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Article · June 2010

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Evaluation of Risk Factors of Cryptosporidiosis in Children A Case-Control Study in Mosul City

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Abstract

Cryptosporidiosis is a significant cause of childhood diarrhea. It is more prevalent in developing countries, it is also associated with high morbidity.A case control study was carried out to study the risk factors concerning cryptosporidiosis in Children suffering from diarrhoea. The children attending two hospitals in Mosul city. The total number of patients was 100 children less than 3 years old. All stool samples were diagnosed by using wet mounts, concentration technique. Staining was done by using Modified Ziehel-Neelsen (hot method) to identify cryptosporidium oocyst in stool samples. The control group consisted of 100 children apparently healthy and of matched age. Among the parameters studied to identify the risk factor, the statistical analysis of data showed that children age (p=0.001), presence of animals (p=0.022) mother age (p=0.006) and feeding pattern (p=0.022) were all significant and be regarded as risk factors for Cryptosporidiosis. These findings indicate that Cryptosporidiosis is prevalent in children in Mosul. These children may be the source of infection for the elder ones in the family. We recommend that health authorities be aware of Cryptosporidium as a cause of diarrhoea in children and that procedure for the diagnosis of this parasite be included in the routine diagnostic procedures for diarrhoeal stool specimens, in all laboratories.

Key words: cryptosporidiosis, risk factors, children, case-control study.

Introduction:

Cryptosporidium parvum is a coccidian protozoan parasite of the intestinal tract that causes severe potentially fatal watery diarrhoea in immunocompromised patients and self-limiting but often prolonged diarrhoeal disease in immunocompetent individuals (1,2). The infective form of cryptosporidium is the thick-walled oocyst which is excreted in large numbers in the faeces during acute infection (3). This parasite can also infect other animal species and thus may be Zoonotic (4). Volunteer studies have demonstrated that the infective dose can be as low as one oocyst (5) and thus infection is easily spread from person to person directly or indirectly.

Cryptosporidiosis is a significant cause of diarrhoeal diseases in both developing and industrialized nations (6) but several epidemiological studies have demonstrated that cryptosporidium is more prevalent in developing countries (5% to > 10%) than in developed countries (<1% to 3%) (7,8,9). In tropical environment, cryptosporidiosis is much more prevalent accounting for 4% to 20% of the cases of childhood diarrhoea, and children less than two years old have the greatest prevalence of cryptosporidiosis (3,5,10). It is associated with high morbidity (7,11,12) and this probably reflects a poorer nutritional status in children in developing countries (13). Transmission of cryptosporidium spp. via contaminated drinking water outdoor and indoor recreational waters and municipal waters is well documented (3). Method for detection of Cryptosporidium spp. In faeces usually involve microscopic examination of stained faecal smears by using Modified Ziehel-Neelsen technique (3). Few studies in Iraq have been done on human cryptosporidiosis especially among children (14,15).

Although diarrhoeal disease is an important cause of morbidity in children but the contribution made to it by cryptosporidium spp. is largely unknown. This study was conducted to detect cryptosporidiosis in children suffering from

diarrhoea and healthy controls, and to determine statistically the possible correlation between cryptosporidiosis and risk factors important for transmission of this disease.

Materials and Methods:

1. Time of Sample's Collection: Stool samples were collected during a period from June 2003 to October 2003.
2. Place of study: The children were attending two hospitals in Mosul city namely Ibn-Al-Atheer and Al-Razi Teaching Hospitals.
3. A case-control study: Was performed to quantify the distribution of cryptosporidium spp. among children suffering from diarrhoea (both acute and chronic).
4. Type of Sample:

The total number of patients involved in this study was 100 children less than 3 years old. Stool samples were diagnosed by using wet mounts, concentration techniques (Zinc-Sulphate flotation and sugar flotation methods), and stained by Modified Ziehel-Neelsen (MZN) staining (16). Cryptosporidial oocysts were examined microscopically in stool samples. The control group (n=100) represented by healthy asymptomatic children of matched age proven negative for parasites by complete stool examination as mentioned for case samples and stained by MZN.

5. Questionnaire:

All the information needed to evaluate the different risk factors from cryptosporidiosis was collected through the use of a questionnaire from which included general information about the studied group.

