden. *E. granulosus* s. s. and *E. canadensis* G6/7 are the most common species utilizing sheep and camels respectively as the intermediates host. *E. ortleppi* and *E. equinus* are rare in North Africa (Deplazes *et al*., 2017; Romig

et al., 2017). In northern arid region of sub Saharan Africa CE is endemic only in Mauritania and Sudan where camels are the most important intermediate hosts. *E. canadensis* (6/7) is the causative agent of CE in this region in livestock and humans whereas *E. granulosus* s. s. and *E. ortleppi* have little contribution to the epidemiology of CE. Very little data on CE exists from Mali, Chad and Niger, with only old sporadic surveys in humans and dogs available (Deplazes *et al*., 2017; Romig *et al*., 2017). Little data is available on CE in West and Central Africa, with older surveys indicating low *Echinococcus* infection in dogs contrary to the high prevalence reported in livestock from Nigeria. Molecular studies are limited in west and central African countries and therefore the agents of CE are unknown (Deplazes *et al*., 2017; Romig *et al*., 2017), apart from a recent study in Nigeria that identified *E. canadensis* (G6/7) as the only species causing CE in camels, cattle and goats (Ohiolei *et al*., 2019). There is scarce data on CE in southern Africa with data available from only South Africa, Zambia and Namibia. Both domestic and sylvatic cycles have been reported in southern Africa. All *E. granulosus* s. l. species have been identified to cause CE or *Echinococcus* infections in wild and domestic carnivores (Wahlers *et al*., 2012; Deplazes *et al*., 2017; Romig *et al*., 2017).

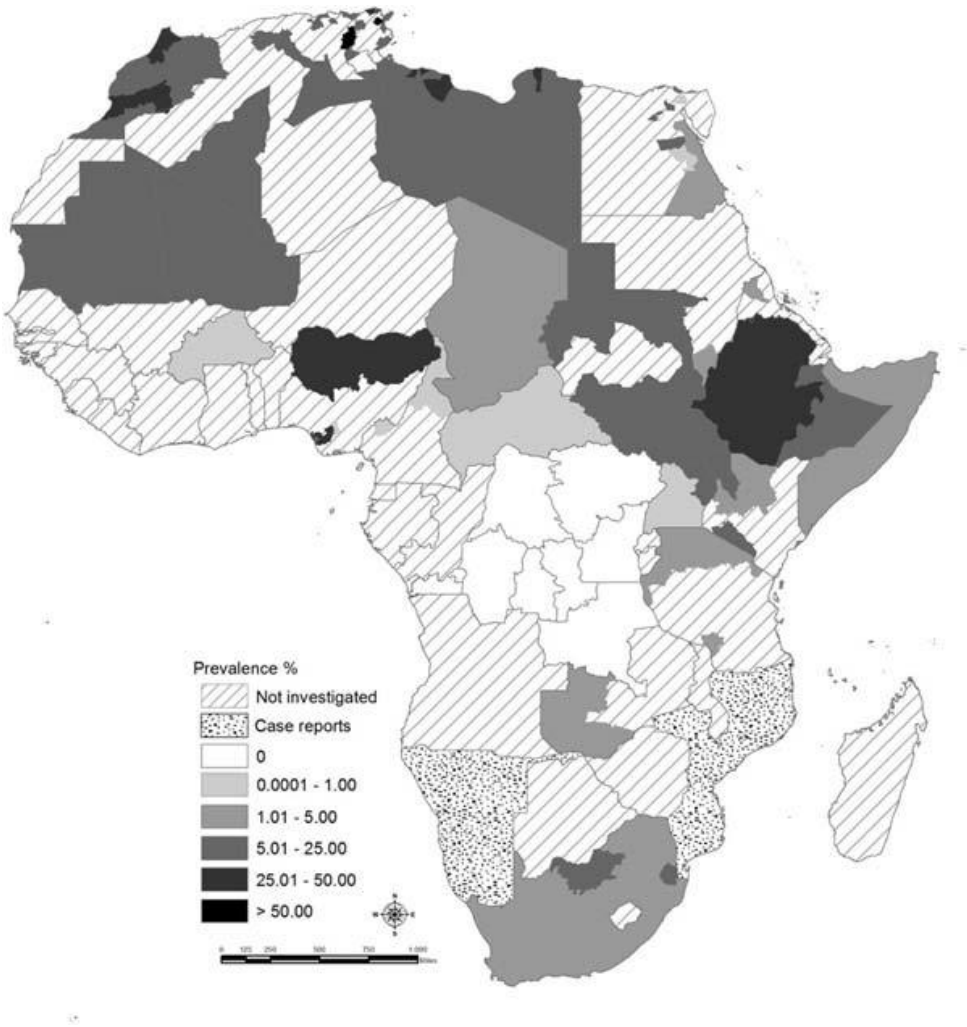

Fig. 2. 3 Distribution of cystic echinococcosis in domestic intermediate hosts in Africa (Deplazes *et al*., 2017).

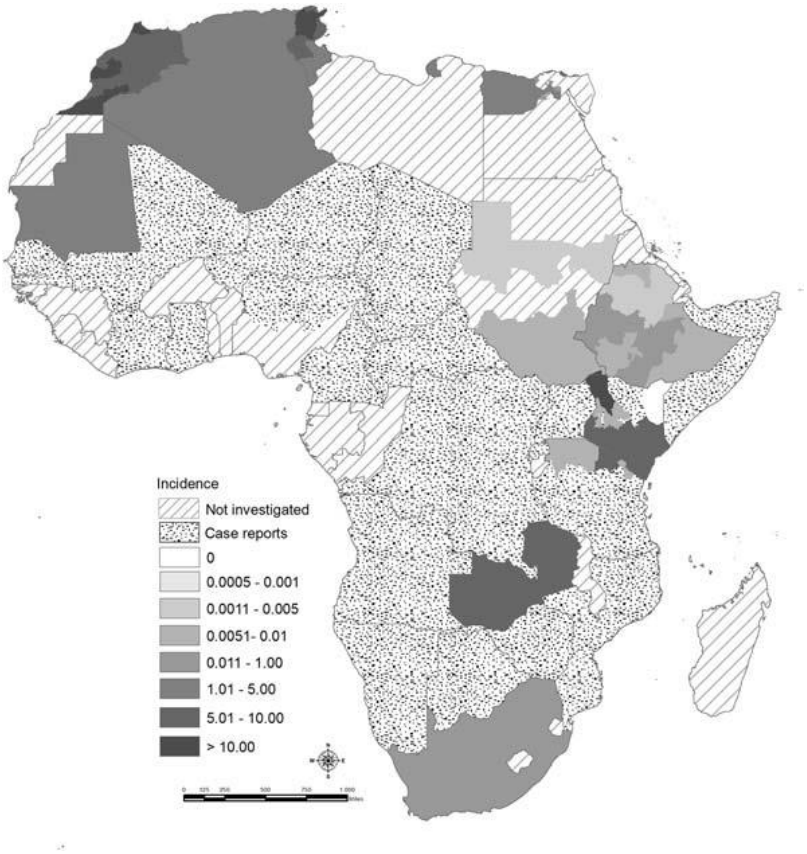

Fig. 2. 4 Current incidence of human cystic echinococcosis in Africa (Deplazes *et al*., 2017).

2.3.2 East Africa

2.3.2.1 South Sudan

Data from epidemiological surveys in South Sudan indicate that human CE prevalence range between 0.0% and 3.5% depending on the community examined (Macpherson *et al*., 1989b; Magambo *et al*., 1996; Magambo *et al*., 1998; Stewart *et al*., 2013). A recent survey in Kapoeta area of South Sudan showed 28/1135 (2.47%) people examined had CE (Zeyhle *et al*., unpublished data). Studies in livestock are limited and shown low prevalence of CE in livestock: 7.1% in both cattle and goats and 2.7% for sheep (Omer *et al*., 2010). *E. canadensis* (G6/7) was identified as

dominant species in cattle, sheep, goats and humans (2 cases from Juba), with only one cyst from cattle identified as *E. ortleppi* (Omer *et al*., 2010). However, in the Kapoeta study, all 38 cysts from 6 individuals were *E. granulosus* s. s. reflecting the situation in the neighbouring Turkana, Kenya (Zeyhle *et al*., unpublished data).

2.3.2.2 Ethiopia

In Ethiopia, several human CE surveys have shown prevalence ranging between 0.0% and 2.9% in different communities (Macpherson *et al*., 1989b; Klungsoyr *et al*., 1993; Assefa *et al*., 2015). High CE prevalence and fertility rate are reported in cattle (Regassa *et al*., 2010; Wahlers *et al*., 2012; Tigre *et al*., 2016). *E. granulosus* s. s. is the dominant species and has been detected in cattle, pigs, goats, camels, sheep (Maillard *et al*., 2007; Romig *et al*., 2011; Hailemariam *et al*., 2012; Tigre *et al*., 2016; Terefe *et al*., 2019). *E. ortleppi* is rare in Ethiopia and was only detected in a pig, sheep and cattle (Tigre *et al*., 2016; Terefe *et al*., 2019), while *E. canadensis* (G6/7) has been reported in camels, cattle and goats where cysts can reach high fertility (50%) in camels and cattle but those from goats are sterile (Hailemariam *et al*., 2012; Tigre *et al*., 2016; Terefe *et al*., 2019). A novel genotype named G_{omo} belonging to *E. granulosus* s. s. cluster was identified from a human patient in southwestern Ethiopia (Wassermann *et al*., 2016).

2.3.2.3 Uganda

In Uganda, CE in humans has been reported from retrospective data, 23 cases in Karamojong, Lango and Acholi communities (Owor and Bitakaramire, 1975). A recent epidemiological study in pastoral and agro-pastoral districts of Uganda showed a prevalence of 1.84% but ranged between 0.45% in Nakapiripirit district and 3.9% in Napak district (Othieno *et al*., 2016). Older CE prevalence data of 1%, 10.5% and 20% in cattle from different parts of Uganda are reported (Owor and Bitakaramire, 1975). High *Echinococcus* infection in dogs (66.3%) was reported in dogs from

Moroto district by post-mortem examinations (Inangolet *et al*., 2010). However a recent study reported much lower prevalence in dogs, 14.4% and 7.4% from Moroto and Bukedea districts respectively, with an overall prevalence of 12.2% (Oba *et al*., 2016). The latter study examined taeniid eggs in faecal samples but the methodology used for differentiating *Echinococcus* eggs from other taeniids was not described (Oba *et al*., 2016). Characterization of cysts from cattle, sheep, goats and camels in Moroto district identified *E. granulosus* s. s. as the dominant species and 3 cases of *E. canadensis* (G6/7) (Chamai *et al*., 2016). In wildlife, *Echinococcus* infections with *E. felidis* were detected in lions, hyena and a warthog and *E. granulosus* s. s. in a warthog (Hüttner *et al*., 2009).

2.3.2.4 Tanzania

Two separate retrospective surveys at Wasso hospital in Tanzania revealed 171 human CE cases were operated between 1990 and 2003 translating to an incidence of 10 cases/100 000/year (Ernest *et al*., 2010), a similar incidence had earlier been reported between 1968 – 1986 (Macpherson *et al*., 1989a). An ultrasound survey for human CE in Maasai communities in northern Tanzania revealed a prevalence of 1% (Macpherson *et al*., 1989a). Five out of ten dogs were infected with *Echinococcus* after necropsy and purgation examinations in northern Tanzania (Macpherson *et al*., 1989a). In livestock, CE data is reported from retrospective surveys, in Ngorongoro district with 63.8% sheep, 34.7% goats and 48.7% cattle slaughtered between 1998 and 2001 infected with CE (Ernest *et al*., 2009). Much lower CE prevalence was recorded in a three years retrospective study (2005 – 2007) in Arusha, shoats (sheep and goats) (6.02%) and cattle (4.2%) (Nonga and Karimuribo, 2009). A recent cross-sectional study at a livestock – wildlife interface area in Ngorongoro, Tanzania showed CE infection in goats and sheep at 22.2% and 16.6% respectively

(Miran *et al*., 2017). So far agents of CE in Tanzania are unknown because genotyping of *Echinococcus* isolates from humans and livestock is not done yet.

2.3.2.5 Rwanda, Burundi and Somali

Data from other East African countries (Rwanda and Burundi) are scanty. A case report of CE in the iliac bone cavity of Somali woman was reported, however the exact location where the infection might be acquired was not documented (Babady *et al*., 2009). A cyst isolate from camel in Somalia was identified as *E. canadensis* (G6/7) (Bowles *et al*., 1992).

2.3.2.6 Kenya

Earlier CE studies in Kenya concentrated in Turkana and Maasailand as these were the only endemic areas known. Studies on *Echinococcus* infection in dogs in Kenya predates back to 1958. Several studies on *Echinococcus* infection in dogs by necropsy in Turkana revealed varying prevalence: 59.1% (Ginsberg, 1958), 70.4% (Nelson and Rausch, 1963) and 39.4% (Macpherson *et al*., 1985). In the first two studies, very few dogs were examined compared to the Macpherson *et al*. (1985) study where 695 dogs from wide spread regions of Turkana were examined. A total of 33% stray dogs in Turkana showed infection with *Echinococcus* after post-mortem examinations (Buishi *et al*., 2006) and a further 26% of owned dogs were copro-antigen positive for *Echinococcus* (Buishi *et al*., 2006). A study conducted in Nairobi revealed 62.7% of dogs had *Echinococcus* worms on necropsy (Nelson and Rausch, 1963), while another study on dogs scavenging around abattoirs in Nairobi showed 72% infection by necropsy (Wachira *et al*., 1994). In Kajiado district (Maasailand) 27.3% of dogs were infected with *Echinococcus* post-mortem examinations (Eugester, 1978). Molecular analysis of adult worm isolates from dogs in Turkana identified 62% of them as *E. granulosus* 'sheep strain' and 38% as camel strain (*E. canadensis* (G6/7) (Wachira *et al*., 1993a).

Earlier studies on CE in livestock from Turkana showed varying prevalence: cattle (15.8 – 30.0%), sheep (7.6 – 13%), goats (3.1 – 15%) and 80% in camels (Froyd, 1960; Gathura and Kamiya, 1990). Ginsberg (1958) observed that more than 30% of cattle, sheep and goats slaughtered in the central abattoir of the Kenya Meat Commission had hydatid cysts. Elsewhere in Maasailand two studies reported nearly similar CE prevalence for sheep (9.5% vs 8.1%), goats (9.0% vs 7.1%) but contrasting CE prevalence in cattle (46.7% vs 8.9%) (Eugester, 1978; Macpherson, 1985). More than a decade later, CE prevalence of livestock in Turkana had not changed: cattle (19.4%), sheep (3.6%), goats (4.5%) and camels (61.4%) according Njoroge *et al*. (2002). Also in Turkana the prevalence of CE in goats was much lower, at 1.82% in a study that used ultrasonography (Njoroge *et al.*, 2000). Two more studies in Maasailand reported CE prevalence of 25.8% in cattle, 16.5% in sheep and 10.8% in goats (Addy *et al*., 2012) and 16% in sheep (Odongo *et al.*, 2018). In central Kenya (Meru and Isiolo), CE prevalence was found to be lower than in both Turkana and Maasailand: 1.92% in cattle, 6.94% in camels, 0.37% in goats and 4.62% in sheep (Mbaya *et al*., 2014).

Case reports of human CE in Kenya began in the 1950s' and were used to estimate incidences of the disease before the introduction of ultrasound scanning and immunological techniques (Nelson, 1986). Case reports included: 119 cases in Kitale hospital (1952 – 1955) by (Wray, 1958), 17/100,000 in south Turkana and 198/100,000 in north Turkana (1976 – 1980) by (French and Nelson, 1982), 163 cases countrywide, 142 being from Turkana 1968 – 1972 (Rottcher, 1973), 40 cases/100,000 persons per annum in Turkana (Schwabe, 1969), and 791 cases in Turkana 1971 – 1975 (O'Leary, 1976). At the Kenyatta hospital, two case reports identified 55 CE human cases between 1955 – 1961 (Nelson and Rausch, 1963) and 105 cases between 1975 –

1984 (Gikenye, 1985). After the introduction of ultrasound scanning and confirmation by serology the prevalence of CE in Turkana was estimated to be 5% to 10% (Nelson, 1986).

Epidemiological surveys based on ultrasound scanning revealed CE prevalence of 5.6% in Turkana and this method was shown to more sensitive than serology (6.6% vs 2.9%) (Macpherson *et al*., 1987). Ultrasound was used to estimate the CE prevalence in major pastoral communities within East Africa and revealed a CE prevalence of between 0.0% and 5.6% among Kenyan pastoral communities. Notably CE was not found among the Rendille, Samburu, Somali and Gabbra communities, while it was 0.1% in Pokot, 1.1% in Maasai, 1.8% in Boran and 0.0 – 5.6% in Turkana communities (Macpherson *et al*., 1989b). Recent ultrasound surveys show that CE prevalence has reduced from 5.6% (1980s') to 1.9 – 3.8% (Solomon *et al*., 2017), attributable to the control programme initiated by African Medical and Research Foundation (AMREF) (Macpherson *et al*., 1986).

Jackals and hyenas were initially thought to be the main definitive hosts of *Echinococcus* in Kenya (Ginsberg, 1958; Wray, 1958; Round, 1962). However, Nelson and Rausch (1963) showed that a domestic cycle was responsible for human infections in Turkana since dogs were heavily infected with *Echinococcus* worms and cattle, sheep, goats and camels had hydatid cysts. This was strongly supported by the fact that there were insufficient large carnivores and wild herbivores in Turkana to maintain the sylvatic cycle of the parasite. It was believed however that in Maasailand, due to the frequency of infection in both wild carnivores and herbivores, the sylvatic cycle was the main transmission route (Macpherson *et al*., 1983; Nelson, 1986). Lions and hyenas have been identified as definitive hosts of *E. felidis* in major national parks in Kenya. However no record of a suitable intermediate host of *E. felidis* has been found yet because wild herbivores are

not available for research. Post-mortem examination of drowned wildebeest in Maasai Mara has revealed infection with *E. granulosus* s. s. (Kagendo *et al*., 2014).

2.4 Burden of cystic echinococcosis

Estimating the burden of CE in any region is critical in the understanding of the public health and economic impacts of the disease in that population. Such information shows the magnitude of the problem, the need for control interventions and provides the means to assess the success of these interventions (Kern *et al*., 2017). The first global estimate of the non-monetary burden of CE was estimated to be 1 million DALYs lost (Budke *et al*., 2006) however this value was thought to be an underestimate (Craig *et al*., 2007). According to Budke *et al*. (2006) the global monetary burden of human CE was estimated at $764 million annually while the livestock-associated monetary annual losses were estimated to be $2.2 billion. In Kenya the economic burden of human CE was estimated in Turkana. The direct cost for 586 surgical patients for the period (1991 – 2011) was US$ 453,154 with an average of US$ 22,658 per year. Annual indirect CE-associated monetary losses due to lost economic opportunities amounted to US$ 4,414 for a herdsman and US$ 1,339 for a house wife (Odero *et al*., 2014).

2.5 Diagnosis of cystic echinococcosis and *Echinococcus* infection

2.5.1 Diagnosis of *Echinococcus* infection in definitive hosts

Diagnosis of *Echinococcus* infection in dogs presents several challenges in that the tapeworm eggs are shed erratically and are indistinguishable from those of other genera of taeniids. Furthermore, gravid proglottids or eggs are not present in faeces during the prepatent phase of infection or can be easily overlooked. The diagnosis of *Echinococcus* relies on a number of techniques including necropsy, arecoline purgation, copro-antigen ELISA and copro-PCR with faecal matter or isolated eggs (Eckert *et al*., 2001; Craig *et al*., 2015).

2.5.1.1 Post-mortem examination (Necropsy)

Examination of adult worms from the intestine of dogs after necropsy has been the gold standard method for evaluation of *Echinococcus* infection in dogs (Eckert *et al*., 2001; Craig *et al*., 2015). Detection of the adult worm in the small intestine of dogs is performed by using sedimentation and counting technique (SCT), intestinal scraping technique and the direct examination technique. Necropsy has 100% specificity in CE endemic areas, but in areas of comorbidity with *E. multilocularis* caution is taken because both species can easily be confused and differentiation of both species is required. The sensitivity of necropsy for detection of *Echinococcus* is high (>97%) but it may fail to detect very low worm burdens (<6 worms) and sedimentation and counting technique is therefore encouraged to increase sensitivity (Craig *et al*., 2015).

2.5.1.2 Arecoline hydrobromide purgation

Arecoline hydrobromide purgation followed by examination of adult worms in the purge is another method for assessing the worm burden in dogs (Eckert *et al*., 2001). Purgation is one the oldest technologies and has been the pre-mortem gold standard for detection of *Echinococcus* infection in dogs for over 100 years. Purgation guarantees 99 – 100% specificity and results are achieved within a short period thus providing epidemiological data and research materials. It has been applied in the successful control programme in New Zealand and in several surveillance programmes (Craig and Larrieu, 2006). Purgation however, has low sensitivity especially in low intensity infections and in situations where the full purge does not occur. It is also difficult to implement in large studies, requires skilled personnel and the purge causes environmental contamination. Purgation is tedious and time consuming because all the purge require processing and examination in the lab. Arecoline is not recommended for use in old, young, sick, pregnant

dogs and in some instances dog owners may fail to consent for their dogs in fear of adverse side effects (Eckert *et al.*, 2001; Craig *et al*., 2015).

2.5.1.3 Serum antibody detection

This method is used to detect serum antibodies (IgG, IgA and IgE) in infected dogs using native or recombinant antigen extracts from adult, protoscolex and oncosphere in ELISA test. Studies have shown that anti-*Echinococcus* antibodies can be detected 2 – 3 weeks post-infection. Although this method has high specificity (90%), it has a low sensitivity (35 -40%) for natural infection in dogs and does not show correlation with worm burden (Gasser *et al*., 1988; Jenkins *et al*., 1990). Another limitation of this method is the persistence of antibodies after the clearance of the adult worms (Craig *et al*., 2003). Further studies on existing and new recombinant antigens are required to improve sensitivity of serum antibody detection in dogs (Carmena *et al*., 2006; Zhang and McManus, 2006).

2.5.1.4 Coproantigen ELISA (Copro-ELISA)

Copro-ELISA detects *Echinococcus* antigens in faecal samples and offers a reliable method in studies seeking to determine prevalence and re-infection rates in dogs (Craig *et al*., 1995; Allan and Craig, 2006; Moss *et al*., 2013). Copro-ELISA utilizes either polyclonal or monoclonal antibodies directed against either somatic or excretory/secretory (ES) antigens (Allan *et al*., 1992; Benito and Carmena, 2005; Morel *et al*., 2013). Copro-ELISA tests for *E. granulosus* have shown good genus specificity (78 – 100%) and sensitivity of (85 – 95%) which corresponds well to the parasite worm burden (Allan *et al*., 1992; Buishi *et al*., 2005). Copro-ELISA can applied in control programmes to confirm infections or re-infections in dogs (Eckert *et al*., 2001). However, it has low sensitivity in low worm burdens (<100) which could give false negative results and cross-reactivity with other taeniids such as *T. hydatigena* in areas of comorbidity (Malgor *et al*., 1997;

Morel *et al*., 2013). Copro-ELISA is a suitable tool for field based studies because Coproantigens remain stable in faecal samples exposed to the harsh environment or in formalin (5‑10%) preserved faecal samples (Allan and Craig, 2006). Three commercial copro-ELISA kits are currently marketed and exhibit varied sensitivity (60%) and specificity (90%) (Huang *et al*., 2014).

2.5.1.5 Copro-PCR

PCR is highly specific and provides a suitable alternative to necropsy and purgation (Craig *et al*., 2015). A number of PCR-based assays demonstrating high specificity have been developed for copro-detection of *Echinococcus* eggs (DNA) in dogs (Cabrera *et al*., 2002; Abbasi *et al*., 2003; Dinkel *et al*., 2004; Stefanić *et al*., 2004; Trachsel *et al*., 2007; Hüttner *et al*., 2009; Boufana *et al*., 2013b). However, some of these methods are species specific (Stefanić *et al*., 2004) and therefore would fail to detect co-infection with other species (Boufana *et al*., 2008), whereas others have, reportedly, the capacity to detect all species of *E. granulosus* s. l. but cannot discriminate them (Abbasi *et al*., 2003). The development of multiplex PCR enables the simultaneous detection of taeniids in definitive hosts (Trachsel *et al*., 2007; Boubaker *et al*., 2013; Liu *et al*., 2015a). Although multiplex PCR showed high sensitivity and specificity to discriminate species of *E. granulosus* s. l., it however exhibited low sensitivity on *Echinococcus* eggs (Boubaker *et al*., 2013). Parasite DNA can be extracted directly from faecal samples (Dinkel *et al*., 2004; Boufana *et al*., 2013b), from isolated taeniid eggs (Stefanić *et al*., 2004; Hüttner *et al*., 2009) and using magnetic capture probe (Isaksson *et al*., 2014; Øines *et al*., 2014). In areas where *Taenia* spp. co-exist with *Echinococcus* spp., PCR on individual eggs is recommended because taeniid eggs can be differentiated by RFLP of PCR amplicons or sequencing of *nad1* gene (Hüttner *et al*., 2009), or through multiplex PCR (Trachsel *et al*., 2007) or by use of species specific primers (Stefanić *et al*., 2004). Other molecular based tools used for diagnosis of *Echinococcus* spp. infection in dogs with

high specificity include the use of loop-mediated isothermal amplification (LAMP) (Salant *et al*., 2012; Ni *et al*., 2014; Wassermann *et al*., 2014) and PCR/dot blot assay (Armua-Fernandez *et al*., 2011).

2.5.2 Diagnosis of cystic echinococcosis in intermediate hosts

Diagnosis of cystic echinococcosis in intermediate hosts is based on post-mortem examinations at abattoirs, ultrasound examination of live small ruminants and use of serology. In livestock, diagnosis of CE mainly depends on necropsy at meat inspection in abattoirs. The presence of cysts in infected viscera is usually detected by visual inspection, palpation (especially in the lungs) and or incision. Cysts present as either single or multiple and are mainly located in the liver or lungs but can be found in other organs such as spleen, kidneys, heart, brain and omentum (Eckert *et al*., 2001). For genetic identification cysts are subjected to molecular tools such as PCR, RFLP, LAMP and DNA sequencing (Eckert *et al*., 2001; Craig *et al*., 2015).

Sheep experimentally infected with *E. granulosus* eggs are reported to develop reactive antibodies within weeks, however natural infections with CE showed reduced sensitivity and this was compounded by cross-reaction with *T. hydatigena* and *T. ovis* infections (Craig *et al*., 2015). The ability of serology to show exposure of *E. granulosus* in sheep makes it an ideal tool for surveillance programme (Eckert *et al*., 2001). Ultrasound was explored in scanning of small ruminant in Turkana, Kenya and in southern Sudan, and showed high specificity (82 – 98%) (Maxson *et al*., 1996; Sage *et al*., 1998; Njoroge *et al*., 2000). However in the presence of metacestodes of *T. hydatigena,* false positive images were observed. The use of ultrasound in diagnosis of CE in small ruminants has been recommended due to its cost effectiveness, while also offering good sensitivity and specificity compared to serological tests (Sage *et al*., 1998; Dore *et al*., 2014).

2.5.3 Diagnosis of cystic echinococcosis in humans

Imaging techniques used for diagnosis of human CE include: ultrasonography (US), computed tomography (CT), standard radiology and magnetic resonance imaging (MRI) (Haliloglu *et al*., 1997; Polat *et al*., 2003). The use of ultrasound in diagnosis of human CE was introduced in the 1980's by Gharbi *et al*. (1981) and has over the years improved diagnosis of CE in humans (Macpherson *et al*., 1987; Perdomo *et al*., 1988; Caremani *et al*., 1997) and was therefore recommended for mass screening of CE in endemic areas (Macpherson *et al*., 2003). Ultrasound scanning offers several advantages, revealing the number of individual cysts, their location, size and stage of cysts. It is portable and therefore suitable for field studies (Eckert *et al*., 2001).

X-ray is the recommended method for diagnosis of pulmonary CE (von Sinner, 1997a; von Sinner, 1997b), but can also be used for screening of CE cysts in the abdomen and bones (Eckert *et al*., 2001). Computed tomography is the main method for scanning cysts in the brain because of its capacity to detect small-sized cysts. However, CT scanning has limited capacity to detect internal septs and floating membranes (Oto *et al*., 1999; Kern *et al*., 2017). Magnetic resonance imaging (MRI) is a specialized method for detection of CE cysts in particular sites not suitable for US and where CE classification is not required. It is used for evaluating floating and detached membranes and in detection of biliary tree involvement (Stojkovic *et al*., 2012; Kern *et al*., 2017).

Immunodiagnostic tools for serum antibody response against CE in humans are applied as confirmatory tests for imaging techniques, or for differential diagnosis of uncertain findings of imaging. Three commonly used serological tests for detection of *E. granulosus* serum antibodies include: ELISA (IgG), the indirect hemagglutination antibody test (IHAT), and the latex agglutination test (LAT). These tests employ native hydatid fluid antigens and therefore lack specificity with many false positives and also suffer from cross-reaction with other helminthic

infections (Craig, 1997; Eckert and Deplazes, 2004; Siles-Lucas *et al*., 2017). These tests are not suitable for CE patients follow-up after treatment because antibodies persist for long even after cysts clearance or removal (Zhang and McManus, 2006). Immunodiagnostic tools are limited in detection of cysts in the brain or eye, calcified cysts and in young children. Recombinant antigens offer better possibility of reducing non-specific reactions than native antigens (Siles-Lucas *et al*., 2017).

2.6 Treatment of cystic echinococcosis in humans

Fig. 2.5 illustrates the World health organisation-Informal Working Group on Echinococcosis (WHO-IWGE) classification of cysts. Clinical management of CE in humans can be achieved by surgery, PAIR techniques (puncture – aspiration – injection – re-aspiration), Chemotherapy and 'watch and wait' approach. The choice of treatment is based on the type of cysts (according to WHO-IWGE classification), cysts location and whether it is a complicated or non-complicated cysts (WHO, 1996; Brunetti *et al*., 2010; Kern *et al*., 2017). Surgery involves complete or partial removal of the cysts (Sayek and Onat, 2001; Buttenschoen and Carli Buttenschoen, 2003). Although surgery presents a good method for removal of cysts from the body, several risks may be involved such as secondary infection due to spillage, recurrence of infection, anaphylactic reactions and post-operative deaths (Brunetti *et al*., 2010; Kern *et al*., 2017). PAIR consists of four steps 1) percutaneous puncture of cysts using US guidance, 2) aspiration of cyst fluid, 3) injection of hypertonic saline solution or alcohol for 10 – 15 min and 4) re-aspiration of the fluid (WHO, 2003a, 2003b). PAIR is the preferred method for treatment of cysts types CE1 and CE3a (WHO-IWGE classification) and can be applied in patients who cannot be operated and for management of surgery relapses or treatment failure. However, PAIR can cause haemorrhage, damage of tissues,

infections and secondary echinococcosis due to spillage and has been linked with anaphylactic shock caused by leakage of cyst fluid and biliary fistulas (Brunetti *et al*., 2010; Kern *et al*., 2017).

