

**The epidemiology and impact of cystic echinococcosis in humans  
and domesticated animals in Basrah Province, Iraq**

By

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This thesis is presented for the degree of Doctor of Philosophy of Murdoch  
University, 2018

## **Declaration**

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Mohanad Faris Abdulhameed

## **Statement of Contribution**

This PhD thesis comprises a number of scientific publications, and each paper has been co-authored by multiple authors. The extent to which the work of others has been used is clearly stated in each chapter and certified by my supervisors.

I declare that I, Mohanad Faris Abdulhameed, have undertaken the majority of the original research presented in these papers and am the principal author of this work.

Mohanad Faris Abdulhameed

### Statement of Contribution (1)

<b>Title of Paper</b>	A Retrospective Study of Human Cystic Echinococcosis in Basrah Province, Iraq
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By signing the Statement of Contribution, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
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- viii. permission is granted for the candidate to include the publication in the thesis; and
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<b>Title of Paper</b>	Cystic Echinococcosis in Edible Offal of Sheep in Basrah, Iraq: Abattoir-based Survey and a Probabilistic Model Estimation of the Direct Economic Losses Due to Hydatid Cyst
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## **Dedication**

I dedicate this Doctoral Thesis to the most precious people in my life, for their everlasting love, full understanding and strong support in my study:

My wife: Rusul ALshetiwi

My sons: Ahmed & Ali

My Daughters: Rawan & Haneen

My parents



## Abstract

Cystic Echinococcosis (CE) is a zoonotic disease caused by the larval stages of *Echinococcus* spp.. The disease results in significant public health effects to the community and economic burden for livestock owners. Although CE is endemic in Iraq, few studies have been undertaken in Basrah Province on the disease's epidemiology in humans and other animals. This study was designed to address these deficiencies.

Medical records from five public hospitals for the period 2005 to 2015 were examined for records of CE. Data on 748 surgical cases of CE were retrieved representing an annual incidence of 4.5 cases per 100,000 people. More females were affected (61.2%) than males (38.8%). Cysts were detected most frequently in the liver (46.3%) and lungs (28.1%) of the patients.

A questionnaire was administered to 50 CE patients who had undergone surgery to describe the characteristics of the patients and their knowledge, attitudes and practices about the disease. The majority of patients were found to adopt poor hygienic practices and had a low level of understanding of how the disease was transmitted. Most specialist medical staff did not inform patients on how to prevent reinfection and the study highlighted the need for improved health education for CE in southern Iraq.

314 livestock farmers were administered a questionnaire to determine their knowledge, attitudes and practices relating to CE in their livestock. Only 86 (27%) farmers owned a dog. 9.8% of respondents ate raw leafy vegetables without prior washing and most (94.3%) did not boil water before using it for domestic purposes. Approximately half of the farmers had a low level of knowledge about CE, especially how it is transmitted and 41.4% did not realize that CE is a potentially dangerous disease to human health.

Eggs of *Echinococcus* were detected in the faecal samples of 10.1% (95% CI 7.1, 13.9) of 335 free-roaming and owned domesticated dogs sampled. A questionnaire was administered to 86 dog-owners to investigate the influence of socio-demographic factors and management and husbandry practices on the knowledge of CE. A multivariable logistic regression model indicated that owners who fed offal had less knowledge about CE (OR=0.17, 95% CI 0.05, 0.53), while keeping a dog tied up was associated with good knowledge (OR=7.0, 95% CI 2.07, 23.78). Dog owners who had a secondary or higher level of education also had better knowledge (OR=5.35, 95% CI 1.65, 17.31) than those with a lower level of formal education.

7.3% (95% CI: 5.4, 9.6) of 631 sheep examined at an abattoir in Basrah contained hydatid cysts. Cysts were detected most frequently in both the livers and lungs of affected sheep (54.3%). The average annual economic loss arising from total or partial condemnation of affected liver and lungs of sheep in Basrah was estimated at US\$72,470 (90% CI;  $\pm$ 11,302) or US\$ 8.79 per affected sheep.

It is concluded that a public health education campaign and a control programme involving worming domesticated dogs and controlling the population of free-roaming dogs should be developed and implemented in Basrah to reduce the impact of CE.

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### **Publications arising from this research**

**Abdulhameed, M. F,** I Habib, S. A Al-Azizz, and I Robertson. 2018. "A retrospective study of human cystic echinococcosis in Basrah province, Iraq." *Acta Tropica* no. 178:130-133.

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### **Conference Presentations (Oral)**

**Mohanad F. Abdulhameed,** Ian D. Robertson, Suzan A. Al-Azizz, and Ihab Habib. Knowledge, attitudes and practices of fifty surgically operated cases of Cystic Echinococcosis from Basrah Province of Iraq. 1<sup>st</sup> Murdoch University Annual Research Symposium. 8<sup>th</sup> November 2017.

### **Poster Presentations**

**Abdulhameed, M. F,** I Habib, S. A Al-Azizz, and I Robertson. (2016). A retrospective study of human cystic echinococcosis in Basrah province, Iraq. Annual Poster Day for year 2016. School of Veterinary and Life Sciences, Murdoch University.



**Abdulhameed, M. F,** I Habib, S. A Al-Azizz, and I Robertson. (2017). Knowledge, Awareness and Practices Regarding Cystic Echinococcosis among Livestock Farmers in Basrah Province, Iraq. WAAVP 2017 26<sup>th</sup> International Conference of the World Association for the Advancement of Veterinary Parasitology. Between 4 -8 September 2017 Kuala Lumpur. Malaysia.

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## List of symbols and abbreviations

$\beta$	Beta (correlation coefficient)
$\chi^2$	Chi-Square
CE	Cystic Echinococcosis
CDC	Centre for Disease Control (in Iraq)
CI	Confidence Interval
df	Degrees of freedom
DALY	Disability-Adjusted Life Year
ELISA	Enzyme-Linked Immunosorbent Assay
FRD	Free-Roaming Dog
GBD	Global Burden of Disease
NGO	Non-Governmental Organizations
N	Number
OR	Odds Ratios
PCR	Polymerase chain reaction
P or P. value	Probability
WHO	World Health Organisation
PAIR	Puncture-Aspiration-Injection-Re-aspiration
YLL	Years of Life Lost
YLLD	Years Lost due to Disability
SPSS	(Software) Statistical Package for the Social Sciences
SD	Standard Deviation
US\$	United States dollar currency

## **Chapter One - General introduction**

### **1.1 Introduction**

Cystic echinococcosis (CE or hydatidosis) is a zoonosis of considerable public health concern and economic monetary burden to livestock owners and the general community (Budke, Deplazes and Torgerson, 2006; Aydın, Adıgüzel and Güzel, 2018). The disease is caused by members of the genus *Echinococcus* which have a two-host life cycle (Eckert and Deplazes, 2004). The adult tape-worm lives in the definitive host, with domesticated dogs being the key host for transmission of the parasite to humans. The egg is immediately infective on passage from the dog and is usually accidentally consumed with contaminated pasture by herbivores, such as sheep, goats and cattle, or omnivores, such as pigs. In these species the egg develops into a cyst, usually in the liver or lungs, and when this is eaten by a dog the lifecycle is complete (Moro and Schantz, 2009; Romig *et al.*, 2017). Humans are accidental hosts in which the cysts develop in the liver, lungs and occasionally the brain (Eckert and Deplazes, 2004). Transmission to humans primarily occurs through ingestion of food or water contaminated with the eggs of *Echinococcus* spp. (Carmona *et al.*, 1998). Humans are regarded as dead-end hosts, however the cysts can have significant health impacts on affected people and usually must be removed by surgery (Brunetti, Kern and Vuitton, 2010; McManus *et al.*, 2012).

Cystic echinococcosis is geographically widespread and is of particular concern in developing countries of Africa, Asia, and South America (Cardona and Carmena, 2013). The disease is re-emerging in regions of China, central Asia, eastern and western European countries and Wales (Eckert, Conraths and Tackmann, 2000; Kern *et al.*, 2003; Buishi *et al.*, 2005; Zhenghuan, Xiaoming and Xiaoqing, 2008). The World Health Organisation (WHO) has classified echinococcosis as one of 17 neglected tropical zoonotic diseases (Campos-Bueno,

LoÁpez-Abente and AndreÁs-Cercadillo, 2000; Torgerson and Budke, 2003; Mableson *et al.*, 2014), because of its chronic nature, long asymptomatic period and costly treatment in humans (Craig *et al.*, 2007). A high incidence of infection with cystic echinococcosis in humans has been associated with rural areas where the parasite is endemic and where livestock are reared and dogs roam freely (Craig *et al.*, 2015; Yuan *et al.*, 2017). Communities that home-slaughter animals, rarely administer anthelmintics to domestic dogs, have a poor level of hygiene, have close contact with dogs, and lack knowledge about hydatid cysts have a higher incidence of disease (Romig, 2003; Battelli, 2009). In endemic areas the incidence rates of CE in humans can reach up to 50 per 100,000 person-years (WHO, 2018) and developing nations tend to have a higher incidence as they cannot implement and fund control programmes in animals (Torgerson and Heath, 2003). The prevalence of echinococcosis in dogs is higher in areas where: there are large numbers of free roaming dogs (FRD) and where owners feed offal or allow their dogs access to it; people have a low level of education; and there is no formal control programme (Oku *et al.*, 2004; Reyes *et al.*, 2012).

The two-major species of *Echinococcus* of medical and public health importance are *E. granulosus* and *E. multilocularis*, which cause cystic echinococcosis and alveolar echinococcosis, respectively in humans (McManus *et al.*, 2003; Agudelo Higueta, Brunetti and McCloskey, 2016). The disease can be life-threatening in humans and result in a significant economic impact to livestock producers (Azlaf and Dakkak, 2006). Although CE is typically asymptomatic in affected livestock, the organs affected with cysts, which are detected on inspection at slaughter, are usually totally condemned resulting in financial loss for the producers. Severe cases in livestock may result in reduced productivity through interference of organ function (Torgerson and Heath, 2003; Scala *et al.*, 2006).



## 1.2 Objectives of this study

The general aims of this project were to undertake a study on CE in Basrah in order to determine the distribution of disease in humans, dogs and sheep, identify putative risk factors for the disease, and estimate the financial impact of the infection in sheep.

The specific aims of the projects outlined in this thesis were to:

1. Analyse data from hospital records on human CE in Basrah province for the period 2005 to 2015 to determine the incidence of disease.
2. Evaluate and describe the characteristics, knowledge, attitudes and practices (KAP) of patients who had undergone surgery for CE.
3. Investigate the knowledge and awareness about the disease and the management and husbandry practices adopted of a group of livestock farmers in Basrah, Iraq.
4. Determine the prevalence of *Echinococcus* in the faeces of domestic and stray dogs and to determine the knowledge of dog-owners about CE and management practices adopted which might increase their dog's risk of infection.
5. Determine the prevalence of hydatid cysts in slaughtered sheep and estimate the financial losses arising from condemnation of sheep offal infected with hydatid cysts.

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## Chapter Two - Literature Review

### 2.1 Historical discoveries of Cystic Echinococcosis (CE)

Diseases have been known to humans for thousands of years with documentation of Epidemic Fever in the Ebers papyrus dating back to 1500 BC (Cartwright and Biddiss, 2000). Hippocrates (~460-377 BC) was the first person to document information about hydatid cysts and indicated “In those whose water stuffed liver opens into the omentum, the belly is filled with water, and they die” (Hosemann et al, 1928 cited in Eckert and Thompson, 2017). Al-Rhazes, a Persian physician, described hydatid cysts in the liver as watery balloons in *circa* AD 900 (Kattan, 1977). At that time it was believed that the cysts were eggs or embryos of an insect or a cystic tumour (Grove, 1990). More than 200 hundred years ago, researchers also reported the presence of cysts in the abdominal cavity of slaughtered goats and pigs, but probably were referring to *Cysticercus tenuicollis* rather than hydatid cysts (Grove, 1990). Philip Jacob Hartmann (1648-1707) had also described a *Cysticercus tenuicollis* with a scolex, the metacestode of *Taenia hydatigena* (Enigk, 1987 cited in Eckert and Thompson, 2017). For many years knowledge about hydatid cysts was lacking. Aelius Galenus 1821-1833 (cited in Grove, 1990) reported that “the liver is very much inclined to produce hydatid cysts in the surrounding fascia”. Hydatid cysts were diagnosed in several patients at that time, but the disease or its cause were not understood, and it was believed the cysts were due to dysfunction of the lymphatic glands (Grove, 1990). In the 17<sup>th</sup> century many researchers described the features of hydatid cysts. For example, Peter Pallas divided hydatid cysts into adherent and non-adherent forms and named the cyst *Taenia hydatigena* (Grove, 1990). The protoscoleces in the hepatic cysts were described in detail by Goeze (Hosemann, 1928 cited in Eckert and Thompson,

2017), and subsequently Batsch (1786) (cited in Grove, 1990) described the hydatid cysts in sheep and named them *Hydatigena granulosa*.

During the 18<sup>th</sup> century advancements were made on the understanding of the life cycle of the parasite in both the definitive and intermediate hosts. Several experimental studies were performed by Carl Theodor von Siebold, Breslau, and Friedrich Küchenmeister to further the understanding of the life cycle of echinococcosis (Tappe *et al.*, 2010). They fed a group of dogs metacestodes of *E. veterinorum* isolated from slaughtered sheep, which led to the discovery of the strobilar stage. Subsequently Von Siebold named the adult worms *Taenia (T.) echinococcus* (Von Siebold, 1853 cited in Eckert and Thompson, 2017). However, a series of further experiments were undertaken to confirm the life cycle of the parasite. The faeces of infected dogs were fed to a group of sheep resulting in the production of fertile liver cysts (Haubne, 1855 cited in Tappe *et al.*, 2010). Rudolf Leuckart fed a group of piglets with eggs of *Taenia echinococcus*, obtained from a dog, and four weeks later detected the presence of small vesicles (0.25 to 0.35 mm) with a laminated layer in the liver of these animals (Eckert and Thompson, 2017). Finally Rudolphi in 1801 suggested the genus name of *Echinococcus*, being derived from the Greek word meaning “spine and berry” (Craig and Larrieu, 2006).

Many human cases of hydatid cysts were reported between 1879 to 1890 in Russia, Siberia and France (Vierordt, 1886 cited in Tappe *et al.*, 2010); although the lesions present in these cases were believed to be a type of tumour. Subsequently German researchers, including Leuckart and Virchow, undertook studies to determine the morphology of the cysts and their contents (Guillebeau, 1890; Hosemann, 1939 cited in Tappe *et al.*, 2010). This resulted in the discovery that the single cyst lesions were caused by *Echinococcus granulosus* and multiple cysts in

the infected organs were caused by *Echinococcus multilocularis* (Eckert and Thompson, 2017).

## **2.2 Taxonomic features of the causative agent (*Echinococcus*)**

*Echinococcus* is the smallest cestode harboured by carnivores and belongs to the order Cyclophyllidea and family Taeniidae (Lavikainen, 2014). Rudolphi classified the genus *Echinococcus* into three species: *E. hominis*; *E. simiia*; and *E. veterinorum* (Grove, 1990). However, initially there was controversy over the naming of the species within the genus. Szidat (1960) (cited in Tappe et al., 2010) called *E. multilocularis* *Alveococcus multilocularis*; however, Rausch and Nelson (1963) refuted this nomenclature because of the presence of morphological differences in both adult worms and larva, including the location of a genital pore and the number of rostellar hooks and proglottids. Subsequently hydatid cysts were classified into the four species: *E. granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli* (Soulsby, 1982; Thompson and Lymbery, 1988). PCR and DNA sequencing have subsequently been used to construct a phylogenetic tree of the genus *Echinococcus*, with intraspecific variation in *E. granulosus* being identified (Thompson, Lymbery and Constantine, 1995; Eckert and Thompson, 1997). Based on molecular characteristics, the species/strains of *E. granulosus* have been taxonomically revised as: *E. granulosus sensu stricto* (G1–G3); *E. equinus* (G4); *E. ortleppi* (G5); *E. canadensis* (G6–G10); and *E. felidis* (Thompson and McManus, 2002; Nakao et al., 2007; Hüttner et al., 2008; Romig, Ebi and Wassermann, 2015). These species can infect a specific type of animal or a range of animals: for example *E. felidis* is restricted to the lion (*Panthera leo*) (Ortlepp, 1934; Hüttner et al., 2008); and *E. shiquicus*, discovered in 2005, is found in Tibetan foxes (*Vulpes ferrilata*) with the pika (*Ochotona curzoniae*) and voles (*Microtus limnophilus* and *Lasiopodomys fuscus*) from the Tibetan plateau being the predominant intermediate



hosts (Boufana *et al.*, 2013; Fan *et al.*, 2016; Wang *et al.*, 2018). This latter species is a “sister species” to *E. multilocularis* (Xiao *et al.*, 2006) and *E. felidis* has been shown to be phylogenetically closely related to *E. granulosus* (Hüttner *et al.*, 2008). In contrast to *E. felidis*, a wide range of hosts are susceptible to *E. granulosus*, including yaks, buffalo, camelids, pigs and equids (Eckert and Deplazes, 2004). The genotype G6/G7 of the pig strain has a wide allopatric distribution, and has been assigned to the species *E. intermedius* and has been reported in pigs and camels as well as in other animals (goats, horses and humans) (Lymbery *et al.*, 2015; Nakao, Lavikainen and Hoberg, 2015). However, it has been suggested that the name *E. intermedius* needs to be reviewed as the morphological traits of the genotypes differ between adult worms from different intermediate species (Nakao, Lavikainen and Hoberg, 2015).

In Eurasia and North America, three subspecies have been suggested for *E. multilocularis*: *E. multilocularis multilocularis*; *E. multilocularis sibiricensis*; and *E. multilocularis kazakhensis* (Rausch, 1967). Only a few substitution sites of a short mitochondrial DNA (MtDNA) have been shown to differentiate the two geographic genotypes M1 (Europe) and M2 (China, Alaska and North America) of *E. multilocularis* (Nakao *et al.*, 2007; Knapp *et al.*, 2011). However, there remains controversy over whether *E. multilocularis* actually displays subspecies variation (Bowles, Blair and McManus, 1992; Bowles and McManus, 1993). In contrast, *E. granulosus* has been shown to be a highly diverse species and it has been recommended that this species should be subdivided further (Thompson, 2008). The diversity of *Echinococcus* species has been recognised in the phenotypic traits which influence their life cycle, host specificity, pathogenicity, antigenicity, sensitivity to chemotherapeutic agents, transmission dynamics, and epidemiology and hence

control methods adopted for CE (Thompson and McManus, 2001; McManus and Thompson, 2003).

### **2.3 Life cycle and host range of *Echinococcus***

Dogs (*Canis familiaris*) and other wild canids serve as the definitive host harbouring the adult worm (*Echinococcus* spp.) in their small intestine, while the larval stage (hydatid cyst) develops in the viscera of the intermediate host which can include domestic livestock, wildlife and humans (Bryan and Schantz, 1989; Binhazim *et al.*, 1992). Dogs become infected after ingesting the offal (usually liver or lung) of an intermediate host that is infected with fertile hydatid cysts (containing viable protoscoleces). In the small intestine of dogs the protoscoleces will develop into mature adult worms, which usually takes between 4 to 12 weeks (Thompson and Lymbery, 1995). An experimental study performed by Lahmar *et al.* (2007) observed that after a group (65) of kennel dogs were orally challenged with ovine hydatid cysts, 58 had adult worms of *Echinococcus* 14 to 34 days after dosing.

The adult tapeworm of *Echinococcus* is very small, measuring only 2 to 7 mm in length and being divided into 2 to 6 segments. It has a scolex which has two rows of hooks and four suckers (Thompson and McManus, 2001). A dog may harbour a large number of worms, between 10,000 and 25,000, in their small intestine. The adult worm can survive and actively shed eggs for more than one year (Torgerson and Heath, 2003). Furthermore, the eggs of *Echinococcus* spp. may remain viable in the environment for up to one year; however abiotic factors affect their viability. A study conducted by Thevenet *et al.* (2005) showed that eggs of *E. granulosus* could survive for 41 months under the natural conditions of an arid climate. Viable eggs have been found in water and damp sand maintained at 30°C for three weeks, for 225 days when maintained at 6°C and for 32 days when maintained between 10 and 21°C (Urquhart *et al.*, 1996). Although the eggs can

survive temperatures up to -50°C (Thompson and McManus, 2001), they are inactivated at temperatures of 45°C and low relative humidity (Federer *et al.*, 2015).

When the eggs are ingested by an intermediate host, they will hatch in the small intestine with subsequent release of an oncosphere which penetrates the intestinal mucosa (Grubor, Jovanova-Nesic and Shoenfeld, 2017). The oncosphere is then carried via the blood to other organs where cysts develop (Zhang, Chen and Wen, 2017). Within one year, the cyst, which is typically filled with fluid, brood capsules and protoscoleces, can reach a size of 5 to 10 cm in diameter (WHO, 1996). Cysts can reach very large sizes before causing any clinical symptoms and the symptoms that develop are usually associated with the pressure effects of the cyst on adjacent tissues/organs (Craig and Larrieu, 2006).

A hydatid cyst consists of two layers: the outer layer is an acellular laminated layer surrounded by a host-derived fibrous capsule; and the inner layer is a nucleated germinal layer which generates the brood capsules and protoscoleces (Golzari and Sokouti, 2014). Morphologically hydatid cysts can either be a uniform shape or an amorphous shape, depending on the species of *Echinococcus*. With *E. granulosus* a single, unilocular cyst or multiseptated cysts with ‘wheel-like’, ‘rosette-like’ or ‘honeycomb-like’ shape is observed (Czermak *et al.*, 2008). In contrast, *E. vogeli* is polycystic in shape (D’Alessandro and Rausch, 2008), with *E. multilocularis* being multi-chambered (Jenkins, Romig and Thompson, 2005). However, the number, size and location of the cysts can impact on the health of the affected intermediate host, with small cysts usually having little impact on the animal’s health while large cysts can result in irreversible damage to the affected organ, and sometimes can result in death (Ghallab and AlSabahi, 2008; Bristow *et al.*, 2012; Simon *et al.*, 2016).

The clinical symptoms of CE are variable and depend on the location and size of the cysts (Moro and Schantz, 2009). For example, hepatic cysts can compress bile ducts, causing obstruction that can manifest as obstructive jaundice, abdominal pain, anorexia, and pruritus (Pakala, Molina and Wu, 2016). While pulmonary cysts can cause chronic cough, dyspnoea, pleuritic chest pain and haemoptysis (Berliner *et al.*, 2016). The rupture of a cyst can potentially be life-threatening due to an anaphylactic reaction (Zhang, Wen, *et al.*, 2011). Ruptured cysts can release viable cystic contents resulting in secondary hydatidosis in the abdominal cavity (da Silva, 2010). Thus infections can sometimes result in sepsis, either due to the primary infection or due to secondary infection from leakage into the biliary ducts (Safioleas *et al.*, 2006; Dolay and Akbulut, 2014). In one study, bacterial superinfection (*Escherichia coli*, *Enterococcus*, and *Streptococcus viridans*) was detected in lung cysts of 7.3% (37/503) of patients diagnosed with CE (García *et al.*, 2010). Four of these patients developed a severe sepsis, with two dying.

The most common organ involved in humans affected with CE is the liver followed by the lungs (60-70% and 20–30%, respectively) (Jenkins, Romig and Thompson, 2005; Filippou *et al.*, 2007). A study of hospital medical records of cases of human CE in Ngorongoro, Tanzania for the period 1990–2003 estimated an annual incidence of 10 cases/100,000 with 78.4% of patients having cysts in the liver and only 2.9% in the lungs, spleen (1.8%), omentum (16.9%), and other sites (4.1%) (Ernest *et al.*, 2010). In contrast to these findings, a study from Turkey reported the presence of cysts in the lung of 71.5% of cases and only 24.4% of cases had cysts in the liver (Gulsun *et al.*, 2010). Pulmonary cysts have been reported to be more common than hepatic cysts in infected children compared with adults (Santivanez and Garcia, 2010). It has been proposed that cysts grow easier and faster in the lungs due to their elastic structure compared with the liver (Kanat, Turk and

Aribas, 2004). Karaoglanoglu *et al.* (2001) observed that the pulmonary cysts of 21.9% of 305 patients were greater than 10 cm in diameter. They also noted a significant positive correlation between the size of the cysts and postoperative complications and the duration of hospitalisation. The lung and liver are the first sites with large capillary beds encountered by the migrating *Echinococcus* oncosphere (hexacanth embryo). The oncosphere pass along the portal vein and first negotiate the hepatic and then the pulmonary filtering system before potentially progressing to other peripheral organs (Kebede *et al.*, 2009).

In humans, the presence of a single cyst in one organ is most commonly reported, with Ernest *et al.* (2010) reporting single cysts in 81.5% of their cases. In another study of 141 patients, more single cysts (67.3%) were present in the liver than the lungs (22.3%) (Aribas *et al.*, 2002). As livestock are the major intermediate hosts for the parasite, counting the number of cysts present in different species and organs and assessing their viability is important to determine the role livestock play in the parasite's lifecycle. In one study conducted in Greece more cysts were found in the lungs of affected sheep, goats and cattle (mean number of cysts of 4.0, 3.8 and 3.7, respectively) than in pigs (0.7 cysts) (Founta *et al.*, 2016). In their study more cysts were found in the lungs than in the liver for sheep, goats and cattle (3.1, 1.1, 2.3, respectively), however, in contrast, pigs were found to have more cysts in the liver (average 3.5 cysts) than the lungs. Also, an abattoir study performed in a regional metropolitan slaughterhouse in Chile involving inspection of 1725 cattle, found that 21.2% were infected with hydatid cysts of which 39.7% were located in the lungs and 24.7% in the liver (Stoore *et al.*, 2018).

The overall prevalence in cattle at a slaughter house at Gondar Elfora, Ethiopia was 20.5% (Abebe, Beyene and Kumsa, 2014). In their study the prevalence was significantly higher in older cattle (22.4%) ( $P < 0.05$ ) than in younger

cattle (15.7%). This would be associated with the time hydatid cysts take to mature plus increased likelihood of exposure to infected pastures.

The fertility of hydatid cysts can be influenced by the infecting strain/genotype for *E. granulosus* (Njoroge *et al.*, 2002; McManus, 2006; Romig *et al.*, 2011). A one-year study of samples collected from abattoirs in Libya revealed that the fertility of hydatid cysts was relatively high in camels (84%) and sheep (80%), however all cysts in cattle were sterile (Elmajdoub and Rahman, 2015). Similarly in a study of 344 buffalo processed in five slaughterhouses in Iran between 2008-2010, only 7.3% of cysts were fertile with 78.75% of fertile cysts viable (Amin Pour, Hosseini and Shayan, 2012).

Another study conducted in two abattoirs in Iran from 2011 to 2013 (Kazerun and Shiraz abattoirs) found a prevalence of hepatic cysts in sheep, cattle, goats and camels of 0.86, 2.1, 0.76, and 15.1%, respectively (Mohamadzadeh *et al.*, 2016). The differences in prevalence between species may be attributed to animal movements, husbandry and management practices, and the number of uncontrolled free roaming dogs (Macpherson *et al.*, 1985; Ibrahim, 2010).

Another study conducted at two abattoirs in the Al Baha region of west Saudi Arabia reported that 67.5, 63.2, 54.8 and 82.1% of infected camels, cattle, sheep and goats contained only single cysts (Ibrahim, 2010). Multiple cysts were also found more commonly in the lungs than in the liver for all species examined. It has not been confirmed what influences the number of cysts developing in different organs, however it has been suggested that it could be associated with the frequency of exposure to eggs and the number of eggs exposed to at each infection event (Otero-Abad and Torgerson, 2013; Pakala, Molina and Wu, 2016).

Viable hydatid cysts are essential for the continuation of the life cycle from the intermediate to the definitive hosts (Daryani *et al.*, 2009). Hydatid cysts have

different fertility rates depending on their size, location and host species infected (Elmajdoub and Rahman, 2015). In sheep the fertility and viability of hydatid cysts is very high, whereas in cattle and pigs the cysts are usually sterile (Thompson, 2017). In a study involving the inspection of animals at an abattoir in Qazvin Province, Iran, 81% of cysts from the liver of sheep were fertile compared to only 1.3% of bovine hepatic cysts (Shahnazi *et al.*, 2013). The fertility of cysts is also influenced by the age of the animal, with fertile cysts being more frequently found in older sheep than younger animals (Scala *et al.*, 2006). A study in North Jordan also reported that cysts in the liver of ewes aged 6 years old or older were more fertile than younger sheep (64.8 to 78.6% compared with 8.7 to 46.2%, respectively) (Abdel-Hafez, Al-Yaman and Said, 1986). This may be associated with a weaker immune system in older animals (Ibrahim, 2010). In western Iran, Dalimi *et al.* (2002) reported that the fertility of cysts in the liver of sheep (39.6%) was higher than for cysts in the lungs (25.2%). Similarly, a study conducted in two slaughterhouses in Saudi Arabia reported a significantly higher viability of protoscoleces in liver (62.2%) than lung cysts (52.7%), in cattle, camels, sheep and goats (Ibrahim, 2010). In contrast, Daryani *et al.* (2009) reported similar levels of fertility of lung (47.1%) and hepatic cysts (39.4%) in sheep.

Transmission of hydatid cysts to humans and livestock most commonly occurs indirectly. Transmission of *Echinococcus* eggs to the intermediate host can result from the indirect ingestion of contaminated pasture, vegetables or water (Carmona *et al.*, 1998). Coprophagic flies can also carry the eggs of *Echinococcus* spp. resulting in mechanical transmission (Patel, 2010). Sheep, goats, cattle and pigs are usually infected after the consumption of grass contaminated with eggs. Dogs and other carnivores usually acquire the infection through a predator-prey relationship or through scavenging from an infected dead animal (Otero-Abad and

Torgerson, 2013). In endemic areas dogs may be fed raw viscera from backyard slaughtered animals, highlighting the role of education in controlling the disease (Otero-Abad and Torgerson, 2013; Craig *et al.*, 2017).

Epidemiological studies have confirmed that cystic echinococcosis can infect a range of hosts (Table 2.1) with members of the families Canidae, Felidae and Hyaenidae acting as definitive hosts and members of Suidae, Cervidae, Camelidae, Giraffidae, Equidae, Hippopotamidae, Elephantidae and Leporidae acting as intermediate hosts (Rausch, 1986; Thompson and Allsopp, 1988; Rausch, 1995).

There are two types of life cycle of *Echinococcus* involving humans: a synanthropic and a sylvatic cycle. The synanthropic cycle focuses on transmission of infection from the domestic dog (*Canis lupus familiaris*) which act as the definitive host in this situation (Otero-Abad and Torgerson, 2013). In Algeria, CE is primarily maintained by a synanthropic cycle involving domestic dogs and livestock (sheep and cattle), where dogs are fed offal discarded from animals slaughtered for human consumption (Kouidri *et al.*, 2012). The same host assemblage is observed in Libya, where livestock are slaughtered in a clandestine and uncontrolled manner, providing dogs with a ready source of infection (Buishia *et al.*, 2005). In Alaska, people can be infected with CE from their sled dogs which are often fed the lungs of wild reindeer (*Rangifer tarandus*) containing cysts (Rausch, 2003). Eckert (1998) reported that, even in the synanthropic cycle, the definitive host (domestic dogs and cats) may acquire infection from infected wild rodents.

The sylvatic life cycle is primarily maintained by the red fox (*Vulpes vulpes*) and the arctic fox (*Vulpes lagopus*), with many species, such as arvicoline rodents (voles, lemmings and muskrats), acting as intermediate hosts (Li *et al.*, 2005). In eastern Finland, the sylvatic cycle of *E. granulosus* involves wolves (*Canis lupus*) as the definitive host and herding reindeer (*Rangifer tarandus tarandus*), elk (*Alces*



*alces*), and wild forest reindeer (*Rangifer tarandus fennicus*) as the intermediate hosts (Hirvelä-Koski *et al.*, 2003). In Europe, an adaptation of the foxes to an urban life has resulted in a change of the balance of sylvatic and synanthropic cycles (Wertheim, 2012) with urban foxes playing an increasing role in the infection of humans (Deplazes *et al.*, 2004). Eckert and Deplazes (2004) highlighted the role that foxes can play in disseminating infection (eggs) over a wide area.

