wjpmr, 2018,4(2), 25-30

SJIF Impact Factor: 4.103

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

PREVALENCE OF CRYPTOSPORIDIOSIS AMONG DIARRHOEIC PATIENT'S ATTENDING TO KOSTI TEACHING HOSPITAL, WHITE NILE STATE, SUDAN

Abdelhakam G. Tamomh[1], Sabah R. Yousif[2], Tesfaye Z. Weldegiorgis[3], Babiker Saad Almugadam[4], Ahmed A. Alfaki[1], Tarteel A. Abdallah[1] and Dr. Mohammed A. Suliman[1]*

[1]Department of Parasitology and Medical Entomology, Faculty of Medical Laboratories Sciences, El Imam El Mahdi University, Kosti, Sudan.
[2]Laboratory Specialist, Kosti Teaching Hospital, Kosti, Sudan.
[3]Department of Public Health, Dalian Medical University, Dalian, China.
[4]Department of Microbiology, Faculty of Medical Laboratories Sciences, El Imam El Mahdi University, Kosti, Sudan.

***Corresponding Author: Mohammed A. Suliman**
Department of Parasitology and Medical Entomology, Faculty of Medical Laboratories Sciences, El Imam El Mahdi University, Kosti, Sudan.

Article Received on 07/12/2017 **Article Revised on 28/12/2017** **Article Accepted on 18/01/2018**

ABSTRACT

Cryptosporidiosis is the disease caused by *Cryptosporidium parvum*, is of great concern because of associated economic losses and the public health significance in humans. The aim of this study was to determine the prevalence of cryptosporidiosis among diarrheic patient's attending to Kosti teaching hospital, White Nile State, Kosti city, Sudan.This representative is descriptive cross-sectional, hospital–based study was conducted from August 2016- April 2017. A total of three hundred stool samples were collected from diarrheic patients, the samples were examined using Formal ether concentration technique and staining techniques and the samples were preserved in schauddins fixative. The overall prevelance of parasite detected by modified ZN stain was 18%, and by trichrome stain 5%. The prevalence of *C.parvum* was higher in female than male. The study showed that modified ZN staining technique is the most sensitive and accurate so will be recommended to be used as first choice in diagnosis of *Cryptosporiduim parvum*. Health education and personal hygiene recommended reduce the disease transmission.

KEYWORDS: Prevelance, Cryptosporidiosis, Diarrhoea, Sudan, ZN stain.

INTRODUCTION

Cryptosporidiosis is one of several parasitic diseases of the mammalian intestinal tract which causes diarrhea. Primary symptoms are acute watery and no bloody diarrhea. Infection is of particular concern in immunocompromised patients. The outbreak of cryptosporidiosis occurred in Milwaukee, 403.000 people were infected through contaminated drinking water and in recent years cryptosporidiosis outbreaks have been reported in some European and American countries.[1] Molecular techniques have shown that *Cryptosporidium parvum* is the predominant species in cryptosporidiosis outbreaks, accounting for 50.8% of cases among 325 water-borne outbreaks of parasitic protozoan diseases worldwide. In stool surveys of patients with gastroenteritis, the reported prevalence of Cryptosporidium was 1-4% in Europe and North America; and 3-20% in Africa, Asia, Australia, South and Central America.[1] Peaks in the prevalence, in developed countries was observed in the late summer and in spring.[2,3] In industrialized countries, the prevalence was high in children under 5 years of age and in young adults. In developing countries, the infection is common in infants less than 1 year, but was rarely seen in adults. Asymptomatic carriage, as determined by stool surveys, generally occurs at very low rates in industrialized countries (<1%), although in day care centers higher rates had been reported.[4,5,6] High rates of asymptomatic carriage 10-30% were common in non-industrialized countries.[1] Seroprevalence rates are generally higher than fecal carriage rates, from 25-35% in industrialized countries up to 68-88% in Russia and 95% in South America.[7,8] Seroprevalence rates increase with increasing age and are relatively high in dairy farmers and day care centre attendants.[7,9,10,11] In two studies which conducted USA showed that people that consumed treated surface water were more likely to show sero conversion during the study period than the people whom consumed well-protected groundwater.[12] During the months of the study, a significant proportion of the population exhibited seroconversion also in the groundwater cities, indicating that Cryptosporidium infections may be relatively common. Illness rates were not increased in the cities supplied with surface water, although infections were more common. The more intense serological response in the residents of the

surface water cities could indicate an increased level of protection from illness. The human feeding trials also indicated a protective effect of a prior infection to illness after low dose exposure, but not against high dose exposure.[12] In both the USA and in Russia, the consumption of drinking water from shallow wells was correlated to a high seroprevalence.[7,19]

