

## The survey of *Cryptosporidium* infection among young children in kindergartens in Anhui province ☆

Jun Lu<sup>a\*</sup>, Chaopin Li<sup>b</sup>, Shan Jiang<sup>c</sup>, Song Ye<sup>a</sup>

<sup>a</sup>Department of Laboratory Medicine, Medical College, Anhui University of Science & Technology, Huainan 232001, Anhui province, China

<sup>b</sup>Department of Pathogenic Organism, Wannan Medical College, Wuhu 241001, Anhui Province, China

<sup>c</sup>Department of Mining Engineering, Huainan Vocational & Technical College, Huainan 232001, Anhui province, China

Received 12 November 2007

### Abstract

**Objective:** To study the *Cryptosporidium* infection among young children in Anhui province, and to explore the best way to diagnose the disease. **Methods:** Stool specimens of 1 204 children were collected; oocysts of *Cryptosporidium* were identified with auramine O-modified staining, acid-fast staining, safranin T and methylene blue staining, and auramine O-modified acid-fast staining. **Results:** The detectable rate of *Cryptosporidium* in four stainings were respectively 2.46%, 1.50%, 1.98% and 3.46%, and there was a higher significant difference in the rate between auramine O-modified acid-fast staining and the others ( $P < 0.005$ ). The detectable rate was significantly lower in urban children (2.14%, 15/684) than in rural ones (5.19%, 27/520). Boys and girls had similar detectable rate (1.99%, 24/1 204 vs. 1.50%, 18/1 204). *Cryptosporidium* infection was usually subclinical, and its major clinical features included benign diarrhea, mild abdominal pain and nausea. **Conclusion:** *Cryptosporidium* infection was relatively common in kindergartens and a higher infection rate was found in rural children. As the majority of the *Cryptosporidium* infections were subclinical, diagnosis is important although difficult.

**Key words:** *Cryptosporidium*; children; kindergarten

### INTRODUCTION

Cryptosporidiosis is a kind of zoonosis caused by *Cryptosporidium*, and diarrhea is its main symptom. The life cycle of *Cryptosporidium* is simple, but the species of hosts of *Cryptosporidium* are numerous. Cryptosporidiosis has been more found overseas<sup>[1-2]</sup>, since first case of cryptosporidiosis discovered in Nanjing city was reported by Hanfan<sup>[3]</sup> in Nanjing Medical University in 1987, this disease were reported over 10 provinces one after another<sup>[4-8]</sup>. In order to get the information of the infection among young children in kindergartens in Anhui province, an epidemiological survey was carried out in some areas of Anhui province.

### MATERIALS AND METHODS

#### Samples

The cluster sampling was conducted according to randomization, a total of 1 204 stool samples obtained from the infants in 29 the infant's schools of 11 cities in Anhui province were collected in 2003, including 641 males and 563 females, aged 3 years to 6 years (the average was 4.39 years).

#### Methods

Case histories were demanded by way of questionnaire on the spot, and detailed information was asked such as: present history, previous history, habits of personal hygiene, status of environmental hygiene, etc.

Fresh stools were collected in time and subsequently processed into round smears (size 1.5 cm<sup>2</sup>) after natural dehydration, and all smears were fixed with methanol and numbered in order. Further examination for oocyst

☆ This study was supported by Natural Science Foundation of Youth of Anhui University of Science & Technology (1999–21)

\*Corresponder author

E-mail address: [cfdxlujun@126.com](mailto:cfdxlujun@126.com)

of *Cryptosporidium* was conducted in laboratory. Four stainings were applied in this survey, including auramine O-modified staining, acid-fast staining, safranin T and methylene blue staining, and auramine O-modified acid-fast staining<sup>[9]</sup>. It was believed Oocysts of *Cryptosporidium* may be found through observation under the microscope.

### Statistical analysis

The data were analyzed by Chi-square test.  $P < 0.05$  was considered as significant difference.

## RESULTS

The detectable results of smear for oocysts of *Cryptosporidium* by four stainings were shown in Tab 1-3.

33/42(78.57%) patients were self-conscious symptoms negative, and others(9/42, 21.43%) were self-conscious symptoms positive, most of them had symptoms in upper or lower digestive tract(8/42, 19.05%), common symptoms occupied 2.38%(1/42).

Tab 1 The *Cryptosporidium* infection among young children in kindergartens in Anhui province

Examination ways	Total number	Positive number	Positive rate(%)
Auramine O-modified staining	1 204	30	2.49
Acid-fast staining	1 204	18	1.50
Safranin T and methylene blue staining	1 204	24	1.99
Auramine O-modified acid-fast staining	1 204	42	2.49

Compared auramine O-modified acid-fast staining with other three stainings,  $P < 0.005$ .

Tab 2 The status of *Cryptosporidium* infection in different sex in children(with auramine O-modified acid-fast staining)

Sex	Total number	Positive number	Positive rate (%)
Male	641	23	3.59
Female	563	19	3.37
Total	1 204	42	3.49

Compared male with female,  $P > 0.05$ .

