Mitt. Österr. Ges. Tropenmed. Parasitol. 17 (1995) 97 – 102 Istituto di Ricovero e Ricerca a Carattere Scientifico (IRRCS), Pavia, Italia (1) Department of Infectious and Tropical Diseases, San Matteo Hospital, Pavia, Italy (2)

An Epidemiological Study on Hepatic Hydatidosis Showing Serologic Findings with Dot Immuno Assay (DIA) and Treatment of Hydatid Cysts with P.A.I.R. Method in Turkana (Kenya)

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Indroduction

Human Hydatidosis is mainly a rural and professional disease rampant among herdsmen, veterinarians and persons who handle and slaughter animals especially in areas of high prevalence. It is becoming increasingly evident that human behaviour plays an important role in the epidemiology of transmission to man.

The present research was carried out to evaluate the sensitivity of serodiagnosis with Dot-Immunoblotting-Assay (DIA) also denominated Dot-Blot-Dye-Assay (DBDA) and the efficiency of P.A.I.R. therapy in patients with hepatic hydatid cysts in the Kenyan region called Turkana. Hydatidosis, caused by larval stage of *Echinococcus granulosus* in the intermediate host (herbivores or man), is characterized by the formation of cysts 65% of them are localized in the liver. Other organs affected are the brain, lungs, bone and more rarely skeletal or cardiac muscle, kidney, spleen, ovary, prostate and thyroid glands.

Materials and methods

Study area and population surveyed

The Turkana region is a vast semi-arid territory extending for 60,000 km² in northwestern Kenya. Most of the 150,000 inhabitants live in rural areas and are nomads or herdsmen. Infection by *E. granulosus* is rampant in both domestic and wild animals. The Turkana are known to have particularly close contacts with their dogs, many of which are infected; some domestic dogs were found to host up to 300,000 adult cestodes. Considering that each cestode releases with faeces one pregnant proglotide containing 400 – 800 eggs every two weeks, it becomes evident that the environment is heavily contaminated. In fact with relevant studies tracing DNA of *E. granulosus* in the most affected areas in Turkana, 40 eggs/gr of soil and 400 eggs/litre of water from open wells and streams were found. In Turkana, jackals and hyenas have access to infection by feeding on unburied human bodies as due to ancient customs of limited use of underground graves, as well as remains of animals that die of famine in periods of draught.

Turkana Research Project

Data were obtained from two groups of persons resident in Turkana, group A consisted of 42 preselected patients with hepatic cysts and group B consisted of 149 subjects, out-patients at Kakuma Hospital and some pupils at a nearby tailoring school. All these individuals underwent:

- 1. Registration with full-name, age, sex, ethnic group, life-style and number of community members
- 2. Measurement of height and weight and determination of body-mass-index.
- 3. Queries about main sources of water and number of dogs owned.
- 4. Analysis of blood specimen to determine blood titers of haemoglobin, eosinophilia, erythrocyte sedimentation rate and alkaline phosphatase.
- 5. Serologic testing for *E. granulosus* which DIA comprising the following steps:
 - Hydatid antigen is spotted on a nitrocellulose stick.
 - The same antigen is dissolved into a pink colloidal dye.
 - 400 ml of serum sample diluted 1 : 2 with a buffer solution and 400 ml of the colloidal dye are incubated with the stick for one hour.
 - The stick is rinsed with tap water and if the serum sample is positive, the antigen spot remains dyed pink. The results obtained with DIA were compared with those obtained with ELISA IgG and RAST IgE.

The 42 preselected patients were underwent ultrasonography to detect the number, size, pattern and location of the cysts, followed by P.A.I.R. therapy which consists of four steps performed with ultrasound guidance:

- P = Percutaneous puncture of the cyst and introduction of a catheter.
- A = Aspiration of hydatid fluid from the cyst. The fluid drained is measured and analysed to detect the percentage of dead and live protoscoleces.
- I = Instillation of alcohol into the cyst. The quantity of alcohol instilled is ¼ of the volume of hydatid fluid drained.
- R = Reaspiration of liquid from the cyst. Every five minutes after instillation of alcohol, small samples of liquid are drained for analysis and after 30 minutes all the liquid in the cyst is slowly drained. Any liquid that collects in the cyst is drained over the following three days after which the catheter is removed. The diameter of the treated cyst is then remeasured.