MATERIALS AND METHODS

This study was conducted in Kosti city, White Nile State. Health services: one teaching hospital, 3 rural hospitals, 20 health center, 34 Health units, 4 Medical clinics. Education services: 140 kindergarten, 82 basic schools, 26 boys, 26 girls and 30 co-education 15 Secondary schools, one university. The most activities are grazing, agriculture, trade and fishing.[16] The design of this study is a descriptive cross-sectional, hospital–based study. It can be described as a prospective study Patients from both sexes with diarrhoea who admitted to Kosti Teaching Hospital between August 2016 and April 2017 The participants will be enrolled after meeting the selection criteria; Approved informed consent, they had diarrhoea and any abdominal symptoms with diarrhoea and had not taken antidiarrhial drugs and antiparasitic drug before the time of collection. 300 samples were collected from kosti Teaching Hospital; specimens collected using systemic sampling technique. Information's were collected according to number, age, sex, water supply, presence and absence of latrine in houses. Questionnaire covering this information was contracted. The patient data records, sample collection, logistic and patient safety issues will be handled according to the protocols set out by the health facilities. The raw data will be stored using two systems: Firstly, the questionnaire papers will be securely stored at a specific place to be used as back-up. Secondly, the data is saved in two electronic packages (Excel and SPSS programmes) for analysis. Within the databases, cases are identified by number only. Data will be recorded and then analysed using Chi- square test by statistical package of social science (SPSS version 16) program. P values < 0.05 will be considered significant for all statistical analysis.

Sample collection and ethics

A diahrraec patients samples were collected from Kosti Teaching Hospital attending to agreement of hospital manager and staff of the hospital laboratory.

Preparation of Schaudinns fixative

70 grams of mercuric chloride were weighted and transferred to heat resistance flask containing 1000 ml of distilled water and mixed well then the flask was placed in container of boiling water to dissolved the mercuric chloride, when it is completely dissolved it left to cool at room temperature, then 95% ethanol is added in ratio of one part of 95% ethanol to two parts of mercuric chloride. 5 ml of glacial acetic acid was added to 95 ml of stock Schaudinns reagent immediately before use of fixative.[13]

Preparation of trichrome stain

1.2grams of chromotrope R2, 0.6 grams of light green S.F and 1.4 grams of phosphotungestic acid were mixed and 2 ml of glacial acetic acid was added and allowed the stain to stay for 30 minutes, then diluted with 200ml of distilled water.[13]

Preparation of modified Ziehl Neelsen stain

Strong carbol fuchsine Dissolve 20 g basic fuchsine in 200 ml absolute methanol and mix on a magnetic stirrer until dissolved. Add 125ml liquid phenol (general purpose reagent (GPR; 80% w/w in distilled water)) carefully until well mixed, and make up to the final volume with 1675 ml deionised water. Mix thoroughly. Filter before use through Whatman No.1 filter paper to remove debris and store in a stock reagent bottle. Label, date and initial. Store the stock reagent in a dark cupboard at room temperature. Commercial supplies are also available. The concentration of basic fuchsine can vary within the acceptable range of 1 to 3%. 1% acid methanol carefully adds 20ml concentrated hydrochloric acid to 1980 ml of absolute methanol and mix. Transfer to a stock reagent bottle, and label, date and initial. Commercial supplies are also available Reagent bottle, label, date and initial. Commercial supplies are also available 0.4% malachite green Add 2g malachite green to 480 ml deionised water and mix on a magnetic stirrer. Filter through Whatman No.1 filter paper into a stock.[13]

Stool sample collection and processing

In clean and dry stool container collect small amount of diahrriac patient stool, first used direct stool examination, and then used concentration technique, and then in clean and dry slides maked smears from fresh sample and fixed by covering the smear by methanol about 3 minute and later stained by ziehl neelsen stain, the remain amount of sample preserved by schauddin fixitive, and later used trichrome stain.

