Original article

Coprological Survey of *Echinococcus granulosus* and Management in Owned Dogs of Southern Tripoli

Imad E. Buishi*, Almabruk R. Fares and Mohamed M. Hosni

Department of Preventive Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Received 20 March 2016 / Accepted 28 April 2016

Abstract

To ascertain the prevalence of *Echinococcus granulosus* in owned dogs in rural areas located southern Tripoli, and to identify some predisposing risk factors including management of dogs and owner practices contributing to perpetuation and transmission of *E. granulosus*. *Taeniid* eggs and other helminth infection in dogs were identified microscopically through faecal flotation method. Infection with *E. granulosus* was determined indirectly via faecal antigen-capture ELISA and copro-PCR. Data on dog management and owner understanding of hydatid disease were collected via questionnaire. Faeces were collected from 149 owned dogs (79 AlKremia; 40 Swani; 30Sedi-sleem). *Taeniid* eggs were found in 11 dog feces.Eggs of hookworms were most prevalent, up to 17.2%.Roundworms *Toxocara canis* was least common, up to 6.1%.Of the 25 dogs found to be *Echinococcus* coproantigen positive, 14 were in Alkremia, 9 in Swani and 2 in Sedi-sleem. Twenty four out of 25 *E. granulosus* coproantigen ELISA-positive dogs were also copro-PCR-positive. The most common dog ration was scrape boiled food and raw meat up to 60(41.4%) and 46 (31.7%) of owners fed scraped boiled food to their dogs as a regular daily food ration. Nearly (41.5%) of owners never deworm their dogs and 45.7% deworm their dogs at irregular interval >6 months. Few dewormed their dogs often enough to ensure they remained cestode free at ≤ 6 months 18 (12.7%), and 32(24%) of owners admitted to left carcases where they were dead without burial, which offer good opportunity for dog scavenging. This study documents some factors that are contributory to the occurrence of *E. granulosus* in dogs in southern Tripoli and identifies the need for introduce an educational components directed towards dog owners.

Key words: *Echinococcus granulosus*; dogs; southern Tripoli; coproantigens; coproPCR

Introduction

Canine echinococcosis caused by *Echinococcus granulosus* infection is endemic in many countries including Libya. The domestic dog is the key definitive hostand the main source of human cystic echinococcosis (CE) worldwide. Diagnosis of *E. granulosus* in domestic dogs is a prerequisite for undertaking epidemiological studies in endemic areas (Jenkins *et al*., 2016). Previous studies on the *E. granulosus* of domestic dogs in Libya are few, and almost exclusively based on detection the adult tapeworms via postmortem examination of small intestine of necropsied dogs and/or microscopic detection of taeniid eggs through faecal flotation (Awan *et al*., 1990; Gusbi *et al*., 1987).However, a more recent study in which faecal samples were screened from northwest Libya owned dogs found that (21.6%) of the dogs had positive result by coproantigen ELISA (Buishi *et al*., 2005a).The *E. granulosus* coproantigen ELISA has been used successfully not only to monitor dog infection but also in control campaigns(Craig *et al*., 2015).

The first and only report of a field study of *E. granulosus* in dogs in Libya using this test collected faeces from owned dogs in three relatively large geographical areas: around Tripoli; Azawia and the other around Alkhums (Leptis-Magna). The authors reported a relatively large proportion of *E. granulosus* coproantigen-positive dogs (Alkhums 24/62 (39%), Tripoli 43/246 (17.5%) and Azawia11/57(19.2%) (Buishi *et al*., 2005a).The prevalence trend of *E. granulosus* infection in Libyan dogs since the 1980s has been not downward to the range of 27.8-40.3% (Packer and Ali, 1986; Gusbi *et al.,* 1987; Awan *et al.*,1990),and therefore echinococcosis remains veterinary-public health problem.