Benzimidazoles (albendazole and mebendazole) and Praziquantel are used for management of CE patients. Benzimidazoles are preferably used for treatment of liver and lung cysts types CE1 and CE3a (Stojkovic *et al*., 2009). Chemotherapy can also be applied during surgery and PAIR to complement cysts removal, sterilize the parasite and to avoid secondary echinococcosis (Brunetti *et al*., 2010), and is recommended for continuous use for several months to achieve success. Praziquantel alone has no parasiticidal effects on CE cysts but has been shown to increase the bioavailability of Benzimidazoles when used in combination, while also preventing secondary echinococcosis (Kern *et al*., 2017). The watch and wait approach is used in management of uncomplicated cysts such as CE4 and CE5 and for CL cysts until their parasitic nature is established (Brunetti *et al*., 2010). The success of watch and wait approach lies on recent advancements in US imaging that allow long term follow-up of CE patients (Junghanss *et al*., 2008).

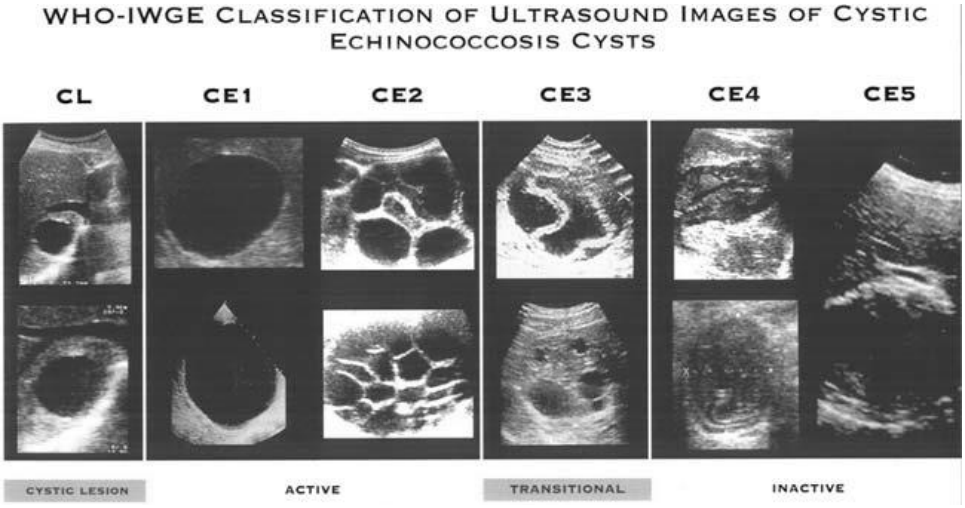

Fig. 2. 5 WHO-Informal Working Group on Echinococcosis standardized classification (WHO/CDS/CSR/APH/2001.6).

2.7 Prevention and control of cystic echinococcosis

Prevention and control of cystic echinococcosis involves measures taken to disrupt transmission in dogs and intermediate hosts. Interruption of the transmission cycle to avoid or reduce dog infection may be achieved by dog population management, eliminating unwanted dogs or by deworming using Praziquantel (Gemmell, 1990). These measures are aimed at reducing/stopping dog infections and consequently reducing human exposure to *Echinococcus* eggs. Methods used for interrupting transmission to dogs may include treatment of dogs with arecoline purgation or use of Praziquantel. These treatment strategies lead to infected dogs expelling adult worms from the small intestine hence reducing worm burden or total elimination of adult worms and therefore reducing transmission. Dog population management is recommended for control of CE and can be achieved through culling of stray/unwanted dogs (although unethical) and birth control though immunocontraception, spaying of bitches and castration of male dogs (Kachani and Heath, 2014; Craig *et al*., 2017).

Control should also target interrupting transmission from infected intermediate hosts and therefore livestock should be thoroughly inspected during slaughter and all condemned infected organs kept away from dogs. Livestock should be slaughtered at abattoirs that are managed by veterinary or public health officers (Craig *et al*., 2017). Vaccination of small ruminants with EG95 antigen based vaccine has shown promising results in both experimental and field trials (Lightowlers, 2006; Larrieu *et al*., 2013; Larrieu *et al*., 2015). Mathematic modelling recommends a combination of the small ruminant's vaccine and deworming of dogs with Praziquantel for effective control of CE (Torgerson and Heath, 2003). Culling of old livestock is also recommended to interrupt transmission because this age group has been shown to carry the highest burden of CE (Craig *et al*., 2017).

Educating people on the transmission cycle and preventive measures of CE is an important component of successful control and prevention. Lack of knowledge on CE and its transmission has been identified as major risk factor for CE infections in humans (Possenti *et al*., 2016; Craig *et al*., 2017). Despite these individual approaches for control and prevention of CE, an integrated scenario where nearly all the control measures are applied is recommended for successful control (Craig *et al*., 2017).

2.8 Zoonotic diseases caused by dog's intestinal parasites

2.8.1 Zoonotic nematode infections

2.8.1.1 Toxocariasis

Human toxocariasis is among the most neglected tropical diseases. This zoonosis is caused by the larval stage of *T. canis* and to a lesser extent *T. cati*, the roundworms of dogs and cats respectively (Schantz, 1989). The medical and public health impact of human toxocariasis is often underestimated due to the non-specific symptoms of this disease that are difficult to diagnose (Chen *et al*., 2018). Clinical syndromes associated with *Toxocara* infections in humans include: ocular larva migrans, visceral larva migrans, covert or common toxocariasis, and neurotoxocariasis (Ma *et al*., 2018). Humans become infected following accidental ingestion of embryonated eggs in soil and contaminated food or by ingestion of encapsulated larvae in improperly cooked tissues of paratenic hosts, such as cattle, sheep and chickens (Overgaauw and van Knapen, 2013; Strube *et al*., 2013). Data on human toxocariasis in Kenya is scanty and therefore the medical and public importance of this disease is unknown. In an earlier study, 7.5% of Turkana nomads tested positive for antibodies against *T. canis* excretory – secretory antigens (TES) (Kenny *et al*., 1995). Two separate studies based on necropsy detected adult worms in 3% of dogs in Nairobi (Wachira *et al*., 1993b) and in one of 42 dogs examined in Turkana (Buishi *et al*., 2006).

2.8.1.2 Ancylostomiasis

Canine hookworms *Ancylostoma caninum, A. braziliense, A. ceylanicum* and *Uncinaria stenocephala* are well known for their detrimental impacts on canine health (Miller, 1968; Georgi *et al*., 1969). In humans, these species are capable of producing cutaneous larva migrans (CLM) or 'ground itch', which is usually self-limiting (Bowman *et al*., 2010). Only *A. braziliense* can cause a more severe form of CLM ("creeping eruptions"), resulting in raised, highly pruritic tracks in humans (Malgor *et al*., 1996) that usually necessitate treatment (Caumes, 2000; Hochedez and Caumes, 2007). *A. ceylanicum* is the only species of canine hookworms capable of causing patent infections in humans, both naturally and experimentally (Traub, 2013). In rare cases *A. caninum* larvae develop into pre-adult, non-patent worms in the human intestine causing eosinophilic enteritis (Landmann and Prociv, 2003).

There is little information on the occurrence and public health significance of zoonotic canine hookworms in sub-Saharan Africa since most reports of CLM are based on cases of tourists and are diagnosed after return to their home countries (Jelinek *et al*., 1994; Bouchaud *et al*., 2000; Blackwell and Vega-Lopez, 2001; Kelkar, 2007; Dhir *et al*., 2010). However, lack of recognition is the most likely reason for this apparent absence in the local population (Ngui *et al*., 2012). Previous studies in Kenya have reported the occurrence of adult hookworms in dogs following post-mortem examination, but the species were never identified either morphologically or by molecular methods (Wachira *et al*., 1993b; Buishi *et al*., 2006).

2.8.1.3 Strongyloidiasis

Strongyloidiasis is an infection of dogs, cats, monkeys and humans caused by *Strongyloides stercoralis*. Humans become infected by skin penetration of larvae passed in dog faeces or when eggs hatch in the small intestines to cause auto-infection (Traub *et al*., 2002). Mild infections in

humans may present as abdominal pain and diarrhoea alternating with constipation while heavy infections may result in fever, liver tenderness, nausea, vomiting, weight loss and severe diarrhoea (Robertson and Thompson, 2002). The prevalence of *S. stercoralis* in humans is much lower than other helminths such as hookworms and *Ascaris lumbricoides* in Kenya. Prevalence of 1.3% in HIV-seropositive individuals and 3.6% sleeping sickness patients has been reported (Walson *et al*., 2010; Kagira *et al*., 2011).

2.8.2 Zoonotic cestode infections

2.8.2.1 Coenurosis

Coenurosis is a parasitic disease that affects intermediate herbivore hosts such as sheep, goats, horses, cattle, camels, deer and pigs and is caused by the metacestode stage (Coenurus cerebralis) of *T. multiceps*. Dogs, jackals, foxes and coyotes are the definitive hosts of *T. multiceps* (Scala *et al*., 2007). The development of the metacestode in the brain or the spinal cord is associated with severe clinical symptoms that include; depression, cycling, blindness, head deviation and ataxia. Infected ruminants scam to death leading to huge economic losses (Avcioglu *et al*., 2011). Coenurosis has been described in different forms of neurological disorders in humans in Italy, Egypt, North America and the United States, South Africa, Europe, India, Brazil, and Israel (Scala and Varcasia, 2006; Lescano and Zunt, 2013) and causes endophthalmitis and retinal detachment (Ibechukwu and Onwukeme, 1991).

2.8.2.2 Sparganosis

Human Sparganosis is a zoonotic disease caused by the plerocercoid larvae (spargana) of the genus *Spirometra*. Cats and dogs are the final hosts of the adult stage and there are two intermediate stages; the first hosted by small crustaceans and the second by vertebrates, usually fish, frogs, snakes, or other reptiles. Humans become infected following the ingestion of raw or undercooked

intermediate hosts, through drinking untreated water containing *Spirometra* larvae, or by using raw flesh in traditional poultices. Larvae do not mature within the human intestine, but become lodged in different tissues resulting in local tissue damage, paralysis, blindness and death. Twenty four human Sparganosis cases were reported in Kenya mainly from the Maasai and Pokot communities (Schmid and Watschinger, 1972). Case reports of human Sparganosis in Maasai community in Narok have surfaced recently (MOH, 2016). In the recent and past cases of human Sparganosis, transmission was linked to consumption of water contaminated by domestic dog or wild carnivore faeces. *Spirometra* spp. eggs are commonly shed by spotted hyenas (74.3%) (Engh *et al*., 2003) and lions (Kagendo *et al*., unpublished data) in Maasai Mara game reserve.

2.8.2.3 Dipylidiasis

Dipylidiasis is a human infection with the dog tapeworm *Dipylidium caninum*. Dogs and cats are the final hosts of *D. caninum* and release gravid proglottids in faeces. Ingestion of eggs by intermediate hosts (mainly fleas and body lice) leads to infection. Within the intermediate host the cestode develop into cysticercoid forms and when the intermediate host is ingested by dogs or cats, the cysticercoids develop into adult tapeworms in the small intestines. Humans become infected after accidental ingestion of infected fleas, body lice or saliva of pets. Young children are the most vulnerable to Dipylidiasis infection. This condition may be asymptomatic or may present as abdominal pain, diarrhea and pruritus. Only few human Dipylidiasis infections have been reported worldwide (Robertson and Thompson, 2002; Craig and Ito, 2007). Although no human Dipylidiasis infections have been reported in Kenya, infections in cats and dogs are documented (Wachira *et al*., 1993b; Buishi *et al*., 2006; Nyambura Njuguna *et al*., 2017).

2.8.3 Zoonotic protozoan infections

2.8.3.1 Cryptosporidiosis

Cryptosporidiosis, caused by apicomplexan protozoans of the genus *Cryptosporidium*, is an enteric disease in humans and animals worldwide. *Cryptosporidium* is considered the second greatest cause of diarrhea and death in children after rotavirus (Ryan and Hijjawi, 2015), and is transmitted via the faecal-oral route, either by consumption of contaminated water or food, or by person to person and zoonotic transmission (Xiao, 2010). Currently 27 species and 40 genotypes of *Cryptosporidium* are considered valid and only 20 species and genotypes infect humans. However, the majority of human infections are due to *C. hominis* and *C. parvum* (Ryan and Hijjawi, 2015). Domestic dogs can transmit *C. canis* and *C. parvum* to humans. *C. canis* can infect both immunocompetent and immunocompromised humans, but the greatest burden is reported in children in developing countries where it is responsible for 4.4% cryptosporidiosis case (Lucio-Forster *et al*., 2010; Ryan *et al*., 2014). In Kenya *C. hominis* and *C. parvum* are responsible for most human infections, although zoonotic species such as *C. canis, C. felis, C. muris* and *C. meleagridis* are also reported in humans (Gatei *et al*., 2002; Gatei *et al*., 2006; Mbae *et al*., 2015).

2.8.3.2 Giardiasis

Giardia duodenalis causes giardiasis in humans and most mammals, and is characterized by diarrhea, abdominal cramps, bloating, weight loss, and malabsorption (Feng and Xiao, 2011). *Giardia* is infectious to a wide range of domestic and wild animals (Thompson, 2004; Appelbee *et al*., 2005). Humans and animals are infected when they come into contact with infectious cysts in faeces in contaminated water, food, and fomites and or by direct physical contact (Feng and Xiao, 2011). Currently up to 8 assemblages (A – H) of *Giardia* are known, A and B for humans and other animals, C and D for dogs, E for artiodactyls, F for cats, G for rodents and H for marine vertebrates

(Caccio *et al*., 2005; Lasek-Nesselquist *et al*., 2010). Giardiasis in humans is caused by Assemblages A and B which are also zoonotic but occasionally humans may be infected with other assemblages (Feng and Xiao, 2011; Heyworth, 2016). Assemblages A and B are the main causes of Giardiasis in children in Kenya (Matey *et al*., 2016; Mbae *et al*., 2016).

The next chapters in this thesis describe and report field and laboratory studies that were conducted to determine the prevalence, distribution and molecular epidemiology of *Echinococcus*, *Taenia* species and other zoonotic intestinal parasites obtained from dogs in four CE endemic areas of Kenya.

CHAPTER THREE: PREVALENCE AND DISTRIBUTION OF ZOONOTIC INTESTINAL PARASITES OF DOGS IN FOUR REGIONS OF KENYA

3.1 Abstract

Dogs are reservoirs of several zoonotic parasites of public health significance worldwide. Intestinal parasites constitute some of the most common infections in animals and humans worldwide. In Kenya, the information on prevalence of zoonotic intestinal parasites of dogs and their role as potential reservoirs of zoonoses is poorly understood. This information is important for development of public health and veterinary strategies for the treatment and control of these zoonoses. This study examined intestinal parasites obtained from dog faecal samples collected in four regions of Kenya (Turkana, Meru, Isiolo and Maasai Mara). The faecal samples were examined using a quantitative method (McMaster) and a qualitative method (zinc chloride flotation-sieving technique). Of the 1,621 faecal samples examined 701/1,621 (43.24%) and 268/1,621 (16.53%) contained at least one parasite by zinc chloride flotation-sieving and McMaster methods respectively. Eleven parasite genera/families were identified namely hookworms, Taeniidae, *Spirometra* spp., Coccidia, *Toxocara* spp., *Trichuris* spp., *Toxascaris leonina*, *D. caninum*, *Anoplocephala* spp., *Mesostephanus* spp. and *Uncinaria stenocephala* (in order of frequency). Hookworms were the most common parasite detected followed by taeniids. This study reports intestinal parasites of dogs which are zoonotic or have zoonotic potential and therefore appropriate control measures such as population control of dogs, deworming, restraining of stray dogs and health education are recommended to avert potential transmission of these parasites to humans.

Keywords: Intestinal parasites; Dogs; McMaster; zinc chloride flotation-sieving technique

3.2 Introduction

Domestic dogs serve as companion animals to humans and contribute greatly to their emotional and social life, both psychologically and physiologically (Paul *et al*., 2010). However, dogs are reservoirs and transmit several zoonotic diseases of public health significance worldwide (Traub *et al*., 2002; Deplazes *et al*., 2011). More than 60 pathogens are known to be potentially zoonotic between dogs and humans (Macpherson, 2005). Intestinal parasites are among the most common infections in animals and humans worldwide (Dado *et al*., 2012; Pullan *et al*., 2014).

Transmission to humans is either by being in contact with infected dogs or being exposed to animal secretions and excretions, consumption of infected water and food (Robertson and Thompson, 2002). Environmental contamination of public areas and water sources with dog's faeces containing infective forms (eggs, larvae, cysts and oocysts) of intestinal parasites poses a risk for human infections. These infective stages can survive in harsh environment for a long time yet remaining infectious to humans. In many rural settings a close human-dog contact is very common especially in livestock keeping communities, and exposes people to high risk of infection with zoonoses transmitted by dogs (Soriano *et al*., 2010b). The transmission of these zoonoses is influenced by poor hygiene and overcrowding, poor veterinary extension services and limited knowledge of zoonoses (Traub *et al*., 2005). Populations at risk of infection with intestinal zoonotic parasites of dogs are small children, pregnant women, the elderly and immunocompromised individuals. Children are vulnerable because of behavioural characteristics such as pica while these other groups are at risk due to their immune status (Overgaauw, 1997a; Irwin, 2002).

High quality diagnosis, drug efficacy trials, monitoring of drug resistance and successful surveillance and control programmes require diagnostic techniques that offer high sensitivity, specificity, accuracy, precision and reproducibility (Banoo *et al*., 2006; Kenyon *et al*., 2009). The

detection of intestinal parasites in the veterinary field relies on several diagnostic techniques namely direct smear, McMaster method and Wisconsin flotation technique (MAFF, 1986). The McMaster method is a quantitative technique recommended by World Association for the Advancement of Veterinary Parasitology (WAAVP) for evaluating successes or failures of anthelmintic drugs treatment programmes due to its low cost and high sensitivity (Wood *et al*., 1995; Coles *et al*., 2006; Pereckiene *et al*., 2007). Over the years several modifications have followed the basic McMaster technique (Gordon and Whitlock, 1939; Whitlock, 1948) and include weight of faeces examined, type of flotation solution used, flotation time and speed, inclusion or exclusion of centrifugation step, time the sample is allowed to settle before microscopy, the design and number of McMaster counting chambers; the counting method and multiplication factors employed (Dunn and Keymer, 1986; MAFF, 1986; Cringoli *et al*., 2004; Pereckiene *et al*., 2007; Karamon *et al*., 2008; Cringoli *et al*., 2010).

Flotation techniques commonly used in diagnosis of intestinal parasites in dogs are flotation in tube and the Wisconsin (Egwang and Slocombe, 1982). Recent improvements of the McMaster method have seen the introduction of superior flotation techniques for faecal egg counts (FECs) such as FLOTAC (Cringoli, 2006; Cringoli *et al*., 2010) and Mini-FLOTAC (Cringoli *et al*., 2013). Generally these flotation techniques have low sensitivity in low intensity infections and are more suited for nematode detection but not other helminths (Bergquist *et al*., 2009; Rinaldi *et al*., 2011). However the success of flotation techniques is dependant on the flotation solution used, for example, saturated sodium chloride (FS2) has been recommended for detection of nematode and cestode eggs whereas saturated zinc sulphate (FS7) or zinc chloride for detection of eggs of trematodes and nematode larvae (Rinaldi *et al*., 2011). Mathis *et al*. (1996) developed a flotation-sieving technique for detecting taeniid eggs and this has been applied in numerous studies on

Echinococcus infections in dogs and wild carnivores (Hüttner *et al*., 2009; Kagendo *et al*., 2014; Conraths and Deplazes, 2015).

In Kenya the knowledge on occurrence of zoonotic intestinal parasites of dogs and their role as potential reservoirs of zoonoses is poorly understood. This information is important for the development of public health and veterinary strategies for treatment and control of these zoonoses. The only available data is based on very few dogs that were examined after necropsy. A study conducted in Nairobi showed infections with *Echinococcus* spp., *Ancylostoma* spp., *D. caninum* and *T. canis* after necropsy (Wachira *et al*., 1993b). Buishi *et al*. (2006) reported infection with *Echinococcus* spp., *T. hydatigena, Ancylostoma* spp., *D. caninum* and *T. canis* in 42 necropsied dogs from Turkana. The work reported herein analysed intestinal parasites in dog faecal samples with emphasises on helminths using zinc chloride flotation-sieving and McMaster techniques. Dog faecal samples were collected from four regions of Kenya; Turkana, Meru, Isiolo and Maasai Mara.

3.3 Materials and Methods

3.3.1 Study area and design

This was a cross sectional study, in which dog faecal samples were collected from the environment (ground) in four study sites namely: Turkana (West, North, Central and South), Narok South (Sekenani, Talek and Ewaso Ng'iro), Meru (Igembe central and Tharaka North) and Isiolo (Oldonyiro, Central, North & South). The study areas are indicated in Fig. 3.1 – 3.5. These study sites were chosen due to existing CE data on livestock, human (Turkana) and wildlife (Maasai Mara) (Addy *et al*., 2012; Mutwiri *et al*., 2013; Kagendo *et al*., 2014; Mbaya *et al*., 2014). The study aimed to compare agents of CE in the different regions reported from livestock, wild carnivores and humans with those in dogs, in order to understand the distribution and transmission

dynamics of *Echinococcus* spp. and factors influencing infections in humans and livestock which are essential for the implementation of control programmes.

3.3.1.1 Turkana

Turkana County is situated in the north-western part of Kenya between longitudes 34° 30’ and 36° 40’ E and latitudes 1° 30’ and 5° 30’ N and covers an area of 68,680.3 km² (Fig. 3.1). Turkana is an arid and semi-arid area which experiences warm and hot climate, with temperatures ranging between 20 °C and 41 °C with a mean of 30.5 °C. The county receives between 150 mm and 400 mm of erratic rainfall annually, with an average precipitation of 250 mm and also with recurring drought, famine and severe water shortage (Turkana County, 2016). The county is divided into 6 sub-counties namely Turkana North, Turkana West, Turkana Central, Loima, Turkana South and Turkana East. The Turkana people are nomads and keep cattle, donkeys, camels, sheep and goats as source of livelihood. Besides the Maasai people, the Turkana are the largest pastoral community in Kenya.

3.3.1.2 Isiolo

Isiolo County is situated in the lower eastern region of Kenya between coordinates 0° 05′ S and 2° N and 36° 50' and 39° 30' E (Fig. 3.2) and covers an area of approximately 25,700 km^2. The county is multi-ethnic and inhabited by the Ameru, Somali, Borana, Turkana, Samburu and Dorobo communities. The county has three sub-counties; Isiolo, Merti and Garbatulla. It is classified into three ecological zones, namely semi-arid, arid and very arid. Generally, the county receives low rainfall of 300 – 500 mm per year, and experiences average temperatures ranging from 12 °C to 28 °C. The county’s average altitude is 200 – 300 meters above the sea level although some parts reach 1000 meters above sea level (www.kenya-information-guide.com/isiolo-county.html). The

backbone of the county's economy is livestock production (cattle, sheep, goats and camels) with over 80 percent of the inhabitants relying on livestock for their livelihoods.

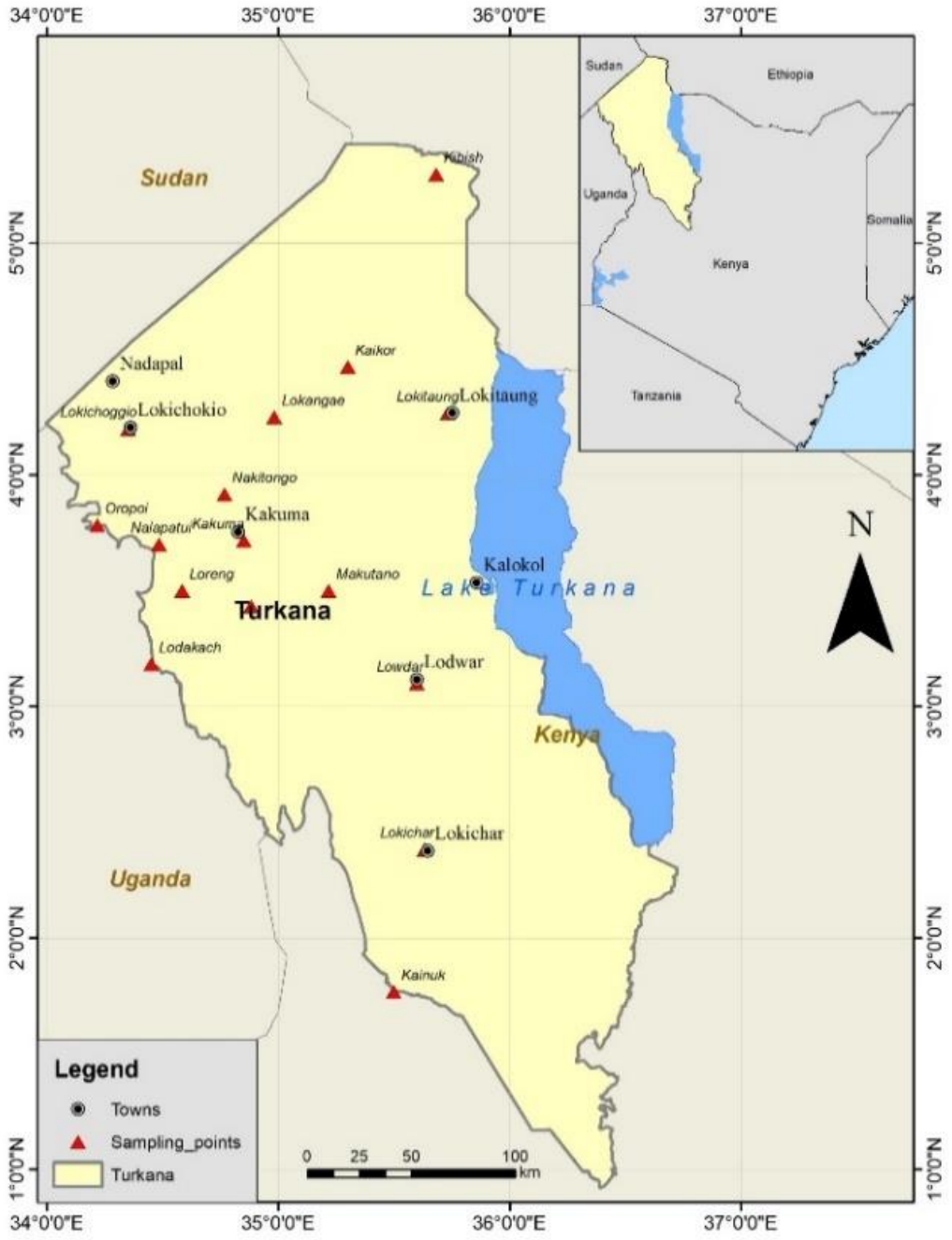

Fig. 3. 1 A map of Kenya showing study areas in Turkana County.

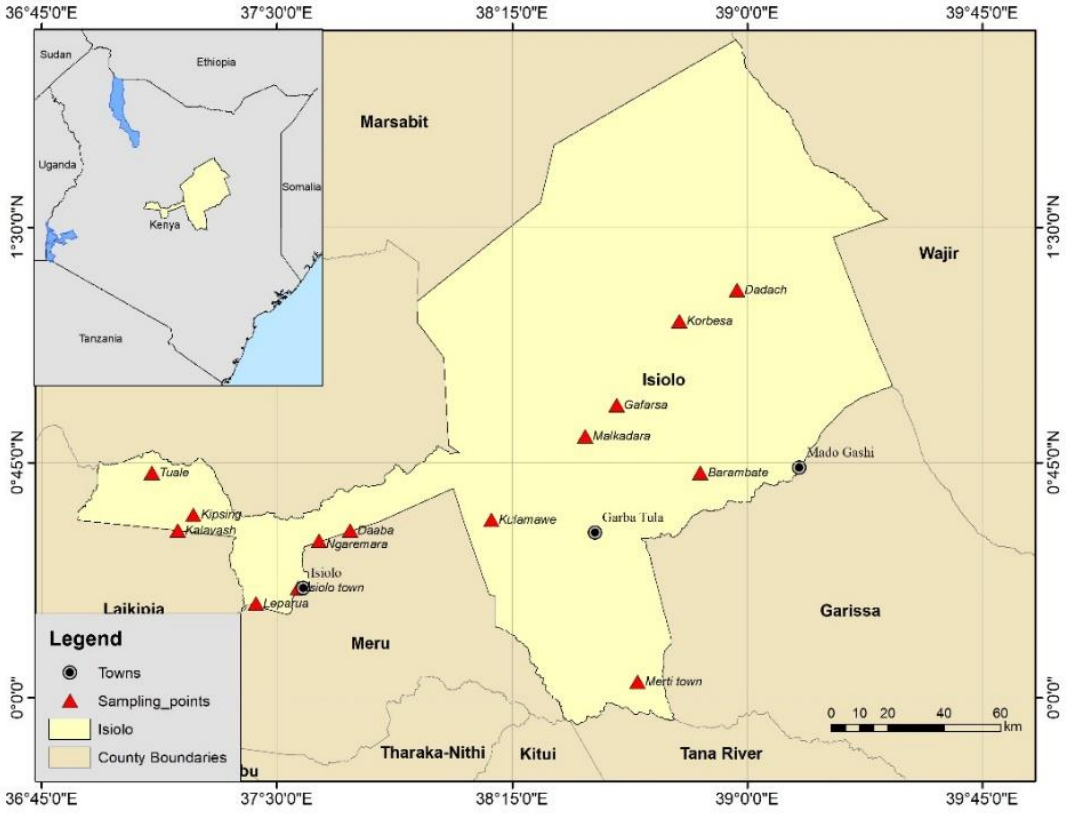

Fig. 3. 2 A map of Kenya showing study areas in Isiolo County.

3.3.1.3 Meru

The county of Meru lies to the east of Mt. Kenya whose peak cuts through the southern boundary. It straddles the equator lying within 0° 6' N and 0° 1' S, and between 37° 5' E and 38° 25' E (Fig 3.3). Meru County occupies a total area of 6,936.2 km^2 out of which 1,776.1 km^2 is gazetted forest. Altitude ranges from 300 m to 5,199 m above sea level. Climate is temperate with average temperature between 16 °C and 23 °C and average annual rainfall of 1600 mm (Meru County, 2015). It is inhabited predominantly by the Meru ethnic community, which are traditionally agriculturalists. The county's economy relies mostly on agriculture and less on livestock

production. Sampling was extended to Tharaka north where animal husbandry and farming are practiced.