Table 2.1 *Echinococcus* species, strains/genotypes and hosts documented with infection of *Echinococcus* in different regions of the world.

Species of <i>Echinococcus</i>	Strain/genotype	Intermediate hosts	Definitive hosts	Regions	References
<i>E. granulosus</i>	Sheep/G1 Tasmanian sheep/G2 Buffalo/G3	Sheep, cattle, pigs, camels, goats, macropods, humans Sheep, humans Buffalo, human	Dog, fox, dingo, jackal, and hyena Dog, foxes Dog	Australia, UK, Tasmania, New Zealand Europe, USA, Africa, Middle East, and South America	Romig, Ebi and Wassermann (2015); Cucher <i>et al.</i> (2016); Deplazes <i>et al.</i> (2017)
<i>E. equinus</i>	Horse/G4	Horses and other equines	Dogs, lions	Europe, UK, Middle East, Africa	Grosso <i>et al.</i> (2012); Wassermann <i>et al.</i> (2015); Romig, Ebi and Wassermann (2015)
<i>E. ortleppi</i>	Cattle/G5	Cattle, humans	Dogs	Africa, Asia, Europe, South America	Cucher <i>et al.</i> (2016)
<i>E. canadensis</i>	Cervid/G8, G10	Human Moose, white-tailed deer, black-tailed deer, and elk	Wolves, coyotes, and Dogs	Africa, Asia, north Europe, North America	Thompson <i>et al.</i> (2006); Himsworth <i>et al.</i> (2010); Schurer <i>et al.</i> (2013); Nakao <i>et al.</i> (2013); Deplazes <i>et al.</i> (2017)
<i>E. intermedius</i>	Pig/G6, G7	Pig, goats, camel, and human	Dogs	Mexico, some countries of south America (Argentina, Brazil, Chile, Peru), Baltic countries	Cruz-Reyes <i>et al.</i> (2007); Soriano <i>et al.</i> (2010); Rodriguez-Prado <i>et al.</i> (2014); Marcinkute <i>et al.</i> (2015); (Nakao <i>et al.</i> , 2013b)

<b>Species of <i>Echinococcus</i></b>	<b>Strain/genotype</b>	<b>Intermediate hosts</b>	<b>Definitive hosts</b>	<b>Regions</b>	<b>References</b>
<i>E. shiquicus</i>	-	Plateau pika	Tibetan fox	China	Xiao <i>et al.</i> (2005); Wang <i>et al.</i> (2018)
<i>E. felidis</i>	-	Warthog, hippopotamus	Africa lion, spotted hyena	Africa	Hüttner <i>et al.</i> (2009); Halajian <i>et al.</i> (2017)
<i>E. vogeli</i>	-	Rodents, humans	Bush dogs	South America	D'Alessandro and Rausch (2008); Soares <i>et al.</i> (2014)
<i>E. oligarthrus</i>	-	Rodents, humans	Wild felids (jaguars, pumas, cougars, ocelots, jaguarondis)	Latin America	D'Alessandro and Rausch (2008); Otero-Abad and Torgerson (2013)

## 2.4 Predisposing factors for Cystic Echinococcosis

An understanding and awareness of the risk factors that predispose humans and other animals for infection with *Echinococcus* is critical for reducing disease incidence (Possenti *et al.*, 2016). Many people, particularly those living in rural areas, have a low education level, own livestock and hence have occupational exposure to domesticated animals and their products and often have contact with dogs, all of which are considered risk factors for the disease (Chalechale *et al.*, 2016; Possenti *et al.*, 2016). However, central to infection of humans and other herbivore/omnivore animals with *Echinococcus* is direct or indirect contact with dogs (or other definitive carnivore hosts) and their faeces. The problem is made worse by the presence of free roaming dogs (FRD), either unrestrained owned dogs or stray (unowned) dogs, which pose a significant human health risk for CE and other diseases, as well as being a community issue through excessive barking, spreading rubbish and causing accidents and being a potential animal-welfare concern (Slater, 2001). By using iGotU (GPS Tracker), Van Kesteren *et al.* (2013) monitored the movement of FRD in southern Kyrgyzstan and found that there was significant environmental contamination with faeces, which potentially increased the risk of infection for villagers. In Iran a higher seroprevalence has been reported in rural residents (38.9%) (Zibaei *et al.*, 2013) compared with only 1.2% in urban residents (Omrani *et al.*, 2017), most likely from increased exposure to eggs from FRD. A study of 350 stray dogs conducted in the Dakahlia governorate of Egypt also reported a significantly higher prevalence of *E. granulosus* in dogs from a rural area (6%) than those from an urban area (3.2%) (Elshazly *et al.*, 2007). Another study undertaken in Nigeria found a higher prevalence of *E. granulosus* (16%) in hunting dogs from a rural area than in companion dogs residing in an urban area

(1.5%). These differences are most likely due to the dogs either being fed or having access to viscera infected with hydatid cysts, thus allowing continuation of the parasite's life cycle (Craig and Larrieu, 2006).

In a study conducted in six regions of Tunisia, although a higher prevalence of *E. granulosus* was found in dogs from rural areas (30%) than those from urban areas (18%), the prevalence was surprisingly similar in dogs sampled near slaughterhouses compared to those more distant (23 and 26%, respectively) (Chaâbane-Banaoues *et al.*, 2016). The inappropriate disposal of affected offal, allowing dogs access to this material, enhances the likelihood of echinococcosis being a problem (Wumbiyya *et al.*, 2017). In addition, inadequate infrastructure and financial resources found in most rural areas means that elimination of endemic zoonoses, such as echinococcosis, can be extremely difficult to achieve (Maudlin, Eisler and Welburn, 2009). A recent study conducted in the north of Iran, found that people living in rural areas were 4.4 times more likely to contract CE than those from urban areas, and this was associated with close contact with dogs and consumption of raw vegetables (Hezarjaribi *et al.*, 2017). In a study conducted in Romania of cases of CE from 2004 to 2010, the incidence in humans from a rural community (5.2 cases per 100,000 inhabitants/year) was higher than in residents from urban communities (3.2 per 100,000 inhabitants/year) (Calma *et al.*, 2011). This probably arose from closer, more frequent contact of rural inhabitants with dogs infected with *Echinococcus* than urban residents (Elshazly *et al.*, 2007).

In contrast, a study area conducted in the Coquimbo region of Chile found that more dogs in peripheral regions of urban environs were copropositive for *E. granulosus* (11.7%) than those from rural sites (3.5%) (Acosta-Jamett *et al.*, 2010). Others have demonstrated that proximity to refuse dumps increases the risk of dogs being infected through scavenging (Bchir *et al.*, 1987). Although human hydatidosis

has traditionally been a public health problem of rural communities, there is increasing opportunity for accidental exposure of urban residents to *E. granulosus* through contact with the faeces of infected foxes or stray dogs (Jenkins and Craig, 1992; Brown and Copeman, 2003). The dogs of recreational pig hunters living in urban centers have also been demonstrated to be a potential public health risk (Thompson *et al.*, 1988). Furthermore urban residents could also be exposed to eggs of *E. granulosus* through direct or indirect contact with wild dog/fox faeces or via coprophagous flies when visiting national parks, reserves or forests/bush areas for recreational purposes (Jenkins, Romig and Thompson, 2005).

Transhumant movement of people and their livestock is also considered a risk factor for transmission of CE in both humans and animals (WHO, 2001). These and other pastoral communities are at greater risk of CE due to: close contact with their animals, in particular dogs; generally poor personal hygiene practices; dietary and cooking preferences; potentially contaminated water sources; poor sanitary conditions; limited access to health care facilities; and generally low socio-economic status (Macpherson, 1994; Macpherson, 1995; Omar and Omar, 1999; Zhou *et al.*, 2000; Zhang, Xing, *et al.*, 2011; Barnes *et al.*, 2017).

Dogs in seminomadic pastoral communities in north-West China have been shown to have a higher prevalence of infection with *E. granulosus* in winter (49.4%) than in summer (18.3%) (Wang *et al.*, 2001). This is most likely linked to access dogs may have to offal through the processing (slaughtering) of carcasses in winter, rather than a true seasonal difference in susceptibility (Tiaoying *et al.*, 2005; Otero-Abad and Torgerson, 2013; Othieno *et al.*, 2016).

Culture and traditional practices can play a large role in the transmission of echinococcosis in different societies. People from many countries, but particularly those located in the Middle East, slaughter animals at home for specific purposes

including celebratory purposes such as weddings or religious festivals, as well as for home consumption (Pednekar *et al.*, 2009). In these situations, offal from the slaughtered animals may be fed directly to dogs or dogs may inadvertently have access to the offal due to the inappropriate disposal of this material (Moro *et al.*, 1997; McManus *et al.*, 2003).

Management and husbandry practices of dogs have also been shown to significantly influence the prevalence of *Echinococcus* in these animals. For example a study in Ethiopia found that none of the surveyed dogs had been dewormed (Gebremichael *et al.*, 2013). Another study in Tibet found that 66% of the respondents fed their dog(s) raw offal and 13% had never medicated their dogs with an anthelmintic agent (Li *et al.*, 2015). Dogs should be regularly treated with anthelmintic drugs to reduce infection in the animals but also to reduce the potential for transmission to humans and other animals (Maas *et al.*, 2014). The life-cycle of *Echinococcus* is such that suitable anthelmintics must be given at least at six-weekly intervals and not at the standard worming interval of three-monthly recommended by many veterinarians (Barnes *et al.*, 2012).

Other factors or practices that increase the risk of infection for humans include: patting/touching the fur of infected definitive hosts; allowing dogs to defaecate in gardens or using their faeces as manure in gardens; gardening without wearing gloves; eating uncooked food (primarily vegetables) from contaminated fields or gardens; drinking contaminated water; or inhaling dust containing tapeworm eggs, possibly during field work (Dowling, Abo-Shehada and Torgerson, 2000; Mastin *et al.*, 2015). In Alaska, dogs living in or close to their owner's house have been shown to increase the risk of transmission of *Echinococcus* eggs to the household (Stehr-Green *et al.*, 1988). Budke *et al.* (2005) reported that women were more likely to be infected with hydatid cysts than men because they were more

likely to undertake potentially risky domestic practices associated with the collection and washing of vegetables and the preparation of food.

Scavenging by dogs near slaughterhouses/processing plants/markets also increases the likelihood of infection in these animals through ingestion of *E. granulosus* infected offal (Wachira *et al.*, 1994; Moro *et al.*, 2004). Dogs from abattoirs located close to a river in Lima, Peru were reported to be 36 times more likely to be infected than those from abattoir centres distant to the river (Reyes *et al.*, 2012). It was proposed that the disposal of offal into the river provided opportunity for dogs to scavenge affected offal.

Frequent slaughter of animals and the associated inappropriate disposal of infected organs can result in a high infection rate in dogs, exposing the human community, as well as grazing herbivores/omnivores, to infection thus perpetuating the lifecycle of *Echinococcus* spp. (Varcasia *et al.*, 2011). There are many factors that lead to infection of humans and other animals with CE and a successful control programme requires a multi-pronged attack addressing these risk factors.

## **2.5 The Economic burden of Cystic Echinococcosis**

Cystic echinococcosis is responsible for a number of negative impacts on humans and livestock (Torgerson, 2003). The annual financial burden of CE on the global livestock industry has been estimated to be up to US\$ 2 billion through both direct and indirect components (Budke, Deplazes and Torgerson, 2006). Direct losses are linked to condemnation of all or part of the carcass or affected organs (Torgerson and Dowling, 2001; Sariozkan and Yalcin, 2009) and indirect costs arise through reduced growth rate, meat-quality, fertility, fleece and milk production (Carabin *et al.*, 2014). In Yugoslavia, hydatid cysts in affected animals were reported to reduce milk yield by 10% and carcass weight by 5% (Polydorou, 1981). While in a municipal abattoir in the Tigary Region of Ethiopia hydatid cysts were



estimated to result in combined losses (direct and indirect) of 366,939.95 birr (approximately US\$ 13,235 per annum (Guadu, Gebremicael and Chanie, 2013). For any disease control programme it is important to quantify the economic losses arising from that disease to ensure the financial viability of disease control (Getaw *et al.*, 2010; Komba *et al.*, 2012), however in zoonotic diseases, such as CE, the human impact of the disease must also be considered.

In humans CE can be treated using medication such as albendazole or mebendazole (Hemphill *et al.*, 2014), however for serious cases or when medication is not successful, surgical intervention is often required, although Puncture-Aspiration-Injection-Re-aspiration (PAIR) is now considered to be a safer alternative (Junghanss *et al.*, 2008). Although surgical intervention is considered the best option for removing cysts, complications and potential death may occur in severe cases and cysts may also recur after surgery (Georgiou *et al.*, 2015). Globally, it is estimated that 6.5% of CE cases relapse after surgery having a prolonged recovery time and 2.2% of surgical patients die (Budke, Deplazes and Torgerson, 2006). Estimating the socio-economic impact of CE on humans is important when developing a control programme, particularly where the disease is endemic and in poor communities.

To assess the burden of a disease on a human population, monetary losses and disability adjusted life years (DALYs) are usually calculated. Monetary losses due to CE can be calculated from the direct costs, which include the costs of diagnostic tests, surgery and post-treatment care (Mastrandrea *et al.*, 2012; Carabin *et al.*, 2014), and the indirect losses associated with lost wages because of inability or reduced capacity to work during hospitalisation and recuperation (Harandi, Budke and Rostami, 2012). The annual financial losses arising from CE in humans has been estimated in Peru at US\$ 2.4 million (Moro *et al.*, 2011), while the annual

economic losses attributable to CE in humans in Iran was estimated to be US\$ 93.4 million (Harandi, Budke and Rostami, 2012). In the early stages of CE the infection is usually subclinical and clinical signs or symptoms may take 5 to 10 years to develop (Brandt, 2003). Consequently it has been estimated that, in addition to the cases diagnosed each year, a further 10% are subclinical resulting in potential underestimation of the economic impact of the disease on a community (Budke, Deplazes and Torgerson, 2006). However, some studies have included financial losses of undiagnosed cases in humans based on the incidence of hospital CE cases and through using screening methods, such as ultrasound (Moro et al., 2011). In Iran, the annual productivity losses for asymptomatic/non-healthcare seeking individuals has been estimated to be approximately US\$ 100 million – approximately 40% of the costs associated with clinical cases (Harandi, Budke and Rostami, 2012).

Disability adjusted life years (DALYs) were first developed in the 1990s and were used in the Global Burden of Disease (GBD) Study to determine the worldwide burden of communicable and non-communicable diseases (Murray and Lopez, 1996) and comprise both years of life lost as a result of mortality (YLL) and the number of years lived with a disability (YLD) (Havelaar *et al.*, 2000). Many researchers prefer using DALYs in order to quantify the price of human health instead of using monetary values. This not only can be used for assessing morbidity and mortality, but also to compare different kinds of interventions to provide an insight into cost-effective methods for increasing human health (McGuire, Henderson and Mooney, 1988).

## 2.6 Distribution of Cystic Echinococcosis

### 2.6.1 Global distribution of Cystic Echinococcosis

Anthropogenic influences, including increased globalisation of animals and animal products, and altered human/animal interfaces are thought to have played a vital role in the global emergence of this pathogenic cestode (Davidson *et al.*, 2012). Expansion of the human population, resulting in a shrinkage of natural habitats and the associated increased urbanization of some wild carnivore species, have resulted in the spread of *Echinococcus* spp. from the traditional rural environs (Carmena and Cardona, 2014). In some parts of the world, such as Central Europe, the Baltic States and the Scandinavian countries, hydatid cysts are less prevalent in humans and other animals (Dakkak, 2010). In contrast a high prevalence has been reported in Russia (Torgerson *et al.*, 2010), and CE is considered to be re-emerging in Wales (Buishi *et al.*, 2005) and Bulgaria (Todorov and Boeva, 1999). Re-emergence in some countries may be as a result of failure of existing control campaigns or socio-economic changes resulting from the collapse of previous political systems (Cardona and Carmena, 2013). Transmission of CE in Europe relies primarily on dogs serving as the definitive hosts and domestic ungulates, including sheep, goats, cattle, buffaloes, horses and pigs, acting as the intermediate hosts. In Table 2.2 the prevalence and global distribution of hydatid cysts is summarised.

Other species of *Echinococcus* have been detected in European countries including *E. granulosus* s.s., *E. equinus*, *E. ortleppi* and *E. canadensis* (Varcasia *et al.*, 2006; Varcasia *et al.*, 2007; Casulli *et al.*, 2008). Since 2000 *E. ortleppi* has only been described in Italy, whereas *E. canadensis* is prevalent in Greece, Italy, Spain and Turkey, along with broad areas of Central and Eastern Europe (Cardona and Carmena, 2013). These species are the only *Echinococcus* known to occur in Slovakia and Lithuania, suggesting that transmission based on the pig-dog cycle is predominant in these geographical areas (Bružinskaitė *et al.*, 2009; Šnábel *et al.*,

2016). The finding that an increasing number of *E. canadensis* G7-infected patients are being reported in Central Europe also highlights the role of the pig strain G7 as a causative agent of human CE, which may have previously been underestimated (Pawłowski and Stefaniak, 2003; Schneider *et al.*, 2010). Human CE remains endemic in Europe with regular recording of sporadic cases (Torgerson, 2017). In Germany CE cases are considered autochthonous with an annual incidence of approximately 0.05 per 100,000 population, being reported from 2001 to 2013. In Austria, the annual incidence of CE has been estimated to be 0.4 per 100,000 inhabitants (Schneider *et al.*, 2010).

Hydatid cysts are endemic in livestock and humans in Africa (Magambo, Njoroge and Zeyhle, 2006; Dakkak, 2010). Although some countries of Africa have undertaken studies to determine the prevalence of hydatid cysts (Dada, Adegboye and Mohammed, 2016), others have not and it is likely that the real burden of infection has been underestimated (Cardona and Carmena, 2013). Molecular studies of CE have been carried out on cysts from production animals in African countries (Algeria, Mauritania, Ethiopia, and Sudan), revealing the presence of *E. granulosus*, *E. ortleppi*, *E. canadensis* and *E. equinus* (Maillard *et al.*, 2007; Omer *et al.*, 2010). In Uganda and Kenya, *E. felidis* has been detected in lions (*Panthera leo*) and warthogs (*Phacochoerus* spp.), although no evidence of infection in humans has been reported (Hüttner *et al.*, 2009). In contrast, a genetic survey of cysts isolated from human patients in Mauritania and Kenya have detected the sheep strain G1, as well as the camel strain G6 (Casulli *et al.*, 2010; Salem *et al.*, 2011). In Egypt, *E. granulosus* have been isolated from sheep and *E. canadensis* from camels and sheep (Amer *et al.*, 2015).

Echinococcosis appears to be widespread in Asia (Wang, Wang and Liu, 2008), with domesticated dogs acting as the major definitive host and a variety of

animals, including sheep, goats, cattle, buffaloes, camels, pigs and yaks, serving as intermediate host species. The parasite is also re-emerging in the former Soviet Republic and would appear endemic in China (Torgerson *et al.*, 2002; Torgerson, Shaikenov and Kuttybaev, 2002). From 1951 to 1990, six provinces in China (Xinjiang, Gansu, Qinghai, Ningxia, Xizang and Nei Monggol) reported a total of 26,065 surgical cases of hydatid disease, most of which were reported in the 1980's with one-third of the patients being children under the age of 15 years (Chai, 1995). An investigation on livestock in the Xinjiang Autonomous territory of China reported a prevalence of hydatid cysts in sheep (9.8%), cattle (8.4%), camels (6.8%) and horses (4.3%) (Qingling *et al.*, 2014).

In South America, CE is a common disease with around 5,000 new human cases reported annually in the five countries of Argentina, Brazil, Chile, Peru and Uruguay (Pavletic *et al.*, 2017). The disease is present at a high prevalence in livestock in this region (Table 2.2). The life cycle of CE is maintained by domestic cycles of transmission involving dogs and herbivores (sheep, swine, cattle, goats, horses and camelids) and multiple species/genotypes including *E. granulosus* (G-G3), *E. ortleppi* (G5) and *E. intermedius* (G6/7) (Deplazes *et al.*, 2017). In Argentina the annual incidence was reported as 0.95 per 100,000 inhabitants between 2005 and 2010 (Moral, 2010). Ultrasound screening has been adopted in Argentina to determine the prevalence of CE in humans (Moral, 2010) and in Rio Negro, an overall prevalence of CE of 7.1% was found on ultrasonography in 2009 (Bingham *et al.*, 2014). In Peru, serological and chest-X rays of humans detected 32 people with hydatid cysts. Unlicensed abattoirs and the lack of veterinary services were attributed to be the main reasons for the parasite's spread in that country (Reyes *et al.*, 2012). In Chile a study of 403 human blood samples and 93 faecal

samples from domesticated dogs detected a seroprevalence of 2.6% and a copro-prevalence of 28%, respectively (Acosta-Jamett *et al.*, 2014).

Table 2.2 Global distribution and prevalence of cystic echinococcosis in livestock and horses.

Region	Country	Study period	Cattle	Buffalo	Sheep	Goats	Camels	Equine	Pigs	References
Europe	Austria	2009	0.1%	-	0.1 %	-	-	-	-	EFSA (2011)
	Bulgaria	2009	5.1%	-	7.0%	10.5%	-	-	0.1%	EFSA (2011)
	Estonia	2004-2005	0.002%	-	-	-	-	-	0.0009%	Marcinkute et al. (2015)
	France	1989	0.1%	-	0.42%	0.4%	-	-	0.009%	Soule, Fabien and Maillot (1995); Umhang <i>et al.</i> (2013b)
		2009-2010	0.0003%	-	0.0004%	-	-	-	-	
	Germany	2009	-	-	0.8%	-	-	-	-	EFSA (2011)
	Greece	2006-2009	4.8%	-	31.3%	8.7%	-	-	1.7%	Founta et al. (2016)
	Lithuania	2005-2006	0.1%	-	-	-	-	-	0.2%	EFSA (2011)
	Italy	2004	0.2%	-	0.3%	-	-	-	-	Manfredi <i>et al.</i> (2011); Capuano <i>et al.</i> (2006)
		2004	-	10.5%	-	-	-	-	-	
	Poland	1995-1997	0.007%	-	18.7%	18.7%	-	-	4.5%	Derylo (1998); EFSA (2011)
2009		-	-	-	-	-	-	0.5%		
Republic Moldova	2000	78.9%	-	82.5%	-	-	-	-	Cardona and Carmena (2013); Chihai <i>et al.</i> (2016)	
	2012	59.3%	-	61.9%	-	-	-	0%		
Romania	2009-2011	43.6%	-	92.9%	-	-	-	37.8%	Mitrea <i>et al.</i> (2012)	

Region	Country	Study period	Cattle	Buffalo	Sheep	Goats	Camels	Equine	Pigs	References	
	Sweden	2009	-	-	-	-	-	-	0.1%	EFSA (2011)	
	Slovenia	2009	0.1%	-	-	-	-	-	0.1%	EFSA (2011)	
	Spain	2010	0.5%	-	0.9%	0.9%	-	-	0.02%	Rojo-Vazquez <i>et al.</i> (2011)	
	Turkey	2003-2007	5.3%	-	-	-	-	-	-	-	Pinar and Neriman (2011); Bekir and Değer (2013); Balkaya and Simsek (2011)
		2009	38.5%	-	28.3%	-	-	20.4%	-	-	
		2012	-	-	-	-	-	-	-	-	
UK	1984-1990	-	-	13%	-	-	-	-	-	Palmer <i>et al.</i> (1996); EFSA (2011)	
	2009	0.4%	-	-	-	-	-	-	-		
Africa	Algeria	1997- 1999	13.9 %	-	-	-	24.8 %	6.0 %	-	Bardonnet <i>et al.</i> (2003)	
	Egypt	2000-2005	6.4%	6.4%	0.3%	0.3%	2.5%	-	0.6%	Haridy <i>et al.</i> (2006)	
	Ethiopia	2010-2011	-	-	-	-	23%	-	-	Debela <i>et al.</i> (2014);Gessese <i>et al.</i> (2015)	
		2011-2012	-	-	9.0 %	1.9%	-	-	-		
Kenya	2011	25.8%	-	16.5%	10.8%	-	-	-	Addy <i>et al.</i> (2012)		



Region	Country	Study period	Cattle	Buffalo	Sheep	Goats	Camels	Equine	Pigs	References
	Libya	2004-2005 2010	15% 10.6%	- -	4.9% 10.5%	2.4% -	- 12.5%	-	-	Kassem, Abdel-Kader and Nass (2013); Elmajdoub and Rahman (2015)
	Mauritania	2010	5.5%	-	6.5%	-	30.1%	-	-	Salem et al. (2011)
	Morocco	2001-2004	22.9%	-	10.5%	1.8%	12.03%	17.8%	-	Azlaf and Dakkak (2006)
	Nigeria	1985	32%	-	24%	42%	-	-	56%	Arene (1985)
	Sudan	2001-2003	6%	-	11%	-	59%	2%	6%	Omer et al. (2010)
	Tanzania	2005-2007	4.2%	-	6.0%	-	-	-	13.2%	Nonga and Karimuribo (2009)
	Tunisia	2003-2010	8.56%	-	16.42%	2.8%	-	-	-	Lahmar <i>et al.</i> (2013)
	Uganda				33.3%	42.5%	-	-	-	Nyero <i>et al.</i> (2015)
Asia	Bangladesh	2008	29.6%	9.19%	16.9%	35.5%	-	-	-	Kabir <i>et al.</i> (2010)
	China	2011-2012 2014-2015	8.4% -	- -	9.8% -	- -	6.8% -	4.3% -	- 9.9%	Qingling et al. (2014); Li <i>et al.</i> (2017)
	India	2007-2008	16.4%	3.8%	11.1%	6.3%	-	-	0.9%	Pednekar et al. (2009)
	Pakistan	2004-2008	5.1%	7.19%	7.5 %	5.4%	-	-	-	Latif <i>et al.</i> (2010)
	Tajikistan	1991-2001	-	-	80%	25.2%	-	-	-	Torgerson <i>et al.</i> (2006)
	Turkmenistan	1978-2000	11.9%	-	51%	-	38%	-	4.9%	Torgerson et al. (2006)

Region	Country	Study period	Cattle	Buffalo	Sheep	Goats	Camels	Equine	Pigs	References
	Uzbekistan	1990-2002	45.5%	-	62.2%	11.1%	35.0%	38.5%	-	Aminjanov and Aminjanov (2004)
South America	Argentina	1997-1999	-	-	3.2%	-	-	-	-	Zanini <i>et al.</i> (2006)
	Brazil	1996-2004	16%	-	18.6%	-	-	-	-	De La Rue (2008)
	Chile	1996–2005	24%	-	11%	6%	-	9%	14%	Acosta-Jamett et al. (2010); Corrêa <i>et al.</i> (2018)
			18.8%	-	-	-	-	-		
	Ecuador	2010	-	-	-	-	-	-	13%	Allaico (2010); Torres (2012)
		2012	0.12%	-	-	-	-	-	0.5%	
Peru	1994-1995	80%	-	87%	-	-	-	68%	Moro et al. (1997)	
Uruguay	2002	12.2%	-	3.8%	-	-	-	-	OPS (2004)	

### 2.6.2 Distribution of Cystic Echinococcosis in the Middle East

Cystic echinococcosis is considered endemic throughout the entire region of the Mediterranean and in all countries of the Middle East (Anderson, Ouhelli and Kashani, 1997) (Table 2.3). Echinococcosis has been reported to be a public health concern in Iran, Iraq, Jordan, Egypt and Turkey as evidenced by the large number of hospitalised cases (Sadjjadi, 2006). A number of factors have been identified contributing to the increase in the disease's prevalence in humans in the Middle East, including poor slaughter-house hygiene, low general public awareness of the disease, inefficient veterinary services and large numbers of free-roaming stray dogs (Dakkak, 2010). The home slaughter of animals (small ruminants particularly) is still common in a number of Middle Eastern countries, and large numbers of animals are often slaughtered for specific festivals such as the Muslim Eid (Othieno et al., 2016). Free roaming dogs have been shown to have high infection rates of *Echinococcus* with a prevalence of 7.9 to 14.3% and 14.2% observed in Palestine and Israel, respectively (Abdel-Hafez and Kamhawi, 1997; Shimshony, 1997). *Echinococcus granulosus* has been reported to be the most dominant species in dogs in Egypt, Jordan and Iran (Ramzy et al., 1999; Al-Qaoud, Craig and Abdel-Hafez, 2003; Shariatzadeh et al., 2015).

Table 2.3 Prevalence of cystic echinococcosis in humans and other animals in Middle Eastern countries.

Country	Period	Humans	Buffalo	Cattle	Sheep	Goats	Camel	References
Egypt	2012-2013	5%	-	0.06%	14.1%	13%	5%	Omar <i>et al.</i> (2013); Abbas (2016)
	2015	-	4.2%	-	-	-	-	
Cyprus	2000-2003	-	-	6.61%	1.53%	-	-	Grosso <i>et al.</i> (2012)
Iran	2009-2015	5.8%	16.5%	8.8%	5.9%	6.4%	32.7%	Khalkhali <i>et al.</i> (2018)
Iraq	1990-1998	-	-	29.8%	64%	35.7%	-	Saeed <i>et al.</i> (2000)
Israel	1988	1.6 %	-	-	-	-	-	Nahmias <i>et al.</i> (1991); Shimshony (1997)
	1996	-	-	-	10%	10%	-	
Palestine	2009	-	-	-	9%	-	-	(El-Ibrahim, 2009)
Jordan	1985-1988	-	-	-	32%	6%	-	Abo-Shehada (1993)
Oman	2010-2013	-	-	0.6%	0.07%	0.03%	5.3%	Al Kitani <i>et al.</i> (2015)
Saudi Arabia	2008-2009	-	-	8.2%	12.6%	6.5%	32.8%	Ibrahim (2010); Al-Qurashi and Bahnas (2012)
	2011-2012	-	-	-	6.8%	2.2%	5.4%	
Syria	2002	4.5%	-	-	-	3%	18.2%	Seimenis and Battelli (2003)
Yemen	2008-2009	-	-	-	3.2 %	2.3 %	-	Muqbil, Al-salami and Arabh (2012); Al-Shaibani, Saad and Al-Mahdi (2015)
	2013	2.8	-	24.5%	19.3%	19.6%	1%	

### **2.6.3 Situation of Cystic Echinococcosis in Iraq and in Basrah Province**

Hydatid cysts have been frequently reported in livestock processed at slaughterhouses in most of the Iraqi provinces. In the north of Iraq the prevalence of hydatid cysts was reported at 12.7% in sheep, 4.8% in goats and 4.3% in cattle (Mero, Jubrael and Hama, 2013). Another study in Basrah province by Murtaza *et al.* (2017) found an overall prevalence of cysts of 14.75% in sheep with a higher prevalence in female sheep (22.9%) than males (7.5%). However most of the scientific research conducted on hydatidosis in Iraq has not investigated risk factors for infection or evaluated the impact, including economic burden, of the disease on the community. Only one study performed by Kadir, Ali and Ridha (2012) has evaluated economic losses from the condemnation of affected viscera of sheep, cattle and goats. In that study, conducted in Kirkuk, an overall annual economic loss of 10,430,000 Iraqi dinars (approximately US\$ 8,800) was estimated.

