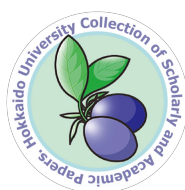




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Cystic echinococcosis in slaughtered animals in Ha'il, Northwestern Saudi Arabia

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Abstract

Meat inspection records of two slaughterhouses were used to determine the prevalence *Echinococcus granulosus* infection among slaughtered animals in Ha'il province, North-western Saudi Arabia. Records showed that from January to December 2015, 149514 animals were examined (126642 sheep, 4347 cattle and 18525 camels). The prevalence of *E. granulosus* was 7.89% (95% CI = 7.74–8.04), 2.76% (95% CI = 2.30–3.30) and 0.51% (95% CI = 0.41–0.62) in sheep, cattle and camels respectively. Hydatid cyst was found strictly in liver and lung. A total of 440 hydatid cysts from sheep were assessed for their fertility and viability: thus, 66.59% were fertile, 12.5% were sterile and 20.90% were purulent or calcified. Among the fertile cysts the protoscoleces were viable in 59.38% of them. In conclusion, the prevalence of slaughtered animal cystic echinococcosis in North-western Saudi Arabia is lower compared to those reported in other regions of the country. Nevertheless, control of stray dog population, deworming of dogs and proper disposal of infected viscera remain crucial to curtail the problem.

Key Words: *Echinococcus granulosus*, slaughtered animals, fertility, Ha'il, Saudi Arabia

Introduction

Cystic echinococcosis (CE) is a zoonosis caused by the larval stage of the cestode *Echinococcus* (*E.*) *granulosus*. This disease leads to medical, veterinary and economic problems and constitutes a public health problem worldwide including in Saudi Arabia^{14,21,23)}. The life cycle of

this helminth includes carnivores, mostly dogs, as definitive hosts and herbivores such as sheep, cattle, goats and camels as intermediate hosts. The improper disposal of dead animals, the access of farm dogs to offal of slaughtered livestock animals, the farmers carelessness to treat their dogs with anti-helminthic, and the grazing of flocks in fields where stray dogs have free access

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increase the exposure of the livestock animals to cystic echinococcosis^{6,8)}. This zoonosis is still affecting the livestock in many regions of the Kingdom of Saudi Arabia (KSA)^{14,18,21-22,35)}. Such infection can lead to economic losses resulting from condemnation of infected organs as well as the decrease of animal productivity in milk meat and wool^{7,32)}. Moreover, infected animals remain potential sources of contamination of *Canidae* and subsequently human and other animals. Currently, only few updated data are available concerning the infection rate of the Saudi Arabia livestock with *E. granulosus*. Indeed, studies of the CE in slaughtered animals were only carried out in Al Baha²¹⁾, Al Madinah Al Munawarah³³⁾, Riyadh²⁾, Asir²²⁾ and Makkah Al Mukarrama^{18,19,35)}. To the best of our Knowledge, no report is available on the prevalence of animal CE in North-western Saudi Arabia. Therefore, the aim of the present study was to determine the prevalence of cystic echinococcosis in slaughtered animals and to assess the fertility and viability rates of animals' hydatid cysts in Ha'il region.

Material and Methods

Study area: Ha'il region is located in northwest of KSA (between 64°25'35" and 29°00' N longitudes and 39°01' and 44°45' E latitudes). It has an area of 103.887 Km². It is characterized by a continental desert climate with hot summers (temperatures typically rise as high as 50°C during day time with diurnal variation of about 25°C) and cool winters (around freezing at night especially at higher altitudes and daytime temperatures nearly always reach 25°C in the sun). Ha'il is located at a high altitude (914 m above mean sea level) with an annual precipitation of 100.6 mm. According to the statistical yearbook of the Ministry of Economy and Planning of Saudi Arabia, 2010, the livestock in Ha'il region is composed of 498295 sheep heads, 64858 goat heads, 5221 cattle heads and 19548 camels heads⁸⁾.

Slaughtered animals' inspection: A descriptive research was conducted from January to December 2015 in the two official slaughterhouses of Ha'il region. Post-mortem examination of the slaughtered animals was carried out by veterinarians through visual inspection of the offal, palpation and incision of visceral organs including particularly the lung, liver, spleen and kidney according to the procedure recommended by FAO/UNEP/WHO (1994)¹⁶⁾.

