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Comparative Aspects on the Fertility and Viability of Hydatid Cysts in Sheep from North Jordan

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Introduction

Hydatidosis is a global cyclozoonotic disease of increasing importance in both humans and animals (Matossian *et al.*, 1977). The disease is endemic or hyperendemic in various Middle Eastern countries including Jordan (Sliman, 1976; Matossian *et al.*, 1977; Dajani and Shihabi, 1979; El-Muhtaseb, 1984; Shennak *et al.*, 1985). Various domestic herbivores were found to act as intermediate hosts for *Echinococcus granulosus* and varying infection rates have been reported in sheep, goats, cattle, camels, pigs and donkeys from Jordan and nearby countries (Pipkin *et al.*, 1951; Babero *et al.*, 1963; Luttermoser and Koussa, 1963; Daily and Sweatman, 1965; Hassounah and Behbehani, 1976; Dajani, 1978; Al-Abbassi *et al.*, 1980; Dajani and Khalaf, 1981; Wajdi and Nassir, 1983; Al-Yaman *et al.*, 1985; Abdel-Hafez *et al.*, 1986). Most of these studies, however, were concerned with fertility rates with regard to the number of infected animals that contained fertile cysts. Occasionally, the number of fertile cysts compared to total cyst load in organs of infected animal was determined (Abdel-Hafez *et al.*, 1986). Although such information is quite important, it is not sufficient for determining the importance of various intermediate hosts in perpetuating the cycle of the disease. The number of protoscolices in each cyst and the viability of such protoscolices are of prime importance for studies pertaining to the role each possible intermediate host could play in the epidemiology of the disease. In the present study, we report on the infectivity rates, number and viability of protoscolices recovered from the lungs and livers of sheep infected with hydatid cysts.

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Materials and Methods

All sheep used in this study were females slaughtered at Irbid slaughterhouse in North Jordan. All animals were indigenous to the area and originated from small farms in various villages scattered around Irbid. The age of each sheep was estimated through the owner of the animal and/or teeth examination. Infected organs were removed immediately following slaughtering and skinning of the animal and brought back to the laboratory within 1 hour. Cysts were measured and counted after freeing them from the surrounding tissue. The hydatid fluid was recovered and the internal membranes were washed 3–6 times either with the hydatid fluid aspirated from the cyst or with sterile phosphate buffered saline (PBS, pH 7.2). The protoscolices were counted using aliquots of 100 μl spread on microscope slides, and were examined under a compound microscope. The average of at least 3 counts was calculated and the number of protoscolices in each cyst was

estimated accordingly. In the first step the viability rate was determined by counting protoscolices that showed actively beating flame cells under the compound microscope. At least 200 protoscolices were counted for each slide and the mean viability rate was calculated after the examination of 3 slides prepared from each cyst. However, this method could not be used for this study and it was ascertained that dead protoscolices are distinctly smaller and brownish in colour than viable ones and the viability rates were based on counting large and small protoscolices.

Results

Table 1 shows the relationship between the diameter of hydatid cyst and the mean number of protoscolices (MNP) in the cysts isolated from the livers and lungs of infected ewes of two different age groups. Obviously, the total MNP per liver cyst increased with increasing cyst diameter regardless of the location of the cysts. However, the MNP in liver cysts was always significantly greater than that in lung cysts of equivalent diameter. For example, the MNP for liver cysts less than 3 cm in diameter was about 2 times that for lung cysts of comparable sizes. This ratio was even greater in cyst sizes between 3–5 cm in diameter in which the MNP per liver cyst was about 3 times that of the lung cysts. These differences were reflected in the total MNP obtained from the two organs. Thus, the MNP per cyst in the livers exceeded 2.5 times that of lung cysts (85.5×10^3 and 33.2×10^3, respectively). Within the cyst size range studied in the livers and lungs (up to 6 cm of diameter), two distinct patterns for protoscolices load in growing cysts were observed. Figure 1 illustrates the total growth rate of the cysts as reflected by the MNP counted for each cyst size category. Evidently, lung cysts showed a slower total growth rate than liver cysts. Both organs showed an initial slow phase which was extended up to cyst size of 4 cm in diameter in case of lung cysts. In the livers, a dramatic increase in the total MNP occurred in cyst sizes between 3–5 cm in diameter. This was followed by a plateau. On the other hand, a steady increase in the protoscolices load per cyst occurred throughout the size range of lung cysts studied (Fig. 1). When the fertility rates were compared with regard to age groups, it appeared clear that the liver cysts in younger age groups (less than 8 years) sustained a significantly higher number of protoscolices per cyst than comparable cyst sizes in the older age group (for all cyst sizes above 2 cm in diameter, Table 1, Fig. 1). Moreover,