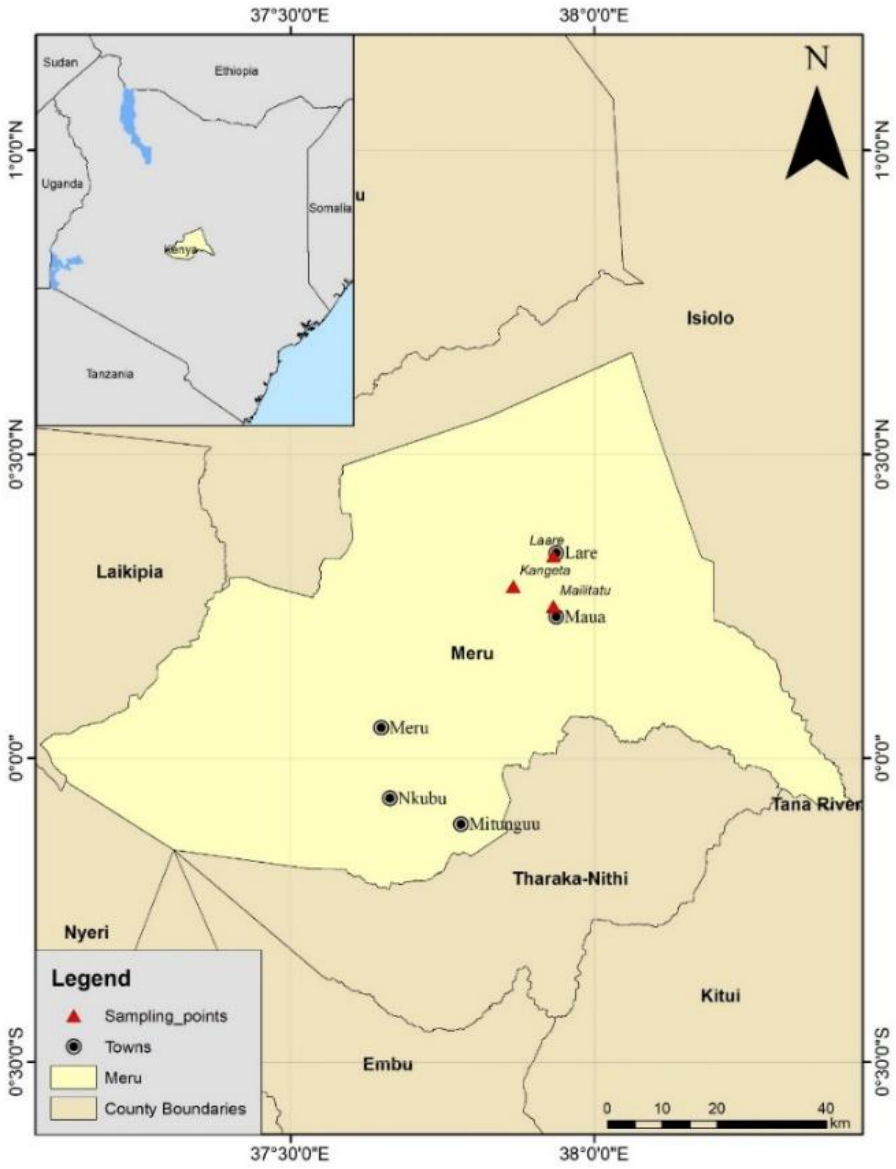

Fig. 3. 3 A map of Kenya showing study areas in Meru County.

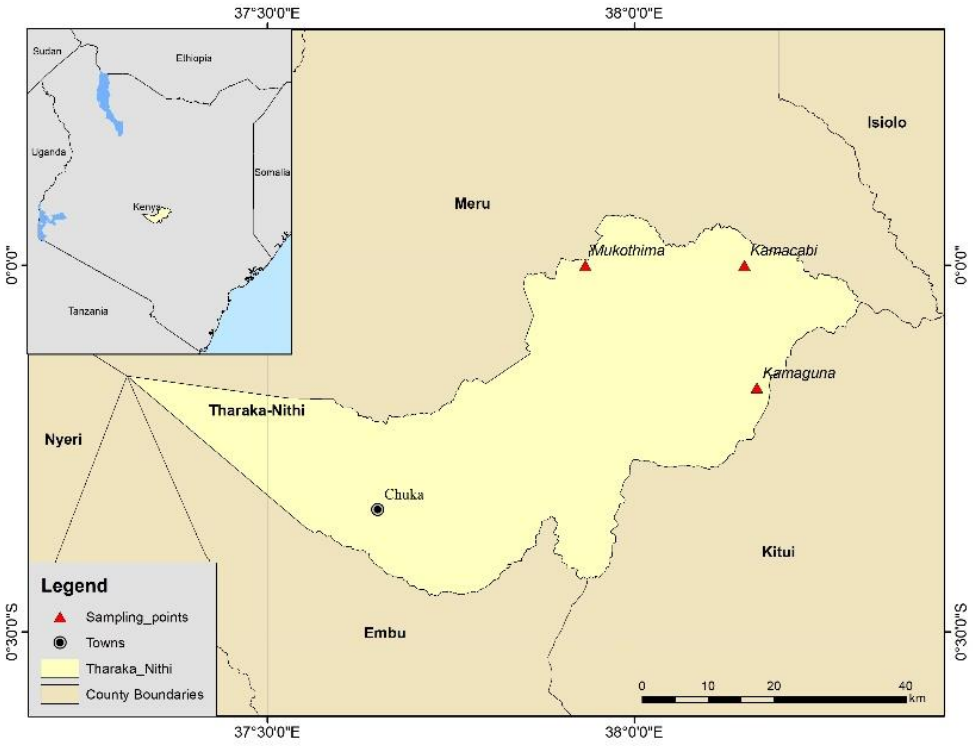

Fig. 3. 4 A map of Kenya showing study areas in Tharaka County (North).

3.3.1.4 Maasai Mara (Narok south)

Narok County is situated in South Rift Valley and shares a border with Tanzania to the South (Fig. 3.5). It covers an area of 17,944 km^2 and is made up of 6 sub-counties namely: Kilgoris, Narok North, Narok South, Narok East, Narok West and Emurua Dikirr. The average temperature range is 12 °C – 28 °C and rainfall range of 500 to 1,800 mm per annum. The county is located on the geographical coordinates; 1° 5’ S, 35° 52’ E (Indigenous Information Network, 2015). Part of the Maasai Mara National Reserve is located within Narok South sub-county. Tourism, wheat farming and livestock keeping (cattle, sheep and goats) are the major economic activities in this county.

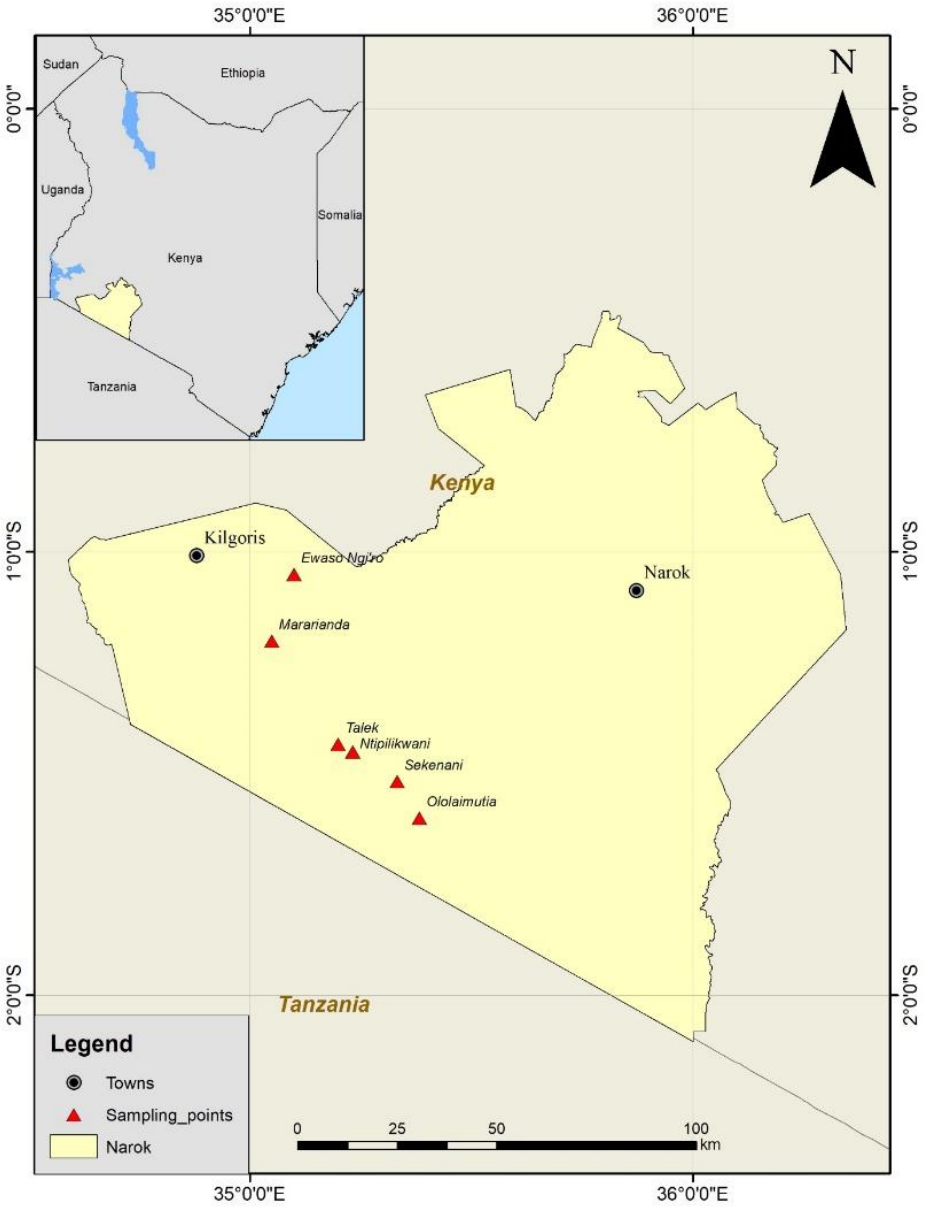

Fig. 3. 5 A map of Kenya showing study areas in Narok County (Maasai Mara).

3.3.2 Study population and dog faecal sampling

Dogs aged three months and above were targeted for this study. Consent was sought from the dog owners or heads of households before sampling (Appendix 1). Fresh dogs' faecal samples from each of the four study sites were collected from the environment around households with dogs, from pathways leading to homes and in rural and urban centres (some of them with slaughter slabs/abattoirs). Dog faecal samples were identified according to field signs as described (Verster, 1965) and in some cases with the help of household family members. To minimize repeated sampling from the same dog, only very fresh samples (up to one day old) were collected.

Faecal samples were aliquoted immediately into two portions: one portion was fixed with 10% formal-saline for intestinal parasites identification, while the second portion was preserved in 80% ethanol for molecular analysis. The fixed faecal samples were transported and stored at the parasitology laboratory of CMR, KEMRI. The sampling period was from September 2013 to June 2016.

3.3.2.1 Sample size calculation

The sample size was determined based on previous prevalence of *E. granulosus* s. l. infection in dogs from Turkana 17.6% – 63.5% (Macpherson *et al*., 1985) and in another study (Buishi *et al*., 2006) which reported *Echinococcus* spp. infection in 26% dogs by Copro-ELISA and 33% by necropsy. In Nairobi 10% of dogs examined had *Echinococcus* spp. worms post-mortem examination (Wachira *et al*., 1993b). The minimum sample size required in this study was calculated using the formula for significant difference for two proportions (two sided) as follows:

$$N = [Z_{\alpha/2} + Z_{\beta}]^2 \times [P_1(1-P_1) + P_2(1-P_2)] \Big/ (P_1 - P_2)^2$$

Where:

P_1 = the proportion of positive specimens in a region e.g. Turkana (P_1 was estimated at 0.3)

P_2 = the proportion of positive specimens in comparator region e.g. Meru (P_2 was estimated at 0.2)

$Z_{\alpha/2}$ = the desired **level of statistical significance** (typically 1.96)

Z_{β} = the **desired power** (typically 0.84 for 80% power)

$P_1 - P_2$ = desired effect size (or difference of clinical importance)

$$N = \frac{[1.96 + 0.84]^2 \times [0.25\,(1 - 0.25) + 0.2(1 - 0.2)]}{(0.25 - 0.2)^2}$$

$$N = \frac{[7.84 \times [0.1875 + 0.16]}{0.0025}$$

$$N = \frac{0.2352}{0.0025}$$

N = 94 in each study site/season

N = 188 dogs/study site for the two seasons

N = 752 dogs was proposed to be sampled from all the study sites for the two different seasons. The underlying hypothesis considered that a sample size of 752 dogs (94 x 4 study sites x 2 two seasons) would be sufficient to detect significant differences of 10 % in prevalence of CE in dogs with 80% power and a 5% significance level. The 10% difference represents the difference between CE infections in any two areas included in the study.

3.3.3 Detection of intestinal parasites in dogs faecal samples by McMaster method

Twenty eight millilitres of saturated sodium chloride solution was transferred into plastic containers and approximately 2 g of faecal material added, stirred and passed through home-made sieves to remove excess debri. The suspension was left to settle and 0.15 ml transferred into the

two chambers of McMaster slide and examined under a microscopy at X100 magnification. The samples were examined for presence of eggs or cysts and the number multiplied by a factor of 100 to determine the eggs/cysts per gram (EPG/CPG) (MAFF, 1986).

3.3.4 Detection of intestinal parasites in dogs faecal samples by zinc chloride flotation-sieving technique

Approximately 2 – 3 g of faecal material was analysed by zinc chloride flotation-sieving technique (Mathis *et al*., 1996) and microscopically examined for the presence of intestinal parasites. Briefly, faecal samples drained of ethanol were transferred into 15 ml falcon tubes, and rinsed with 8 – 12 ml of distilled water. Four parts of saturated zinc chloride solution (specific gravity = 1.45) were mixed with one part of the faecal pellet, and, after flotation, the top layer containing helminth eggs was filtered through two sequential sieves of 50 µm and 22 µm (Franz Eckert GmbH, Germany) respectively using distilled water. In order to dislodge the retained helminth eggs the second sieve (22 µm) was inverted and washed off with distilled water via a funnel connected to a 15 ml falcon tube. After centrifugation, excess distilled water was removed and the pellet containing helminth eggs stored in 2 ml microcentrifuge tubes containing 70% ethanol. The concentrate/pellet was then examined microscopically for the presence of helminth eggs.

3.3.5 Data analysis

The prevalence and exact binomial confidence intervals (95% CI) were calculated for each study site. Mean eggs per gram (EPG) of faeces were calculated by the arithmetic mean. STATA version 12.0 (STATA Corporation, College Station, Texas, USA) was used for all statistical analyses.

3.3.6 Ethical approval

This study was approved by the Scientific Ethics Review Unit (SERU) and the Animal care and use committee at KEMRI (SSC. No. 2658) (Appendix 3). Permission to conduct this study was also obtained from the Department of Veterinary Services, Kenya (Appendix 4).

3.4 Results

3.4.1 Detection of intestinal parasites by McMaster and zinc chloride flotation-sieving

A total of 1,621 were collected from the 4 study sites: Turkana (524), Meru (303), Isiolo (294) and Maasai Mara (500) (Table 3.1). Of the 1,621 faecal samples examined 701/1,621 (43.24%) and 268/1,621 (16.53%) contained at least one parasite by zinc chloride flotation-sieving and McMaster methods respectively (Table 3.2). Eleven parasite genera/families were detected by the McMaster method namely hookworms, Coccidia, *Toxocara* spp., *Toxascaris leonina*, Taeniidae, *Trichuris* spp., *Spirometra* spp., *D. caninum*, *Anoplocephala* spp., *Mesostephanus* spp. and *Uncinaria stenocephala* (Table 3.3). The zinc chloride flotation-sieving technique identified only 8/11 of the parasites genera/families reported by McMaster method but failed to detect *Anoplocephala* spp., *Mesostephanus* spp. and *Uncinaria stenocephala* (Table 3.4). However, both methods reported hookworm as the most common parasite in the dog faecal samples (Table 3.3 and 3.4).

3.4.2 Multiple infections with intestinal parasites in dog's faecal samples from four regions of Kenya

Single parasite infections were detected in 599/1621 (36.95%) and 246/1621 (15.18%) faecal samples using zinc chloride flotation-sieving and McMaster methods respectively. Co-infections of 2 parasites were reported in 93/1,621 (5.73%) using zinc chloride flotation-sieving technique and 22/1,621 (1.36%) by McMaster method. The McMaster method failed to detect more than 2 infections in faecal samples while zinc chloride flotation-sieving technique detected multiple infections involving 3 parasites in 6/1,621 (0.37%), and 4 parasites in 3/1,621 (0.19%) (Table 3.2).

Table 3. 1 Study areas and sites for faecal samples collection

Study sites	Area	No. of dog faecal samples collected
Turkana	West	185
	North	87
	Central	162
	South	90
Total		**524**
Maasai Mara	Sekenani	183
(Narok South)	Talek	214
	Ewaso Ng'iro	103
Total		**500**
Isiolo	Oldonyiro	114
	Central	93
	South	24
	North (Merti)	63
Total		**294**
Meru	Igembe Central – Ndoleli	93
	Laare	101
	Tharaka North	109
Total		**303**
Overall		**1621**

Table 3. 2 Multiple infections with intestinal parasites in dog's faecal samples from CE endemic areas of Kenya

No. of parasites	No. of faecal samples	Prevalence %	No. of faecal samples	Prevalence %
	zinc chloride flotation-sieving		McMaster	
0	920/1621	56.76	1353/1621	83.47
1	599/1621	36.95	246/1621	15.18
2	93/1621	5.73	22/1621	1.36
3	6/1621	0.37		
4	3/1621	0.19		

3.4.3 Intensity of parasitic infections in faecal samples

The intensity of infection was only reported for McMaster method, Coccidia had the highest intensity (OPG = 632.73), taeniids (EPG = 500), hookworms (EPG = 301.05), *Toxocara/Toxascaris* (EPG = 287.5), *Anoplocephala* (EPG = 150), *Trichuris, Spirometra, D. caninum, U. stenocephala* and *Mesostephanus* spp. all had EPG of 100 (Table 3.3).

3.4.4 Prevalence of dog intestinal parasites as determined by McMaster and zinc chloride flotation-sieving technique

The overall prevalence of hookworm, taeniids and *Spirometra* was higher by zinc chloride flotation-sieving technique and these were significantly different when compared to McMaster method of detection ($p<0.001$). The prevalence of Coccidia was however highest by McMaster compared to zinc chloride flotation-sieving technique (3.39% vs 1.85%; $\chi^2=870.3$, $p<0.001$) respectively (Table 3.3 and 3.4).

Table 3. 3 Prevalence and intensity of intestinal parasites detected in dog faecal samples by the McMaster method

Parasite species	Study sites				Total	Prevalence	95% CI	Mean
	Turkana	Isiolo	Meru	Maasai Mara				
	n = 524	n = 294	n = 303	n = 500	1621	%		EPG/OPG
Hookworm	30	5	50	104	189	11.65	10.14 – 13.32	301.05
Coccidia	15	16	3	21	55	3.39	2.67 – 4.39	632.73
Toxocara/Toxascaris	3	0	10	12	24	1.48	0.95 – 2.19	287.5
Taeniidae	5	0	0	4	9	0.56	0.25 – 1.05	500
Trichuris spp.	0	0	0	4	4	0.25	0.07 – 0.63	100
Spirometra spp.	0	0	1	2	3	0.18	0.04 – 0.54	100
Dipylidium caninum	1	0	1	0	2	0.12	0.01 – 0.44	100
Anoplocephala spp.	0	1	0	1	2	0.12	0.01 – 0.44	150
Uncinaria stenocephala	0	0	1	0	1	0.06	0.00 – 0.34	100
Mesostephanus spp.	0	0	1	0	1	0.06	0.00 – 0.34	100

Table 3. 4 Prevalence of intestinal parasites detected in dog faecal samples by the zinc chloride flotation-sieving technique

Parasites	Study sites					Prevalence %	95% CI
	Turkana n = 524	Meru n = 303	Isiolo n = 294	Maasai Mara n = 500	Total n = 1621		
Hookworms	62	136	58	234	490	30.23	27.99 – 32.53
Taeniidae	113	7	24	34	178	10.98	9.49 – 12.60
Spirometra spp.	0	1	0	55	56	3.45	2.62 – 4.46
Toxocara/Toxascaris	5	16	2	17	40	2.47	1.77 – 3.34
Coccidia	9	3	6	12	30	1.85	1.25 – 2.63
Trichuris spp.	0	6	0	11	17	1.05	0.61 – 1.67
Dipylidium caninum	1	3	0	0	4	0.25	0.07 – 0.63

3.5 Discussion

The overall prevalence of intestinal parasites of dogs in this study was (701/1621) 43.24% which is in agreement with findings from other studies worldwide; 41.5% in USA (Gates and Nolan, 2009), 43.3% in Nigeria (Ayinmode *et al*., 2016), 45.2% in Zambia (Nonaka *et al*., 2011), 43.3% in Cuba (Puebla *et al*., 2015), and 45.7% in Slovak republic (Szabová *et al*., 2007). In this study up to 11 families/genera of intestinal parasites were identified and included: hookworms, Taeniidae, *Spirometra* spp., Coccidia, *Toxocara* spp., *Trichuris* spp., *T. leonina*, *D. caninum*, *Anoplocephala* spp., *Mesostephanus* spp. and *U. stenocephala* (in order of frequency). Although the results can be compared to those of other studies, it is worth noting that different factors are likely to influence the results of one study to the other. These factors may include sampling procedure (necropsy vs copromicroscopy), varying ecological and climatic conditions, the sample size, differences in detection techniques, and the endemicity of a particular parasite (Robertson *et al*., 2000). Furthermore the number of different species identified, the differences in prevalence from one study to another and also differences in the most prevalent parasites detected might also depend on the diagnostic technique used (Mateus *et al*., 2014).

This study reported intestinal parasites in dog faecal samples that have zoonotic potential, mainly nematodes and cestodes. Some of the zoonotic cestodes identified included taeniids (*Echinococcus* and *Taenia* spp.), *Spirometra* spp., *Anoplocephala* spp. and *Dipylidium caninum*. Molecular characterization of taeniids are discussed in details in chapters five (*Echinococcus* spp.) and six (*Taenia* spp.) of this thesis. The prevalence of taeniids in this study is comparable with a study conducted in Argentina (Soriano *et al*., 2010b). Proglottids of taeniids are not regularly voided in faeces and therefore coprodiagnosis techniques are likely to miss 40 – 45% of infections (Lillis, 1967).

The genus *Spirometra* occurs worldwide, but is most common in China, Korea, Japan and North America. In some parts of China, human Sparganosis is considered a major public health problem where more than 1,000 human infections are known (Liu *et al*., 2015b; Hong *et al*., 2016). Human infections are common in Asia due to feeding of food containing raw snails, frogs, or snakes (Lescano and Zunt, 2013; Liu *et al*., 2015b). Human Sparganosis cases are rare in Africa and mainly reported as case reports, such as in the Maasai and Pokot communities in Kenya (Schmid and Watschinger, 1972) and recently in Narok County (MOH, 2016). In Ethiopia and South Sudan 3 and 34 case reports respectively were observed during Guinea worm eradication campaigns (Eberhard *et al*., 2015). Elsewhere in northern Tanzania 62.5% of 216 individuals tested positive for anti-sparganum IgG antibodies (Kavana *et al*., 2016). In Kenya human Sparganosis infections are linked to consumption of water contaminated by faeces of domestic dogs or wild carnivores. *Spirometra* spp. eggs are commonly reported in faeces of spotted hyenas (74.3%) (Engh *et al*., 2003) and lions in Maasai Mara game reserve (Kagendo *et al*., unpublished data). Human cases of Sparganosis coincide with infection in dogs and wild carnivores in Maasai Mara. Nelson *et al*. (1965) suggested that wild animals may acts as reservoirs of such as zoonotic cestodes in this region. Wild herbivores can serve as second intermediate hosts and once preyed by dogs and wild carnivores maintain the life cycle of *Spirometra* spp. (Nelson *et al*., 1965; Muller-Graf, 1995). *Spirometra* spp. were highly prevalent in faecal samples of lions and hyena in Tanzania and Zambia (Muller-Graf, 1995; Muller-Graf *et al*., 1999; Berentsen *et al*., 2012). There are however few studies reporting occurrence of *Spirometra* in dogs in Africa. *Spirometra* spp. infections in dogs in this study (3.45%) was closer to that reported in Japan (3.6%), but much lower than in endemic areas of China (up to 77%) (Liu *et al*., 2015b).

Dipylidiasis is a human infection with the dog tapeworm *D. caninum*. Over 120 cases of human Dipylidiasis have been reported mainly in children from India, Russia, Japan Brazil and Chile (Narasimham *et al*., 2013; Sarvi *et al*., 2018). No human Dipylidiasis infections have been reported in Kenya so far. Post-mortem examination of intestinal contents of dogs after necropsy revealed 45% of dogs infected with *D. caninum* in Nairobi (Wachira *et al*., 1993b) and in Turkana (Buishi *et al*., 2006). Faecal examination revealed that 8.7% of domestic cats had eggs of *D. caninum* in Thika (Nyambura Njuguna *et al*., 2017). The prevalence reported in this study was much lower than in other studies in Kenya, owing to the expected differences between post-mortem results compared to faecal examination for cestodes (Villeneuve *et al*., 2015). Results from other African countries reveal variations in the prevalence of *D. caninum* in dogs, in Zambia (2.2 – 2.4%), 8.6% in Gabon and 5.83 – 14.8% in Nigeria and 22.4 – 34.3% in Ethiopia (Chidumayo, 2018). Proglottids of *D. caninum* are not regularly voided in faeces and therefore coprodiagnosis techniques are likely to miss up to 95% of infections (Lillis, 1967).

This study identified nematodes of both medical and veterinary importance that included: hookworm, *T. canis*, *T. leonina*, *T. vulpis* and *U. stenocephala*. Hookworms were the most common intestinal parasites reported in dogs which is in agreement with results of other studies in sub-Saharan Africa (Chidumayo, 2018). Majority of the faecal samples were collected from dogs of age > 1 year, which may explain why hookworms were the dominant species as opposed to *T. canis* which commonly occurs in puppies and young dogs aged ≤ 6 months (Mandarino-Pereira *et al*., 2010). Detailed descriptions of hookworm prevalence and species identified in dogs from this study are discussed in chapter four of this thesis. The detection of *U. stenocephala* in a dog in this study was rather unusual as this hookworm species is adapted to temperate and subarctic regions (Traversa, 2012). However, *U. stenocephala* was recently reported in dogs in Tanzania although

the authors postulated that this species might have been introduced by dogs imported from Denmark (Merino-Tejedor *et al*., 2018). Although the presence of *U. stenocephala* was not confirmed by molecular methods, the eggs of this species are generally larger than those of other canine hookworms (Kalkofen, 1987).

Toxocara spp. infections in humans cause ocular larva migrans, visceral larva migrans, covert or common toxocariasis, and neurotoxocariasis (Ma *et al*., 2018). Human toxocariasis occurs worldwide (Lee *et al*., 2010) but is common in subtropical and tropical regions affecting the poorest communities of developing countries (Overgaauw, 1997a; Macpherson, 2013). The burden of toxocariasis is unknown or likely to be underestimated in endemic areas because the data available is based on serodiagnosis in humans which faces several challenges such differentiating exposure, past and current infections, choice of antigens and cross-reactivity with other parasites (Fisher, 2003; Poulsen *et al*., 2015; Holland, 2017). Data on human toxocariasis in Kenya is scanty and therefore the relative contribution of domestic dogs and cats to human infection is unknown. In an earlier study 7.5% of Turkana nomads tested positive for antibodies against *T. canis* excretory – secretory antigens (TES) (Kenny *et al*., 1995). The prevalence of *T. canis* in this study was in the range of that reported in two previous studies in Nairobi (3%) (Wachira *et al*., 1993b) and in Turkana (2.38%) (Buishi *et al*., 2006). However these two earlier studies reported presence of adult worms obtained after necropsy.

In Africa studies reporting the prevalence of *T. canis* in dog faecal samples are few apart from in Nigeria and Ethiopia where extensive research on gastrointestinal parasites in dogs have been reported. The prevalence reported in this study is comparable with studies in Ethiopia (3.06%) (Chidumayo, 2018). The findings of this study were different to those of other studies that reported *T. canis* as the most common intestinal parasites in dogs. The prevalence of *T. canis* varied among

different countries and parts in the same country, they ranged between 3.3 - 95% in Nigeria, 21.82 - 39.79% in Ethiopia, 7.9% in South Africa, 11% in Zambia, 13.7% in Tanzania, 5.8 - 18.8%, 34.35% in Cameroon, 58.5% in Gabon and 63.63% in Madagascar (Chidumayo, 2018). The frequency and burden of *T. canis* are normally higher in young dogs and puppies than older dogs because they also acquire infections via transmammary and/or transplacental routes (Overgaauw, 1997b; Deplazes *et al*., 2011). *Toxascaris leonina* is thought to occur rarely as compared to *T. canis* (Xhaxhiu *et al*., 2011; Mateus *et al*., 2014), as was the case in this study but this is contrary to findings of Beiromvand *et al*. (2013).

Trichuris vulpis is a nematode that inhabits the large intestine of domestic and wild canids and has a worldwide distribution (Traversa, 2011). The zoonotic potential of *T. vulpis* is not well understood because only case reports of human infections exists, where diagnosis was based on detection and measurement of eggs in human faeces. Thus such diagnosis might have been erroneous since morphological characteristics of the adult worm or molecular tools were not used (Dunn *et al*., 2002). Furthermore measurements of eggs are inadequate to differentiate between *T. trichiura* and *T. vulpis* (Traversa, 2011). In this study *Trichuris* spp. were only detected in Maasai Mara and Meru, coincidentally in counties with highest prevalence of hookworms. The eggs of this parasite undergo development under favourable rainfall and humidity conditions, thus more prevalent in Maasai Mara and Meru and less frequent in drier and hot conditions of Turkana and Isiolo (Maya *et al*., 2012). An association of biological and epidemiological factors favours the coexistence of both *T. vulpis* and hookworms (Fontanarrosa *et al*., 2006; Traversa, 2011). Infection with *T. vulpis* in dogs is much higher in older dogs than younger ones because long exposure to the infective eggs in the environment for long periods and also because this parasite is not transmitted via transmammary and transplacental routes like hookworms and *T. canis* (Schantz, 1999). The

prevalence of *Trichuris* spp. in this study can be compared with that reported in other studies in Ethiopia (0.6%) and Nigeria (0.5%), however, in other studies the prevalence ranged between 3.7 – 31.8% in Nigeria, 3.3 – 7.95% in Ethiopia, 6 – 7.9% South Africa and 49.5% in Gabon (Chidumayo, 2018). The lower prevalence could be explained by the heavy eggs of *T. vulpis* (SG 1.13) that could have been missed by flotation techniques. The appropriate method for their detection is based on centrifugation (Zajac *et al.*, 2002; Dryden *et al.*, 2005). However the use of flotation solution with higher specificity gravity such as zinc chloride as was the case in this study also increases the recovery of *Trichuris* spp. eggs from faecal samples (Kochanowski *et al*., 2014).

3.6 Conclusions

Hookworms were the most common parasite detected in this study. Some of the parasites detected in dog faecal samples in this study have zoonotic potential and therefore appropriate control measures such as population control of dogs, deworming, restraining of stray dogs and health education are recommended to avert the possible transmission of these parasites to humans.

