

Incidence of Cystic Echinococcosis in Libya: I. Seroprevalence of Hydatid Disease in Sheep and Goats Naturally Exposed to the Infection in the North Midland Region

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Abstract: Hydatid disease is one of the most and serious public health and veterinary problems in Libya and other North African countries. Thirteen rural villages of two main districts bordered to each other at the north midland of the country namely, Misrata which is almost agricultural area and about 200 km east of Tripoli and Sirt which is almost pasture area and about 500 km east of Tripoli, were included in the current study. Incidence of cystic echinococcosis was investigated serologically using serum samples collected from 2651 animals of three groups; young sheep under two years old (240), adult sheep over two years old (2082) and adult goats over two years old (329). Antigen B prepared from camel crude hydatid cyst fluid together with ELISA were used for detection of total IgG antibodies against hydatid cysts in the collected serum samples. 1377/2651 serum samples from all animal groups of both districts gave overall ELISA seropositive result of 52%. The overall ELISA seropositivity for each group of animals was 55/240 (23%), 1235/2082 (59%) and 87/329 (26%) for young sheep, adult sheep and goats (all adults) respectively. In Misrata district, the overall seropositivity was 729/1243 (59%) and among the villages it was 43% from Saso and 78% from Tawergha; while in Sirt district, the overall seropositivity was 648/1408 (46%) and among the villages it was 25% from El-Gbeba and 63% from El-Arbaien. Statistical analysis showed no significant differences in the rate of seropositivity between the three groups of animals which was 23, 59 and 26%, in young sheep, adult sheep and adult goats respectively and between the two district areas which was 59% in Misrata and 46% in Sirt. Also statistical analysis showed no significant differences in the rate of seropositivity between the different age animal groups which was 23% for young sheep and 68% for adult sheep 7- <10 yrs old and in the case of goats, it was 24% for goats 2- <4 yrs old to 29% for goats 7+ yrs old.

Keywords: Antigen B, Cystic Hydatidosis, ELISA, Goats, Misrata, Sheep, Sirt

Introduction

Cystic Echinococcosis (CE) is one of the most geographical widespread zoonotic diseases that occur in all inhabited continents, with variable levels of endemicity ranged from endemic to hyper endemic. The greatest prevalence of the disease in human and animal hosts has been reported from the countries of the temperate zones, including several parts of the Mediterranean regions (including North African

countries), Russia, Central Asia, China, Australia, parts of America (especially South America) and East Africa, (Grosso *et al.*, 2012). The distribution of *E. granulosus* is more prevalent in rural communities where there is a close contact between dogs, the definitive hosts and various domestic ruminants including sheep, goats and others which act as the intermediate hosts for the parasite (Eckert and Deplazes, 2004). In Libya, CE is a serious economic and public health problem where the common sheep/dog cycle is usually considered as the major

source and important cycle for human infection as it has been reported elsewhere (Gusbi *et al.*, 1987b; Ibrahim and Gusbi, 1997; Thompson and McManus, 2001; McManus, 2002; Office International des Epizootics, 2008). However, livestock infection on the other hand leads to economic losses and the feeding of domestic and stray dogs with offal discarded from animals slaughtered for human consumption helps to maintain the life cycle of *E. granulosus* (Dalimi *et al.*, 2009). Sheep, goats and camels are the major reservoirs for the larval stage (Hydatid cysts) of *E. granulosus* in Libya; they play an important role in the maintenance and transmission of CE (Ibrahim and Gusbi, 1997; Kachani *et al.*, 1997). In terms of establishing the levels of infection in livestock, abattoir data from slaughtering animals is currently the only reliable indicator of the existence of the disease in domestic animals (Al-Qureishy, 2008; Lotfi *et al.*, 2010; Kumsa and Mohammedzein, 2014). The intermediate hosts for *E. granulosus* produce a significant immune response against the infection and the parasite on the other hand develops highly effective strategies to protect itself from the host defences and to avoid clearance (Zhang *et al.*, 2003), however, the production of such antibodies are important for the development of serodiagnostic assays.