Formal ether concentration technique

Formal ether concentration technique is used. About one gram of faeces is placed in a container; 4-7 ml of 10% formal saline is added, then emulsified and sieved using a fine sieve. The sieved sample is transferred to 15 ml centrifuge tube, about two ml of diethyl ether is added, shacked gently for few seconds, centrifuged, the faecal debris is released from the surface of the tube then the supernatant is discarded. The sediment is mixed by means of Pasteur pipette and transferred to a microscopic slide, covered with cover glass and examined microscopically using 10x and 40x objectives. The number of eggs is calculated and recorded as a number of eggs per gram of faeces.[14]

Trichrome staining technique

Make a thin smear of the faeces on a slide. Fixed it in schaudinns fixative for 1 hour at room temperature (the smear must not be allowed to dry during staining) Immersed the slide in a container of ethanol iodine solution for 2-5 minutes followed by 1 minute rinsed in

two containers of 70% v/v ethanol. Stained in a container of trichrome stain for 10, minutes. Rinsed briefly in the acidified ethanol solution, followed by brief rinses in two containers of 95% v/v ethanol. Immersed the slide in a container of absolute ethanol for 2-5 minutes. Cleared the smear by immersing the slide in a container of xylene or toluene for 3 minutes.[13]

Modified Ziehl Neelsen staining technique

Wear protective clothing and disposable gloves. Fix the air-dried smear in methanol for 3 minutes. Immerse or flood the slide in cold strong carbol fuchsin and stain for 15 minutes. Rinse the slide thoroughly in tap water. Decolorize in 1% acid methanol for 10 –15 seconds. Rinse the slide in tap water. Counter stain with 0.4% malachite green for 30 seconds. Rinse the slide in tap water. Air-dry the slide. Examine for the presence of oocysts by scanning the slide systematically using the ×40 objective lens of a bright field microscope. Confirm the presence of oocysts under the oil immersion objective lens.[14]

RESULT

Three hundred stool sample were collected and screened for *cryptosporidium parvum using* Ziehl Neelsen staining technique and Trichrome staining technique. The numbers of infected cases for in *cryptosporidium parvum* stool samples were 0 (0%) using formal ether concentration technique and 54 (18%) using modified Ziehl Neelsen staining technique and 15 (5%) using Trichrome staining techniques; Table (1).

Table 1: The number and percentage of infected and non infected cases with *cryptosporidium parvum* using the formal ether concentration technique, Trichrome staining technique and Modified Ziehl Neelsen staining technique.

Techniques / Cases	Formal ether concentration technique	Trichrome staining technique	Modified Ziehl Neelsen staining technique
Infected cases	0 (0%)	15 (5%)	54 (18%)
Non infected cases	300 (100%)	285 (95%)	246 (82%)
Total	300(100%)	300 (100%)	300 (100%)

Prevalence of infection according to age group

The age of patient's were grouped into three groups; age group one, age group two and age group three which represent the age of 1–25, 26–50, over 50 years respectively; (Table 2).

Table 2: The number and percentage of infected cases wit*h cryptosporidium parvum* using the Ziehl Neelsen staining technique and Trichrome staining techniques correlated with age group.

Techniques / Age groups	Modified Ziehl Neelsen staining technique	Trichrome staining technique
1-25yrs	11 (20.3%)	2 (13.4%)
26-50	25 (46.4%)	9 (60%)
Over 50yrs	18 (33.3%)	4 (26.6%)
Total	54 (100%)	15 (100%)

Prevalence of infection according to sex

Out of 300 stool samples examined, 169(56.3%) were male and 131(43.7%) were female; Table (3).

Table 3: Thes number and percentage of infected cases with *cryptosporidium parvum* in relation to sex using the Modified Ziehl Neelsen staining technique and Trichrome staining techniques.

Techniques / Sex	Modified Ziehl Neelsen staining technique	Trichrome staining technique
Male	24 (44.5%)	6 (40%)
Female	30 (55.5%)	9 (60%)
Total	54 (100%)	15 (100%)

Prevalence of infection according to present or absent of latrine

Out of 300 stool samples investigated 235(78.4%) have latrines and while 65 (21.6%) without latrines; Table (4).

Table 4: The number and percentage of infected cases with *cryptosporidium parvum* according to the latrine facility using the Modified Ziehl Neelsen staining technique and Trichrome staining techniques.

Techniques / Cases	Modified Ziehl Neelsen staining technique	Trichrome staining technique
Present	7 (12.97%)	5 (33.3%)
Absent	47 (87.03%)	10 (66.7%)
Total	54 (100%)	15 (100%)

Prevalence of infection according to sources of drinking water

Out of 300 stool samples examined from patient's drink from pipe were 258(86%), from canals were 25 (8.3%) and from donkey cart were17 (5.6 %); Table (5)

Table 5: The number and percentage of infected cases with *cryptosporidium parvum* in according to sources of drinking water using the Modified Ziehl Neelsen staining technique and Trichrome staining techniques.