CHAPTER FOUR: MOLECULAR IDENTIFICATION OF ZOONOTIC HOOKWORMS IN DOGS FROM FOUR COUNTIES OF KENYA

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4.1 Abstract

All canine hookworms are known to be zoonotic, causing infections ranging from transient skin irritations to prolonged 'creeping eruptions', eosinophilic enteritis and even patent intestinal infections. There is little information on canine hookworm species and their public health significance in sub-Saharan Africa. This study determined the prevalence and species of hookworms in dogs from different climatic zones of Kenya. Dog faecal samples were collected from the environment and hookworm eggs were isolated by zinc chloride flotation-sieving then subjected to DNA extraction. Polymerase chain reaction (PCR) assays targeting the internal transcribed spacer (ITS) 1 and 2, 5.8S and 28S ribosomal RNA of *Ancylostoma* spp. and *Uncinaria stenocephala* were performed, and hookworm species were identified by PCR restriction fragment length polymorphism (PCR-RFLP) or DNA sequencing. Hookworm eggs were detected by microscopy in 490/1621 (30.23%, 95% CI 27.99 –32.53) faecal samples. The prevalence of hookworms was high in counties receiving higher rainfall (Narok 46.80%, Meru 44.88%) and low in those with a more arid climate (Isiolo 19.73%, Turkana 11.83%). In a subset of 70 faecal samples, *Ancylostoma caninum* (n = 59) was the most common species, followed by *A. braziliense* (n = 10) and *A.* cf. *duodenale* (n = 1). This study reports for the first time the detection of *A.* cf. *duodenale* in dog faeces and zoonotic hookworm species in Kenyan dogs. These findings emphasize the need for control measures such as enforcing laws for restraining stray dogs, regular deworming of dogs, and public health awareness programmes aimed at informing communities on outdoor use of footwear.

Keywords: Hookworm species; *Ancylostoma caninum; Ancylostoma braziliense; Ancylostoma duodenale*; Dogs; Kenya

4.2 Introduction

The canine hookworms *Ancylostoma caninum, Ancylostoma braziliense, Ancylostoma ceylanicum* and *Uncinaria stenocephala* are well known for their detrimental impacts on canine health. *Ancylostoma caninum* is a leading cause of acute, potentially fatal haemorrhagic enteritis in young puppies and causes chronic iron-deficiency anaemia in older dogs (Miller, 1968; Georgi *et al*., 1969). However, in addition to their veterinary significance, all four canine hookworm species have zoonotic potential. In humans, these species produce cutaneous larva migrans (CLM) or 'ground itch', which is usually self-limiting (Bowman *et al*., 2010). Only *A. braziliense* can cause a more severe form of CLM ('creeping eruptions'), resulting in raised, highly pruritic tracks in humans (Malgor *et al*., 1996), which usually necessitate treatment (Caumes, 2000; Hochedez and Caumes, 2007). *Ancylostoma ceylanicum* is the only species of canine hookworms capable of causing patent infections in humans, both naturally and experimentally (Traub, 2013). This hookworm is the agent of an emerging zoonosis that is being increasingly detected during molecular-based epidemiological surveys in the Asia Pacific region of Cambodia (Inpankaew *et al*., 2014), Malaysia (Ngui *et al*., 2012), Thailand (Traub *et al*., 2008; Jiraanankul *et al*., 2011), Laos (Sato *et al*., 2010; Conlan *et al*., 2012), Solomon Islands (Speare *et al*., 2016; Bradbury *et al*., 2017), and Australia (Koehler *et al*., 2013; Smout *et al*., 2017). In rare cases, *A. caninum* larvae develop into pre-adult, non-patent worms in the human intestine, causing eosinophilic enteritis (Landmann and Prociv, 2003). It is likely that this zoonosis is grossly underdiagnosed due to the obscure and non-specific clinical signs that often accompany the infection coupled with the challenges of diagnosis (Bradbury and Traub, 2016).

There is little information on the occurrence and public health significance of zoonotic canine hookworms in sub-Saharan Africa, since most reports of CLM are based on cases of tourists

diagnosed after returning to their home countries (Jelinek *et al*., 1994; Bouchaud *et al*., 2000; Blackwell and Vega-Lopez, 2001; Kelkar, 2007; Dhir *et al*., 2010). However, lack of recognition is the most likely reason for this apparent absence in the local population (Ngui *et al*., 2012). Previous studies in Kenya have reported the occurrence of adult hookworms in dogs following post-mortem examination, but the species were never identified either morphologically or by molecular methods (Wachira *et al*., 1993b; Buishi *et al*., 2006). However, in a recent study in Tanzania, four zoonotic hookworm species (*A. caninum, A. braziliense, A. ceylanicum* and *U. stenocephala*) were detected in dogs (Merino-Tejedor *et al*., 2018). As the frequency of hookworm infection is associated with climatic conditions, and is usually more frequent in regions with higher rainfall because of better conditions for soil larvae, the sites for this study were regions of Kenya within different climatic zones. This study reports the estimated prevalence and species of zoonotic hookworms in dogs from four different ecological regions of Kenya using molecular methods.

4.3 Materials and methods

4.3.1 Dog faecal samples collection

Single canine faecal samples were collected from the environment in four counties of Kenya as described in sections 3.3.1. and 3.3.2. The faecal samples (~20 – 30 g) were preserved in 80% ethanol immediately after collection.

4.3.2 Isolation of hookworm eggs

Briefly, the ethanol was drained, 2 ml (~2 g) of stool were transferred into 15 ml falcon tubes and the sample was rinsed with 8 ml of distilled water. Helminth eggs were recovered using the zinc chloride flotation-sieving technique (specific density: 1.45) (Mathis *et al*., 1996). The concentrate/pellet was examined microscopically for the presence of helminth eggs (section 3.3.4).

4.3.3 DNA extraction

A total of 78/490 microscopically positive faecal samples were selected randomly for DNA extraction. All the faecal samples selected had parasite intensity of at least 300 eggs per gram, based on the McMaster method (results not shown here), and these included 20 from three study sites (Turkana, Meru, Narok) and 18 samples from Isiolo. The eggs from each sample were settled by centrifugation at 8000 rpm for 1 minute, and the excess water was removed by decanting. The QIAamp® DNA Stool Mini Kit (Qiagen) was used to extract total DNA according to the manufacturer's protocol, with slight modification. Briefly, the eggs were lysed through three rounds of freezing (−80°C for 30 minutes) and thawing (80°C for 15 minutes). DNA was eluted in 50 µl elution buffer and stored at −20°C awaiting PCR processing. Positive control genomic DNA for *A. caninum* and *A. ceylanicum* was provided by Prof. Rebecca Traub (University of Melbourne, Australia).

4.3.4 Detection of host species DNA

To identify the host (source) of faecal sample containing *A. duodenale* eggs, genomic DNA was extracted from the faecal sample as described by Dinkel *et al*. (1998). Briefly, 0.5 g of dog faecal sample was emulsified in 1 ml of distilled water (1:2) and 1.5 ml of the suspension transferred into 15 ml falcon tubes. To the faecal suspension, 108 µl of 1M KOH and 30 µl of 1 M dithiothreitol (DTT) were added, thoroughly mixed and incubated at 65 °C for 30 min. The potassium hydroxide was neutralized by adding 270 µl of 2 M Tris-HCl (pH 8.3) and 40.5 µl of (25%) HCl. Host genomic DNA was isolated by adding 1950 µl Phenol-Chloroform-Isoamylalcohol solution (25:24:1) to the sample, thoroughly mixed and centrifuged at 10,000 g for 10 min. The upper aqueous DNA phase (~1700 µl) was transferred into a new 15 ml falcon tube, then 5.4 ml of binding buffer and of 30 µl Prep-A-Gene®Matrix added followed by incubation for 1hr at 37 °C with

maintaining constant rotation. The suspension was centrifuged for 10 min at 10,000 g, supernatant discarded and pellet resuspended in 1 ml binding buffer then transferred into a new 1.5 ml microcentrifuge tube and centrifuged at 16,000 g for 30 s. The supernatant was discarded and the pellet rinsed three times in wash buffer with centrifugation at each step for 30 s at 16,000 g. The pellet was dried in a 45 ℃ incubator for 1 hr and resuspended in 50 µl of nuclease free water then incubated at 50 ℃ for 15 min. The suspension was centrifuged at 16, 000 g and 40 µl of the supernatant transferred into a new 1.5 ml microcentrifuge tube and stored at – 20 ℃ until use.

A nested PCR was performed to amplify the canid and felid specific cytochrome b gene using primer pairs and methods previously described by Hüttner *et al*. (2009) and Kagendo *et al*. (2014). Primers specific to domestic dog *cob* were used in the secondary PCR. The nested PCR assay was carried out using primers (pancrocF 5' – TCA TTC ATT GA(C/T) CT(C/T) CCC AC(C/T) CCA – 3' and pancrocR 5' – ACG GTA (A/G)GA CAT A(A/T)C C(C/T)A TGA A(G/T)G – 3') for primary PCR and primers lupus for cob 5′ - CAT CTA ACA TCT CTG CTT GAT G – 3′ and lupus rev cob 5′-CTG TGG CTA TGG TTG CGA ATA A-3′. PCR was carried out in a final reaction volume of 50 µl, containing 5 µl genomic DNA, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1 × DreamTaq™ Green Buffer (Thermo Fisher Scientific) and 1.25 units of DreamTaq Green DNA Polymerase (Thermo Fisher Scientific). The cycling conditions included an initial denaturation at 94 ℃ for 5 min, followed by 40 cycles of 94 ℃ for 30 s, 55 ℃ for 30s, 72 ℃ for 30s and a final extension step at 72 ℃ for 5 min. In the primary PCR 5 µl of total host DNA was used and 2 µl of the product used as template in the nested PCR (Hüttner *et al*., 2009; Kagendo *et al*., 2014). PCR products were resolved on 2% agarose gel and visualized under UV transilluminator following ethidium bromide staining.

4.3.4.1 Polymerase chain reaction of ITS 1 & 2, 5.8S and 28S rRNA

Single PCRs were carried out using primers RTGHF1 (5'-CGT GCT AGT CTT CAG GAC TTT G-3') and RTGHR1 (5'-CGT TGT CAT ACT AGC CAC TGC-3') targeting the internal transcribed spacer (ITS)-1, 5.8S and ITS2 regions yielding a 544 bp PCR product for *A. caninum*, *A. ceylanicum* and *A. duodenale* and 552 bp for *A. braziliense* (Traub *et al*., 2004). With negative samples, a second PCR was performed using the primer pair NC1 (5'-ACG TCT GGT TCA GGG TTC TT-3') and NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') to amplify an approximately 310 bp region of the internal transcribed spacer-2 (ITS-2), 5.8S and 28S ribosomal RNA gene (Gasser *et al*., 1993). PCR was done in a final reaction volume of 50 µl, consisting of 5 µl genomic DNA, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1 × DreamTaq™ Green Buffer (Thermo Fisher Scientific) and 1.25 units of DreamTaq Green DNA Polymerase (Thermo Fisher Scientific). The cycling conditions were as described in section 4.3.4, except that the number of cycles was increased from 40 to 50. Ten microliters of the PCR products were detected on 2% agarose gel, then DNA bands visualized following staining with ethidium bromide (Fig. 4.1).

4.3.4.2 Amplification of cytochrome C oxidase 1 *(cox1)*

A single PCR reaction of the mitochondrial cytochrome C oxidase 1 (*cox1*) was performed on hookworm isolate identified as *A. duodenale* by the ITS regions PCR. The forward primer AceyCOX1F (5′-GCT TTT GGT ATT GTA AGA CAG-3′) and reverse primer AceyCOX1R (5′-CTA ACA ACA TAA TAA GTA TCA TG-3′) amplified a fragment of 377 bp (Inpankaew *et al*., 2014). The 50 µl PCR reaction mix consisted of 5 µl genomic DNA, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 µM of each primer, 1 × DreamTaq™ Green Buffer (Thermo Fisher Scientific) and 1.25 units of DreamTaq Green DNA Polymerase (Thermo Fisher Scientific). The cycling conditions were identical as those of the ITS 1 and 2 regions PCR except the annealing temperature which was

increased to 58 °C. Ten microliters of the PCR products were detected on 2% agarose gel, then DNA bands visualized following staining with ethidium bromide.

4.3.4.3 Species identification by Restriction Fragment Length Polymorphism (RFLP)

Restriction digests were performed on the 545 bp PCR product only, using *Hin*FI and *Rsa*I restriction endonucleases (New England Biolabs) as previously described (Traub *et al*., 2004). Briefly, 10 µl of the PCR product was digested separately in a 20 µl final volume of consisting 5 units enzymes, 1 × CutSmart® Buffer (NEB). The digests were incubated overnight at 37 °C, and separated on 3% agarose gel to identify hookworm species (Palmer *et al*., 2007). PCR products from confirmed *A. caninum* and *A. ceylanicum* were digested alongside the test samples as positive controls.

4.3.4.4 Sequencing of PCR products

Thirty five PCR products generated by NC1 and NC2 primers (15) RTGHF1 and RTABCR1 (20) were purified using the Wizard® SV Gel and PCR Clean-Up kit (Promega, USA) and sequenced with reverse primers RTABCR1 (5'-CGG GAA TTG CTA TAA GCA AGT GC-3') or NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') respectively at GATC Biotech AG, Germany. The *cox1* PCR product was also purified and sequenced using both primers (AceyCOX1F and AceyCOX1R). The chromatograms were manually checked and edited using GENtle v. 1.9.4 (http://gentle.magnusmanske.de) and species were confirmed through identity search of the National Centre for Biotechnology Information (NCBI) reference sequences, using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997).

4.3.5 Data analysis

The prevalence and exact binomial confidence intervals (95% CI) were calculated for each study site. Statistical differences between the study sites were assessed using Fisher's exact Chi-square test on SPSS version 22.0. Significance was indicated by a probability of $P < 0.05$.

4.4 Results

4.4.1 Prevalence of hookworm eggs in dog faecal samples

Hookworm eggs were detected in 490/1621 [30.23%; 95% CI 28.01–32.59%] faecal samples by microscopy. Narok county (Maasai Mara region) had the highest prevalence (46.80%; 95% CI 42.37– 51.28%) (234/500) followed by Meru (44.88%; 95% CI 39.22–50.67%) (136/303), Isiolo (19.73%; 95% CI 15.43– 24.84%) (58/294) and Turkana (11.83%; 95% CI 9.25– 14.98%) (62/524) (Table 4.1). There was a significant difference in hookworm prevalence in the four counties ($p < 0.0001$).

4.4.2 Molecular species identification

PCR products were obtained from 70/78 samples with either of the two sets of primers; eight DNA samples failed to yield a product with either of the two PCR methods (Table 4.1 and Fig. 4.1). The first PCR successfully amplified DNA from 55/78 faecal samples, whereas 15/78 PCR products were obtained with the second PCR (NC1/2 primers). Hookworm species in all 55 amplified products from the first PCR were identified by RFLP. The restriction profiles following digestion with both endonuclease *Rsa*I and *Hin*FI are shown in Table 4.2 and Fig. 4.2. Hookworm species were identified by sequencing of all 15 PCR products (second PCR). To further confirm the RFLP results, 20 of 55 PCR products (first PCR) were selected randomly for sequencing. Hookworm species identified included *A. caninum* (n = 59), *A. braziliense* (n = 10) and *A.* cf. *duodenale* (n =

1) (Table 4.1). Neither of the detection methods gave indications of multiple species infections (e.g. double peaks). All *A. caninum* (n = 25) and *A. braziliense* (n = 10) sequences were 100% identical to references DQ438071 and DQ438061 (e Silva *et al*., 2006), respectively. All the nucleotide sequences obtained from *A. braziliense* and *A. caninum* isolates were identical for each species, and therefore only representative sequences for each species were deposited in GenBank, under accession numbers MG271917, MG271918 and MG271920. The *A.* cf. *duodenale* sequence showed 99% identity with *A. duodenale* accession number EU344797 (Wang *et al*., unpublished data), with three single nucleotide polymorphisms (SNPs) occurring at ITS 1 (2) and ITS 2 (1) regions. This nucleotide sequence was deposited in GenBank under accession number MK271367. Amplification and partial sequencing of mitochondrial cytochrome c oxidase I (*cox1*) confirmed the *A. duodenale* isolate. The nucleotide sequence of this isolate was 99% identical to the reference sequence accession number AJ417718 (Hu *et al*., 2002) and was deposited in GenBank under accession number MK288015.

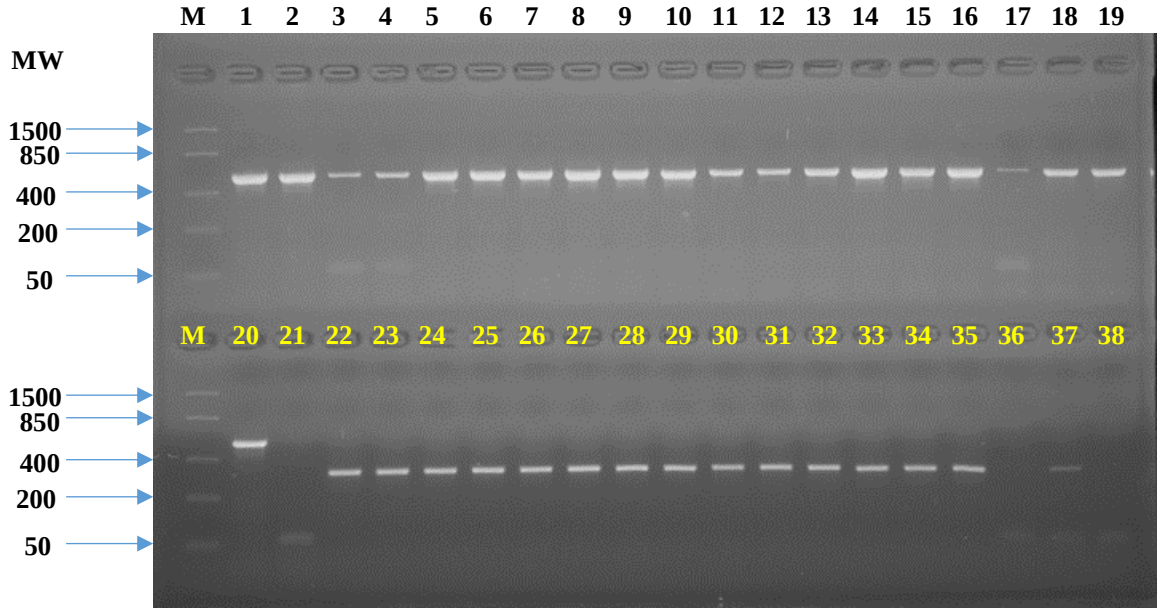

Fig. 4. 1 Hookworm PCR of ITS 1 & 2, 5.8S and 28S rRNA.

PCR products were separated on 2% agarose gel and stained with ethidium bromide. M: is the molecular weight marker, lanes 1 – 19: correspond to the 545 bp PCR (1st PCR), lane 20: represents a positive control for 1st PCR, lane 21: represents a negative control for 1st PCR. Lanes 22 – 35: correspond to 310 bp PCR (2nd PCR), lane 36: represents a negative sample by PCR (2nd PCR), lane 37: represents a positive control (2nd PCR) and lane 38: represents a negative control (2nd PCR).

4.4.3 Geographical distribution of hookworm species

A. caninum was the most common species in all study sites and the only species detected in Narok (Maasai Mara). *A. braziliense* was common in Turkana with 8/20 positive faecal samples, but was only found in one faecal sample each from Isiolo and Meru. *A.* cf. *duodenale* was detected in only one faecal sample from Turkana (Table 4.1 and Fig 4.3).

Table 4. 1 Prevalence of hookworm eggs in dog faeces and molecular species determination

Study sites	No. of faecal samples	Samples with hookworm eggs	Prevalence (%)	95% CI	DNA extracted	PCR-RFLP/DNA sequencing	Samples with *A. caninum*	Samples with *A. braziliense*	Samples with *A.* cf. *duodenale*
Turkana	524	62	11.83	9.25 – 14.98	20	18	9	8	1
Isiolo	294	58	19.73	15.43 – 24.84	18	13	12	1	0
Meru	303	136	44.88	39.22 – 50.67	20	20	19	1	0
Narok	500	234	46.80	42.37 – 51.28	20	19	19	0	0
Total	**1621**	**490**	**30.23**	**27.99 – 32.53**	**78**	**70**	**59**	**10**	**1**

Table 4. 2 Restriction profile of RTGHF1-RTABCR1 PCR products following digestion with restriction endonucleases (*Rsa*I & *Hin*FI)

Restriction endonuclease	Species	Identified band sizes (bp)
*Rsa*I	*A. caninum*	276, 268
	A. braziliense	434, 77, 41[a]
	A. ceylanicum	276, 268
	A. duodenale	276, 268
*Hin*FI	*A. caninum*	216, 187, 85, 49, 7[a]
	A. braziliense	220, 198, 85, 49
	A. ceylanicum	301, 194, 49
	A. duodenale	216, 187, 85, 49, 7[a]

[a] Product too small to visualize on gel

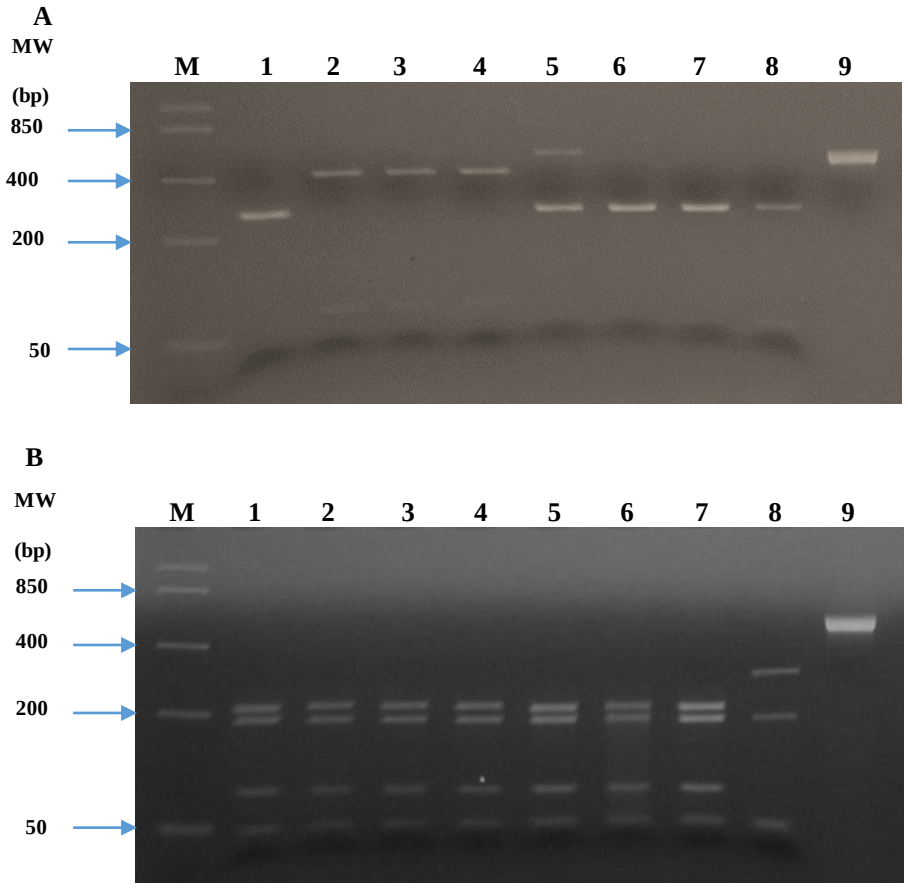

Fig. 4. 2 RFLP of PCR product RTGHF1-RTABCR1 following digestion with restriction endonucleases *Rsa*I (Panel A) and *Hin*FI (Panel B).

Digests were separated on 3% agarose gel and stained in ethidium bromide. M: is the molecular weight marker, lane 1: represents *Ancylostoma duodenale* (TNU 331), lanes 2 – 4: represent *Ancylostoma braziliense*, lanes 5 & 6: represent *Ancylostoma caninum*, lane 7: represents *Ancylostoma caninum* positive control, lane 8: represents *Ancylostoma ceylanicum* positive control, lane 9: represents uncut PCR product.

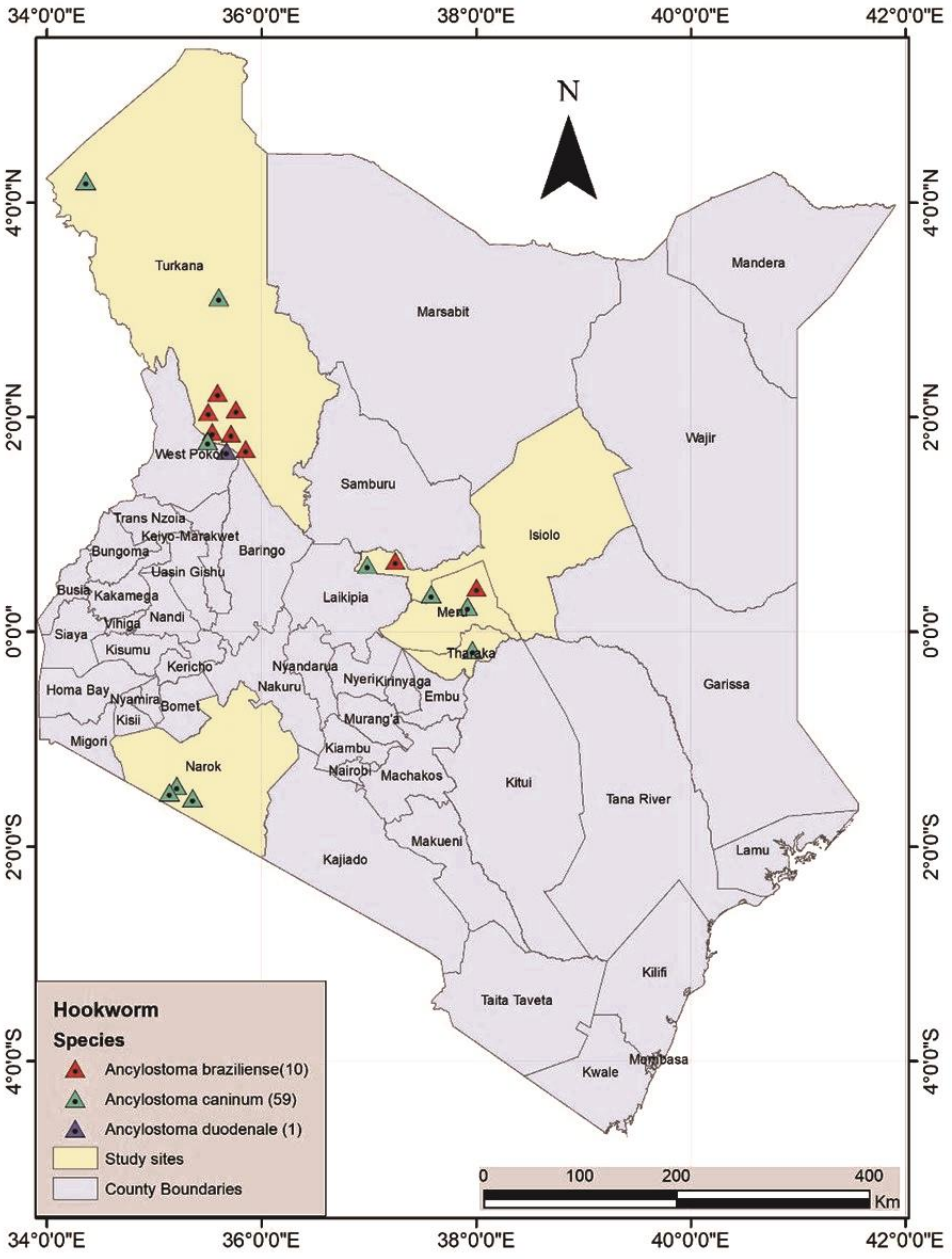

Fig. 4. 3 Distribution and frequency of hookworm species within the study sites.

4.5 Discussion

This study is the first to report the prevalence and species of hookworms from dog faecal samples in Kenya. The overall prevalence of hookworms in this study (30.23%) is in the range of prevalence reported from Nigeria (34.6%) (Sowemimo, 2009) and Gabon (34.8%) (Davoust *et al*., 2008). Within Kenya, there are only two previous studies available for comparison, both with a small number of dogs examined from single locations. One study was done in Nairobi, where 88% of 156 dogs were found to be infected (Wachira *et al*., 1993b). The second study was done in Turkana, giving a prevalence (11.8% of 42 dogs; (Buishi *et al*., 2006) that is remarkably close to data in this study (11.9%). However, these studies were based on post-mortem examination, which would be expected to give higher sensitivities due to the identification of pre-patent infections. Also, hatching of hookworm eggs in the faeces before collection is likely to decrease the sensitivity of the method used in this study, as is desiccation of eggs in the environment (Levecke *et al*., 2011).

Narok and Meru counties had far higher prevalence than Isiolo and Turkana counties. This may be attributed to the hot (> 40°C) and dry conditions (annual average rainfall 250 mm) in Turkana and Isiolo, which are known to negatively affect the survival of hookworm larvae in the environment (Crompton, 2001; Mudenda *et al*., 2012), while high-rainfall conditions in Meru and Narok are favourable for larval development in contaminated soil. Eggs of hookworms do not develop below 15°C and larvae prefer shady and moist areas with temperatures at or above 30°C, only being killed at temperatures above 45°C. Larvae also survive for longer periods at lower temperatures (20–25°C) than at temperatures above 30°C (Udonsi and Atata, 1987; Brooker *et al*., 2006; Pullan and Brooker, 2012).

In this study three hookworm species, namely *A. caninum, A. braziliense* and *A.* cf. *duodenale*, were identified in canine faecal samples. *Ancylostoma caninum* was the most common

species, found in 84.3% of positive faecal samples, an observation also reported in Australia (Palmer *et al*., 2007), Brazil (Coelho *et al*., 2011; Oliveira-Arbex *et al*., 2017), China (Liu *et al*., 2015c), India (Loukas *et al*., 2005; George *et al*., 2016) and Thailand (Traub *et al*., 2008). The failure to amplify hookworm DNA in eight samples could be as a result of microscopy misdiagnosis of hookworm eggs with other related nematode eggs, such as *Trichostrongylus* and *Oesophagostomum*, which are morphologically similar to hookworm eggs (Jozefzoon and Oostburg, 1994; Ziem *et al*., 2006). Occasionally mite eggs are also confused with hookworm eggs (Werneck *et al*., 2007). Genomic DNA extracted from faecal samples is known to contain PCR inhibitors, which results in false negatives (Traub *et al*., 2004; Repetto *et al*., 2013). Furthermore, low egg counts are also linked with limited or undetectable DNA by PCR (Palmer *et al*., 2007; Hu *et al*., 2016).