Almost all serological tests developed for immunodiagnosis of CE cases have been able to detect antibodies against the infection with considerable differences between the various tests both in specificity and sensitivity but some of them found to be insensitive and nonspecific and therefore, they are useless. Such tests include Complement Fixation Test (CFT), Latex Agglutination Test (LAT), Cassoni Intradermal Test (CIT) and Indirect Haemagglutination Test (IHAT). More sensitive and specific techniques like Enzyme Linked Immunosorbent Assay (ELISA), Indirect Immune Fluorescence Antibody Test (IFAT), Immunoelectrophoresis (IEP) and Immunoblotting (IB) have been replaced the old ones in routine laboratory application (Lightowlers and Gottestine, 1995; Nasrieh and Abdel-Hafez, 2004). Serological tests are potentially important for epidemiological studies, confirmation of infection situation, treatment and the monitoring of control programs. Among the above serological tests, ELISA for detection of IgG antibodies was the most commonly used and considered to be highly sensitive and specific technique in detecting anti-hydatid antibodies (Wattal *et al.*, 1986; Haniloo *et al.*, 2005).

The sensitivity and accuracy of any serological test used for detection of anti-hydatid antibodies in serum samples found to be depends on the composition, concentration and stability of the antigen in use. Hydatid Cyst Fluid (HCF) from different animals has been used most frequently as a source of *E. granulosus* antigens and its components have been extensively investigated for their applicability in serological tests mainly in the

human cases. Much less researches have been directed towards the development of immunodiagnostic techniques for *E. granulosus* infection in domestic animals; therefore, the development of highly specific and sensitive serological diagnostic test for the detection of CE infection in livestock would represent a major advance as it would help governments to monitor animals imported into their countries which are free from hydatidosis and it would help other countries where control schemes for the disease are in operation (McManus, 2014).

Serological approaches based on the detection of specific antibodies in infected animal sera using ELISA and other techniques have been assessed principally in sheep cases (Lightowlers, 1990; Craig, 1997; Ibrahim *et al.*, 2002). Furthermore, ELISA has been performed using HCF for the serodiagnosis of CE in camels and donkeys and the obtained result was 100% sensitivity for both animals and 97.6 and 70.5% for camels and donkeys respectively (Mahmoud *et al.*, 2008) and in a recent study using ELISA with *E. granulosus* secreted antigen, Okolugbo *et al.* (2014) reported 96.4 and 70.5% sensitivity and 80 and 76% specificity for camels and cattle respectively. Njeruh and Gathuma (1990) reported 100% specificity and 91% sensitivity using HCF in ELISA for natural hydatidosis in sheep and goats.

E. granulosus antigen B (AgB) which is one of the major lipoprotein components of HCF, found to be highly immunogenic molecule and has been comprehensively used in immunodiagnosis of human CE using ELISA technique. The obtained results showed high levels of sensitivities and specificities depends on the origin of the cyst fluid (Sadjjadi *et al.*, 2007; Rahimi *et al.*, 2011; Reiterova *et al.*, 2014). It has already been shown that AgB preparation derived from sheep and camel hydatid cyst fluid in ELISA may also be successfully adapted for the serodiagnosis of CE in sheep with specificity up to 100% and sensitivity 90% (Kanwar and Kanwar, 1994). AgB-ELISA has been evaluated for its seroreactivity against sera from naturally infected sheep as confirmed at post mortem examination and the obtained results showed that, native antigen B preparation from camel hydatid cyst fluid gave the highest sensitivity 92% with 99% specificity comparing to the other antigens from different sources (Ibrahim *et al.*, 1996). Furthermore, ELISA method and AgB preparation from camel hydatid cyst fluid have been also tested against sera from camel naturally infected with CE as confirmed at slaughter and the reported result was 97% sensitivity and 99% specificity (Ibrahim *et al.*, 2002). However, these results indicates that, ELISA based on serum antibody detection to AgB from camel hydatid cyst fluid could be developed for immunodiagnosis of CE in naturally infected livestock.

All the previous studies on the prevalence of CE in livestock were concentrated on the post-mortem

examination at the abattoirs only and no reports were available on the seroprevalence of CE in live animals (ant-mortem) in Libya. The aim of this serological work was designed to determine the occurrence of cystic hydatidosis in sheep and goats using ELISA together with antigen B derived from camel hydatid cyst fluid in the North Midland region including Misrata and Sirt districts.

