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The Epidemiology and Risk Factor of Cryptosporidium Parvum in Najaf Governorate

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Abstract. Cryptosporidiosis is a common cause of diarrhea in children. Morbidity rates are higher in underdeveloped nations, where it is more common. The risk factors for cryptosporidiosis in children with diarrhoea were studied in a case control study .The children in Al-Najaf city, who attend two hospitals were the sample of the this study . There were 89 patients under the age of three in the sample . The wet mounts, concentration approach was used to diagnose all stool samples that were submitted for testing .In order to find *Cryptosporidium* oocysts in stool samples, researchers used the Modified Ziehel-Neelsen staining technique. The control group included 61 children who seemed healthy and were the same age as the experimental group .The statistical analysis of data revealed that the P- value of age of children was (p=0.002), the P- value of the presence of animals in the environment was (p=0.032), the age of the mother (p=0.005), and the feeding patterns (p=0.021) had all of these factors been significant and should be considered a risk factors for Cryptosporidiosis. These data show that children in Al-Najaf city are at risk for Cryptosporidiosis. The senior members of the family may be at risk of infection due to the presence of these children. We urge that health authorities become awareness of *Cryptosporidium* as a source of diarrhoea in children and that a protocol for diagnosing this parasite be included in all labs' normal diagnostic testing for diarrhoeal stool specimens.

Keywords. Risk factor, *Cryptosporidium* Parvum, Children**.**

INTRODUCTION

Humans and animals all throughout the globe suffer from diarrhea and gastroenteritis due to the parasite illness cryptosporidiosis. Intracellular apicomplexan parasite *Cryptosporidium* infects the epithelial cells of its host's microvillus border in the gastrointestinal system, where it causes intestinal inflammation (1).Disrupting the function of the small intestine's epithelium may produce moderate or severe diarrhea with other stomach problems. In "immunocompetent hosts, cryptosporidiosis" is considered to be a self-limiting illness that resolves on its own.Nevertheless, in immunocompromised persons, it may lead to long-term and perhaps life-threatening sickness. The oocysts that cause human illnesses and environmental pollution may be found in many animals, especially cattle, that have been infected with *Cryptosporidium*. *Cryptosporidium* is capable of infecting virtually all animals. *Cryptosporidium* is easily transmitted through the fecal-oral route, and the oocysts' resistance to infection is a major factor in this. *Cryptosporidium* infective stage, which is highly infectious and resistant to severe climatic conditions as well as many disinfectants (3) Numerous epidemics of *Cryptosporidium* infections have already been identified across both developed and the developing nations across the globe .The aim of the study *Cryosporidium parvum* is a

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parasite of the digestive system that produces watery diarrhoea that may be life-threatening. In immunocompromised patients, and in immunocompetent persons, self-limiting but often protracted diarrhoeal illness. During an acute infection, the thick-walled oocyst is the infectious form of *Cryptosporidium* and is expelled in high quantities in the feces .This parasite has the potential to spread to other animals, making it a potential Zoonotic hazard. As little as one oocyst may be infective, and so illness can transfer from person to person either directly or indirectly, volunteer studies have shown. In both developing and developed countries, cryptosporidiosis is a major cause of diarrheal illness (4). *Cryptosporidium*, however, is more common in underdeveloped nations (5% to > 10%) than in wealthy ones (1% to 3%), according to various epidemiological studies . Cryptosporidiosis is more widespread in tropical environments, accounting for 4% and 20% of the cases of infantile diarrhea.Cryptosporidiosis is more common in infants less than two years old. Children in undeveloped nations are more likely to die from it, which may be due to a lack of nutrition. It's well-known that *Cryptosporidium* spp. may spread via contaminated drinking water, whether it's through outdoor or indoor activities, or municipal sources. *Cryptosporidium* spp. detection method In faeces, the most common method is to use the modified Ziehel-Neelsen technique to examine stained faces smears. Research on human cryptosporidiosis, particularly among youngsters, is scarce in Iraq.Diarrheal diseases in children are common, but the role of *Cryptosporidium*. in contributing to their morbidity is still mainly understood(5). Children with diarrhoea and healthy controls were studied to see whether they had cryptosporidiosis, and if so, whether or not there was any statistical evidence linking cryptosporidiosis to the risk factors that may lead to its spread. .A case control study was conducted to determine the risk variables for cryptosporidiosis in children with diarrhoea in Najaf, Iraq. Children currently being treated at three hospitals in Al-Najaf namely : Al-Hakim, Al-Sadr and Al-Zahra. There were 89 children there age was less than three years. The wet mounts, concentration approach was used to diagnose all stool samples that were submitted for testing. To detect *Cryptosporidium* oocysts in stool samples, the modified Ziehel-Neelsen (hot technique) staining was used. The control group comprised of 61 children who seemed to be in good health and were of comparable age.