Ancylostoma caninum is the most widely distributed hookworm species in the tropics, subtropics and in warm temperate areas in southern Europe (Galanti *et al*., 2002; Rinaldi *et al*., 2006). This is not surprising given the biological advantage of *A. caninum* in relation to the other hookworm species in the ability of its larvae to undergo transmammary transmission and arrested development in tissue during seasons unfavourable for its survival in the environment. Although eosinophilic enteritis caused by *A. caninum* has not been reported in humans in Kenya, this study provides a basis for future investigation. A recent study in India detected *A. caninum* eggs in human stool; however, the authors could not provide evidence that these eggs were the result of natural infection or an inadvertent passage (George *et al*., 2016). Moreover, as *A. caninum* and *A. duodenale* are closely related and appear to have recently diverged, it is possible that the ITS region of the rRNA is too conserved to allow confident differentiation of the two species (Traub *et al*., 2007).

Ancylostoma braziliense was the second most abundant species of canine hookworms in this study (found in 14.3% of faecal samples). This parasite is limited to tropical and subtropical regions, including Central and South America, the Caribbean, south-eastern USA, Africa, Malaysia, and northern Australia (Del Giudice *et al*., 2002; Schaub *et al*., 2002). It is responsible for causing 'creeping eruptions' caused by cutaneous larva migrans, often reported in travellers returning from endemic areas (Bowman *et al*., 2010). Two patients in the UK and two in France were treated for cutaneous larva migrans after visiting Kenya (Bouchaud *et al*., 2000; Blackwell and Vega-Lopez, 2001; Kelkar, 2007; Dhir *et al*., 2010). Furthermore, 32% of 44 patients treated for cutaneous larva migrans at the Hospital for Tropical Diseases, London, originated from Africa (Blackwell and Vega-Lopez, 2001), whereas in Germany 10.2% of patients treated for creeping eruption at a travel-related-disease clinic had visited East Africa (Jelinek *et al*., 1994). Hookworm related cutaneous larva migrans has been reported in other areas where *A. braziliense* is endemic in dogs and cats (Hochedez and Caumes, 2007; Bowman *et al*., 2010). Human cases of cutaneous larva migrans in Kenya are limited or not documented; therefore, the public health significance of this hookworm may be underestimated, possibly due to misdiagnosis for other skin conditions, as observed elsewhere (Mahdy *et al*., 2012).

Ancylostoma cf. *duodenale* was characterized by DNA sequencing in a single faecal sample originating from Turkana. However, in the absence of adult worms to confirm this finding, it is postulated that this observation could be a result of coprophagy of human stools containing *A. duodenale* eggs, which is common in the area (personal observation). Furthermore, detection of *E. felidis* (Chapter 5) and *T. saginata* (Chapter 6) in dog faeces in this study confirms that dog coprophagy is common. The mechanical transmission of human parasites by dogs has been demonstrated by the passive passage of viable *T. gondii* oocysts after feeding on cat faeces (Lindsay

et al., 1997; Frenkel *et al*., 2003), and passage of *A. lumbricoides* eggs in dog faeces (Joshi and Sabne, 1977; Traub *et al*., 2002). Dogs were also involved in transient passage of other canid parasites, such as *T. canis*, as a result of coprophagy (Fahrion *et al*., 2011); this phenomenon has often led to an overestimation of the occurrence of patent helminth infections in dogs (Nijsse *et al*., 2014). Although the ITS region is thought to be too conserved for accurate differentiation of *A. caninum* and *A. duodenale* (Traub *et al*., 2007), this is unlikely to be the case in this study because the isolate was confirmed as *A. duodenale* by amplification and partial sequencing of the cytochrome c oxidase gene. The possibility that this isolate originated from human faeces as a result of misidentification as dog faeces was ruled out after detecting the latter's DNA from the faecal sample. This finding is interesting because human infections with *A. duodenale* are rare in Kenya and have been identified through morphological characterization (Miller, 1970; Macpherson and Craig, 1991). Moreover, recent molecular-based studies have identified *Necator americanus* as the only hookworm species infecting humans in Kenya (Arndt *et al*., 2013; Easton *et al*., 2016).

The zoonotic canine hookworm *A. ceylanicum* was not reported in this study. However, only 70 faecal samples of the 490 microscopically positive hookworm samples were characterized by PCR-RFLP and/or DNA sequencing. The failure to detect this species could be attributed to the inability of the PCR to detect mixed infections (George *et al*., 2016), especially where the primary hookworm *A. caninum* is dominant, and would necessitate the use of species-specific primers (Merino-Tejedor *et al*., 2018). Additionally, despite co-infection with different hookworm species, eggs are not shed equally. Dogs experimentally infected with *A. caninum* excreted more eggs than those infected with *A. braziliense*; however, more adult worms of the latter were detected than those of *A. caninum* (Loukas *et al*., 2005; Dias *et al*., 2013). *Ancylostoma ceylanicum* has been reported in humans, cats and dogs in African countries, including Sierra Leone, Madagascar and

South Africa (Traub, 2013; Ngcamphalala *et al*., 2019). *Ancylostoma ceylanicum* was recently detected in four dog faecal samples in Tanzania, and this was only possible after using species-specific primers to show a coinfection with the dominant *A. caninum* (Merino-Tejedor *et al*., 2018). This observation confirms the postulation that *A. ceylanicum* may be prevalent in dogs in Kenya, as both countries experience similar climatic conditions. In previous post-mortem studies in Kenya, *A. ceylanicum* might have been overlooked due to the morphological similarities with *A. braziliense*, which was reported from Turkana (Wachira *et al*., 1993b; Buishi *et al*., 2006; Traub, 2013).

4.6 Conclusions

The findings of this study highlight the need to consider the public health importance of domestic dogs as reservoirs of zoonotic ancylostomiasis. As environmental hygiene is poorly regulated in Kenya, public areas, including beaches, parks, shared water sources (humans and animals) and playing grounds for children, can be significant sources of such zoonoses. Ideal effective control measures against zoonotic ancylostomiasis would include regular deworming of community dogs by integrated anthelmintic treatment, with ongoing dog population control and vaccination programmes implemented by the county governments in collaboration with the department of veterinary services and other partners. The public should be educated in relation to responsible pet ownership (prohibiting free roaming), the importance of at least monthly deworming protocols, and appropriately disposing of dog faeces as advocated by the Tropical Council for Companion Animal Parasite (TroCCAP) “Guidelines for the diagnosis, treatment and control of canine endoparasites” (www.troccap.com). The public and tourists should also be educated on basic hygienic principles, including the use of protective footwear in parks, playgrounds or beaches.

CHAPTER FIVE: MOLECULAR CHARACTERIZATION OF *ECHINOCOCCUS* SPECIES AND HAPLOTYPES IN DOGS FROM FOUR REGIONS OF KENYA

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https://www.ncbi.nlm.nih.gov/pubmed/ 29773136

5.1 Abstract

Cystic echinococcosis is endemic both in livestock and humans in many parts of Kenya. However, very little data exists on *Echinococcus* infections in dogs, and therefore their role in maintaining the transmission cycles and environmental contamination with eggs of *Echinococcus* species is unknown. The study aimed to establish the prevalence, distribution and haplotypes of *Echinococcus granulosus* sensu lato causing infection in dogs in Kenya. A total of 1,621 dog faecal samples were collected from the environment in four different regions and examined microscopically for the presence of taeniid eggs. Up to 20 individual taeniid eggs per faecal sample were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and sequencing of the NADH dehydrogenase subunit 1 (*nad1*) gene. *Echinococcus* haplotypes were determined by sequencing of cytochrome C oxidase 1 gene *(cox1)*. Eleven percent (178/1,621) of faecal samples had taeniid eggs, while 4.4% (71/1,621) contained *Echinococcus* spp. eggs. Area-wise, the faecal prevalence of *Echinococcus* spp. was 9.2% (48/524) in Turkana, 4.0% (20/500) in Maasai Mara, 0.7% (2/294) in Isiolo and 0.3% (1/303) in Meru. *E. granulosus* s. s. was the dominant *Echinococcus* taxon, followed by *E. canadensis* (G6/7) that was detected in 51 and 23 faecal samples, respectively. *E. ortleppi* was detected in only 5 faecal samples. This study reports for the first time, the presence of *E. felidis* eggs in two dog faecal samples from Maasai Mara. Mixed infections of these taxa were also found in faecal samples, including: *E. granulosus* s. s. and *E. canadensis* (G6/7) (n = 7), *E. granulosus* s. s. and *E. ortleppi* (n = 1) and all three species (n = 1). Of the 12 and 8 haplotypes identified, 6 and 2 were novel for *E. granulosus* s. s. and *E. canadensis* (G6/7) respectively. The dog data presented here confirm the differences in diversity and abundance of *Echinococcus* spp. between regions of Kenya, corresponding well with previously published data from livestock, and tentatively suggest a role of domestic dogs as a link between domestic and sylvatic cycles of *Echinococcus* spp.

Keywords: Cystic Echinococcosis; *Echinococcus* spp.; *Echinococcus felidis*; Dogs; Kenya

5.2. Introduction

Cystic echinococcosis (CE) is an infection in humans and herbivores caused by the larval stage of the dog tapeworms *Echinococcus granulosus* sensu lato (s. l.). At least five species constitute *E. granulosus* s. l. namely *E. granulosus* s. s. (G 1 – G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6–G10) and *E. felidis* (Nakao *et al*., 2013b; Nakao *et al*., 2013c; Romig *et al*., 2017). The *E. canadensis* (G6 – G10) cluster is still unresolved, some authors have proposed *E. canadensis* for genotypes G6, G7, G8 and G10 (Moks *et al*., 2008) or further subdivision of G8 and G10 to *E. canadensis* based on nuclear DNA and *E. intermedius* for genotypes G6, G7 and G9 (Saarma *et al*., 2009). Further a recent review of this cluster has suggested three species namely; *E. intermedius* (G6/7), *E. borealis* (G8) and *E. canadensis* (G 10) (Lymbery *et al*., 2015). Recently two distinct species were proposed for G6/7 and G8/10 clusters based on sequencing of mitochondrial genes (Addy *et al*., 2017b) and nuclear genes (Laurimae *et al*., 2018), but opposed by Yanagida *et al*. (2017) after finding shared alleles within these two clusters in regions where different mitochondrial DNA linkages occur sympatrically. In addition the status of G_{omo} as a distinct species needs a solution (Wassermann *et al*., 2016). These species are mainly maintained in the domestic cycle and use the dog as the definitive host, except for *E. felidis* that was only isolated from wildlife and principally uses lions as definitive hosts. *Echinococcus canadensis* G8 and G10 have also sylvatic cycles that are maintained by wolf and cervids (Lavikainen *et al*., 2003; Lavikainen *et al*., 2006; Moks *et al*., 2008). Diagnosis of *Echinococcus* infection in dogs presents several challenges since the tapeworm eggs are shed erratically and are indistinguishable from eggs other genera of taeniids. The diagnosis relies on a number of techniques including necropsy, arecoline purgation, copro-antigen ELISA and copro-PCR with faecal matter or isolated eggs (Eckert *et al*., 2001; Craig *et al*., 2015).

While data on the prevalence of different *Echinococcus* spp. in intermediate hosts (herbivores) are available from all endemic regions (Deplazes *et al*., 2017) and have provided valuable information on the geographical distribution of the parasites and the role of different livestock species for transmission, there are comparatively little data on the infection of dogs with defined species of *E. granulosus* s. l. However, such data would be particularly important to estimate the relative infection pressure to intermediate hosts and humans, and their proportion of the environmental contamination. Even in Kenya where extensive research on CE has been carried out, recent insight into the situation in dogs is still lacking. Over the years, more epidemiological data on *Echinococcus* has been generated using metacestodes isolates from intermediate hosts (and to some extent from humans). In Kenya, studies on dogs started as early as in the 1960s mainly in Turkana, but a few others in Maasailand and Nairobi (Gathura and Kamiya, 1990; Wachira *et al*., 1993a; Wachira *et al*., 1994; Buishi *et al*., 2006). Only one of these studies did differentiate the isolated worms into the various *Echinococcus* taxa (Wachira *et al*., 1993a). Additional data on livestock CE have recently been reported from other regions of Kenya including Maasailand, Meru and Isiolo (Addy *et al*., 2012; Mbaya *et al*., 2014) and in wildlife from six conservation areas (Kagendo *et al*., 2014). Collectively, these recent data revealed the presence of all species of *E. granulosus* s. l. except *E. equinus*, and are suggestive scenarios for links between domestic and wildlife transmission patterns.

Studies on the genetic variability and population structure of *E. granulosus* s. l. in dogs are limited worldwide (Parsa *et al*., 2012; Konyaev *et al*., 2013; Boufana *et al*., 2014; Boufana *et al*., 2015a; Shariatzadeh *et al*., 2015; Spotin *et al*., 2017). This is because the main focus of most genetic diversity studies has been on characterization of metacestodes from intermediate hosts (livestock and humans). This could partly be due to the ethical issues and challenges related to

acquiring adult tapeworms for characterization. The situation is not different in Kenya where many isolates from humans and livestock but only a few adult tapeworms have been characterized (Boufana *et al*., 2015a; Addy *et al*., 2017a; Addy *et al*., 2017b). It is important to establish the genetic variability of *E. granulosus* s. l. in dogs and compare with existing data from intermediate hosts.

The present study assessed the *Echinococcus* spp. infection in dogs in areas where CE data on livestock, human (Turkana) and wildlife (Maasai Mara) had recently been reported (Addy *et al*., 2012; Mutwiri *et al*., 2013; Kagendo *et al*., 2014; Mbaya *et al*., 2014). The study further established genetic variability of *E. granulosus* s. s. and *E. canadensis* (G6/7) isolates from individual eggs. This study was necessary to compare agents of CE in the different regions reported from livestock, wild carnivores and humans with those in dogs. This information is important to understand the distribution and transmission dynamics of *Echinococcus* spp. and factors influencing infections in humans and livestock and is essential for the implementation of control programmes. This study presents the current situation of *Echinococcus* spp. infections and haplotypes in dogs in Turkana, Meru, Isiolo and Maasai Mara regions of Kenya, and indicates aspects of domestic – wildlife transmission dynamics that were previously unknown.

5.3 Materials and methods

5.3.1 Faecal sample collection

Dog faecal samples were collected from the environment in four counties of Kenya as described in sections 3.3.1. and 3.3.2. The faecal samples (~20 – 30 g) were preserved in 80% ethanol immediately after collection.

5.3.2 Isolation of taeniid eggs, microscopy examination and DNA extraction

Approximately 2 – 3 g of faecal materials were analysed by a zinc chloride flotation-sieving technique (Mathis *et al*., 1996) and microscopically examined for the presence of taeniid egg (section 3.3.4). Individual taeniid eggs were picked using a micro-pipette under the microscope (Magnification × 40 or 100) and transferred into 0.2 ml thin wall PCR tubes containing 10 µl of 0.02 M NaOH and lysed at 99 °C for 10 min. A previous study (Kagendo *et al*., 2014), showed that analysing 20 individual taeniid eggs per sample was sufficient to detect mixed infections since the eggs were picked randomly and therefore in this present study, the number of eggs analysed per sample was limited to 20. A small proportion of taeniid positive samples (n = 23) did not yield the targeted 20 individual eggs for PCR.

5.3.3 Polymerase chain reaction of *nad1* gene

A nested PCR targeting part of the *nad1* gene was performed. In the primary PCR primers 5' – TGT TTT TGA GAT CAG TTC GGT GTG – 3' and 5' – ATA TCA AAG TAA CCT GCT ATG CAG – 3' (Hüttner *et al*., 2008) were used in a 25 µl reaction consisting of 1 × DreamTaq Green Buffer (20 mM Tris-HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% (v/v) Nonidet P40, 0.5% (v/v) Tween 20) (Thermo Scientific), 0.2 mM dNTPs, 0.25 µM of each forward and reverse primer, 2 mM $MgCl_2$ and 0.625 U of DreamTaq Green DNA Polymerase (Thermo Scientific) and 2 µl of the taeniid lysate as DNA template. The secondary PCR was performed under similar conditions and the primers were 5' – CAG TTC GGT GTG CTT TTG GGT CTG – 3' and 5' – TCT TGA AGT TAA CAG CAC GAT – 3' and resulted in a product of 545 – 552 bp (Hüttner *et al*., 2008). In both PCRs cycling conditions included 5 min of initial denaturation at 94 °C, followed by 40 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min and a final extension step at 72 °C for 5 min.

5.3.4 Restriction Fragment Length Polymorphism of *nad1* amplicons

Restriction digestion was performed on the PCR amplicons in a 20 µl volume reaction containing 10 µl of the secondary PCR product, 0.5 µl (5 U) *Hph*I endonuclease (Thermo Scientific), 1 × Buffer and 7.5 µl of nuclease free water. The digestion reactions were performed overnight in an incubator at 37 °C. The restriction fragments were resolved on 3% agarose gel then visualized under UV transilluminator following staining with ethidium bromide. To identify *Echinococcus* species known positive controls of *E. granulosus* s. s., *E. ortleppi*, *E. canadensis* (G6/7) and *E. felidis* were similarly digested and resolved alongside the test samples.

5.3.5. Purification and sequencing of nested PCR products

Nested PCR products that presented unclear banding patterns were purified using the High Pure PCR Product Purification Kit (Roche, Germany) following the manufacturer's protocol. The PCR product were sequenced using nested reverse primer at GATC Biotech AG, Germany. The chromatographs were manually checked and edited using GENtle v. 1.9.4 (http://gentle.magnusmanske.de) and species confirmed through identity search on Basic Local Alignment Search Tool (BLAST) to the National Centre for Biotechnology Information (NCBI) reference sequences (Altschul *et al*., 1997).

5.3.6 Extraction and amplification of host species specific DNA

Host (dog) genomic DNA was extracted from the faecal samples as described by Dinkel *et al*. (1998) (section 4.3.4). A nested PCR was performed to identify the carnivore origin of two faecal samples that contained *E. felidis* eggs. The nested PCR amplifies the canid and felid specific cytochrome b gene *(cob)* using the primers and conditions previously described by Hüttner *et al*. (2009) and Kagendo *et al*. (2014) (section 4.3.4). The PCR products were separated on 2% agarose gel and visualized under UV transilluminator following ethidium bromide staining.

5.3.7 Haplotype network analysis

5.3.7.1 Cytochrome C oxidase 1 (*cox1*) PCR

PCR based on cytochrome C oxidase 1 (*cox1*) was performed on taeniid eggs that were identified as *Echinococcus* spp. (section 5.3.4). Out of 283 *Echinococcus* spp. egg lysates only 238 were available for *cox1* PCR. An additional 106 taeniid eggs were picked from 10 faecal samples that had *Echinococcus* eggs only and their haplotypes analysed by *cox1* PCR. The entire mitochondrial *cox1* was amplified in three overlapping parts, i.e. the five prime region, the middle region and the three prime region. This was because of the limited genetic material in a single taeniid egg to obtain the entire *cox1* gene (1608/9 bp) in a single PCR reaction.

The five prime region was obtained in a nested PCR reaction in which forward primer *cox1* A and reverse primer *cox1* f nest rev were used in the primary PCR. The nested reaction was performed using forward primer *cox1* B and reverse primer *cox1* walkLH (Table 5.1). In a 25 µl reaction the primary PCR consisted of 1 × PCR reaction buffer (50 mM Tris/HCl, 10 mM KCl, 5 mM $(NH4)_2SO4$, pH 8.3/ 25°C, Roche, Germany), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer, 0.625 units FastStart Taq DNA Polymerase (Roche, Germany) and 2 µl of taeniid egg lysate. Similar conditions were used for the secondary PCR but the final volume was adjusted to 50 µl. The cycling conditions were 5 min of initial denuration at 95°C, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min and a final extension at 72°C for 5 min. The middle region sequences were overlapping with both the 5' and 3' regions and was amplified in a nested PCR using forward primer *cox1* G and reverse primer *cox1* I for the primary PCR, forward primer *cox1* b for and reverse primer Efel b were used in the secondary PCR. The same conditions as described for the 5' region was used except that the $MgCl_2$ concentration was reduced to 1.5 mM, the annealing temperature was 52.5°C for the primary PCR and number of cycles reduced to 25

and 35 in primary and secondary respectively (Table 5.1). The 3' region of *cox1* gene was amplified in a nested PCR where in the primary PCR forward primer *cox*nest B and *cox1* CNEW were used. The nested PCR utilized forward primer Efel C and reverse primer *cox* D. PCR and cycling conditions for the 3' region were identical to those used for the 5' region except the annealing temperature which was adjusted to 50°C (Table 5.1). The secondary PCR products were purified using the High Pure PCR Product Purification Kit (Roche, Germany) following the manufacturer's protocol (section 5.2.5).

5.3.7.2 Sequence analysis and haplotype network construction

E. granulosus s. s. and *E. canadensis* (G6/7) *cox1* DNA sequences were first viewed and manually edited using GENtle v. 1.9.4 http://gentle.magnusmanske.de. The amino acid sequences were inferred based on the flatworm mitochondrial genetic code (Nakao *et al*., 2000). Population diversity indices (number of haplotypes, haplotype diversity and nucleotide diversity) were calculated using DnaSP 5.1 (Librado and Rozas, 2009), while Arlequin 3.1 (Excoffier *et al*., 2007) was used to conduct neutrality tests: Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) indices for each species. The haplotype networks were computed by TCS 1·21 software with 95% connection limit (Clement *et al*., 2000).

Table 5. 1 *Echinococcus* spp. haplotype *cox1* primers and PCR conditions

Region of *cox1* gene	Name	Primers (5'-3')	Size (bp)	PCR conditions
5' region	*cox1* A *cox1* f nest rev	TTACTGCTAATAATTTTGTGTCAT ACCCAACAGTAAACATATGATGAC	1010	annealing temp 55°C, 40 cycles, 2 mM $MgCl_2$
	cox1 B *cox1* walkLH	GTTAGTTTTGACTGTACGTTTTCA ATCAACACATAAACCTCAGG	801	annealing temp 55°C, 40 cycles, 2 mM $MgCl_2$
Middle region	*cox1* G *cox1* I	CTGTTTTGTTATTGGTTACGTTGC ACRTAATGAAAATGAGC	568	annealing temp 52.5°C, 25 cycles, 1.5 mM $MgCl_2$
	cox1 b for Efel b	TGATCCGTTAGGTGGTGGTGATCC ATACCAGTAACACCTCCAAACGTA	411	annealing temp 52.5°C, 35 cycles, 1.5 mM $MgCl_2$
3' region	*cox*nest B *cox1* CNew	GTTTACTTGATTGTATATGTTATT ATACTTTAAAAAACTCCGTTAAGC	781	annealing temp 50°C, 40 cycles, 2 mM $MgCl_2$
	Efel C *cox* D	TCTAGTGTTAATGTTAGTGATCCA GACTAATAATCAACTTAGACTTAC	669	annealing temp 50°C, 40 cycles, 2 mM $MgCl_2$

R represents nucleotide A or G

5.4 Results

5.4.1 Taeniid egg isolation and identification

A total of 1,621 faecal samples were collected from Meru (303), Maasai Mara (500), Isiolo (294) and Turkana (524), details of which have already been described in Table 3.1. Microscopic analysis identified 178/1,621 (11%) faecal samples from the four study sites containing taeniid eggs, from which 3,352 eggs were isolated for PCR. The presence of taeniid eggs differed across the four

study sites 2.3% (7/303), 6.8% (34/500), 8.2% (24/294) and 21.6% (113/524) in Meru, Maasai Mara, Isiolo and Turkana respectively (Table 5.2). Positive PCR amplification of the *nad1* gene was achieved from 685 eggs isolated from 118 out of the 178 taeniid positive samples (on microscopy, Fig. 5.1). The RFLP diagnosis and DNA sequencing revealed 357/685 as eggs of *Taenia* spp. from 79/118 faecal samples (Chapter 6), 283/685 eggs as *Echinococcus* spp. representing 71/118 PCR positive taeniid faecal samples. The identity of 45 eggs from 23/118 faecal samples could not be determined due to low yield of PCR products for RFLP or sequencing. A total of 33/118 faecal samples contained eggs of both genera (Table 5.2 and Fig. 5.2).

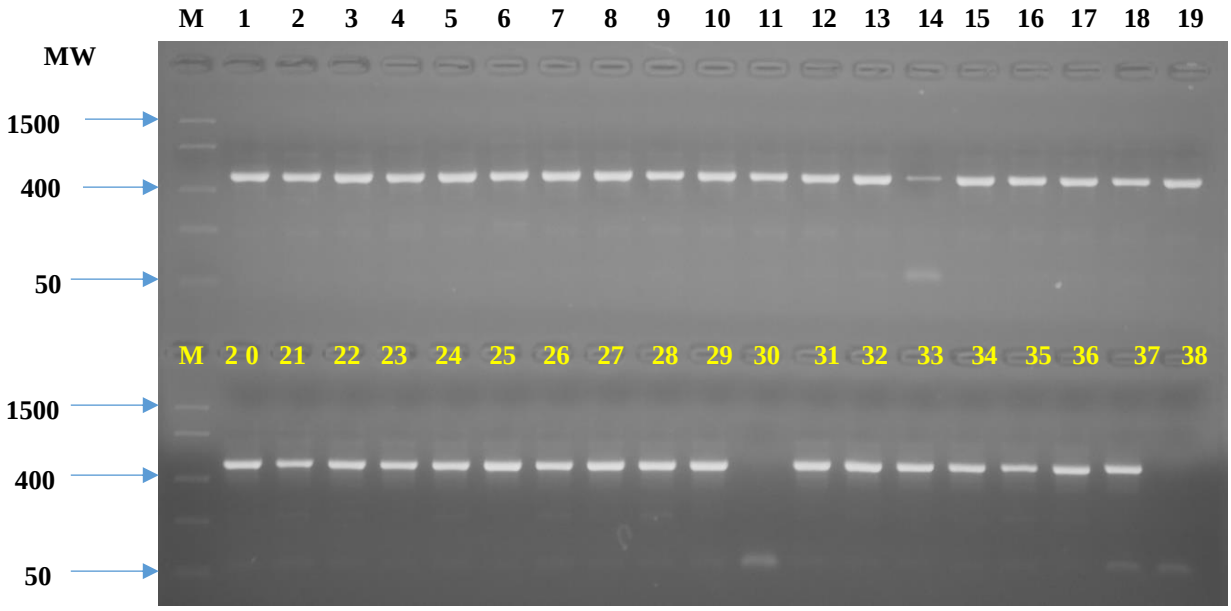

Fig. 5. 1 PCR of *nad1* gene on lysed taeniid egg lysates.

PCR products were separated on 2% agarose gel and stained with ethidium bromide. Lane M: is the molecular weight marker, lanes 1 – 29, 31 – 36 showing taeniid eggs positive by PCR, lane 30; represents negative taeniid egg by PCR. Lanes 37 and 38 represent positive and negative controls respectively.

5.4.2 Eggs of *Echinococcus* spp.

Differential diagnosis of the 283 *Echinococcus* eggs revealed *E. granulosus* s. s. (n = 166), *E. canadensis* (G6/7) (n = 108), *E. ortleppi* (n = 6) and *E. felidis* (n = 3) (Table 5.2). The biogeographic distribution of *Echinococcus* spp. eggs was *E. granulosus* s. s. in Turkana, (77/184), in Maasai Mara (86/92), in Meru (2/2), in Isiolo (1/5); *E. ortleppi* in Turkana (4/184), in Maasai Mara (2/92); *E. canadensis* (G6/7) in Turkana (103/184), in Isiolo (4/5), in Maasai Mara (1/92) while *E. felidis* was found only in Maasai Mara (3/92).

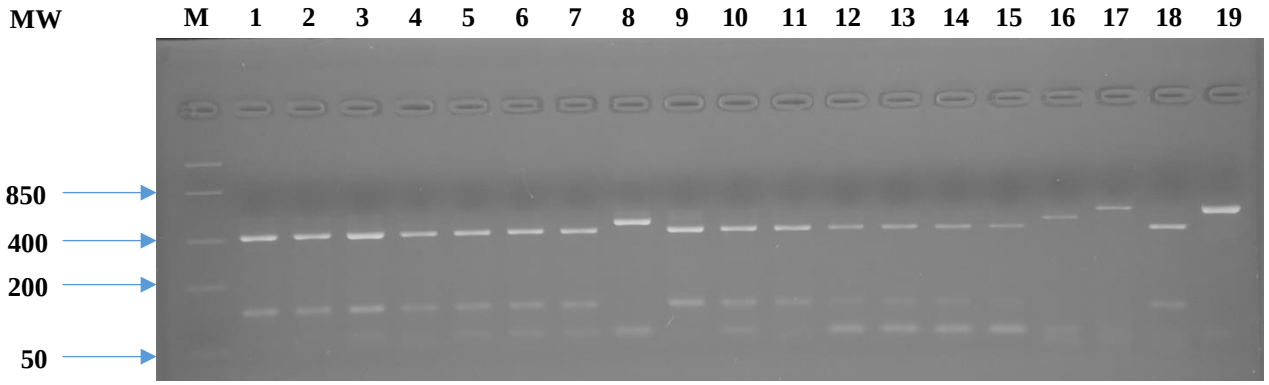

Fig. 5. 2 PCR-RFLP following restriction digestion with *Hph*I

Digests were separated on 3% agarose gel. Lanes M: is the molecular weight marker, lanes 1 – 7, 9 – 15: represent *E. canadensis* (G6/7), lane 8: represents *E. granulosus* s. s.; lane 16: represents *E. granulosus* s. s. positive control; lane 17: represents *E. ortleppi* positive control; lane 18: represents *E. canadensis* (G6/7) positive control; lane 19: represents uncut PCR product (negative control).

Table 5. 2 Numbers and species identities of taeniid eggs isolated from dog faecal samples in CE endemic areas of Kenya

Study site	No. of faecal samples	Faecal samples with taeniid eggs	n taeniid eggs examined[a]	n PCR positive taeniid eggs[b]	*Taenia* spp. eggs (n)[c]	*Echinococcus* spp. eggs (n)[d]
Turkana	524	113 (21.6%)	2121	305	103	*E. g* (77), *E. o* (4), *E. c* (103)
Maasai Mara	500	34 (6.8%)	661	213	114	*E. g* (86), *E. o* (2), *E. c* (1), *E. f* (3)
Isiolo	294	24 (8.2%)	468	155	133	*E. g* (1), *E. c* (4)
Meru	303	7 (2.3%)	102	12	7	*E. g* (2)
Total	**1621**	**178 (11%)**	**3352**	**685**	**357**	***E. g* (166), *E. o* (6), *E. c* (108), *E. f* (3)**

Key:

E .g	*E. granulosus s. s.*
E. o	*E. ortleppi*
E. c	*E. canadensis* (G6/7)
E. f	*E. felidis*
[a]	Number of taeniid eggs picked and screened by nested PCR
[b]	Number of taeniid eggs positive by nested PCR
[c]	Number of taeniid eggs identified as *Taenia* spp. by RFLP
[d]	Frequency of *Echinococcus* spp.