In addition, slaughter data has frequently reported high prevalence levels of hydatid cystsin slaughtered sheep from different sheep-rearing areas of Libya, particularly from the eastern parts (Ibrahem and Craig 1998) and lesser extend in northwest parts (Elmajdoub and Rahman, 2015;Buishi *et al*., 2012). These data suggest that the infection pressure of *E. Granulosus* in dog population may not equally in urban dogs as in rural dogs (Buishi and Fares, 2014). Although the worm burden of *E. granulosus* in necropsied owned domestic dogs is less than 200tapeworms, this is not the case in the stray dogpopulation when burdens were >1000 (Awan *et al.,* 1990; Buishi *et al*., 2005a).

Similar findings was demonstrated by a number of studies conducted elsewhere have reported a high prevalence in most of the stray dog populations, with much higher worm burdens than those seen in domestic owned dogs(Jenkins and Morris 2003; Jenkins *et al.*,2006; 2008).In rural areas where dogs and sheep interact, dogs may transmit *E. granulosus* infection to sheep, providing a potential risk for transmission of infection to rural dogs.During the study we also collected data through an owner questionnaire, on the feeding and deworming of rural dogs, other practices undertaken by owners, such as slaughter, and also dog owners' knowledge regarding the transmission of hydatid disease. A combination of coproantigen and

***Corresponding Author:** Imad E. Buishi. Department of Preventive Medicine. Faculty of Veterinary Medicine, University of Tripoli. P.O. Box 13662 Tripoli, Libya. Tel:+218 214628422, Fax:+218 21 4628421. Email: imadbuishi@hotmail.com

copro-PCR detection strategies was employed in this survey of *E. granulosus* conducted in a relatively small area around Tripoli of Libya-aknown endemic area of human CE (Aboudaya 1985; Shambesh *et al*., 1999) in order to estimate the prevalence level of *E. granulosus* and describe the potential risk factors resulted from dog management and practices of owners.

Materials and methods

Study sites/ questionnaire

Dog owners living in rural areas of southern Tripoli were randomly selected from veterinary practice records and invited to take part in the study. For each property visited, a questionnaire was administered relating to detailed information such as owner name, information on; dog ownership (number of dogs currently owned), management of these dogs, dog demographics (age, sex, type), whether owners slaughter livestock on their farms and if so, whether fed slaughter offal to their dogs. Anthelmintic treatment frequency, the nature of dog food and whether dogs were confined or not, we also sought information about the level of understanding dog owners had on the transmission pathway of human cystic echinococcosis. However, not all questions were answered by all interviewees. In total 149 dogs were registered. All questionnaire data were entered into Microsoft Access.

Collection and examination of faecal samples

Samples were taken from the available owned dogs (*n*=149). Faecal samples were collected rectally and/or taken from the ground around the homestead, Each sample was divided in to two parts; a part of faeces were subjected to a faecal flotation test using standard methodology with saturated sodium nitrate as the flotation medium. Eggs were visualized microscopically and where possible identified to species level through their morphology. The rest part of faecal sample, 2 gram were placed in either saline buffer (phosphate buffered saline (PBS) with Tween 20) or 80% ethanol, and initially stored frozen at -20^{o}C until sent to the University of Salford, England, where they were stored at -80^{o}C for coproantigen and copro-PCR testing.

Taeniid egg identification

Taeniid egg isolation was performed using a flotation and sieving Method (Mathis *et al*., 1996). Briefly, 8 mL of zinc chloride solution (1.45 g/mL) were added to 2 g of each faecal sample. The samples were homogenized and centrifuged at 1000gfor 20 min. The supernatant was passed through 41- and 21-µm mesh sieves. The Taeniid eggs were collected from the 21-µm mesh and re-suspended in water in a 10-mL tube. Egg identification was carried out using an inverted microscope.

Echinococcus coproantigen ELISA

Supernatants of 149 dog faecal samples were aliquoted and tested for the presence of coproantigen using an ELISA standardized against *Echinococcus* adult somatic antigens (Allan *et al*., 1992; Craig *et al*., 1995). All samples were tested using the same reagents in the same 'batch', with each sample tested in duplicate in adjacent wells. For controls, defined faecal panel of necropsy dog samples was available as described in Buishi *et al*. (2005a).

