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# FIELD INVESTIGATION ON CYSTIC HYDATID INFECTION IN MAN AND CAMEL IN TAMBOOL TOWN AND KHARTOUM STATE HOSPITALS SUDAN

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## ABSTRACT

*Echinococcosis/hydatidosis is a zoontic disease. The disease is caused by adult worms and larval (metacestode) stages of the taeniid cestode Echinococcus granulosus. The life cycle is completed into two hosts. The final host usually carnivore e.g. dog, and the intermediate host usually herbivorous and man.* ***The aim*** *of this study is to evaluate the fertility of cysts and viability of protoscoleces in man and camel hydatid cysts in order to determine the prevalence of their fertility and to study the association with variables typical of host and of the parasite.* ***Material and methods:*** *Across-sectional and observations study was done in biological material (the fluid from human and camel hepatic and lung hydatid cysts). The viability criteria used were ovoid form invaginated scolices and intact calcareous corpuscles. The present of vibrating movements, and the absence "vital" staining, the cysts were grouped in diameter cysts from small cysts to very large size of cysts. Descriptive statistics were for the calculation of the prevalence of fertility, analytical statistics for comparison of groups, and multivariate analysis for examination of the association between cyst fertility and clinical variables.* ***Results:*** *A total of 99 cysts with less than 2 cm 5 (7.8%), from2 - 6 cm 52 (81.2 %,) medium diameter 6-10cm 37(57.8% ), and more than10 cm 5(7.8), and prevalence of general fertility 68%,sterial cysts was 6%, supporative was 10% and calcified was15%. Most of camel fertile cysts had medium size (81.2%), while the lowest rate belongs to the small cysts (7.8%). There was significant relationship between fertility of the cyst, type of infected organs, type of animals and size of cyst. Association with location, type, and diameter of the cyst, the viability Protoscolecess was assessed by microscopic observation of biological feature represented by muscular movements, flame cell activity in 0.1% aqueous eosin stain.* ***Conclusion****: The differences between prevalence rates, the fertility of hydatid cysts and diversity sites localization observed in humans and camels. Fertility is associated with the type of cyst, location type, type of animal and size of cyst.*

**KEYWORDS:** *hydatidosis, Field investigation, hydatid cyst, Sudan*

## INTRODUCTION

Cystic *Echinococcosis* (CE)/ Hydatidosis is one of important zoontic diseases caused by larval stage of the dog tapeworm, *Echinococcus* granulosus. Hydatid disease is most extensively found in East and North Africa. India, Australia, New Zealand, South America, and Middle East including Iran. [1 -9].The rate of the infection in carnivores such as dog and herbivores such as sheep, camels is significant. Also human accidentally infections. [10 -16], Hereby, hydatidosis is a major health – economic problem, that has become one of the WHO`s active plans for controlling the disease [17].

Larval in man caused hydatid disease, adult worms are only seen in definitive host, dogs, and they cannot develop in man. When the ova are ingested by a suitable intermediate host, they hatch in the duodenum and the onc0sphere migrates to the blood stream where it is carried to the liver and others organs of the body. Here it develops into hydatid cyst which consists of an outer thick laminated cyst wall and an inner layer, from the inner layer brood capsules are produced which contain protoscoleces. Fertile hydatid cysts with viable protoscoleces in intermediate hosts are important factors in transferring infection, and stray dogs that wandering around abattoirs; spread the disease due to feeding on infected organs. These factors which differ according to the geographical situation, host and type of infected organs, affect on Echinococcus cycle persistence [17]. Therefore the aim of this survey was to evaluate the fertility of hydatid cysts and viability of their protoscoleces based on the site, size and type of cysts in slaughtered animals in Tambool, central eastern region of Sudan.