Cystic echinococcosis has been reported to be endemic in humans in Iraq, with most cases being recorded in the northern, central and southern provinces of the country (Jarjees and Al-Bakeri, 2012; Faraj and Muhsin, 2013; Abu Tabeekh and Thuwaini, 2015). According to the Iraqi CDC (2012), the number of cases of hydatidosis in humans has increased dramatically since 2000 (Figure 2.1). From 2011 to 2015 4,769 human CE cases were recorded by the Communicable Diseases Control Center (Parasitology and Helminthology Units) in Iraq (Saheb *et al.*, 2017). This may be due to the presence of a large population of free roaming dogs in the cities/villages, resulting in heavy environmental contamination with eggs (Saeed *et al.*, 2000; Bajalan, 2010). The strategic implementation of a control programme to eliminate or reduce the number of free roaming dogs, as well as owned domesticated dogs, has not been implemented in Iraq (Al-Shabbani, 2014). Consequently the number of new cases of CE in humans has increased. In addition, Saida and

Nouraddin (2011) reported an incidence risk in the north of Iraq of 6.3/100,000 persons/year. They also observed that more females were infected with CE and proposed that this arose from the routine household chores undertaken which would increasing their exposure to eggs of *Echinococcus*. Maktoof and AbuTabeeekh (2015) estimated an incidence rate of surgical cases of hydatid cysts at 3.2 cases/100,000 people per year in Basrah involving patients ranging from 12 to 40 years. An epidemiological study conducted by Thamir, Abood and Halboosh (2015) between 2000 to 2005, reported that 508 patients had undergone surgery to resect hydatid cysts in Basrah, with slightly more females (54%) undergoing surgery than males (46%).

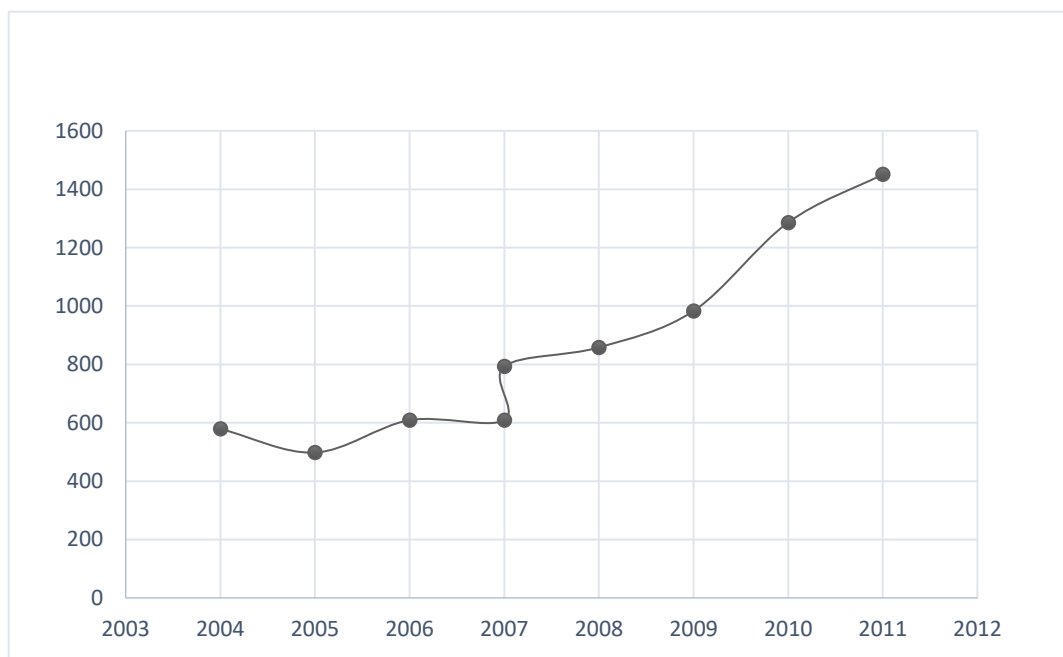


Figure 2.1 Number of patients with Cystic Echinococcosis reported by CDC (2012).

Basrah province is located in the south of Iraq and is surrounded by the three countries of Kuwait, Iran and Saudi Arabia (Figure 2.2). The human population in Basrah was estimated at 2.4 million in 2014, with 20.1% of this rural ([http://www.ncciraq.org/images/infobygov/NCCI Basra Governorate Profile.pdf](http://www.ncciraq.org/images/infobygov/NCCI_Basra_Governorate_Profile.pdf)).

In Basrah, the number of farmers and animals has been estimated at 2,618 and 134,395, respectively (data obtained from the Veterinary Hospital of Basrah, Ministry of Agriculture based on the last census of livestock conducted in Basrah in 2015).

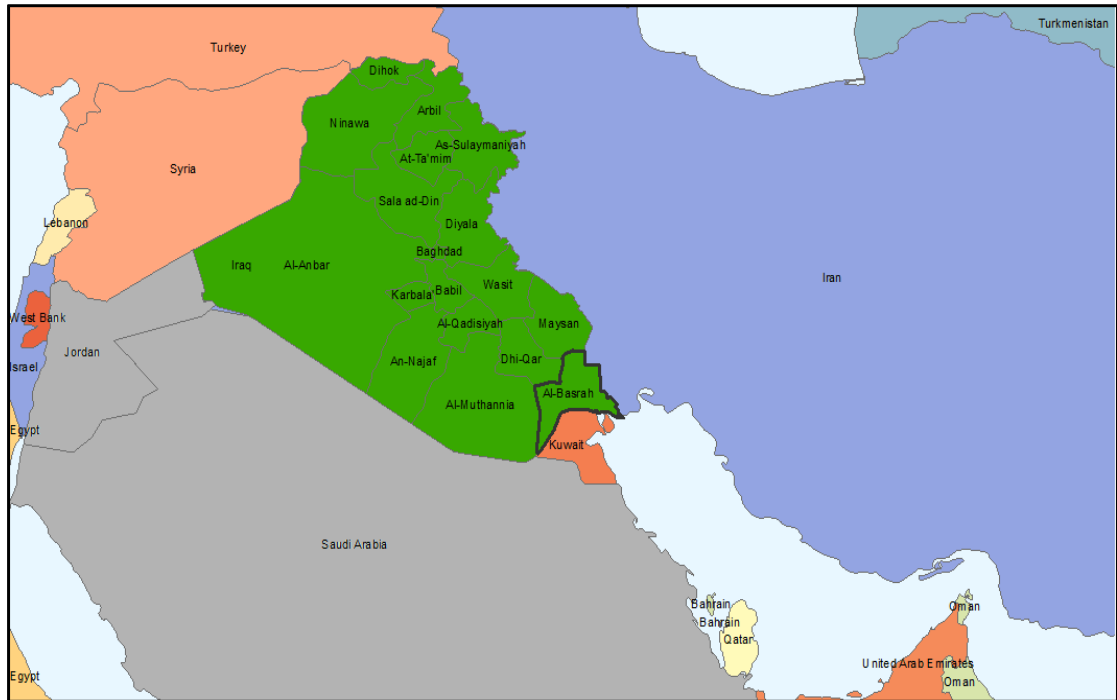


Figure 2.2 Map of Iraq and Basrah province (Basrah demarcated within the dark border) - created with Arc GIS version 10.4.

The main veterinary hospital of Basrah has implemented an annual programme involving the culling of stray dogs from public places in the province (Abu Tabeekh and Thuwaini, 2015). Although culling these FRD can reduce the likelihood of infection of humans and other animals with hydatid cysts, the number of cases in humans has still escalated (Ali, 2011; Murtaza et al., 2017). Furthermore the benefit in culling FRD on hydatid disease control has been questioned, as well as raising potential animal-welfare concerns (Dalla Villa *et al.*, 2010).

The detection of CE in humans and other animals in Iraq is summarised in Table 2.4. However, there is a lack of recent and epidemiological sound data on the

situation of hydatidosis in Basrah, as well as in other Iraqi provinces. Consequently, the study outlined in this thesis was designed to address these deficiencies.



Table 2.4 Prevalence of cystic echinococcosis reported in humans and livestock in Iraq.

Province	Period	Humans	Buffalo	Cattle	Sheep	Goats	References
Al-Muthana	2005	-	-	-	13.8		Mallah (2005)
Basrah	2008-2015	-	-	-	22 %	-	Abu Tabeekh and Thuwaini (2015)
	2016	-	-	-	14.7	-	Murtaza et al. (2017)
Diyala	2013-2015	19.3%	-	-	-		Al-Ezzy and Abood (2016)
Duhok	2009-2010	-	-	10.58%	9.9%	6.2%	Meerkhan and Abdullah (2012 )
Erbil	2008-2009	0.8%	-	7.7%	11.1%	1.6%	Saida and Nouraddin (2011)
Karbala	2012	-	1.7%	1.1%	1.2%	1.4%	Al-Nassir (2014); Jawad, Sulbi and Jameel (2018)
	2016-2017	-	-	1.8%	1.9%	2.4%	
Kirkuk	2009-2010	-	-	1.7%	0.7%	-	Kadir, Ali and Ridha (2012)
	2016	-	-	3.1%	-	-	Al-Mahmood <i>et al.</i> (2017)
Najaf	2015-2016	-	1.0%	1.5%	1.0%	-	Alatabi, Al-dujaily and Al-mialy (2017)
Nasiriya	2007	-	6.7%	7.0%	5.9%	5.9%	Thweni and Yassen (2015)
Mosul	2008-2009	-	-	0.5%	2.0%	0.5%	Jarjees and Al-Bakeri (2012)
Sulaimaniya	2006	2.5%	0.2%	-	1.5%	0.5%	Mohamad, Al-taie and Amin (2008);Hama, Mero and Jubrael (2014)
	2013	5.1%	-	-	-	-	

## 2.7 Control of Cystic Echinococcosis

Control of CE is designed to eliminate infection in the definitive hosts and/or to prevent transmission of the hydatid cysts from the intermediate to the definitive hosts. Dosing dogs and foxes with anthelmintic drugs, either directly or through baits, has helped reduce the presence of adult *Echinococcus* worms in the definitive hosts (Lembo *et al.*, 2013; van Kesteren *et al.*, 2015). One of most effective drugs that has been used in the definitive hosts is praziquantel at a dose of 5.5 mg/kg (Jiang *et al.*, 2017). In Europe, anthelmintic laced baits have also been used to reduce the prevalence of *E. multilocularis* in foxes with the aim of reducing transmission to humans and other animals and to preserve the ecological balance without needing to cull these animals (Hegglin and Deplazes, 2013). In China a programme involving deworming every dog in endemic areas with praziquantel tablets or baits every month was purported to have had a good success rate in reducing the prevalence (Andersen *et al.*, 1991). Praziquantel has also been used with arecoline hydrobromide to accelerate the destruction of the eggs of *Echinococcus* (Christofi *et al.*, 2002). This procedure was implemented in New Zealand and Tasmania, Australia, resulting in eradication of echinococcosis (Craig *et al.*, 2007). Experimentally, praziquantel has also been used to successfully damage the wall of hydatid cysts in mice (Urrea-París *et al.*, 2001), and it has been proposed that it could be prescribed to patients with hydatidosis to help degenerate the wall of the cysts in these patients, especially if it was administered in combination with albendazole (Jamshidi *et al.*, 2008). Garcia *et al.* (2011) highlighted the increased cysticidal efficacy of this combination in a trial of 32 infected patients.

Central to the control of hydatid cysts is involvement of the community. Education of the general public, by increasing their awareness about hydatid disease,

how the life cycle is completed and risk factors for infection, including avoiding eating raw vegetables without first washing them and fencing vegetable gardens to prevent access by dogs, is a key component of disease control (Heath *et al.*, 2006; Adanir and Tasci, 2013). Targeted education of the rural population to prevent raw viscera being accessed by dogs and providing a location for the centralised slaughtering of animals is recommended for rural and pastoral areas (Heath *et al.*, 2006). Jiang *et al.* (2017) considered that educational efforts to change human practices, including stopping the feeding of raw viscera to dogs, periodic treatment of dogs with praziquantel and improved sanitary conditions in slaughterhouses would help control the disease. Knowledge and the understanding of how zoonotic diseases of dogs are transmitted by the general public are key features of a public educational campaign. In Emin County, Xinjiang, China a control programme on echinococcosis was undertaken between 2007 to 2013 and focused on several activities including: health education; mass-community screening by ultrasound; patient treatment; monthly treatment of dogs with praziquantel; and inspection of offal at slaughterhouses. This programme had a remarkable impact reducing the prevalence of cysts in sheep by around 70% during the first three years of the campaign (Yang *et al.*, 2015). Similarly in Morocco, when over 11,000 people were screened during two 10-day ultrasound surveys for CE, the participants were educated about the parasite's life cycle and the impact the disease potentially had on human health (Kachani *et al.*, 2003). Such programmes require input from the general public, schools, veterinarians and staff from human hospitals, and follow a One Health principle (Kahn, 2011; Bidaisee and Macpherson, 2014). If people are aware of the potential dangers of hydatid cysts, they can then personally take action to protect or minimise themselves from being infected. Also to break the life cycle of echinococcosis, dogs should not be fed the viscera of slaughtered animals and

further control can be implemented if home slaughter of sheep and other livestock is restricted (Craig *et al.*, 2017).

Vaccination against echinococcosis has been proposed to further the control of hydatid disease, in conjunction with the inspection of animals at slaughter, improving husbandry and hygiene practices, and regular deworming dogs (Craig *et al.*, 2017). A vaccine, EG95, has been developed for the immunisation of the parasite's intermediate hosts (Chow *et al.*, 2004). According to Woollard *et al.* (2001) immunisation of sheep with three truncated regions (EG95-1, EG95-2, and EG95-3) of hydatid cysts elicited antibodies that could lyse the coat of the parasite. EG95 has been trialled in New Zealand, Australia and Argentina, with a high level of protection (96 - 100%) reported one year after vaccination (Lightowlers *et al.*, 1999). In Australia, Tammar wallabies (*Macropus eugenii*), which are common intermediate hosts of *E. granulosus*, have also been vaccinated with EG95. A 100% protection was reported and vaccination of such wildlife would help reduce the sylvatic phase of the disease (Barnes *et al.*, 2009), although it would be potentially challenging to attain a wide coverage of at-risk intermediate hosts.

Larrieu *et al.* (2013) undertook a field trial of the EG95 vaccine against cystic echinococcosis in sheep in four regions of Rio Negro, Argentina. A total of 3146 lambs from 79 farms received two injections at 30 and 60 days of age with a third booster injection given when the animals were approximately 1–1.5 years of age. The vaccine successfully protected sheep up to 3 years of age and reduced the prevalence of hydatid cysts in sheep from 26 to 7.8%. Another trial was conducted in the western area of Rio Negro province by Larrieu *et al.* (2015) and involved vaccination of 2725 weaned lambs with the EG95 vaccine. The vaccination campaign was continued for 6 years from 2009 to 2015. Although the vaccine resulted in a decrease in the prevalence of cysts from 56.3 to 21.1%, this reduction

was not statistically significant, with the authors attributing this to low power from insufficient animals included in the study. A study conducted by Heath *et al.* (2012) on cattle involved immunising 6 month-old calves twice one month apart with EG95 at five times the dose recommended for sheep. This resulted in 90% protection against infection with hydatid cysts which was maintained for 12 months. Administering a third immunisation at 12 months resulted in 99% protection for a further 11 months.

Research into the efficacy of other vaccines against hydatid cysts has also been undertaken with a range of antigens showing promise to protect humans and other animals against infection. Kouguchi *et al.* (2007) isolated the antigen EMY162 from *E. multilocularis* which showed promise as a potential vaccine agent for alveolar echinococcosis in humans. An S3Pvac-phage vaccine that was trialled in pigs, was shown to significantly reduce the prevalence of cysts by 56.1% in these animals (Morales *et al.*, 2011). Although there are promising developments in the vaccine field, the cost of the vaccines (US\$ 1.8-1.9 per dose) is beyond the reach of many rural communities, plus they also require time, effort and suitable infrastructure to be administered in the field (Larrieu *et al.*, 2015; Craig *et al.*, 2017).

Some vaccines have also been trialled in dogs to protect them against infection with *Echinococcus*. These vaccines have included the inactivated contents of hydatid cysts administered orally to dogs to either prevent them from harbouring adult worms or to reduce the number of eggs produced (Petavy *et al.*, 2008). Turner, Berberian and Dennis (1933) used antigens prepared from *E. granulosus* protoscoleces and demonstrated a reduction in the number of worms in the vaccinated dogs. Immunization of dogs through oral administration of irradiated protoscoleces has also resulted in a decrease in the number of adult worms and a reduction in the development of worms to the gravid stage (Aminzhanov, 1980;

Movsesijan and Mladenovic, 1970). These vaccines potentially show promise and could be used in conjunction with other preventive measures in hydatid control programmes (Zhang and McManus, 2008), however further work is required to confirm their efficacy and the costs may prevent use in developing nations where the parasite is endemic.

## **2.8 Conclusions**

Cystic echinococcosis constitutes a major public health problem in most developing countries and is re-emerging in some regions of developed nations. The parasite's life cycle highlights the diversity of animals involved, with canids acting as the definitive host and a range of omnivores and herbivores serving as intermediate hosts. Although CE is endemic in Iraq, little research has been conducted on the disease. Understanding a disease's distribution, risk factors and economic impacts is a key part when developing a control programme. Therefore, the study outlined in this thesis was developed to improve the knowledge about CE in humans, livestock (sheep) and dogs in the province of Basrah in Iraq as well as assessing the current knowledge, attitudes and practices adopted by livestock and dog owners in the province.

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## **Chapter Three - A Retrospective Study of Human Cystic Echinococcosis in Basrah Province, Iraq**

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### **3.1 Preface**

This chapter consists of a paper published in *Acta Tropica* 2018, 178:130-133, entitled “A Retrospective Study of Human Cystic Echinococcosis in Basrah Province, Iraq”. The text of this chapter is the same as the published version of the manuscript except for minor modifications, such as referencing and formatting. This chapter provides information to highlight that CE is prevalent in humans in Basrah and is an important health problem. The study was undertaken by examining hospital medical records for the period 2005 to 2015 from six public hospitals in Basrah Province for cases of CE patients.

### **3.2 Statement of contribution**

As first author on this manuscript, **MFA** conceived the idea and conducted the survey in Iraq (Basrah province) and wrote an initial draft. **IH** was involved in manuscript administration, statistical analysis, reviewing and edition. **SA** helped obtain permission from the health department of Basrah for accessing data at the hospitals. **IR** supervised the project, obtained ethics approval and reviewed and

edited the manuscript. All authors critically reviewed and approved the final version of the manuscript.

**MFA: 60%**

### **3.3 Abstract**

Human cystic echinococcosis (CE) is a parasitic zoonosis with serious clinical burden and constitutes a challenge to public health in endemic areas worldwide. We performed a retrospective study to investigate the occurrence of CE in patients at six hospitals in Basrah province, Iraq. In the current study setting, data retrieval and validation of the quality of hospital records was very challenging considering the difficult situation Iraq is unfortunately facing. Hospitalization records were reviewed from January 2005 to December 2015. A total of 748 cases of human with CE were diagnosed and operated in Basrah hospitals, equivalent to an annual clinical incidence of approximately 4.5 cases per 100 000 people. Hospital records show that CE affected more females (61.2%) than males (38.8%). Descriptive review of recorded CE cases in the surveyed hospitals revealed that more cases were reported in the age group of 21–30 years than in the other age groups. Based on the reviewed recorded clinical reports, cysts were mainly found in the liver (46.3%) and lungs (28.1%) of the patients. Hospital reports demonstrate that females had more hepatic cysts (63.9%) than males (36.1%). This study found that CE continues to pose a threat to public health in Basrah, and there is a need for more epidemiological investigations of CE in humans in order to determine risk factors and the economic impact of the disease in this province of Iraq.

### **3.4 Introduction**

Human cystic echinococcosis (CE) is caused by the larval stage of *Echinococcus granulosus*, a helminth belonging to the cestode group. A number of herbivorous and omnivorous animals act as intermediate hosts of *Echinococcus*.

They become infected by ingesting the parasite eggs in contaminated food and water, and the parasite then develops into larval stages in the viscera. Carnivores act as definitive hosts for the parasite and host the mature tapeworm in their intestine. They are infected through the consumption of viscera of intermediate hosts that harbor the parasite (Budke, Deplazes and Torgerson, 2006). CE is principally maintained in a dog–sheep–dog cycle. Humans are an accidental intermediate host for this parasite, and are infected through ingestion of eggs released from dogs or other canids (Eckert and Deplazes, 2004). CE demonstrates a high predilection for the liver and lung. Clinical symptoms vary depending on the size and position of the cysts in the organ (Eckert *et al.*, 2001; McManus *et al.*, 2003). In the liver, the pressure effect of the cyst can produce symptoms of obstructive jaundice and abdominal pain. Involvement of the lungs produces chronic cough, dyspnoea, pleuritic chest pain, and haemoptysis (Larrieu and Frider, 2001; Schantz, 2006).

The World Health Organization identifies human CE as one of the most important neglected zoonoses, as the disease continues to pose a serious socio-economic problem in many parts of the world (Budke, Deplazes and Torgerson, 2006). CE is highly endemic in most of the countries of the Mediterranean basin, including North Africa and the Middle East. The high endemicity of echinococcosis in the Mediterranean region has been attributed to many risk factors, such as a lack of adequate public health education, insufficient application of control programmes, and the common practice of home slaughter of small ruminants (Dakkak, 2010).

The zoonotic nature and the serious clinical burden of CE make it important from public health and economic perspectives worldwide. High annual incidences of CE have been reported in Levant, the Persian Gulf, and Middle East countries (Sadjjadi, 2006) and the costs associated with the disease have a great impact on affected individuals, their families, and the community as a whole (McManus *et al.*,

2003). For example, in Morocco, 1700 human surgical cases of CE (5.5 cases per 100 000 inhabitants) were recorded in 2003 and the average cost for surgical intervention was US \$1500 per case (Azlaf and Dakkak, 2006). The overall annual cost of CE in Iran was estimated at US\$232.3 million (95% CI US\$103.1–397.8 million), including both direct and indirect costs (Rokni, 2009).

Human CE is endemic in Iraq, and the disease has been recognized from the number of patients that were admitted to the hospitals and treated surgically (Jarjees and Al-Bakeri, 2012; Faraj and Muhsin, 2013; Maktoof and AbuTabeekh, 2015). Higher number of cases of human CE have been recorded in the southern provinces of Iraq (Abdul Ameer, Al-Hassani and Benyan, 2013; Thweni and Yassen, 2015), and in particular Basrah province (Thamir, Abood and Halboosh, 2015). Despite the substantial burden of the disease, national surveillance programmes for CE do not exist in Iraq. The fragile health services in Iraq, after years of international economic sanctions and ongoing political and ethnic conflicts, are challenging for any organized efforts to combat endemic tropical and zoonotic diseases (Barnett-Vanes *et al.*, 2016).

In common with several other neglected zoonoses, little is known on the prevalence and incidence of human CE in Iraq, and the resulting lack of awareness generates little interest for developing and implementing appropriate control programmes. The aim of this study was to compile data from hospital records on human CE in Basrah province, as an attempt to characterize aspects of the disease in an endemic setting in southern Iraq.

### **3.5 Material and methods**

#### **3.5.1 Study area and population**

Basrah is the third largest province in Iraq and lies in south of the country and borders Iran, Kuwait, and Saudi-Arabia. Its economy is largely dependent on the

oil industry; some of Iraq's largest oil fields are located in the province, and most of Iraq's oil exports leave from there. Basrah province has a desert climate with great temperature variations between the seasons and the highest temperature reaches 50°C in the summer. Basrah is in a fertile agricultural region, with major products including rice, maize corn, barley, pearl millet, wheat, dates, and livestock. The province had an estimated population of 1.5 million in 2012, with 20.1% of this rural (NGO, 2015).

In Basrah province, there are 14 public and 5 private hospitals primarily located in urban area. Only 8 of these hospitals offer surgical services, and of these 6 (5 public and 1 private) are equipped with technical facilities and skilled surgeons to perform human CE operations on human cases of CE. The study outlined in this manuscript was conducted at these 6 hospitals (Al-Sadar general hospital, Al-Basrah general hospital, Al-Faihaa general hospital, Al-Shifa general hospital, Al-Mawana general hospital, and Abin-Albaitar private hospital).

### **3.5.2 Data collection**

Clinical records of the 6 hospitals were reviewed for the 11 year period from 2005 to 2015. Data retrieval was very challenging as: the majority of the records were handwritten paper-based reports; there was no universal data collection style or form; and there was no system in place to validate the quality of the hospital clinical records. The following data were extracted from the records and entered into excel sheets; age, gender, occupation, residency (urban vs. rural), and localization of cysts. Descriptive data analysis was performed using the software STATA Ver.11 for Windows (Stata Press, College Station, TX, USA). Categorical variables were analyzed using the chi-square test for independence at a critical probability of  $P < 0.05$ .

### **3.5.3 Ethics statement**

This research study was approved by Human Ethics Review Committee of Murdoch University, Perth, Australia (Permission number: 2016/034). Official written approval forms the Ministry of Health in Iraq and from Basrah Health Directorate were obtained before commencement of the field work.

### **3.6 Results**

Reviewing the available clinical records revealed that a total of 748 human patients with CE were diagnosed and surgically operated at the 6 hospitals in the 11-year period from 2005 to 2015 (Table 3.1) giving an average of 68 cases per year. Using 2012 population data for Basrah (United Nations Development Programme (Iraq Country Office), 2013) as an estimate of the hospital's catchment area, an annual clinical incidence of 4.5 cases/100 000/year was calculated. The highest number of CE cases (12.7%, 95% confidence interval (CI): 10.4, 15.3) was recorded in hospital reports of the year 2014, while the lowest (4.3%, 95% CI: 2.9, 5.9) was in 2005.

The age and gender distributions of patients with CE are summarized in (Table 3.1). The largest share of patients with CE (26.9%) was in the 21–30 year-age-group. Based on our data, cystic echinococcosis was recorded more in females (61.2%) than males (38.8%). Housewife was the most frequently reported occupation listed for cases revealed from the hospitals records and represented 53.9% of CE cases. The average age of patients with CE was 34.6 years (SD 16.6 years), and the median 32 years (Table 3.2). The youngest patient operated on was only 6 months of age and the oldest 85 years.

The distribution of cysts in the body is summarized in (Table 3.3). The two anatomical sites most commonly affected were the liver (46.3%)

and lung (28.1%). Surprisingly, the reviewed hospital data indicate that 15.7% of CE cases among female patients were recorded in ovaries. Recorded cases in females had more hepatic cysts (63.9%) than males (36.1%), and both genders were equally affected by pulmonary cysts (Table 3.3). Our data show that the majority of CE cases featured with single organ involvement and with solitary cysts. Cysts were found in multiple organs of only 3.2% of patients and livers of male patients was commonly (7/24) involved (Table 3.3). Involvement of various atypical organs was evident, for instance 6 patients received surgery in the neck and 4 patients in vertebral column. Three patients received surgery due to cyst in brain, spinal cord, legs, thigh and pelvic region. Two cases were reported with cysts in the testicles, scrotum, pancreas, shoulders, and thorax vertebrate.

Table 3.1 Age and gender of human CE patients in Basrah, Iraq between 2005- 2015.

<b>Age (years)</b>	<b>Female (%)</b>	<b>Male (%)</b>	<b>Total (%)</b>
<10	17 (42.50)	23 (57.50)	40 (5.3)
11-20	75 (62.50)	45 (37.50)	120 (16.04)
21-30	120 (59.70)	81 (40.30)	201 (26.87)
31-40	88 (61.1)	56 (38.9)	144 (19.25)
41-50	73 (68.87)	33 (31.13)	106 (14.17)
51-60	51 (64.56)	28 (35.44)	79 (10.56)
61-70	32 (64.00)	18 (36.00)	50 (6.68)
>70	2 (25.0)	6 (75.0)	8 (1.07)
<b>Total</b>	<b>458 (61.23)</b>	<b>290 (38.77)</b>	<b>748 (100.00)</b>



Table 3.2 Occupation, gender and age distribution of human CE patients in Basrah, Iraq, between 2005-2015.

<b>Occupation</b>	<b>Number (%)</b>	<b>Female (N)</b>	<b>Male (N)</b>	<b>Age (Mean ± SD)</b>
Child (< 12 yrs)	22 (2.94)	9	13	4.26 ± 1.68
Public Servant	49 (6.55)	11	38	37.93 ± 11.26
Housewife	403 (53.88)	403	0	37.90 ± 15.25
Retired	10 (1.34)	0	10	59.6 ± 10.27
Student	70 (9.36)	33	37	14.52 ± 5.60
Teacher	2 (0.27)	2	0	34.00 ± 5.65
Unemployed	192 (25.67)	0	192	36.23 ± 15.15
<b>Total</b>	<b>748 (100.00)</b>	<b>458</b>	<b>290</b>	<b>34.58 ± 16.64</b>

Table 3.3 Distribution of single and multiple cysts in different organs in 748 human patients with CE in Basrah, Iraq, between 2005 and 2015.

<b>Organ</b>	<b>Number of CE cases (%)<sup>a</sup></b>	<b>Sex</b>	<b>Frequency (%)<sup>b</sup></b>	<b>Number of multiple cysts</b>
Liver	346 (46.26)	Female	221 (63.87)	2
		Male	125 (36.13)	7
Lungs	210 (28.07)	Female	105 (50.00)	1
		Male	105 (50.00)	3
Ovary	72 (9.63)	Female	72 (100.00)	1
		Male	0 (0.0)	0
Kidney	32 (4.27)	Female	18 (56.25)	1
		Male	14 (43.75)	0
Abdominal Cavity	17 (2.27)	Female	8 (47.06)	2
		Male	9 (52.94)	2
Spleen	10 (1.34)	Female	5 (50.00)	1
		Male	5 (50.00)	0
Chest cavity	9 (1.20)	Female	5 (55.56)	0
		Male	4 (44.44)	0
Others	41 (5.48%)	Female	16 (39.02)	4
		Male	25 (60.97)	0
<b>Total</b>	<b>748 (100.00)</b>			<b>24 (3.2%)</b>

<sup>a</sup> The denominator is the total number of human CE cases.

<sup>b</sup> The numerator is the number of CE cases per affected organ.

### 3.7 Discussion

Cystic echinococcosis is a cosmopolitan zoonosis, with highly endemic areas in regions of South America, China, the Middle East and North Africa (Dar and Alkarmi, 1997). Iraq is an important endemic focus of CE where several species of intermediate host (e.g. sheep, goats, camels, cattle and buffalo) are commonly infected with *E. granulosus* (Thweni and Yassen, 2015). This study demonstrated that CE poses an important threat to human health in south of Iraq, with a high incidence of hospitalized cases (4.5 cases/100 000/year). Such incidence estimate should be treated with caution, as data retrieval and quality validation of clinical records was very challenging in the surveyed 6 hospitals in Basrah, considering the difficult situation Iraq is unfortunately facing. The physical infrastructure of the health system in Iraq has deteriorated as a result of over 20 years of under-investment, poor management, war and conflict. There is no effective health information system, and reliable and high-quality epidemiological data is generally scarce in Iraq (Alwan, 2004). Compared to our results, a lower incidence of CE was reported in neighboring Iran with 1.18-3 cases per 100 000 population in different regions of the country (Rokni, 2009). Also, lower annual incidence rates have been reported in some Arab countries including Jordan (2.3/100 000/year) (Al-Qaoud, Craig and Abdel-Hafez, 2003), Libya (4.2/100 000/year) (Shambesh *et al.*, 1999), and in different governorates in Egypt (0.80–2.60 per 100 000/year) (Kandeel *et al.*, 2004). Although the data obtained from the hospital records are the most dependable source of information on human hydatidosis in many countries, it is likely that the true incidence of infection is higher due to underdiagnosing and underreporting

of cases (Craig *et al.*, 2007). In future work, it is important also to consider the impact of health-care migration in the study area, i.e. that a number of patients with CE going to other hospitals in nearby regions to be operated, and the vice versa where patients come from neighboring regions to be operated in Basrah hospitals. The presented information in this research on the rate of surgically operated patients illustrates the magnitude of the public health burden of human CE in Basrah and provides evidence of the high infection pressure with *E. granulosus* in the environment in southern Iraq.

