EPIDEMIOLOGY OF ECHINOCOCCOSIS IN NEPAL

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Abstract. Echinococcosis and hydatidosis is a parasitic zoonotic disease of human and animals. This disease has created public health and environment problems in all urban areas of Nepal. Based on the three year study (1993 - 1995) it has been revealed that the epidemiological cycle (indigenous) of *Echinococcus granulosus* parasite is dog-pig-dog cycle and human aequire infection accidentally through infected dog stool. However, this study has proved also the epidemiological cycle like dog-sheep-dog, dog-goat-dog and dog-buffalo-dog. This study was supported by International Development Research Centre (IDRC), Ottawa, Canada.

INTRODUCTION

This study had two parts; one on animals slaughtered and canine part of study and the other one was human part of the study. Using an ELISA coproantigen test, the highest prevalence of *E. granulosus* (5/88=5.7%) was seen in domestic dogs from an area of Kathmandu city. The carcasses of the animals were examined and hydatid cysts were found in water buffalo 5% (153/3,065), goat 3% (55/1,783), sheep 8% (12/150) and pig 7% (10/143) in 17 different abattoirs in Kathmandu.

In dog ecology studies, the number of dogs per household was 1.2%; 13% fed butchers waste or other raw meats/offal to dogs and 34% of the time dogs were kept confined. From the community health survey study 134 households (HH) had responded and answered that 20% HH dogs fed on raw meat and offal organs.

Human serum samples tested by ELISA assay method of which 113 (14.1%) screened positive (OD > 0.500). During 1994/95 (up to July) 120 human cases of hydatidosis operated in different hospitals of Kathmandu and cysts were in liver (55%), lungs (43%) or kidney (2%) and other organs (1%).

It was perceived from the study that the public health and environment problems are due to lack of slaughterhouse and waste disposal system, too many street dogs, food spoilage, poor sewage drainage, poor garbage pick up, poor hygiene and sanitation in meat marketing centres, lack of meat inspection act and poor knowledge of disease transmission in butchers and meat seller communities.

Major intervention approaches are needed for approval of the Meat Inspection act, construction of simple slaughterhouses, continuous epidemiological surveillance both in humans and animals, carrying out of mass awareness and health education programs, and finally research and development work with international cooperation and collaboration.

EPIDEMIOLOGICAL BACKGROUND

Echinococcosis was first investigated in Nepal (Joshi, 1973) when echinococcal cysts were found in buffalo, goats, sheep and pigs laughtered in Kathmandu. A later, preliminary study on echinococcosis in Kathmandu (Joshi, 1984) indicated that there had been 47 cases of echinococcosis amongst the 30,792 operations performed in the city's three hospitals between 1985 and 1990. Of these 47 patients, 26 were male and 21 female and most had cysts in the liver (55%) or lungs (43%). There was no active screening or case finding procedure for infection in Nepal at the time. A 1, 147 cases were found at a late stage or during surgery for other purposes. Ten of the cases were fatal (Joshi, 1984; 1985a).

Five per cent (153/3,065) of water buffalo, 3% (55/1,783) of goat, 8% (12/150) of sheep and 7% (10/143) of pig carcasses examined in 77 small abattoirs in Kathmandu between May and September 1991 also carried hydatid cysts (Joshi; 1995). Thirty (10%) of 291 canine stools collected in the vicinity of the abattoirs and examined by stool concentration methods were found positive for taeniid eggs, but adult *E. granulosus* were not recorded (Joshi, 1995).

Epidemiologically there are seven genetically distinct biological strains of *E. granulosus* which are buffalo, sheep, horse, cattle, camel, pig and monkey strains. Besides these there are other strains like lion and Tasmanian strains. The *E. granulosus* parasite needs two hosts to complete its life cycle like final host (fox, dog, cat, jackal) and intermediate host (buffalo, sheep, horse, cattle, camel, pig, monkey and human) The main epizootic chains prevalent globally are as follows:buffalo-dog-buffalo-dog-buffalo, sheep-dog-sheep, cattle-dog-cattle, horse-dog-horse, camel-dog-camel, pig-dog-pig, monkey-dog-monkey.