6. The Pilot study:

At the beginning of this work, a pilot study was conducted in the hospital from which the sample was decided to be collected. The objective of the pilot study was to get an idea about the rate of cases so as to determine the sample size that should be collected during the proposed period for data collection. The pilot sample was collected during the first two weeks of the study period and consisted of 10 cases and 10 controls. In the view of pilot study, the proposed sample size was determined (with the number of each sample of cases and controls to be collected)

7. Statistical Analysis of Data:

Computer feeding and statistical analyses were carried out using Pentium IV computer. Odds ratio (OR) is defined as the ratio of the odds the cases were exposed to the odd that controls were exposed. Odds ratio was calculated from the 2x2 tables (17, 18) as follows:

	Cases	Controls
Risk factors present (Exposed)	a	b
Risk factors absent (Not Exposed)	c	d
Odds of exposure	a / c	b / d

$$OR = \frac{ad}{bc}$$

The interpretation of the value of OR was as follows:

If OR = 1 it means that the exposure is not related to the disease. When OR > 1 then this indicate that the exposure is positively related to the disease. If OR < 1, it means that the exposure is negatively related to the disease or protective (18).

The 95% C.I for OR was calculated using Miettinen's test based approach where 95% C.I = OR ($1 \pm 1.96 / \chi$). C.I for the lower value = OR ($1 - 1.96 / \chi$), and C.I for the upper value = OR ($1 + 1.96 / \chi$). Where χ is the square root of $\chi 2$ value.

Chi square ($\chi 2$) test for contingency tables and Z-test were used to find the statistical association or differences between cases and controls for the presence or absence of significance i.e. p-value of <0.05 (19). An important part of the statistical analysis in the present study is dealt with the assessment of the significance of the risk factors through the use of the backward logistic regression model aiming to identify risk factors (independent variables) that may predict the occurrence of cryptosporidiosis (dependent variable) in children (20).

Results:

Characteristics related to gender, residence, M.Z.N, presence of animals, mother education are shown in table 1. The gender shows no significant difference between case and control groups. The presence of the animals was the only variable that carried a significant difference between the two groups (OR=3.083, p-value=0.001) it means that the presence of animals was three times more prone to get cryptosporidiosis. The residence was recorded in more than half of the cases (rural) as well as controls pointing to no significant relationship (p=0.171) between cryptosporidiosis and such factors. Concerning the laboratory method used for identification of the etiological agent (M.Z.N), it showed no significant difference between cases and controls.

The distribution of the study population according to age was statistically studied among the parameters of children's age (in months) and their mother's age (in years). Table 2 demonstrates no significant difference (p=0.104, p=0.061) between case and control groups among these parameters respectively.

The types of feeding which include (breast, bottle, mixed of both, breast and solid, bottle and solid, solid only and solid with mixed) were analyzed statistically in connection with cryptosporidiosis among case and control groups, as shown in table 3. The data showed that in the analysis of possible risk factors, feeding pattern was a significant factor overall.

In order to identify factors that help to predict the occurrence of cryptosporidiosis, the background logistic regression model was used after standardization of the data, to reassess the importance of in particular factors previously showed a statistical significant association with cryptosporidiosis by the use of $\chi 2$ and t-testing.

These factors included animals, and feeding. For these factors a special equation was modeled with the total number of each sample of cases and controls being (100). Table 4 shows predictors that significantly increased the risk of cryptosporidiosis including animals (p=0.010), and feeding pattern (p=0.000).

Discussion:

To obtain more concise information in this field, stool from 100 children suffering from diarrhoea and 100 apparently healthy controls were screened for possible risk factors of cryptosporidiosis.

Among the parameters studied in the present work, only the presence of animals showed a highly statistical significance and therefore considered as risk factor. It has been stated from a recent study in Iraq, that the presence of animals among case and control (200 subject) groups showed a higher infection rate than the absence of animals, and the high percentage (71%) in case subjects may assign the cause that children are in continuous contact with infected animals and their faeces make them more exposed to the infection (15).In a case-control study for the detection of cryptosporidium diarrhea in early childhood from Guinea Bissau (West Africa) during 1994, the study clearly showed that, domestic animals like (cows, goats, sheep and horses) are the major source of human infection(21). It has been suggested that companion animals such as rodents, puppies and kittens are also reservoirs for infections (22). This long list of animals reported to be infected with cryptosporidium spp. Lead to the view that Zoonotic transmission accounts for more human infections.

In human the prevalence of cryptosporidiosis ranged from 1% to 37.3% and seemed to be higher in the studies that targeted infants and children under seven years old (3), however, some studies which were done for the detection of cryptosporidium among diarrhoeic and asymptomatic children in Jedda (Saudi Arabia), and Kuwaiti children showed that the highest prevalence was among children less than two years of age (23,24). Age of children is considered as a risk factor, however, it is possible that the infection rate would have been higher if more than one stool specimen had been collected from each child because of the intermittent nature of oocyst excretion with this parasite (3,25). It has been reported that outpatient studies showed significantly higher prevalence's than those where inpatient were included which may have resulted from the presence of cryptosporidium oocysts in asymptomatic children, some of whom could be considered carriers who act as important reservoirs of

the organism and finally a potential source of infection (3).