Many potential risk factors for human CE may be prevalent in Basrah governorate. A high prevalence (22%) of hydatid cysts has been reported in domestic sheep slaughtered in Basrah abattoirs (during 2006–2007), in addition 14.7% of stray dogs in Basrah city were infected with *E. granulosus* (Maktoof and AbuTabeekh, 2015). Dogs with little or no veterinary attention and especially those found to be free- roaming and living in close proximity to people and their livestock are a crucial factor for maintaining the CE infection cycle (Buishia *et al.*, 2005; Acosta-Jamett *et al.*, 2010). Interestingly, a high infestation rate of 80% was found in dogs roaming around Basrah abattoir, which is hypothesized to be due to the frequent access of stray dogs to hydatid cysts discarded during meat inspection (Maktoof and AbuTabeekh, 2015).

Although environmental conditions in Basrah province include a hot and arid climate, its location within the Persian Gulf, results in high humidity and rainfall. The province receives an average of 152 mm of rainfall between the months of October and May (Hadeel, Jabbar and Xiaoling, 2011). Less than half of the people connected to the public water network have water available for the full day. Covered canals are the source of waste

water disposal for 26.7% of Basrah inhabitants (NGO, 2015). In the absence of plentiful potable water and widespread environmental contamination with dog feces, it is highly possible that drinking water is contaminated with *Echinococcus* eggs. A study in Jordan indicated that contaminated open source drinking water was an important risk factor for human CE infection (Dowling, Abo-Shehada and Torgerson, 2000). In Iraq, the provision of clean water supplies declined in quantity and quality over the last two decades. Water supply and sewage treatment plants were damaged during the wars, and raw sewage is released into the rivers directly (Alwan, 2004). Water accessibility challenge and unhygienic domestic waste disposal might be hypothesized as among the causes of higher number of CE recorded in Basrah.

Before discussing the key descriptive findings of the present retrospective study, it is important to state that there were some key limitations and challenges with respect to data retrieval, management, integration, and, most importantly, data quality issues that are present in the surveyed hospitals in Basrah. Our study was not designed to evaluate the information system quality in the surveyed hospitals, but we think it is important to highlight this experience from a challenging field work in Iraq as it might impact the validity of other investigations for endemic diseases, beyond CE. We noted, absence of an up to date and detailed data dictionaries, data models, and there was no available information on how data and process flows within each hospital structure. In all of the surveyed hospitals there were no regular data audits and controls. Variation in data collection elements was evident in some hospitals records over multiple years. Iraq faces enormous challenges in rebuilding the infrastructure,

strengthening management; there is virtually no information technology nor an effective health information system tackling the main causes of the rise in communicable and non-communicable diseases (Alwan, 2004).

Based on the hospitalization data summarized in this study, the age distribution of human CE cases showed that higher infection rate was recorded in individuals aged between 21 and 30 years. The age groups with the highest proportion of cases varies between regions: in Turkey most cases appeared in 41–50 (22.7%) year age group (Gulsun *et al.*, 2010), and in Tunisia most CE cases were aged 30–44 years (Lahmar *et al.*, 2009), while in Egypt about one-third of the cases were  $\leq 20$  years of age (Kandeel *et al.*, 2004).

In the analysis of gender distribution of CE recorded cases in the present study; females had a higher occurrence of CE (61.2%) than males. Similar findings were reported in Tunisia (Bchir *et al.*, 1991), Jordan (Dowling, Abo-Shehada and Torgerson, 2000), Iran (Hajipirloo *et al.*, 2013), and China (Zhang *et al.*, 2015). This may arise from the fact that females may appear more affected due to possible increased exposure, but also the result may derive from a bias on females accessing more the health services.

In the present retrospective analysis, hospital records show that the anatomical distribution of cysts was highest in the liver (46.26%) followed by lungs (28.07%). The result was similar to previous findings that the majority of cases presented as isolated liver cysts (AL-Barwari *et al.*, 1991; Pawlowski, 2001; Zhang *et al.*, 2015). In the present study, most of the patients with primary CE have single organ involvement and harbor a solitary cyst, and this is in accordance with findings of other research (Aribas *et al.*, 2002; Talaiezadeh and Maraghi, 2006). A very questionable finding in the present

study was the high reporting of CE in ovary. On paper, it is obvious that the hospital reports indicate that the female patients went through surgery; but in reality, it was difficult to retrieve past data on the differential diagnostic conditions for these cases.

Existing literature indicates that the incidence of hydatid cyst in the female reproductive system is rare and constitutes less than 0.5% of all hydatid cysts (Abike *et al.*, 2011; Vural *et al.*, 2011; Sharma *et al.*, 2012). It is very difficult to differentiate hydatid cysts from other ovarian lesions that may appear to be mostly cystic (cystadenoma, cystadenocarcinoma) on the basis of imaging findings alone (Sharma *et al.*, 2012). This finding warrants further investigations through active clinical surveillance, in order to rule out any possibility of imperfect differential diagnosis, or bad reporting on paper.

### **3.8 Conclusion**

This is the first retrospective analysis of human CE cases in Basrah. In view of our findings, human CE is prevalent and presents a public health burden in Basrah. Quality of health care has progressively deteriorated in Iraq over the last two decades; there is no effective hospitals information system tackling endemic diseases as CE. Added to that, there is a shortage of diagnostic supplies, equipment, and training of staff, there has also been a continuing depletion of experienced professionals at all levels. This might have impacted the differential diagnostics skills for some neglected zoonotic diseases and might be a hidden reason behind the very questionable finding in the present study on the high reporting of CE in ovary. The findings of this study highlight the need for more extensive epidemiological investigations of CE in human to determine the prevalence and economic impact of the disease in Basrah Province and Iraq.

### 3.9 Acknowledgements

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## **Chapter Four - Neglected Zoonoses and the Missing Opportunities for One Health Education: The Case of Cystic Echinococcosis among Surgically Operated Patients in Basrah, Southern Iraq**

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### **4.1 Preface**

In this chapter the results of a questionnaire survey administered to 50 patients with CE are reported. The survey was designed to evaluate their knowledge, attitudes and practices relevant to CE. The study demonstrated that the patients generally had a low level of knowledge about CE; they mostly adopted poor hygiene practices; and medical practitioners failed to advise patients on how to prevent further infection. This study highlighted the need for a health education programme for the general community of Basrah, as well as the need for further education of the medical profession. The text of this chapter is the same as the published version of the manuscript except for minor modifications, such as referencing and formatting. The contents of this chapter has been published in the journal of Disease, 7 (1):1-10, in a manuscript titled “Neglected Zoonoses and the Missing Opportunities for One Health Education: The Case of Cystic Echinococcosis among Surgically Operated

Patients in Basrah, Southern Iraq". The text of this manuscript is the same as that submitted for publication except for minor referencing and formatting modifications.

#### **4.2 Statement of contribution**

**MFA** and **SA** administrated the questionnaire survey to the 50 surveyed patients in Basrah province. **MFA** validated and analysed the data and wrote the initial manuscript draft. **IR** supervised the project, obtained the necessary ethics approvals and reviewed and edited the manuscript. **IH** also oversaw the project and reviewed and edited the manuscript. All authors critically reviewed and approved the final version of the manuscript.

**MFA: 70%**

#### **4.3 Abstract**

Cystic echinococcosis (CE) is recognised as a neglected disease of public health concern throughout the world, particularly in low and middle-income countries. The objectives of this study were to describe the characteristics, attitudes, knowledge, and practices of some Basrah province residents diagnosed with CE. Using a questionnaire survey, we interviewed 50 surgically operated cases of CE from Basrah. The cases comprised of 31 females and 19 males, of which 74% originated from rural areas. The questionnaire contained 30 questions and focused on gathering the demographic characteristics of the patients and capturing their overall knowledge, attitudes, and practices relating to CE. Approximately half of the participants reported slaughtering livestock at home for their families' consumption, 78% indicated the presence of a large number of stray dogs roaming freely about their village, 86% reported that they never boiled water prior to drinking it, and 26% reported not washing vegetables prior to eating them. A large proportion of the participants (72%) had not heard of hydatid disease before getting sick and over half (57%) were not aware how the disease was transmitted from animals to humans.

This study highlights a gap in health education efforts regarding CE in southern Iraq, with a lack of counselling of patients on how to prevent reinfection. An intensive control programme imperatively should be implemented and founded on health education to reduce CE disease in Basrah community.

#### **4.4 Introduction**

Cystic echinococcosis (CE) is a neglected disease of public health significance throughout the world, especially in low and middle-income countries (Deplazes *et al.*, 2017). The disease is caused by a tapeworm belonging to the genus *Echinococcus* which is transmitted between carnivores (dogs and wolves: the definitive hosts) and primarily livestock (the intermediate hosts), with humans accidentally acquiring the infection, usually through consuming food or water contaminated with eggs shed by the definitive host (Romig *et al.*, 2017). Once ingested by an intermediate host the eggs hatch in the small intestine releasing oncospheres which penetrate the intestinal wall and circulate in the bloodstream to finally lodge in a vital organ where the cyst (s) slowly grow over a period of several years (Acha and Szyfres, 1987). Approximately 70% of cysts in humans are found in the liver and 20% in the lungs (Torgerson and Budke, 2003; Eckert and Deplazes, 2004).

The highest prevalence of CE in humans is typically found in sheep-raising rural communities, as sheep are considered the most important intermediate host. also, the emergence of human echinococcosis may be attributed to a change in the local ecology and increasing urbanization resulting in greater exposure of people to infected dogs (Craig *et al.*, 2007; Moro and Schantz, 2009). Several studies have highlighted the role of socio-demographic characteristics; including age, gender, occupation and level of education, as important factors in the transmission of

echinococcosis to humans (Heidari *et al.*, 2011; Zhang *et al.*, 2015; Galeh *et al.*, 2018).

In Iraq, CE is regarded a major public health concern (CDC, 2012). The highest numbers of human cases have been reported in the provinces of central and southern Iraq including Basrah, Nasiriyah, and Muthana (Al-Yasari and Al-Shaiely, 2013; Maktoof and AbuTabeekeh, 2015; Thweni and Yassen, 2015). In our recent research, hospitalization records of Basrah hospitals from January 2005 to December 2015 were reviewed, and an annual clinical incidence of CE of 4.5 cases per 100 000 people was estimated (Abdulhameed *et al.*, 2018b). In general, treatment of echinococcosis in the humans is a costly and complicated and needs a prolong post-operative health care (Kern *et al.*, 2017). Hence, it is important to ensure that people living in endemic communities receive appropriate health education on how to protect themselves from infection. Understanding the level of knowledge and awareness of the disease in previously infected individuals is an important step in identifying potential gaps in knowledge prior to developing health educational programmes. In an endemic setting, such as in Iraq, failure to understand these gaps results in an increased burden on the public health system. The objectives of this study were to describe the characteristics, attitudes, knowledge and practices of a cohort of patients who had undergone surgery as a result of CE in Basrah, Iraq.

## **4.5 Subjects and Methods**

### **4.5.1 Study setting**

Basrah is the third largest province in Iraq and lies in the south of the country bordering Iran, Kuwait, and Saudi-Arabia. The human population in Basrah was estimated at 2.4 million in 2014, with 20.1% of this rural (NGO, 2015). The province contains almost 140,000 livestock (data obtained from the Veterinary Hospital of Basrah, Ministry of Agriculture based on the last census of livestock



conducted in Basrah in 2015). In Basrah 6 (5 public and 1 private) hospitals have the facilities and skilled surgeons to perform operations on human cases of CE.

#### **4.5.2 Case recruitment and questionnaire administration**

This research was approved by the Human Ethics Review Committee of Murdoch University, Perth, Australia (Permission number: 2016/034). Official written approval to review hospital records and to contact patients was obtained from the Ministry of Health in Iraq and from the Basrah Health Directorate. Of six hospitals in Basrah from which previous incidence data had been collected (Abdulhameed *et al.*, 2018b), four permitted researchers to interview hospitalized CE patients after surgical intervention or to contact patients who had undergone surgery for CE from 2014 to 2016. The hospitals were visited four times a week from May to July 2016, and 19 current patients recruited.

A total of fifty participants were administered a questionnaire either at the hospital (face-to-face) for the 19 current patients or via telephone interview for the 31 discharged patients. Prior to administering the questionnaire, a verbal explanation of the study was given and oral consents from all participants were obtained. The parents/guardians of two female patients (4 and 10 years old) were interviewed on behalf of the children. The questionnaire contained 30 questions and focused on demographic characteristics of the patients and their knowledge, attitudes, and practices relating to CE. The questionnaire was written in English and translated into the Arabic language, by a native researcher from Iraq, and it was back-translated from Arabic to the English language to verify the clarity of translation. For all interviewed patients, the questionnaire was administered by the same person (the first author) to ensure consistency over time. A copy of the questionnaire can be obtained from the corresponding author. The instrument for data collection

(questionnaire) was based on a set of questions derived from a prior study where the instrument has been validated before (Abdulhameed *et al.*, 2018a)

#### 4.5.3 Data analysis

Questionnaire data were entered into a spreadsheet (Microsoft Excel, 2013) and descriptive analyses undertaken using Statistical Package for the Social Sciences version 20. Frequencies, percentages, and 95% confidence intervals (CI) for responses were calculated.

#### 4.6 Results

Sixty cysts had been surgically excised from the 50 participants with half of the cysts (51.7% - 31/60) in the liver and 28.3% (17/60) in the lungs (Table 4.1). There was no significant difference in the location of cysts between females and males. Most patients only had one organ affected (82%), 16% had cysts in two organs, and only one patient (2%) had three organs affected.

Table 4.1 Anatomical sites of hydatid cysts as reported by the patients (n= 50).

<b>Organ involved*</b>	Female	Male	Total (%)
Lung	10 (25.6) *	7 (33.3) *	17 (28.3)
Liver	18 (46.1) *	13 (61.9) *	31 (51.7)
Urinary bladder	1 (2.5)	1 (4.7)	2 (3.3)
Spleen	4 (10.2) *	0	4 (6.7)
Kidney	2 (5.1) *	0	2 (3.3)
Ovary	2 (5.1)	0	2 (3.3)
Pancreas	1 (2.5)	0	1 (1.7)
Intestine	1 (2.5)	0	1 (1.7)
Total	39 (65.0)	21 (35.0)	60
<b>Number of organs affected in patients</b>			
One	24 (77.4)	17 (89.4)	41 (82.0)
Two	6 (19.3)	2 (10.5)	8 (16.0)
Three	1 (3.2)	0	1 (2.0)
Total	31 (62.0)	19 (38.0)	50

\*as some patients had more than one organ affected the total number of organs involved was > 50

The age of the CE patients ranged from 4 to 72 years (median: 39.5, Std: 14.8) (Table 4.2). Ten percent of the patients reported having another family member (not surveyed) also diagnosed with CE. Nearly three-quarters of the patients surveyed (74% - 37/50) originated from a rural area, 42% had only obtained a primary school level of education and 24% had never been to school. Approximately half (54%) of the patients (87% of female patients) undertook domestic duties, 16% of all patients were farmers (42% of male patients) and 12% were unemployed.

Table 4.2 Results of socio-demographic characteristics of 50 Cystic Echinococcosis patients in Basrah Province.

Factor	Category	N	Percentage (95% CI)
Gender	Female	31	62 (47.2, 75.3)
	Male	19	38 (24.7, 55.8)
Age of patients (years)	< 10	2	4 (0.5, 13.7)
	11-20	5	10 (3.3, 21.8)
	21-30	10	20 (10.0, 33.7)
	31-40	10	20 (10.0,33.7)
	41-50	14	28 (16.2, 42.5)
	51-60	6	12 (4.5, 24.3)
Has any other member in your family been diagnosed with CE?	> 61	3	6 (1.3, 16.5)
	Yes	5	10 (3.3, 21.8)
Location	No	45	90 (78.2, 96.7)
	Rural	37	74 (59.7, 85.4)
Education level	Urban	13	26 (14.6, 40.3)
	Never went to school	12	24 (13.1, 38.2)
	Literacy class only	5	10 (3.3, 21.8)
	Primary	21	42 (28.2, 56.8)
	Secondary	10	20 (10.0, 33.7)
Occupation	College	2	4 (0.5, 13.7)
	Public servant	2	4 (0.5, 13.7)
	Farmer	8	16 (7.2, 29.1)
	Housewife	27	54 (39.3, 68.2)
	Student	2	4 (0.5, 13.7)
	Unemployed	6	12 (4.5, 24.3)
	Other	5	10 (3.3, 21.8)

Table 4.3 summarise the practices adopted by the patients. Twenty of the surveyed patients (40%) owned one or more dogs. Of these, six reported allowing their dog(s) to roam freely, while five reported tying up their dog (s) within 50 metres of their house. Forty percent of dog-owners never allowed their dog access to

the kitchen or food preparation area, and 50% never allowed their dog access to water storage containers. Sixty percent of dog-owners reported feeding raw offal to their dog(s).

A significant number of patients (78%;  $p = 0.03$ ) reported the presence of a large number of dogs roaming freely about their village. Approximately half (48%) of the participants had slaughtered livestock at home, and no-one had contacted a veterinarian when they had observed/detected a cyst or lesion characteristic of CE in the viscera of the slaughtered animal(s). While no significant difference existed, these trends align with results published in prior research. The majority (86%) of the participants reported that they never boiled water prior to drinking it; however, most participants (90%) did store their water in covered containers. Additionally, a significant portion of the respondents (72%,  $p = 0.01$ ), reported receiving their water from a reverse osmosis system. The majority (86%) of the participants reported that they never boiled water prior to drinking it; however, most participants (90%) did store their water in covered containers. 26% of participants never washed vegetables prior to eating them, 8% rarely washed vegetables, and 40% reported that they sometimes washed vegetables prior to eating them.

Table 4.3 Descriptive results of patient's practices toward cystic echinococcosis in Basrah Province, Iraq.

<b>Question</b>	<b>Response Categories</b>	<b>N</b>	<b>Percentage (95%CI)</b>
Do you own a dog (s)?	Yes	20	40 (26.4, 54.8)
	No	30	60 (45.2, 73.6)
If you own a dog is it tied up?	Yes	6	30 (11.9, 54.3)
	No	14	70 (45.7, 88.1)
If dog is tied up, where is it tied up?	Far (>50 m) from my dwelling	1	16.7 (0.4, 64.1)
	Near (<50 m) to my dwelling	5	83.3 (53.9, 99.6)
Does your dog have access to the kitchen area/food preparing area?	Never	8	40 (19.1, 63.9)
	Rarely	2	10 (1.2, 31.7)
	Sometimes	6	30 (11.9, 54.3)
	Often	2	10 (1.2, 31.7)
	Always	2	10 (1.2, 31.7)
Does your dog have access to containers used to store drinking water for people?	Never	10	50 (27.2, 72.8)
	Rarely	2	10 (1.2, 31.7)
	Sometimes	7	35 (15.4, 59.2)
	Often	1	5.0 (0.1, 24.9)
	Always	0	0.0 (0.9, 16.8)
How often do you feed your dog raw offal (for example liver or lung)?	Never	8	40 (19.1, 63.9)
	Rarely	1	5 (0.1, 24.9)
	Sometimes	5	25 (8.7, 49.1)
	Often	4	20 (5.7, 43.7)
	Always	2	10 (1.2, 31.7)
Have you seen stray dogs in your neighbourhood over the last week?	Yes	39	78 (64.0, 88.5)
	No	11	22 (11.5, 36.0)
Do you own livestock?	Yes	22	44 (30.0, 58.7)
	No	28	56 (41.3, 70.0)
Do you slaughter livestock at your home?	Yes	24	48 (33.7, 62.6)
	No	26	52 (37.4, 66.3)

<b>Question</b>	<b>Response Categories</b>	<b>N</b>	<b>Percentage (95%CI)</b>
Do you always call an inspector (Vet or meat inspector) if there is a cyst in the slaughtered carcass?	Yes	0	0.0 (0.0, 14.3)
	No	24	48 (27.4, 69.1)
What is the main source of family drinking water?	Reverse Osmosis (RO)	36	72 (57.5, 83.8)
	Tanker	9	18 (8.6, 31.4)
	Tap water	4	8 (2.2, 19.2)
	Well	1	2 (0.1, 10.6)
Do you boil water before drinking it?	Never	43	86 (73.3, 94.2)
	Some of the time	4	8 (2.2, 19.2)
	Most of the time	2	4 (0.5, 13.7)
	All the time	1	2 (0.1, 10.6)
How is your family's drinking water stored?	Stored in covered containers	45	90 (78.2, 96.7)
	Stored in uncovered containers	5	10 (3.3, 21.8)
How often do you eat leafy vegetables without first washing them?	Never	13	26 (14.6, 40.3)
	Rarely	4	8 (2.2, 19.2)
	Sometimes	20	40 (26.4, 54.8)
	Often	4	8 (2.2, 19.2)
	Always	9	18 (8.6, 31.4)
For lettuce and other leafy vegetables, how would you prepare them as part of your salad?	Wash it under running water only	36	72 (57.5, 83.8)
	Soak in water in the sink	10	20 (10.0, 33.7)
	Wash it with detergent	3	6 (1.3, 16.5)
	Peel outer leaves & eat the rest	1	2 (0.1, 10.6)

In Table 4.4 the patient's knowledge about hydatid cysts and their attitudes toward handling potentially infected organs of slaughtered animals are outlined. A significant number of participants (72%;  $p = 0.02$ ) had not heard about hydatid cysts prior to their surgery. Additionally, 57% were not aware how the disease was transmitted. A significant number of patients (70%;  $p = 0.04$ ) reported that they had not received any information on how they may have become infected with CE. Furthermore, a significant number of participants (86%;  $p = 0.01$ ) reported that they had not received any advice on methods to prevent reinfection by hydatid cysts from medical staff at the where they were operated on. Surprisingly, half (50%) of the participants who deemed organs unsuitable for human consumption in livestock they slaughtered would consider feeding those affected organs to their dog(s). Similarly, over 81% of the respondents would consider throwing organs unsuitable for human consumption into their uncovered home garbage. A significant proportion of the participants would definitely not burn affected organs (58%;  $p = 0.02$ ), and 58% would not bury any organs which were considered unsuitable for human consumption.

Table 4.4 Results of the descriptive analyses for the knowledge and attitudes of patients infected with cystic echinococcosis in Basrah Province.

<b>Variable</b>	<b>Category</b>	<b>N</b>	<b>Percentage (95% CI)</b>
Before getting sick, had you heard about cystic echinococcosis or hydatid cyst disease?	Yes	14	28 (16.2, 42.5)
	No	36	72 (57, 83.8)
How do you believe cystic echinococcosis or hydatid cyst disease can be caught?	Not sure	8	57 (28.9, 82.3)
	Dog	1	7.14 (0.2, 33.9)
	Food	4	28.57 (8.4, 58.1)
	Water	1	7.14 (0.2, 33.9)
Did your doctor, nurse or other medical staff explain how you became infected with a hydatid cyst?	Yes	15	30 (17.9, 44.9)
	No	35	70 (55.4, 82.1)
Did your doctor, nurse or other medical staff explain ways of how to protect you from further infection?	Yes	7	14 (5.8, 26.7)
	No	43	86 (73.3, 94.2)
Would you feed organs not suitable for human consumption to your dog?	Would definitely consider doing it	10	50 (27.2, 72.8)
	Might consider doing it	7	35 (15.4, 59.2)
	Would definitely not do it	3	15 (3.2, 37.9)
Would you throw organs not suitable for human consumption into a communal open rubbish area?	Would definitely consider doing it	13	54.17 (32.8, 74.4)
	Might consider doing it	9	37.50 (18.8, 59.4)
	Would definitely not do it	2	8.33 (1.0, 27.0)



<b>Variable</b>	<b>Category</b>	<b>N</b>	<b>Percentage (95% CI)</b>
Would you burn organs not suitable for human consumption?	Would definitely consider doing it	1	4.17 (0.1, 21.1)
	Might consider doing it	9	37.50 (18.8, 59.4)
	Would definitely not do it	14	58.33 (36.6, 77.9)
Would you bury organs not suitable for human consumption?	Would definitely consider doing it	2	8.33 (1.0, 27.0)
	Might consider doing it	8	33.33 (15.6, 55.3)
	Would definitely not do it	14	58.33 (36.6, 77.9)

## 4.7 Discussion

The purpose of this descriptive study was to sketch socio-demographic characteristics, household practices and attitudes in 50 patients with CE. Among the CE patients enrolled in this study, cysts were most commonly reported in the liver and lungs, as has been reported by others (Kebede *et al.*, 2009).

In this study, more patients were females. This is in line with a previous finding from a retrospective study in Iraq where we revealed that CE affected more females (61.2%) than males (38.8%) (Abdulhameed *et al.*, 2018b). Females may be more frequently exposed to the infection than males due to being involved with activities including feeding of dogs and preparing food for the family. A higher occurrence of CE in females has similarly been reported in other countries including Jordan, Tunisia and Iran (Al-Qaoud, Craig and Abdel-Hafez, 2003; Lahmar *et al.*, 2009; Hajipirloo *et al.*, 2013).

In this survey, 40% of the patients affected with CE owned one or more dogs and of these 60% reported feeding them raw offal. The close association of people with dogs, combined with feeding offal, enhances the likelihood of the transmission of this zoonotic tapeworm along with environmental contamination (Schantz *et al.*, 2003; Possenti *et al.*, 2016).

The majority of patients (78%) reported the presence of large numbers of dogs roaming freely in their village. Stray or free-roaming dogs are considered a major source of CE for humans (Kayouèche *et al.*, 2009). A study conducted by Buishia *et al.* (2005) in Tripoli, Libya reported that 25.8% of stray dogs had *E. granulosus*, primarily arising from access to offal and scavenging from dead animals. It is important that the veterinary services in Basrah undertake steps to reduce the number of stray dogs which constitute a major public health concern, not only for echinococcosis but other zoonotic diseases such as rabies and toxocariasis

(Kilic *et al.*, 2006; Macpherson, 2013). A control programme should also be developed and implemented involving dosing domestic and stray dogs with anthelmintic containing praziquantel. In Western China, stray/unwanted were culled by police while registered dogs were treated monthly with praziquantel over a four year period (Zhang *et al.*, 2009). This program resulted in a significant reduction in the prevalence of infected dogs from 18.6 to 0%.

Around 50% of the participants reported slaughtering animals at home for household consumption. Additionally, 100% ( $p < 0.01$ ) of the respondents who slaughtered their own animals report not calling a veterinarian or meat inspector if they find cysts in the animal tissue. Other regional studies have highlighted the common practice of slaughtering animals by households in or near their homes (Nasrieh *et al.*, 2003; Galeh *et al.*, 2018). However slaughtering animals in an abattoir, under the supervision of a veterinarian, reduces the opportunity for the completion of the *Echinococcus* life cycle through ensuring appropriate disposal of affected offal (Ito *et al.*, 2003), as well as reducing exposure of humans to other zoonotic pathogens (Jiménez *et al.*, 2002).

Although RO purchased water was commonly used among the interviewees in this study, yet 86% of them reported they never boiled water prior to drinking it, and most (90%) did store water in covered containers. The RO water in the study area is not based on home-installed systems; it is sold by whole distributors using industrial size RO units. Uncontrolled distribution, transportation and storage conditions could work as a window for post-treatment contamination of the water supply with CE and other pathogens. Keeping water safe and away from free-roaming dogs would help reduce the potential transmission of disease, as reported by others (Moro *et al.*, 2008). Studies in Jordan by (Dowling, Abo-Shehada and Torgerson, 2000) and Kenya (Craig *et al.*, 1988) established that contaminated

drinking water was a risk factor for human CE, with *Echinococcus* eggs detected in the water used by both people and livestock. Consequently, treatment of water prior to drinking is an important process to minimise the risk of disease transmission.

In this study, 26% of participants never washed vegetables prior to consumption. A similar study in Jordan involving 55 patients infected with CE, revealed that, in addition to contact with animals through their occupation, many also consumed raw vegetables (Yaghan, Bani-Hani and Heis, 2004). Eating unwashed vegetables is a risky practice potentially increasing exposure to *Echinococcus*, as well as other canine zoonotic diseases (Qaqish *et al.*, 2003; Federer *et al.*, 2016). A study in Turkey identified a variety of canine parasite eggs on unwashed vegetables, including *Taenia* spp. (3.5%), *Toxocara* spp. (1.5%) and *Ascaris lumbricoides* (1.0%) (Kozan *et al.*, 2005). A low awareness and less knowledge of eating unwashed vegetables is considered as an important factor for possibly acquiring the cystic echinococcosis in humans (Aydın, Adıgüzel and Güzel, 2018).

As eggs from *Echinococcus* can survive for nearly 41 months in an arid climate under ideal environmental conditions (Thevenet *et al.*, 2005), and the high prevalence of infection in dogs (Beiromvand *et al.*, 2013) and large free-roaming dog population, it is critical that the general public is made aware of the risk of echinococcosis from the consumption of potentially contaminated food or water.

It is not surprising that the majority (72%) of participants (patients) in the current study had not heard of CE prior to their hospitalisation, but it was of concern that (57%) did not know the mode of transmission hydatid cysts, even after surgery for the condition. This is consistent with the results of other questionnaire surveys that have been conducted in Libya and Morocco (Buishia *et al.*, 2005; El-Berbri *et al.*, 2015), which found most respondents have limited to no knowledge about

echinococcosis and how it is transmitted. Currently, there is no control programme for echinococcosis or educational campaign in Basrah, putting the community at a disadvantage regarding this important health issue.

The current study found that most participants followed poor practices concerning the disposal of offal unsuitable for human consumption. Offal from slaughtered livestock that is unsuitable for human consumption needs to be disposed of by burning, burying or rendering to break the life cycle of *Echinococcus* (Wachira, Macpherson and Gathuma, 1991; Acosta-Jamett et al., 2010). Unfortunately few respondents were aware of the risks associated with dogs and other carnivores having access to raw offal and how it can be involved in the transmission of *Echinococcus*. This outcome is similar to that reported in a survey of communities in Jordan, where waste from slaughtered animals was disposed in a manner allowing it to be accessed and eaten by domestic or stray dogs (Qaqish et al., 2003).

It is necessary for the Health Department of Basrah, in collaboration with the veterinary authorities, to develop and implement educational programmes on echinococcosis for farmers, pet owners and the general public in the province. Such programmes should provide information on: the need for regular deworming of dogs; improved hygiene for food preparation; slaughtering animals at their homes, including strict guidelines on how to dispose of infected offal; the life cycle of *Echinococcus* and how to stop it developing; and practices to minimize infection from dogs.

#### **4.8 Conclusions**

A questionnaire was administered to 50 patients with CE in Basrah province, Iraq. Many patients owned dogs, there were large numbers of free-roaming dogs reported in the vicinity, animals were slaughtered at many of the patient's homes for

consumption, and few participants: washed vegetables prior to eating them; understood how echinococcosis was transmitted to humans; or disposed of affected offal in a safe manner. It is strongly recommended that a control programme for CE is implemented to reduce the disease in humans, livestock and dogs and an educational campaign developed for the general public to reduce the incidence of this preventable disease.

#### **4.9 Acknowledgments**

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## **Chapter Five - Knowledge, Awareness and Practices Regarding Cystic Echinococcosis among Livestock Farmers in Basrah Province, Iraq**

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### **5.1 Preface**

As the knowledge of patients with CE was low (Chapter 4), a survey was conducted to determine the knowledge and practices of livestock owners in Basrah. The findings of this study are presented in this chapter, the contents of which were published in the *Journal of Veterinary Science* 2018, 5: 1-10, in a manuscript titled “Knowledge, Awareness and Practices Regarding Cystic Echinococcosis among Livestock Farmers in Basrah Province, Iraq”. The text of this manuscript is the same as that submitted for publication except for minor modifications, such as referencing and formatting. A structured questionnaire was developed and delivered to 314 farmers to assess their knowledge about CE and to identify management and husbandry practices that are potential risk factors for infection with hydatid cysts in their livestock. The study, as with that of patients with CE, identified: a lack of awareness and knowledge of CE; adoption of practices and management of dogs that would increase the risk of infection and transmission of *Echinococcus*; and the need for a control programme to be developed and implemented focusing on improving education of livestock and dog owners.

