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ORIGINAL ARTICLE

Epidemiological study of *Echinococcus granulosus* in sheep in the Gharb plain (North-West of Morocco)

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Abstract Hydatidosis is considered endemic in Morocco and caused serious health problems in ruminants. The aim of this research is to present an epidemiological study on echinococcosis in sheep. The study was carried out between April 2016 and May 2017. A total number of 1600 sheeps was examined via autopsy for *Echinococcus granulosus* in various slaughterhouses in the Gharb plain of Morocco. The Results of the study showed that the prevalence of cystic echinococcosis was 7.63%. The infection was not significant among sheep which age is < 1 year (2.67%), while it increases among those which age is from 1 to 3 years old (8.63%). It also grows significantly among sheep which age is over 3 years old (11.71%). Parasitized at 62%, the liver is the most affected organ followed by the lungs (38%). Protoscoleces are only found in liquid cysts and contribute to their fertility which is estimated at 66.66% in the liver and 57.74% in the lungs. In addition, the prevalence of calcified cysts is 12.24% in the liver and 21.11% in the lungs. Examination of the fertile cysts revealed a high level of Protoscoleces viability, whether it's in the liver (65.93%) or in the lungs (59.30%). This study also shows how the evolution of echinococcosis is closely linked to sex and age.

Keywords Sheep · Hydatid cysts · *Echinococcus granulosus* · Protoscoleces · Viability · Fertility · Gharb plain · Morocco

Introduction

Hydatidosis or echinococcosis is one of the most frequent zoonotic diseases in the world, particularly in the endemic areas represented by all sheep-farming countries, mainly in the regions with limited economic resources (Otero-Abad and Torgerson 2013). It is related to the development of the larval form of the dog's taenia, *Echinococcus granulosus* (*EG*), class Cestoidea, family Taeniidae in various organs (Chaligiannis et al. 2015; Scioscia et al. 2016).

In Morocco, this disease represents a real scourge for livestock and public health (Chebli et al. 2017). In 2012, 1627 human surgical cases (5.2 cases per 100,000 inhabitants) were recorded for the whole country (Delm 2012). The cost of treatment was estimated to be approximately US$ 1700 and US$ 3200 for simple and repeat cases respectively; a financial burden to the health sector (Anderson et al. 1997).

Sheep are considered as intermediate hosts with a highly reception that develop in their internal organs, usually in their livers and lungs, hydatid cysts which become fertile and potentially infective for the dogs. It is by feeding the viscera of parasitic ruminants that the dog is infested by the Echinococcus (Tapeworm). The adult worm lives in the small intestine of the carnivore (definitive host) and the intermediate larval stage develops in the internal organs of many mammalian species (intermediate hosts), including humans, who contract the infection by accidental ingestion of tapeworm eggs. Access to infected offal appears to be one of the most commonly identified risk factors for canine

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infection with *E. granulosus* (Carmona et al. 1998; Moro et al. 1999; Buishi et al. 2006; Acosta-Jamett et al. 2015).The transmission of (*EG*) primarily maintained with many wild canids as definitive hosts and livestock animals as intermediate hosts (Scioscia et al. 2016).

In 2015, the WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) estimated that echinococcosis accounted for 19.300 deaths and approximately 871.000 disability-adjusted life years (DALYs)[1] annually in the world. Annual costs attributable to cystic echinococcosis are estimated at $ 3 billion by estimating case treatment and losses for livestock industry (WHO/echinococcosis/mediacentre/factsheets; 2017).

In recent years, many research projects have been conducted in different parts of Morocco to collect as much data as possible on the pathogen (*EG*) and to plan a strategy for controlling this disease. In the same context, we carry out this investigation to determine the profile of hydatidosis and its pathogen, as well as assess the epidemiological situation in Gharb plain of Morocco.

Materials and methods

Study area

The study was carried out in many slaughterhouses with an average of two visits per month for each slaughterhouse. 1600 carcasses of sheep were subjected to post-mortem inspection for hydatid cysts in the viscera. In the ante-mortem examination, age and sex were recorded for each animal. The study environment is known for its warm and temperate climate. During the summer season, there is less rainfall than in winter. This climate is classified as Csa by the Köppen–Geiger climate card (warm temperate climate to warm, dry weather). The average temperature in Kenitra is 18.4 °C and the average annual rainfall is 570 mm.