Results

Information obtained from the questionnaires led to the conclusion that the major risk factor for hydatidosis in Turkana is ingestion of contaminated water from waterholes. In fact 92.9% of the subjects in group A used waterholes as their main water sources, while among members of group B, with mean seropositivity of 20%, 20.3% used water from waterholes, 43.9% used water from closed or protected wells, 35.2% had access to tap water (Fig. 1). Another important risk factor was found to be belonging to the Turkana tribe, living as nomads and keeping lifestock as major way of life. The percentages of nomads in our samples of the population were 97.6% in group A and 76.5% in group B (Fig. 2). On the other hand (Tab. 1) correlation between hydatidosis and the number of dogs each person owned was found not to be statistically significant (Chi square = 2.49, grades of liberty = 3 and P = 0.48).

Table 2 shows the results obtained from three serologic tests specific for *Echinococcus sp.* DIA, being sensible to blood titers of both IgG and IgE, showed positivity in 78.6% of the patients in group A and in 22.1% of those in group B. ELISA and RAST registered lower percentages of positivity in group A, but in group B ELISA IgG registered positivity of 24.8% while RAST IgE registered 14.1%.

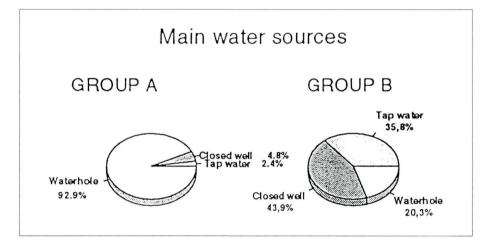


Figure 1

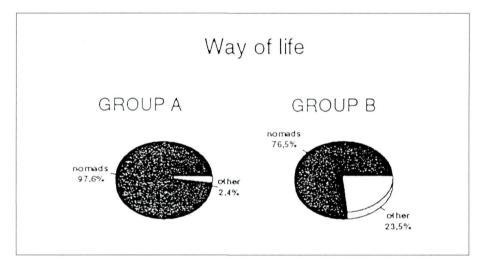


Figure 2

Dividing our patients in four age groups (< 14, 14 - 24, 25 - 40 and > 40) and comparing the results to the serologic tests and Body Mass Index (BMI), we found that children younger than 14 had relatively high incidence rates of hydatidosis and tended to show lower BMI than the other age groups. Values of BMI tended to increase with age in both groups but rise in antibody production was evident only in group B (Tab. 3).

Under ultrasonography the patterns of cysts were distinguished according to the classification by ANGELINI et al. (9) and percentages of each cyst pattern were calculated (Tab. 4). 45.6% of the cysts contained daughter cysts, 26.3% of them were simple cysts, 17.5% had the water-lily sign (collapsed endocysts floating in the fluid) and only four cysts had calcified pericysts.

In the 42 patients forming group A, 59 cysts were found and treated with P.A.I.R. The cyst diameters decreased only slightly after treatment, from 84.2% of the cysts 1 – 2 litres of hydatid fluid were drained, 12.3% contained > 2 litres and 3.5% contained < 1 litre. Five minutes after instillation of alcohol into the cysts, the percentage of live protoscolices in fluid drained from the cyst decreased from 64.9% to 1.7% while that of dead protoscolices rose from 29.8% to 92.9% (Tab. 5).

None of our patients manifested any undesirable side effects (for example manifestations of allergy or the much dreaded

anaphylactic shock) and all catheters were removed after the third day. In the follow-up period the treated patients showed an increase of antibodies during the first three months followed by a steady decrease. In order to avoid reinfection the patients were advised to abstain from ingestion of water from unprotected water sources.

Discussion

Although all patients in group A had confirmed presence of hydatid cysts in the liver, none of the tests used showed 100% seropositivity; it was therefore necessary to use both serologic tests and ecotomography in order to make accurate diagnosis of hydatidosis. Of the three tests used DIA was the most adapted one for use in Turkana region as it is sensitive to both IgG and IgE, it gives results within one hour and was the simplest and cheapest to carry out.

In group B seropositivity rose with age, as older patients proved to have higher antibody levels due to prolonged exposure to infection and higher risks of reinfection. Children under the age of 14 had relatively low BMI probably due to dysphagia caused by compression of oesophagus by posterior hepatic cysts.

Table 1: Number of dogs owned by members of groups A and B.

Number of dogs	Group A	Group B
0	22	81
1	9	22
2	6	16
> 2	5	30
Total number	42	149

Table 2: Comparison of results obtained with the 3 serologic tests used.

