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COMPARISON OF TWO PARASITOLOGICAL STAINING TECHNIQUES IN DIAGNOSIS OF CRYPTOSPORIDIOSIS AMONG DIARRHOEIC PATIENT'S ATTENDING TO KOSTI TEACHING HOSPITAL, WHITE NILE STATE, SUDAN

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ABSTRACT Background: Cryptosporidiosis is the disease caused by Cryptosporidium parvum, is associated with economic losses and the public health significance in humans. The aim of this study was to detect C.parvum and compare between modified ZN staining technique and trichrome staining as a parasitological techniques for recovery of cryptosporidiosis among diarrheic patient's attending to Kosti teaching hospital, White Nile State, Kosti city, Sudan.This representative is a comparative study was conducted from August 2016- April 2017. Methods: 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. Results: The overall results of parasite detected by modified ZN stain was 18%,and by trichrome stain 5%. The detection of C.parvum was higher in female than male. Conclusion: The study showed that modified ZN staining technique is the most sensitive and accurate so will be recommended to be use as first choice in diagnosis of Cryptosporiduim parvum.

KEY WORDS : Cryptosporidiosis, modified ZN staining technique, Trichrome staining technique, Parasitological techniques.

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 and infection is of particular concern in immunocompromised patients. In Milwaukee, 403.000 people were infected through contaminated of water and also cryptosporidiosis outbreaks reported in Europe and America [1]. Molecular techniques have shown that Cryptosporidium parvum is the predominant species in cryptosporidiosis, accounting for 50.8% of cases among 325 water-borne parasitic diseases worldwide. In stool examination of patients with gastroenteritis, the reported frequency 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 [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, 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 [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].

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Materials and Methods :

This study was conducted in Kosti city, White Nile State. The design of this study is a comparative 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.The Schaudinns fixative, the trichrome stain and modified Ziehl Neelsen stain prepared as described in [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.

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 stool samples 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 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 Trichrome staining technique and Modified Ziehl Neelsen staining technique.

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

Frequency 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 with 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%)

Frequency 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 stainin g 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%)

Frequency of infection according to present or absent of latrine:

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%)

Frequency of infection according to sources of drinking water:

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%)

Frequency 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%)

Detection of C.parvum according to present or absent of mucus and blood in stool sample:

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 stainingTechnique	Trichrome staining technique
Present	4 (7.4%)	2 (13.3%)
Absent	50 (92.6%)	13(86.7%)
Total	54 (100%)	15 (100%)

4. Discussion:

Three hundred stool samples were collected and screened for Cryptosporidium parvum using modified Ziehl Neelsen staining technique and trichrome staining technique. The overall frequency 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 recovery 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 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 detection 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.

Conclusion:

The routine parasitological examinations in developing countries should include permanent staining techniques such as modified ZN staining technique to detect the parasitic disease early and improve the health status of diarrhoeic patients. Also in this study the most sensitive and accurate technique is modified ZN staining technique so we recommend to be used as first choice in diagnosis of Cryptosporiduim parvum.

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