Assessment of cyst fertility and protoscoleces viability among infected slaughtered animals: A random sample of infected organs from slaughtered animals was investigated for cyst fertility and protoscoleces viability. Small cysts less than 5 mm in diameter were not included in this study because it was difficult to differentiate them from other metacestode lesions. Thus, hydatid fluid of each cyst was collected individually in a sterile container and the germinal layer was extracted and washed in a Normal saline solution to retrieve potential protoscoleces. One drop of the collected hydatid fluid was examined microscopically (40×) for the presence of protoscoleces. A cyst is considered fertile if one or more protoscoleces were detected under microscope. The cyst which contained no protoscolex as well as calcified cysts were considered as unfertile cysts. To test the viability of the detected protoscoleces, one drop of hydatid fluid was mixed with one drop of aqueous solution 0.2% eosin (W/V) and examined microscopically (40×) according to the protocol of Daryani *et al.*¹¹⁾. Viable protoscoleces do not take the stain up whereas the dead ones do.

Data management and analysis: Collected slaughtered animal's data were entered into a Microsoft Excel data base and then analyzed using the SPSS V. 17 statistical software. Prevalence was calculated as percentage value. Statistical association of *E. granulosus* prevalence with animal species and season (winter [December, January and February], Spring [March, April

Table 1. Prevalence of *E. granulosus* infection among slaughtered animals in Ha'il region according to the animal species

Species	No. examined	Infected number	Prevalence (%)	95% CI	χ^2	P-value
Camels	18525	95	0.51	0.41–0.62	1499.51	<0.0001
Cattle	4347	120	2.76	2.30–3.30		
Sheep	126642	9994	7.89	7.74–8.04		
Total	149514	10209	6.82	6.70–6.95		

Table 2. Seasonal prevalence (%) of cystic echinococcosis in slaughtered animals in Ha'il region

Animal species	Season	Combined prevalence (%)		95% CI	χ^2	P-value
		a/ b	Percentage (%)			
Camels (n = 18525)	Summer	28/5367	0.52	0.35–0.76	2.43	0.487
	Autumn	24/5696	0.42	0.27–0.63		
	Winter	26/4005	0.64	0.43–0.96		
	Spring	17/3457	0.49	0.29–0.80		
Cattle (n = 4347)	Summer	27/974	2.77	1.87–4.06	2.58	0.461
	Autumn	38/1629	2.33	1.67–3.22		
	Winter	23/803	2.86	1.86–4.33		
	Spring	32/941	3.40	2.37–4.82		
Sheep (n = 126642)	Summer	2558/31401	8.14	7.84–8.45	4.89	0.180
	Autumn	3050/39391	7.74	7.48–8.01		
	Winter	2927/36933	7.92	7.65–8.20		
	Spring	1459/18917	7.71	7.33–8.09		

a/b: no. of infected animals/no. of examined animals

and May], summer [June, July and August] and Autumn [September, October and November]) was analyzed using χ^2 test. A statistically significant association between variables is considered to exist if the *p-value* is < 0.05.

Results

Prevalence of cystic echinococcosis in slaughtered animals

During the study period, a total of 149514 animals were slaughtered in the two abattoirs. Among them, there are 4347 cattle (2.90% of the slaughtered animals), 18525 camels (12.40% of the slaughtered animals) and 126642 sheep (84.70% of the slaughtered animals). Sheep were the most commonly slaughtered animals in this studied area.

The prevalence of hydatid cyst was 0.51% (95/18525) for camels, 2.76% (120/4347) for cattle and 7.89% (9994/126642) for sheep (Table 1). CE infection prevalence was significantly different among host species (Table 1, $P < 0.0001$) when infection combined. The prevalence by host species showed that sheep have higher prevalence than cattle and camels. Thus sheep were significantly more likely to be infected than cattle and camels (Table 1). The results demonstrated an absence of seasonal variation in CE infection prevalence for each host species as well as when infection combined. No significant variation of the CE prevalence was reported between the four seasons of the year with a *p-value* > 0.05 (Table 2). Unfortunately, the absence of data in the abattoir veterinary records concerning the number of hydatid cyst in each infected organ, prevent us to analyze the intensity of infection of this disease.

Table 3. The intensity of infection with *E. granulosus* larvae in sheep

Site	1-5 cysts	6-10 cysts	11-20 cysts	>20 cysts	Total
	Number (%)	Number (%)	Number (%)	Number (%)	
Lung	10 (52.63)	4 (21.05)	3 (15.78)	2 (10.52)	19 (35.18)
Liver	24 (68.57)	9 (25.71)	2 (5.71)	0 (0.0)	35 (64.81)
Total	34 (62.96)	13 (24.07)	5 (9.25)	2 (3.70)	54

Table 4. Fertility and viability rate of collected hydatid cysts from slaughtered sheep in Ha'il region