## **5.2 Statement of contribution**

As first author on this manuscript, **MFA** travelled to Basrah province to perform the questionnaire survey. **SA** obtained permission from the main veterinary hospital of Basrah to allow the survey to be conducted. **MFA** and **IH** were involved in the manuscript administration, statistical analysis, reviewing and editing. **IR** supervised the project and the analysis, obtained the necessary animal ethics approval and reviewed and edited the manuscript. All authors critically reviewed and approved the final version of the manuscript.

**MFA: 65%**

## **5.3 Abstract**

Cystic echinococcosis is an endemic neglected parasitic zoonosis in many of the Middle East countries. The disease poses remarkable economic burden for both animals and humans. In this study, we conducted a questionnaire survey among livestock farmers in Basrah province, south of Iraq, in order to evaluate their knowledge and awareness about CE, and to understand some of the risky practices that could contribute to spread and persistence of such disease. Overall the interviewed participants ( $n= 314$ ), 27.4% owned dogs on their farms. Among farmers owning dogs, 76.7% (66/86) never tied up their dogs, and 43% (37/86) indicated feeding uncooked animal viscera to their dogs. The majority (96.5%) of the farmers indicated that they did not de-worm their dogs at all. From within all respondents, 9.8% (31/314) indicated eating raw leafy vegetables without washing. Added to that, 32% of the interviewee indicated that they source water for domestic use from a river; meanwhile 94.3% (296/314) of them do not boil water before using it for domestic purposes. Half of the interviewed livestock farmers in Basrah were not aware about how humans get infected with CE disease, and 41.4% (130/314) did not even realize that CE is a dangerous disease to human health. Almost 1/3 of the

respondents who owned dogs on their farms viewed that de-worming of their dogs is a low priority practice. This study highlights the gap in knowledge and awareness about CE among the study population. Risky practices associated with dog keeping management and food and water handling practices were identified. The insight from this research could be used to improve the delivery of a health education message relevant to cystic echinococcosis control at the human-animal interface in Iraq.

#### **5.4 Introduction**

Cystic echinococcosis (CE) is a neglected zoonotic disease caused by the tapeworm *Echinococcus granulosus*. The disease is commonly reported in the Middle East and North and East Africa, Russia, Central Asia, China, South America (Harandi, Budke and Rostami, 2012; Deplazes *et al.*, 2017). In such endemic regions, CE poses a remarkable One Health challenge due to the economic losses in animals combined with the high risk of morbidity in humans (Torgerson and Macpherson, 2011 ). At a global level, it has been estimated that there are more than one million human CE cases with a disease burden between 1 and 3.6 million disability-adjusted life years (DALYS) (Budke, Deplazes and Torgerson, 2006).

CE is particularly highly endemic in most of the countries of the Mediterranean basin, including North Africa and the Middle East. In Libya, the incidence rate of human CE was estimated at 4.2 cases per 100,000 inhabitants (Tashani *et al.*, 2002). In Iraq, the incidence has been reported at 6.3 per 100,000 inhabitants (Saida and Nouraddin, 2011). In Egypt, the incidence rate of human CE varied between 1.3 and 2.6 cases per 100,000 inhabitants (Kandeel *et al.*, 2004). An incidence rate has been reported in Tunisia to be as high as 12.6 per 100,000 inhabitants (Chahed *et al.*, 2010). Human infection in endemic regions is influenced

by different biotic and abiotic factors, and also depends on a number of behavioural and socio-economic variables (Macpherson, 2005).

In Iraq, previous studies have confirmed that human CE is an important endemic disease (Jarjees and Al-Bakeri, 2012; Faraj and Muhsin, 2013), with annual economic losses estimated at 189,406 US\$ in North of Iraq (Saida and Nouraddin, 2011). Several human cases with CE have been reported in the southern provinces of Iraq (Abdul Ameer, Al-Hassani and Benyan, 2013; Thweni and Yassen, 2015), and in particular in Basrah province (Sawady and Al-Faddagh, 2012). In Basrah, the incidence rate of human CE has been estimated as 5.6 cases per 100,000 inhabitants (Thamir, Abood and Halboosh, 2015). The prevalence of *Echinococcus granulosus* in stray dogs in Basrah recorded at 14.7%, while the prevalence of hydatid cysts in slaughtered sheep in Basrah was reported as 22% (Maktoof and Abu Tabeekh, 2015).

Transmission of cystic echinococcosis is influenced by socio-economic and culture conditions of a community. For instance, home slaughtering practice, improper disposal of internal organs of livestock to dogs, neglected deworming of dogs, feeding dogs with condemned offal are all considered among the common practices associated with increasing prevalence and risk of exposure of domestic animals to cystic echinococcosis (Buishi *et al.*, 2005; Ibrahim, 2010; Romig *et al.*, 2011). Added to that, the low level of education is also speculated to be a risk factor in human CE (Seimenis, 2003). Dogs are the major source of infection to humans, and the majority of documented human CE cases caused by *E. granulosus* with a life cycle that occurs mainly within a rural setting between sheep and shepherd dogs (Craig and Larrieu, 2006)

In order for a control program of CE to be efficient, it is important to evaluate the level of knowledge about the disease, awareness regarding the

preventive measures, and risky practices that spread the disease within the community. To the best of our knowledge, there have been no published studies, neither in Basrah nor in other Iraqi provinces, to assess knowledge, awareness, and practices regarding CE. Therefore, we conducted this survey to investigate the disease-related knowledge, awareness and practices among livestock farmers in Basrah, south of Iraq. The insight from this research could be used to develop health promotion tools and to improve the delivery of efficient health education message relevant to CE control at the human-animal interface in Iraq.

## **5.5 Materials and Methods**

### **5.5.1 Study area**

The study subjects were livestock farmers in Basrah province. Basrah is the third largest province in Iraq and lies in the south of the country and borders Iran, Kuwait, and Saudi-Arabia. Basrah is in a fertile agricultural region, with major agriculture and livestock production. The province had an estimated population of 2,403,301 million, in which rural-urban proportion is distributed at 20.1% -79.9%, respectively (NGO, 2015).

### **5.5.2 Study design**

Throughout this cross-sectional study, 320 farmers were enrolled from 20 villages distributed over the six counties of Basrah (Abu Al-Kasib, Al-Midaina, Al-Qurnah, Al-Zubair, Shat Al-Arab, and Al-Basrah). The sample size was based on 95% confidence limits at the precision of 5% and assuming response distribution of 30%. It was not possible to execute a random sampling approach from the study subjects, given to political and tribal conflicts in Iraq during the field work period (March to July 2016). Hence, we adopted in this study a convenience targeted sampling approach. After coordination with local veterinary authorities, the inclusion criterion for the targeted sampling was the possibility of gaining a secured



access to the targeted villages, based on security situation assessment at the time of the study. Local veterinarians and tribal leaders in each village assisted in introducing the research team to the farmers' community and helped in explaining the purpose of this survey. In each village, farmers were chosen using a rolling sample method in which the first select farmer provided information about the next available farmer in the area until the required number of respondents had been achieved. Out of 320 farmers, those were approached by the research team; only six farmers were not willing to participate, hence in total 314 farmers accepted to fill in this questionnaire data collection tool during a face-to-face interview visits to their farm.

### **5.5.3 Questionnaire design and administration**

The study instrument was a questionnaire which comprised of three sections with approximately 30 questions. Part 1, was related to socio-demographic characteristics; part 2, was related to practices towards CE prevention; and, part 3, concerned with knowledge and awareness about CE infection and transmission sources. The questions were either closed-ended or dichotomous, and the questionnaire was pre-tested by the authors to allow for improvements. Prior to the interview, a verbal consent form was obtained from each participant. The questionnaire survey was administrated in the local language (Arabic). A copy of the questionnaire could be obtained from the corresponding author. Data were coded and stored in Microsoft Excel (Windows 2000) sheets, and descriptive data analysis was performed using STATA software (v.14, Texas, USA).

### **5.5.4 Ethics statement**

This study was approved by the Human Ethics Review Committee of Murdoch University, Perth, Australia (Permission number: 034/ 2016). Official

written approval from the Ministry of Health in Iraq and the Basrah Health Directorate were obtained before commencement of the field work.

## **5.6 Results**

### **5.6.1 Socio-demographic characteristics of the study population**

Age of the interviewed farmers ranged from 18 to 84 years (Mean  $45.3 \pm 2.1$  Standard deviation), and the vast majority (99.1%) were males (Table 5.1). Regarding farmers' level of education, 50% of the interviewed farmers had completed primary school, while 25% of them were unable to read and write. Working as a farmer was a secondary occupation in almost 20% of the interviewee (Table 5.1). Cattle, alone or integrated with sheep and buffalo, was the main livestock species owned by the farmers interviewed in this study (Table 5.1).

### **5.6.2 Practices towards CE prevention**

Among all interviewed farmers, 27.4% (86/314) indicated that they own dogs on their farms (Table 5.2). Our result highlights some undermined dog management practices. For instance, among farmers owning dogs, 76.7% (66/86) never tied up their dogs. Added to that, 43% (37/86) of the interviewed farmers indicated that they feed uncooked animal viscera to their farm dogs (Table 5.2). The majority of the farmers (95.3%) answered that they tend to leave dog fecal droppings unattended on the ground of the farm wherever they are. The interviews also revealed that the vast majority of the farmers (96.5%) do not de-worm their dogs.

More than half (60%) of the farmers indicated that their dogs frequently have close contacts with livestock on their farms. The questionnaire results also highlighted some unsanitary food and water handling practices among the interviewed farmers (Table 5.2). Worth highlighting that among all the respondents, 9.8% (31/314) indicated eating raw leafy vegetables just after peeling the outer

leaves but without considering any kind of washing with water. Almost third (32%) of the livestock farmers indicated that they source water used for domestic purposes from a nearby river, and 94.3% (296/314) of them answered that they do not boil such water sourced from the river (Table 5.2).

### **5.6.3 Knowledge and awareness about CE infection and transmission sources**

The interviews revealed that 40.8% (128/314) of the study subjects did not know about the possibility of transmission of certain diseases (zoonoses) between livestock and humans. However, 70.7% (222/314) of the farmers answered that they had heard previously about CE disease, despite that 41.4 (130/314) of the farmers did not know that CE is a dangerous disease to human health (Table 5.3). Almost half of the interviewed livestock farmers in Basrah were not aware of how humans acquire the infection with CE. Added to that, 64.4% (202/314) of them were not aware that livestock animals could also get infected and act as carrier for the parasite causing CE. Almost one third of the farmers who owned dogs answered that they regard de-worming of their dogs as a low priority practice to do and failed to appreciate the importance of de-worming for either dogs or human health (Table 5.3).

Table 5.1 Socio-demographic characteristics of livestock farmers (N= 314) participated in CE knowledge, awareness and practices survey in Basrah, Iraq.

<b>Variables</b>	<b>Category</b>	<b>N</b>	<b>%</b>
County	Abu Al-Kasib	64	20.4
	Al-Midaina	47	15.0
	Al-Qurnah	16	5.1
	Al-Zubair	44	14.0
	Shat Al-Arab	112	35.7
	Al-Basrah	31	9.8
Age (years)	<20	16	1.9
	21-30	36	11.5
	31-40	77	24.5
	41-50	91	29.0
	51-60	67	21.3
	61-70	32	10.2
	>71	5	1.6
Gender	Male	311	99.1
	Female	3	0.9
Residence	Urban	63	20.1
	Rural	251	79.9
Education level	Illiterate (cannot read and write)	78	24.9
	Primary	158	50.3
	Secondary	67	21.3
	University	11	3.5
Principle occupation	Butcher	3	0.9
	Civil servant	32	10.2
	Farmer	245	78.1
	Policeman	19	6.1
	Soldier	2	0.6
	Teacher	7	2.2
	Retired	6	1.9
Livestock ownership*	Cattle	87	27.7
	Cattle & sheep	56	17.8
	Cattle & buffalo	55	17.5
	Sheep	35	11.2
	Buffalo	33	10.5
	Buffalo, cattle & sheep	14	4.5
	Other mixed livestock farms	34	10.8

\* Multiple answers allowed.

Table 5.2 Descriptive results of livestock farmers' practices relevant to CE prevention and control.

Theme	Variables	Category	N	%
Dog ownership (N= 314) *	Do you own dog on your farm?	Yes	86	27.4
		No	228	72.6
Dog management practices (N= 86) **	Do you tie up your dog?	Yes	20	23.3
		No	66	76.7
	How long you keep your dog tied?	In the day time	17	85.0
		Only for the night	3	5.0
	Do you let your dog access your house?	Never	48	55.8
		Rarely	7	8.2
		Sometime	15	17.4
		Often	7	8.1
		Always	9	10.5
	Do you feed your dog uncooked animal viscera?	Yes	37	43.0
		No	49	57.0
	How do you handle droppings/stools of your dog?	Leave it where they are	82	95.3
		Bury it	1	1.2
		Throw in water canal	1	1.2
		Throw in agriculture field	2	2.3
	How often do you wash hands after handling or feeding your dog?	Never	16	18.6
		Rarely	0	0.0
		Sometime	12	14.0
		Often	15	17.4
		Always	43	50.0
	Do you regularly de-worm your dog?	Yes	3	3.5
		No	83	96.5

<b>Theme</b>	<b>Variables</b>	<b>Category</b>	<b>N</b>	<b>%</b>
	How often does your dog have contact with farm livestock?	Never Rarely Sometime Often Always	21 2 11 19 33	24.4 2.3 12.8 22. 38.4
	How often do you or your family members play with you dog?	Never Rarely Sometime Often Always	41 1 18 19 7	47.7 1.2 20.9 22.1 8.1
Food and water sanitary handling practices (N= 314) *	How frequently do you wash hands before eating?	All the time Most of the time Some of the time Do not wash hands	213 91 6 4	67.8 29.0 1.9 1.3
	How do you eat food in your home?	By hands only With cutlery only By both hand and cutlery	96 57 161	30.6 18.1 51.3
	How do you clean raw vegetables before eating them?	Do not wash (peel outer leaves) Rinse using tap water Soak in water in the sink With detergent With river water With well water With RO <sup>y</sup> treated water	31 144 86 42 4 2 5	9.8 45.9 27.4 13.4 1.3 0.6 1.6

Theme	Variables	Category	N	%
	What is the source of water for your domestic use?	River	100	32.0
		Tap water	191	61.0
		Well	5	1.3
		RO <sup>¥</sup> treated water	18	5.7
	Do you boil water for domestic use before drinking it?	Yes	18	5.7
		No	296	94.3

% was calculated among all interviewed farmers ( $N= 314$ ).

\*\* % was calculated among farmers indicated that they own dogs on their farms ( $N= 86$ ).

¥ RO, Reverse osmosis treated water.

Table 5.3 Descriptive results of livestock farmers' (N= 314) knowledge about and awareness of sources of infection with CE.

<b>Theme</b>	<b>Variables</b>	<b>Category</b>	<b>N</b>	<b>%</b>
Knowledge about CE	Do you know that some disease could be transmitted from livestock to human?	Yes	186	59.2
		No	128	40.8
	Did you hear about echinococcosis disease?	Yes	222	70.7
		No	92	29.3
	Do you know if echinococcosis disease can be dangerous to human health?	Yes	184	58.6
		No	130	41.4
	Have you ever seen hydatid cysts like these pictures in the organs of animals?	Yes	117	37.3
		No	190	60.5
		Not sure	7	2.2
Awareness of CE sources of infection	Do you know how humans are infected by hydatid disease?	Does not know	161	51.3
		Contaminated food	50	15.9
		Contaminated hands	61	19.4
		Contaminated water	20	6.4
		Eating raw food	9	2.9
		Contact with dogs faeces	13	4.1
	Are you aware that buffalo, cattle, sheep and goats can be infected with hydatid disease?	Yes	100	31.8
		No	202	64.4
		Not sure	12	3.8
	Are you aware that it could be dangerous to eat raw vegetables contaminated with dog faeces?	Yes	225	71.7
		No	89	28.3



<b>Theme</b>	<b>Variables</b>	<b>Category</b>	<b>N</b>	<b>%</b>
	To what level do you consider de-worming of your dogs as a priority for your dog's health?	Essential	11	12.8
		High priority	10	11.6
		Low priority	29	33.7
		Medium priority	6	7.0
		Not a priority	30	34.9
	To what level do you consider de-worming of your dogs as a priority for your family health?	Essential	13	15.1
		High priority	11	12.8
		Low priority	29	33.7
		Medium priority	8	9.3
		Not a priority	25	29.1

## 5.7 Discussion

In this study, and despite several obstacles due to political and security instability in Basrah province, we were able to administer face-to-face questionnaires with 314 livestock farmers. Convenience cluster sampling was undertaken in this study as it is easy to undertake and is inexpensive (Aday, 2006; Dohoo, Stryhn and Martin, 2012). A true random stratified sample would require more time and resources and would be difficult to apply because of the lack of a suitable sampling frame. To the best of the authors' knowledge, this is the first study in Iraq to collect data on livestock farmers' knowledge about CE, awareness regarding the preventive measures against such important neglected zoonosis, and risky practices that contribute to spread and persistence the disease. The population interviewed in in this study was livestock farmers, which we believe is an important target for any one health education campaign aiming at raising awareness on CE at the human-animal interface. A sound understanding of the epidemiology of CE in livestock-raising communities is a key factor in limiting the transmission cycle to humans (Possenti *et al.*, 2016)

Several potential risky practices have been underlined among the livestock farmers community interviewed in this study, notably practices related to dog management on farms. Almost three-quarters of farmers never tie up their dogs while on farm. This finding reflects a poor awareness among the farmers in Basrah regarding the role of dogs in CE transmission. A study from Libya demonstrated that untying dogs was a significant risk factor for increasing the positivity of detecting *Echinococcus granulosus* in dogs' faecal samples (Buishia *et al.*, 2005). In addition to being not tying up their dogs, the majority of the interviewed farmers also did not de-worm their dogs, and half of the farmers indicated feeding uncooked viscera to their dogs. Such risky practices have been among the most important factors that

increase contamination of the environment with faeces containing *Echinococcus* eggs (Possenti et al., 2016). Similar findings have been documented in other CE endemic settings. A study in Sardinia (Italy) reported that the majority of the interviewed farmers use raw offal, after home slaughtering, for feeding their dogs (Varcasia et al., 2011). In addition, a study in Tibet demonstrated that feeding dogs with uncooked viscera is a risk factor for increasing the likelihood of human infection with *E. granulosus* (Li et al., 2015).

Our study also revealed that the majority of the interviewed livestock farmers tend to leave dogs feces unattended on the farm ground. Such unhygienic practice might carry potential risk mainly for children who tend to play and crawl on the ground. Added to that, this would also increase the chance of dog feces contamination of other crops, accidentally or as a fertilizer. It has been reported that *Echinococcus* eggs that deposited in the soil could stay viable for up to a year (Acosta-Jamett et al., 2010). On another aspect, more than half of the dog's owner revealed that their dogs have frequent contact with the livestock animals on their farms. Similar to our finding, a study in Portugal indicated that dogs have close contact with livestock among approximately 60% of the interviewed farmers (Mateus et al., 2016). Positive copro-antigen results were mainly reported in working dogs such as hunting, guard or shepherd dogs that presumably are more likely to roam freely (Otero-Abad and Torgerson, 2013; Asfaw and Afera, 2014).

In highly endemic areas it is quite possible for individuals to contract CE through indirect transmission of echinococcosis through contaminated food or water (Torgerson, 2014; Chaâbane-Banaoues et al., 2015). Our results show that approximately 10% of the respondents indicated eating raw leafy vegetables without washing. Although not a consistent finding, there are studies that indicate an association of CE human infection with homegrown vegetables, which presumably

have been contaminated by dog feces (Harandi *et al.*, 2011). One third of the farmers interviewed in our study reported using water supplied from a nearby river. Unsafe water supply has been also found to be associated with infection with CE, and this may be due to water contamination with dog faeces (Yang *et al.*, 2006; Torgerson, 2014). Adequate hygienic handling practices and heat treatment (cooking food or boiling water) should contribute to minimizing the risk of foodborne echinococcosis.

The result of this study indicates that CE seems to be not a familiar disease to the livestock farmers' community in Basrah, south of Iraq. Around 40% of the farmers did not know how CE disease could be transmitted to the human, and even did not realize that CE is a dangerous infection to human health. In Jordan, a neighbouring country to Iraq, awareness regarding CE in rural communities was higher (86%) compared to what we experienced among the livestock farmers interviewed in this study in Basrah (Qaqish *et al.*, 2003). In our study, half of the interviewed farmers were not aware of how a human can acquire the disease and more than 60 % of the farmers were not aware that livestock could also get infected with CE. Compared to our finding, a study in Morocco concluded even a lower level of awareness about CE, where only 20% of the interviewees realized that dogs play a role in the transmission of CE in humans and animals (El-Berbri *et al.*, 2015). Collectively, these findings call for an urgent need to strengthen the health education strategy among rural communities and livestock farmers in Iraq. It was quite worrisome to reveal that most of the livestock farmers interviewed in this study had a negative attitude toward deworming their dogs, as the majority did not realize the benefits of deworming dogs for both animals and human's health. We hypothesize that the cost and the availability of anthelmintic is probably one of the main reason of their quite low frequency of use among farmers in Basrah.

## **5.8 Conclusions**

After decades of war, sanctions and political instability, Iraq has been under socio-economical-political challenges and therefore, the level of education, communication among communities and general healthcare is low resulting in poor education and health indicators of the population in the study area. This study highlighted the low knowledge about CE in livestock farmers in Basrah. The awareness of the interviewed farmers regarding specific CE preventive measures was generally poor. Risky practices concerning management of dogs on farms and the handling practices for human food and water handling practices were identified. In Basrah, the annual incidence of human echinococcosis has been estimated at 5.6 cases per 100,000 inhabitants (Thamir, Abood and Halboosh, 2015) and the prevalence of *E. granulosus* in stray dogs in the Province has been recorded at 14.7%, while the prevalence of hydatid cysts in slaughtered sheep was 22% (Maktoof and AbuTabeeh, 2015). The insight from this research could be used to develop health promotion tools and to improve the delivery of a better health education strategy relevant to cystic echinococcosis control in Iraq.

## **5.9 Acknowledgments**

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## **Chapter Six - Prevalence of taeniid eggs in the faeces of owned and free-roaming dogs in Basrah, Iraq, and the knowledge of dog owners on cystic echinococcosis**

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### **6.1 Preface**

The larval stages (metacestodes) of some taeniids of carnivores, particularly *Echinococcus* spp., can result in severe disease for humans and livestock. In this chapter 335 faecal samples from free-roaming and owned dogs were examined for taeniid eggs and a questionnaire administered to dog-owners to assess their knowledge of hydatid disease and the management and practices adopted which could result in the spread of the parasite. The contents of this chapter will be submitted to the journal “Japanese Journal of Infectious Disease”. The text of this manuscript is the same as that which will be submitted for publication except for minor modifications, such as referencing and formatting.

### **6.2 Statement of contribution**

As first author on this manuscript, **MFA** performed the laboratory work and administered the questionnaire survey to the dog owners. **MFA** undertook the statistical analysis under the guidance of **IR**. **SA** provided advice on the tests to use

in the laboratory. **IR** supervised the project, obtained animal ethics approval and reviewed and edited the manuscript. **IH** also reviewed and edited the manuscript and provided input into the study design. All authors critically reviewed and approved the final version of the manuscript. **MFA: 65%**

### **6.3 Abstract**

The larval stages (metacestodes) of some taeniids of carnivores, particularly *Echinococcus* spp., can result in severe disease for humans and livestock. The prevalence of taeniid eggs by direct examination of faecal smears in 335 free-roaming dogs (FRD) and owned domesticated dogs in Basrah Province was 10.1% (95% CI 7.1, 13.9). A structured questionnaire was administered to 86 dog owners to investigate the influence of socio-demographic factors and management and husbandry practices on their knowledge of cystic echinococcosis (CE). The results of a multivariable logistic regression analysis revealed that dog owners who fed offal had less knowledge of CE (OR=0.17, 95% CI 0.05, 0.53), while keeping a dog(s) tied up was associated with good knowledge (OR=7.0, 95% CI 2.1, 23.8). Dog owners who had a secondary or higher level of education also had better knowledge (OR=5.4, 95% CI 1.7, 17.3). It was concluded that an educational campaign should be developed for dog owners in Basrah to reduce the risk of disease in both humans and other animals.

### **6.4 Introduction**

Although dogs play an important and beneficial role in society (Robertson *et al.*, 2000), they can carry a range of zoonotic diseases, including parasites (Joffe *et al.*, 2011; Deplazes *et al.*, 2011). Transmission of canine parasites to humans or other animals can occur through direct contact with the dogs or via indirect contact with contaminated food or water (Martinez-Moreno *et al.*, 2007).

Dogs are considered the principal definitive host for several parasites belonging to the family Taeniidae, including *Taenia* and *Echinococcus* spp. (Soulsby, 1982; Lavikainen, 2014). Taeniids have a wide global distribution and utilise a range of mammalian species in their life-cycles resulting in a potential risk for human health and reduce productivity of livestock (Lee *et al.*, 2016; Schneider-Crease *et al.*, 2017). Canine taeniids require two hosts to complete their life cycle. The adult worm resides in the intestinal passage of the dogs (definitive hosts), while the larval stage is either a hydatid cyst, cysticercus or coenurus in the tissue of the relevant intermediate host (livestock, humans or other animals) (Rausch, 1994b; Hoberg *et al.*, 2000; Chervy, 2002; D'Alessandro and Rausch, 2008). Eggs are passed in the faeces of the definitive hosts and are accidentally ingested by the intermediate host with contaminated food or water (Lawson and Gemmel, 1983).

The eggs of taeniids are not easily differentiated on morphology (Trachsel, Deplazes and Mathis, 2007) as they all contain a hexacanth or six-hooked embryo (Wardle and McLeod, 1952; Smyth and Smyth, 1969; Thompson and Lymbery, 1995). Taeniid eggs can survive on pasture, in soil and in water following contamination with infected dog faeces and subsequently wind and wildlife can disperse the eggs over long distances (Torgerson *et al.*, 1995). After ingestion by the intermediate host, the eggs hatch in the small intestine and penetrate the intestinal wall (Smyth and Smyth, 1969; Thompson and Lymbery, 1995). Subsequently they are dispersed by the circulatory system to organs such as the liver and lungs where they develop into cysts or metacestodes (larval stage) (Gemmell, 1966; Garrido, de Aluja and Casas, 2007).

Most of the canine taeniids are medically and economically important in parasitized humans and livestock (Baneth *et al.*, 2016) with the larval stages of *Echinococcus* spp. (hydatid cysts) (Zhang and McManus, 2006) and *T. multiceps*

(*Coenurus cerebralis*) (El-On *et al.*, 2008) being of particular significance. Although infection in the intermediate host can be asymptomatic, it can result in severe pathological changes to the affected organ(s) or even death of the animal/human (Moro and Schantz, 2009). Inspection of carcasses at slaughterhouses is an effective, quick and inexpensive means for determining infection in livestock (Abdulhameed *et al.*, 2018a); however, knowledge of the carriage of taeniids in dogs is a key aspect of understanding the risk and biological transmission of these parasites to humans and other animals.

Dogs are infected with taeniid tapeworms after consuming the larval stages (usually the liver or lungs) in cattle, buffalo, sheep, goats and pigs. The adult worms then develop in the small intestine within 1 to 3 months (Thompson and Lymbery, 1995). Many laboratory techniques have been developed to determine the prevalence in dogs, including serological assays, detection of antigen in the faeces (coproantigen), observing worms at necropsy or direct examination of eggs in the faeces (Eckert, 2003; Benito and Carmena, 2005; Barnes *et al.*, 2012).

Human behaviour can help maintain the domestic life cycle of taeniid tapeworms through the feeding of dogs with affected viscera, the failure to appropriately dispose of organs with cysts, allowing dogs to wander and potentially scavenge from carcasses and rubbish and a failure to regularly administer suitable anthelmintics to dogs (Moro *et al.*, 1994; Alishani *et al.*, 2017). Educational programmes, especially for poor communities, have been highlighted to be essential components of control programmes for echinococcosis in humans (Craig *et al.*, 2007). Prior to the implementation of any control programme, it is important to determine the prevalence of the disease/infection and the knowledge of the animal-owners about the disease and how to control it.

In this study, direct smears of faeces from stray and owned domesticated dogs in Basrah province of Iraq were examined to determine the prevalence of taeniid tapeworms. A questionnaire was also administered to dog-owners to assess their knowledge of cystic echinococcosis (CE) and the management practices adopted which could increase the risk of parasite transmission.

## **6.5 Materials and methods**

### **6.5.1 Study area**

This study was performed in Basrah Province, south Iraq. The province has extremely hot weather during the summer, with a mean temperature of 37.4 °C and a maximum temperature of 45 °C. The lowest mean summer temperature is 29.2°C. The annual humidity is less than 50% and remains below 30% during the daytime (Hadeel, Jabbar and Xiaoling, 2011).

### **6.5.2 Study design, questionnaire survey and sampling of dogs**

The study was performed from March to July 2016. Of 314 livestock owners who were interviewed to assess their knowledge, awareness and practices toward CE (Abdulhameed *et al.*, 2018b), 86 owned at least one dog (total of 122 dogs from 16 villages in the six districts of Abu Al-Kasib, Al-Midaina, Al-Qurnah, Al-Zubair, Shat Al-Arab, and Al-Basrah. A questionnaire was administered to the 86 dog-owners and included questions on management practices adopted (whether the dog was restrained or not; administered anthelmintics; fed offal; and how faeces from the dogs were disposed of; and the age and sex of the dogs). The level of knowledge on hydatid disease/echinococcosis was evaluated based on the answers to six questions: have you heard of hydatid cysts?; how can the disease be transmitted to humans?; have you ever seen a lesion of hydatid cyst in slaughtered animals? (a respondent was shown pictures of infected liver and lungs); do you know if buffalo, cattle, sheep or goats can be infected with hydatid cysts?; are you aware it can be



dangerous to eat raw vegetables contaminated with dog' faeces?; and do you know if acquiring hydatid cysts can be dangerous to the human health? Correct responses were allocated a value of 1 and incorrect/not-known answers given a value of 0. The results were then summed for the six questions for each respondent. Participants with a knowledge score greater than the median (4) were considered to have good knowledge, while those participants with a score  $\leq$  the median were categorized as having poor knowledge.

Approximately 25-50 g of fresh faeces (n=122) were collected directly from the ground where the owned dogs were fed, slept or housed. In addition, 213 fresh canine faecal samples of free-roaming dogs (FRD) were collected off the ground from areas where these dogs clustered within a radius of three kilometres of the 16 villages surveyed. To avoid the potential for duplicate samples from the same dog in a location, stool samples were inspected to ensure that no other samples of a similar consistency and color were collected from the location on days of sampling. All stool samples were collected using a sterile disposable wooden spatula and placed in a container and labelled with information about the owned domesticated dog (age, sex, owner name) or FRD location prior to sending on ice to the Parasitology Laboratory at the Veterinary College, Basrah University. Samples were examined by microscopy on the same day as submission to the laboratory. Ten thin and ten thick slides were prepared from each faecal sample and examined under light microscopy at 4X, 10X and 40X to identify eggs of taeniids. Taeniid eggs were identified based on their ovoid shape and the presence of oncospheres (hexacanth embryo) (Lapage, 1965; Soulsby, 1982).

### **6.5.3 Ethical statement**

The questionnaire surveys and faecal collection were approved by the Human Ethics Committee (034/2016) and Animal Ethics Committee (R2755/15), respectively at Murdoch University, Perth, Australia.