5.4.3 Estimated prevalence of *Echinococcus* spp. in dogs

Echinococcus spp. were detected in 71/1,621 (4.4%) faecal samples representing 48 (9.2 %) in Turkana, 20 (4.0 %) in Maasai Mara, 2 (0.7 %) in Isiolo and 1 (0.3 %) in Meru (Table 5.3). Among these *Echinococcus* spp., *E. granulosus* s. s. was the most abundant species in dogs. Faecal samples from Turkana dogs harboured 3 different *Echinococcus* spp. namely *E. granulosus* s. s. (32), *E. canadensis* (G6/7) (21) and *E. ortleppi* (4). In Isiolo, the faecal samples contained *E. granulosus* s. s. (1) and *E. canadensis* (G6/7) (1), while the only positive faecal sample from Meru showed infection with *E. granulosus* s. s. Maasai Mara exhibited the highest diversity with four *Echinococcus* spp. namely; *E. granulosus* s. s. (17), *E. canadensis* (G6/7) (1), *E. ortleppi* (1) and *E. felidis* (2) identified in dog faecal samples. In total, *E. granulosus* s. s. was found in 51/71 faecal samples, while *E. canadensis* (G6/7), *E. ortleppi* and *E. felidis* were found in 23/71, 5/71 and 2/71 faecal samples, respectively. Co-infections involving *E. granulosus* s. s. and *E. canadensis* (G6/7) were observed in seven and one faecal samples from Turkana and Maasai Mara, respectively. *E. granulosus* s. s. and *E. ortleppi* infection was recorded in one faecal sample from Turkana. One faecal sample from Turkana showed infection with the three taxa, *E. granulosus* s. s., *E. ortleppi*, and *E. canadensis* (G6/7).

5.4.4 Estimated prevalence of *Echinococcus* spp. within study sites

The prevalence of *Echinococcus* spp. infection in dogs differed within different regions in the same study sites. *Echinococcus* spp. infection was 2% in Turkana north, 8.8% in Turkana west, 11.1% in central and 14.4% in Turkana south. (Table 5.4). Three sites areas were examined in Maasai Mara and included: Sekenani, Talek and Ewaso Ng'iro and accounted for 1.6%, 4.2% and 7.8% respectively (Table 5.4). Tharaka north reported no case of infection but 0.5% was recorded in Igembe central of Meru. Of the four sub divisions in Isiolo *Echinococcus* spp. was reported only

in two that is central (1.1 %) and Merti (1.6 %), both Isiolo south and Oldonyiro divisions reported no infections in dogs. However only 24 faecal samples were examined from Isiolo south (Table 5.4). *Echinococcus* spp. infection hot spots within the study sites included; Lokangae, Lodwar, Lokichar and Kainuk all in Turkana and Ewaso Ng'iro and Tipilikwani in Maasai Mara, where many taxa of *Echinococcus* were present. Host species specific PCR detected dog DNA from one of the two faecal samples with *E. felidis* eggs.

Table 5. 3 Faecal prevalence of *Echinococcus* species in CE endemic areas of Kenya

Study site (n samples)	Samples positive for *Echinococcus* spp.	Samples positive for *E. granulosus s. s.*	Samples positive for *E. ortleppi*	Samples positive for *E. canadensis* (G6/7)	Samples positive for *E. felidis*
Turkana (n = 524)	48 (9.2 %)	32 (6.1 %)	4 (0.8 %)	21 (4.0 %)	0 (0.0 %)
Meru (n = 303)	1 (0.3 %)	1 (0.3 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Isiolo (n = 294)	2 (0.7 %)	1 (0.3 %)	0 (0.0 %)	1 (0.3 %)	0 (0.0 %)
Maasai Mara (n = 500)	20 (4.0 %)	17 (3.4 %)	1 (0.2 %)	1 (0.2 %)	2 (0.4 %)
Total n = 1621	**71 (4.4 %)**	**51 (3.1 %)**	**5 (0.3 %)**	**23 (1.4 %)**	**2 (0.1 %)**

n = Number of faecal samples

Table 5. 4 Variation of estimated faecal prevalence of *Echinococcus* spp. infection in subdivisions of CE endemic areas of Kenya

Study area	No. of faecal samples	*Echinococcus* spp.	Prevalence (%)
Turkana			
West	171	15	8.8
North	101	2	2
Central	162	18	11.1
South	90	13	14.4
Total	**524**	**48**	**9.2**
Meru			
Igembe Central	194	1	0.5
Tharaka North	109	0	0
Total	**303**	**1**	**0.3**
Isiolo			
Oldonyiro	114	0	0
South	24	0	0
Central	93	1	1.1
Merti	63	1	1.6
Total	**294**	**2**	**0.7**
Maasai Mara			
Sekenani	183	3	1.6
Talek	214	9	4.2
Ewaso Ng'iro	103	8	7.8
Total	**500**	**20**	**4**

5.4.5 Identification of *Echinococcus* spp. haplotypes

Only 53 out of 344 taeniid eggs yielded a PCR product with at least of one the three regions of *cox1* (five prime, middle and three prime regions). Of these, 38 had been identified by *nad1* PCR assay while the remaining 15 eggs were part of the additional 106 eggs that were freshly picked. The 53 eggs were from 11 faecal samples which originated from Turkana (6) and Maasai Mara (5).

5.4.5.1 *E. granulosus* s. s. haplotypes

Cytochrome C oxidase 1 sequences of 32 *E. granulosus* s. s. eggs from 7 faecal samples (Maasai Mara, 5 & Turkana, 2) were obtained. For haplotype analysis each region of the *cox1* gene was

analysed independently and included; 10 sequences for 5'region (672 bp long), 24 sequences for middle region (338 bp) and 18 sequences for the 3'region (599 bp). Four haplotypes were identified for each of the three regions of the gene (EgKd01 – EgKd12). Information on the frequency, identity and distribution of *E. granulosus* s. s. haplotypes is provided in Table 5.5. Thirteen polymorphic sites were identified in *cox1* sequence of *E. granulosus* s. s., they included 4, 5 and 4 for the 5', middle and 3' regions respectively. Majority of the substitutions did not result into amino acid sequences change (8 of 13), while 5 of 13 substitutions resulted into changes in amino acid sequences. The 12 haplotypes (EgKd01 – EgKd12) for *E. granulosus* s. s. are represented by sequences with accession numbers MH311673 – MH311682, MN249173 and MH311683 respectively (Table 5.5).

5.4.5.2 *E. canadensis* (G6/7) haplotypes

Cytochrome c oxidase 1 sequences from 21 eggs of *E. canadensis* (G6/7) from four Turkana faecal samples were obtained. The 5', middle and 3' regions of *E. canadensis* (G6/7) were represented by 12, 15 and 17 *nad1* sequences respectively in this study. These sequences resulted to 4 haplotypes (EcKd01 – 04) for the 5' region and 2 haplotypes for each of the middle (EcKd05 – 06) and 3' region (EcKd07 – 08). Information on the frequency, identity and distribution of *E. canadensis* (G6/7) haplotypes is provided in Table 5.6. Five polymorphic sites were observed for *cox1* of *E. canadensis* (G6/7), 3 for the five prime region and one for each of middle and three prime regions. Three of the substitutions resulted into amino acid sequences changes (non-synonymous substitution) while in the other 2 substitutions there were no changes of the amino acid sequences (synonymous substitution). The 8 haplotypes (EcKd01 – EcKd08) for *E. canadensis* (G6/7) are represented by sequences with accession numbers MH311684 – MH311691 respectively (Table 5.6).

Table 5. 5 *Echinococcus granulosus* sensu stricto haplotypes detected in dog faecal samples

	Haplotype	Accession number	No. of sequences	Identity	Geographical distribution	Reference
1	EgKd01	MH311673	5/10	EG01 Acc. No. JQ250806	Worldwide	(Yanagida *et al.*, 2012),
2	EgKd02	MH311674	3/10	EgK42	Kenya	Ebi unpublished
3	EgKd03	MH311675	1/10	Novel, G12C substitution from EG01 Acc. No. JQ250806		(Yanagida *et al.*, 2012),
4	EgKd04	MH311676	1/10	EgK23 and EgK32	Kenya	Ebi unpublished
5	EgKd05	MH311677	1/24	EG02 Acc. No. JQ250807	Worldwide	(Yanagida *et al.*, 2012)
6	EgKd06	MH311678	21/24	EG01 Acc. No. JQ250806	Worldwide	Yanagida *et al.*, 2012)
7	EgKd07	MH311679	1/24	Novel, T948C, EgA85 KX020405	Armenia	Ebi unpublished
8	EgKd08	MH311680	1/24	Novel, substitution of T756C, T776C EgA82 KX020402, & haplotype NGR Acc. No. MG322623	Armenia and Iran	Ebi unpublished, Nikpay unpublished
9	EgKd09	MH311681	14/18	EG01 Acc. No. JQ250806	Worldwide	(Yanagida *et al.*, 2012)
10	EgKd10	MH311682	1/18	Novel, substitution of C1528A, EgA84 KX020404	Armenia	Ebi, unpublished
11	EgKd11	MN249173	2/18	Novel, substitution of C1234T ARG1 acc. No. KY766882	Argentina	(Kinkar *et al.*, 2017)
12	EgKd12	MH311683	1/18	Novel, substitution of G1297A IND2 acc. No. KY766891	India	(Kinkar *et al.*, 2017)

Table 5. 6 *Echinococcus canadensis* (G6/7) haplotypes detected in dog faecal samples

	Haplotype	Accession number	No. of sequences	Identity	Geographical distribution	Reference
1	EcKd01	MH311684	1/12	Identical to several haplotypes	Wide (Kenya, Sudan, Iran Russia, Mongolia, Argentina)	(Konyaev *et al*., 2012; Konyaev *et al*., 2013; Ito *et al*., 2014; Addy *et al*., 2017b; Kinkar *et al*., 2018)
2	EcKd02	MH311685	9/12	Haplotype Ec13 acc. No. KX010842	Kenya	(Addy *et al*., 2017b)
3	EcKd03	MH311686	1/12	Haplotype Ec06 acc. No. KX010835	Kenya	(Addy *et al*., 2017b),
4	EcKd04	MH311687	1/12	New haplotype, substitution A635C from haplotype Ec03, acc. No. KX010832		(Addy *et al*., 2017b)
5	EcKd05	MH311688	14/15	Identical to AB208063	Wide (Africa, Europe, South America and Asia)	(Nakao *et al*., 2007)
6	EcKd06	MH311689	1/15	Identical to haplotypes Ec05, acc. No. KX010834 and Ec11, acc. No. KX010840	Kenya	(Addy *et al*., 2017b)
7	EcKd07	MH311690	16/17	Identical to several haplotypes	Africa, Europe, South America, Asia	(Konyaev *et al*., 2012; Konyaev *et al*., 2013; Ito *et al*., 2014; Addy *et al*., 2017b; Kinkar *et al*., 2018)
8	EcKd08	MH311691	1/17	New haplotype, substitution for C1059A from sequence AB208063.		(Nakao *et al*., 2007)

5.4.5.3 Diversity and neutrality indices

The haplotype diversity of *E. granulosus* s. s. and *E. canadensis* (G6/7) sequences was generally low albeit for the 5' region. Thus for *E. granulosus* s. s. 0.6364 ± 0.128 and 0.4545 ± 0.170 for *E. canadensis* (G6/7) (Table 5.7 and 5.8) respectively. The middle and three prime regions showed lower haplotype diversity for both species. The nucleotide diversity for both *E. granulosus* s. s. and *E. canadensis* (G6/7) sequences were low and ranged from 0.00020 ± 0.00017 for *E. canadensis* (G6/7) and 0.00135 ± 0.00043 for *E. granulosus* s. s Table 5.7 and 5.8 respectively. The neutrality indices Tajima's D and Fu's Fs were negative for all regions of both species but were not significant.

Table 5. 7 Diversity and neutrality indices of *E. granulosus s. s.* based on *cox1* sequences for the five prime, middle region and three prime of the gene.

Part of *cox1*	n	Hn	Hd ± SD	π ± SD	Tajima's D	Fu's Fs
5' region	10	4	0.6364 ± 0.128	0.00135 ± 0.00043	-1.10317	-1.872
Middle region	24	4	0.2300 ± 0.110	0.00118 ± 0.00071	-1.99611	-1.813
3' region	18	4	0.3801 ± 0.134	0.00102 ± 0.00042	-1.37975	-1.177

Table 5. 8 Diversity and neutrality indices of *E. canadensis* (G6/7) based on *cox1* sequences for the five prime, middle region and three prime of the gene.

Part of *cox1*	n	Hn	Hd ± SD	π ± SD	Tajima's D	Fu's Fs
5' region	12	4	0.4545 ± 0.170	0.00131 ± 0.00054	-0.37851	-0.895
Middle region	15	2	0.1333 ± 0.112	0.00039 ± 0.00033	-1.15945	-0.649
3' region	17	2	0.118 ± 0.101	0.00020 ± 0.00017	-1.16387	-0.748

5.5 Discussion

This study reports the prevalence and distribution of *Echinococcus* spp. infections in dogs from four different regions in Kenya based on faecal samples that were collected from the environment. It presents for the first time data on *Echinococcus* spp. from dogs in Isiolo, Meru and Maasai Mara and current figures on the situation in Turkana. Prevalence of *Echinococcus* spp. infection in dogs reported here was lower than previous figures from Kenya. For example, in a study conducted two decades ago, 42/58 (72%) of dogs examined at necropsy in Nairobi (Kiserian, Rongai and Dagoretti) were found with *Echinococcus* adult worms (Wachira *et al*., 1994), while a prevalence of 27.3% in dogs in Maasailand (Kajiado district) was reported by Eugester (1978). Similarly, previous prevalence accounts in dogs in Turkana showed higher values: 39.4% at necropsy (Macpherson *et al*., 1985), 33% at necropsy and 26% by copro-antigen detection (Buishi *et al*., 2006). The lower prevalence figures in this study are explained by the lower sensitivity of detection method used in this study (eggs in faeces) that was intended to obtain individual eggs for further examination, compared to the previous studies that used copro-antigen, necropsy and detection of adult worms in the small intestines of dogs. Prevalence figures between these studies are therefore not comparable, and no conclusions on temporal trends can be drawn. Taeniid eggs from 60 faecal samples could not be amplified by PCR and therefore not identified, which further contributed to a decreased prevalence of *Echinococcus* infection in dogs.

The decrease in prevalence could also be attributed to prepatent infections and periodic shedding of *Echinococcus* eggs during patent infections, desiccation of taeniid eggs due to extreme temperatures (Wachira *et al*., 1991; Trachsel *et al*., 2007; Hüttner *et al*., 2009) and the cross-sectional approach used in this study. The earlier surveys in Turkana were conducted immediately after major droughts, during which an abundance of livestock carcasses were available to dogs

(Macpherson *et al*., 1985; Wachira *et al*., 1990) which was not the case in the present study. The difference of infection frequency in dogs between the various study regions corresponds well with data from livestock: in the most recent surveys, prevalence of CE in livestock in Turkana was reported as 19.4% in cattle, 3.6% in sheep, 4.5% in goats and 61.4% in camels (Njoroge *et al*., 2002). In Meru and Isiolo it was only 1.92% in cattle, 6.94% in camels, 0.37% in goats and 4.62% in sheep (Mbaya *et al*., 2014). In Maasailand CE prevalence was 25.8% in cattle, 16.5% in sheep and 10.8% in goats (Addy *et al*., 2012).

A number of PCR-based tools demonstrating high specificity have been developed for copro-detection of *Echinococcus* eggs (DNA) in dogs (Cabrera *et al*., 2002; Abbasi *et al*., 2003; Dinkel *et al*., 2004; Stefanić *et al*., 2004; Trachsel *et al*., 2007; Boufana *et al*., 2013b). However, some of the PCR methods are species specific (Stefanić *et al*., 2004) and therefore would fail to detect co-infection with other species (Boufana *et al*., 2008), whereas others methods have, reportedly, the capacity to detect all species of *E. granulosus* s. l. but cannot discriminate them (Abbasi *et al*., 2003). The development of multiplex PCR enables the simultaneous detection of taeniids in definitive hosts (Trachsel *et al*., 2007; Boubaker *et al*., 2013; Liu *et al*., 2015a). While Boubaker *et al*. (2013) multiplex PCR showed high sensitivity and specificity to discriminate species of *E. granulosus* s. l., it has however exhibited low sensitivity on *Echinococcus* eggs. Parasite DNA can be extracted directly from faecal samples or from isolated taeniid eggs. In areas where *Taenia* spp. co-exist with *Echinococcus* spp., PCR on individual eggs is recommended because taeniid eggs can be differentiated by RFLP or sequencing (Hüttner *et al*., 2009), or through multiplex PCR (Trachsel *et al*., 2007) or by use of species specific primers (Stefanić *et al*., 2004). The approach chosen in the present study allowed the identification of mixed infection with different *Echinococcus* and *Taenia* (Chapter 6) taxa, based on visually identified eggs, which was

considered more informative compared to PCR results from direct faecal DNA extraction. As a trade-off, PCR on individual taeniid eggs was preferred in the present study although it offers considerably lower sensitivity, as *Echinococcus* eggs are not shed regularly and would be missed during the prepatent phase of *Echinococcus* infection. In case of further studies the combined application of copro-antigen tests (for more realistic prevalence estimation) and PCR of eggs would be desirable (Deplazes *et al*., 2003).

Based on the number of prevailing *Echinococcus* spp. and abundance in localities, some areas were identified as hot spots for infection in dogs (and certainly high risk for intermediate host and human infections). For example, the risk of abundance of *Echinococcus* taxa, (*E. granulosus* s. s., *E. ortleppi* and *E. canadensis* (G6/7) in Lokangae, could be seen in the high CE infection in humans (5/150 people positive on ultrasonography – 3.3%) at the time of faecal sampling (Zeyhle, personal communication). The observation in Lokangae is an old persisting scenario, since older studies by Macpherson *et al*. (1985) found *Echinococcus* spp. prevalence as high as 64.7% in dogs at necropsy. Lokangae and most parts of Turkana West included in this study are of typical rural setting where transmission is likely to be influenced by home slaughter, access to offal by dogs, poor hygiene due to shortage of water and close human – dog interactions (Buishi *et al*., 2006). Another rural hot spot identified was Tipilikwani village of Talek in Maasai Mara. The village covers an area of approximately 1 km^2 and *E. granulosus* s. s. eggs were detected in most of the *Echinococcus* positive faecal samples. There, CE was recently detected in 3/133 residents (2.3%) by ultrasonography (Zeyhle, personal communication) with two of the positive individuals living in a homestead with an *Echinococcus* positive dog. In small-town settings such as in Lodwar (Turkana Central), Lokichar and Kainuk (Turkana South), transmission of CE in humans is likely to be affected by improper disposal of slaughter offal in abattoirs, and by free roaming dogs from

neighbouring villages. Prevalence of *Echinococcus* infection in dogs in these areas were comparable to previous observations of 28.6% in Turkana South and 16.7% in Turkana Central by Macpherson *et al*. (1985). In Ewaso Ng'iro (Maasai Mara), there were three slaughter houses whose hygiene and condemned offal disposal practices were clearly inadequate, and this could be linked to the rate of infection in dogs. The contribution of urban abattoirs to the transmission of *Echinococcus* spp. infection has been investigated in Kenya and elsewhere (Wachira *et al*., 1994; Reyes *et al*., 2012; Chaâbane-Banaoues *et al*., 2016; Thapa *et al*., 2017). Seventy two percent of dogs scavenging around abattoirs in Nairobi were found infected with *Echinococcus* spp. at necropsy (Wachira *et al*., 1994). In Lima, Peru, infection in dogs around urban abattoirs could be linked to 9. 3% of CE cases in humans (Reyes *et al*., 2012).

E. granulosus s. s. was the most abundant species reported in the present study, confirming evidence from humans and livestock in Kenya presented in previous studies (Wachira *et al*., 1993a; Dinkel *et al*., 2004; Magambo *et al*., 2006; Hüttner *et al*., 2009; Casulli *et al*., 2010; Romig *et al*., 2011; Addy *et al*., 2012; Mutwiri *et al*., 2013; Mbaya *et al*., 2014; Odongo *et al*., 2018). It was the only taxon present in dogs from all four study sites in both rural and urban settings. *E. granulosus* s. s. is known as the major cause of human CE (88%) in the world (Alvarez Rojas *et al*., 2014) and particularly (83 – 100%) in Kenya (Wachira *et al*., 1993a; Dinkel *et al*., 2004; Hüttner *et al*., 2009; Casulli *et al*., 2010; Romig *et al*., 2011; Mutwiri *et al*., 2013). Similarly, all characterised livestock CE studies in Kenya identified *E. granulosus* s. s. as the predominant taxon in cattle, sheep and goats (Wachira *et al*., 1993a; Dinkel *et al*., 2004; Hüttner *et al*., 2009; Romig *et al*., 2011; Addy *et al*., 2012; Mbaya *et al*., 2014; Odongo *et al*., 2018). It was indicated that the slaughter of cattle away from resource poor rural settings could mitigate the transmission of *Echinococcus* to dogs in the livestock-raising areas (Addy *et al*., 2012), but poor abattoir conditions in some urban areas

mean that dogs around those abattoirs could act as infection sources for humans. In addition, *E. granulosus* s. s. is able to reach fertility in all livestock ruminants, particularly in sheep (which are commonly slaughtered at home) (Njoroge *et al*., 2002; Addy *et al*., 2012; Mbaya *et al*., 2014) and may aid transmission to dogs. Sheep are often the preferred source of meat in Maasailand (Macpherson *et al*., 1985; Addy *et al*., 2012) and this coupled with home slaughter, high cyst fertility, lack of stringent meat inspection, poor abattoir conditions and the high numbers of roaming dogs (an observation during the study) may explain the abundance of *E. granulosus* s. s. in the present study.

E. canadensis (G6/7) was the second most common species in this study and was reported in Turkana, Isiolo and Maasai Mara. A similar trend was observed before in the works of Wachira *et al*. (1993a) where 38% of isolated *Echinococcus* adult worms in dogs were of the camel strain, second to the sheep strain (62%). *E. canadensis* (G6/7) is also the second most important agent of human CE in Turkana after *E. granulosus* s. s., accounting for between 15-17% of characterised cases there (Casulli *et al*., 2010; Mutwiri *et al*., 2013) compared to 11% of characterised cases globally (Alvarez Rojas *et al*., 2014). The frequency of *E. canadensis* (G6/7) in dogs is related to the high prevalence in camels (61.4%) in Njoroge *et al*. (2002) and goats (30% to 92%) in Wachira *et al*. (1993a), Dinkel *et al*. (2004), and Hüttner *et al*. (2009). *E. canadensis* (G6/7) has high fertility in camels (85%) (Njoroge *et al*., 2002) and in goats (16%) (Varcasia *et al*., 2007) and 3 cysts out 4 (Addy *et al*., 2012). These factors coupled with the preference for home slaughter of goats for meat in Turkana (Njoroge *et al*., 2002) may support and enhance the transmission in dogs.

E. ortleppi, although rare in this study, was reported for the first time in dogs in Kenya. *E. ortleppi* in Kenya was first detected in a pig isolate (Dinkel *et al*., 2004) and later in cattle from Isiolo (Mulinge unpublished data) and Maasailand (Addy *et al*., 2012). It was the study by Mbaya

et al. (2014) that shed light on Meru and Isiolo as major foci of *E. ortleppi* where it was isolated from cattle, goats and sheep in a total of 51/258 (19.8%) cysts examined. Interestingly, in the present study, *E. ortleppi* was not detected in Meru or in Isiolo among the 597 faecal samples examined. This discrepancy to livestock data remains unexplained; possibly, transmission is focal around areas of frequent cattle home slaughter, which have not been covered in this survey. The general rarity of *E. ortleppi* in Maasai Mara (in both dogs and livestock) may be explained by the slaughtering of cattle in distant abattoirs (Addy *et al*., 2012). The detection of *E. ortleppi* in Turkana is rather surprising considering that it had never been found in any of the other hosts in the area; however, the coverage of intermediate hosts in Turkana is insufficient compared to other regions of Kenya (Wachira *et al*., 1993a; Dinkel *et al*., 2004; Hüttner *et al*., 2009; Romig *et al*., 2011; Mutwiri *et al*., 2013). The only available studies in Turkana by Njoroge *et al*. (2002) have shown 19.4% of cattle slaughtered in three centres, Lokichogio, Kakuma and Lodwar had CE (taxa not determined). Although the fertility of cysts in cattle from that study was high with 50.7% in Lokichogio and 42.9% in Kakuma (Njoroge *et al*., 2002), indicating the involvement of the cattle-adapted *E. ortleppi*, the epidemiology of this parasite in Turkana remains to be unravelled.

This study reports for the first time the occurrence of *E. felidis* in domestic dogs. *E. felidis* so far had only been isolated from wild mammals and is thought to be restricted to wildlife transmission (Hüttner *et al*., 2009; Kagendo *et al*., 2014; Halajian *et al*., 2017). Isolation of *E. felidis* from domestic dog faeces was therefore a surprising observation. For one of the faecal samples, the domestic dog was confirmed by host specific PCR. This sample was taken in Oloolaimutia community in the vicinity of the Maasai Mara National Reserve. The second sample was collected in Ewaso Ng'iro, an urban centre distant (~80 km) from the National Reserve. Though the host species of this sample could not be established by molecular means, it is likely to

be from a domestic dog considering the environment. The involvement of a domestic intermediate host remains unlikely, as several studies in the Maasai Mara areas failed to detect *E. felidis* in livestock (Wachira *et al*., 1993a; Hüttner *et al*., 2009; Addy *et al*., 2012) and (Kagendo unpublished data). On the other hand, data on the intermediate host range of *E. felidis* is still scanty and the only reported accounts were from warthogs in Uganda (Hüttner *et al*., 2009) and Namibia (Romig *et al*., 2017) and in a most recent account from hippopotamus in South Africa (Halajian *et al*., 2017).

In the Maasai Mara National Reserve, studies on wild herbivores are restricted to the examination of 493 drowned wildebeests showing no *E. felidis* infection (Kagendo *et al*., 2014). The finding of *E. felidis* in faecal samples from domestic dogs in this study may be due to an infection acquired by scavenging on carcasses of wild intermediate hosts in the National Reserve, which is plausible at least for the dog from Oloolaimutia. However, as dogs are known to be coprophagous and may feed on faeces of other carnivores, the presence of *E. felidis* eggs in dog faeces may be explained by this route. In the absence of any detection of gravid worms in a dog's intestine, these findings do not permit confirmation of dogs as hosts of *E. felidis*. However, the suitability of domestic dogs as definitive hosts needs to be further explored. As *E. felidis* belongs to the same clade as *E. granulosus* s. s. (Hüttner *et al*., 2008), pathogenicity for humans cannot be ruled out even though no human case has ever been detected. This requires a careful evaluation of this species epidemiology and its potential for a switch to domestic transmission.

The mixed infections with *E. granulosus* s. l. taxa observed in the present study add to the global understanding of such occurrences in dogs. Generally, dual infection in definitive hosts with *Echinococcus* taxa have been shown in studies from Iran, China, Kazakhstan and Canada (Stefanić *et al*., 2004; Xiao *et al*., 2006a; Zhang *et al*., 2006; Beiromvand *et al*., 2011; Jiang *et al*., 2012; Schurer *et al*., 2014; Shariatzadeh *et al*., 2015). The mixed infections in dogs with different

Echinococcus taxa demonstrate frequent access to offal infected with the different taxa of *Echinococcus* (Lymbery, 2017). Mixed taxa infection as explained by Lymbery (2017) enables cross fertilization, which should stimulate further research in this study region on nuclear gene markers of these parasites, in order to detect possible hybridizations and estimate the extent of gene flow between these taxa.

E. granulosus s. s. haplotypes displayed varied geographical distribution, half of the haplotypes reported for the first time in this study were the least found. Two haplotypes were reported earlier in Kenya (Ebi unpublished data) and occurred less in this study. A rare haplotype in this study showed a wide geographical distribution being found in Africa, Europe, South America and Asia (Nakao *et al*., 2010; Yanagida *et al*., 2012; Boufana *et al*., 2015a; Alvarez Rojas *et al*., 2016; Kinkar *et al*., 2018). The last three haplotypes showed worldwide distribution and where the most common in this study (Nakao *et al*., 2010; Casulli *et al*., 2012; Yanagida *et al*., 2012; Boufana *et al*., 2014; Boufana *et al*., 2015a; Alvarez Rojas *et al*., 2016; Kinkar *et al*., 2018). The presence of a common haplotype in this study with worldwide occurrence is an indication that each *E. granulosus* s. s. populations may have originated from a common ancestor (Yanagida *et al*., 2012). Furthermore, Boufana *et al*. (2015a) reported a common lineage for *E. granulosus* s. s. isolates of canids of wide geographical locations. *E. canadensis* (G6/7) showed less geographical distribution compared to *E. granulosus* s. s. Two novel haplotypes were found rarely in this study, while three haplotypes were only reported in Kenya before and rare apart from one which was very common (Addy *et al*., 2017b). Two of three haplotypes that showed wide global distribution were the most common in this study but one of them occurred once (Konyaev *et al*., 2012; Konyaev *et al*., 2013; Ito *et al*., 2014; Addy *et al*., 2017b; Kinkar *et al*., 2018).

Low haplotype diversities compared to other genetic diversity studies were observed from this study. Boufana *et al.* (2015a) reported similar findings for definitive hosts isolates as compared to isolates derived from intermediate hosts but is contrary to findings in the Middle East in which high haplotype diversity was reported in isolates from stray dogs (Shariatzadeh *et al.*, 2015). The low haplotype diversity could be due to sexual reproduction of the adult tapeworms in the small intestine of definitive hosts where self-fertilization is likely to occur and is known to promote homozygosity which favours low genetic variability (Lymbery, 2017). The short life span of the adult tapeworm in the small intestines of definitive hosts is also thought to affect genetic variability (Boufana *et al*., 2015a). The low haplotype and nucleotide diversities could also be explained as lack of population expansion as observed for *E. equinus* in United Kingdom (Boufana *et al*., 2015b). It could also be argued that the sample size used in this study was small to make any conclusive findings. The neutrality indices (Tajima's D and Fu's Fs) for all the parts of *cox1* were negative for both *E. granulosus* s. s. and *E. canadensis* (G6/7) sequences which might be explained as result of population expansion.

5.6. Conclusions

The study has shed light on the *Echinococcus* spp. infection in the definitive host to corroborate the extensive knowledge in livestock in Kenya. Turkana and Maasai Mara (Maasailand) remain highly endemic areas for cystic echinococcosis with *E. granulosus* s. s. and *E. canadensis* (G6/7) as the most important agents of *Echinococcus* infection. Isolation of *E. felidis* in faeces of domestic dogs exposes a new aspect of the 'African lion strain' and highlights its little understood epidemiology. There is therefore the need for comprehensive studies to unravel the wildlife-domestic transmission overlap hypothesis in biodiversity rich settings like in the Maasai Mara. This

study provides important baseline data on *Echinococcus* spp. infection in dogs that is useful in designing control strategies.