	Number of examined organs	Fertile cyst		Unfertile cyst	Purulent/ Calcified cyst	Total of collected cyst
		Viable	Not viable			
Liver	35	141	59	44	73	317
Lung	19	33	60	11	19	123
Total	54	174	119	55	92	440

Livestock hydatid cyst characterization

During the study period a total of 440 hydatid cysts were collected from 54 organs (liver [n = 35] and lung [n = 19]) of 46 slaughtered sheep (infected liver [n = 27], infected lung [n = 11] and simultaneous infection of liver and lung [n = 8]). Most of infected organs (62.96%) harbored 1-5 cysts each, 24.07% had 6-10 cysts, 9.25% had 11-20 cysts and 3.7% had more than 20 cysts (Table 3).

All these collected hydatid cysts were assessed for their fertility and the viability of protoscoleces. Among them, 123 (27.95%) were from lungs and 317 (72.05%) from livers. No cyst was collected from spleen or kidneys. Among the 440 collected hydatid cysts, protoscoleces were detected in the hydatid fluid of 293 hydatid cysts which corresponds to an overall fertility rate of 66.59%. For all organs with more than one hydatid cyst, at least one was fertile with the detection of protoscoleces. Examined hydatid cysts of the lungs had a higher fertility rate than those of the liver. Indeed, this rate was of 63.09% and 75.60% in liver and lung organs respectively. Out of the 147 remaining cysts, 55 (37.41%) were sterile and 92 (62.58%) were calcified or purulent (Table 4). The viability of the protoscoleces detected in the 293 fertile cysts was assessed. The overall viability rate in the examined fertile cysts was 59.38% (174/293). This viability rate

was higher in liver cysts (44.47%) than in lung ones (26.82%).

Discussion

Certainly, it is crucial to monitor zoonosis and get updated data concerning the prevalence of the disease in both human and animals in order to follow up its epidemiologic aspects. During the present study the incidence of CE in livestock at Ha'il abattoirs was found to be 6.82%. Our finding was lower than those reported in Iran, Ethiopia and Tunisia where the prevalence of animal cystic echinococcosis was 9% (for cattle and buffaloes), 32% (for cattle) and 16.42% (for sheep) respectively^{1,4-5,25}. The difference could most likely be due to the variation in the agro ecology of the study areas, the slaughtering system (as backyard slaughtering) as well as the animal husbandry systems. Moreover, prevalence of animal CE in Ha'il is the lowest one compared to those reported in Al Baha, Al Taif, Asir and Jeddah with 10.26%, 12.91%, 14.58%, 42.43% respectively^{19,21,22,33,35} (Figure 1). Nevertheless, it was higher than those reported in Riyadh (1.06%) and Al Madinah Al Munawarah (0.19%)^{2,33}.

The relatively low prevalence of CE in Ha'il region could most likely be the result of both: (i) the effort conducted in this region to control this

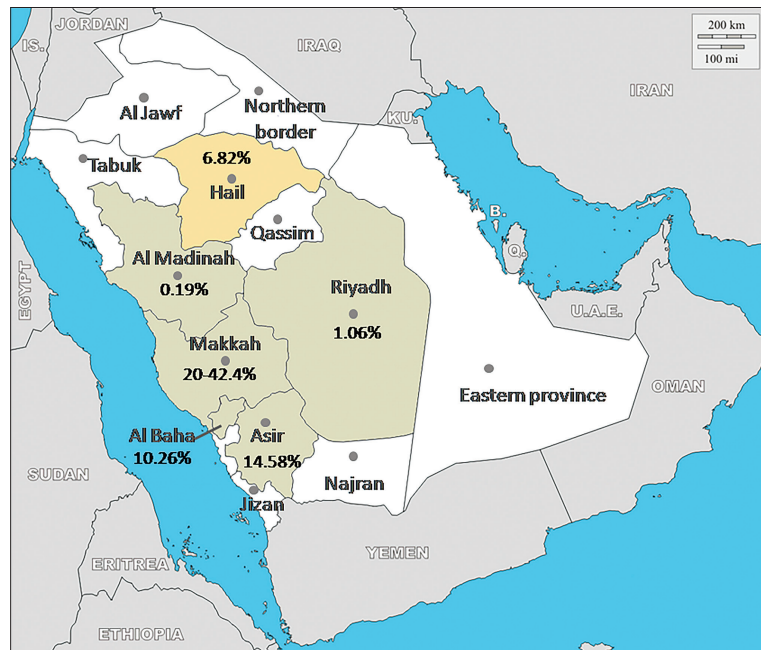


Fig. 1. Prevalence of CE among slaughtered animals in some Saudi Arabia provinces according to literature review (Riyadh²⁾, Makkah Al Mukarramah^{18,19,35)}, Al Baha²¹⁾, Asir²²⁾, Al Madinah Al Munawarah³³⁾) and in Ha'il region according to our study.