E. granulosus specific Polymerase Chain Reaction

The method of Abbasi *et al*. (2003) modified by Boufana *et al*., (2008) was used with the following primers: Eg1121a 5'-GAATGCAAGCAGCAGATG-3' and Eg1122a 5'GAGATGAGTGAGAAGGAGTG-3'. The PCR was performed in a final volume of 25µl containing 10Mm KCI, 1.5Mm MgCI2, 200Mm (each) dNTPs (Bioline, UK) 0.04Mm of the amplification primers, 2.5 units Taq DNA polymerase (BioTaq, Biolin, UK) 1% deionized formalide and target DNA. The PCR cycles were as follow: 5 min. at 95°C, and a final elongation step at 72°C for 10 min. PCR products were then visualized on 1.5% agarose gel, containing 1µg of ethidium bromide per ml. A 1kb plus DNA ladder (Gibco BRL®) was used to determine the size of the products.

Results

Questionnaire

A total of 173 dog owners in southern Tripoli area were identified randomly and agreed to take part in the survey. Of these, 19 (11%) owned dogs were not available for sampling at time of visit. On another 5 (2.9%) respondent refused to cooperate in control their dogs; and sampling of dogs was not possible. Of the remaining149 owners completed questionnaires giving a compliance rate of (86.1%); a run-through not everyone provided answers for all questions (Table1).

Backgrounds of studied population

The dogs included in the study came from a variety of farms located southern Tripoli, but ~ 67% were from properties where sheep were raised. The average number of dogs per property was two. Dogs are used mainly for guarding purposes and thus 62 respondents (46.6%) admitted to allow their dogs to roam freely, only 50 (37.6%) of a total of 133 respondents partially confined dogs i.e. kept chained during the day but they are allowed to roam freely at night time. Only 8 out of 149 dogs (5.3%) allowed access to the house.

Dog food

Almost all dogs (73%) were fed on scraped boiled food exclusively or as part of their daily food (Table 1). Of those fed on mixed food, 41.3% were fed raw meat, of which 26.8% of owners fed sheep offal. Home slaughter was undertaken by almost of the owners (93.2%) and between 16.4% and 37.4% of owners either left slaughter offal of sheep in open or feed to their dogs (Table 1). The most commonly identified offal component fed was lungs. In addition, 68.5% of owners thought their dogs could have access to carcasses of dead sheep and 11.1% of owners also fed slaughtered offal containing hydatid cyst(s) to their dogs.

Taeniid egg identification

The faeces of 11 dogs (7.4%) were found to contain eggs of taeniid tapeworms. Nine dog faeces (6%) were contain roundworm eggs identified as *Toxocara canis*, three of those (2%) was identified as mixed infection of both taeniid and *T. canis*. *Dipylidium caninum* were not found.

Coproantigen ELISA detection

A total of 149dog faecal samples from southern Tripoli; 79 from Alkremia, 40 from Swani and 30 samples from Sedi-Sleem were tested. An overall positive *E. granulosus* coproantigen result was obtained for 25 (16.77%); 14 (18%) of the Alkremia samples, 9 (23%) of Swani and 2 (7%) of the Sedi-Sleem samples (table 2).

CoproPCR

All samples were found to be negative for the presence of *Taeniid* eggs on direct microscopic examination, and were tested negative by coproantigen ELISA, were not included in the copro-PCR assay. Therefore, twenty five faecal extracts positive in the coproantigen ELISA, were subjected to an *E. granulosus* coproPCR. Twenty four of thesamples confirmed as PCR positive result for *E. granulosus* DNA. One coproantigen ELISA positive sample were appeared negative by PCR test (Figure1).

