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NATURAL CRYPTOSPORIDIUM INFECTION OF THE STOMACH AND INTESTINE IN LABORATORY MICE

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ABSTRACT

Two species of cryptosporidium infect laboratory mice (*Mus musculus*) namely *C. muris* and *C. parvum* which inhabitant of gastric and intestinal mucosa respectively *C. parvum* and in rare occasions *C. muris* are pathogenic for humans particularly in immunocompromised individuals. The animals harbor cryptosporidium organism could be a potential sources of infection for humans and susceptible species.

In the present study to survey the occurrence of cryptosporidium in the laboratory mice, tissue sections stained with Haematoxylin and Eosin and Mepacrine staining methods were carefully examined and the positive specimens were further stained with Modified Ziehl Neelsen technique recommended for identification of cryptosporidial organisms. 100 conventional and BALB/C laboratory mice from six research and educational centers were studied for the presence of cryptosporidium in the gastric and intestinal mucosa. Sixty-three out of hundred (63%)) of animals examined were infected with cryptosporidium organisms: gastric cryptosporidiosis due to *C. muris* nine out of hundred (9%), intestinal cryptosporidiosis caused by *C. parvum* thirty-four out of hundred (34%) and both cryptosporidiosis of stomach and intestine twenty out of hundred (20%). With the exception of gastric cryptosporidiosis, cryptosporidiosis of intestine is very mild to moderate. It is concluded that a high percentage of laboratory mice harbors cryptosporidium organisms. The infected animals not only could be a source of infection for laboratory animals but also in many instances such the animals may not a suitable one for research purposes.

Key words: Cryptosporidium, natural infection, laboratory mice

INTRODUCTION

C. muris and *C. parvum* that infect laboratory mice (*Mus muculus*) were primarily described by Tyzzer (1907, 1910, 1912). *C. muris* infects mice, is only slightly pathogenic in laboratory animals as was reported in laboratory mice (Ozkul and Aydin 1994), wild house mice (Chalmers et al, 1994 and 1997), house rats (Iseki, 1986), cattle (Upton and Current 1986, Anderson, 1987), in camel (Fayer et al. 1991) and recently in human (Clemente et al. 2000). *C. parvum* is pathogenic in SCID and athymic mice (Mead et al, 1991) and has shown to have a wide host range across mammalian species including humans (Dubey, Speer, Fayer, 1990). Neonates of mammalian species and immunodeficient individuals are most susceptible to the infection Cross transmission of *C. muris* and *C. parvum* occurs between different species of mammals (Dubey, Speer, and Fayer, 1990).

In the present study, for the first time, the occurrence of gastric and intestinal cryptosporidiosis is

reported in the laboratory mice from Iran and the rate of infection and pathological findings have also been considered.

MATERIAL AND METHODS

One hundred mature conventional (68 cases) and Balb/C mice (32 cases), from both sexes were obtained from six research and educational centers, euthenized humanity by diethyl ether and necropsied. A part of samples taken from gastric and intestinal tissues were fixed in a ten percent buffered formalin solution (pH=7.2) and another part was immediately frozen and kept at -70 °C. The formalin-fixed tissues were processed m an Autotechnlcon tissue processor (Autotechnicon, USA), paraffin blocks were made and cut with a Jung microtome (Jung, Germany). Tissue sections were stained routinely with Harris Haematoxylin and Eosin method (Luna, 1968). Five to six microns frozen sections were cut with a Slee Cryocut (Slee, England) from frozen tissue samples, air-dried, fixed in a ten percent buffered formalin solution for 3 minutes and stained with Mepacrine method primarily introduced by Ungureanu and Dontu (1992) for screening of cryptosporidium in fecal smears. The procedure was followed as recommended by Ungureanu and Dontu. The formalin fixed-ftozen sections were stained with 0.5 percent aqueous Mepacrine solution for five minutes, rinsing with tap water, decolorizing with one percent hydrochloric acid in 97 percent ethyl- alcohol for 1 -2 minutes, rinsing with tap water, counterstaining with one percent $KMnO_4$ in distilled water for 30 seconds and mounting with 50 percent glycerin in phosphate-buffered saline (pH=7.2). Finally the sections were examined using an Olympus fluorescent microscope (Olympus, Japan). The positive specimens were further stained with Modified Ziehl Neelsen technique (Garcia et. al, 1985).