Materials and Methods

Study Area

Thirteen villages of two districts, 200 and 500 km (from their city centre) east of Tripoli the capital called Misrata and Sirt respectively; the first is a large semiarid and fertile agricultural region with a population of about 500,000 people and the second is mostly pasture region with a population of about 97,000 people (Fig. 1). These two districts are located in the coastal strip of the Mediterranean Sea and were particularly selected for this study for two reasons first, they are fairly known areas for rearing and grazing large number of domestic animals due to their favourable weather condition which helps pastoralism and second, stray, sheep-guard and farm dogs which are the main sources for the infection with hydatid disease to livestock animals are frequently observed in large numbers in the study areas.

Serum Samples Collection and Processing

Blood samples were collected from randomly selected animals by jugular vein punctures using suitable syringes and needles and from approximately 1/3 of each flock or herd. A total of 240 young sheep < 2 years old, 2082 adult sheep over 2 years old and 329 goats (all adults) over 2 years old were included in the present study. The animal age was determined based on dentition (teeth number, size and location on the animal jaws) and owner's information.

Blood samples were collected in 15 ml disposable tubes, allowed to clot for few hours at room temperature, the clot was then removed and the remaining sample was left under the same condition for another one hour before centrifuged at 2000 g for five minutes. Finally serum samples were carefully drawn off and transferred to 1.5 ml microcentrifuge plastic tubes (Eppendorf) in 1 ml aliquots and stored at -20°C until used. 10 serum samples from sheep and goats heavily infected with cystic hydatidosis in liver and lungs as assessed at post-mortem examination were used as a positive control and 14 serum samples from sheep and goats that reared under controlled conditions (dogs free place) and did not show any sign of cystic hydatidosis at post-mortem examination were also used as a negative control.

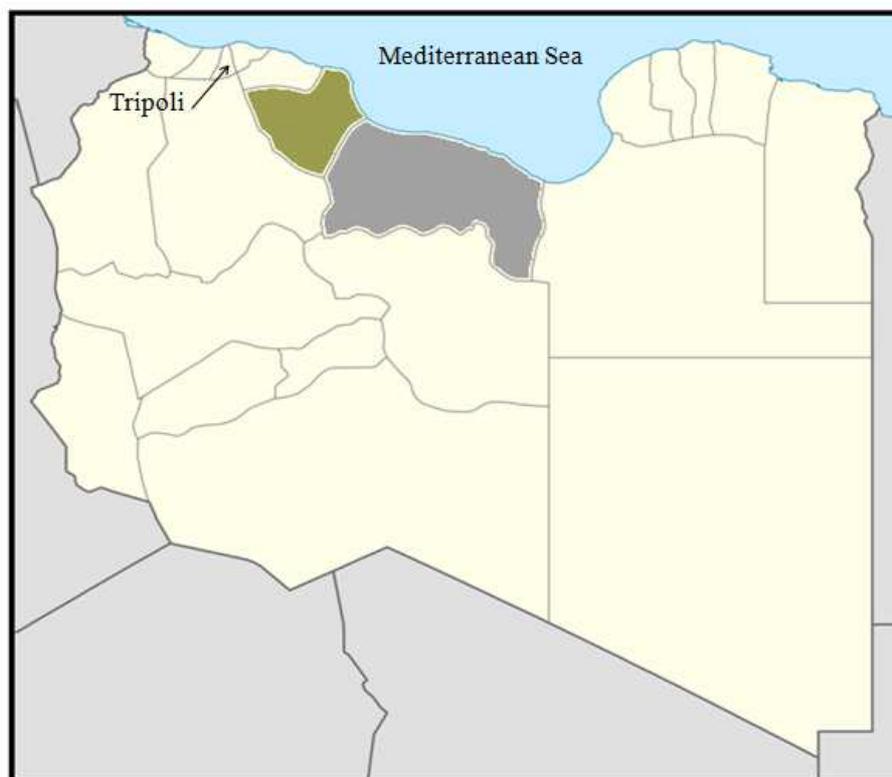


Fig. 1. Map of Libya showing the two study districts, Misrata (■) and Sirt (■)