MATERIALS AND METHODS

Study Sample

The stool samples were collected. It was conducted at three hospitals in Al-najaf city, namely Al-Hakim, Al-Sadr, and Al-Zahra Hospitals, where the children were receiving treatment. Using a case-control study, researchers determined the prevalence of *Cryptosporidium* among children with diarrhea "both acute and chronic".

Study Place and Sitting

It was conducted at three hospitals in Al-najaf city, namely Al-Hakim, Al-Sadr, and Al-Zahra Hospitals, where the children were receiving treatment

Inclusion and Exclusion Criteria

Stool sample Selection and exclusion samples: Samples of patients suffering from diarrhea and samples that contained urine and tap water were excluded

Data Collection

The date on which the sample was obtained between September 2020 and January 2021, stool samples were collected. It was conducted at three hospitals in Al-najaf city, namely Al-Hakim, Al-Sadr, and Al-Zahra Hospitals, where the children were receiving treatment. Using a case-control study, researchers determined the prevalence of *Cryptosporidium* among children with diarrhea "both acute and chronic". There were 89 children under the age of three that participated in this research as a sample.Stool samples were identified using wet mounting, concentration procedures Zinc-Sulphate extraction as well as sugar flotation techniques), including Modified Ziehel-Neelsen

(MZN) staining. stools were analysed under a microscope for the presence of the parasite *Cryptosporidium* Parvum. The control group (n=61) consisted of healthy, asymptomatic children of the same age who had been proved negative for parasites by a comprehensive stool examination and stained with MZN. In order to get the data required to assess the various cryptosporidiosis risk factors, a questionnaire was used to gather basic demographic data about the participants. The preliminary investigation and pilot studies were finalized at the hospital where samples were to be taken for this project's first phase. It was the goal of the pilot research to gain a sense of the rate of instances in order to establish the sample size that should be gathered within the specified time period of data collection. During the first two weeks of the investigation, a pilot sample of 10 patients in addition to 10 controls was tested and treated. The suggested sample size was calculated based on the number of case and control groups to be gathered for the research. The process through which data are statistically analysed with a Dell PC was used for computer feeds and statistical analysis. The odds ratio (OR) is the proportion of the cases' odds to the odds of the controls. Using the 2x2 tables, the odds ratio was determined as follows:

TABLE 1. The calculation the odds ratio, by comparing the probabilities of exposure of cases with those of the control group.

	Cases	Controls
Risk Factors Present (Exposed)	a	b
Risk Factors Absent (Not Exposed)	c	d
Odds of Exposure	a/c	b/d

The Odd Ratio can be calculated in the following equation

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

OR's value was interpreted as follows: There is no link between exposure and illness if OR = 1. If OR is more than 1, then the link between the exposure and the illness is strong. If the OR is less than 1, it indicates that the exposure has a negative or protective effect on the illness. According to Miettinen's test-based method, the 95 percent Confidence Interval . for OR is 95% C.I = OR $(1 \pm 1.96 / \chi)$. Lower C.I = OR $(1 - 1.96 / \chi)$, higher C.I = OR $(1 + 1.96 / \chi)$. In this case, χ is equal to the square root of χ^2. The p-value of 0.05 was utilized to determine whether or not there was a statistically significant relationship or difference between the cases and controls using the Chi χ^2 test for contingency tables and the Z-test. Using a backward logistic regression model to identify risk factors "independent variables" that may predict the presence of cryptosporidiosis (the dependent variable) in children, an essential portion of the statistical analysis in this research is dealt with.

