

Detection of *Cryptosporidium* oocysts in calves and children in Mosul, Iraq

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Summary

This study was carried out during May-October 2010 to determine the prevalence of *Cryptosporidium* spp. in calves and children in rural areas around Mosul, north of Iraq. A total of 78 faecal samples were taken from calves which were no more than one year old. Information about age, consistency of faeces (as diarrheic or normal) and contact with human were recorded. At the same time, 95 stool samples were taken from children aged one month to 12 years old suffering from diarrhea and living in the same farms where the samples of calves have been collected. All samples were stained with modified Ziehl-Neelsen acid-fast stain and Auramine O techniques to detect the presence of *Cryptosporidium* oocysts. Results revealed that the prevalence of *Cryptosporidium* in calves and children were 43.56% and 18.9%, respectively. Comparison of results obtained with the two stain techniques showed no significant difference and being sensitive.

الكشف عن أكياس بيض طفيلي *Cryptosporidium* sp. في العجول والأطفال في مدينة الموصل , العراق

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الخلاصة

أجريت هذه الدراسة لتحديد نسبة انتشار طفيلي *Cryptosporidium* sp. في العجول والأطفال في المناطق الريفية حول مدينة الموصل , شمال العراق للفترة من شهر آذار وحتى نهاية شهر تشرين الأول 2010 . أخذت 78 عينة براز من عجول لا تزيد أعمارها عن سنة واحدة , وسجلت المعلومات حول العمر وقوام البراز (طبيعي أم إسهال) وكذلك التماس مع الإنسان , وبنفس الوقت , جمعت 95 عينة براز من أطفال يعانون من الإسهال تتراوح أعمارهم بين شهر واحد واثنى عشرة سنة ويعيشون في نفس المزارع التي جمعت منها عينات براز العجول . جميع العينات صبغت بصبغة (زِيل نلسن) المحورة وتقنية Auramine O للكشف عن أكياس بيض الطفيلي . أظهرت النتائج أن نسبة الخمج بهذا الطفيلي في العجول والأطفال كانت 43.5 % و 18.9 % على التوالي . كما بينت النتائج عدم وجود فرقا معنويا مهما بين طريقتي الصبغ , إضافة إلى كفاءة وحساسية كلا الطريقتين المتبعتين للكشف عن أكياس بيض طفيلي *Cryptosporidium* sp. .

Introduction

Members of the genus *Cryptosporidium* are eukaryotic organisms, including obligate and intracellular parasites. *Cryptosporidium* has a complex life cycle, including both sexual and asexual reproduction, an auto-infectious cycle, and the ability to complete its development within a single host (1). The transmission form is a robust, environmentally resistant oocyst, excreted in the stool, which can exist for long periods of time in the environment. Because animals, in particular domesticated livestock, are its primary host, human infection is usually zoonotic (2,3). Those at greatest risk are immunocompromised adults and children, especially in patient with AIDS, children in day care, travelers to endemic regions, dairy or cattle farm workers or their families or contacts, household contacts of cases or carriers, and possibly owners of infected dogs or cats or their neighbors (4).

Cryptosporidium is related to direct zoonotic and this parasite infects human directly from animals (5). *Cryptosporidium parvum* is the major species seen in humans and animals. *C. parvum* type I (*C. hominis*) is found in humans, and *C. parvum* type II (*C. bovis*) is found in humans and animals (especially ruminants) (6). As well as causing morbidity and mortality in animals, cryptosporidiosis in lambs and calves is considered to be an infection source for

humans (6). Infected calves can eliminate a large number of oocysts in their faeces and contaminate fresh food, drinking and recreational water causing infection affecting humans and other animals (7). The presence of *Cryptosporidium* spp. in faeces of calves and children has been reported in other countries in the world (6,7,8, 9,10) and studies on risk factors have also been carried out, usually focusing on management factors at the herd level, derived from questionnaire gathered from farmers (11). The major clinical sign in cryptosporidiosis is gastroenteritis that is self-limiting. But in persons who are immunocompromised, it may cause chronic diarrhea (12).