zoonosis and to minimize the contact of livestock with infected dogs and (ii) the dry climate of Ha'il which is unsuitable for the spread of this disease. Indeed, the exposure of *E. granulosus* eggs to sunlight and high temperatures leads to their desiccation within few hours and subsequently they become unable to hatch while consumed by the intermediate hosts^{11,28)}. By comparing the prevalence of CE between animal species in Ha'il region, we highlight that sheep were the most infected species followed by cattle. The camels had the lowest infection rate. The same result was already reported by Daryani *et al.*¹¹⁾ and Fakhar & Sadjjadi¹⁵⁾. The high prevalence rate in sheep may be explained by either their feeding habit (by grazing, sheep are more exposed than other animal to pick cestode eggs)¹⁵⁾ or the difference in *E. granulosus* genotypes abundance and their specificity to the host. Indeed, *E. granulosus* genus has an extensive genetic variation with 10 different genotypes (G1–G10) including G1 and G2 as sheep strains, G3 and G4 as bovid strains, G5 and G6 as horse and camels strains respectively³¹⁾.

Beside, Pestechian *et al.*³⁰⁾ reported that, in Iran, the sheep strain is the most prevalent (74.24%) followed by the bovid strain (22.72%), while the camels strain (which was recently classified as an independent species, *E. canadensis*) is the less prevalent (3.03%). Unfortunately, lack of data concerning the *E. granulosus* genotypes circulating in Saudi Arabia prevents us to support this hypothesis. Further investigations are needed to identify the genotypes of *E. granulosus* in this region¹²⁾. No significant seasonal variation for prevalence of animal cystic echinococcosis was found in the present study. The absence of association between season and cystic echinococcosis prevalence could be explained by the chronicity of CE infection and animals remain infected throughout their lives⁷⁾.

Data on the fertility and viability of hydatid cysts in various livestock animals play an important role in providing credible indicators of the importance of each livestock as a possible source of infection of definitive hosts. Usually, depending on the host species, the size and location of larval stage, hydatid cysts may have

different rates of fertility. In this regard, a number of studies have been conducted to estimate the fertility and viability rates of protoscoleces in a variety of slaughtered animals^{11,13,34}. The present study showed that most of infections (66.59%) in sheep were fertile. This finding on cyst fertility was similar to those previously recorded in Saudi Arabia¹⁹ and elsewhere²⁶. On an organ basis, the highest fertility rate was seen in the lung rather than the liver. This result was in agreement with that reported by Kedir *et al.*, 2013 in Ethiopia²⁴. The softer consistency of lung tissue compared with other organs possibly favors the development and fertility of cysts. However, our finding is in contrast with reports from Iran and Tunisia where the highest fertility rate was observed among hepatic compared with pulmonary cysts^{25,26}. This discrepancy could most probably explained by the difference in *E. granulosus* strains circulating in these countries.

Regarding human CE in Ha'il region and based on hospital records, only five cases of human CE were recorded in 2015 (unpublished data). This incidence was almost similar to that reported by the Ministry of Health (MOH) in Riyadh region which was 6 and 7 cases in 2006 and 2007 respectively²⁷. It is important to highlight that due to the very slow process of the hydatid cyst growth, the asymptomatic period is too long and CE might be diagnosed 20 to 25 years post-infection²⁹. Consequently, the number of reported cases does never reveal the current state of the disease. According to the Saudi Ministry of Health reports (2006–2013)²⁷, the reported Incidence Rate of CE in human is 0.03–0.04/100.000 inhabitants. This incidence is lower than those reported in Chile (1.4–1.8/100,000), Spain (2.1/100.000), Italy (2.4/100.00) and Tunisia (12.7/100.000)^{3,9,17,20}. This low incidence rate of CE in Saudi Arabia compared to others countries worldwide is most likely due to its climatic features which are unsuitable for the propagation of the cestode gathered with the success of control programs established by the KSA health

authorities.¹⁰

In conclusion, this study provides preliminary baseline data useful for further investigations. We found that livestock cystic echinococcosis is less prevalent in Ha'il slaughterhouses compared to the other regions of the kingdom. Also, the high fertility rate of hydatid cysts collected from sheep implies that this species is still important as a potential source of infection to dogs. Further investigations such as the genotyping of the circulating *E. granulosus* strains are crucial to monitor this important zoonosis.

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Conflicts of interests

This work has no conflict of interest

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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