Statistical Analysis

Data were double entered and crosschecked with EpiData software .Descriptive summary measures of frequency and central tendency of participants' characteristics were computed as appropriate. Pearson's chi-square test was employed in the univariable analysis to examine the associations between some parameters and *Cryptosporidium* by computing crude odds ratios (cORs) with 95% confidence intervals (CIs). Multivariable logistic regression was subsequently carried out through stepwise elimination to adjust for confounders, and adjusted ORs (aORs) with 95%CIs were calculated. $P \leq 0.05$ were considered statistically significant. Statistical analyses were done with the SPSS statistical package.

RESULTS

Gender, domicile, M.Z.N, animal presence, and mother education are all given in the first table (2). Gender does not seem to vary much between the two groups wich resulted in no significant difference betweem case and grops . A significant difference was found between the groups with animals and the two groups (OR=3.744, p-value=0.0011), which suggests that the presence of animals was three times more likely to result in cryptosporidiosis than the absence of animals.

TABLE 2. The study population and background of data distribution with results . Chi-squared used with one Df and M.Z.N: Modified Ziehel-Neelsen method for cryptosporidiosis.

Parameter	Case (n=89)	Control (n=61)	χ2-Value*	P-Value	OR	95% C.I (OR)
			CHGender			
Male	46	28	0.731	0.398	1.271	0.74-2.23
Female	43	33				
			Residence			
Rural	64	39	1.880	0.182	1.529	0.85-2.76
Urban	25	22				
			M.Z.N**			
Positive	25	18	0.024	0.881	0.961	0.53-1.74
Negative	64	43				
			Presence of Animals			
Positive	33	10	11.361	0.0011	3.744	1.6-5.97
Negative	66	51				

More over half of the patients (rural) and controls had their place of residence registered, and the results showed no correlation (p=0.183) between cryptosporidiosis and these factors. The laboratory approach employed to identify the etiological factor (M.Z.N) revealed no significant differences between cases and controls. The age distribution of the sample population was investigated statistically using the parameters of the children 's age which was in months and the age of their mother (in years). Between the case and control groups, these parameters show no significant difference, the P- value was (p=0.116, p=0.069) in Table (3).

TABLE 3. Population of the research distributed according to their age group.

Parameter	Case (n=100) Mean ± SD	Control (n=100) Mean ± SD	t-Value	P-Value
Children Age (months)	36.88±18.19	41.29±18.66	1.640	0.116
Mother Age (years)	28.16±8.96	33.24±6.95	1.893	0.069

Case and control groups were compared statistically on the forms of feeding "bottle, breast,breast and solid, mixed of both, mixed of both breasts and solids, bottle and solid, solid alone, and solid with mixed" that they used, as indicated in table (4). According to the findings, feeding pattern was a major factor overall in the examination of potential risk variables.

TABLE 4. The prevalence of cryptosporidiosis in children varies depending on the type of feeding they get . *The two-tailed test was conducted using (d.f = 1). ** The d.f = 6 data set was subjected to a two-tailed test.

Type of Feeding	Case No (%)	Control No (%)	χ2-Value*	P Value	OR	95% C.I (OR)
Breast	7	0	7.261	0.006	---	---
Bottle	8	0	9.498	0.003	---	---
Breast & Bottle	7	1	2.918	0.091	3.71	0.83-17.53
Breast & Solid	25	6	11.782	0.002	3.88	1.82-8.57
Bottle & Solid	25	2	23.901	0.003	12.78	4.75-34.81
Solid	14	51	95.360	0.001	0.04	0.03-0.05
Breast & Bottle & Solid	4	1	2.813	0.098	5.22	0.82-32.73
Total	89	61	98.502**	0.003	---	---

For the purpose of identifying risk factors for cryptosporidiosis. After the data had been standardized, the background logistic regression model was employed to analyze the data. By using χ2 and testing, we may reevaluate the significance of several parameters that previously demonstrated a statistically significant connection with cryptosporidiosis. Animals and food were two of these considerations (factors).

In order to account for these variables, a unique equation was constructed using the total number of cases and controls in each sample (89) and (61). Cryptosporidiosis is more likely to occur in animals (p=0.012) and in people who eat a variety of feeding patterns (p=0.0002) as in table (5).