Table 1 Relationship between Hydatid Cyst Diameter and the Mean Number of Protoscolices in Two Age Groups of 51 Infected Ewes from North Jordan

Cyst diameter (cm)	Mean total number of protoscolices ($\times 10^3$) per cyst of					
	Liver			Lung		
	less than 8 years	8 years or more	Total	less than 8 years	8 years or more	Total
<2	2.1(6)	12.6(15)	9.6(21)	3.4(8)	8.1(6)	5.4(14)
2–2.9	37.3(13)	22.5(35)	26.5(48)	7.1(13)	18.7(31)	15.3(44)
3–3.9	144.3(12)	67.7(19)	97.3(31)	40.8(8)	28.4(26)	31.3(34)
4–4.9	246.6(14)	162.0(6)	221.3(20)	75.0(1)	89.6(12)	88.5(13)
5–5.9	291.2(6)	158.0(2)	232.9(8)	–	120.7(6)	120.7(6)
Total	145.7(51)	46.1(77)	85.8(128)	17.2(30)	39.1(81)	33.2(111)

Figures in parenthesis refer to number of cysts examined.

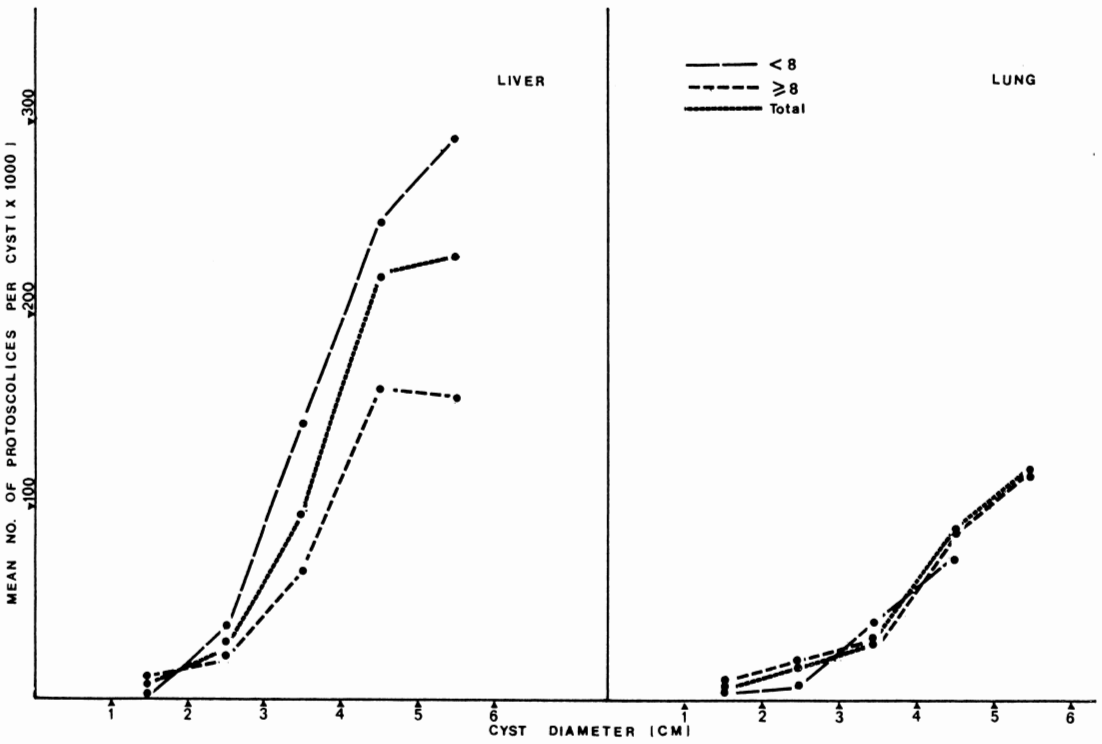

Fig. 1 Relationship between cyst diameter and mean number of protoscolices for liver and lung cysts in two age groups of ewes infected with hydatid cysts. Number of cysts examined in each size category is provided in Table 1.

while the protoscolices load of cysts in ewes less than 8 years of age did not show a plateau in cyst sizes above 4 cm in diameter, older age groups showed a distinct plateau in comparable cyst sizes. These observations were quite different in the case of lung cysts in which there was no significant difference between the two age groups throughout the cyst size range studied, and the mean number of protoscolices continued to rise in both groups (Fig. 1).