CHAPTER SIX: DIVERSITY OF *TAENIA* AND *HYDATIGERA* (CESTODA: TAENIIDAE) IN DOMESTIC DOGS IN KENYA

(This chapter has been published in Parasitology Research, 2020)

https://www.ncbi.nlm.nih.gov/pubmed/

6.1 Abstract

Taenia species of domestic dogs can cause cysticercosis and coenurosis in a wide range of intermediate hosts including humans. Most taeniids of dogs are globally distributed, but some wildlife-transmitted species can be specific for certain regions. Generally, little information exists on the species composition and frequency in most regions of the world, which impairs risk assessment and control strategies. This study determined the range of taeniid species in dogs in four widely spaced areas of Kenya by genetic identification of eggs in faeces collected from the environment. Individual taeniid eggs were characterized by nested polymerase chain reaction of NADH dehydrogenase subunit 1 and cytochrome C oxidase 1 genes, restriction fragment length polymorphism and partial sequencing. Overall 79/1621 (4.9%) faecal samples contained eggs of *Taenia* or *Hydatigera* (8.0% in Turkana, 4.8% in Isiolo, 3.8% in Maasai Mara and 1.3% in Meru). *Taenia hydatigena* and *T. multiceps* were the most frequent, found in 36 and 15 samples, respectively. Other eggs found in the faeces belonged to *T. serialis* (sensu lato), *T. madoquae* (the first record in domestic dogs), *T. ovis*, *T. saginata* and *Hydatigera taeniaeformis*. Polymorphism of *nad1* sequences revealed 22 and 8 haplotypes of *T. hydatigena* and *T. multiceps*, respectively. The results show the involvement of dogs in both domestic and sylvatic transmission cycles. In addition to the species range, this study provides data on the intraspecific diversity of *T. hydatigena* and *T. multiceps* in Kenya, which will serve as baseline information for further studies into cysticercosis and coenurosis in livestock and humans in the region.

Keywords: *Taenia*/*Hydatigera* species; Haplotypes; Dogs; Kenya

6.2 Introduction

The family Taeniidae (Eucestoda: Cyclophyllidea) consists of four genera: *Taenia* Linnaeus, 1758, *Echinococcus* Rudolphi, 1801, *Hydatigera* Lamarck, 1816 and *Versteria* Nakao et al., 2013 All taeniid cestodes complete their life cycles in two mammalian hosts; carnivorous or omnivorous definitive hosts that harbour the adult tapeworm in the small intestine, and herbivorous or omnivorous intermediate hosts in which the larval stages (cysticerci or coenuri, strobilocerci, and the various forms of *Echinococcus* metacestodes) are localized in the musculature, visceral organs or central nervous system depending on the cestode species.

Various taeniids are of veterinary and economic importance in resource poor communities and / or are associated with human diseases (Hoberg, 2002). The domestic dog is a definitive host of several *Taenia* spp. that infect livestock, namely *T. hydatigena* (Pallas, 1766), *T. multiceps* (Leske, 1780) and *T. ovis* (Cobbold, 1869), which may lead to substantial losses to the livestock industry (Oryan *et al*., 2012; Scala *et al*., 2015; Asmare *et al*., 2016). Much of these losses occur in the context of traditional livestock husbandry in resource poor countries where the economic impact is difficult to quantify. In addition, several dog taeniids have zoonotic potential as agents of coenurosis (*T. multiceps* (Leske, 1780), *T. serialis* (Gervais, 1847)) and cysticercosis (*T. crassiceps* (Zeder, 1800)) (Deplazes *et al*., 2019). Globally, nearly 50 *Taenia* spp. are known; the majority are present in sub-Saharan Africa, whose cestode diversity – particularly in wild mammals – is still poorly investigated and many more species await description. Many of these wildlife-associated species are known or suspected to occur in domestic dogs, but the knowledge on the host range of African taeniids is still inadequate (Loos-Frank, 2000; Hoberg, 2006; Lavikainen *et al*., 2008; Terefe *et al*., 2014).

Accurate identification of taeniids causing infections in every endemic locality is critical for understanding the epidemiology and development of intervention strategies. Identification has previously relied on morphological characteristics of cysticerci/coenuri and adult tapeworms (Verster, 1969; Rausch, 1994). However, taeniid eggs are morphologically indistinguishable even between genera and therefore require molecular methods for identification. The use of molecular tools (mitochondrial and/or nuclear markers) to detect and characterise taeniid DNA in recent times have shed much light on the understanding of *Taenia* diversity (Allsopp *et al*., 1987; Kedra *et al*., 2001; Liu *et al*., 2011). Using morphologic and genetic data has led to re-evaluation of previously described taeniids in different endemic areas and linked phenotypic characteristics with intra-species genetic variations (McManus, 2006; Christodoulopoulos *et al*., 2016; Varcasia *et al*., 2016).

Presence or absence of individual taeniid species in any particular area are closely linked to environmental and cultural factors (climate, livestock husbandry, presence of wildlife). The current study was therefore performed in four widely spaced areas that represent diverse environments within Kenya. This study focusses on species of *Taenia* and *Hydatigera*, as the presence of *Echinococcus* spp. in these areas has already been addressed in Chapter 5.

6.3 Materials and methods

6.3.1 Faecal sample collection, isolation of taeniid eggs and microscopy examination

Faecal samples were collected from the ground (environment) and preserved immediately in 80% ethanol (section 3.3.2). Taeniid eggs were isolated from approximately 2 – 3 g of faecal samples using zinc chloride flotation-sieving technique (Mathis *et al*., 1996), this method is described in

details in section 3.3.2. Individual taeniid eggs (1-20) were picked using a micro-pipette under the microscope (Magnification × 40 or 100) for molecular analysis as described in section 5.3.2.

6.3.2 DNA extraction, *nad1* nested PCR-RFLP and sequencing

DNA was obtained from proglottids in one faecal sample using DNeasy Blood & Tissue Kits (Qiagen) and by lysing taeniid eggs in 10 µl of 0.02 M NaOH at 99 °C for 10 min. The proglottis was chopped into tiny pieces and subjected to DNA extraction according to manufacturer's instructions. A nested PCR targeting part of the *nad1* gene was performed to detect *Echinococcus* and *Taenia* spp. eggs as reported in section 5.3.3. PCR products were digested in a 20 µl reaction comprising of 10 µl of PCR products, 5 units *Hph*I endonuclease (Thermo Scientific), 1 × Buffer B (provided in the kit) and nuclease free water. The digestion reactions were incubated overnight at 37 °C and the digest resolved on 3% agarose gel electrophoresis. The bands were visualized under UV following ethidium bromide staining (section 5.3.4). This PCR-RFLP method differentiated eggs of *E. granulosus* s. l. from *Taenia* spp. except between *Taenia* spp. and *E. felidis*. Amplicons of suspected *Taenia* spp. or *E. felidis* isolates at this stage were purified using the High Pure PCR Product Purification Kit (Roche, Germany) and sequenced with the nested reverse primer at GATC Biotech AG, Germany (section 5.3.5). PCR products of 3 – 5 eggs were sequenced for each *Taenia* positive sample and if more than one species was detected additional PCR products of eggs from the same faecal sample were sequenced.

6.3.3 Amplification of cytochrome C oxidase 1 (*cox1*) gene and DNA sequencing of amplicons

A further 106 taeniid eggs representing 10 faecal samples were screened by nested PCR targeting the middle region of cytochrome C oxidase 1 (*cox1*) gene as described in section 5.3.7.1. PCR products were resolved on 2% agarose gel and visualized under UV following ethidium bromide staining as described in section 4.3.5. Amplicons 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 (section 5.3.5).

6.3.4 Data analysis

To identify *Taenia* spp., the DNA sequences were first viewed and manually edited using GENtle v. 1.9.4 http://gentle.magnusmanske.de. *Taenia* spp. were identified by comparing sequences with those available in the National Centre for Biotechnology Information database (NCBI) using the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul *et al*., 1997). Using the DNA sequences of identified *Taenia* spp. (*T. hydatigena* and *T. multiceps*), the amino acid sequences were inferred based on the flatworm mitochondrial genetic code (Nakao *et al*., 2000). The population diversity indices (number of haplotypes, haplotype diversity and nucleotide diversity) were calculated using DnaSP 5.1 (Librado and Rozas, 2009). The population genetics package Arlequin 3.1 (Excoffier *et al*., 2007) was used to conduct neutrality tests thus Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) indices for each species. Sixty-five *T. hydatigena nad1* gene partial sequences (377 bp) from this study and 24 from NCBI database were used for haplotype analysis; FJ518620 (Liu *et al.*, 2011), GQ228819 and NC012896 (Jia *et al*., 2010), JN831267 – JN831273, JN831275 – JN831278, JN831280 – N831284, JN831286 – JN831289 (Hao *et al*., unpublished data). For *T. multiceps* 46 *nad1* gene partial sequences (350 bp) from this study and 6 sequences from the NCBI database were used for haplotype analysis; FJ495086 (Liu *et al*., 2011), GQ228818 and NC012894 (Jia *et al*., 2010), KC794809 – KC794811 (Hao *et al*., unpublished data). The haplotypes networks were computed by TCS 1·21 software with 95% connection limit (Clement *et al*., 2000). For phylogeny, multiple sequence alignment was performed by MAFFT online platform (http://mafft.cbrc.jp/alignment/server) and trees were constructed using the Neighbour-Joining method (Saitou and Nei, 1987) utilizing Maximum

Composite Likelihood method (Tamura *et al*., 2004) in MEGA v.7.0 (Kumar *et al*., 2016). *E. felidis nad1* sequence MG271925 (Mulinge *et al*., 2018) was used as the out-group.

6.4 Results

6.4.1 Eggs of *Taenia* spp.

A total of 357 taeniid eggs from 78 faecal samples were identified as *Taenia* spp. by PCR-RFLP (Fig. 6.1). A representative 182/357 PCR products (181 eggs and 1 proglottid) from 63 faecal samples were sequenced. A total of 20 taeniid eggs that were PCR positive from 15 faecal samples were not sequenced due to cost, therefore the *Taenia* spp. in these samples is unknown. Five *Taenia* spp. were identified in each study site except in Meru where only two *Taenia* spp. were found (Table 6.1). *Taenia* spp. identified from the 182 sequences included: *T. hydatigena* (in Turkana – 47, Maasai Mara – 24, Isiolo – 1), *T. multiceps* (in Isiolo - 29, Maasai Mara – 24, Turkana – 2), *T. madoquae* (in Isiolo – 8, Maasai Mara – 8, Turkana – 6), *T. serialis* (in Isiolo – 10, Meru – 6, Maasai Mara – 2, Turkana - 1), *T. ovis* (in Isiolo – 9, Turkana – 1), *T. saginata* (in Maasai Mara - 3), *H. taeniaeformis* (in Meru - 1) (Table 6.1). A phylogenetic tree was constructed for the *Taenia* spp. isolates (Fig. 6.2), and sequences were compared with the following deposits in the NCBI database, showing 99 – 100% identity on the 453 bp fragment: *T. hydatigena* FJ518620 from China (Liu *et al*., 2011), *T. multiceps* FJ495086 from China (Liu *et al*., 2011), *T. serialis* AB731674 from Australia (Nakao *et al*., 2013a), *T. ovis* AB731675 from New Zealand (Nakao *et al*., 2013a), *T. madoquae* AB731726 from Kenya (Nakao *et al*., 2013a), *T. saginata* AY684274 from Korea (Jeon *et al*., 2007) and *H. taeniaeformis* AB745096 from Belgium (Nakao *et al*., 2013a). Representative sequences of the *Taenia* spp. were deposited in the Genbank under the accession numbers *T. ovis* (MH287111), *T. saginata* (MH287112), *T. serialis* (MH287113 - MH287115), *T. madoquae* (MH287116 - MH287124) and *H. taeniaeformis* (MH287125).

Table 6. 1 Identification and frequency of *Taenia* spp. in dog faecal samples from CE endemic areas of Kenya

Study site (n faecal samples)	Faecal samples with taeniid eggs	Taeniid eggs examined by PCR	PCR positive taeniid eggs	*Taenia / Hydatigera* spp. eggs (n) (RFLP)	*Taenia / Hydatigera* spp. (identified no. eggs) (*nad1* sequencing)
Turkana (n=524)	113	2121	305	103	*T. hy* (47), *T. mu* (2), *T. se* (1), *T. ma* (6), *T. ov* (1), ND (46)
Maasai Mara (n=500)	34	661	213	114	*T. hy* (24), *T. mu* (24), *T. se* (2), *T. ma* (8), *T. sg* (3), ND (53)
Isiolo (n=294)	24	468	155	133	*T. hy* (1), *T. mu* (29), *T. se* (10), *T. ma* (8), *T. ov* (9), ND (76)
Meru (n=303)	7	102	12	7	*T. se* (6)[a], *H. t* (1)
Total (n=1621)	**178**	**3352**	**685**	**357**	***T. hy* (72), *T. mu* (55), *T. se* (19), *T. ma* (22), *T. ov* (10), *T. sg* (3), *H. t* (1), ND (175)[b]**

KEY

T. hy	*Taenia hydatigena*	*T. ov*	*Taenia ovis*
T. mu	*Taenia multiceps*	*T. sg*	*Taenia saginata*
T. se	*Taenia serialis (sensu lato)*	*T. ma*	*Taenia madoquae*
H. t	*Hydatigera taeniaeformis*	*ND*	*Not determined by nad1 sequencing*

[a] *one of the T. serialis sequences was obtained from a proglottis, five from eggs*

[b] *155 Taenia or Hydatigera spp. eggs (PCR products) were not sequenced from the 63 faecal sample (represented by 181 eggs sequenced), 20 eggs from 15 faecal samples were not sequenced*

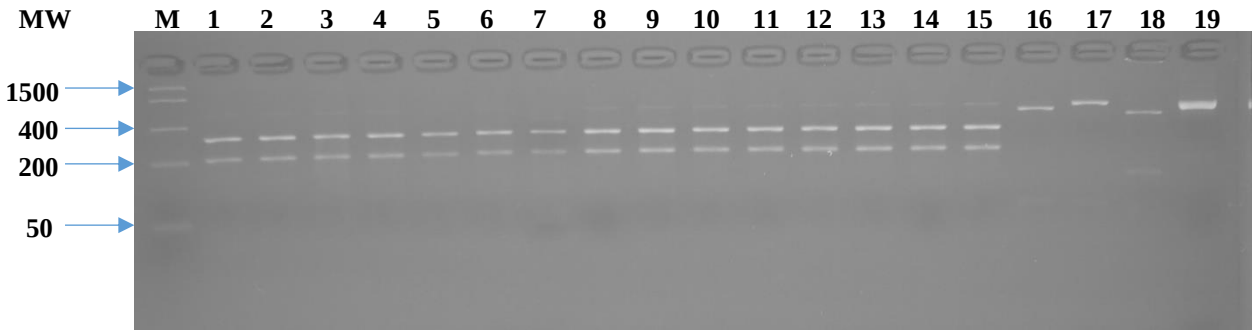

Fig. 6. 1 PCR-RFLP for *nad1* PCR products following restriction digest with *Hph*I.

Digests were separated on 3% agarose gel electrophoresis and stained with ethidium bromide. Lane M: is the molecular weight marker; lanes 1 – 15; represent *Taenia* spp. eggs, lanes 16 – 18: represent positive controls for *E. granulosus* s. s., *E. ortleppi* and *E. canadensis* (G6/7) respectively. Lane 19: represents uncut PCR product (negative control).

6.4.2 Detection of *Taenia* positive faecal samples

Taenia spp. eggs were detected in 78/1621 (4.8%) faecal samples distributed across the study sites: Turkana (8.0%), Isiolo (4.8%), Maasai Mara (3.8%) and Meru (1.3%). Overall *T. hydatigena* and *T. multiceps* were found in most of the *Taenia* positive faecal samples, thus 36/78 and 15/78, respectively. The other *Taenia* spp. were sparsely found in faecal samples and included: *T. serialis* (n = 7), *T. madoquae* (n = 5), *T. ovis* (n = 4), *T. saginata* (n = 1) and *H. taeniaeformis* (n = 1). The distribution of these *Taenia* spp. in the faecal samples are described in Table 6.2.

Table 6. 2 Frequency of faecal samples with *Taenia* / *Hydatigera* spp. eggs according to nested PCR-RFLP and sequencing of *nad1* in CE endemic areas of Kenya

Study site (n faecal samples)	Microscopy	RFLP	*nad1* sequencing						
	n samples with taeniid eggs	n samples with *Taenia* / *Hydatigera* spp.	n samples with *Taenia* / *Hydatigera* spp.	*T. hydatigena*	*T. multiceps*	*T. serialis*	*T. madoquae*	*T. ovis*	*T. saginata*	*H. taeniaeformis*
Turkana (n = 524)	113	42 (8.0%)	27	23	2	1	2	1	0	0
Maasai Mara (n = 500)	34	19 (3.8%)	19	12	6	1	2	0	1	0
Isiolo (n = 294)	24	14 (4.8%)	13	1	7	2	1	3	0	0
Meru (n = 303)	7	4 (1.3%)	4[c]	0	0	3	0	0	0	1
Total (n = 1621)	**178**	**79**	**63**	**36**	**15**	**7**	**5**	**4**	**1**	**1**

[c] one faecal sample had proglottids but no taeniid eggs

6.4.3 Detection of faecal samples with mixed infections of *Taenia* spp.

Mixed infections were reported in 6 faecal samples; two from Turkana; *T. hydatigena* and *T. serialis* (n = 1) and *T. hydatigena* and *T. multiceps* (n = 1), one faecal sample from Isiolo harboured *T. madoquae* and *T. ovis* eggs. In Maasai Mara three faecal samples had mixed *Taenia* taxa; two had eggs of *T. hydatigena* and *T. multiceps* while one faecal sample showed *T. hydatigena* and *T. madoquae* mixed infection.

6.4.4 Analysis of *nad1* haplotypes

6.4.4.1 *Taenia hydatigena* haplotypes

Of 72 *T. hydatigena* sequences, 65 entered haplotype analyses based on sequence quality. Twenty-three polymorphic sites were observed in the *nad1* 377 bp sequence of *T. hydatigena* in which 17 and 6 were synonymous and non-synonymous changes respectively. These substitutions resulted in 22 haplotypes (ThKd01 – ThKd22), represented by accession numbers (MG282256 – MG282277) respectively. Twenty haplotypes were new (ThKd02 – ThKd04, ThKd06 – ThKd22) and 2 (ThKd01 & ThKd05) had been reported previously: ThKd01 (accession number MG282256) was identical to the sequence accession number JN831273 (from a goat in China) (Hao *et al*., unpublished data) and was rare with only one sample from this study belonging to this haplotype, ThKd05 accession number MG282260 was identical to reference sequence FJ518620 (China) (Liu *et al*., 2011) and was the most frequent haplotype in this study (16 out of 65 eggs). *T. hydatigena* haplotypes identified in this study were unevenly distributed in the three endemic areas and were of varied frequencies (Fig. 6.3A). Most haplotypes were area-specific, only ThKd02, ThKd03 and ThKd05 were detected both in Turkana and Maasai Mara. Diversity was similar in Turkana (17 haplotypes among 40 eggs) and Maasai Mara (7 haplotypes among 24 eggs).

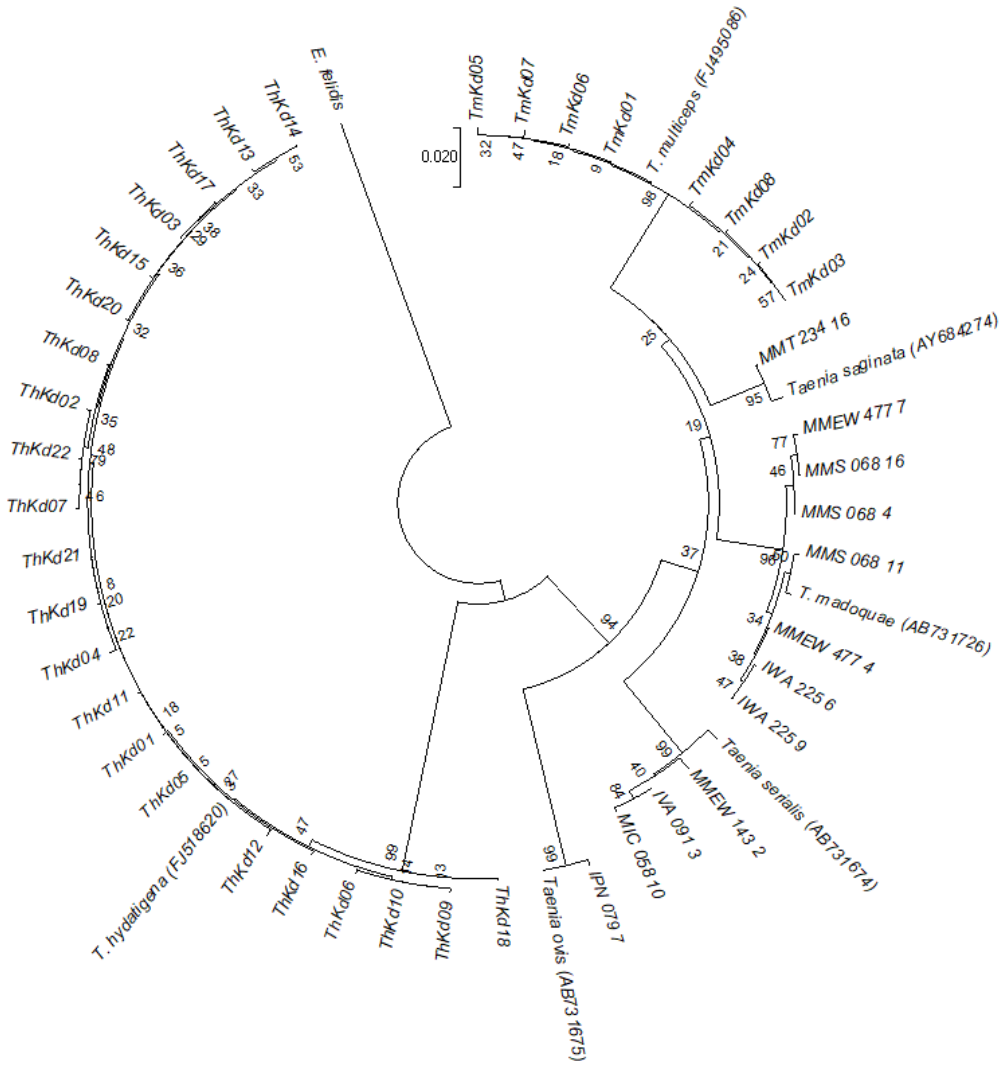

Fig. 6. 2 Phylogenetic analysis of *Taenia* species based on 453 bp mitochondrial nad1 gene sequences. The tree was inferred using Neighbour-Joining method and Maximum Composite Likelihood method in MEGA v7. *Taenia* species, *T. hydatigena* and *T. multiceps* haplotypes from this study are shown alongside reference sequences

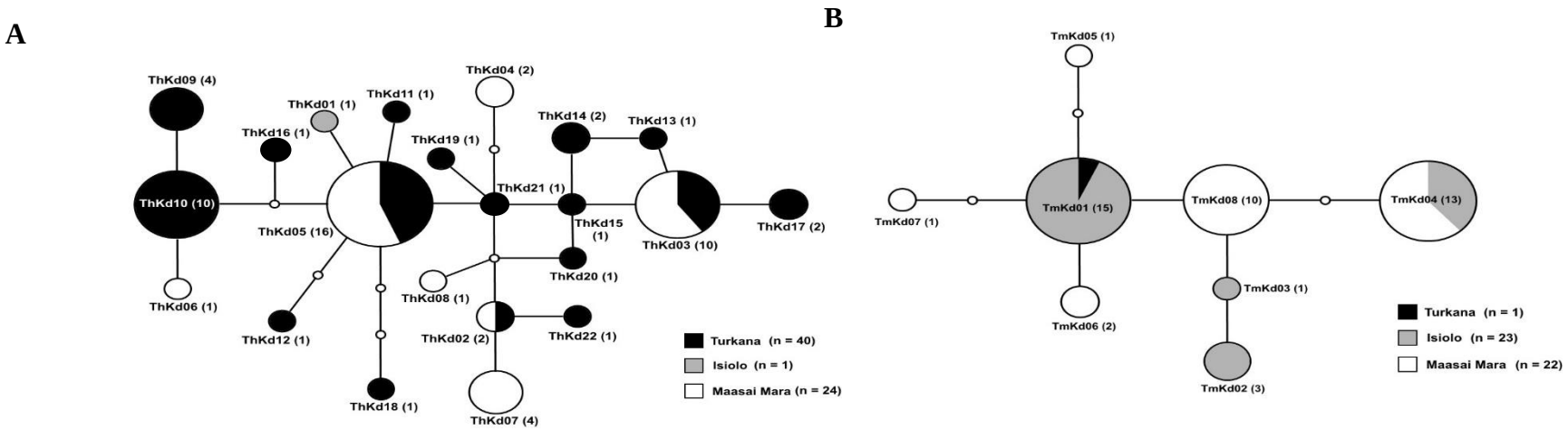

Fig. 6. 3 Haplotype network of intraspecific microvariation observed in *Taenia hydatigena* (A) and *Taenia multiceps* (B) based on the mitochondrial *nad1* gene, 377 bp and 350 bp respectively. The circle size is relative to haplotype frequency, in parenthesis are number of isolates belonging to each haplotype. The black spots on branches (small circles) indicate mutational steps.

6.4.4.2 *Taenia multiceps* haplotypes

Of 55 *T. multiceps* sequences, 46 entered haplotype analyses based on sequence quality. Ten polymorphic sites were observed for *T. multiceps* isolates, six were parsimony informative while the remaining four were non-informative. A total of eight haplotypes were identified TmKd01 – TmKd08 represented by accession numbers MG282278 – MG282285 respectively. Six haplotypes TmKd02 – 07 were novel and are reported for the first time in this study. Haplotype TmKd01 accession number MG282278 was identical with *T. multiceps* haplotype 1 KC794810 (from a goat in China) (Hao *et al*., unpublished data) and was the most abundant haplotype (15 out of 46 eggs) in the present study. Haplotype TmKd08 accession number MG282285 was identical to reference sequence KC794809 (from a goat in China) (Hao *et al*., unpublished data), and was present in 10 out of 46 eggs. The distribution and relative abundance of the *T. multiceps* haplotypes are described in Fig. 6.3B. Six of the eight *nad1* haplotypes of *T. multiceps* were area-specific.

6.4.5 Frequency of haplotypes in faecal samples

The majority of the haplotypes (16/22 for *T. hydatigena* and 5/8 for *T. multiceps*) originated from one faecal sample each. For *T. hydatigena*, haplotypes ThKd10, ThKd03 and ThKd05 were detected in 5, 6 and 7 faecal samples, respectively. For *T. multiceps*, haplotypes TmKd08, TmKd04 and TmKd01 were present in 2, 3 and 5 faecal samples respectively. More than one haplotype was only found in four faecal samples for *T. hydatigena* (two and three haplotypes) and in two samples for *T. multiceps* (two haplotypes).

6.4.6 Diversity and neutrality indices of *T. hydatigena* and *T. multiceps*

The haplotype diversity for *T. hydatigena* was 0.8913± 0.023, nucleotide diversity of 0.00854± 0.00053, neutrality indices were negative Tajima's D value of -1.04704 and Fu's Fs -9.854 (Table 6.3). The haplotype diversity for *T. multiceps* was 0.776± 0.032, nucleotide diversity of 0.00534±0.00050, neutrality indices were negative Tajima's D value of -0.51658 and Fu's Fs -0.783 (Table 6.3).

Table 6. 3 Diversity and neutrality indices of *T. hydatigena* and *T. multiceps* isolates based on *nad1* sequences 377 bp and 350 bp, respectively

	n	Hn	Hd ± SD	π ± SD	Tajima's D	Fu's Fs
T. hydatigena	65	22	0.8913± 0.023	0.00854± 0.00053	-1.04704	-9.854
T. multiceps	46	8	0.776± 0.032	0.00534±0.00050	-0.51658	-0.783

n, number of isolates; Hn, number of haplotype; Hd, haplotype diversity; π, nucleotide diversity

6.5 Discussion

This study identified *Taenia*/*Hydatigera* species from faecal samples of domestic dogs collected environmentally in four widely spaced areas in Kenya. The study also reports the haplotypes of the two most common *Taenia* species, *T. hydatigena* and *T. multiceps* based on the analysis of partial mitochondrial *nad1* gene. For decades, research of Taeniidae was focussed on the genus *Echinococcus* due to its medical importance and public health impact. While the knowledge on geographical distribution and intrageneric diversity of *Echinococcus* has rapidly expanded due to the application of molecular characterization methods (Deplazes *et al*., 2017; Romig *et al*., 2017), such knowledge on other taeniid genera is still largely based on data from the pre-molecular period, which were summarized two decades ago (Loos-Frank, 2000). While a new taxonomic concept for the previous catch-all genus *Taenia* has now been established (Nakao *et al*., 2013a), there is still insufficient data on the number and identity of the species within the genera *Taenia* (sensu stricto), *Hydatigera* and *Versteria*. Where data exist, they usually focus on the presence and characterization of species with medical or economic importance (Ale *et al*., 2014; Christodoulopoulos *et al*., 2016). In particular, studies on wildlife-transmitted cestodes are scant, and the application of molecular techniques led to the unexpected discovery of new species even in the comparatively well-researched northern hemisphere (Haukisalmi *et al*., 2011; Haukisalmi *et al*., 2016). Metacestodes of wildlife taeniids (*Taenia*, *Hydatigera* and *Versteria*) may be more relevant for public health than currently acknowledged. Recently reviewed human cases of cysticercosis and coenurosis originated from Europe and North America only, which is certainly a detection bias (Deplazes *et al*., 2019).

Sub-Saharan Africa is a hotspot of taeniid cestodes both in domestic animals and in wildlife; of 39 '*Taenia*' species recognized two decades ago, 20 occur in Africa, and 15 of these are parasites

of wild mammals (Loos-Frank, 2000). According to the widely accepted 'out of Africa' hypothesis even globally distributed species like *T. solium* and *T. saginata* had initially evolved in Africa as wildlife parasites (Hoberg *et al.*, 2001). However, even data on economically important livestock-associated taeniids are sporadically scattered across Africa, and the number of 15 described wildlife-associated non-*Echinococcus* taeniids is certainly the tip of the iceberg: a recent small study on *Taenia* in spotted hyenas in a single location in Ethiopia found four genetic lineages of *Taenia*, only one of which could be related to a described species (Terefe *et al*., 2014), and only one of seven *Taenia* mitochondrial sequences of wild mammal origin in Namibia could be linked to a known species (Wassermann and Aschenborn, unpublished data).

Of the seven species detected in dog faeces in this study, three depend on livestock as intermediate hosts (*T. hydatigena, T. multiceps, T. ovis*), three are wildlife cestodes (*T. serialis*, *T. madoquae*, *H. taeniaeformis*) and one is apparently of human origin (*T. saginata*). Their distribution and frequencies in the four study areas clearly reflect the different cultural and economic situations there, in particular livestock husbandry and slaughtering practices.