### **6.5.4 Statistical analyses**

Questionnaire data were entered into a spreadsheet (Microsoft Excel, 2013) and statistical analyses undertaken using SPSS version 20. The test prevalence and 95% confidence intervals (95% CI) for parasitism with taeniids were calculated in the sampled domestic and stray dogs from the six sampled districts. Univariable analyses were initially performed using a Chi-square test to compare knowledge about Cystic Echinococcosis (CE) disease with socio-demographic characteristics of the respondents and the management practices adopted. Odds ratios (OR) and their 95% CI were also calculated. Factors with a  $P \leq 0.25$  in the univariable analyses were offered to a multivariable binary logistic regression model using a backwards stepwise method. Variables were retained in the final logistic regression model if the likelihood ratio test was significant ( $P \leq 0.05$ ). The goodness of fit of the final model for the data was assessed with the Hosmer and Lemeshow goodness-of-fit test (Hosmer and Lemeshow, 2000).

## **6.6 Results**

### **6.6.1 Estimation of the prevalence of taeniid tapeworm**

Taeniid eggs were detected overall in 10.1% (95% CI 7.1, 13.9) of the 335 faecal samples examined (Table 6.1). The prevalence for the FRD (10.8%; 95% CI 7.0, 15.8) was comparable to that for domesticated dogs (9.0%; 95% CI 4.6, 15.6) ( $P = 0.6$ ). There was also no significant difference in the prevalence between districts ( $P=0.42$ ).

### **6.6.2 Socio-demographic characteristics of dog owners**

All 86 dog owners who participated in this study were males with a mean age of 45.6 years (SD: 12.1). Approximately one-quarter of the dog-owners (23.3%) had never received any formal education, and 50% had only attained a primary school level of education. Most dog owners worked solely as farmers (75.6%) with the remainder also working as public servants.

### **6.6.3 Influence of socio-demographic characteristics and dog management practices on knowledge of cystic echinococcosis**

The association between knowledge about CE by the dog owners and their socio-demographic characteristics and management practices adopted are summarised in Table 6.2. A respondent who had a secondary or higher level of education was more likely to have a better level of knowledge on CE than owners who had only attended primary school or had not received any formal schooling. Owners who fed offal to their dogs or who didn't restrain/confine their dogs had lower levels of knowledge (Table 6.2). In addition, the majority of dog owners 95.3% (82/86) reported that they did not pick up and dispose of their dog's faeces but left them where they were deposited.

In the final logistic regression model (Table 6.3), respondents who had at least attended secondary school or who restrained/confined their dogs were more knowledgeable of CE (OR=5.4, 95% CI 1.7, 17.3; OR= 7.0, 95% CI 2.1, 23.8, respectively). In contrast, respondents who fed uncooked offal to their dogs had lower knowledge about CE (OR=0.17, 95% CI 0.05, 0.53). The model represented a good fit of the data (Hosmer-Lemeshow goodness-of-fit test,  $p = 0.48$ ).

#### **6.6.4 Identification of risk factors for detection of taeniid tapeworm in dogs**

There was no significant association between observing eggs of taeniids in the faecal samples and the management and husbandry practices adopted for the owned dogs (all P values > 0.25 in the univariable analyses), and consequently a multivariable logistic regression model was not generated.

Table 6.1 Test prevalence of eggs of taeniids identified from the faecal samples of owned domesticated and free-roaming dogs surveyed.

Category	Total number sampled	Test prevalence (95% CI)	Odds ratios (95% CI)	P-value
Free roaming dogs	213	10.8 (7.0, 15.8)	1.2 (0.57, 2.6)	0.6
Domestic dogs	122	9.0 (4.6, 15.6)	1.0	
Total	335	10.1 (7.1, 13.9)		

Table 6.2 Univariable analyses of the dog owner's knowledge of cystic echinococcosis and their socio-demographic characteristics and management practices adopted (n = 86).

Variable	Categorised knowledge		OR (95% CI)	P-value
	Good	Poor		
Age (years)				0.84
≤ 30	25 (33.3)	50 (66.7)	0.88 (0.23, 3.27)	
> 30	4 (36.4)	7 (63.6)	1.00	
Education level completed				0.007
Secondary & Tertiary	13 (56.5)	10 (43.5)	3.82 (1.40, 10.39)	
Primary school or never attended school	16 (25.4)	47 (74.6)	1.00	
Main occupation				0.96
Farmer	22 (33.8)	43 (66.2)	1.02 (0.36, 2.90)	
Public servant	7 (33.3)	14 (66.7)	1.00	

<b>Variable</b>	<b>Categorised knowledge</b>		<b>OR (95% CI)</b>	<b>P-value</b>
Do you feed your dog uncooked viscera or internal organs?		1		
Yes	20 (54.0)	7 (46.0)	5.23 (1.98, 13.79)	<0.001
No	9 (18.4)	40 (81.6)	1.00	
Have you treated your dog (s) with antiworming medicines?				
No	28 (33.7)	55 (66.3)	1.02 (0.09, 11.72)	0.98
Yes	1 (33.3)	2 (66.7)	1.00	
Do you ever keep your dog(s) tied up outside?				
Yes	13 (65.0)	7 (35.0)	5.8 (01.98, 17.05)	<0.001
No	16 (24.2)	50 (75.8)	1.0	
What do you usually do with the stools/droppings of your dog(s)?				
Leave where they are.	27 (33.0)	55 (67.0)	1.0	0.48
Dispose of them in water canal, nearby stream, a nearby agriculture field or bury them.	2 (50.0)	2 (50.0)	2.04 (0.27, 15.25)	

Table 6.3 Multivariable logistic regression model of factors associated with the respondent's knowledge on cystic echinococcosis.

<b>Variable</b>	<b><math>\beta^*</math></b>	<b>S.E.#</b>	<b>P-value</b>	<b>Odds ratios (95%CI)</b>
Constant	0.802	0.55	0.15	-
Dog restrained/confined tied up	1.949	0.623	0.002	7.0 (2.07, 23.78)
Dog free roaming	-	-	-	1.0
Dog is fed offal	-1.751	0.572	0.002	0.17 (0.05, 0.53)
Dog not fed offal	-	-	-	1.0
Attained a secondary level of education or higher	1.677	0.599	0.005	5.35 (1.65, 17.31)
Had either only attended primary school or had never attended school	-	-	-	1.0

\* **correlation coefficient**  
# **S.E Standard Error.**

## 6.7 Discussion

In this study, the overall prevalence of eggs of taeniid tapeworms in the sampled dogs was 10.1%. This was lower than the results of other canine faecal surveys conducted in Baghdad and Duhok province of Iraq, where eggs of *Taenia* and *Echinococcus* spp. were detected in 29.1 and 13.7%, respectively of stray dogs sampled (Hasson, 2014; Hadi and Faraj, 2015; Muhamed and Al-barwary, 2016). A high prevalence of *Taenia* spp. (87%) was also observed in stray dogs from Kalar city of Sulaimani province (north governorate of Iraq) (Bajalan, 2010). Studies conducted in other countries where taeniid tapeworms are endemic have also reported high prevalences: for example 34.4% in farm dogs from Qinghai Province of China (Guo *et al.*, 2014); and 73.3% in domestic dogs from Ngorongoro (northern Tanzania) (Swai *et al.*, 2016).

Taeniid eggs are spherical in shape, of between 25 to 45  $\mu\text{m}$  in diameter, covered with a thick shell, brown in colour, and radially striated. Inside each egg is an oncosphere (or hexacanth embryo), containing three pairs of hooks (OIE, 2014). However microscopic examination of canine stool samples has low reliability for identification of *Echinococcus* infection due to the similar morphology of all taeniid eggs (Deplazes, Dinkel and Mathis, 2003; Barnes *et al.*, 2012). Modern laboratory methods have been developed to differentiate the species of taeniids in dogs including antigen and DNA-based techniques. Copro-antigen ELISA and copro-DNA PCR are considered gold standard tests to detect *E. granulosus* and to discriminate them from other *Taenia* species carried by dogs (Allan and Craig, 2006; Huang *et al.*, 2007; Villeneuve *et al.*, 2015; Chaâbane-Banaoues *et al.*, 2015; Buishi, Fares and Hosni, 2015). It is recommended that future studies should use a PCR or ELISA to diagnose infections with *Echinococcus* to more accurately determine their real prevalence.



Management and husbandry practices of dog owners and their knowledge of CE were also assessed in the current study. Not surprisingly feeding uncooked offal and not restraining dogs were identified as risk factors for parasitism, as has been reported by others (Craig *et al.*, 2015). The logistic regression analysis for owner knowledge highlighted improved knowledge in dog owners who restrained or confined their dog(s) (OR= 7.0, 95% CI 2.1, 23.8). In contrast, those who fed offal were more likely to have a lower level of knowledge (OR=0.17, 95% CI 0.05, 0.53). Similar findings have been reported elsewhere in Tanzania, Ethiopia and Uganda (Ernest *et al.*, 2009; Nyakarahuka, 2011; Omandang *et al.*, 2018). Ziadinov *et al.* (2008) also observed that dogs which were restrained had a lower prevalence of *Echinococcus* (11%) compared with FRD (26%), most likely due to the latter population's potential for access to the larval stages presented in intermediate hosts.

In the present study, the respondents who had a secondary school or higher level of education (OR=5.4, 95% CI 1.7, 17.3) had a better knowledge of CE than those who attended only primary school or who had never received any formal education. A similar recent study in Uganda demonstrated that a low knowledge level on CE was present in agro-pastoral and poorly educated people (Omandang *et al.*, 2018). Similarly, a study conducted in Qinghai Province, China demonstrated that humans with alveolar echinococcosis were more likely to have a lower educational level compared to non-infected controls (Schantz *et al.*, 2003). Such findings highlight the need for developing and implementing educational campaigns suitable for dog-owners in Basrah (Abdulhameed *et al.*, 2018b). A key component of an educational campaign should be improving the knowledge of farmers and dog owners about CE and the parasite's life cycle. Confining/restraining, limiting access to offal and regular six-weekly administration of a suitable anthelmintic incorporating praziquantel are essential components for control of the parasite in

dogs (Torgerson and Heath, 2003; Craig *et al.*, 2017). Control of the parasite in dogs will significantly reduce the risk of disease transmission to humans (Moro and Schantz, 2006). In La Rioja, Spain, a control programme, which included elimination of FRD, suitable disposal of offal from slaughtered sheep, disposal of dead sheep in pits, and treatment of domestic dogs with praziquantel at 45-day intervals, resulted in the prevalence of *E. granulosus* declining in dogs from 7.0% in 1987 to just 0.2% in 2000 (Jiménez *et al.*, 2002).

In the current study, the majority of dog owners (95.3%) reported that they left the faeces of their dogs where they were deposited. Failure to remove and bury or dispose of dog faeces increases the risk of infection in humans and other livestock (Reyes *et al.*, 2012). A similar finding was observed by (Wumbiya *et al.*, 2017) who reported that 95% of 353 pastoralists from South Sudan never disposed of their dog's faeces. Again these findings highlight the need for a multi-pronged approach to the diseases control with development and implementation of an educational campaign a key requirement for a successful outcome.

## **6.8 Conclusions**

A relatively high (10.1%) prevalence of taeniid eggs was found in this study in the faeces of owned and FRD in Basrah. A low level of knowledge and awareness of CE by dog owners was found with few owners deworming their dogs, restraining them, or appropriately disposing of their dog's faeces. There is a need to improve awareness of dog owners about CE in Basrah through developing an educational programme which should include information on control of the FRD population, regular use of anthelmintics and restricting access of dogs to offal and carcasses.

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## **Chapter Seven - Cystic Echinococcosis in Edible Offal of Sheep in Basrah, Iraq: Abattoir-based Survey and a Probabilistic Model Estimation of the Direct Economic Losses Due to Hydatid Cyst**

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### **7.1 Preface**

Cystic echinococcosis results in a significant economic loss for livestock producers through reduced production and condemnation of organs at abattoirs. Understanding these losses and the epidemiological features of CE are important when evaluating control programmes. In this chapter the results of a survey to determine the prevalence of hydatid cysts in slaughtered sheep in Basrah are reported and the economic burden of the disease is estimated. A questionnaire was also administered to veterinarians to obtain historical data on the presence of hydatid cysts from 2008 to 2015 in licensed slaughterhouses in Basrah Province. The contents of this chapter have been published in the *Journal of Parasite Epidemiology and Control* 2018, 3: 43-51. The text of this chapter is the same as that published except for minor modifications, such as referencing and formatting.

### **7.2 Statement of contribution**

**MFA** conducted all the field work in Basrah province. **SA** helped obtain permission to access samples and historical data at the abattoir. **MFA & IH** undertook the

statistical analysis and reviewed and edited the manuscript. **IR** supervised the complete project, was responsible for the animal and human ethics approval and finalised the reviewing and editing of the manuscript. All authors critically reviewed and approved the final version of the manuscript.

**MFA: 60%**

### **7.3 Abstract**

Cystic echinococcosis (CE) is a highly endemic parasitic zoonosis in Iraq with substantial impacts on livestock productivity and human health. The objectives of this study were to study the abattoir-based occurrence of CE in edible offal of sheep in Basrah province, Iraq, and to estimate, using a probabilistic modeling approach, the direct economic losses due to hydatid cysts. Based on detailed visual meat inspection, results from an active abattoir survey in this study revealed detection of hydatid cysts in 7.3% (95% CI: 5.4; 9.6) of 631 examined sheep carcasses. Occurrence of hydatid cysts was significantly higher ( $P < 0.001$ ) in carcasses from female and older aged (>1 years) animals. Post-mortem lesions of hydatid cyst were concurrently present in both livers and lungs of more than half (54.3% (25/46)) of the positive sheep. Direct economic losses due to hydatid cysts in edible offal were estimated using data from government reports, active abattoir survey completed in this study, and expert opinions of local veterinarians and butchers. A Monte-Carlo simulation model was developed in a spreadsheet utilizing Latin Hypercube sampling to account for uncertainty in the input parameters. The model estimated that the average annual economic losses associated with hydatid cysts in the liver and lungs of sheep marketed for human consumption in Basrah to be US\$72,470.93 (Std Dev: 48,593.85) = (Iraqi Dinar 84,948,104 (Std Dev: 56,960,159)). These estimates suggest that CE is responsible for considerable livestock-associated

monetary losses in the south of Iraq. These findings can be used to inform cost-benefit analyses of different regional CE control program options in Iraq.

#### **7.4 Introduction**

Livestock diseases can impact animal production and adversely affect the security of the human food supply chain. Added to that, many livestock diseases are zoonotic in nature and can infect humans via several routes. Parasitic zoonoses continue to pose serious concerns at the human-animal interface in many developing countries (McManus *et al.*, 2003). Cystic echinococcosis (CE) is an important parasitic infection impacting both animal and public health, notably throughout the Middle East and North Africa. The disease is caused by the genus *Echinococcus*; notably by *Echinococcus granulosus*. Livestock (e.g. sheep, cattle, goats, and pigs) can acquire the infection indirectly from grass and water contaminated by the eggs of *E. granulosus*, which are excreted with faeces of dogs (Deplazes *et al.*, 2004; Craig *et al.*, 2007). In humans, the disease is most commonly found in rural communities and among people involved in raising sheep, as a result of the sheep's role as an intermediate host of the parasite and the presence of working dogs that are allowed to eat the offal of infected sheep. Ingested eggs can develop into the parasite's larval stage (metacestoda) in the livestock's internal organs (Brunetti, Kern and Vuitton, 2010). The hydatid cysts grow slowly, with minimal clinical signs observed in livestock animals, and typically are only discovered during routine meat inspection. In the majority of animals harbouring hydatidosis, the cysts are most commonly located in the liver and/or lungs (Craig *et al.*, 2007).

The economic cost of CE in livestock can be divided into direct costs (mainly the loss of revenue through the condemnation of offal) and indirect costs (reduction in growth, fecundity and milk production of infected animals). At the abattoir, detecting hydatid cysts during routine meat inspection will usually lead to

condemnation of the infested offal (mainly livers and lungs). Fertile cysts (with viable protoscoleces) in livestock offal are very important for continuation of the sheep-dog transmission cycle (Torgerson, 2003). A study in Jordan, estimated the annual loss of edible livers due to hydatidosis at around US\$850,000 annually (Torgerson and Dowling, 2001).

In Iraq, the small ruminant sector is very important for sustaining the country's food security. There are presently an estimated 7-8 million sheep in Iraq contributing a valuable source of meat, and providing income and job security to people working across the agricultural sector (Jarjees and Al-Bakeri, 2012; Thweni and Yassen, 2015). The fragile veterinary services in Iraq, after years of international economic sanctions and on-going political and ethnic conflicts, is very challenging for setting organized efforts to combat endemic livestock diseases. Various studies have reported the prevalence of hydatidosis in the livestock in different regions of Iraq (Saida and Nouraddin, 2011; Jarjees and Al-Bakeri, 2012; Maktoof and Abu Tabeekh, 2015; Thweni and Yassen, 2015). However, none of these studies considered a detailed estimation of the direct or indirect economic losses incurred by such endemic livestock disease. Estimation of such financial losses is largely handicapped by poor record keeping and reporting systems at abattoirs in Iraq, thus limiting the value of any economic evaluation using retrospective abattoir data. Added to that, in Iraq there are widespread informal traditional butcheries with their on-site slaughter slabs, where freshly slaughtered meat and offal are sold directly to the public without any kind of meat inspection. Given the fact that traditional informal meat markets are widespread in Iraq, they should be considered alongside with the formal meat market (where animals are slaughtered and inspected at an official abattoir) in order to enumerate the realistic economic impact of CE in sheep.

The sheep industry is an important segment of the agriculture sector in Basrah, the second biggest province in Iraq after the capital Baghdad (Maktoof and Abu Tabeekh, 2015). In this study, we conducted an active abattoir survey at the central abattoir of Basrah, located in the south of Iraq, to determine the abattoir-based occurrence of hydatid cysts in sheep slaughtered for human consumption. In parallel to this active abattoir survey, we developed a probabilistic model to estimate the direct financial loss from offal condemnation due to hydatid cysts in slaughtered sheep. We interviewed local veterinarians and butchers to gather expert-opinions for populating some of the model variables. This model-based approach could be extended to other regions in Iraq and might be applicable to similar developing country settings where the infrastructure of abattoir data collection system is lacking.

## **7.5 Materials and methods**

### **7.5.1 Study setting**

This study was conducted in Basrah province located in the south of Iraq at latitude 30° 34' N and longitude: 47 °46' E. Basrah is in a fertile agricultural region and contributes to the Iraqi agriculture sector through its large agricultural and livestock industries (Maktoof and Abu Tabeekh, 2015). The study was carried out over a period of six months (between May and October 2016), at the central governmental abattoir in Basrah province.

### **7.5.2 Post-mortem abattoir inspection and cyst viability examination**

A cross-sectional survey was conducted at the abattoir in order to determine the prevalence, organ distribution, viability and economic implications of hydatid cysts in the slaughtered sheep. Both the meat inspection and laboratory work were carried out personally by the first author of this study. Regular visits were made to the central governmental abattoir in Basrah, and in total 631 sheep were examined



by detailed post-mortem inspection for the presence of hydatid cysts. All animals were from native flocks, and none were imported. The post-mortem examination procedure employed visual examination, palpation and systematic incision of each carcass and visceral organs, particularly the lung, liver, spleen, kidney and heart (Herenda *et al.*, 1994). The numbers of hydatid cysts per animal and per organ were recorded, in addition to information on the age and sex of each examined animal. The age of each animal was estimated based on the dentition formula as used by (Kumsa, 1994).

On detection cysts were carefully incised and examined for the presence of protoscoleces, and cysts with protoscoleces were characterized as fertile cysts. Fertile cysts were subjected to a viability test where fluid of the cysts was poured into a petri dish and 3-4 drops of 0.1% aqueous eosin solution were added to the sediment and left for one minute. A drop of the stained sediment was then placed onto a microscopic slide, covered with a cover slip and examined for amoeboid like peristaltic movements under a microscope at  $\times 40$  magnification (Smyth and Barrett, 1980).

### **7.5.3 Analysis of abattoir survey**

Abattoir survey data were recorded into a MS Excel sheet, and all descriptive analyses were made using the software STATA (version 11, SE for Windows). The abattoir-based occurrence (frequency) of hydatid cysts was calculated as the number of affected animals (with post-mortem lesion) divided by the total number of inspected animals. Multivariable logistic regression analysis was used to determine the association of the occurrence of hydatid cysts in the offal with the sex and age of the slaughtered sheep. The effect of confounding interaction between animals' age and sex was investigated by observing the change in the estimated odds ratios of the variables that remain in the model once a non-

significant variable is removed. When the removal of a non-significant variable led to a change of >25% of any parameter estimate, that variable was considered a confounder and was not removed from the model.

#### **7.5.4 Model-based estimation of direct economic losses due to offal condemnation**

During the abattoir visits we noticed that hydatidosis were recorded in the abattoir daily inspection reports only if there was a severe case of profuse (multiple cysts) organ infestation that lead to “total organ condemnation”. Localized cysts were typically trimmed off the affected offal and then the organs and carcasses were collected by the attending butcher. After removal of these localized cysts the remaining organ entered the domestic market; however, no record of these events was included in the daily abattoir inspection reports. Given such limitation in the recording system, we built a probabilistic model based on the results from the active abattoir survey (as described above), combined with expert-opinions of local veterinarians and butchers working in Basrah.

#### **7.5.5 Model objective and scope**

The objective of the model was to estimate the direct economic loss from the condemnation of livers and lungs of the slaughtered sheep. The model scope was to cover both formal and informal meat markets as a sizable proportion of animals are slaughtered without official abattoir inspection (e.g. by traditional butcheries). The model focused on losses arising from partial or total condemnation of livers and lungs as these organs are economically more valuable than other visceral organs, and also because of their high involvement in hydatid infestation as was revealed from the active abattoir survey. The model notation, variable description and parameterization are described in Table 7.1.

### **7.5.6 Model implementation and software**

Uncertainty and variability of the economic variables were incorporated in the model using probability distributions of parameters as described in Table 7.1. The model was run for 50,000 iterations using a Monte Carlo simulation approach (Latin Hypercube sampling) in the commercial software @Risk version 7.5, Palisade Corporation, running as add-in to Microsoft Excel©. Sensitivity analysis was undertaken, using a stepwise linear regression of the estimated costs against the input parameter values, to assess the impact of each input parameter on the overall cost estimate.

### **7.5.7 Model imputes and data sourcing**

- a) *Retrospective abattoir data- number of sheep slaughtered at Basrah abattoir:*

Official records were only available for eight years between 2008 and 2015. The mean and standard deviation of these data were used to set parameters for a Normal Distribution describing variability in the annual number of sheep slaughtered at Basrah abattoir (Vose, 2008);

- b) *Active abattoir survey data - completed during this study:*

Results from inspection of slaughtered sheep ( $n= 631$ ) were used to provide input parameters for a Beta Distribution describing uncertainty in the prevalence of hydatid cyst in the lung and livers of slaughtered sheep (Vose, 2008).

- c) *Expert opinions of the local meat hygiene veterinarians:* We interviewed all ( $n= 6$ ) meat hygiene veterinarians working in meat control at the abattoir and markets of Basrah. The veterinarians provided their expert opinion on the percentage of sheep slaughtered for human consumption at Basrah abattoirs, as compared to the proportion of sheep slaughtered at informal markets (out of the control of the official meat inspection system). Then the opinions of all the interviewed veterinarians were combined using a Discrete distribution which is a general type of function used to describe a variable that can take one of several

explicit discrete values  $\{x_i, \% \text{ of all sheep slaughtered at the abattoir}\}$  and where a probability weight  $\{p_i\}$  is assigned to each value of the expert opinion and was based on the number of years of experience that each veterinarian spent working in local meat hygiene. It was not necessary to normalize the probability weights  $\{p_i\}$ , as @RISK automatically rescaled them to ensure they summed to one (Vose, 2008).

- d) *Expert opinions of local butchers:* Butchers ( $n= 23$ ) attending the Basrah abattoir were interviewed for their input of the market price (namely minimum, most likely, and maximum) of liver and lungs (price per organ, as it is sold as a whole organ rather than sold per kilogram). The butchers were also requested to provide their opinion regarding their valuation of the reduction percentage (minimum, most likely, maximum) in market revenue due to partial trimming of hydatid cysts from affected livers and lungs. A PERT distribution was used to describe the model parameters summarizing the price variables based on the combined butchers' expert opinion (Vose, 2008).

Table 7.1 A probabilistic model used to estimate the direct economic losses due to hydatid cysts in livers and lungs of sheep marketed for human consumption at Basrah, south of Iraq.

<b>Notation</b>	<b>Variable description</b>	<b>Distribution parameterization and model formula</b>	<b>Source of information</b>
$P_{ss\_in}$	Probability of having sheep slaughtered at abattoir (with official meat inspection)	RiskDiscrete ( $\{x_i\}, \{p_i\}$ ) = RiskDiscrete ( $\{10\%, 24\%, 3\%, 25\%, 2\%, 10\%\}, \{10, 5, 30, 10, 5, 25\}\}$ )*	Expert-opinion (veterinarians ( $n=6$ ) interviews) on % of sheep slaughtered in abattoir, out of the total sheep slaughtered for human consumption in Basrah
$N_{ss\_in}$	Number of sheep slaughtered per annum at abattoir	Normal (Mean $N_{ss\_in}$ , St.Dev $N_{ss\_in}$ ) =RiskNorm (9011, 2831)	Retrospective data from abattoir records for numbers of sheep slaughtered from 2008 to 2015
$P_{ss\_out}$	Probability of having sheep slaughtered elsewhere than the abattoir (without official meat inspection)	Probability rule of subtraction; = $1 - (P_{ss\_in})$	Model equations
$N_{ss\_out}$	Number of sheep slaughtered per annum elsewhere from abattoir	= $(N_{ss\_in} \times P_{ss\_out}) / P_{ss\_in}$	Model equations

<b>Notation</b>	<b>Variable description</b>	<b>Distribution parameterization and model formula</b>	<b>Source of information</b>
$P_{H\_Lung}$	Probability of having hydatid cyst in lung of a slaughtered sheep	Beta ( $\alpha_1, \alpha_1$ ); where $\alpha_1 = N$ of sheep with post-mortem hydatid cyst in lung + 1 $\alpha_2 = N$ of sheep with post-mortem hydatid cyst in lung – total inspected sheep + 1 = RiskBeta (30, 603)	Active abattoir survey data (generated in this study)
$C_{Lung}$	Retail price of a sheep lung sold at local market	Pert (Minimum, Most likely, Maximum) = RiskPert (10.000, 15.000, 17.000)	Expert-opinion (in Iraqi Dinars): Butchers ( $n= 23$ ) interview
$D_{H\_lung}$	Probable percentage of decline in the retail price of a trimmed lung due to hydatid cyst	Pert (Minimum, Most likely, Maximum) = RiskPert (5.000, 8.000, 10.000)	Expert-opinion (in Iraqi Dinars): Butchers ( $n= 23$ ) interview
$CD_{H\_lung}$	Estimated retail price of a trimmed (downgraded) whole sheep lung due to hydatid cyst	$C_{Lung} - D_{H\_lung}$	Probability distribution of the difference between two distributions

<b>Notation</b>	<b>Variable description</b>	<b>Distribution parameterization and model formula</b>	<b>Source of information</b>
$P_{H\_Liver}$	Probability of having hydatid cysts in the liver of a slaughtered sheep	Beta ( $\alpha_1, \alpha_1$ ); where $\alpha_1 = N$ of sheep with post-mortem hydatid cyst in liver + 1 $\alpha_2 = N$ of sheep with post-mortem hydatid cyst in liver – total inspected sheep + 1 = RiskBeta (42, 591)	Active abattoir survey data (generated in this study)
$C_{Liver}$	Retail price of a sheep liver sold at local market	Pert (Minimum, Most likely, Maximum) = RiskPert (10.000, 15.000, 17.000)	Expert-opinion (in Iraqi Dinars): Butchers ( $n= 23$ ) interview
$D_{H\_liver}$	Probable percentage of decline in the retail price of a trimmed liver due to hydatid cysts	Pert (Minimum, Most likely, Maximum) = RiskPert (5.000, 8.000, 10.000)	Expert-opinion (in Iraqi Dinars): Butchers ( $n= 23$ ) interview
$CD_{H\_liver}$	Estimated retail price of a trimmed (downgraded) whole sheep liver due to hydatid cysts	$C_{Liver} - D_{H\_liver}$	Probability distribution of the difference between two distributions
$Total_{LLL}$	Total estimated annual cost due to hydatid cysts in the lungs and liver of a slaughtered sheep	$= (N_{ss\_in} \times P_{H\_Lung} \times CD_{H\_lung})$ $+ (N_{ss\_out} \times P_{H\_Lung} \times CD_{H\_lung})$ $+ (N_{ss\_in} \times P_{H\_Liver} \times CD_{H\_liver})$ $+ (N_{ss\_out} \times P_{H\_Liver} \times CD_{H\_liver})$	Product of model distributions: output estimated after 50,000 iterations using a Monte Carlo simulation

### **7.5.8 Model assumptions**

The following underlying assumptions were considered while conceptualizing this model-based estimation.

- a) The prevalence of hydatid cysts in the lungs and liver of sheep slaughtered at informal markets was similar to that observed in the sheep slaughtered at Basrah abattoir;
- b) Local butchers at the informal markets will trim off any visible hydatid cyst from the liver or lungs before selling it to the consumer.

## **7.6 Results**

Results of meat inspection in the abattoir survey indicated that 7.3% (95% CI: 5.4; 9.6) of the examined 631 sheep carcasses had one or more hydatid cysts. The occurrence of hydatid cysts was higher in the carcasses of female animals (Table 7.2). However, the two-way interaction between animals' age and sex was statistically significant ( $P=0.004$ ), indicating a confounding correlation; as females are sent to slaughter older than male sheep.

As indicated in Table 7.3, the majority of the cysts were located in the livers and lungs, with only one cyst detected outside these organs in the spleen. Hydatid cysts were concurrently present in the livers and lungs of more than half (54.3% (25/46)) of the positive sheep. In total, 37.1% (98/264) of the liver and lung cysts examined were viable.

The probability distribution ( $P_{ss\_in}$ ) describing the aggregated expert opinions of veterinarians ( $n=6$ ) working in meat hygiene and inspection indicated that on average only 11% (Median: 10%, Standard Deviation: 7.3%) of the total sheep in Basrah are slaughtered at the certified abattoir (with official meat inspection). Hence, the majority of sheep meat and offal in Basrah come from carcasses of animals slaughtered informally out of the abattoir. The average



simulated number of sheep slaughtered elsewhere from abattoir (Nss\_out) in Basrah was estimated at 103,026 per annum (Median: 90,036; Std Dev: 65,241.45).

The monetary value of the results was calculated at an exchange rate of 100,000 Iraqi Dinars=US\$85.30 (September – 2017). Based on the aggregated opinions of the local butchers (n=23), the simulated retail market price of the liver and lungs was estimated at an average of US\$12.70 (Std Dev: 0.32) (=14,881 Iraqi Dinars; Std Dev: 371) per sheep. The model estimated that the average retail price of a trimmed whole sheep liver or lung due to the presence of hydatid cysts US\$4.50 (Std Dev: 0.36) (=5,286 Iraqi Dinars; Std Dev: 424). The model estimated that the annual economic losses (considering both formal and informal markets) associated with hydatid cysts in the livers and lungs of sheep (Total LLL) lost/condemned in Basrah to be an average of US\$72,470 (90% Confidence Interval (CI);  $\pm 11,302$ ) (=84,948,104 Iraqi Dinars; 90% CI;  $\pm 132,499$ ) (Figure 7.1.A). The mean proportion of this monetary value of losses due to hydatid cysts in the livers and lungs was estimated as 0.42% (90% CI;  $\pm 0.002$ ) of the annual sheep product value (PV AL) in Basrah province (Figure 7.1.B). The normalized regression coefficient values in Fig. 7.2 illustrate the impact of uncertain parameters on the overall costs; under the present model conditions, the input variable on the probability of having sheep slaughtered at the official abattoir became most important in determining the overall costs. The regression estimates for this variable (having sheep slaughtered at the official abattoir) resulted in a negative coefficient value ( $-0.61$ ), indicating that this input variable.