METHODOLOGY

The sampling frame comprised all the households in two wards, No. 19 and No. 20, of Kathmandu where considerable butchering and meat selling occur. In focusing on the household as the unit of analysis, we have assumed that households have some degree of control over exposure of their members to *Echinococcus* eggs, and that household practices are not overwhelmed by general contamination in the community. A complete list of all the households in each ward was obtained, houses were numbered, and a simple, using a list of computer generated random numbers, was selected. Based on an expected prevalence of infected households of 10%, and a desire to be within 5% of the true prevalence, 95% of the time, a sample size of 150 (82 for Ward 19 and 68 for Ward 20) was deemed adequate (Martin *et al,* 1987). Families were to have lived in the house for at least five years in order to be eligible, and a set of rules was devised for the field workers to select alternates should it not be possible to interview the pre-selected family. At this first visit, field workers asked six questions about occupation, family size, and dog ownership; the self-designated head of the family was then given a numbered identification card and all family members five years old or older were asked to attend a temporary health clinic which would be arranged in their ward.

Blood sample collection

Blood sample collection was also done by two team members after the household family was examined by the physician. Every evening serum was separated from the blood samples and stored in the freezer at the National Zoonoses and Food Hygiene Research Center (NZFHRC) laboratory. In addition, minor medical care and medication for poor patients was provided by the center at the clinic free of charge.

Laboratory testing

Eight hundred and four sera were tested in Nepal using a commercially produced enzyme-linked immunosorbent assay (ELISA)(LMD Laboratories, Carlsbad, CA, USA) and an automated reader at 450 nm. All sera were re-tested using the same test at the Centers for Disease Control in Atlanta, Georgia, USA. Households were classified as positive if at least one person in that household was classified as positive.

Hydatid case investigation

Checklists for collection of data from Bir Hospital, Kanti Children's Hospital and Tribhuvan University Teaching Hospital and Patan Hospital were developed. Case review forms for the surgeons were also developed and surgeons were interviewed. Morbidity and mortality records for all hydatidosis operations at the three hospitals were collected continuously and tabulated. Human cases admitted in the four hospitals were being followed up by the team. Seventeen patients were successfully operated on at Bir Hospital and the hydatid fluid was collected and sent to Dr Peter Schantz at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA for further analysis.

Hospital-based active case finding

Area hospitals were contacted and training courses for physicians on recognizing and treating cases of hydatid disease were arranged. Arrangements were made to do ELISA teasting of sera from people who were suspect cases based on clinical examination.

After the completion of training programs in different hospitals, including Tribhuvan University (TU) Teaching Hospital, Scheer Memorial Hospital, Banepa, Bir Hospital, Patan Hospital and Kanti Children's Hospital serum samples from suspected human cases of hydatid disease were sent to NZFHRC labotory for testing.

Twenty-three samples were received from March to September 1995 from the hospitals and private nursing homes. Detailed results are listed in Table 1 along with the enzyme linked immunosorbent assay (ELISA) tests results for echinococcal antibodies.

Canine study

Epidemiological design. In Kathmandu, the area of wards no. 19 and 20 called the project *"target area"*, was intensively monitored. The population of owned and street dogs in the target area was presumed to be

at a high risk of acquiring the parasite. The dogs were collared and tagged (red nylon collars for owned dogs and yellow for street dogs, secured by two numbered metal tags), given rabies vaccine, infected with praziquantel, sampled rectally and photographed. No praziquantel was given without sampling. During sampling, one sampling form designed for owned dogs was filled out per household. The information about the street dogs was gathered on sampling forms designed for ownerless dogs.

The dog populations of Kathmandu were divided into three categories of risk for echinococcosis (high, medium and low), based largely on their closeness to the so-called "target area" where most livestock was slaughtered (the nearer the higher the risk) but also on whether they were domestic (*ie* owned) or street (the street dogs being assumed to be at higher risk because of their greater access to the offal and other waste from slaughtering).

Coproantigen ELISA test. All fecal samples were tested using a corproantigen ELISA based on hyperimmune rabbit serum raised against adult *E. granulosus* (proglottis) somatic antigen. The supernatant fractions were prepared by mixing one g fecal material with 1.5 ml of 5% formol-saline containing 0.3% Tween 20 (Sigma). At this stage, the samples were often kept at room temperature for several days before being processed further. Once at the laboratory, the samples were shaken vigorously by hand and centrifuged at 2000*g* for 20 minutes. The supernatant was stored in 1.5 ml aliquots at 4 °C until the test was performed.

The mean optical density (OD) for the negative controls was the mean reading of the duplicate wells for each control. The cut-off level used, 0.072 was this mean plus three SD.