Despite the obvious differences in the number of protoscolices sustained in the two different organs studied in sheep, there was no significant difference between the percent viability of protoscolices obtained from these two locations (Fig. 2). The mean viability rate in the livers and lungs was 66% and 71%, respectively.

Discussion

This study has focused on the degree of fertility and viability of hydatid cysts recovered from the lungs and the livers of infected sheep with regard to the number of protoscolices per cyst. This report complements our earlier finding regarding the significance of sheep as a major intermediate host for the perpetuation of the life cycle of *Echinococcus granulosus* in Jordan (Abdel-Hafez *et al.*, 1986). In the previous report, fertility was assessed on the basis of the percentage of animals with fertile cysts among all cysts recovered from infected animals. Although such information is quite important, the true representation of fertility in terms of the importance of disease transmission should be determined by the number of viable protoscolices that the cysts can sustain and that could be passed on subsequently. Although significant differences exist between the livers

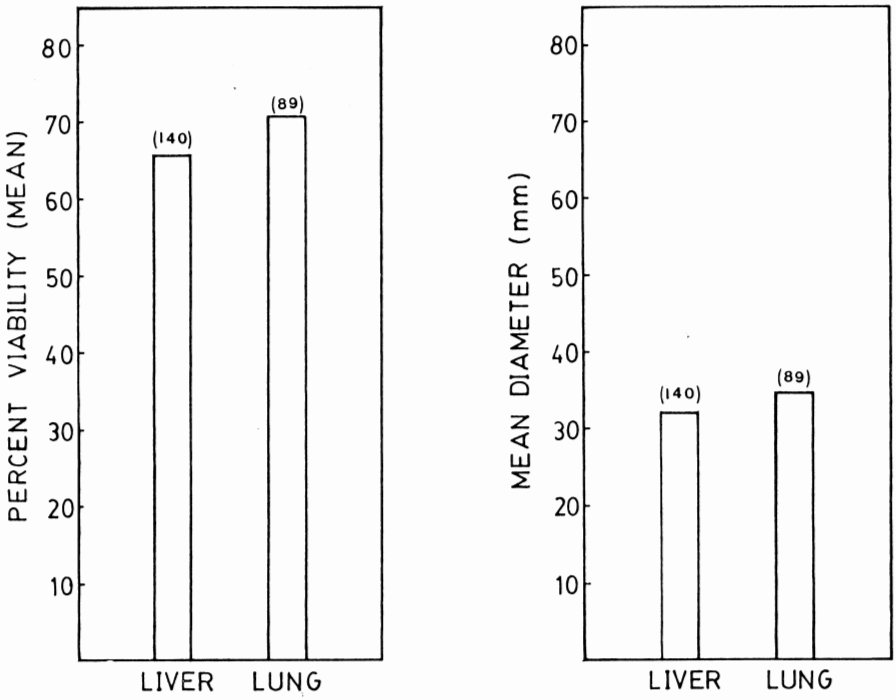

Fig. 2 Mean diameter and viability rates of hydatid cysts from liver and lung of infected ewes from North Jordan. Number in parenthesis represents the number of cysts examined.

and lungs as major sites for hydatid cysts in sheep, the two organs sustain a large number of protoscolices (Table 1). This fact, together with the high rate of viability of protoscolices recovered from either of the two organs (Fig. 2), reinforces the importance of the sheep in the endemicity of hydatidosis in Jordan.