Table 7.2 Occurrence of hydatid cysts in relation to sex and age of sheep slaughtered at Basrah abattoir (n= 631, between May and October 2016).

Slaughtered sheep characteristics	Number examined	Number positive	Occurrence (%)	Odd Ratio (95% CI)	P. value
Sex				1	
Male	418	13	3.1	5.71 (2.94;	< 0.001
Female	213	33	15.5	11.11)	
Age				1	
Young (< 1 yr)	294	9	3.1	3.91 (1.85;	<0.001
Adult (> 1 yr)	337	37	11.0	8.24)	

Table 7.3 Organ level distribution and viability of hydatid cysts in sheep slaughtered at Basrah abattoir (n= 631, between May and October 2016).

Organs	Number (%) of positive organs	Total cysts recovered	Number (%) of viable cysts
Liver	16 (34.8)	37	7 (18.9)
Lung	4 (8.7)	8	0
Liver & lung	25 (54.3%)	218	91 (41.7)
Spleen	1 (2.2)	1	0
Kidney	0	0	0
Heart	0	0	0
Total	46 (7.2)	264	98 (37.1)

Figure 7.1 Monte Carlo simulation output representing the distribution of the estimated annual direct economic losses due to hydatid cysts in livers and lungs of sheep marketed for human consumption at Basrah, south of Iraq.

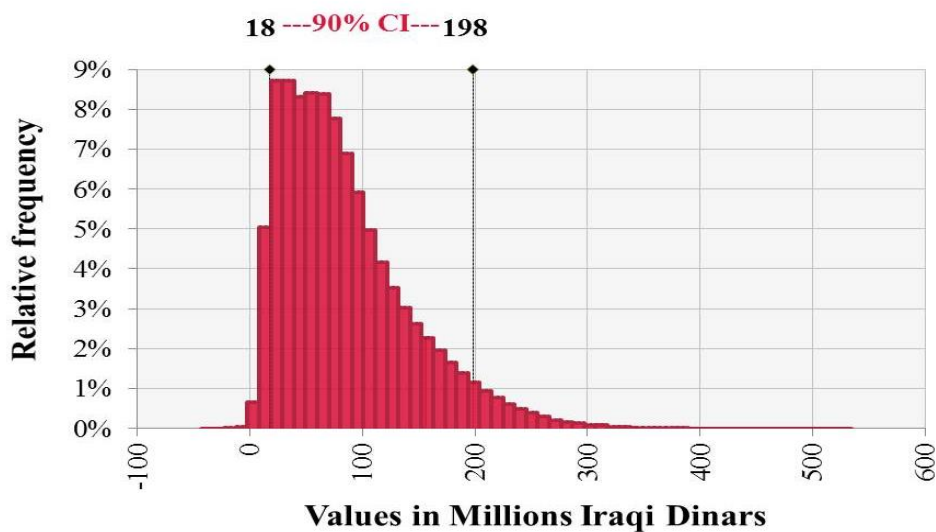
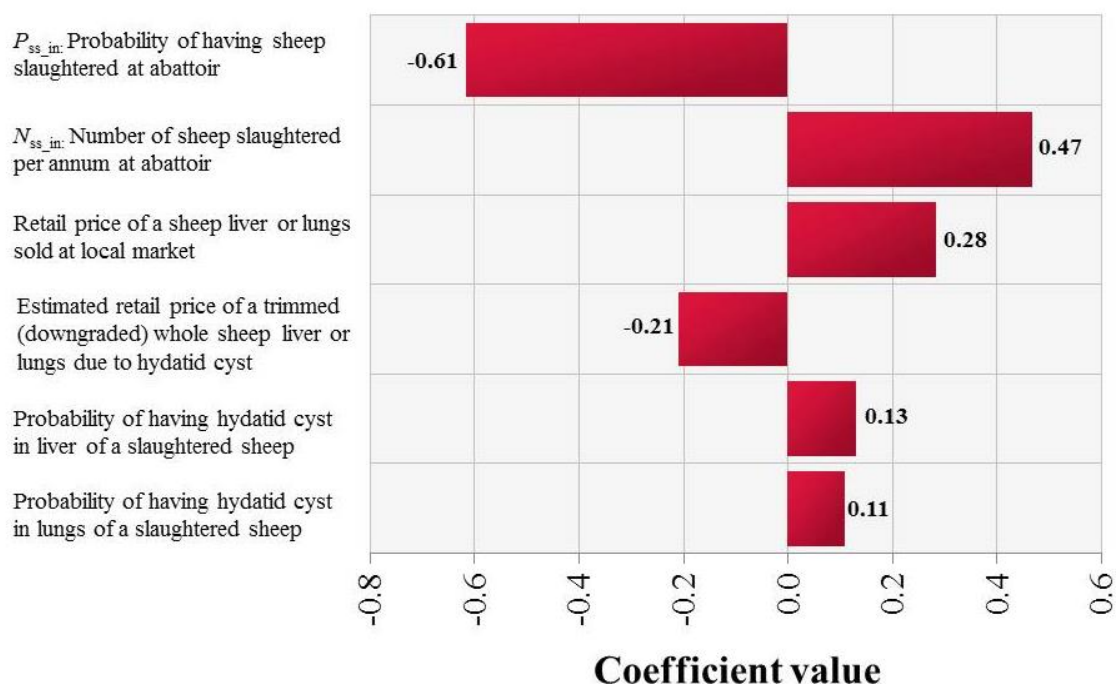


Figure 7.2 Estimated normalized regression coefficients showing the association between uncertain parameters and total estimated annual direct economic losses due to hydatid cysts in livers and lungs of sheep marketed for human consumption at Basrah, south of Iraq.



### 7.7 Discussion

Studies at the abattoir level provide valuable data on prevalence and pathology profiles of diseases encountered during meat inspection which could be utilized in the assessment disease burden or for future planning of livestock disease control strategies (Ahmadi and Meshkehkar, 2011). Cystic echinococcosis is an endemic disease in Iraq and still poses significant health consequences in humans (Sawady and Al-Faddagh, 2012). A total of 748 cases of human with CE were diagnosed and operated in Basrah hospitals from January 2005 to December 2015, equivalent to an annual clinical incidence of approximately 4.5 cases per 100 000 people (Abdulhameed *et al.*, 2018). In this study, our active abattoir survey focused on inspection of sheep carcasses slaughtered in Basrah, the second biggest province in Iraq. The study focused on sheep, given the fact that consumption of lamb is traditionally important in Iraq, where it is widely preferred to beef. The results of the

present study indicate that hydatid disease is relatively prevalent (7.3%) in sheep in Basrah. This result is comparable to a recent finding from an active abattoir survey (12 months) done in Erbil, in the north of Iraq, where a CE prevalence of 9.1% was reported in 2556 slaughtered sheep (Hassan *et al.*, 2016). Regionally our prevalence was also comparable to that (8.7%) reported by Al-Khalidi (1998) in Libya, however it was lower than that reported by Yousefi, Asna Ashri and Montazeri (2007) in Iran (14.7%), while variable rates have been reported in different regions of Morocco (7.4% in Rif and 11.1% in Middle Atlas) (Azlaf and Dakkak, 2006). Our results signify the importance of sheep as an intermediate host of *E. granulosus* and the potential important role they play in the transmission of the parasite in the southern region of Iraq.

The present abattoir-based survey in Basrah revealed a significantly higher occurrence of hydatid cysts in carcasses of females and older aged (>1 years) sheep. The association between CE and animal sex has been variable, with some studies finding no difference and others finding that females were more likely to be infected than males (Daryani *et al.*, 2007; Ibrahim, 2010). The most consistent risk factor for intermediate host infection and hydatid cyst burden is animal age, with older animals being more likely to be infected and generally harbouring more protoscolices than younger animals (Kebede *et al.*, 2009; Marshet *et al.*, 2011). This is expected as cyst persistence is generally life-long and as such cyst burden represents an on-going infection pressure over time.

Based on our survey data, it was obvious that the most frequently condemned organs in sheep slaughtered in Basrah were the livers and lungs. In line with our findings, previous research confirmed that in the Middle East the most common location of hydatid cysts in sheep was the liver, followed by the lungs (Al-Yaman *et al.*, 1985; Abo-Shehada, 1993; Kamhawi *et al.*, 1995; Saeed *et al.*, 2000),

but for cattle and camels it was the reverse (Al-Yaman et al., 1985; Kamhawi et al., 1995; Ahmadi, 2005). Interestingly, 37.1% of the cysts in the livers and lungs were fertile, viable cysts. The proportion of animals with fertile, viable cysts is an important indicator of the significance of a species as an intermediate host (Gebremeskel and Kalayou, 2009). In our study, the majority of infected sheep (54.3%) had hydatid cysts in both the liver and the lungs, similar to that which has also been reported elsewhere (Yildiz and Gurcan, 2003; Kebede et al., 2009) This is explained by the fact that lungs and liver possess the first great capillary sites encountered by the migrating *Echinococcus* oncosphere which adopts the portal vein route and primarily negotiates the hepatic and pulmonary filtering systems sequentially before any other peripheral organ is involved (Kebede et al., 2009).

Another goal of our study was to estimate the direct economic losses due to hydatid cysts in livers and lungs of sheep marketed for human consumption in Basrah, Iraq. This is the first study to produce a comprehensive estimate of the annual direct economic burden of CE in a livestock sector in the Iraqi setting. In this study, the Monte-Carlo simulation technique has been applied in the model using a Latin Hypercube sampler method to represent the uncertainty surrounding the input parameters and is particularly suitable for estimating economic costs when accurate epidemiological data are scarce (Majorowski *et al.*, 2001; Torgerson, 2003). We attempted to develop a simple model that is tailored to the local situation in Basrah, which we believe is very similar to the situation in several regional and developing countries where informal (outside formal abattoirs) markets are common, and where also records keeping is not yet well established in a systematic way in the official abattoirs. Added to that, and in order to make the estimates more robust, we used local expert opinions based on interviews with veterinarians and butchers. Expert opinion can be and has been used to address the critical lack of data existing for

prevalence and incidence of many global animal diseases and is a cost effective method of obtaining an estimate in resource poor countries (Garabed *et al.*, 2009; Brookes *et al.*, 2017).

The probabilistic model used in this study estimated an average annual direct economic loss of US\$72,470.93 (Std Dev: US\$48,593.85) (considering both formal and informal markets) due to the visible hydatid cysts in livers and lungs of sheep marketed for human consumption in Basrah. This estimated figure for only direct losses in sheep represents the potential impact that this disease has on the agricultural industry in Iraq. Given the scarcity of data availability in the study setting, this model estimation was limited to only estimating direct economic loss. Hence, our estimation should be regarded as a partial overview of the estimate of the economic burden. The importance of the contribution of direct versus indirect losses to the total economic burden varies according to livestock species, animal population sizes, and disease prevalence (Torgerson, 2003). Bingham *et al.* (2016) estimated that the annual direct costs associated with CE in sheep in Rio Negro Province, Argentina in 2010, using officially reported slaughter values, was an average of US\$15,000. That study also indicated that indirect costs (production losses) represented 96.4% of the total annual livestock-associated losses, when government-reported slaughter statistics were used. Added to that, a study analysing the economic impact of CE in livestock in Spain concluded that indirect losses account for almost 99% of the total cost association with CE, whereas the direct losses were negligible (Benner *et al.*, 2010). However, this could vary in lower to middle income developing country in the Middle East. For instance, an economic evaluation in Ahwaz, south-western Iran combining both direct (liver and lung abattoir condemnation) and indirect losses (meat, milk, fleece, and infertility) estimated an economic loss of US\$123,490.0 based on the market prices in 2008

(Ahmadi and Meshkehkar, 2011). In Jordan, where echinococcosis is highly endemic, Torgerson and Budke (2003) estimated that the loss of edible liver was over US\$850,000 whilst total losses to the livestock industry in the whole country were over \$3.5 million annually. Direct comparison between different studies on economic losses is difficult due to a lack of standardized methods for estimating costs, differences in chain logistics from farm-to fork across different settings, and also due to inherent socioeconomic patterns and periods of valuation (Benner et al., 2010).

Although the current model developed in this study was limited only to sheep slaughtered in Basrah; we believe that the proposed model-based approach could be easily extended to a wider national assessment of the annual economic burden of CE in livestock in Iraq. However, to achieve such a goal, it is important to re-enforce adequate meat inspection procedures in abattoirs, and to enable improved capacities for proper record keeping of abattoir data, with the ultimate development of a centralized regional and national digital recording of such data (Yibar, Selcuk and Senlik, 2015). Otherwise the actual prevalence and epidemiologic characterization of the diseases encountered during meat inspection of slaughtered animals may be underestimated.

The economic model could be improved by combining both direct and indirect losses. Although a number of studies have suggested that CE is responsible for livestock production losses, these losses are difficult to accurately estimate due to the lack of available experimental data (Bingham et al., 2016). The sensitivity analysis (Figure 7.2) of the input-output relationships between model variables highlights the total model uncertainty could be reduced by gathering more data on the baseline estimate of the percentage of sheep slaughtered at abattoir (with official

meat inspection) out of the total sheep slaughtered for human consumption in Basrah.

The results of this study demonstrate that CE is relatively prevalent and represents an important economic problem in Basrah province, Iraq. In addition, because this is the first study to examine the direct monetary burden of CE in Basrah, the second biggest province in Iraq after the capital Baghdad; it adds valuable information to the existing body of knowledge about CE in this region. CE is responsible for considerable losses of edible offal resulting in monetary losses to the livestock sectors in the south of Iraq. Therefore, it is important to continue efforts to combat CE in this region of Iraq. Stakeholders and policymakers can use these data to better allocate resources for combatting CE in this region.

## **7.8 Acknowledgements**

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## **Chapter Eight - General Discussion**

The studies presented in this thesis were designed to investigate the epidemiological and public health features of CE in Basrah. Prior to this research, little information was available on CE in the province, and no ongoing control programme had been developed and implemented. The thesis reports the results of five studies into CE disease which involved: performing a study examining historical hospital records of patients with CE for the period 2005 to 2015; administering a questionnaire to 50 patients with CE to determine their knowledge, attitudes and practices with respect to the disease; administering questionnaires to livestock and dog owners to also determine their knowledge, attitudes and practices with respect to CE; investigating the presence of hydatid cysts in slaughter sheep to assess the economic burden of the disease in these animals; and determining the prevalence of *Echinococcus* in a sample of domesticated and free-roaming dogs.

### **8.1 Significant results of CE in humans and animals from Basrah**

Dogs are the main source of infection of humans for CE; however, prevention and control against infection is extremely challenging in regions where the parasite is endemic (Craig *et al.*, 2015; Gavidia *et al.*, 2008). In Iraq CE is a widely distributed disease of livestock resulting in financial losses through its effect on livestock production. Furthermore, the disease constitutes a major public health problem, primarily because of the absence of a national control programme (Al-Shabbani, 2014; Al Saeed and Almufty, 2016). Any future control programme should focus on a range of aspects including methods to: reduce the prevalence of infection in the definitive host (dogs) through the strategic use of anthelmintics (Addis, 2017); reduce exposure of dogs to the metacestode larval stage (hydatid cysts) in intermediate hosts through inspection of carcasses at approved abattoirs and preventing dogs from accessing infected offal (Thompson, 2017); increase the

immunity of livestock through immunisation programmes (Heath *et al.*, 2012); and improve the knowledge of dog and livestock owners, along with the general public, on the risk factors for infection to mitigate against the parasite completing its life cycle (Possenti *et al.*, 2016).

In the current study (Chapter 6) the prevalence (apparent/test) of taeniid tapeworms was estimated at 10.1% (95% CI 7.1, 13.9) using direct microscopic examination of faecal samples collected from 335 domesticated and free-roaming dogs from the five districts sampled in Basrah province. Determining the prevalence in the definitive host is important when evaluating the potential risk of infection for humans and livestock and when developing a control programme for the parasite (Lustigman *et al.*, 2012; Cable *et al.*, 2017). Of particular concern in the current study was the similar test prevalence observed in free-roaming dogs (10.8%) and owned domesticated dogs (9.0%). This highlights the exposure of dogs, most likely through scavenging, to affected organs of the parasite's intermediate hosts, but also the large potential for shedding of eggs resulting in contamination of the environment and hence a source of infection for humans and livestock (Alvarez Rojas, Mathis and Deplazes, 2018). In Ngorongoro (Tanzania) approximately 40% of farmers fed domestic dogs viscera of slaughtered animals and more than 90% admitted not deworming their dogs. These are important factors resulting in the high prevalence of taeniids (73.2%) in the local domesticated dogs (Swai *et al.*, 2016). Also, in a study using a coproantigen test on faecal samples collected from domesticated dogs in urban and rural areas of north-central Chile, positivity with *E. granulosus* was associated with the home slaughter of animals (Acosta-Jamett *et al.*, 2010). Such findings support the need for education in disease control programmes to: improve management and husbandry practices adopted by both dog-owners and livestock producers (Merino *et al.*, 2017); and the concurrent use of effective

anthelmintics in dogs and management (control) of the dog population (Torgerson, 2006).

The prevalence (7.3%; 95% CI: 5.4; 9.6) of hydatid cysts in sheep slaughtered at the licensed Basrah abattoir in the current study (Chapter 7) was lower than that reported in studies undertaken in Erbil province (north Iraq) and Basrah (11.1 and 14.7%, respectively) (Saida and Nouraddin, 2011; Murtaza *et al.*, 2017). Sheep, the major intermediate host for *Echinococcus*, provide a reliable indicator of infection in dogs, and periodic surveillance of slaughtered sheep is a simple and inexpensive method of evaluating the effectiveness of control programmes for the disease (Cabrera *et al.*, 2003; Craig *et al.*, 2007; Zhang *et al.*, 2015). Total or partial condemnation of affected organs at the abattoir, in conjunction with disposal of these organs by deep burial, burning or rendering, is essential to reduce the access by dogs to the cysts (Mastin, 2005). In China strengthening meat inspection at approved slaughterhouses, in conjunction with restricting the feeding of offal to dogs and prohibiting the sale of affected offal, contributed to a marked reduction in the parasite's prevalence in sheep (Yang *et al.*, 2015).

In the current study female sheep were found to have a significantly higher prevalence of hydatid cysts (15.5%) than male sheep (3.1%) (Chapter 7). This was most likely associated with the older age of females slaughtered at the abattoirs, providing a greater opportunity for exposure to *Echinococcus* eggs on infected pasture (El Berbri *et al.*, 2015). Focusing abattoir surveillance on the older sheep processed would enhance the likelihood of detecting infection. Reducing the prevalence and incidence in sheep is a key component of disease control and implementing vaccination programmes against CE and minimising access of dogs, and importantly their faeces, to the fields/areas where sheep are grazed or housed



should be included in any disease control programmes implemented (Eckert and Deplazes, 2004). In addition, the inclusion of an education programme on the identification of cysts in organs and correct disposal of them is a key component of the multi-pronged One-Health approach to the control of CE.

When developing any disease control programme, evaluating the costs and benefits of disease control are critical for determining which control practices, if any, to implement, and to compare between alternative control options. The current (Chapter 7) and other studies (Benner *et al.*, 2010; Moro *et al.*, 2011) have highlighted the financial impact of hydatid cysts on the animal industry and the rural community through the partial or total condemnation of infected organs (liver, lung or other organs). In the current study, the direct annual economic losses associated with hydatid cysts in the livers and lungs of sheep marketed for human consumption in Basrah was estimated at US \$72,470.93, equivalent to US\$ 8.79 loss per affected sheep. However the real loss in livestock would be greater if the indirect losses arising from reduced live weight (5 – 10% reduction), fecundity, wool (10 - 40% reduction) and milk production (7 – 10% reduction) and reduced meat quality were incorporated into the economic evaluation (Polydorous, 1981; Torgerson and Dowling, 2001; Battelli, 2009; Melaku, Lukas and Bogale, 2012). The higher impact of the disease from combining both direct and indirect costs was evident in a study of slaughtered sheep at a municipal abattoir in Iran where the loss from each affected sheep was estimated at US\$ 22.50 (Ahmadi and Meshkehkar, 2011). However, these losses are those only borne by the livestock industry and losses also arise because of the impact of the disease on human health. The total economic impact of CE should be evaluated by the medical and veterinary authorities in Basrah when evaluating future disease control options.

Prevention and control of CE infection in humans is dependent upon control of the disease in both the definitive and intermediate hosts. Humans acquire CE either through direct contact with infected dogs or indirectly through ingestion of food or water contaminated with eggs of *Echinococcus* (Pakala, Molina and Wu, 2016). In the current study the annual clinical incidence detected in humans (4.5 cases per 100,000 people – Chapter 3) was lower than that reported for the north of Iraq (6.4 cases per 100,000 people) (Saida and Nouraddin, 2011), but higher than that reported from north-west Iran (1.2 - 3 cases per 100,000 population) (Rokni, 2009). Socioeconomic status and behavioural habits of a community play an important role in the transmission of echinococcosis to humans due to: the adoption of poor hygienic practices and unsanitary living conditions; close contact with animals; the existence of a large number of uncontrolled dogs; and the frequent slaughtering of animals outside licensed abattoirs with the subsequent inappropriate disposal of infected organs (Schantz *et al.*, 1995; El-Berbri *et al.*, 2015). To reduce the threat of CE to humans residing in Basrah there is a need to implement a control programme adopting public health measures. To be successful such a control programme requires support from the national and provincial Governments, non-government organisations and the general community (Craig *et al.*, 2017).

This study found a higher proportion of cases of CE in females (61.2%) than in males, similar to that reported in Tanzania (Ernest *et al.*, 2010). This may be due to their domestic activities related to food preparation and cleaning that women undertake in the surveyed region, along with their responsibility of “looking after” domesticated animals, including the feeding of owned dogs. These practices would increase the likelihood of contact with infected dogs or with contaminated environments or fomites (Macpherson, 2005). Ensuring that educational material on how to prevent infection is available to, or specifically designed for, women in

Basrah province would be essential in reducing the incidence of disease within the region.

Further studies should be conducted on CE in humans in Basrah to determine the impact of any disease control methods adopted. However, it is likely that the current study, which used hospital records, has underestimated the real incidence of CE in Basrah as sub-clinical or mild cases would not have been included. Furthermore, due to the slow growth of cysts it is likely that the incidence of disease may initially increase, even when a control programme is implemented (Schantz, 1999), as cysts enlarge resulting in infected people presenting with clinical symptoms.

## **8.2 Risk factors for cystic echinococcosis infection**

Transmission of CE is associated with various risk factors, including human behaviour and habits, management and husbandry of livestock and domesticated dogs, and environmental factors (Wen, 2016). Identifying and understanding these factors is very important when developing and implementing control programmes for CE to reduce the possible transmission of the disease to humans and other animals. In the current research, a case study of 50 patients with CE (Chapter 4), found that approximately half (48%) of the participants reported slaughtering livestock at home for their families' consumption. Sadjjadi (2006) similarly reported the common practice of slaughtering animals within the backyard of houses in Arab countries, particularly during specific celebrations such as "Eid festival" (Al-Abri *et al.*, 2017). It is important to encourage residents to have animals slaughtered at licensed abattoirs, where they would undergo formal inspection by trained meat inspectors, and subsequent "safe" disposal of affected organs to reduce the access of dogs to such organs (Alvseike *et al.*, 2018).

Approximately three quarters (78%) of the CE patients reported the presence of a large number of FRD near their village. Free-roaming dogs can disseminate the eggs of *Echinococcus* spp. resulting in wide-spread environmental contamination, and hence are a potential threat to both public health and the health of other animals (Kamiya *et al.*, 2006). Control of the FRD population through programmes such as Animal Breeding Control (ABC) or Capture-Neuter-Vaccinate-Release (CNVR) has been used widely in other countries to ethically control and reduce the dog population and to reduce the risk of the transmission of zoonotic diseases, including echinococcosis (Tenzin *et al.*, 2015). As faeces from FRD have the potential to contaminate water and food sources, information on the need to boil drinking water, wash vegetables prior to eating and wash hands after handling dogs or potentially contaminated soil should be included in educational material produced for the general public (Gebremichael *et al.*, 2013; Tiaoying *et al.*, 2005).

The study of patients with CE reported in Chapter 4 and the survey of livestock owners in Chapter 5 highlighted the general low awareness and poor knowledge of the community about CE. Others (El-Berbri *et al.*, 2015; Aydın, Adıgüzel and Güzel, 2018) have similarly highlighted a low level of awareness about CE. Due to this general low level of awareness, education is a key component of reducing the incidence in humans, livestock and dogs. The key role of knowledge about how a disease is transmitted was evident in the current study where a survey of dog owners showed that those who fed offal to their dogs were less knowledgeable of CE (OR=0.17, 95% CI 0.05, 0.53), in contrast owners who restrained (tied up) their dogs were more likely to have good knowledge about the disease (OR=7.0, 95% CI 2.07, 23.78). Feeding viscera and failure to confine/restrain dogs have also been highlighted by others as risk factors for the transmission of CE to humans (Possenti *et al.*, 2016; Li *et al.*, 2015). In the current

and other studies, few dog-owners regularly dewormed their dogs (Omadang *et al.*, 2018; Varcasia *et al.*, 2011). Others (Eckert and Deplazes, 2004; van Kesteren *et al.*, 2015) have reported the success of dosing dogs with suitable anthelmintics at six-weekly intervals, and some countries and regions, such as New Zealand and Tasmania, have successfully eradicated the parasite through sustained application of anthelmintics to dogs combined with the implementation of educational programmes (Moro and Schantz, 2009).

### **8.3 Limitations of the present study**

As with most research studies, there were some limitations in the study. Data collected as part of the retrospective survey of CE patients did not include the address of the patients, for confidentiality reasons, and hence associations between residence (urban or rural) and infection could not be investigated (Chapter 3). Future studies should collect such information and also include a suitable comparative group to enable risk factors for human infection to be confirmed. In the future, as more medical records are computerised, larger studies could be conducted, and importantly local risk factors for infection investigated. Improvement in the medical record system would also reduce the significant amount of time spent in retrieving records for analysis.

It was challenging to collect samples from FRD and the samples collected in the current study may not be a true representation of this component of the dog population. Enumeration of the FRD population, as has been undertaken in other countries (Tiwari *et al.*, 2018), is also necessary to determine the potential impact on human health of this population. More faecal samples, from both owned and FRD, should be collected and tested with more sensitive assays, such as ELISA's or PCR's (Varcasia, Garippa and Scala, 2004). This would provide a more accurate estimate of the prevalence of infection in the definitive host and help evaluate the

necessity for control of the dog population. However such testing is expensive and requires equipment which is currently lacking or difficult to access in Iraq. Further studies should also be undertaken to confirm the species/strains/genotypes of *Echinococcus* affecting the dogs and livestock in the region, as these can vary in their pathogenicity for humans and hence public health importance (Dinkel *et al.*, 2004).

The study in livestock, the intermediate hosts for *Echinococcus*, should be expanded to include more sheep, including those slaughtered in “back-yards” or small processing plants, and to also include other livestock species such as cattle, buffalo and goats (Getachew, Almaw and Terefe, 2012; Chaligiannis *et al.*, 2015). Expanding the number of species examined would provide further evidence of the importance of the different livestock in the life-cycle of the parasite. Others have reported that goats are less frequently infected with hydatid cysts, most likely because of their browsing habits as compared to the grazing habits of sheep, resulting in less frequent ingestion of vegetation contaminated with dog’ faeces (Fallah *et al.*, 2010; Chalechale *et al.*, 2016). In addition, it is important to determine the proportion of cysts that are fertile/viable in the different species, as this provides a reliable indicator of the importance of each species as a potential source of infection for dogs (Assefa *et al.*, 2015). Variation in the cyst’ fertility/viability in different species may be related to the immune response of the host or differences in the infecting strains of *E. granulosus* (Arene, 1985; McManus, 2006; Assefa *et al.*, 2015).

#### **8.4 Recommendations to control cystic echinococcosis**

The goal of any control programme against CE is to reduce: the morbidity, mortality and production losses in affected livestock; the prevalence and incidence of infection in dogs; and the incidence and severity of disease in humans (Craig *et*

al., 2017). From the studies undertaken as part of the research reported in this thesis, it can be inferred that the occurrence of CE in humans is mainly influenced by risk factors associated with the demographic and sociological characteristics of the humans, such as education level, and their environment (human behaviour, animal management and practices adopted). A control programme is more likely to be successful if there is collaboration between groups, including the medical and veterinary staff and the general public, to identify the most appropriate way to break the parasite's life cycle in cost-effective and practical ways (Marcotty *et al.*, 2013). A One Health (OH) approach to control CE in Basrah is recommended. The OH concept has been advocated for zoonotic diseases by the WHO and US CDC (Centers for Disease Control & Prevention) to optimise the health outcomes in both humans and other animals (WHO, 2018; CDC, 2014). The OH approach requires an integrated approach involving a multidisciplinary team of stakeholders including epidemiologists, veterinarians, medical healthcare staff, ecologists, urban planners, structural and environmental engineers, geologists, hydrologists, climatologists, geospatial scientists, botanists, parasitologists and microbiologists (Dórea *et al.*, 2014; Lebov *et al.*, 2017). One of the challenges facing Iraq when implementing a OH disease control approach is a lack of trained experts, and training and up-skilling of locals will be required for this approach to be successful.

Preventive and control measures that have been adopted to control CE in other locations have included: avoiding contact with dog faeces; hand washing and improved sanitation; reducing the FRD population; administering anthelmintics to dogs; preventing access of dogs to offal from intermediate hosts; safe disposal of infected offal; and initiation of a health education programme (Craig *et al.*, 2017). Also dog faeces should be disposed of in a way to diminish contamination of the environment by eggs of the parasite (Nijssse *et al.*, 2015). Such a comprehensive OH

approach to disease control is more likely to reduce the level of parasitism in humans, livestock and dogs than a non-unified approach.

## **8.5 Targeted intervention measures in dogs**

Dogs are the main definitive host of *Echinococcus* and controlling the population size and administering anthelmintics to them could help break the parasite's life cycle and thus reduce the incidence in humans (Otero-Abad and Torgerson, 2013). Praziquantel is considered to be the most effective anthelmintic to treat echinococcal infections in dogs and has been used widely for over thirty years (Gemmell and Johnstone, 1981). For effective control, it is critical that regular treatment with praziquantel is undertaken at no-longer than six-weekly intervals (Cabrera *et al.*, 1996; Gemmell *et al.*, 2001; Craig and Larrieu, 2006; Lembo *et al.*, 2013). Others have shown the impact of the regular use of praziquantel (Zhang *et al.*, 2009; Irabedra *et al.*, 2016) and it has been incorporated into successful eradication campaigns in New Zealand and Tasmania (Craig and Larrieu, 2006). Regular dosing of owned dogs is practical through the provision of the anthelmintics to owners or administration by animal health workers/veterinarians; however, control of the parasite in FRD can be more challenging. Use of praziquantel laced baits in China was effective in reducing the prevalence of *Echinococcus* in FRD, although it was costly and time-consuming to implement (Yu *et al.*, 2017; Yang *et al.*, 2009). Regular baiting of areas in Basrah where FRD congregate is recommended in conjunction with population control.

### **8.5.1 Dog population management**

Managing the size of the dog population (especially FRD) could help reduce the risk of disease transmission from these animals to humans and livestock (Craig *et al.*, 2017). A comprehensive dog population management programme should include control of both owned and FRD and should be applied simultaneously with



health education programmes and the dosing of dogs with anthelmintics. Control of FRD can be undertaken through the removal of animals and/or by fertility control. Before implementing a control programme the number of owned and FRD should be enumerated to ensure sufficient resources are available for such a programme, and a variety of methods have been used and evaluated to enumerate FRD (Tiwari et al., 2018). In conjunction with population enumeration of FRD, owned dogs should be counted, a formal registration system of dogs established and restrictions placed on the number that can be owned (FAO, 2014).