Adult parasites. Arrangements were made with the city authorities for researchers to take fecal samples and the small intestines from the poisoned dogs prior to their burial, up to 9 hours post-mortem. The fecal samples were kept at room temperature in 5% formal saline. Each intestine was identified with a tag matching the number on the fecal sample vial and injected with 10% formalin before being immersed in the same solution. After fixation for 55-60 days, the intestines were opened longitudinally and examined in detail for the presence of *E. granulosus* adults as well as nematodes and other cestodes. Any dubious worm-like material was examined by light microscopy. *Echinococcus* adults were fixed in 70% ethanol and stained with aceto-carmine.

RESULTS

Most human specimens (n=9) came from Bir Hospital, the largest hospital in Nepal. TU Teaching hospital sent five and Scheer Memorial, four. Specimens were tested and distribution of results by gender and age of positive specimens are presented in Table 1. There were six specimens testing negative for echinococcal antibodies and seventeen positive. Among the positive, nine (53%) were male and eight (47%) were female. The age group 35 and older had the highest number of positive (13.8%) among both genders.

Table 1

Distribution of hospital specimens by age and gender of positive human cases by ELISA for echinococcal antibodies.

Age group	Gender			ELISA	Surgical findings
	M	F	Total	OD range	
0-9	1	0	1	2.0	Hydatid confirmed
10-14	0	0	0	-	-
15-24	1	0	1	1.32	Hydatid confirmed
25-34	1	1	2	1.9-2.0	Hydatid confirmed
35+	6	7	13	0.555-2.0	Hydatid confirmed
Total	9	8	17		

Hospital hydatid case investigation 1995 from Kathmandu Valley and Banepa.

Twenty-three samples were received from March to September 1995 from hospitals and private nursing homes.

The prevalence of individuals sero-positive for *E. granulosus* in blood samples from area hospitals and blood bank was 27 (11.7%) of 230 for the hospitals and 24 (10.6%) of 227 for the blood bank. Statistically significant differences between various hospitals and between hospitals and the blood bank were seen. A total of 115 (14.3%) screened ELISA positive.

The automated readings indicated 12 (1.7%) coproantigen-positive samples out of the 696 dogs tested. Four of the 67 samples for which no questionnaires or sampling forms were available were found positive; three of these were from stools collected on the municipality and found to be infected at necropsy (used as a positive control). Of the 629 samples, 530 were first samples and 99 were samples taken 3 months post-treatment. Only eight (1.5%) of the first samples were coproantigen positive, five from domestic dogs in the target area (5.7% of samples from these dogs) and three (1.8%) from the veterinary clinics. Although, in those areas where infections were detected, prevalence of infection was higher in dogs fed raw food than in those not fed raw food (12.2% *vs* 0% in the target area and 3.8% *vs* 1.4% in the veterinary clinics) these differences were not statistically different (p=0.10). Of the eight dogs infected, five, all from the target area, were said to have access to the street (for 2-22 h/day) and were also observed on the streets (Baronet *et al,* 1994).

Most of the 20 dogs examined post mortem were infected with hookworm (85%) and/or *Dipylidium caninum* (85%) . Some carried *Toxocara* spp (35%), *Taenia* spp. (15%) and/or *E. granulosus* (15%). The identification of adult *E. granulosus* found in three of the dogs was based on gross morphology, size and shape of the uterus and the posterior position of the genital pore. This is the first recorded observation of adult *E. granulosus* in Nepal. Only the fecal sample of one of the three *Echinococcus*-infected dogs from Kathmandu was coproantigen positive and no more than five worms were recovered from this dog. Only one worm was found in the intestine of each of the other two dogs.

There were 1,000 households listed for Ward 19 and 1,100 for Ward 20. Of the 150 households selected, we were successful in obtaining sera and questionnaire information from 134. Most households (97.8%) included at least one literate person (defined as being able to read a local newspaper).

Table 2

Household management of dogs in Kathmandu, 1994 (n=31).

Dogs fed raw meat and/or organ	29.0%
Dogs defecate inside house	38.7%
Dogs allowed in food preparation area	32.3%
Dogs allowed in dining area	29.0%
Dogs allowed into the street	61.3%
Dogs sleeps in the house	93.5%

Selected dietary practices and indications of knowledge of disease transmission are listed in Table 3.

Table 3

Household knowledge and activities related to possible *E. granulosus* transmission in Kathmandu, Nepal, 1994 (n=134).

Eat raw meet	65.7%
Have seen cysts in meat	24.0%
Eat cysts	3.2%
Feed to dogs	12.9%
Throw into garbage	67.7%
Have heard of hydatid disease	3.0%
Have heard of disease from raw meat	17.2%
Have heard of disease from dogs	56.7%

Finally, respondents were asked what they thought were the most important public health problems being in their area. These are listed in Table 4.