With regard to the development pattern of the cysts, liver cysts showed an increase in the number of protoscolices as the cyst increased in size until 5–6 cm in diameter. This was different in the case of lung cysts wherein a steady increase in cyst size was accompanied by a continued increase in the mean number of protoscolices (Fig. 1). The plateau phase recognized in liver cysts was obvious in the older age group (>8 years) which was reflected in the growth curve of both age groups. As this phase did not occur in the lungs, the difference in the developmental pattern between the two organs might be a reflection of the type of tissue in which the cyst is located. Liver cysts over 2 cm in diameter recovered from the older age group did not sustain as high a number of protoscolices as those from the younger age group (Fig. 1). This may indicate that older animals contain older cysts in which some of the protoscolices have disintegrated as the cyst is aging. Interestingly, liver cysts of smaller size (less than 2 cm in diameter) recovered from the older age group contained a greater number of protoscolices than comparable cysts from the younger age group.

Similar studies pertaining to the fertility and viability of hydatid cysts in other domestic animals which can act as intermediate hosts for *E. granulosus* is urgently needed. Of particular importance is the situation of imported and indigenous cattle in Jordan with regard to infection rate with hydatid cysts. Indigenous cattle have a high infection rate (Al-Yaman *et al.*, 1985; Abdel-Hafez *et al.*, 1986) in contrast to imported ones which displayed low fertility in terms of both the percent of fertile cysts

recovered and the mean number of protoscolices per cyst (data not shown). If such cattle are truly resistant or unsuitable hosts for the growth of hydatid cysts, then breeding such highly infected indigenous cattle with the imported ones is of obvious value in decreasing the susceptibility of local cattle to infection with hydatid cysts. Indeed different fertility rates in intermediate hosts such as cattle from different origins have been reported (Thompson *et al.*, 1984). Moreover, studies of the fertility and viability rates of protoscolices in other intermediate hosts is of obvious value in determining other key hosts for the perpetuation of the disease cycle and is important when one is considering an overall control programme of the disease in Jordan.

Summary

Hydatid cysts from infected ewes of various age groups were studied and the fertility and viability of protoscolices were analyzed in their livers and lungs. In cysts smaller than 5 cm in diameter, the number of protoscolices per cyst increased as the size of the cyst increased regardless of the location. However, the liver cysts seemed to sustain a greater number of protoscolices than lung cysts of equivalent diameter. Moreover, liver cysts obtained from the younger age group (<8 years) sustained a significantly higher number of protoscolices than those of equivalent size obtained from ≥8 year old ewes. There was no significant difference between the viability rates of protoscolices separated from the two organs. The liver seems to be a more suitable organ for the development of hydatid cysts than the lung.

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北ヨルダン産ヒツジに寄生する包虫の生殖と生育

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種々の年齢グループの雌ヒツジに寄生する包虫について，肝および肺内の原頭節の生殖能と生育能をしらべた．直径5㎜以下の包虫内の原頭節数は寄生部位に関係なく包虫の大きさに比例して増加した．しかし肝内の包虫は同じ大きさの肺内の包虫より多数の原頭節を保有する傾向が見られた．また，8才以下の若いヒツジの肝内の包虫は，8才以上のヒツジの肝から得た同じ大きさの包虫より有意に多数の原頭節をもっていた．肝の包虫内の原頭節の生育率と肺の包虫内の原頭節のそれとの間に有意差は認められなかった．包虫の発育には肺より肝の方が好適であるように思われた．