Study animals and sampling methods

During a 12 months period (from May 2016 to April 2017) Post-mortem examination was carried out immediately after slaughtering of the sheep. This study was conducted on males and females at different ages. The presence or absence of the cyst was established by palpation and incision of each organ. The recovered cysts were completely emptied of their liquid by aspiration using a syringe. The cysts that contained hydatid sand were considered as fertile, and those which did not contain them as sterile. Fertile cysts were subjected to a viability test; the viability of the protoscoleces were assessed using 0.1% aqueous solution of eosin staining; all protoscoleces that retained staining were considered dead while unstained protoscoleces were considered viable (Dalimi et al. 2002; Moazeni and Nazer 2010). The study is finalized by performing histological sections of hydatid cysts to determine its different components.

Data analysis

The data collected from antemortem, postmortem and laboratory findings were entered into a Microsoft excel worksheet and analysed using the SPSS 20, the prevalence of EC was calculated as the number of EC infected individuals divided by the total number of Examined animals and was than multiplied by 100. Chi square (χ^2) test was applied for comparison of infection rate with regard to the factors like age, sex and seasons. ($P < 0.05$) was considered significant.

Results and discussions

Prevalence of *Echinococcus granulosus* infection in sheep

Out of the 1600 sheep examined, 122 had hydatid cysts in lungs and lever, giving an overall prevalence of (7.63%). Comparing this prevalence with the ones found in previous surveys in Morocco, we find that it is much lower than the one recorded by Azlaf and Dakkak (2006), who found a value of (11.14%) in the middle atlas or by Belkourati (2010) who found (11.13%) in a survey conducted in Sidi Kacem (west of Morocco). On the other hand, our result percentage is higher than the one recorded by Azlaf and Dakkak (2006) who reported a value of (1.42%) in the south and (2.66%) in the center of the country. Compared to other countries, we notice that our infestation rate is lower than the one recorded in Egypt by Osman et al. (2014) (8.06%). However, our result is significantly higher than the one recorded by Almalki et al. (2017) viz (1.06%) in Riyadh city, Saudi Arabia.

The lower prevalence in (Gharb plain) compared to other locations could be attributed to the region and the mode of rearing, variations in feeding behaviors of the animals and the Raising awareness against zoonotic disease as well as to the progressive generalization of echinococcosis vaccines in rural areas.

Seasonal prevalence of hydatid cyst in slaughtered sheep

The results in Fig. 1 showed that the highest prevalence was recorded in spring (26.23%) followed by winter

[1] One disability-adjusted life year (DALY) can be thought of as one lost year of "healthy" life.