## RATIONALE

Hydatidosis is known to occur in Sudan in man as well as animals, but the extent of the disease in human population is not known. This is mainly due to the nonspecific clinical presentation and the nonexistence of a sensitive and specific diagnostic methods, a part from confirmation at operation. Therefore this study was undertaken to study the epidemiology of the disease among the human population and to extract antigenic constituents from hydatid cysts in man and attempt to analyses them and use them as targets for different serological tests for the laboratory diagnosis of hydatidosis in man

## OBJECTIVES

### General objective:-

- To carry out the field investigation of hydatidosis/*Echinococcus* in Tambool area (The Central Region) and man at hospitals of Khartoum state.

### Specific objectives:-

To collected the cysts from internal organs (liver, lungs and other organs) and removed the tissue by scalpel, washed by normal saline five times, aspirate the fluid by syringing in tube 50ml and centrifuged to separate the protoscoleces and large particle.

## MATERIALS AND METHODS

### Study Design:-

It is an observational, cross sectional study, was done in biological material (the fluid from human and camel hepatic and lung hydatid cysts).

### Study Area:-

The study was conducted in Tambool town market (Central Eastern of Sudan) which is located 150Km South East of Khartoum. Al butane area is part of the Central rain lands that provides good grazing for camels, sheep, goats, and cattle stretches from the Ethiopian border in the East to Gezira State in the West roughly occupying the area between isohyets 400 and 700mm. It comprises 120,000 square kilometers and lies between latitude 13.5∘–17.5∘ N and longitude 32.4∘–36.0∘ E. It is situated in the rich savanna environment, and second area at hospitals in Khartoum state, and other private clinics. Specimens were taken from patients who have been already operated or suspected to have hydatidosis.

### Sample Technique:-

The samples collected from 200 slaughtered camels, and fourteen cases were aspirated (table 4); (10 liver aspirates, 2 lung aspirate, one abdominal aspirate and a spleen aspirate). Removed hydatid cysts were counted and classified according to the type. Macroscopic characteristics (calcified, suppurative and active), and size (small < 2cm), medium (2 – 6), large (6 -10) and very large (> 10). The fluid of active cysts was aseptically aspirated and transferred separately into tubes. After being washed with normal saline, the cyst fluid was centrifuged at 500rpm for 5 minutes. The precipitate of each sample was observed under a light microscope for cysts. Cysts with no protoscoleces were considered as sterile. Using staining with an aqueous solution of 0.1% eosin together with considering the motility of flame cell, viability of protoscoleces was assessed in fertile cysts. Viable protoscoleces did not take stain up whereas the dead ones did.

**Study period:** the survey was carried out from November 2011 - July 2013

Data was collected by attendant the patient or suspected patient for hydatidosis to the hospital and administered questionnaire information included information of (number, age, sex, marital status,

education, level-economic). And for camel at Tambool slaughter house.

## Ethical Consideration:-

Ethical approval for the study was obtained from the Ethical Committee of the federal ministry of health, and permission was provided from all hospitals where investigation was conducted. in addition and informed consent was obtained from each participant prior to interview.

## Statistical Analysis:-

Data were analyzed by using the Statistical Package for Social Science (SPSS). Presented in tables and figure.

## RESULTS

The study included a total number of 200 slaughtered camel cysts 53 (26.5), (Table 1) and 14 aspirated from (lung and liver) patients 4 (28.6%).

**Table 1: The infection rate of hydatid cyst in camels slaughtered at Tambool during 2011 - 2013**

| Animal species | No. of animal examined | No. of infected animal | Rate of infection | Total no. of cysts counted | Location of cysts | |
|---|---|---|---|---|---|---|
| | | | | | Lung | Liver |
| Camels | 200 | 53 | 26.5% | 99 | 90/ 90% | 9/ 9% |

Fourteen cases were aspirated (table 2);

**Table 2: Aspiration for hydatid cyst among human**

| Location | Lung | Live r | Spleen | Abdominal | Total |
|---|---|---|---|---|---|
| Positive | 2 | 2 | | | 4 |
| Negative | 3 | 5 | 1 | 1 | 10 |
| Total | 5 | 7 | 1 | 1 | 14 |

(10 liver and2 lung aspirates, one abdominal aspirate and a spleen aspirate). Out of the 8 male samples, 2 were found positive while out of the 6 female samples, 2 were found positive. One from the two positive females aged 16 years with a large cyst (9.8x9.6x8.9cm) in the right lobe. This was detected by CT scan. The other female was 42 years old with a lung hydatid cyst.