The present study was investigated to determine the prevalence of *Cryptosporidium* in calves and children in rural areas around Mosul city and also to understand its epidemiology by using two microscopic methods Ziehl-Neelsen acid-fast and Auramine O techniques.

Materials and methods

Since May to October 2010, samples were obtained from cattle farms in rural areas around Mosul ,Iraq. A total of (78) fresh stool samples were collected directly from diarrheic calves no more one year old, using sterile latex gloves. Information about age, consistency of faeces (as diarrheic or normal) and contact with human were collected and recorded. At the same time, a total of (95) stool samples were also taken from children aged one month to 12 years suffering from diarrhea and living in the same farms where the samples of calves have been collected. Each sample was collected in a dry and clean plastic container labeled with child's name, age, living location and stored at 4°C until they were processed.

The samples were submitted to sedimentation of oocysts by centrifugation, in which the sediment was fixed with methanol for 5 minutes and then used for the smearing procedure (13). The stool were smeared into glass slides and stained using the modified Ziehl Neelsen (14) and Auramine O techniques (15). The samples stained by the Ziehl Neelsen technique were examined under light microscopy (100 X). The Auramine O stained smears were analyzed by fluorescence microscopy, after a previous screening (100 X) .

A sample was considered positive for cryptosporidiosis, if an oocyst was identified based on morphology including optical properties, internal structure, size, and shape. In the Auramine O method, samples were studied with dark field microscope and *Cryptosporidium* oocysts had fluorescent property.

Statistical analysis was performed using analysis of one-way ANOVA analysis with the help of SPSS computer software (16).

Results

The results of this study confirmed that cryptosporidial infection was present in calves in rural areas around Mosul. Out of (78) fecal samples examined (34) (43.56%) samples were positive for *Cryptosporidium* table (1) .The results appear that infection in cattle is highly age-dependent ,the young calves showing highest prevalence and intensity of shedding the organism table (1). Out of (95) stool samples examined for children suffering from diarrhea (18) (18.9%) samples were positive for *Cryptosporidium* sp. ,highest prevalence was in the age group of one – three years (45.4%) table (2).

This study also revealed the significant association between infection with *Cryptosporidium* and diarrhea.

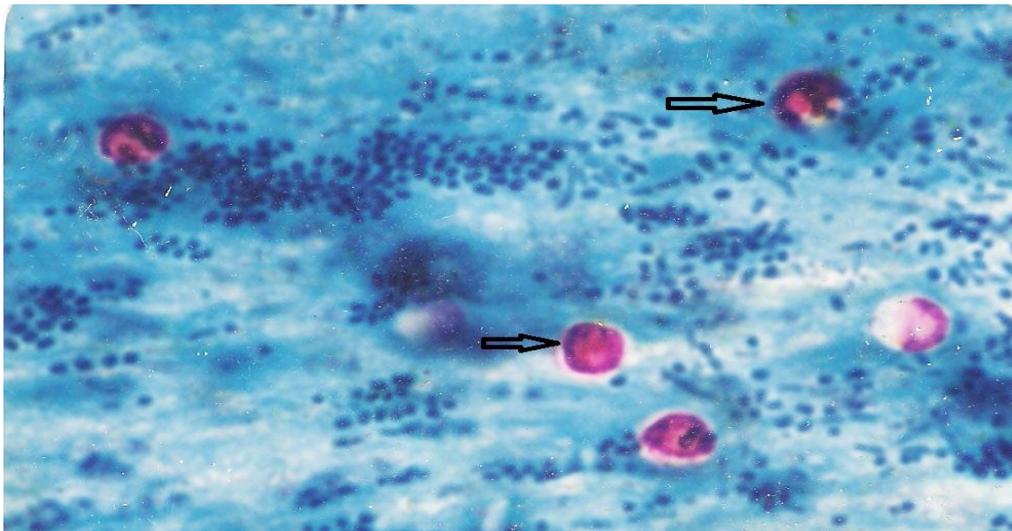
In this present study, there are no significant differences between Auramine O stain and Ziehl Neelsen technique, both are specific for diagnosis of *Cryptosporidium* in prepared faecal samples

The appearance of detected *Cryptosporidium* oocysts after staining with modified Ziehl - Neelsen as bright red spherical oocysts with a diameter of 5.2 x 4.4 µm figure (1).