TABLE 5. A backward logistic regression model was developed to predict the presence of cryptosporidiosis. (In the equation as a variable)

Parameter	B	SE(B)	Sig.	Exp(B)	95% C.I for Exp(B)
Animals	1.025	0.401	0.012	2.813	1.274-6.172
Feeding Pattern	1.167	0.202	0.0002	3.134	2.219-4.6347
Constant	-6.853	1.321	0.0002	0.002	---------

DISCUSSION

To get more comprehensive information in this sector, stool samples from 89 diarrhoea-affected children and 61 seemingly healthy controls were tested for putative cryptosporidiosis risk factors. In this study only the presence of animals was found to be statistically significant among the criteria tested in this research, and as a result, it was classified as a risk factor. According to a recent research conducted in Iraq, cases and controls with animals revealed a greater infection rate than those without animals cause(6,7) . The large proportion of cases (72% of them) suggests that youngsters are more susceptible to infection since they are constantly in touch with diseased animals and their feces. A case-control study from Guinea Bissau"West Africa" in 1994 looked for early children cases of *Cryptosporidium* diarrhea.Domestic animals such (cows, goats, sheep, and horses) are indeed the primary cause of human infection, according to the research. Domestic pets such as rats, puppies and kittens have also been linked to the spread of disease. According to this extensive list, zoonotic transmission is to blame for the majority of human illnesses caused by *Cryptosporidium* spp. From one to three-and-a-half percent, the prevalence of cryptosporidiosis in humans varied from one to three-and-a-half percent. *Cryptosporidium* has been found in children under two years old in Jedda (Saudi Arabia) and Kuwait. However, it's plausible that the infection rate would have been greater if each kid had more than one feces sample obtained, even if age is considered a risk factor. Because of the parasite's irregular oocyst discharge. Outpatient studies have been shown to have a greater prevalence than those that included inpatients. This might be the outcome of asymptomatic youngsters having *Cryptosporidium* oocysts. It's possible that some of these people may be considered carriers of the bacterium, and so a possible source of infection(8,9).The mother's age is also considered a risk factor for cryptosporidiosis, according to the CDC. The risk of infection among children whose moms were between the ages of 25 and 35 was significantly greater than that of other age groups(10,11). As a possible explanation, it's possible that so many of these busy moms are raising a large number of kids in the same family or perhaps many families. Overcrowding in tiny spaces, such as bathrooms or living rooms, may be a breeding ground for germs(12,13).Carriers and sick people may transfer disease in these situations, even if they don't show symptoms. In this research, feeding pattern was shown to be a significant risk factor (p=0.002). In many places of the globe, infection rates differed between breast, bottle, and both combination feeding methods.Addis Ababa researchers found no indication of *Cryptosporidium* infection in the breast-feeding group in a study of children under five years of age(14.15). The risk of *Cryptosporidium* infection in infants who drank from a bottle and ate solid food was higher than in children who solely drank breast milk. Breastfeeding is known to have a protective impact on immunocompetent youngsters. For this reason, host protection against *Cryptosporidium* is thought to be dependent on mucosal immunity. The role of cloistral " immunoglobulin" in humans has been the focus of attention(16,17).A research curried out earlier in Al- Najaf Governorate found that artificial feeding and early weaning were risk factors for malnutrition in infants. There are several illnesses in which malnutrition has a significant impact on their length and severity. Another factor in the development of this issue is diarrhoea(18,19).More infants were breast-fed in 2021 than in 2020, according to a study from other governorate in Iraq. There has to be a good diagnostic of the oocyst of *Cryptosporidium* in Najaf. For improved identification of the causative agent of diarrhoea in children, which is usually overlooked in all hospital labs. This is especially critical now that the medication nitazoxanide is available for treatment(20,21).

ETHICAL APPROVAL

Written informed consent was obtained from the Department of Health and patient for publication of this paper.

FINANCIAL SUPPORT

Financial support from the researchers themselves

CONCLUSION

This multicountry cohort study confirmed the association of *Cryptosporidium* infection with stunting in Three sites highlighting the significance of cryptosporidiosis as a risk factor for poor growth. We observed that the rate, age of onset, and number of repeat infections varied per site; future interventions should be targeted per region to maximize success.

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