From 8 patients in Khartoum state there were 2 patients positive for hydatid disease. One of 2 patients from Elgedarif state had hydatid cyst in the liver while one 14 year old boy from Elgezira state had hydatid cyst in the lung. All cases from southern and western Darfur were negative for hydatid cyst

## EXAMINATION OF CYST FERTILITY & VIABILITY OF PROTOSCOLECES

The pressure of the cyst fluid was reduced by using a sterile hypodermic needle. Then the cyst wall was transformed to sterile container and examined microscopically 40 x for the present of protoscoleces. Similarly, the germinal layer was put in glycerin between two microscopic glass slides and examined for the presence of protoscoleces. Cysts which contained no protoscoleces as well as heavily supurative or calcified were considered infertile. Fertile cysts were subject to viability test, drop of the sediment containing protoscoleces was placed on the microscopic glass slide and covered with a cover slip and observed for amoebioed like peristaltic movements with 40x objective. The viability of protoscoleces was tested for each fertile cyst per human camel and organ. For clear vision a drop of 0.1aqueous eosin solution was added to equal volume of protoscoleces, of hydatid fluid on slide with the Principle that viable protoscoleces should completely or partially exclude the dye, while the dead ones taken up **(Figure).**

## Figure legend:-

**Figure:** shows the viable protoscoleces by staining with an aqueous solution of 0.1% eosin

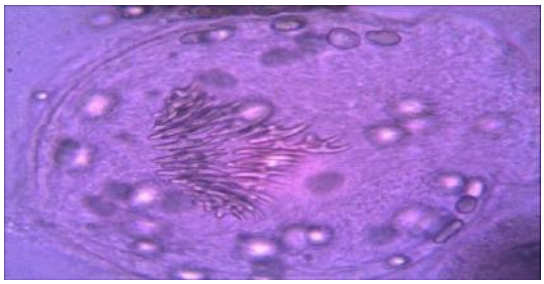

**Figure 1:** shows the viable protoscoleces stain by staining with an aqueous solution of 0.1% eosin. .

Most of camel fertile cysts had medium size (81.2%), while the lowest rate belongs to the small cysts (7.8%). There was significant relationship between fertility of the cyst, type of infected organs, type of animals and size of cyst,

Also significant difference was observed between viability of camel (lung and liver) cyst protoscoleces (64.9% and 4.0%) respectively. The highest and lowest viability rate seen in small and high size of the cyst. Calcified cysts 15 (15.1) and suppurative cysts 10 (10.1) were the most common type of cysts (15.1%), and sterile were least (5.1%), fertile and suppurative cysts allocated the highest percentage of infection. The highest rate of fertility was revealed in camel lung (64.6%), and the lowest in the liver (4.0%). Most of sterile, suppurative and calcified cysts were found in camel lung (30.3%), and least were found in the liver (5.1%) the fertility cysts rate and viability of protoscoleces show in (table 3)

**Table 3: Characteristic of human and camel hydatid cysts**

| Intermediate host | Organ involved | Total nom. of cyst | Average of cyst/organ | Tt.num. of fertile cyst | T.num. of sterile cyst | T.num.of. supurative cyst |
|---|---|---|---|---|---|---|
| **Human** | Liver | 2 | 1 | 2 / 16.7% | 3 / 25% | 2 / 16.7% |
| | Lung | 2 | 1 | 2 / 16.7% | 2 / 16.7% | 1 / 8.3% |
| **Camel** | Liver | 9 | 2 | 64 /64.6% | 5 /5.1% | 21/21.2% |
| | Lung | 90 | 2 | 4/4.0% | 1/1.0% | 4/4.0% |