Cryptosporidium oocysts appear ring or ovoid shaped and exhibit a characteristically bright apple-green fluorescence against a dark background, measuring 4–5 µm in diameter when stained with Auramine O figure (2).

Table 1 : Prevalence of *Cryptosporidium* oocysts in calves in Mosul city

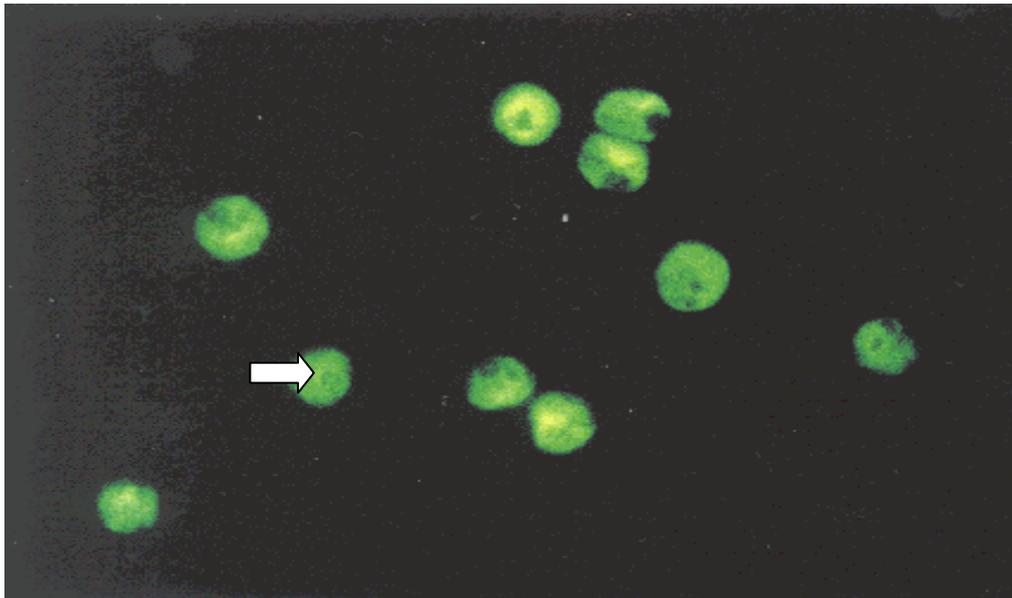
Age	Number of animals examined	Positive cases		ZN	AO	Clinical signs
		N.	%			
1 – 2 days	1	2	50	+	+	Diarrhea
1 – 2 weeks	9	5	55.5	+	+	Diarrhea
2 weeks – 1 month	18	6	33.3	-	-	Diarrhea
1 – 2 months	22	11	50	-	-	Diarrhea
Up to 1 year	25	10	13.3	-	-	Diarrhea
Total	78	34	43.56			



Figure(1): The oocysts of *Cryptosporidium* sp. Staining with modified Zeihl-Neelsen 100X

Table 2: Prevalence of *Cryptosporidium* oocysts in children in Mosul city

Age	Number of children examined	Positive cases		ZN	AO	Clinical signs
		N.	%			
1 – 6 month	39	4	10.2	+	+	Diarrhea
6 – 12 month	28	7	25	+	+	Diarrhea
1 – 3 years	11	5	45.4	-	-	Diarrhea
3 – 6	10	1	10	-	-	Diarrhea
6 – 12	7	1	14.2	-	-	Diarrhea
Total	95	18	18.9			



Figure(2): The oocysts of *Cryptosporidium* sp. Staining with Auramine O by Fluorescent microscope 100X

Discussion

Cryptosporidium sp. is a common intestinal pathogen of animals and humans. It may have an important economic impact on farms and cause potentially zoonotic infections (17).