Preparation of Antigen B

Crude hydatid cyst fluid of *E. granulosus* was collected from liver or lungs of camels which showed multiple cysts infection as confirmed at post-mortem. Hydatid cyst fluid was aspirated under sterile conditions and clarified by centrifugation at 2000 g for 15 min to remove the protoscoleces and any other solid materials, the supernatant was then collected and stored at -20°C until used. Antigen B enriched fraction was prepared essentially as described previously by Rogan *et al.* (1991); on the basis of the original method of Oriol *et al.* (1971). 100 ml of the clarified stored hydatid supernatant fluid which containing the main lipoproteins namely antigen B and antigen 5, was first dialyzed overnight at 4°C against 0.005 M acetate buffer pH 5.0. The fluid was then centrifuged at 15000 g for 30 min at 4°C and the precipitate pellets were collected and dissolved in 10 ml of 0.2 M Phosphate Buffer (PBS) pH 8.0, boiled in a water bath for 15 min and re-centrifuged at 20000 g for 1 h at 4°C. The supernatant containing antigen B was assayed for protein concentration and reactivity and stored in aliquots at -20°C until use.

Enzyme Linked Immunosorbent Assay (ELISA)

Antigen B at protein concentration 7 µg/ml was used at optimal working dilution 1/200 using 0.05M bicarbonate/carbonate buffer (BCB), pH 9.6 to coat (100 µl/well) polystyrene microtitre plates (Immulon 1, Dynatech, USA), incubated overnight at 4°C. Plates were washed three times with 0.1% PBS, pH 7.4, mixed with 0.05% Tween 20 (T20) to remove unbound antigens and blocked with 100 µl/well of 0.3% PBS/T20 for one hour at room temperature. Blocking solution was removed and animal serum samples to be tested were diluted at 1/200 in 0.3% PBS/T20 and 100 µl/well of each sample was added in duplicate to each plate. Control positive and negative serum samples were included on each plate and treated on the same way of the tested sheep and goats' sera. After washing three times as above, 100 µl/well of donkey anti-sheep IgG (whole molecule) alkaline phosphatase conjugate (Sigma) at the optimal dilution 1/30000 with 0.3% PBS/T20 was added and incubated for 1.5 hrs at RT. Following conjugation treatment and washing as above, all plates were developed using 100 µl/well of p-nitrophenyl phosphate (p-NPP, Sigma) substrate at 5mg/5ml with 1M diethanolamine buffer, pH 9.8, for 30 min in the dark. ODs values were recorded at 405 nm using an automatic plate reader (Dynatech MR5000, UK). The ELISA cut-off value was measured based on the mean ODs values of the control negative serum samples plus 3 standard

deviations (3 SD). Tested serum samples were considered to be positive or negative by comparing their mean ODs readings with the cut-off absorbance values.

Statistical Analysis

The obtained results were analyzed statistically using analysis of variance (ANOVA) test to find out any significant differences in the rate of seropositivity between the two districts in general, between sheep and goats and between the animal age groups.

Results

A total of 2651 serum samples collected from live young sheep (240), adult sheep (2082) and adult goats (329) were serologically tested in ELISA using partially purified native antigen B preparation for the detection of total IgG anti-hydatid antibodies. Seropositive results were calculated according to the positive-negative cut-off value which was 0.154. The overall seropositivity for the two districts was 1377/2651(52%) and for each group of animals, the rate of seropositivity was 55/240 (23%) for young sheep, 1235/2082 (59%) for adult sheep and 87/329 (26%) for goats (Table 1 and Fig. 2). In Misrata district, the overall rate of seropositivity was 59% and among the animal groups it was 24, 65 and 21% for young sheep, adult sheep and goats respectively (Fig. 3). In the individual villages, the total rate of seropositivity was between 43% from Saso village and 78% from Tawergha, while among the animal groups, the rate of seropositivity for young sheep was ranged between 21% from Tummena and 26% from El-Krareem, for adult sheep it was between 48% from Saso and 82% from Saddon and for goats it was 0% from Saddon and Tummena and 29% from Saso (Table 2). In Sirt district, the overall rate of seropositivity was 46% and among the animal groups it was 22, 53 and 28% for young sheep, adult sheep and goats respectively (Fig. 3). In the individual villages, the total rate of seropositivity was between 25% from El-Gbeba and 63% from El-Arbaien, while among the animal groups, the rate of seropositivity for young sheep was ranged between 15% from Harrawa and 27% from El-Gordabia, for adult sheep it was 28% from El-Gbeba and 72% from El-Arbaien and for goats it was 13% from El-Gbeba and 41% from Harrawa (Table 3). Age group seropositivity was 23% for young sheep and between 43% and 68% for adult sheep 2-< 4 and 7-< 10 yrs respectively and between 24% and 29% for goats 2-< 4 and 7+ respectively (Table 4 and Fig. 4). Analysis of variance (ANOVA) showed no significant differences in the overall seropositivity between the two districts ($p = 0.425$) and between the animal age groups ($p = 0.235$).