## DISCUSSION

The diagnosis of CE mainly depends on radiological and immunological procedures. Imaging methods are sometimes limited by small size of the lesion and atypical images which are not easy to be distinguished from abscesses or neoplasm. Routine laboratory diagnosis of CE is dependent on detection of specific antibody response. Serum is generally used for detection of specific antibody although some studies show the detection of antibody in urine might also be a good alternative [18]. The prevalence of CE in human in areas of extensive farming reaches up to 5 % [19]. Therefore, (CE) is particularly important in developing countries, where many nomadic rural inhabitants live under poor sanitary conditions without adequate supplies of clean water and in close proximity to their domestic animals [20]. *Echinococcosis* is a disease closely related to certain jobs: butchers, farmers, shepherds, and the geographic distribution of the disease follows the curve of the infection in shepherds and is determined by the level of pastoral hygiene [21].

In Sudan several studies documented the endemicity of cystic *Echinococcosis* in different part of the country 22-25]. The result from this survey is lower than those observed, in the same region, [24], who reported prevalence of 44.6%, 6.9% and 3.0% in camel, sheep and cattle, respectively. The infection rate reported (16.1%) is likely reflecting the true epidemiological situation in camel since only one camel infected in age group less than three years. However, the prevalence of *E. granulosus* is known to be positively correlated with age [26], [1].

Cystic *Echinococcus* (CE) occurs in all continents including circumpolar, temperate, tropical and subtropical and zones ranks in some areas as the leading disease of public health significance [19]. Camel appear to play the major role for the maintenance of *E. granulosus* in Central Sudan, for about 26.5% of camels examined in this study were found to be infected with cystic *Echinococcus* with high fertility rate (68%). Moreover, molecular characterization indicated that camel strain (G6) is more prevalent in this area. Since it had been found in camel, however, in all countries where camel has been reported as an intermediate host, it was too

important for the local maintenance of the life cycle [19. For all examined hydatid cysts, there were significant relations between the type of animals and infected organ with type of cysts. In addition the highest percentage of infection belonged to sterile cysts while calcified cysts possessed the least (Table 6). The presence of protoscoleces either attacked to the germinal layer epithelium in for of browed capsule or either presence in the cyst fluid was considered as indicative of fertility [27]. The viability of protoscoleces was assessed by the mobility of flame cells together with the staining with 0.1aqueous eosin solution [28]. Furthermore infertile cysts were further classified as sterile characterized by their smooth inner lining usually with slightly turbid fluid in its content. Typical calcified cyst produce a gritty sound feeling up on incision [29, 30]. In agreement with our findings [31], in northwestern Ethiopia, reported the sterile cysts as the most prevalent type of removed hydatid cysts [32], [31], Additional [32],in Arak province of Iran, found calcified cysts as the least type among all hydatid cysts [31], however opposite to our finding, in Sardinia (Italy) calcified cysts were observed as the most prevalent [33]. In our statistical analysis showed a significant relation of cysts, fertility and their size. Most of fertile hydatid cysts had a medium size in camel lungs (table 6).

In one case of human cystic *Echinococcosis* in the Netherlands the Argentina molecular analysis of *Echinococcus* granulosus isolates encountered from man intermediate host showed for the first time that the camel strain genotype (G6) is infective for human [34]. Another study carried out in Mauritania identified the camels strain genotype (G6) in two human samples.

## CONCLUSION

In conclusion can be said that fertility rate of liver and lung hydatid cysts of camel and viability of protoscoleces is considerable. In addition the fertility of hydatid cysts in human was low but they had high viability rate of protoscoleces. Fertility is associated with the type, location and the size of cyst.

## Competing interests:-

The authors declare that they have no competing interests.

## Author's contribution:

Albadawi collected hydatid cyst samples from Tambool slaughter house, and examined macroscopic and microscopic of the cysts. Ahmed edited and helped with experimental designed, Nawal analyzed the sequences and designed the study, All authors read and approved the final version of the manuscript.

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