Table 4

Perceived health problems in study area in Kathmandu, Nepal, 1994.

	no.	%
Lack of good drinking water	44	(32.8)
Lack of toilets	3	(2.2)
Slaughter house waste	6	(4.5)
Street dogs	5	(3.7)
Food spoilage	2	(1.5)
Poor sewage drainage	17	(12.7)
Poor garbage pickup	44	(41.8)
Other	1	(0.7)

The distribution of cases and non-cases by age and sex, and the rates of gastro-intestinal (GI) problems, lung problems, "other" health problems, and whether or not the individual had been hospitalized in the last month are displayed in Tables 5, 6.

Table 5

Distribution of age and sex of the study population.

Age group	Total	M	F
0-14	9(31.25%)	8(12.5%)	1(1.5%)
15-above	55(85.9%)	20(31.25%)	35(54.6%)

Table 6

Factors associated with sero-positive reaction to *E. granulosus* in individuals in a population in Kathmandu, 1994.

Factor	Cases	Non-cases	RR	p-value*
Average age (SD)	31(18)	31(19)		
Sex (% Male)	28M	123	1.01	0.9221
Hospitalized in last month (% yes)	none			
GI problems (% yes)	3(4.6%)	34	0.41	0.1360
Lung problems (% yes)	1(1.1%)	10	0.48	0.6965
Other health problems (% yes)	5(7.8%)	65	0.34	0.0105

* P-value for all variables except age are based on a Yates-adjusted x^2; the p-value for the difference in ages is based on a Student's *t*-test

The relationship of household serological status with dog ownership and various dog-care and dietary practices are listed in Table 7.

Table 7

Selected risk factors associated with households with at least one person sero-positive to *E. granulosus* in a population of Kathmandu, Nepal 1994.

	Pos house (n=)	Neg house (n=)	RR	p-value
Own dog (n=31)	13	18	1.27	0.4849
Feed raw meat to dog	2	7	0.44	0.3060
Dog defecates in house	7	5	1.85	0.2720
Dog allowed in food prep/area	4	6	0.93	0.5966
Dog allowed in dining area	3	6	0.73	0.4171
Dog sleeps inside	11	18	0.38	0.1670
People eat raw meat	28	60	0.77	0.3670
People eat buffalo meat	45	81	1.43	0.7124
Have seen cysts	10	21	0.93	0.9744
Have heard of hydatid disease	1	3	0.71	0.5613
Think people can get disease from dogs	22	54	0.67	0.1286
Think people can get disease from handling raw meat	8	15	0.99	0.8353

p-values are based on Yates corrected χ^2 or Fisher's Exact test (for small sample sizes)

DISCUSSION

The following intervention programs for echinococcosis/hydatidosis need to be implemented:

1. Measures for inspecting and controlling infected imported animals.
2. Control of stray/community dog population.
3. Registration of pet/owned dogs.
4. Testing with arecoline of all registered dogs in the endemic areas.
5. Treatment with droncit (praziquantel) of:
   (a) all imported dogs.
   (b) all (infected) dogs in the endemic areas.
6. Meat inspection for hydatid cysts of all animals slaughtered (food animals).
7. Mass awareness, enlightening, training, seminar/ workshop, publicity by radio, TV, newspapers, posters, etc.
8. Surgical inspection for hydatid cysts in all human surgical cases.
9. Continuous epidemiologically surveillance in both humans and animals (food animals and dogs).

ACKNOWLEDGEMENTS

This research was supported by the International Development Research Centre (IDRC) in Ottawa and the National Zoonoses and Food Hygiene Research Centre (NZFHRC) in Kathmandu. Special thanks is due to Dr Don de Savigny from IDRC who actively facilitated this work. We are also grateful to Dr PS Craig, Dept. of Biological Sciences, University of Salford, England, Dr David Waltner-Toews, University of Guelph, Canada and Dr Peter M Schantz, CDC, Atlanta, Georgia, USA. Dr Dominique Baronet and DR Bhatta were instrumental in organizing the initial stages of the community health survey. Finally, we wish to thank the following staff members from the NZFHRC : Mr Alan R Wald (Consultant), Mr CK Gurung, Mr PR Bista, Mr DB Thapa, Mr Bobi Thapa and Mr SP Bhandari. Special thanks go to the Chairmen from Wards 19 and 20 of Kathmandu Municipality, Hospital Directors, Physicians and Surgeons, Veterinary Hospitals, Veterinary Surgeons from private veterinary clinics.

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