Serologic test	% of positive results in Group A	% of positive results in Group B
DIA	78.6	22.1
ELISA IgG	62.9	24.8
RAST IgE	76.2	14.1

Table 3: Coparison of percentages of positive results obtained with DIA in the different age groups with mean values of BMI.

	% of Positivity	BMI values
Group A		
< 14 age group	92	15.06
> 14 < 24	77	19.58
> 25 < 40	70	18.13
> 40	71	18.19
Group B		
< 14 age group	24	17.62
> 14 < 24	16	19.56
> 25 < 40	32	20.24
> 40	38	18.92

Surgical removal of hepatic cysts is a world wide used therapy which can safely be substituted with the above described P.A.I.R. procedure, especially in areas like Turkana where incidence rates are high (198/100,000), post-surgery recurrence occurs in 30% of operated patients, surgical equipment may be scarse and surgical treatment is impossible in about 50% of the patients (24).

In previously severely striken areas elsewhere in the world, for example Iceland before 1960 or Tasmania in more recent times, effective control measures drastically reduced the number of human cases of hydatidosis (12). These measures included health education, legislation forbidding access of dogs to animal offal and medical treatment of all dogs. Each region has to determine the main risk factors leading to human infection and the relevant control measures to be carried out. The main risk factor in Turkana is ingestion of contaminated water from unprotected water sources, like waterholes and streams. Other risk factors are inadequate health education, poor sanitary conditions especially in rural areas and close contact with dogs and lifestock.

Acknowledgements

This work received financial support from Direzione Scientifica of the "Instituto di Ricovero e Ricerca a Carattere Scientifico (IRRCS)", Pavia. The physicians (Prof. C. Filice and M. Tinelli) from the Department of Infectious and Tropical Diseases of San Matteo Hospital, Pavia, worked in collaboration with colleagues at Kakuma Hospital and members of the African Medical and Research Foundation (AMREF).

Summary

Hepatic hydatidosis in Turkana affects about 20% of the population with cases of individuals having three or four cysts at the same time. Effective control measures have still to be implemented in order to reduce the incidence of infection. DIA is suitable particularly for serodiagnosis on a large scale in rural areas white ultrasound scanning may be used for confirmation of the diagnosis and for visualizing the therapeutical procedure of percutaneous drainage and alcolholization of hepatic cysts denominated P.A.I.R.

Key words

Echinococcus granulosus, hepatic hydatidosis, Turkana (Kenya), epidemiology, diagnostic methods, treatment.

Table 4:

Type of cysts found with ultrasound scanning in group A patients.

Type of cyst	Number	Percentage
univesicular cysts	15	26.30%
with daughter cysts	26	45.60%
with water-lily sign	10	17.50%
with calcified pericysts	4	7.00%
apparently solidified	0	0.00%
missing data	2	3.60%

Table 5: Parameters after treatment with P.A.I.R

% of	before P.A.I.R.	after P.A.I.R.
cysts with live protoscolices cysts with dead protoscolices	64.9% 29.9%	1.7% 92.9%

Zusammenfassung

Eine epidemiologische Studie über das Vorkommen und die Bekämpfung der zystischen Echinokokkose der Leber in Turkana (Kenya)

Die zystische Echinokokkose der Leber in der Region Turkana (Kenya) betrifft etwa 20% der Bevölkerung. Einzelne Individuen weisen drei bis vier Zysten gleichzeitig auf. Zur Reduktion dieser hohen Inzidenz ist die Einführung verläßlicher Kontrollmethoden erforderlich. Der DIA (Dot-Immunoblotting-Assay) eignet sich für eine breitbasige serologische Diagnostik in ländlichen Gebieten, während Ultraschalluntersuchungen zur Bestätigung der Diagnose sowie zur optischen Kontrolle bei der Durchführung der P.A.I.R.-Therapie (perkutane Punktion der Zysten und anschließende Alkoholinstillation) verwendet werden können.

Schlüsselwörter

Echinococcus granulosus, hepatische Hydatidose, Turkana (Kenya), Epidemiologie, Diagnostik, Therapie.

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und Parasitologie

Jahr/Year: 1995

Band/Volume: 17

Autor(en)/Author(s): Lanyero Komakec Jaqueline, Tinelli M., Tinelli C.

Artikel/Article: An Epidemiological Study on Hepatic Hydatidosis Showing Serologie Findings with Dot Immuno Assay (DIA) and Treatment of Hydatid Cysts with PAIR. Method in Turkana (Kenya). 97-102