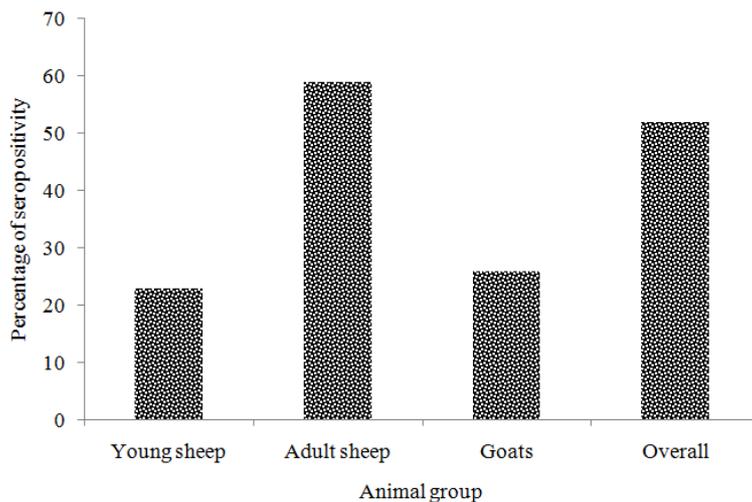


Fig. 2. The total rate of ELISA seropositivity for CE in each group of animals and the overall from both districts

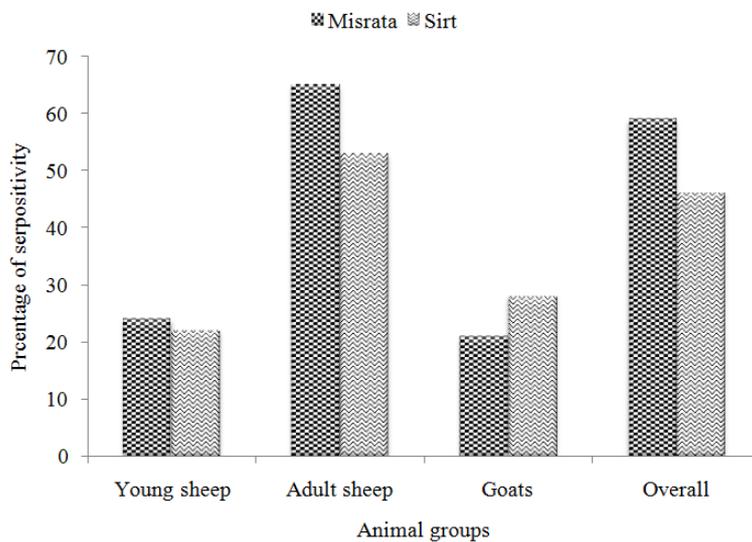


Fig. 3. The total seroprevalence rate of CE in each group of animals and the overall as a comparison between the two districts

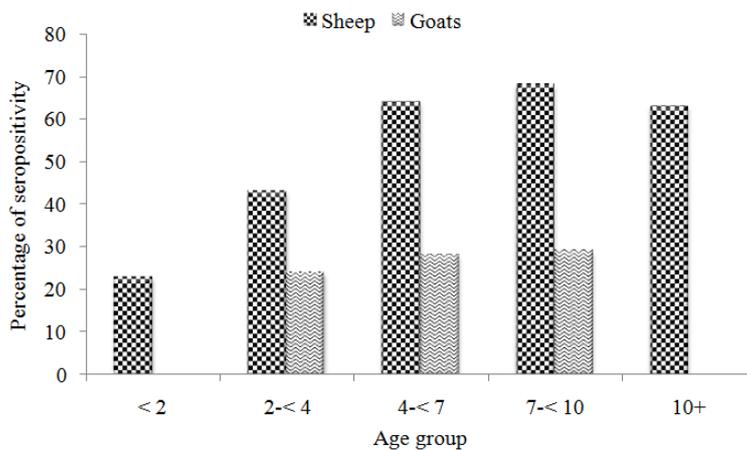


Fig. 4. Age-group seroprevalence result of CE in sheep and goats

Table 1. The total and the overall of ELISA seropositive results for CE in the three groups of animals from both districts