Mother age is also regarded as a risk factor for cryptosporidiosis. A highly significant rate of infection was detected among children belong to mothers (25-35 years old) in comparison to other groups of lower or higher ages (15). This might be due to the fact that such an active group of mothers of child-bearing ages might have more than one child or even groups of children within the same family. This situation coupled with overcrowding might serves as a source of infection especially among those sharing small living rooms or bathrooms. In such conditions even a symptomatic carriers or one infected person may spread infection to others (3).

Feeding pattern overall is considered a risk factor in this study (p=0.001). Differences were observed between breast, bottle, and both mixed type of feeding in the rate of infection in different parts of the world (15, 21). A study of cryptosporidiosis in children under-five years of age in Addis Ababa showed no evidence of cryptosporidium infection among breast-feed group (26). Children used bottle and solid type of feeding showed a high rate of cryptosporidium infection than children feed on breast milk only (15, 21, 27). The protective effect of breast feeding in immunocompetent children is well documented (28). Because mucosal immunity is believed to be important in host defense against cryptosporidium, attention has been centered on the effect of colostral immunoglobulin in humans (21). A study from Mosul showed that artificial feeding and sudden weaning were considered as risk indicators of malnutrition where the duration and the severity of many diseases are greatly affected by malnutrition (29). Furthermore, diarrhoea plays a role in the development of this problem. Based on report from Mosul (30) it has been found that fewer children were breast-fed in 1995 (10%) than in 1993 (23%).

We conclude that an effective diagnosis for cryptosporidium oocyst is needed which goes frequently unrecognized in all hospital laboratories for better recognition of the etiological agents of diarrhoea in children. This is particularly important since there is now an effective drug nitazoxanide, available for therapy (31).

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Table 1: Background of the study population

Parameter	Case (n=100)	Control (n=100)	χ2-Value*	P-Value	OR	95% C.I (OR)
	No (%)	No (%)				
Gender						
Male	52 (52.0)	46 (46.0)	0.720	0.396	1.27	0.73-2.22
Female	48 (48.0)	54 (54.0)				
Residence						
Rural	73 (73.0)	64 (64.0)	1.877	0.171	1.52	0.84-2.77
Urban	27 (27.0)	36 (36.0)				
M.Z.N**						
Positive	28 (28.0)	29 (29.0)	0.025	0.876	0.95	0.52-1.75
Negative	72 (72.0)	71 (71.0)				
Presence of animals						
Positive	37 (37.0)	16 (16.0)	11.321	0.001	3.08	1.60-5.94
Negative	63 (63.0)	84 (84.0)				

* $\chi 2$ test was used with (d.f = 1).
** M.Z.N: Modified Ziehel-Neelsen technique for cryptosporidiosis.

Table 2: Distribution of the study population according to age.

Parameter	Case (n=100)	Control (n=100)	t-Value	P-Value
	Mean ± SD	Mean ± SD		
Children Age (months)	35.85±18.99	40.28±19.41	1.631	0.104
Mother Age (years)	29.12±8.80	31.23±6.91	1.886	0.061

Table 3: Distribution of cryptosporidiosis in children according to the type of feeding**.**

Type of Feeding	Case	Control	χ2-Value*	P-Value	OR	95% C.I (OR)
	No (%)	No (%)				
Breast	7 (7.0)	0 (0.0)	7.254	0.007	---	---
Bottle	9 (9.0)	0 (0.0)	9.424	0.002	---	---
Breast & Bottle	7 (7.0)	2 (2.0)	2.909	0.088	3.69	0.82-16.52
Breast & Solid	28 (28.0)	9 (9.0)	11.971	0.001	3.93	1.81-8.54
Bottle & Solid	28 (28.0)	3 (3.0)	23.860	0.001	12.57	4.55-34.73
Solid	16 (16.0)	85 (85.0)	95.230	0.001	0.03	0.02-0.07
Breast & Bottle & Solid	5 (5.0)	1 (1.0)	2.749	0.097	5.21	0.74-36.68
Total	100(100.0)	100(100.0)	98.501**	0.001	---	---

* $\chi 2$ test was used with (d.f = 1).
** $\chi 2$ test was used with (d.f = 6).

Table 4: Backward logistic regression model for the occurrence of cryptosporidiosis. (Variable in the equation)

Parameter	B	SE(B)	Sig.	Exp(B)	95% C.I for Exp(B)
Animals	1.015	0.399	0.010	2.760	1.263-6.034
Feeding Pattern	1.148	0.188	0.000	3.151	2.178-4.558
Constant	-7.735	1.266	0.000	0.000	---------