Techniques / Cases	Modifie Ziehl Neelsen staining technique	Trichrome staining technique
Pipe	45 (83.4%)	8 (53.4%)
Canal	7 (12.9%)	5 (33.3%)
Donkey cart	2 (3.7%)	2 (13.3%)
Total	54 (100%)	15 (100%)

Prevalence of infection according to family occupation

Out of 300 stool samples examined families of patient's farmer were 122(40.6%), employee were 59 (19.6%), laborers were 73 (24.3 %) and others 46 (15.4 %); Table (6).

Table 6: The number and percentage of infected cases with *cryptosporidium parvum* in according to family occupation using the Modified Ziehl Neelsen staining technique and Trichrome staining techniques.

Techniques / Cases	Modifie Ziehl Neelsen staining technique	Trichrome staining technique
Farmer	24 (44.43%)	6 (40%)
Employee	6 (11.1%)	3 (20%)
Labourer	13 (24.07%)	4 (26, 7%)
Others	11 (20.4%)	2 (13.3%)
Total	54 (100%)	15 (100%)

Prevalence of infection according to present or absent of mucus and blood in stool sample

Out of 300 stool samples investigated 77 (25.6%) have blood and mucus and while 223(74.3%) without blood and mucus; Table (7).

Table 7: The number and percentage of infected cases with a *cryptosporidium parvum* ccording to the mucus and blood using the Modified Ziehl Neelsen staining technique and Trichrome staining techniques.

Techniques / Cases	Modified Ziehl Neelsen staining Technique	Trichrome staining technique
Present	4 (7.4%)	2 (13.3%)
Absent	50 (92.6%)	13(86.7%)
Total	54 (100%)	15 (100%)

Distribution of other intestinal parasite detected by concentration technique

Out of 300 stool samples investigated found 154(51.3%) patients infected with other intestinal parasite and while 146(48.7%) were non-infected; Table (8).

Table 8: The number and percentage of infected cases with other intestinal parasite detected by formal ether concentration technique.

Formal either c. technique	Frequencey & percentage
G.lamblia	77 (25.7%)
E.histolytica	60(20%)
H.nana	17(5.7%)
Negative	146(48.6%)
Total	300(100%)

DISCUSSION

Three hundred stool samples were collected and screened for *Cryptosporidium parvum* using modified Ziehl Neelsen staining technique and trichrome staining technique. The overall prevalence of Cryptosporidiosis was detected by modified Ziehl Neelsen staining technique 54 (18%) in table (1) this result is agreement with the results obtained by,[1] whom found the prevalence of cryptosporidiosis were (3-20%).

Also the high prevalence of parasite was detected in age group 26-50 years 25 (46.4%) using modified Ziehl Neelsen stain in table (2), this result was agreement with (9, 11-7) whom found that the infection increase with the same age group and disagree with[15] whom found that 31.5% of all children less than 2 years of age are infected with the parasite. The high prevalence was detected in female 30 (55.5%) more than male 24 (44.5%) by modified Zeihl Nelson staining technique in table (3), this may due to female directly contact with children infected with cryptosporidiosis and working in farms. this result were disagreement with Park *et al*,. 2006 ,which is found the high infection in male (1.9%) more than female (1.2%). The greater number of parasite was detected in patients who haven‘t latrine in their houses, table (4). This may due to personal hygiene and defecation in the open and contamination of food or water which aid in the transmission of disease.

High prevalence is found in people whom consumed pipe water 45(83.4%) in table (5) this may due to water purification station not clearing periodically and not sanitation by optimum method, this result agreement with[12] whom found in pipe water consumed people because consumed treated surface water were more likely to infected than the people that consumed well protected ground water.

There was high prevalence among farmers 24 (44.4%) in table (6) this may due to directly contact with farms soil that fertilised or contaminated by waste product of animals containing oocysts of cryptosporidium parvum, this result agreed with the result obtained by[17] whom found that the parasite is present on more than 90% of dairy farms. When used formal ether concentration technique the cryptosporidium oocyst was not detected because the oocyst is clear and acid fast, but other parasites found in formal ether concentration technique are *Giardia lamblia* 77 (25.7%), *Entameoba histolytica* 60 (20%) and *Hymenolepis nana* 17 (5.8%) in table (8).