District	Young sheep	Adult sheep	Goats	Overall
Misrata	31/130 (24%)	685/1050 (65%)	13/63 (21%)	729/1243 (59%)
Sirt	24/110 (22%)	550/1032 (53%)	74/266 (28%)	648/1408 (46%)
Overall	55/240 (23%)	1235/2082 (59%)	87/329 (26%)	1377/2651 (52%)

Table 2. ELISA seropositive results for CE in the three groups of animals from Misrata district

Villages	Young sheep	Adult sheep	Goats	Total
Saddon	4/16 (25%)	45/55 (82%)	0/2 (0%)	49/73 (49%)
El-Krareem	6/23 (26%)	35/60 (58%)	3/16 (19%)	44/99 (44%)
Saso	9/38 (24%)	100/207 (48%)	6/21 (29%)	115/266 (43%)
Eddafnia	7/29 (24%)	156/265 (59%)	4/22 (18%)	167/316 (53%)
Tummena	5/24 (21%)	137/191 (72%)	0/2 (0%)	142/217 (65%)
Tawergha	-	212/272 (78%)	-	212/272 (78%)
Overall	31/130 (24%)	685/1050 (65%)	13/63 (21%)	729/1243 (59%)

Table 3. ELISA seropositive results for CE in the three groups of animals from Sirt district

Villages	Young sheep	Adult sheep	Goats	Total
Harrawa	2/13 (15%)	49/86 (57%)	9/22 (41%)	60/121 (50%)
El-Gbeba	4/18 (22%)	22/80 (28%)	2/15 (13%)	28/113 (25%)
Sirt centre	2/10 (20%)	30/70 (43%)	5/22 (23%)	37/102 (36%)
Abuhady	3/14 (21%)	36/82 (44%)	11/40 (28%)	50/136 (37%)
El-Arbaien	4/21 (19%)	205/285 (72%)	23/65 (35%)	232/371 (63%)
El-Gordabia	3/11 (27%)	50/92 (54%)	6/22 (27%)	59/125 (47%)
Jarif	6/23 (26%)	158/337 (47%)	18/80 (23%)	182/440 (41%)
Overall	24/110 (22%)	550/1032 (53%)	74/266 (28%)	648/1408 (46%)

Table 4. Age-group ELISA seropositive results for sheep and goats naturally exposed to the infection with cystic hydatidosis

Age-group	No. of sera tested	No. positive samples (%)
Sheep < 2	240	55 (23)
Sheep 2-< 4	564	243 (43)
Sheep 4-< 7	645	415 (64)
Sheep 7-< 10	489	334 (68)
Sheep 10+	384	243 (63)
Goats 2-< 4	114	27 (24)
Goats 4-< 7	156	43 (28)
Goats 7+	59	17 (29)
Total	2651	1377 (52)

Discussion

Cystic hydatidosis, hydatid disease and cystic echinococcosis are terms used to describe human and animal infection with the larval metacestode stage, the hydatid cyst of *Echinococcus granulosus* tape worm. The availability of data on the prevalence of hydatid disease in ovine animals is very important as it provides a good indication of the extent of local environmental contamination with *E. granulosus* eggs and could help in prevention and control programmes.

Abattoir data on the prevalence of CE in sheep and goats in Misrata and Sirt of Libya are very limited, however, these two districts were involved in a general abattoir survey on CE in livestock carried out by Gusbi *et al.* (1987b; 1990) and Ibrahim and Craig (1998) and the obtained results were 8.3 and 15.8% for sheep and 1.6 and 3.8% for goats respectively.

The available data from Misrata abattoirs showed that, 16.75% of examined sheep at post-mortem were infected with hydatid disease, (Elmajdoub *et al.*, 2007) and in a recent study from the same area, Elmajdoub and Rahman (2015) conducted further study on the prevalence of CE in sheep and reported 10.52% of the examined animals were infected. Data from Sirt abattoirs showed that, 4.9% of sheep and 2.4% of goats examined at post-mortem were found to be infected with hydatid disease (Kassem *et al.*, 2013).