In all centers studied, the animals were kept in the translucent polycarbonate mouse boxes, and supplied with wood shave bedding water and mouse feed chew obtained from local producers with the exception of the BAB/C mice from center No. 3, all animals examined were a symptomatic and appeared healthy. The BALB/C mice simultaneously had helminthes infestation. They hunched in a corner and showed clinical signs of growth retardation, anorexia and diarrhea.

RESULTS AND DISCUSSION

Careful microscopic examination of tissue sections stained with Harris H &E method showed that 63% of animals examined were infected with cryptosporidium organisms; intestinal cryptosporidiosis due to *C. parvum* was 34%, gastric cryptosporidiosis caused by *C. muris,* was 9% and simultaneously both gastric and intestinal cryptosporidiosis was 20% (table 1).

The infected glandular structures of the stomach were dilated. The epithelial cells were flattened attenuated and degenerated and numerous fairly basophilic cryptosporidium organisms varying in size and shape were present within lumen of the glands or lying on the glandular epithelium (Fig.1). The inflammatory reactions were very mild to negligible and only the number of lymphocytes of the lamina propria was slightly increased. Frozen formalin-fixed tissue sections stained with Mepacrine method, showed oocysts as yellow green fluorescent discs against a dark background
(Figs.5, 6).

With modified Ziehl Neelsen method, the oocysts appeared as red discs against a blue background (Fig.2). The cryptosporidium organisms found in the intestinal tissue were smaller than those found in the

stomach, they were basophilic and located within parasitoferous vacuoles formed by microvilli of the intestinal epithelium (Fig.3). In all cases examined only ileal portion of the intestine was infected and most]y, only a few cryptosporidium organisms were present.

The staining characteristics of the cryptosporidium organisms (*C. parvum)* found on mucosa of the intestine stained with Mepacrine and Ziehl Neelsen Methods (Fig.4) as similar to those described for gastric cryptosporidiosis. Tissue changes of intestine were negligible.

Table 1. The occurrence of cryptosporidium in the gastric and intestinal tissues of the laboratory mice

Center number	Type of animal	No	Stomach (%)	Intestine (%)	Stomach and Intestine (%)	Total (%)
1	Conventional	32	1 (3.12)	13 (40.6)	14 (43.8)	28 (87.5)
2	BALB/C	10	0 (0.0)	4 (40.0)	2 (20.0)	6 (60.0)
3	Conventional	8	1 (12.5)	4 (50.0)	0 (0.0)	5 (62.5)
3	BALB/C	10	0 (0.0)	3 (30.0)	0 (0.0)	3 (30.0)
4	Conventional	10	3 (30.0)	1 (10.0)	2 (20.0)	7 (70.0)
5	Conventional	10	3 (30.0)	2 (20.0)	1 (10.0)	6 (60.0)
5	BALB/C	10	1 (10.0)	4 (40.0)	1 (10.0)	6 (60.0)
6	Conventional	10	0 (0.0)	2 (20.0)	0 (0.0)	2 (20.0)
Total (%)		100	9 (9.0)	34 (34.0)	20 (20.0)	63 (63.0)

DISCUSSION

In this paper, for the first time the cryptosporidiosis of laboratory mice and rate of infection (Table1) were reported from Iran. The characteristics of the organisms found in the tissue sections of stomach and intestine of the infected animals are similar to those reported originally by Tyzzer (Tyzze 1907, 1910, 1 912). The diameters of *C. muris* found in the gastric glands were larger than *C. parvum* found on intestinal mucosa. In spite of substantial number of *C. muris* organisms in gastric glands no significant inflammatory responses were seen, except only the lymphocyte of the lamina propria were slightly increased and the infected mice showed no clinical signs and gross lesions. The findings are in concordance with those described by other workers (Tyzzer, 1910, Ozkul and Aydin, 1994).