In the present study, the overall prevalence of *Cryptosporidium* in calves was (43.56%). These results agreed with studies carried out in calves in Kars province, Turkey have reported a prevalence of 42.8%. The rate of infection was 30.3% in calves with diarrhea and 10% in healthy calves (6). According to another study carried out in young calves in New Zealand, (10) have reported a prevalence of 28% with PCR. For the first time in Argentina, infection to *Cryptosporidium* was reported in 17% of calves with <30 days old (7).

Results appear that infection in cattle is highly age-dependent table (1), with young calves showing the highest prevalence and intensity of shedding the organism (18). Therefore, these young animals can shed the oocyst of *Cryptosporidium* which has a wide host range and is considered to be potentially zoonotic (10).

Out of (95) stool samples examined for children suffering from diarrhea (18) (18.9%) samples were positive for *Cryptosporidium* sp. table (2). Oocysts excretion is often intermittent and occurs in small quantities, thus, prevalence would be undoubtedly higher if more than one stool sample from each child were examined (8). Children with cryptosporidiosis are indistinguishable from non-*Cryptosporidium* gastroenteritis children with respect to their age, nutritional status, severity of diarrhea or metabolic disturbance (19).

In the developing world, the association of *Cryptosporidium* with acute and persistent diarrhea in children is striking. Several cross sectional studies in children with diarrhea suggest that cryptosporidiosis is endemic in developing world with prevalence of up to 26% in Mexican and 16.5% in Brazilian children with diarrhea (4). Prevalence in African countries, Central and South American countries, Asian countries is greater than in Europe and North America (20). In Pakistan and Indonesia the parasite has been found in 10.3 and 8.2% of children with diarrhea respectively (19,21).

Highest prevalence was in the age group of one – three years (45.4%) table (2). *Cryptosporidium* has been reported in persons from three days to 95 years of age. But data suggest that young children are more susceptible to infection(15). This corresponds to the weaning period when the children are exposed to contaminated environment, food and water. Similar observations were made in Indonesia, Pakistan and Ghana where children less than 2 years old were found to be the most susceptible (19). In contrast, higher age group (4.5 years) as found to be the most susceptible in Kuwait(22).

The most important risk factor for cryptosporidiosis in children is contact with a person suffering from diarrhea (12). Other possible methods of transmission are association with dogs and cats, consumption of untreated milk, water supply and dummies (15). Thus, the major routes of transmission associated with this parasitosis should be assessed further investigation. The clinical findings and the course of illness do not help in indicating the source of patients' infection. *Cryptosporidium* lacks host specificity (12) and thus is a potential zoonosis (9). The role of *Cryptosporidium* in causing disease in infected patients is unclear, but diarrhea has been experimentally induced in lambs and calves (17).

This study also revealed the significant association between infection with *Cryptosporidium* and diarrhea. There are much data about cryptosporidial human infection in the world especially in children with or without diarrhea. (23) showed that intestinal parasitic infection is a common health problem among children in Saudi Arabia and *Cryptosporidium* was reported from 8.3% inpatients and 2.3% outpatients. (24) revealed that infection with *Cryptosporidium* species and *Entamoeba histolytica* were more prevalent in patients with acute diarrhea than in healthy control subjects, for all ages and specifically for those 0-12 months of age in Dhaka, Bangladesh .

In this present study, there are no significant differences between Auramine O stain and Ziehl Neelsen technique, both are specific for diagnosis of *Cryptosporidium* in prepared faecal samples and the prevalence rate of cryptosporidiosis was seen to be higher when this stain was used (25).

It was concluded that young calves and children play an important role in maintaining infection in nature and represent an important risk factor for transmission of infection to other humans and animals.

Further studies on characterization and subtyping of human, animal and environmental isolates are required to evaluate the public health significance of *C. parvum* infection in livestock, the exact sources and routes of transmission and population dynamics of *Cryptosporidium* . Control measures based on such knowledge shall be useful in reducing morbidity and mortality due to cryptosporidiosis in children.

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