Owners knowledge

The activities by dog owners contributing the transmission of hydatid disease were; never deworm dogs (59/142 respondents; 41.5%), and frequent deworming of dogs was undertaken only by (18/142respondents; 12.7%). Feeding offal to dogs were practicing by26.8% (39/145) of owners compared to 31.7% (46/145) not fed offal to dogs. Improper dispose of livestock offal was admitted to practice by the majority of interviewers(94/128; 73.4%).Disposal of slaughter offal either by burring and/or burning were conducting only by the minority of dog owners (13/128; 10.2%). Washing hands frequently (38/149; 25.5%) were regarded as less important. Keeping dogs confined was regarded as least important; dogs kept to roam freely by (112/133; 84, 2%) of owners, only (21/133; 15.8%) confined their dogs. Although another 2(1.3%) respondents considered that no prevention measures needed to be taken, because they considered that there is non-risk of infection.

Table 1. Survey of dog management and human activity data collected from 149 questionnaires dog owners in eastern Tripoli

Question (number responding to the question)	Number of positive responses (% of respondents to question)
Farms containing livestock(103)	
Sheep only	35 (34)
Sheep and others	68(66)
Keeping dogs confined (133)	
Never confined	62 (46.6)
Partial confined	50 (37.6)
Full confined	21 (15.8)
Dog food (145)	
scraped/boiled food only	46 (31.7)
scraped/boiled/raw sheep meat	60(41.4)
Feed offal	39 (26.9)
Anthelmintic treatment (Deworming)(142)	
Never deworm	59 (41.5)
Regular deworming interval≤6months	18(12.7)
Infrequent deworming at >6months	65 (45.8)
Home slaughter performed (139)	
Slaughter offals (128)	
Fed to dogs	21 (16.4)
Left in open/scrub	94(73.4)
Buried/burnt	13 (10.2)
Disposal of dead sheep (133)	
Removal from/incinerate in premises	101 (76)
Retain on premises	32 (24)
Dog access to dead carcasses (102)	
Dog access to the house (8)	
Personal hygiene following contact with dogs-washing hands frequently(38)	

Table 2. Numbers of canine faecal samples analysed from the three study villages in Tripoli together with point estimates of the *Echinococcus* coproprevalence (%) and coproPCR. Confidence intervals are not shown.

Village	Proportion of total samples	Number of samples	Number of Coproantigen-positive (%)	Number of CoproPCR-positive (%)
Alkremia	0.53	79	14 (17.7)	13 (16.5)
Swani	0.27	40	9 (22.5)	9 (22.5)
Sedi-sleem	0.20	30	2 (6.7)	2 (6.7)
Total	0.100	149	25 (16.8)	24 (16.1)

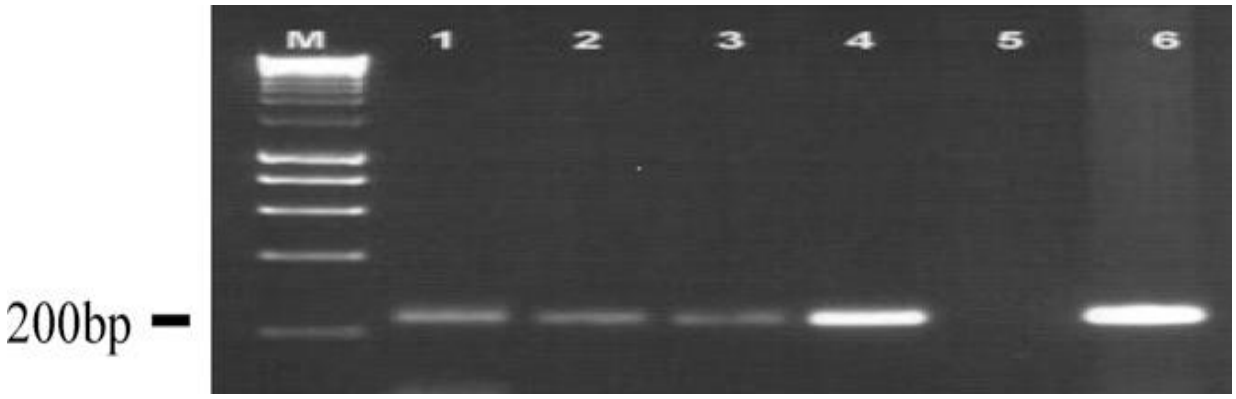

Figure 1. Detection of *Echinococcus granulosus* DNA in eggs piked fecal samples using G1 PCR.Lane M, DNA marker, lanes 1–4, represent copro DNA extracted from 1 gram of feces, lane 5, negative control, lane 6, positive control.