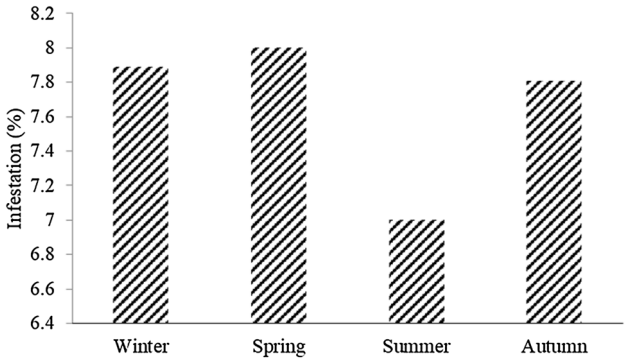

Fig. 1 Mean seasonal prevalence of hydatid cyst in slaughtered sheep

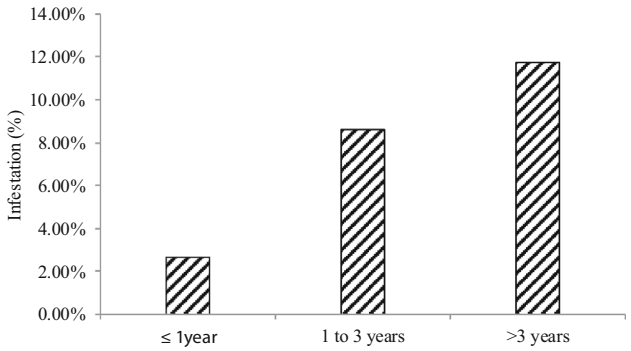

Fig. 2 Mean Prevalence of hydatid cyst according to age of slaughtered sheep

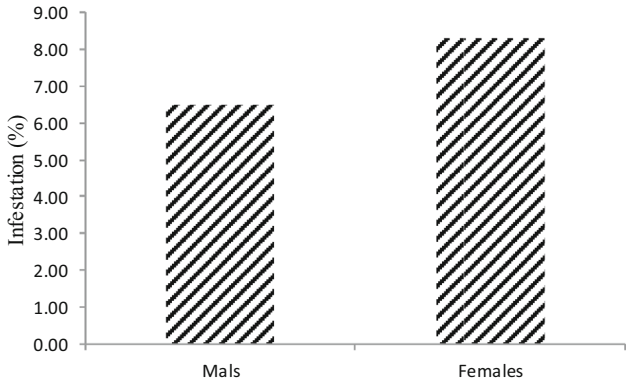

Fig. 3 Mean prevalence of hydatid cyst per sex of slaughtered sheep

(24.59%) and autumn (20.49%), while the lowest prevalence was reported in summer (28.69%). This study shows that the rate of echinococcosis infestation according to the season is not significant, ($P = 0.05$). These results are consistent with those found by Elmajdoub and Rahman (2015) among sheep slaughtered in Libya. In comparison, Daryani et al. (2007) reported significant differences in the prevalence of infestation between winter and autumn in Iran. Moreover, Ibrahim (2010) found significant differences in infection rates between autumn and spring in Saudi Arabia. These variations could be explained by changes in climatic factors in each country, such as rainfall, temperature, humidity, and the nature of the pasture (Ibrahim 2010; Elmajdoub and Rahman 2015; Almalki et al. 2017).

Hydatidosis in sheep according to age

The age of the host has been largely recognised as an infection determinant for many farm species (Islam et al. 2003; Lahmar et al. 2007; Christodoulopoulos et al. 2008).

The results obtained showed a proportional increase in hydatid cyst infestation depending on the age of the autopsied sheep. Indeed, sheep over than 3 years old reveled an infestation rate of (11.71%), 8.63% in animals of 1–3 years, while lambs did not exceed 2.67% (Fig. 2). The very low rate of infestation in lambs is due to the slow development of hydatid cysts, as they need 4–13 months to have a few millimeters in size.

Hydatidosis in sheep according to sex

According to the results obtained and illustrated in Fig. 3, the rate of infestation by echinococcosis in females (68.03%) (83/122) is higher than in males (31.97%) (39/122). This difference seems to be very closely related to the age of the animals, since in general, males are slaughtered when young (< 1 year), unlike females, which are usually slaughtered for reasons of reform at a later age. Furthermore, during the gestation periods, immunity in females becomes low, thus favoring the risk of infestation.

Several surveys of hydatidosis in sheep in different countries have produced results similar to ours; e.g. China (Ming et al. 1992) eastern Libya (Tashani et al. 2002), and Iran (Daryani et al. 2007). However, a study in Ethiopia found that small male ruminants were significantly more susceptible to infection than females (Erbeto et al. 2010).

Localization of Hydatid cysts

Hydatidosis is usually associated with the presence of cysts in the liver; the examination of cyst location indicated that the liver is the most infected organ followed by the lung, the prevalence was 62 and 38%, respectively. Similar results were reported by Bardonnet et al. (2003) in Algeria, Azlaf and Dakkak (2006) in the Rif region of Morocco, Haridy et al. (2006) in Egypt, Ibrahim (2010), and Toulah et al. (2012) in Saudi Arabia. These preferential localisations can be explained by the migration of embryophores. Indeed, when leaving the intestine, the embryophore wins the liver, then the right-side heart, then the lungs and left-side heart from which they can move to other organs and

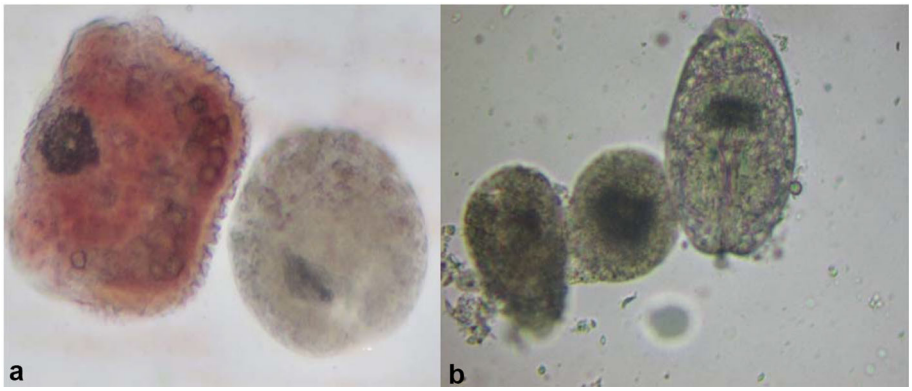

Fig. 4 Representative photomicrographs for viability test; **a** dead protoscoleces after staining with 0.1% eosin; **b** viable protoscoleces after staining with 0.1% eosin (× 100)

tissues by aortic way (Al-Khalidi 1998). In contrast Azami et al. (2013) found that the infestation of the lungs is greater than in the liver among slaughtered sheep in Iran.