Infection with the *C. parvum* was mild in the infected mice and no clinical signs, gross lesion and significant tissue changes were present. Experimentally induced *C. parvum* , cryptosporidiosis in mice from different ages, sexes and strains produced no clinical diseases (Tarazona, et. al, 1998). These findings indicate that, in spite of susceptibility of mice to cryptosporidial infection, the infected animals remains asymptomatic. However, cryptosporidiosis in athymic (nude) BALB/C mice and SCID mice resulted in persistent and widespread infections and diarrhea, followed by death (Dubey et. al 1990 and Mead et. al, 1991).

In contrast, the euthymic conventional and BALB/C mice used in this stud), showed no clinical manifestation of infection. But, because of simultaneous infection of the animals was accompanied by clinical manifestations so-called "sick mice".

Paraffin tissue sections stained with Harris H & E method revealed endogenous stages of cryptosporidium life cycle (Fig.1), where as staining tissue sections with Modified Ziehl-Neelsen Method,

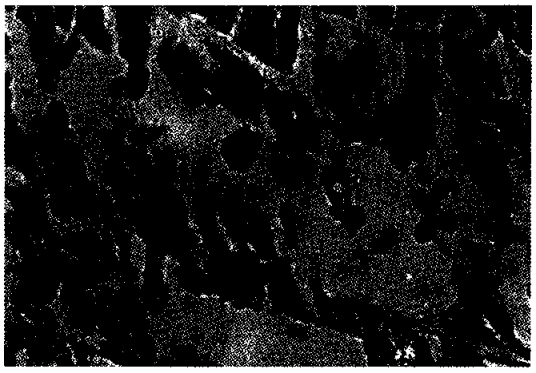
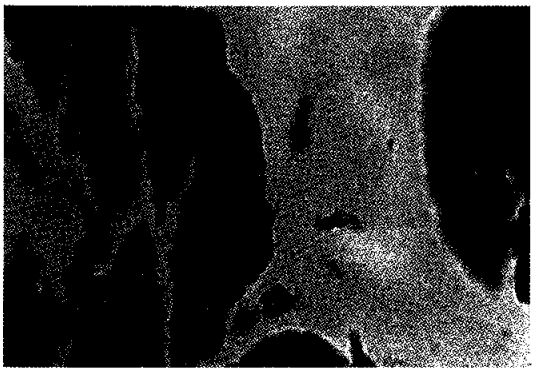


Fig.1: A histological section of gastric mucosa infected with *Cryptosporidium muris* in small white laboratory mice. Many cryptosporidial organisms are seen within distended

Fig.2: Gastric mucosa infected with *C. muris* stained with Modified Zeihl Neelsen Method. Round to oval acid- fast cryptosporial organisms are present.



Fig·.3: Histological section from intestine infected with *C. parvum*. Two basophilic cryptosporidial organisms were seen within parasitoforous vacuoles (H and E x 1,000).

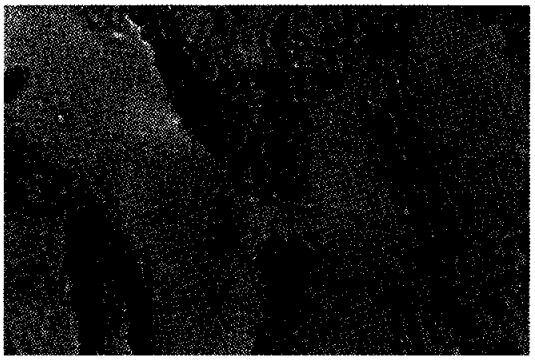


Fig.4: Modified Zeihl Neelsen stained tissue section from intestine infected with *C. muris* the acid fast organisms lodged on the surface of intestinal epithelium (Modified Modified Zeihl Neelsen x1,000)