Discussion

The overall canine echinococcosis prevalence in the study area was estimated as 16.4% (range 16.1% coproPCR and16.7%coproantigen ELISA). One of 25*E.granulosus*coproantigen ELISA positive dog was found negative by PCR. This may have been because of the small amount of faecal sample sent for testing (<1 g) or may be that during the extraction process some DNA was lost and if the amount of starting material is small the PCR may appear negative. Failure to detect DNA in faecal sample by PCR could be a 'false' negative result, or because of the relatively low egg output due to small number of worms in that dog faecal sample. In the absence of eggs in the sample that was used to extract the DNA probably little or no cellular parasite tissue would be released during the growing phase resulting in a negative PCR result. By contrast during the growing period of adult worm, higher concentration levels of coproantigen might be produced as a result of high metabolic activity during the early phase of the prepatent infection period, which could be detected by coproantigen ELISA (Jenkins *et al.*, 2014).

This limitation inherent to the PCR test, so, eggs essentially need to be present for the primers to amplify sufficient non-degraded DNA to give a positive PCR result. Although, it has been reported that some coproantigen ELISA-positives are PCR-negative because, faeces contains substances that inhibit the Taq polymerase in the PCR reaction (Abbasi *et al*., 2003). However, in the absence of eggs or segments of *E. granulosus* in the faeces of the dogs, false-positive reactions in either the coproantigen or coproPCR test cannot be excluded. Nonetheless with high sensitivity and specificity of copro-PCR assay can be considered as a useful complementary tool, giving supportive information that can confirm coproantigen ELISA results.

In addition to estimate the copro-prevalence level of *E. granulosus*, the current study was aimed to describe management of dogs and owner practices contributing to the perpetuation and transmission of *E. granulosus*. In previous studies, risk factors for canine echinococcosis have been investigated in Libya (Buishi *et al*., 2005a) and worldwide (Otero-Abad and Torgerson, 2013).The most commonly identified risk factors are those relating to access to infected material (infected offal); factors associated with variability in infection after ingestion of infectious material; and factors associated with removal of infection, such as a history of anthelmintic treatment (Craig *et al*., 2015). In the current study, many practices by owners toward their dogs were found to be potential risk related with positive result. Keeping dogs confined appeared to be regarded as unimportant as 84% of owners were not confined dogs, only small proportion of the interviewers16% appeared to keep their dogs chained 'fully controlled' and were not allowed to roam freely. This is of concern; particularly most owners did not deworm their dogs frequent enough ≤ 6 months to ensure they are free of *E. Granulosus* infection and nearly two-third of dog owners fed raw meat and offal

and/or admitted their dogs had opportunities to access dead sheep carcasses.

In other studies a combination of purgation and coproPCR results were used to address the potential risk factors for of *E. granulosus* infection (Ziadinov *et al*., 2008). In the study we have use a combination of coproantigen test and copro-PCR to evaluate canine prevalence status with *E. granulosus*, and describe the risk factors. As a part of the current epidemiological study, in view of many owners feeding offal to their dogs,the presence of eggs of taeniid cestodes in their faeces was expected. However, what was surprising in view of the number of owners who fed slaughter sheep offal and/or raw sheep meat to their dogs, on microscopical examination of faeces, few dogs had eggs of the *Taenia* species in their faeces.

Eleven dogs were found to be infected with Taeniid eggs, indicating consumption of sheep offal in the recent past. However, therelatively low number of dogs (represent only 7.4% of total samples tested) with taeniid eggs in their faeces could be explained; either the dogs being uninfected or if infected, the tapeworms were immature. However, a literature search for recent published data highlighted possible drawback of faecal flotation tests; in similar study using flotation technique to detect taeniid eggs in positive necropsied dogs, taeniid eggs were seen only in 4/7 (57.1%) of dogs with confirmed taeniid cestode infection, indicating infections in 43% of infected dogs would have been missed (Adolph *et al*., 2013). However, those authors did not stated if the taeniid worms in the undetected infections were immature or gravid; nevertheless, these data suggested that centrifugation was not included in the flotation method. Likewise in our study a number of *Taenia*-infected dogs probably may not have been detected and/or overlooked.