ACKNOWLEDGMENTS

The authors thank the El Imam El Mahdi University and Kosti Teaching Hospital Administrations, especially all the laboratory staff of Medical Laboratory department for supporting this research.

REFERENCES

1. Current WL, Garcia LS. Cryptosporidiosis. *Clin. Microbial. Rev*, 1991; 4(3): 225-258.
2. Chappell CL, Okhuysen PC, White AC. Cryptosporidium parvum: infectivity, pathogenesis and the host-parasite relationship. In: Thompson RCA, Armson A Ryan UM (Eds.) Cryptosporidium: from molecules to disease. Elsevier, Amsterdam, the Netherlands, 2004; 19-49.
3. Casemore, D. Epidemiological aspects of human cryptosporidiosis. *Epidemiol. Infect*, 1990; 104: 1-28.
4. Lacroix C, Berthier M, Agius G, Bonneau D, Pallu B, Jaquemin JL . Cryptosporidium oocysts in immunocompetent children: epidemiological investigations in the day care centres of Poitiers, France. *Eur. J. Epidemiol*, 1987; 3: 381-385.
5. Frost FJ, Muller T, Craun GF, Calderon RL, Roeffer PA. Paired city Cryptosporidium serosurvey in the southwest USA. *Epidemiol. Infect*, 2001; 126: 301-307.
6. Park JH, Kim H.J, Guk SM, Shin EH, Kim JL, Rhim H.J, Lee SH, Chai JY. A. Survey of cryptosporidiosis among 2.541 residents of 25 coastal island in Jeollanam-Do (Province), Republic of Korea. *Korean J Parasitol*, 2006; 44: 367-372.
7. Egorov A, Frost F, Muller T, Naumova E, Tereschenko A, Ford T. Serological evidence of Cryptosporidium inections in a Russian city and evaluation of risk factors for infections. *Ann. Epidemiol*. 2004; 14: 129-136.
8. S. Tzipori, J.R. Baker, R. Muller, D. Rollinson (Eds.). Advances in Parasitology: Opportunistic Protozoa in Humans, *Academic Press, London*, 1998; 40.
9. Zu SX, Fang GD, Fayer R, Guerrant, R. L. Cryptosporidiosis - Pathogenesis and Immunology. *Parasitol. Today*, 1992; 8(1): 24 -27.
10. N.D. Levine, Phylum II Apicomplexa, in: R.R. Lee, S.H. Hutner, E. C. Bovee (Eds.). An Illustrated Guide to the Protozoa, Allen Press, Lawrence, 1985; 322–374.

11. Roberts WG, Green PH, MAJ, Carr M, Ginsberg AM. Prevalence of cryptosporidiosis in patients undergoing endoscopy: evidence for an asymptomatic carrier state. *Am. J. Med,* 1989; 87: 537-539.
12. Frost FJ, Kunde TR, Muller TB, craun GF, Katz LM, Hibbard AJ, Calderon RL. Serological responses to Cryptosporidium antigens among users of surface vs. groundwater sources. *Epidemiol. Infect,* 2003; 131: 1131-1138.
13. Cheesbrough, M. Medical laboratory manual for tropical countries. 2nd edition. British university, 1987.
14. Cheesbrough, M. District laboratory practice in tropical countries. Part 1. 3rd, Cambridge university, 1999.
15. P.C. Okhuysen, C.L. Chappell, M.M. Marshall, G. Widmer, S. Tzipori Infectivity of a Cryptosporidium parvum isolate of cervine origin for healthy adults anti gamma interferon knockout mice, *J. Infect. Dis,* 2002; 185: 1320–1325.
16. www.whitenilestate.gov.sd, 2016.
17. Sorvillo, F. J., L. E. Lieb, P. R. Kerndt, and L. R. Ash. Epidemiology of cryptosporidiosis among persons with acquired immunodeficiency syndrome in Los Angeles County. *Am. J. Trop. Med. Hyg*, 1994; 51: 326–331.
18. W.R. Mac Kenzie, N.J. Hoxie, M.E. Proctor, M.S. Gradus, K. A. Blair, D.E. Peterson, J.J. Kazmierczak, D.G. Addiss, K. R. Fox, J. B. Rose. A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply, *N. Engl. J. Med*, 1994*;* 331: 161–167.
19. Frost FJ, Muller T, Craun GF, Lockwood WB, Calderon RL. (2002). Serological evidence of endemic waterborne Cryptosporidium infections. *Ann. Epidemiol,* 2012; 12: 222-227.