Previous studies based on abattoir data suggested that, cystic hydatidosis in livestock animals in Libya in general may have significantly increased by time depending upon the magnitude of contamination of the environment and the absence of any control programmes (Ibrahim and Craig, 1998; Elmajdoub *et al.*, 2007; Ekhnefer *et al.*, 2012).

Apart from the several techniques used for the immunodiagnosis of CE in humans, non have been successfully adapted for the diagnosis of the disease in live animals, however, the serodiagnosis remained the only ante-mortem way to investigate the prevalence of the disease in livestock in endemic areas especially when controlled abattoirs are infrequent or not available like in Libya. Also post-mortem examination method has been shown to have limitations since small cysts and to some extent large cysts are missed during palpation and when this happens, such animals are recorded as uninfected (Macpherson and Miller, 2003).

In serology, the detection of circulating antigens in sera is less sensitive than antibody detection which remains the method of choice (Zhang *et al.*, 2003). Several serological assays have been used for the immunodiagnosis of CE but showed some cross-reactions with other Taeniid cestodes including, *T. hydatigena* and *T. ovis* (Yong *et al.*, 1984; Lightowlers and Gottstein, 1995; Sbihi *et al.*, 2001), however, such assays considered to be not useful for the diagnosis of the disease. ELISA assay on the other hand has been the technique which received most attention as an immunodiagnostic method for various parasitic infections and it was the most sensitive serological test for the diagnosis of hydatid disease. The technique is relatively easy to use, can be performed in poorly equipped laboratories and can be used for large-scale screening of populations in which hydatidosis is endemic (Zarzosa *et al.*, 1999; Akalin *et al.*, 2014).

ELISA technique using a variety of antigens have been applied to the immunodiagnosis of CE in animals (Yong *et al.*, 1984; Sadjjadi *et al.*, 2007; Fallah *et al.*, 2014) and found to be a sensitive approach for the diagnosis of the disease and able to detect antibody responses in infected animals even when the cysts are small and as such will give a more dependable result on the status of CE (Tabar *et al.*, 2010; Okolugbo *et al.*, 2014).

An improved ELISA was developed using partially purified antigen B from camel hydatid cyst fluid to detect anti-hydatid antibodies in sheep sera and the obtained result gave a sensitivity of 90% and a specificity of 99% (Ibrahim *et al.*, 1996). In more recently; studies on cystic hydatidosis in human using ELISA with AgB of sheep origin showed sensitivities of 84.37 and 88.3% and specificity of 96.7% (Tabatabaie *et al.*, 2013; Tenguria *et al.*, 2013); these findings suggested that, AgB-ELISA might be of use and promising tool for the primary serodiagnostic screening of CE in ovine animals and however, it has been chosen as the best serological technique for investigating the incidence of CE in sheep and goats in the present study.

The present work is the first comprehensive seroepidemiological study which aim is to determine the extent of CE in sheep and goats in this part of the country. The recorded results, however, showed that, the overall seropositivity was as high as 59% in adult sheep comparing to 23 and 26% in young sheep and goats respectively and the reason for such high seropositivity in adult sheep may be due to the extended age of the animals which sometimes reaches 10 yrs or over, this could be attributed to that aged animals have longer exposure time to the eggs of *E. granulosus* compared to young animals, therefore, the risk of acquiring infection would be high (Kebede *et al.*, 2009; Ripoche *et al.*, 2009; Desta *et al.*, 2012; Getachew *et al.*, 2012). It has been reported in other studies that, there was a positive

correlation between the rate of infection with hydatid disease and the age of the animals (Khan *et al.*, 2013; Al-Shaibani *et al.*, 2015). Furthermore, the lower prevalence of CE seen in small ruminants during abattoir investigations might be due to the fact that old sheep and goats are rarely slaughtered in abattoirs and under veterinary control. Thus the real CE prevalence might be underestimated, given that only data from young animals are reported (Manfred *et al.*, 2011). However, in Libya, the rate of infection with adult worms of *E. granulosus* in dogs has been reported as high as 40.3% in stray dogs, 34.8% in sheep dogs and 21.6% in farm or domestic dogs (Gusbi, 1987a; Buishi *et al.*, 2005). During our visit to the animal flocks we noticed that, 3-10 dogs/flock were frequently in close contact with the livestock feed stuff, drink freely from the same water holes and sometimes uses the same shelter for sleeping especially in summer when the temperature is very high and in winter when it is raining; therefore, this relationship between the dogs and the livestock may be also responsible for increasing the chance of animals getting infection with CE and therefore, the higher rate of seropositivity obtained in the present study reflects the situation.