Potential additional means of *E. granulosus* transmission to dogs, apart from being fed sheep offal, is through scavenging the dead animal carcases. Livestock animals particularly sheep in many parts of Libya are commonly infected with cysts of *E. granulosus* (Buishi *et al.*, 2012; Elmajdoub and Rahman, 2015),and our questionnaire data showed that most dead animals are left in the open where they were died without burial, which provides a potential, easily accessible source of *E. granulosus* infection for unconfined dogs. The lack of knowledge about *E. granulosus* and its transmission mode among dog owners may explain the frequent practice of feeding livestock offal to dogs; nevertheless, whenever dogs are fed hydatid-infected sheep offal there is always a risk of infection (Jenkins *et al*., 2014; Buishi *et al.*, 2006). While direct microscopic examination part of this study was being conducted, a number of dog faecal samples found to be infected with *Taeniid* eggs, suggesting this dog as having eaten sheep offal in the past. It should be noted here that access to infected offal have been identified by a number of studies as risk factors for infection of domestic dogs (Bchir *et al*., 1987; Moro *et al.*, 1999;Shaikenov*et al.*, 2003; Budke *et al*., 2005; Buishi *et al*., 2005b, 2006; Ziadinov *et al*., 2008; Acosta- Jamett *et al*., 2010; Mastin *et al*., 2011).

Furthermore, domestic dogs pose the largest risk of human infection due to their close association with humans (Budke *et al*.,2005).Our data have identified 59 (41.5%)of the dog owners admitted to not deworm dogs, this could be a reflection to the lack of owners knowledge about hydatid disease transmission. Not feeding offal to dogs and attention to good personal hygiene following contact with dogs were regarded as less important for the control of hydatid transmission to humans by a reasonable proportion of interviewers. In previous studies, a lack of recent anthelmintic dosing has been identified as a risk factor for dog infection (Craig *et al*., 1995; Buishi *et al*., 2005a;Acosta-Jamett *et al*., 2010).Consequently, the effect of anthelmintic treatment on prevalence rates of *E. granulosus* infection have been investigated in dog population from rural and urban areas of Libya, in that study, the copro-positive pre-treatment of dogs was significantly decreased from 21.6% before praziquental treatment to 9% post-treatment in 5 months intervals (Buishi and Fares., 2014).This result strongly support the importance of regular deworming of dogs ≤ 6 months which can be useful for designing a control schemes based on treatment of infection in dogs.

Two major limitation in the current study ;First the present survey was directed specifically towards *E. granulosus* infection in rural and peri-rural dogs residing in southern Tripoli, which commonly receive less veterinary supervision than other dogs residing in urban parts. And therefore, the observed prevalence may not reflect the true estimate of *E. granulosus* infection status. Second limitation is that despite efforts to sample dogs per rectum whenever possible, some faecal samples were collected from the ground around the household due to the free-roaming behaviour of the dogs. Therefore the possibility of mismatch the individual dog identification to its coproantigen status cannot be definitively excluded. It is also likely that some of the present analyzed data perhaps not related to the dogs for which particular questionnaire data were collected. This problem of sampling strategy would be expected to reduce the study power, but not necessary will result directional bias.

As the true estimate of the epidemiological situation of *E. granulosus* infection in owned dog population is more likely to be associated with the detection method/s of use (Craig *et al*., 2015).In this study we have used multi techniques such as combinations of microscopic analysis of collected faecal samples, copro-ELISA and copro-PCR; to assess *E. granulosus* infection status in dogs. The coproantigen prevalence would be expected to relate broadly to the prevalence of canine infection in a population, and can therefore act as a useful approximation of the overall levels of transmission. Whilst the use of PCR assay was employed to confirm previously identified positive-coproantigen faecal samples from 25 dogs from southern Tripoli, the copro-PCR was positive in 96% (24/25) and thus confirmed that there was a very high probability that these coproantigen-positive dogs were indeed positive for *E. granulosus* DNA, and probably therefore also egg positive.