T. hydatigena was, overall, the most abundant species in this study, but was almost exclusively detected in Turkana and Maasai Mara where nomadic and semi-nomadic pastoralism – with associated unsupervised home slaughter - is still common. It is a globally ubiquitous species, mainly associated with domestic animals including dogs, ruminants and pigs, but also known from wild mammals. Cysticercus tenuicollis, the metacestode of *T. hydatigena* causes significant economic loss due to condemnation of infected organs such as liver and/or death of young animals due to traumatic hepatitis (Radfar *et al*., 2005; Nourani *et al*., 2010; Scala *et al*., 2016). Presence of *T. hydatigena* in this study correlates with the abundance of *Echinococcus* in dogs and livestock in these areas (Njoroge *et al*., 2002; Addy *et al*., 2012; Mbaya *et al*., 2014) (See Chapter 5). This

is attributable to the shared transmission pattern between the *Echinococcus* spp. and *T. hydatigena*. *T. hydatigena* seems persistent in these areas even in older records of high prevalence in dogs in Turkana, e.g. 89.5% (Jenkins *et al*., 1991) and 66.7% (Buishi *et al*., 2006). Lower prevalence were observed in Rabat, Morocco (12.3%) (Pandey *et al*., 1987) and in Lusaka, Zambia (17.64%) (Islam and Chizyuka, 1983). The low prevalence of *T. hydatigena* in dogs in Rabat may be due to the urban settings of that particular study and is contrary to recent studies which have reported high infections of dogs with the related *Echinococcus* spp. in Morocco (Deplazes *et al*., 2017). In Kenya, *T. hydatigena* was also found in silver-backed jackals (*Canis mesomelas*) (Zhang *et al*., 2007).

T. multiceps was the second most frequent dog tapeworm in this study and was common in Maasai Mara and Isiolo, though rare in Turkana. The zoonotic potential of *T. multiceps* indicates that the observed abundance in dogs could pose a serious public health concern in those areas. Worldwide, dogs and wild canids including jackals, foxes and coyotes are the definitive hosts of *T. multiceps*, while the intermediate hosts include large herbivores and pigs (Varcasia *et al*., 2013; Varcasia *et al*., 2015). The metacestode stage, Coenurus cerebralis, is a cerebral or non-cerebral parasite, that cause coenurosis in small ruminants (Scala *et al*., 2007). The development of the metacestode in the brain or the spinal cord is associated with severe clinical symptoms that include circling, blindness, head deviation and ataxia. Infected ruminants scam to death which leads to considerable economic losses (Avcioglu *et al*., 2011). Transmission of this parasite is caused by feeding of dogs with coenurus cysts of livestock after opening the skull or by dogs scavenging unattended carcasses (Scala and Varcasia, 2006). Besides, the cerebral forms of coenurosis, *T. multiceps* also causes non-cerebral metacestodes in small ruminants in the Middle East and Africa (Varcasia *et al*., 2012; Oryan *et al*., 2014; Christodoulopoulos *et al*., 2016). *T. multiceps* is likely to be widely distributed in Kenya and is also known from other countries in the region such as

Tanzania (Miran *et al*., 2015; Hughes *et al*., 2019), Ethiopia (Asmare *et al*., 2016), and Egypt (Desouky *et al*., 2011; Amer *et al*., 2017). The status of coenurosis in livestock and humans is undocumented in Kenya, clearly due to lack of recording. This data should raise awareness for *T. multiceps* as a cause of human disease, and the collection of data from livestock and human infections is urgently required.

T. ovis was the third domestically transmitted species found in this study, although it appears to be much rarer, found only in Isiolo and Turkana. Lifecycle and host range are similar to *T. hydatigena* and *T. multiceps*. Metacestodes develop in the cardiac, diaphragmatic and skeletal muscle causing cysticercosis. Disease burden in livestock mainly stems from condemnation of infested carcasses, thus, economic losses can be high in some regions (Eichenberger *et al*., 2011; De Wolf *et al*., 2014). Dogs infected with *T. ovis* have been associated with outbreaks of cysticercosis in sheep flocks in Canada (Soehl, 1984), England (Eichenberger *et al*., 2011) and China (Shi *et al*., 2016). In China and mainland Australia *T. ovis* infection is a threat to the sheep industry (Jenkins *et al*., 2014; Zheng, 2016), red foxes and dingoes were identified as important definitive host in Australia (Jenkins *et al*., 2014). No data on *T. ovis* cysticercosis is available for Kenya. The infrequency of this species in this study may indicate rarity of the disease in livestock, but survey data is required to make any further conclusion. The detection of *T. hydatigena, T. multiceps* and *T. ovis* in domestic dogs is attributed to frequent home slaughter, dog's access to offal and dead carcasses of livestock, failure to deworm dogs, poor offal disposal in abattoirs and owners' poor health education in these endemic areas (Craig *et al*., 2017). The absence of these livestock-associated taeniids in Meru can be attributed to the lesser importance of traditional livestock husbandry, and to better veterinary infrastructure in abattoirs.

T. serialis is of worldwide distribution and uses the domestic dog as definitive host in addition to wild canid species. In most regions, lagomorphs (e.g. hares) are the intermediate hosts, although in Africa rodents (and primates) could be involved to a larger extent. *T. serialis* had in the past been synonymized with two other coenurus-forming taxa from Africa, *T. brauni* and *T. glomerata* due to lack of relevant morphological differences, although the latter two were tentatively retained as a subspecies (*T. serialis brauni*) based on possible differences in host range (Verster, 1969). In the absence of further morphological and molecular taxonomic treatment of this cluster this study refrains from any speculations and retains isolates here as *T. serialis* (sensu lato). Coenuri of *T. serialis* (sensu lato) are rare in humans worldwide and are mostly located subcutaneously or intramuscularly; however, almost 30 human cases of 'African type coenurosis', allocated to *T. brauni* and *T. glomerata*, have been reported from Rwanda, Uganda, Nigeria and Congo (Deplazes *et al*., 2019). In addition to dogs as definitive hosts, the adult tapeworms of *T. serialis* were characterized in the golden wolf (*Canis anthus*) in Kenya (Allsopp *et al*., 1987; Zhang *et al*., 2007), and the coenuri seem to occur in a variety of rodents and primates from all major regions of Africa (Verster, 1969; Schneider-Crease *et al*., 2013; Chanove *et al*., 2019). In this study *T. serialis* was the only species present in all four study areas, indicating a wide (and largely unknown) intermediate host species range that certainly differs between the study areas with their divergent environmental conditions.

Taenia madoquae was described as a wildlife parasite of silver-backed jackals (*C. mesomelas*) and small antelopes (*Madoqua guentheri*) in Turkana area, Kenya and in Somalia; its cysticerci are found in the skeletal muscles (Pellegrini, 1950; Jones *et al*., 1988; Zhang *et al*., 2007). This is the first time that this parasite was found in dogs, or any domesticated animal. It was present in Turkana, Isiolo and Maasai Mara, where both jackals and *Madoqua* species are abundant. The

infrequent, but widespread presence in dogs indicates a regular exchange of parasites between wildlife and domestic animals, in this case either by dogs scavenging on wild animal carcasses (e.g. road kills) or by feeding on remains of hunted antelopes.

Hydatigera taeniaeformis uses felids, viverrids and, to a lesser extent, canids as definitive hosts, while rodents mainly rats are the intermediate hosts (Loos-Frank, 2000; Lavikainen *et al*., 2016). Infections of domestic dogs seem to be rather rare (Zibaei *et al*., 2007; Dyachenko *et al*., 2008; Karamon *et al*., 2016). A single human case of larval infection is known (Deplazes *et al*., 2019). The genus was recently partially revised based on morphology and molecular data (Lavikainen *et al*., 2016), although very few isolates from Africa were available for that study. This species was only found in one faecal sample in Meru, confirming that dogs are unusual hosts for this parasite. It is intriguing to note, however, that in Meru only small-mammal-transmitted species were found, pointing at rodents or hares as a regular source of food.

Isolation of *T. saginata* eggs in dog faecal matter is rather an unusual observation. *T. saginata* uses humans as definitive hosts and cattle as intermediate hosts. It can be assumed that *T. saginata* eggs from dog faeces were a result of dogs feeding on human faeces (Staebler *et al*., 2006), since open defecation is common due to low toilet coverage. Although no data exist on the presence of *T. saginata* in humans from this area, previous records indicate high infections in livestock (Onyango-Abuje *et al*., 1996) which points at an intense transmission cycle.

Both *T. hydatigena* and *T. multiceps nad1* sequences showed high haplotype diversity, low nucleotide diversity and negative values for Tajima's D test signifying rapid demographic expansion. Additionally, the Fu's Fs values indicated rare haplotypes which are characteristic of purifying selection events that may have occurred in the past. Similar observations were made for

T. hydatigena and *T. multiceps* populations in the Mediterranean region (Boufana *et al*., 2015c; Varcasia *et al*., 2016).

The majority of haplotypes has not been published before (20/22 haplotypes of *T. hydatigena*, 6/8 haplotypes of *T. multiceps*), which is likely to reflect the paucity of deposited sequences rather than the uniqueness of the Kenyan parasite populations. As an interesting result, very few haplotypes of both parasites were shared by two or more study areas. This is indicative of a very limited exchange of livestock between these areas, and it may be interesting to initiate studies on metacestodes from livestock, using longer sequences with better epidemiological resolution, to challenge this hypothesis.

This study did not attempt to describe the genetic structure of the *Taenia* populations and compare them with other regions of the world, as the short sequences used in this study (due to moderate DNA quality after extraction from single eggs) were not suitable for that purpose. However, diversity indices of *T. multiceps* in this study area were at a comparable level with studies from Europe, western Asia and other parts of Africa (Rostami *et al*., 2013; Al-Riyami *et al*., 2016; Christodoulopoulos *et al*., 2016), indicating a long presence of these parasites in the region.

6.6 Conclusions

Overall, *T. hydatigena* and *T. multiceps* were the most common species in dogs. Livestock-transmitted species were most frequent in areas of traditional pastoralism. The presence of wildlife cestodes in dogs indicates a regular parasite transmission interface between wildlife and domestic animals. *Taenia madoquae* was found for the first time in domestic dogs. In agricultural / high rainfall areas (Meru), the cestodes found were exclusively transmitted by small mammals. The predominance of area-specific haplotypes indicates infrequent livestock exchange between these

areas. The study forms the basis for further research of dog parasites, to elucidate transmission routes of diseases in humans and livestock associated with these parasites.

CHAPTER 7: GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 General conclusions

The aim of this study was to determine the prevalence and distribution of *E. granulosus* s. l., *Taenia* spp. and other zoonotic intestinal parasites in dogs from CE endemic areas of Kenya. The study also aimed to identify *Echinococcus* and *Taenia* spp. and their haplotypes in dogs. In this study 11 families/genera of intestinal parasites were identified, which included: hookworms, Taeniidae, *Spirometra* spp., Coccidia, *T. canis*, *T. vulpis*, *T. leonina*, *D. caninum*, *Anoplocephala* spp., *U. stenocephala* and *Mesostephanus* spp. Hookworms were the most common parasite detected followed by taeniids. Although the zinc chloride flotation-sieving technique is usually recommended for detection of taeniid eggs, this study has demonstrated that it can applied in detection of other parasites as well. Majority of the parasites detected in this study have zoonotic potential and therefore appropriate control measures such as population control of dogs, deworming, restraining of stray dogs and health education are recommended to avert transmission of these parasites to humans.

All three hookworm species (*A. caninum*, *A. braziliense* and *A.* cf. *duodenale*) identified from dog faecal samples can cause infections in humans. This study reported for the first time the detection of *A. duodenale* in dog faeces which was linked to coprophagy of human stool containing *A. duodenale* eggs. The zoonotic canine hookworm *A. ceylanicum* responsible for patent infections in humans was not reported in this study but could still be present in dogs in Kenya after being found to infect dogs in Tanzania and South Africa.

The prevalence and distribution of *Echinococcus* spp. from dogs in Isiolo, Meru and Maasai Mara is reported for the first time. The prevalence of *Echinococcus* spp. infection in dogs reported here was lower than in previous studies Nairobi, Kajiado and Turkana. The differences in

prevalence is strongly linked to the methodology used in this study compared to the previous studies. However, the differences in infection frequency in dogs among the various study regions corresponds well with data from livestock in these endemic areas. Areas identified as hot spots for *Echinococcus* spp. infection in dogs were mapped out and are therefore considered high risk for intermediate host (livestock) and human infections. They included Lokangae, Lodwar, Lokichar and Kainuk in Turkana county and Tipilikwani and Ewaso Ng'iro in Maasai Mara (Narok county).

Four *Echinococcus* spp. were identified in dogs' namely *E. granulosus* s. s., *E. ortleppi*, *E. canadensis* (G6/7) and *E. felidis*. *E. granulosus* s. s. was the most abundant species and was reported in dogs from all four study sites. The dominance of *E. granulosus* s. s. in dogs also corresponds to infections with this species in humans and livestock. *E. canadensis* (G6/7) was the second most common species in this study and was reported in Turkana, Isiolo and Maasai Mara. This species is fairly distributed in the country, infecting all livestock species, while human infections are known only from Turkana. *E. canadensis* (G6/7) is common in camel rearing areas and in the absence of camels, goats are the preferred intermediate hosts. The present study, reported *E. ortleppi* for the first time in dogs in Kenya. *E. ortleppi* is a rare species in Kenya and has been reported in livestock, a pig, camels and donkeys. However, the distribution of this species in Kenya seems uneven with a major foci in Meru and Isiolo counties. The study also reported for the first time the occurrence of *E. felidis* in domestic dogs. *E. felidis* had so far only been isolated from wild mammals and is thought to be restricted to wildlife transmission. The detection of *E. felidis* in faecal samples from domestic dogs may be due to an infection acquired by scavenging on carcasses of wild intermediate hosts in the National Reserve or through coprophagy as dogs are known to be coprophagous and may feed on faeces of other carnivores. The presence of *E. felidis* eggs in dog faeces without the adult or gravid worms, this findings alone are insufficient to confirm domestic

dogs as definitive host of this species. This study also observed mixed infection with *Echinococcus* species which demonstrate that dogs have frequent access to offal infected with the different taxa of *Echinococcus.*

The study also reports on novel, rare and globally distributed haplotypes of *E. granulosus* s. s. and *E. canadensis* (G6/7) haplotypes. The common haplotype observed in this study occurs worldwide shows that *E. granulosus* s. s. populations have originated from a common ancestor. The low haplotype and nucleotide diversities observed could be due to sexual reproduction of the adult worms, lack of population expansion, the short life span of the adult worm or due to the small sample size.

Seven *Taenia*/*Hydatigera* spp. were identified and reported from dogs. The *Taenia*/*Hydatigera* spp. included *T. hydatigena, T. multiceps* and *T. ovis* that are transmitted primarily between small ruminants and dogs. *T. serialis, T. madoquae,* and *H. taeniaeformis* that are transmitted in sylvatic cycles were also reported. This study reports for the first time in Kenya the detection of *T. saginata* in dog faeces that was assumed to be as result of dogs feeding on human faeces infected with this species. Predominance of *T. hydatigena,* particularly in Maasai Mara and Turkana roughly correlates with abundance of other members of Taeniidae family such as *Echinococcus* in dogs and livestock in these areas. This observation is attributable to the shared transmission pattern between these two taxa (*Echinococcus* spp. and *T. hydatigena*). *T. multiceps* was the second most frequent dog tapeworm in this study and causes Coenurosis in small ruminants and humans. The presence of *T. multiceps* in dog faeces could pose serious disease burden to humans where coenurosis may manifest in several neurological disorders.

T. serialis was the only species present in all four study areas and was more frequent in Meru and Isiolo. Cerebral coenurosis due to *T. serialis* infections are rare in humans globally,

although the adult tapeworms of *T. serialis* has previously been reported in the golden wolf (*C. anthus*) in Kenya. In the present study *T. madoquae* was found in Turkana, Isiolo and Maasai Mara, previously the adult tapeworms of this species were reported from silver-backed jackal (*C. mesomelas*) in Turkana. *T. madoquae* was reported for the first time in domestic dogs. The presence of this species in Isiolo and Maasai Mara is not surprising considering the close proximity of human settlements with wildlife. This study reports for the first time in Kenya the presence of *H. taeniaeformis* in dog faeces. Human infections with *H. taeniaeformis* strobilocercus-type larvae or the adult tapeworm are rare worldwide. Detection of *T. serialis, T. madoquae* and *H. taeniaeformis* in domestic dogs in those endemic regions confirms the active wildlife-domestic transmission interphase.

Sequencing data of both *T. hydatigena* and *T. multiceps* demonstrated that these populations are going through rapid demographic expansion. Majority of the haplotypes for both species were rare which is characteristic of purifying selection events that may have occurred in the past. Perhaps these features are characteristic of the widely distributed cestodes, *T. hydatigena* and *T. multiceps*. Many of the *T. hydatigena* & *T. multiceps* haplotypes were novel, while the remaining haplotypes were have been previously reported and had wide geographical distribution. The relatively short sequences obtained from egg isolates in the present study did not allow of drawing much inference into the population structure of *T. hydatigena* or *T. multiceps*. The predominance of area-specific haplotypes indicates infrequent livestock exchange between these areas. The study forms the basis for further research of dog parasites, to elucidate transmission routes of diseases in humans and livestock associated with these parasites.

7.2 Recommendations

1. The role of domestic dogs as reservoirs of zoonotic parasites of public health importance should be considered and control measures to avert transmission of zoonoses are recommended. These measures should include dog population management (sterilizing the male & spaying females), enforcing laws for restraining stray dogs, regular deworming of dogs, and public health educational awareness programmes. Environmental contamination with dogs faeces should be discouraged and maintaining high standards of hygiene in public areas, proper disposal of dog faeces and use of protective footwear encouraged.

2. To improve the sensitivity of copro-detection of *Echinococcus* and other taeniids in dogs, copro-antigen ELISA is recommended. This will enhance detection of *Echinococcus* during prepatent and patent periods when eggs are not shed regularly. The use of real-time PCR is recommended to increase the sensitivity of PCR in detection of *Echinococcus* or *Taenia* spp. eggs. Extraction of DNA from faeces is also recommended in instances were individual taeniids eggs fail to yield a PCR product.

3. In order to determine the population structure of *Echinococcus* and *Taenia* species with certainty, sequence of analysis of more and full mitochondrial genes such as cytochrome C oxidase, NADH dehydrogenase and nuclear genes is recommended.

4. Additional studies are required to further understand the epidemiology of *E. felidis* and *Taenia* species, more specifically the domestic-wildlife cycle overlap.

5. Future studies on *Taenia* spp. are recommended to establish the burden of diseases in humans and livestock associated with these parasites.

6. The collection of faecal samples from the rectum of individual dogs is recommended to associate the results with other demographic factors such as sex, age and study areas.

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APPENDIX 1: CONSENT FORM

MOLECULAR EPIDEMIOLOGY OF *ECHINOCOCCUS GRANULOSUS* AND *TAENIA* SPECIES IN DOGS FROM CYSTIC ECHINOCOCCOSIS ENDEMIC AREAS IN KENYA

Principal Investigator: Erastus Mulinge Kakundi. **Supervisors:** Dr. David Odongo, Dr. Thomas Romig, Prof. Japhet Magambo, Prof. Sammy Njenga. P.O Box 19464-00202 Nairobi, Tel: 020-2722541 Ext 3339, or 0722 935320

What is cystic echinococcosis?
You are invited to participate in this research project that aims to understand the importance of cystic echinococcosis and other intestinal parasite infections in dogs in your community. Cystic echinococcosis is a disease with severe consequences' in human body. The disease is caused by the dog tapeworm (*E. granulosus*) and transmitted by dogs when they are fed with infected livestock organs such as liver and/lungs. Dogs in turn release infective eggs in their faeces into the environment, where animals are infected while grazing, human are also accidentally infected from direct contact with infected dogs or from contaminated water or soil.

The purpose of the study
In this study we intend to identify cases of these infections in dogs in this community as well as identifying the factors that may be placing people at risk of contracting the diseases. This information will be used to estimate the degree of infection in dogs, areas where dogs are infected and the type of the parasite they are infected with, as these are important aspects in control programmes of the disease.

Procedures involved
You have been selected for participation in this study because you own dog(s) that might be infected by CE and other intestinal parasite infections. Therefore taking part in this study will involve collecting faecal specimen from your dog(s) that will be used to test if they are infected with CE or intestinal parasite infections. Stool specimen collection is a routine medical procedure, non-invasive and therefore dog(s) will not experience any pain. If you agree your dog(s) to participate in this research, we will ask you some questions on your dogs, livestock and (in some cases wildlife), related to the spread of the disease. The faecal specimen will be transported to the Centre for Microbiology Research laboratory in KEMRI, Nairobi for analysis and storage. If you give us permission, *E. granulosus* isolates we may get from the dog may be shipped and sequenced at the Parasitology Department University of Hohenheim, Germany.

Participation
Please understand participation in this study is voluntary and you have the right to withdraw your consent or discontinue participation at any time without penalty. Additionally, you are free to accept or decline your dog(s) specimen being examined at the Parasitology Department University of Hohenheim, Germany in the event that *E. granulosus* isolates are detected from it.

Confidentiality
A coded identification number will be assigned to each household and used only for the purpose of sample tracking. Privacy will be maintained in all published and written data resulting from the

study.

Who to contact
If you have questions about your rights as a dog(s) owner or participant in the study or you are dissatisfied at any time with any aspect of this study, you may contact the investigators on the contacts given above or The Secretary KEMRI ERC office. P.O Box 54840-00200, Nairobi Tel. 2722541 Ext. 3307.

……
Dog(s) owner/Participant name

……
Signature/thumb print Date

……
Name of witness Signature Date

……
Name of interviewer Signature Date

APPENDIX 2: QUESTIONNAIRE

MOLECULAR EPIDEMIOLOGY OF *ECHINOCOCCUS GRANULOSUS* AND *TAENIA* SPECIES IN DOGS FROM CYSTIC ECHINOCOCCOSIS ENDEMIC AREAS IN KENYA

Household questionnaire

Study site: a) Turkana b) Maasai Mara c) Isiolo d) Meru

Location/area: ……...

1. Education of household owner

a) Primary b) Secondary c) Technical d) University e) Not attained formal education

I. Questions regarding dogs

1. How many dogs do you have? ……..

a) 1 -5 b) 6-10 c) more than 10

2. Sex of the dog (s)

a) Male b) female

3. Age a) 3 - 12 months b) 13 – 24 months c) 25 – 36 months d) 37 – 48 months e) Above 48 months

4. What is the main reason for keeping your dog(s)

a) guarding b) herding c) hunting

5. How often is your dog(s) allowed to roam

a) never b) always c) sometimes

6. Do you see free-roaming dogs in the neighbourhood?

a) Always b) Sometimes c) Never

7. What kind of food do you feed your dog(s)

a) Food cooked at home b) raw meat c) Lungs d) Do not feed them at all

8. How many times do you feed your dog(s) in a day

a) Once b) Twice c) Three d) Never

9. How do you dispose your waste, including dog faeces?

a) Open dump b) Closed dump c) Burning d) left scattered in the compound

10. Do you de-worm your dog(s)

a) Yes b) No

11. If yes how often

a) After three months b) After six months c) Once a year

II. Questions regarding livestock

12. Do you keep domesticated animals?

a) Yes b) No

13. If yes which ones?

a) Cattle b) Goats c) Sheep d) Pigs d) Donkeys e) Camels

14. Do you slaughter at home?

a) Yes b) No.

15 When you slaughter your animals are there dogs present near the area of slaughter?

a) Yes b) No

16. How do you dispose off viscera (condemned meat) of slaughtered animals?

a) Bury b) Burn c) Throw away d) Feed to dogs

17. Have you seen offal of cystic echinococcosis (CE) when you slaughter your animals (showing pictures), a) Yes b) No

18. Has anyone in your household been diagnosed with CE? a) Yes b) No

19. Do you feed dogs with CE, a) Yes b) No

20. May you indicate your knowledge of what cause the cyst you see on your animals?

a) Do not know b) water c) worms

21 How do you dispose off livestock carcasses?

a) Incineration b) burial c) leave it unattended d)feed it to dogs e) feed on by human

III Questions regarding water as a source of infection

22. What is the source of your drinking water?

a) Pond b) river c) borehole d) Tap water e) spring

23. Do you treat drinking water?

a) Yes b) No

If yes how

a) boiling b) using chlorine (water) c) others

24. Do you handle/touch dog(s)?

a) Yes b) No

if yes do you wash/clean your hands after handling dog(s)

a) Yes b) No

25. Do you have separate water sources for human and livestock/dogs

a) Yes b) No

IV Questions regarding wildlife

26. What species of wild animals do exist in this area?

a) Hyenas b) Jackals c) Zebra d) Elephants d) Lions e) None

27. Have you seen any of these species close to your house or your animals?

a) Yes b) No

28. What of the named species come closer to your house more often?

a) Hyenas b) Jackals c) Zebra d) Elephants d) Lions e) None

29. Have you seen your dog(s) interacting with wild animals?

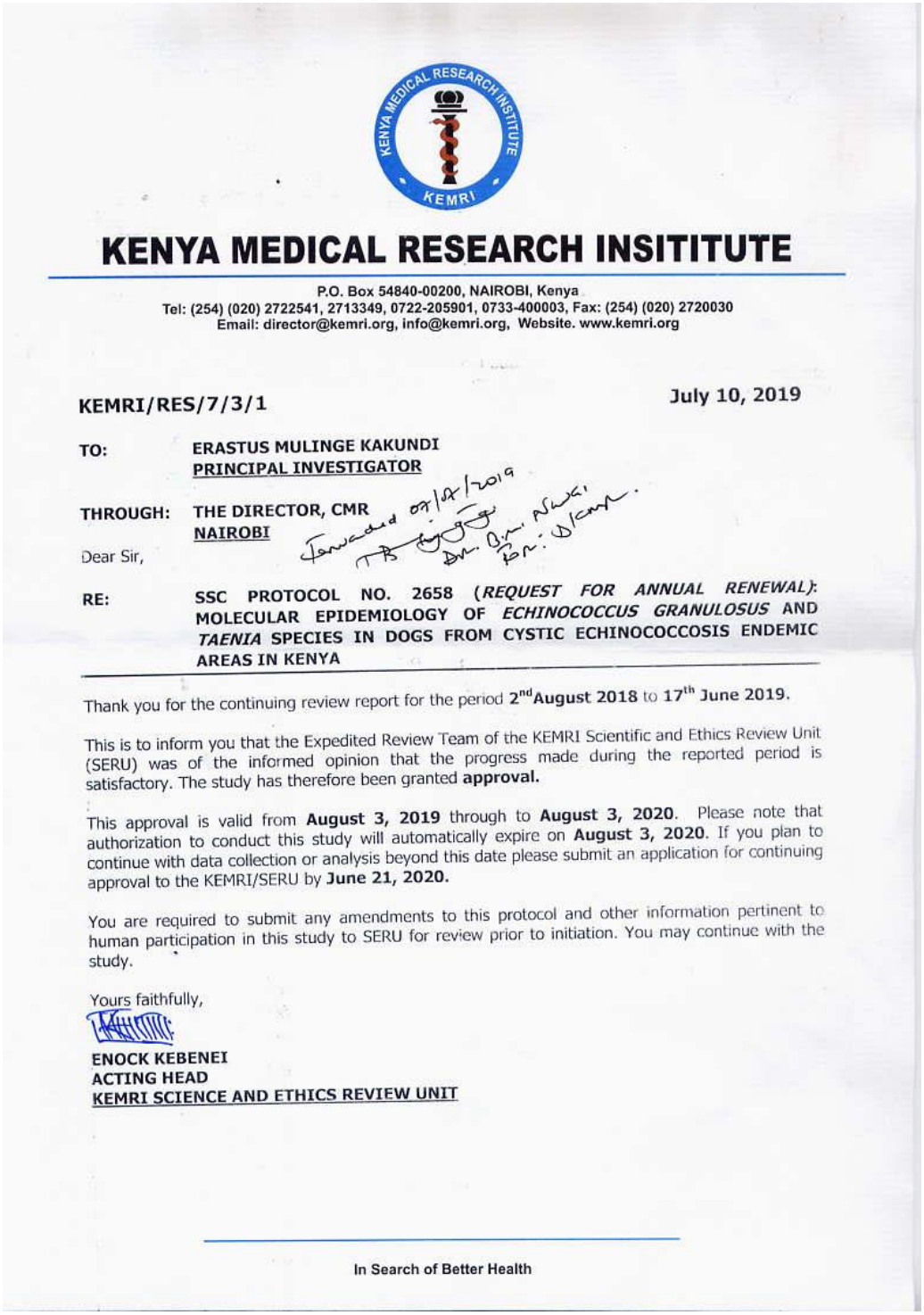
a) Yes b) No

…….

30. With what species have you seen your dog(s) interacting?

a) Hyenas b) Jackals c) Zebra d) Elephants d) Lions e) None

APPENDIX 3: ETHICAL APPROVAL FROM KEMRI SCIENCE AND ETHICS REVIEW UNIT



KENYA MEDICAL RESEARCH INSITITUTE

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Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **July 10, 2019**

TO: **ERASTUS MULINGE KAKUNDI**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CMR**
NAIROBI

Forwarded 07/08/2019

Dear Sir,

RE: **SSC PROTOCOL NO. 2658 (*REQUEST FOR ANNUAL RENEWAL*): MOLECULAR EPIDEMIOLOGY OF *ECHINOCOCCUS GRANULOSUS* AND *TAENIA* SPECIES IN DOGS FROM CYSTIC ECHINOCOCCOSIS ENDEMIC AREAS IN KENYA**

Thank you for the continuing review report for the period 2nd **August 2018** to 17th **June 2019.**

This is to inform you that the Expedited Review Team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval.**

This approval is valid from **August 3, 2019** through to **August 3, 2020**. Please note that authorization to conduct this study will automatically expire on **August 3, 2020.** If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the KEMRI/SERU by **June 21, 2020.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to SERU for review prior to initiation. You may continue with the study.

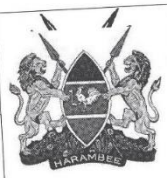
Yours faithfully,

ENOCK KEBENEI
ACTING HEAD
KEMRI SCIENCE AND ETHICS REVIEW UNIT

In Search of Better Health

APPENDIX 4: ETHICAL CLEARENCE FROM DEPARTMENT OF VETERINARY SERVICES

MINISTRY OF AGRICULTURE, LIVESTOCK AND FISHERIES

Telegrams: "VETLAB", Kabete Telephone: 020 – 2067641 and 020-2700705 Fax: 020-2026212 E-mail: cvfokabete@yahoo.com, director@dvskabete.go.ke		DEPARTMENT OF VETERINARY SERVICES, VETERINARY RESEARCH LABORATORIES, PRIVATE BAG, 00625 KANGEMI
When replying, please quote: **REF: MEAT/VOL/XIV/51** *All correspondences should be addressed to:* The Director of Veterinary Services *Parcel by rail:* Nairobi Station		**Date: 25TH JUNE, 2013**

ERASTUS MULINGE

RE: PERMIT LETTER FOR SAMPLING OF DOG FAECES FOR Echinococcus/Taeniid eggs

Please refer to your request email on permit letter for sampling of dog faeces for research (PhD proposal) for Erastus Mulinge. This Department has no objection for your sampling of the same provided you will share findings with this office.

DR. JAMES KARITU
FOR: DIRECTOR OF VETERINARY SERVICES

cc: Eberhard Zeyhle
Project Manager Turkana
AMREF