Nature of Hydatid cysts in sheep

The nature of Hydatid cysts (fertile and viability of Protoscoleces) is a credible indicator of the importance of each livestock as a possible source of end-user infection, particularly dogs.

Fertility of Hydatid cysts

In 98 livers and 60 lungs (in cases of a mixed infection, the organs were counted separately), fertility was confirmed by the presence of Protoscoleces, the results obtained are shown in Table 1.

Generally, the Hydatid cysts have different fertility rates depending on their size and location and the type of host (Elmajdoub and Rahman 2015). According to our results exhibited in Table 1, the liver cysts are more fertile (66.95%) than pulmonary cysts (50.42%) while sterile cysts are higher in the lungs (33.61%) than at the liver level (26.31%). Also, we recognize a significantly higher rate of calcified cysts in the lungs (15.96%) than in the liver (6.73%). The same conclusion was reached by Boudguigue (2009) in the region of Sidi KACEM with a fertility rate of 54.6% (Dalimi et al. 2002; Ibrahim 2010; Elmajdoub and Rahman 2015). The presence of non-fertile cysts (calcified or degenerate) indicates that the animal has had an old infestation or has a better defense with early calcification of cysts and faster evolution to non-infesting stages.

The Protoscoleces viability

According to the hepatic or pulmonary localization, the study of the viability of Protoscoleces, Fig. 4 revealed a viability rate of Protoscoleces in the liver of 65.93% higher than that in pulmonary cysts (59.30%) (Table 2).

Comparing our results with those recorded in other countries, we notice that they do not match those recorded in Egypt by Osman et al. (2014) or in Iran by Shahnazi et al. (2016) who reported a high viability rate at the level of the lungs, [respectively (75%) and (55.3%)] than in the liver [respectively (66.7%) and (46.49%)]. However, research done in Mauritania and Tunisia respectively by Ould-Ahmed Salem et al. (2010) and Lahmar et al. (2013) support our results.

The histology of hydatid cysts in sheep

A histological section that was performed on hepatic and pulmonary cysts to study their structure allows us to distinguish a layer on which protoscoleces are fixed is the germinal layers (proliferous membrane) surmounted by a second parietal layer; is an anhistic cuticle (Fig. 5).

Table 1 Fertility of Hydatid cyst recovered from liver and lungs of slaughtered sheep

Infected organs	No. of infected organs	No. of examined cysts	Liquid hydatid cyst		Calcified cysts
			Fertile cysts	Sterile cysts	
Lever	98	342	229 (66.95%)	90 (26.31%)	23 (6.73%)
Lungs	60	238	120 (50.42%)	80 (33.61%)	38 (15.96%)

Table 2 Viability rate of Hydatid cysts in sheep

Parasitized organ	Average volume of Hydatid cyst (ml)	Average number of protoscoleces (ml)	Average number of live protoscoleces (ml)	Viability rate of protoscoleces (ml) [%]
Liver	20	91	60	65.93
Lungs	20	86	51	59.30

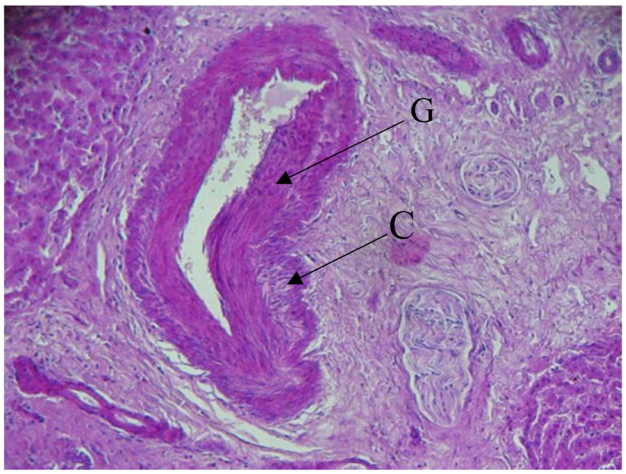

Fig. 5 Histological section of sheep liver with echinococcosis (× 100). C: anhistic cuticle. G: germinal layers

Conclusion

It is concluded that Hydatidosis in sheep in Gharb plain of Morocco is characterized by the dominance of hepatic localisations followed by lungs localisations. The high rates of fertility of hydatid cysts and viability of protoscoleces are worthy of consideration because it is the fertile cysts with living protoscoleces that preserve transmission of the disease. The prevalence of hydatid cysts and other liver and lungs lesions in sheep need more joint work with medical and public health researchers in human zoonosis in and out slaughterhouses. Our investigation provides preliminary information for the future monitoring of these potentially harmful parasitic diseases.

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