Principally, *E. granulosus* occurring commonly in sheep and domestic dogs, a recent study of hospital records (Hosni *et al.*, unpublished data) has ascertained that transmission of hydatid disease to humans in Tripoli is still occurring. However, the current study used parasitological and coprological diagnostic tools, indicated that *E. granulosus* has a high infection pressure in the surveyed territory (southern Tripoli) and has adapted itself perfectly to the region, and being transmitted at high levels between dogs and sheep. This situation highlights the needs to be closely collaboration between veterinary and public health authorities to monitor the parasite transmission. An adequate steps need to be taken to control the parasite in Libya through an educational program targeted dog owners and farmers focused to change practices towards dogs; especially in dog restraint, and regular deworming of dogs as the key elements of control measures.

References

Abbasi I, Branzburg A and Campos-Ponce M (2003). Copro-diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence. *Am J Trop Med Hyg*.69:324–330.

Allan JC, Craig PS and Garcia-Noval F (1992). Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. *Parasitol*. 62:347–355.

Aboudaya MA(1985). Prevalence of human hydatidosis in Tripoli region of Libya. *Int J Zoo*. 21: 304-307.

Acosta-Jamett G, Cleaveland S, Bronsvoort BMD, Cunningham AA, Bradshaw H and Craig PS (2010). *Echinococcus granulosus* infection in domestic dogs in urban and rural areas of the Coquimbo region, north-central Chile. *Vet Parasitol*. 169: 117–122.

Adolph C, Little. S and Thomas. J (2013). Prevalence of covert infections of intestinal infections in dogs. In: *Proceedings of the American Association of Veterinary Parasitologist's 58th Annual Meeting, Western Chicago River, Nor Illinois.*

Awan MA, Gusbi, AM and Beesley WN (1990). Echinococcosis in Libya.III. Further studies on the prevalence of *Echinococcus granulosus* in dog. *Ann Trop Med Parasitol*. 84: 473-475.

Bchir A, Jaiem A, Jemmali M, Rousset JJ, Gaudebout C and Larouze B (1987). Possible existence of an urban cycle of *Echinococcus granulosus in* central Tunisia. *Trans R Soc Trop Med Hyg*. 81: 650.

Boufana BS, Campos-Ponce M, Naidich A and Buishi I (2008). Evaluation of three PCR Assays for the identification of the sheep strain (Genotype 1) of *Echinococcus granulosus* in canid feces and parasite tissues. *Am J Trop Med Hyg*.78:777–783.

Budke CM, Campos-Ponce M, Qian W and Torgerson PR, (2005). A canine purgation study and risk factor analysis for echinococcosis in a high endemic region of the Tibetan plateau. *Vet Parasitol*. 127: 43–49.

Buishi IE, Njoroge E, Zeyhle E, Rogan MT and Craig PS (2006). Canine echinococcosis in Turkana (north-western Kenya): a coproantigen survey in the previous hydatid-control area and an analysis of risk factors. *Ann Trop Med Parasitol.* 100: 601–610.

Buishi IE, Njoroge EM, Bouamra O and Craig PS, (2005a). Canine echinococcosis in northwest Libya: assessment of coproantigen ELISA, and a survey of infection with analysis of risk-factors. *Vet Parasitol*. 130: 223–232.

Buishi, IE, Walters T, Guildea Z, Craig PS, and Palmer S (2005b). Reemergence of canine *Echinococcus granulosus* infection, Wales. *Emerg Infec Dis.* 11:568–571.

Buishi IE, Fares AR, and Elzlitni R (2012). A retrospective analysis abattoir data for cystic echinococcosis (CE) in livestock in Tripoli. *Libyan Vet Med J.* 3: 137-145.