The high rate of seropositivity reported in the present study reflects that, the incidence of hydatid disease in sheep and goats is of a considerable economic problem in Libya and therefore, serious action must be taken by the government to reduce the possibility of transferring the disease indirectly to human through infected dogs which is subsequently affect the human health.

According to the data from Libyan abattoirs, the infection rate with hydatid disease in lambs (young sheep) was slightly lower 15.8%, (Ibrahim and Craig, 1998), compared to that obtained in the present serological study 23%, this may be because during routine meat examination, it is very difficult or impossible sometimes to recognize very small cysts (< 5 mm) in infected organs which may stimulate antibody production against the infection that can virtually be detected by serological methods.

Studies on the prevalence of CE in sheep from different countries of the world reported to be between 10.6 and 75%, (Dalimi *et al.*, 2002; Azlaf and Dakkak, 2006; Scala *et al.*, 2006; Christodouloupoulos *et al.*, 2008; Kebede *et al.*, 2009; Acosta-Jamett *et al.*, 2010; Omer *et al.*, 2010; Oryan *et al.*, 2012). This wide range of variations could be due to the variations in the environmental conditions, the way of animal raising, strains of *E. granulosus* (McManus, 2006; Ibrahim, 2010). Our results on the seroprevalence of CE in adult sheep 59% and in goats 26%, does agree with that reported elsewhere (Njoroge *et al.*, 2002; Umur, 2003; Azlaf and Dakkak, 2006); however, the explanation for these differences may not be due to the susceptibility of each animal to the infection, but rather due to the grazing habits, as goats were always seen grazing mostly on the

upper parts of the plants and not so close to the ground like sheep and therefore, the risk of goats getting infection by *E. granulosus* eggs is probably lower.

We reported that, seropositivity was increased with the animal age, for example, it was 23% in young sheep, 68% in adult sheep between 7 to < 10 yrs old, 63% in adult sheep over 10 years old. For goats, the rate of seropositivity between the different age groups was almost the same i.e., 24, 28 and 29% in 2-< 4 yrs old, 4-< 7 yrs old and over 7 yrs old respectively, these observations suggests that, transmission of CE can be occurred at any age of the animals with variable degree of infectivity and also agreed with the abattoir data that previously reported (Ongling *et al.*, 2014). The slight differences in the total rate of seropositivity between both districts in general and between villages of each district could be due to sharing the same environmental conditions, grazing methods and human behaviour.

The obtained data in general confirms that, the epidemiological situation of CE in both districts and all villages can be ranged between endemic to hyper endemic. These results indicated that, there have been no sufficient control programmes in the country in the past decades and therefore, it is highly recommended the importance of implementing effective measures including de-worming of sheep and farm dogs using praziquantel drug every 6 weeks after they have access to livestock offal that may contain hydatid cysts. Burning or buried of dead animals or animal viscera to prevent dog's access to it, destroying of stray dogs, build fences around vegetable and fruit gardens and around children's play areas to keep dogs and other canids, away from the premises, personal hygiene are essential in reducing the chances of spreading the disease.

Conclusion

Cystic echinococcosis is considered to be an important ailment which may greatly affect the public health and economy especially in the hyper endemic countries. The present study provides a comprehensive view of the epidemiological situation of CE in the studied districts and thus, may serve as baseline data for future extended surveys to comprise other areas in the country. The obtained results evidently indicates that, incidence of CE in both districts can be reached a hyper endemic level. Sheep and goats can play an important role in the transmission and maintenance of *E. granulosus* life cycle. Outlining control measures should include de-worming of shepherd dogs and elimination of stray dogs as well as public health education to ensure effective reduction of disease incidence in livestock.

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Authors' Contributions

Mohamed M. Ibrahim: Designed the research plan and organized the study, participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

Badereddin B. Annajar: Contributed to the writing of the manuscript, made significant comments on the article.

Wafa M. Ibrahim: Participated in co-wrote and organizes sections of the article and edited the overall manuscript.

Conflict of Interest

This is an original work and its contents have not been published before. The corresponding author confirms that, the co-authors have read and approved the manuscript and no ethical issues involved.

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