Tab 3 The status of *Cryptosporidium* infection in urban infants and rural young children(with auramine O-modified acid-fast staining)

Sex	Total number	Positive number	Positive rate (%)
Urban infants	684	15	2.14
Rural infants	520	27	5.19
Total number	1 204	42	3.49

Compared male with female,  $P > 0.01$ .

## DISCUSSION

Cryptosporidiosis is a kind of zoonosis caused by *Cryptosporidium*<sup>[10]</sup>, and diarrhea is its main symptom. *Cryptosporidium* is a kind of parasitic protozoan living within the cell, it can infect the function of intestine of mankind and other vertebrates. The infants were prone

to infect *Cryptosporidium* result in hypoevolutism because their immunological functions were quite limited<sup>[3]</sup>. Through analyzing situation of infection caused by *Cryptosporidium* in the infant's schools in Anhui province, we found the detection rate of oocysts of *Cryptosporidium* in infant groups as much higher. Our investigational results showed that 42 of 1 204 stool samples were *Cryptosporidium* positive, the detection rate was 3.49% being far lower than those in the developing countries and similar to previous reports<sup>[11-14]</sup>. Between the percentage of males(3.59%) and that of females (3.37%) there was no notable difference found between them( $P > 0.05$ ). This showed that the occurrence of cryptosporidiosis seemed to have no obvious relationship with gender. Its cause may be that the immune organs, such as thymus gland, have not completely developed, and that the infant's bodies were not capable of producing sufficient antibody to neutralize *Cryptosporidium* so that the latter multiplied causing chronic infection.

In Tab 3, the percentage of infants in urban area was 2.14% and those in rural areas were 5.19%. This showed there was a notable difference between them( $P < 0.01$ ). The causes may be as follows; in recent years, with the improving of the living standard and the development of economy, people have cut off the mode of spread of *Cryptosporidium* infecting man from stool-mouth or hand-mouth. The gap between in city and in village has been enlarged obviously. The condition in villages was still unsatisfactory so that the infants were infected from some foods including oocysts of *Cryptosporidium*<sup>[15]</sup>. Therefore, the infectious rate of *Cryptosporidium* in rural infants was quite high.

Furthermore, we found there was higher significant difference in the rate between auramine O-modified acid-fast staining than found with others( $P < 0.005$ ). Auramine O-modified acid-fast staining is the best staining<sup>[3]</sup>, as it only requires a generic microscope (no fluorescence microscope). And there are a lot of other particles that could influence the specificity of staining, so some smears could not be well judged. The application of auramine O-modified acid-fast staining can overcome the shortcomings of false positive and false negative results and raise detection rate of oocyst of *Cryptosporidium*.

To sum up, existing cases of cryptosporidiosis among the young children in kindergartens in Anhui province, were especially found in rural infants. Because people are generally sensitive to cryptosporidiosis and lack the effective preventive methods, controlling reservoir is pivotal. We suggest that the young children in kindergartens should be periodically surveyed in a large scale. When the therapy depending on antibiotics is no longer efficient to patients with chronic diarrhea, we must

analyze age, immune functions of patients and exclude possibilities of other correlated illnesses. It is necessary to think of the existence of cryptosporidiosis. Therapeutics of expelling *Cryptosporidium* should be employed in order to avoid recurrence of the illness.

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## DNA repair genotypes and phenotypes and cancer susceptibility

Qingyi Wei

Department of Epidemiology, The University of Texas, M.D. Anderson Cancer Center

The role of DNA repair in the etiology of cancers has been well illustrated in several hereditary syndromes, in which an inherited defect in DNA repair and related biological processes is associated with extraordinarily high incidence of cancer. For example, patients with xeroderma pigmentosum (XP) have germline mutations in genes involved nucleotide excision repair (NER) coupled with more than 1000-fold increased risk of UV-induced skin cancers. However, such associations between inherited DNA repair defect and risk of cancer have not been apparent in the general population. In the study of the role of DNA repair capacity phenotype and genotypes as markers for susceptibility to head and neck, a tobacco-induced cancer, in about 800 patients with head and neck cancers and 800 cancer-free matched controls, we found that reduced DNA repair capacity as measured by both reduced host-cell reactivation, increased levels of induced DNA adducts, reduced levels of gene and protein expression, enhanced mutagen sensitivity, and adverse genotypes, we have demonstrated that suboptimal DNA repair capacity for removing DNA damage induced by benzo[a] pyrene diol epoxide, an ultimate tobacco carcinogen, is associated with increased risk of head and neck cancers. Furthermore, we found that such a suboptimal repair phenotype has a genetic basis, because some common single nucleotide polymorphisms (SNPs) in the genes involved in NER repair pathways are associated with the variation in the phenotypes. These findings provide a rationale that by genetic screening for functional SNPs, it may be feasible to identify at-risk populations who can be targeted for primary prevention.