Buishi IE and Fares AR (2014). Assessing the changes in the coproantigen prevalence and natural re-infection rates of *E. granulosus* infection in domestic dogs from rural and urban areas of Libya. *Libyan J Vet Med Sci*.1: 19-35.

Craig PS, Gasser RB and Parada L (1995). Diagnosis of canine echinococcosis: Comparison of coproantigen and serum antibody testing with arecoline purgation in Uraguay. *Vet Parasitol*.56:293–301.

Craig PS, Mastin A, Kesteren F and Boufana B (2015). *Echinococcus granulosus*: Epidemiology and state-of-the-art of diagnostics in animals. *Vet Parasitol*. 2:1- 17.

Elmajdoub LO and Rahman WA (2015). Prevalence of hydatid cysts in slaughtered animals from different areas of Libya. *Open J Vet Med*.5:1-10.

Gusbi AM, Awan MAQ and Bessley WN (1987). *Echinococcus granulosus* in dogs with particular reference to the role of the dog in Libyan society. *Ann Trop Med Parasitol*. 81: 29-34.

Ibrahem MM and Craig PS (1998). Prevalence of cystic echinococcosis in camels (*Camelus dromedarius*) in Libya. *J Helminth*. 72: 27-31.

Jenkins DJ and Morris B (2003). *Echinococcus granulosus* in wildlife in and around the Kosciuszko National Park, south-eastern Australia. *Aust Vet J*.81:59–63.

Jenkins DJ, Allen L and Goullet M (2008). Encroachment of *Echinococcus granulosus* into urban areas of Queensland. *Aust Vet J.* 86:294–300.

Jenkins DJ, Lievaart JJ, Boufana B, Lett WS, Bradshawb H and Armua Fernandezc M (2014). *Echinococcus granulosu* sand other intestinal helminths: current status of prevalence and management in rural dogs of eastern Australia. *Aust Vet J*.92:292–298.

Jenkins DJ, McKinlay A, He D and Morris B (2006). Detection of *Echinococcus granulosus* coproantigens in faeces of naturally infected rural domestic dogs in southeastern Australia. *Aust Vet J*.84:12–16.

Mathis P, Deplazes J, and Eckert (1996). An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. *J Helminthol.* 70:219–222.

Mastin A, Brouwer A, Fox M, Craig PS, Guitian J, Li W and Stevens K (2011). Spatial and temporal investigation of *Echinococcus granulosus* coproantigen prevalence in farm dogs in South Powys, Wales. *Vet Parasitol.* 178: 100–107.

Moro PL, Bonifacio N, Gilman RH, Lopera L, Silva B. Takumoto R, Verastegui,M and Cabrera L (1999). Field diagnosis of *Echinococcus granulosus* infection among intermediate and definitive hosts in an endemic focus of human cystic echinococcosis. *Trans R Soc Trop Med Hyg*. 93: 611–615.

Otero-Abad, B and Torgerson PR (2013). A systematic review of the epidemiology of echinococcosis in domestic and wild animals. *PLoSNegl. Trop. Dis*. 7: 2249.

Packer DE and Ali TM (1986). *Echinococcus granulosus* in Libya. *Ann Trop Med Parasitol*. 80: 137-139.

Shaikenov BS, Torgerson PR, Usenbayev AE, Baitursinov KK, Rysmukhambetova, T, Abdybekova AM and Karamendin KO (2003). The changing epidemiology of echinococcosis in Kazakhstan due to transformation of farming practices. *Acta Trop*. 85: 287–293.

Shamesh MA, Craig PS and Macpherson CNL (1999). An extensive ultrasound serologic study to investigate the prevalence of human echinococcosis in Northern Libya. *Am J Trop Med*. 60:462-468.

Ziadinov I, Mathis A, Trachsel D, Rysmukhambetova Abdyjaparov TA, Kuttubaev OT, Deplazes P and Torgerson PR (2008). Canine echinococcosis in Kyrgyzstan: using prevalence data adjusted for measurement error to develop transmission dynamics models. *Int J Parasitol*. 38: 1179–1190.