Reduction in the number of dogs can be undertaken in a number of ways. Although euthanasia is an effective procedure to rapidly reduce the dog population in endemic regions, it requires approval from local governments, poses potential health risks to the workers involved in the culling and should use methods acceptable to the local community and which are safe (Wang *et al.*, 2014; Kachani and Heath, 2014; Craig et al., 2017). However on-going population control is necessary to prevent other dogs from filling the niche left by the removed dogs (Taylor *et al.*, 2017). Alternative non-lethal procedures for population control include catching and rehoming and fertility control through ABC or CNVR programmes (Larrieu and Zanini, 2012; Kachani and Heath, 2014). Capture-Neuter-Vaccinate-Release programmes offer the advantages that they can: be applied with both free-roaming and owned dogs; focus on both males and females; and overcome the issue of other dogs migrating into an area where dogs have been culled from (Jackman and Rowan, 2007). Sterilization of female dogs, rather than males, is considered more cost-effective (Reece and Chawla, 2006), although sterilisation of males does offer the advantages of reducing aggressive behaviour and roaming (Kachani and Heath, 2014). Although CNVR programmes are costly, time-consuming and require an organised approach to maximise their impact (FAO,

2014), they have frequently been sponsored by international aid organisations such as the World Society for Protection of Animals (WSPA), the Humane Society International (HSI) and Vets Beyond Borders (VBB) (WSPA, 2009; DOL/HSI, 2009; OIE, 2011; FAO, 2014), and such sponsorship would be essential for implementation in Basrah due to a lack of funds, infrastructure and qualified personnel. Importantly, these organisations have developed guidelines on: habitat control and hygiene improvement (sanitation of food and water); legislative measures (responsible dog ownership); reproduction control (animal birth control); and coordination of multiple stakeholders; which ensures that programmes are manageable, sustainable and cost-effective. It is important to fully understand and objectively measure the dynamics of the dog population before embarking on any dog population management programme.

### **8.5.2 Public educational programmes**

Cystic echinococcosis is a serious zoonotic disease of public health importance among low income and developing countries. A good level of health literacy in the community has many advantages by empowering individuals to understand diseases and health-related disease risks and to promote healthy behaviours (Kickbusch, 2001). In the current study, most of the participants surveyed (Chapters 4 and 5) had a poor level of knowledge of CE, highlighting the need to develop and implement a health education programme on echinococcosis within the region. This programme should focus on both dog and livestock owners and the general public. Dog owners should be advised to: prevent their dogs wandering through tying them up or confining them to a dog-proof fenced area; not feed offal that has hydatid cysts to their dogs; pick-up and dispose of their dog's faeces, ideally daily, to minimise environmental contamination; treat their dogs with anthelmintics containing praziquantel every six weeks; and wash their hands after

handling their dogs (Schurer *et al.*, 2015; Ibrahim, Annajar and Ibrahim, 2016; Solomon *et al.*, 2017). As the current study found a poor level of management (suitable disposal) of dog faeces by dog owners (Chapter 6), education on safe disposal methods should be included in any educational programme to improve environmental sanitation. Ideally, these faeces should be buried deeply or bagged and disposed of in the municipal rubbish system (EPA, 2001). Handling of dog faeces should be minimised, and protective measures including using bags, gloves, and “pooper scoopers” or “sani-scoops” encouraged (Penakalapati *et al.*, 2017). Fencing the land around a house is very useful to prevent FRD and other dogs defecating close to an area where people are residing or children playing (Sprenger, Green and Molento, 2014), however this will be an expensive option and beyond the means of most households in Basrah.

Livestock owners should be advised to: prevent access of dogs to grazing fields or to areas where feed is stored; and ensure that infected offal from home-slaughtered stock is burnt or buried so that dogs cannot access it. Ideally, livestock should be slaughtered and processed in licensed abattoirs (Larrieu, 2017; Craig *et al.*, 2017). The general public, including dog and livestock owners, should be advised to: wash their hands after touching or feeding animals, and in particular dogs; wash vegetables before eating them with clean water; boil water for drinking, if it is not chlorinated or from a safe source; and prevent access of stray or non-treated dogs to their yards, gardens or house (Larrieu, 2017; Craig *et al.*, 2017). These aspects, along with information on the life cycle, preventive measures and symptoms of CE, should be covered in a health education programme, plus clinical diagnostic tools should be offered by the medical profession to at-risk members of the population to ensure the disease is detected promptly prior to the onset of more serious clinical signs and symptoms (Kachani *et al.*, 2003).

As this study (Chapter 4) found that most patients who had been operated on for CE had not received an explanation on how they acquired the infection and what to do to protect themselves and their families from future infection, the medical profession should also be targeted for an educational campaign to ensure they are aware of the risks of infection and what information they can provide to their patients and their families on how to reduce the risk of infection. In Tibet, as the majority of dog owners had low literacy levels and many children did not attend schools, a health education campaign was conducted as a part of a control programme involving the use of laminated colour pages illustrated with cartoons about the disease (Heath *et al.*, 2006). These illustrations provided information on the parasite's life cycle and methods to prevent infection including: not touching dogs; not feeding dogs raw liver or lungs; dosing dogs with praziquantel; and washing hands before eating. Similar simple messages should be incorporated into future educational campaigns in Iraq.

Children are an important group who should be targeted for including in any health programme to control CE, as was demonstrated in Chile (Alvarez Rojas *et al.*, 2016). In that programme students were shown various images of echinococcosis and advised on how to implement preventive measures against infection with the parasite. In 1989 a health education programme on preventing the transmission of *E. granulosus* to people in Wales was also introduced to schools, with a specific target group of children aged between 9 and 11 years (Lloyd, Walters and Craig, 1998). Based on the benefits of visual learning, the programme used a video film in classrooms to impart knowledge, as well as providing home exercises and information leaflets on methods to reduce the disease risk and how to prevent infection. Another effective idea for children was developed by Walters (2004), who used the puppet characters "Supersoap and Gooley Germ" as part of a health

education programme intervention to educate primary school aged children (6 to 12 years) about potential infection with *Giardia* and *Cryptosporidium*. Simple inexpensive interventions and game-play could be adopted in Basrah, and it is recommended that the Ministry of Education works closely with the Medical Health Directorate and the Veterinary Directorate in Basrah to develop suitable health education material on CE for both primary and secondary school pupils. Such material, which should highlight the importance of hygiene, would also provide benefits in reducing the incidence of other zoonotic and non-zoonotic diseases (Stull, Brophy and Weese, 2015; Matilla *et al.*, 2018).

A OH education programme is very important for disease risk mitigation at the human-animal interface (Berrian *et al.*, 2018) and specific information should be provided to at-risk or target groups (Craig *et al.*, 2017). In the surveyed location, information on the need to wash raw vegetables should be specifically provided to women who are responsible for food preparation in the home (Sanli *et al.*, 2011). In contrast, the need for awareness about hygienic practices, such as washing hands after touching dogs or soil, should be directed to all representatives of the community (Baneth *et al.*, 2016). Discussions and regular meeting with farmers to disseminate information regarding CE in livestock, as well as humans, should be instigated. Farmer groups could be used to raise the awareness about the disease, similar to that used in other disease control programmes (Robertson *et al.*, 2010). Simple booklets, explaining disease transmission and preventive measures, have been used successfully to control other livestock diseases (Leslie, 2012), and similar educational materials should be developed on CE for farmers and the community in Basrah.

Information suitable for raising the awareness about CE in the general public of Iraq could be provided through a variety of means including radio broadcasts,

bill-boards, posters, pamphlets, newspapers, television, mobile phone messages, interviews, seminars/presentations, lectures and conferences (Halton *et al.*, 2013; Sterneberg-van der Maaten *et al.*, 2016). A strategic plan should be developed and implemented by the Public Health Directorate in Iraq, which has a branch in each province of Iraq including Basrah, to address this serious disease.

### **8.5.3 Targeted intervention measures in livestock**

#### **8.5.3.1 Targeting livestock at abattoirs**

Livestock are important intermediate hosts for echinococcosis and preventing access of dogs to affected organs from these animals is one of several key methods to break the parasite's life-cycle (Oksanen and Lavikainen, 2015). Central to preventing infection of the canine definitive hosts is the safe disposal and/or destruction of infected offal (Moro *et al.*, 2004; Economides and Christofi, 2000). This can be done through deep burial or incineration, particularly when animals are slaughtered in licensed abattoirs where carcasses and viscera are inspected by qualified meat inspectors (Ibrahim, Annajar and Ibrahim, 2016). However, illegal or home slaughtering of livestock can be an issue, particularly if affected organs are disposed of in a manner to enable consumption by dogs. Home slaughtering should be discouraged, or the people involved in the slaughter educated on how to identify hydatid cysts and how to dispose of them in a manner to prevent access by dogs (Omadang *et al.*, 2018). Although banning home slaughtering is an option, the cultural practices of the local Iraqis, who have home-slaughtered animals for many generations would be difficult to prevent and banning may result in the practice continuing but affected organs not being reported or disposed of appropriately (Sadjjadi, 2006). Although the ideal would be to have all carcasses and associated viscera inspected by veterinarians and/or meat inspectors, this may be difficult and expensive to implement initially. However abattoirs in Iraq also need improving to ensure they have adequate infrastructure, sanitation and trained meat

inspectors to ensure production of wholesome and safe products for the community (Joshi *et al.*, 2003; Cook *et al.*, 2017; Craig *et al.*, 2017). This would also help improve food safety for the general community, as well as reducing the likelihood of the lifecycle of echinococcosis being completed.

Surveillance through inspection of animal carcasses in the slaughterhouses is also a valuable monitoring programme for CE, as well as other diseases. Such monitoring, not only helps to further understand the epidemiology of the parasite, but also provides information on the effectiveness of control measures against the parasite (Vuitton *et al.*, 2014). Not only should the prevalence of infection be confirmed but the viability of the cysts detected should also be undertaken, including confirmation of the species of *Echinococcus* affecting the livestock through the use of molecular tools (Thompson and McManus, 2001). This will help underpin the future development of vaccines and treatment against this parasite in Iraq (McManus and Thompson, 2003; Craig *et al.*, 2007).

#### **8.5.3.2 Livestock traceability**

An important component of producing meat safe for human consumption is traceability of animals to identify farms and/or regions where the disease is present or at a higher prevalence (Heath *et al.*, 2006; Assefa *et al.*, 2015). Tracing the movement of animals between provinces and from adjoining countries into Iraq is important to ensure that infected animals/farms/regions are identified promptly to prevent reintroducing the parasite into areas where control and/or eradication programmes have been implemented (Gemmell *et al.*, 2001). Some nations, such as Australia, require each large ruminant to be identified with a unique ear tag with a radio frequency identification device as part of the National Livestock Identification Scheme (Trevarthen, 2006; Neethirajan, 2017). Although this offers significant advantages for tracing livestock and their movements, the cost is likely to be too

great to implement in Iraq at this stage. However, alternatives, such as requiring movement permits, could be an inexpensive means to enable trace-back to the source of origin and to minimise transfer of diseases to other livestock or humans. This would, however, require the establishment of a property identification code system (Mennecke and Townsend, 2005; Bowling *et al.*, 2008) currently not present in Iraq. Implementing formal movement and property and animal identification processes would offer the advantage of being able to trace animals and potentially implement control programmes for many diseases, as well as CE.

#### **8.5.3.3 Improving immunity of intermediate hosts through vaccination**

Vaccination against CE in intermediate hosts has also been undertaken to control the disease. The EG95 vaccine was developed from components of the oncosphere (Heath and Lawrence, 1996; Lightowers *et al.*, 1996). Larrieu *et al.* (2015) undertook a field trial of the vaccine in Argentina where weaned lambs received two injections one month apart and a single booster injection one year later. A significant reduction (62%) in the prevalence of *E. granulosus* was observed. Although the EG95 vaccine has shown to induce 95 to 100% protection against *E. granulosus* in sheep, it is expensive and would currently be beyond the means of most Iraqi farmers (Pan *et al.*, 2017; Valizadeh *et al.*, 2017).

### **8.6 Future research**

#### **8.6.1 Molecular epidemiological studies**

Other researchers have used molecular tools to better understand the transmission and epidemiology of *Echinococcus* and to confirm the species/strains affecting different hosts (Thompson and McManus, 2002; McManus and Thompson, 2003; Thompson, 2008). *Echinococcus* has been recognised as a cryptic genus with high genotypic and phenotypic variability resulting in differences in morphology, development and host specificity (including infectivity/pathogenicity for humans)



(Romig, Ebi and Wassermann, 2015; Ito *et al.*, 2017; Mulinge *et al.*, 2018). This variability has important implications for the design and development of vaccines, diagnostic techniques and therapeutic drugs against the parasite (McManus, 2002; Sharma *et al.*, 2013; Romig, Ebi and Wassermann, 2015).

Recently various molecular tools, such as restriction fragment length polymorphism (RFLP), PCR-RFLP and mitochondrial DNA sequencing, have been used to differentiate strains of *Echinococcus* (Chaâbane-Banaoues *et al.*, 2016; Ito *et al.*, 2017). Furthermore coproantigen and copro-PCR tests, which are sensitive diagnostic techniques, have been developed to detect *Echinococcus* eggs or antigens in the stools of dogs (Zhang and McManus, 2006). The application of molecular tools has revealed different genotypes of *E. granulosus* from humans, sheep, cattle, buffalo and camels (Thompson *et al.*, 2006; Kia *et al.*, 2010; Pour, Hosseini and Shayan, 2011). Based on new data collected from molecular studies, along with the biological and epidemiological characteristics of the species and strains, the species within the genus *Echinococcus* have been taxonomically revised (Craig *et al.*, 2015). However, these molecular techniques are expensive and time-consuming and require modern laboratory facilities and trained technicians (Zhang and McManus, 2006; Tappeh, Hanifian and Diba, 2012). Although this information is useful in confirming sources of infection, using them in Iraq is unlikely to be warranted, and focus should initially be on implementing simple diagnostic methods so that cost-effective control measures can be adopted to prevent infection of people, livestock and dogs.

### **8.6.2 Further studies required on CE disease in humans and other animals**

A case-control study of human cases of CE should be undertaken using a control group matched for age and sex with the cases to identify key risk factors for infection, as has been undertaken in Spain (Campos-Bueno, López-Abente and Andrés-Cercadillo, 2000). Such a study would help identify specific local

practices/habits linked to infection so that control programmes could be developed specifically for the local situation.

Further work is also required to ensure that cases of CE are diagnosed promptly, and the use of portable ultrasonography and serological tests should be considered for screening the general population to identify asymptomatic cases (Gavidia *et al.*, 2008). The classification issued by the WHO-IWGE (World Health Organization-*Informal Working Group on Echinococcosis*) allows the distinction into active (CE1 and CE2), transitional (CE3) and inactive (CE4 and CE5) cyst stages (WHO-IWGE, 2003). Most inactive uncomplicated cysts, which are localised in the livers of CE patients (CE4 and CE5), do not need treatment and should only be monitored regularly with ultrasound using a “watch-and-wait” approach (Lissandrin *et al.*, 2018). Nevertheless, early diagnosis of asymptomatic populations could significantly reduce the costs of treatment by replacing the need for surgery required in advanced cases, with anthelmintics, such as albendazole, which are effective in early cases (Brunetti, Kern and Vuitton, 2010; Chebli *et al.*, 2017).

Cystic echinococcosis can have a significant socio-economic effect, not only on an affected individual and their family, but also to the community (Harandi, Budke and Rostami, 2012). Undertaking a thorough economic study on the burden of CE to the community should be undertaken in Basrah. In conjunction with this, a control programme should be developed and implemented (Moro *et al.*, 2011) using the OH approach. Studies in the abattoirs should be expanded to examine all carcasses and viscera of cattle, buffalo, goats and sheep slaughtered in Basrah to identify lines of animals with a high prevalence. This would allow focused control or treatment programmes to be implemented to reduce the parasite’s prevalence.

## 8.7 Conclusions

In conclusion, this study described and investigated CE in humans and other animals in Basrah, Iraq. Cystic echinococcosis was shown to be present at a high prevalence in the sampled dogs and sheep in the province. The study highlighted several risk factors which could contribute to the spread of the disease. Based on the findings it is recommended that a control programme is developed and implemented in Basrah to reduce the incidence of the infection in humans, livestock and dogs. This control programme should be based on a One Health approach through adopting health education, the treatment of owned and free-roaming dogs with anthelmintics, management of the population of FRD, and ensuring that all animals are slaughtered and processed in official licensed abattoirs.

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## Appendices

### Appendix. 1 CE patient's questionnaire to determine their knowledge, attitudes, and practices (KAP) of cystic echinococcosis.

#### A. Part 1:

Date: \_\_\_ / \_\_\_ / \_\_\_

1. Name of patient: .....
  2. Age of patient (in years): .....
  3. Gender: Male  Female
  4. Full address
    1. District .....
    2. Subdistrict .....
    3. Village .....
    4. Rural  or Urban
  5. Hydatid lesion was diagnosed as: Hepatic  Alveolar  others please specify
  6. Has any other member in your family/household/neighbourhood also been diagnosed with hydatid cysts?  
Yes (>> if yes go to (Q7))  No  Not sure
  7. If yes in Q6, do you know when that happened? In the past .....
- (record in months)



8. What is the highest level of education you have completed?
1.  Never went to school
  2.  Elementary
  3.  High school
  4.  College
  5.  Higher education (professional or post-graduate)
  6.  Religious schooling only
  7.  Literacy classes only
9. What is your current occupation? .....
10. How long have you been in this job? .....

**B. Part 2:**

1. Do you own a dog(s)?
  1. Yes
  - b. NO (go to next page)
2. Why do you own a dog (s)?
  1.  Guarding my house
  2.  Guarding my livestock
  3.  Hunting
  4.  As a pet
  5.  Other reason please specify (.....)
3. How many dogs do you own?
4. Is your dog tied up?
 

Yes  No
5. If yes in Q 4, where is it tied up?
  1.  Far (>50 m) from my dwelling
  2.  Near (<50 m) to my dwelling

6. For the following situations please rate the frequency of the event occurring using the scale of never, rarely, sometimes, often or always?

1. Does your dog have access to the kitchen area/food preparing area?

1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

2. Does your dog have access to the dining/eating area?

1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

3. Does your dog have access to containers used to store drinking water for people?

1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

4. How often do you feed your dog uncooked offal (for example liver or lung)?

1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

7. Have you seen stray dogs in your neighbourhood over the last week?

1.  Yes > if yes go to (Q8)                      b.  No > if no go to (Q9)

8. How many stray dogs do you usually see in your neighbourhood on average each week?

9. Have you seen foxes in your neighbourhood over the last week?

1. Yes > if yes go to (Q10)                      b. No > if no go to (Q11)

10. How many foxes do you usually see in your neighbourhood on average each week?

11. Do you raise any of the following livestock?

1.  Sheep

2.  Goats

3.  Cattle

4.  Buffalo

5.  Horses

12. How frequently do stray dogs have access to your livestock?

1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

13. Do you slaughter livestock at your home?

Yes  No

14. If yes in Q 13, how often do you slaughter the following livestock at your home in a month?

1.  Sheep 1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

2.  Goats 1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

3.  Cows 1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

4.  Buffaloes 1. Never 2. Rarely 3. Sometimes 4. Often 5. Always

15. Do you always call an inspector (Vet or meat inspector) if there is a cyst in the slaughtered carcass?

Yes  No

16. For parts or organs that are not kept for human consumption as judged by you or by a specialist what would you do in the following situations? Please use the choices of would definitely consider doing it, might consider doing it or definitely would not do it.

1. Feed organs not suitable for human consumption to my dog:

1 – Would definitely consider doing it

2 – Might consider doing it

3 – Would definitely not do it

2. Throw organs not suitable for human consumption in the home garbage

1 – Would definitely consider doing it

2 – Might consider doing it

3 – Would definitely not do it

3. Throw organs not suitable for human consumption outside my home in an open garbage area:
  - 1 – Would definitely consider doing it
  - 2 – Might consider doing it
  - 3 – Would definitely not do it
4. Burn organs not suitable for human consumption
  - 1 – Would definitely consider doing it
  - 2 – Might consider doing it
  - 3 – Would definitely not do it
5. Bury organs not suitable for human consumption
  - 1 – Would definitely consider doing it
  - 2 – Might consider doing it
  - 3 – Would definitely not do it

**Part 3**

1. What is the source of your family's drinking water?

1.  Tap water
2.  Reverse Osmosis water
3.  Tanker-truck water
4.  Water from dug well
5.  Water from surface water/spring

2. Do you boil water before drinking it?

- 1-  Yes all the time
- 2-  Yes most of the time
- 3-  Yes about half the time
- 4-  Yes but only some of the time
- 5-  No never

3. How is your family's drinking water stored? (tick all that apply)

1.  Stored water in covered containers (e.g., bottle, narrow-neck jug)
2.  Stored water in uncovered containers (e.g., pitcher, bucket)

4. How often do you eat leafy vegetables without first washing them?

1. Never      2. Rarely      3. Sometimes      4. Often      5. Always



3. *What was the worst impact of having hydatids on your life?*

.....  
.....  
.....  
.....  
.....

**Appendix. 2 Questionnaire survey of Knowledge, awareness, and Practices associated with cystic echinococcosis cysts to farmers in Basrah, Iraq**

Date: \_\_\_ / \_\_\_ / \_\_\_

**Part 1: Demographic information**

- 1- What is your name? .....
- 2- Gender:            Male     Female
- 3- What is your age or date of birth? .....
- 4- What is your full address?
  - 1. District .....
  - 2. Subdistrict .....
  - 3. Village .....
  - 4. Urban     Or Rural
- 5- What is the highest level of education you have completed?
  - 1. Never been to a school
  - 2. Elementary
  - 3. High school
  - 4. College
  - 5. Higher education (professional or post-graduate student)
  - 6. Religious schooling only
  - 7. Literacy classes only

6- What is your current occupation?

.....

7- How long have you been in this job? .....

**Part 2: Practice**

1- Do you keep livestock?

Yes  No

2- What kinds of animals do you have?

- 1. Buffalo
- 2. Cattle
- 3. Sheep
- 4. Goats
- 5. Camel
- 6. Horse/donkey

3- Do you own a dog(s)?

Yes  No

4- If yes in Q3, how many dogs do you have?

( )

5- If yes in Q3, how old is your dog(s)?

Dog No.	Age

6- If yes in Q3, what is the gender of your dog(s)? (faecal collection)

Dog No.	Age

7- Do you ever keep your dog(s) tied up outside?

Yes       No

8- If yes in Q7, how long do you keep your dog(s) tied up outside?

1. Dog tied up all the day
2. Dog tied up in the night only
3. Dog tied up in the morning only
4. Other please specify .....

9- Does your dog enter your house?

1. Never    2. Rarely    3. Sometimes    4. Often    5. Always

10- Do you feed your dog uncooked viscera or internal organs?

Yes       No

11- What do you usually do with the stools/droppings of your dog(s)?

1. Leave where they are
2. Bag it, tie it, and bin it
3. Dispose it in water canal or nearby stream.
4. Throw into a nearby agriculture field.
5. Other please specify .....

12- Do you wash your hands after handling, touching, and feeding or giving drinking water to your dog(s)?

Yes       No

13- If yes in Q 12, how often do you wash your hands?

1. Never    2. Rarely    3. Sometimes    4. Often    5. Always

14- Have you treated your dogs with anti-wormers during the last 3 months?

Yes       No       cannot remember

15- Does your dog have contact with your livestock?

Yes       No



16- If yes in Q 15, how often does your dog have contact with your livestock?

1. Never      2. Rarely      3. Sometimes      4. Often      5. Always

17- Does your dog have close contact or play with you or your family?

Yes     No

18- If yes in Q 17, how often does your dog have contact or play with you or your family?

1. Never      2. Rarely      3. Sometimes      4. Often      5. Always

19- In the last 12 months have you requested a veterinarian to inspect the carcass of an animal slaughtered at home?

Yes     No

20- Do you wash your hand before eating?

Yes     No

21- If yes in Q 20, how frequently do you wash them?

1. All the time
2. Most of the time
3. Some of the time
4. Rarely

22- Do you eat your food with?

1. Your hand
2. Utensils (e.g. spoon)
3. Both hand and utensils (e.g. spoon)

23- Do you usually wash or clean leafy vegetables before eating them?

Yes     No

24- If yes in Q23, what do you use for washing leafy vegetable?

1. Only remove outer leaves and visually dirty parts and eat the rest
2. Rinse it using running tap water
3. Soak it in water in the sink
4. Wash it with detergent and then rinse it under running water
5. Other please specify .....

25- Do you boil your water before drinking it?

Yes  No

26- What is the source of your family's domestic use water?

1. River
2. Tap water
3. Well
4. Reverse osmosis (RO)

27- What is the source of your family's drinking water?

1. River
2. Tap water
3. Well
4. Reverse osmosis (RO)

### **Part 3: Knowledge and attitudes**

1- Have you ever heard about zoonotic diseases?

Yes  No

2- Have you ever heard of a disease called hydatid cysts or Echinococcosis?

Yes  No

3- If yes in Q2: how can the disease be transmitted to humans? (**route of infection hydatid cyst to people**)

1. Contaminated food
2. Contaminated water
3. Contaminated hands
4. Eating raw food
5. Contact with dogs
6. Other please specify .....

4- Have you ever been infected with hydatid cysts?

Yes  No  Not sure

5- If yes in Q4, were you treated or not?

Yes  No  Not sure

6- If treated, what treatment did you receive?

.....  
.....  
.....

7- Do you know if buffalo, cattle, sheep or goats can be infected with hydatid cysts?

Yes  No  Not Sure

8- Have you ever seen cysts like these in the pictures in the organs of any animals?

Yes  No  Not sure

9- Are you aware it can be dangerous to eat raw vegetables contaminated with dog's faeces?

Yes  No

10- Do you know if acquiring hydatid cysts can be dangerous to the health of humans?

Yes  No

11- Is the regular de-worming of your dogs an important aspect of your dogs' health?

1. Not a priority
2. Low priority
3. Medium priority
4. High priority
5. Essential

12- Is the regular de-worming of your dogs an important aspect to protect you and your family's health?

1. Not a priority
2. Low priority
3. Medium priority
4. High priority
5. Essential

**Part 4: open questions**

1- In your experience what have veterinary staff done with stray dogs and foxes in your village over the past two years?

.....  
.....  
.....

2- What information, if any, have veterinary staff told you about worming your dog?

.....  
.....

3- If veterinary or medical staff have held a meeting about zoonotic diseases and hydatids what information did they tell you?

.....  
.....  
.....

**Appendix. 3 Questionnaire for estimating the economic burden of hydatid cysts in livestock infected in Basrah, Iraq – Veterinary staff and abattoir attendees' expert opinion**

Date: .... / .... / ....

Abattoir name: .....

Abattoir address: .....

<b>Part one: Abattoir veterinary staff interview (expert opinion):</b>
--

- 1) What is your name? .....
- 2) What is your current position? .....
- 3) How many years have you been working at this abattoir? .....
- 4) How many years have you been working in veterinary meat inspection?  
.....
- 5) What is the minimum, most likely, and maximum number of sheep slaughtered in a typical month at Basrah abattoir?

Minimum	Most likely	Maximum

- 6) What is the minimum, most likely, and maximum number of sheep detected with hydatid cysts in a typical month at Basrah abattoir?

Minimum	Most likely	Maximum

7) What percentage of hydatid cysts are found in the following organs in sheep (please make sure percentages add up to 100%)?

- a. Liver [    %]
- b. Lung [    %]
- c. Spleen [    %]
- d. Kidney [    %]

8) Based on your experience, what percentage of the following organs are totally condemned because of hydatid cysts in sheep?

- a. Liver [    %]
- b. Lung [    %]
- c. Spleen [    %]
- d. Kidney [    %]

9) In your opinion what percentage of sheep slaughtered for human consumption, are slaughtered in licensed abattoirs in Basrah?

- Percentage [            ]

10) What is the minimum, most likely, and maximum number of goats slaughtered in a typical month at Basrah abattoir?

Minimum	Most likely	Maximum

11) What is the minimum, most likely, and maximum number of goats detected with hydatid cysts in a typical month in Basrah abattoir?

Minimum	Most likely	Maximum

12) What percentage of hydatid cysts are found in the following organs in goats? (Please make sure percentages add up to 100%)

- a. Liver [    %]
- b. Lung [    %]
- c. Spleen [    %]
- d. Kidney [    %]

13) Based on your experience, what percentage of the following organs are totally condemned because of hydatid cysts in goats?

- a. Liver [    %]
- b. Lung [    %]
- c. Spleen [    %]
- d. Kidney [    %]

14) In your opinion what percentage of goats slaughtered for human consumption, are slaughtered in licensed abattoirs in Basrah?

- Percentage [            ]

15) What is the minimum, most likely, and maximum number of cattle slaughtered in a typical month at Basrah abattoir?

Minimum	Most likely	Maximum

16) What is the minimum, most likely, and maximum number of cattle detected with hydatid cysts in a typical month at Basrah abattoir?

Minimum	Most likely	Maximum

17) What percentage of hydatid cysts are found in the following organs in cattle?

(Please make sure percentages add up to 100%)

- a. Liver [    %]
- b. Lung [    %]
- c. Spleen [    %]
- d. Kidney [    %]

18) Based on your experience, what percentage of the following organs are totally condemned because of hydatid cysts in cattle?

- a. Liver [    %]
- b. Lung [    %]
- c. Spleen [    %]
- d. Kidney [    %]

19) In your opinion, what percentage of cattle slaughtered for human consumption, are slaughtered in licensed abattoirs in Basrah?

- Percentage [            ]

20) What is the minimum, most likely, and maximum number of buffalo slaughtered in a typical month at Basrah abattoir?

Minimum	Most likely	Maximum

21) What is the minimum, most likely, and maximum number of buffalo detected with hydatid cysts in a typical month at Basrah abattoir?

Minimum	Most likely	Maximum

22) What percentage of hydatid cysts are found in the following organs in buffalo?

(Please make sure percentages add up to 100%)

- a. Liver [ %]
- b. Lung [ %]
- c. Spleen [ %]
- d. Kidney [ %]

23) Based on your experience, what percentage of the following organs are totally condemned because of hydatid cysts in buffalo?

- a. Liver [ %]
- b. Lung [ %]
- c. Spleen [ %]
- d. Kidney [ %]

24) In your opinion, what percentage of buffalo slaughtered for human consumption, are slaughtered in licensed abattoirs in Basrah?

- Percentage [ ]

**Part two: Butchers interview:**

1. Over the past one year, what has been the minimum, usual, and maximum price for a kilogram of the following sheep offal?

Type of organ	Minimum price	Usual price	Maximum price
Liver			
Kidney			



Spleen			
Lung			

2. What is the minimum, usual and maximum reduction in the market price of sheep offal per Kg because of partial condemnation from hydatid cysts?

Type of organ	Lowest price	Usual price	Highest price
Liver			
Kidney			
Spleen			
Lung			

3. Over the past one year, what has been the minimum, usual, and maximum price for a kilogram of the following goat's offal?

Type of organ	Minimum price	Usual price	Maximum price
Liver			
Kidney			
Spleen			
Lung			

4. What is the minimum, usual and maximum reduction in the market price of goat offal per Kg because of partial condemnation from hydatid cysts?

Type of organ	Lowest price	Usual price	Highest price
Liver			
Kidney			
Spleen			
Lung			

5. Over the past one year, what has been the minimum, usual, and maximum price for a kilogram of the following cattle offal?

Type of organ	Minimum price	Usual price	Maximum price
Liver			
Kidney			
Spleen			
Lung			

5. What is the minimum, usual and maximum reduction in the market price of cattle offal per Kg because of partial condemnation from hydatid cysts?

Type of organ	Lowest price	Usual price	Highest price
Liver			
Kidney			
Spleen			
Lung			

6. Over the past one year, what has been the minimum, usual, and maximum price for a kilogram of the following buffalo offal?

Type of organ	Minimum price	Usual price	Maximum price
Liver			
Kidney			
Spleen			
Lung			

7. What is the minimum, usual and maximum reduction in the market price of buffalo offal per Kg because of partial condemnation from hydatid cysts?

Type of organ	Lowest price	Usual price	Highest price
Liver			
Kidney			
Spleen			
Lung			