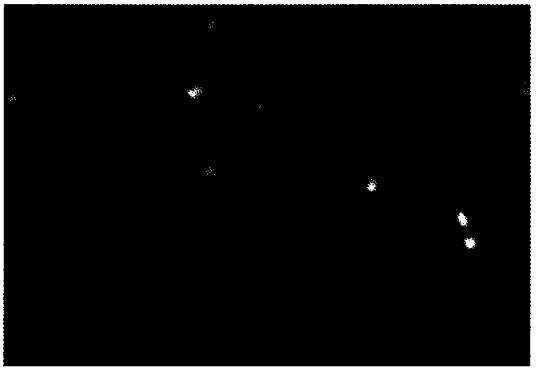


Fig.5: A frozen section from infected gastric tissue stained with Mepacrine staining method. Many yellow-green fluorescent organisms are present (Mepacrine staining x400)

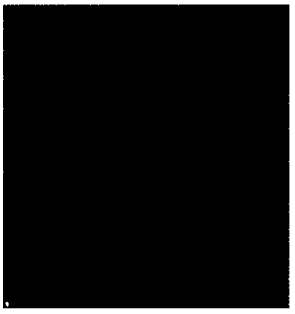


Fig.6: A higher magmficatron from fig 5. (Mepacrine staining method x 1,000).

only oocysts were detectable (Figs.2, 4). The results obtained in our study is in agreement with those presented by Garcia et. al, (1985) for screening of cryptosporidium oocysts in fecal smears. Staining the frozen sections with Mepacrine method was satisfactory and the results are as similar as to those described by Ungureanu and Dontu (1992). The organisms in paraffin sections failed to take Mepacrine dye. In my opinion, since Haematoxylin and Eosin method could stain most of the endogenous stages of cryptosporidium life cycle in tissue sections, it is a satisfactory technique to identify cryptosporidium organisms.

It is concluded that a high percentage of laboratory mice harbors *C. parvum* and *C. muris*. In my opinion the endogenous stages of organisms are easily detectable in tissue sections stained with H and E method, provided careful examination of tissue sections Such infected asymptomatic animals not only could be a source of infection for laboratory personals and susceptible laboratory animals, but also in many instances the animals are not suitable for research purposes.

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REFERENCES

Anderson, B.C. (1987). Abomasal cryptosporidiosis in cattle. Vet. Pathol. 24: 235-238.

Chalmers, R.M., Sturdee, A.B., Bull, S.A. Miller A and Wright S F (1997) The prevalence of *Cryptosporidium parvum* and *Cryptospondium muris* in *Mus domesticus*, *Apodemus sylvaticus* and *Clethronomy glareolus* in an agricultural system. Parasitol. Res. 83: 478-482.

Chalmers, R.M., Sturdee, A.P., Casemore, D.P., Curry, A., Miller. A., Parker N. D and Rrchmond T.M. (1994). *Cryptosporidium muris* in wild house mice (*Mus musculus*). 1st report in the U.K. Eur. J. Parasitol. 30:151-155.

Dubey, J.P., Speer, C.A. and Fayer, K. (1990). Cryptosporidiosis of man and animals. C.R.C. Press, Boca Ratton. FL.

Clemente C.M., Caramori, C.A., Padula, P. and Rodrigues, M.A (2000) Gastric cryptosporidiosis as a clue for the diagnosis of acquired immunodeficiency syndrome. Arq. Gastroentrol. 37:180-182.

Fayer, R., Philips, L., Anderson, B.C., and Bush, M. (1991). Chronic cryptosporidiosis in a Bactrian camel (*Camelus bactrionus*). J. Zoo. Wildlife. Med. 22. 228-232.

Garcia, L.S., Bruckner, D.A., Brewer, T.C. and Shimizu, R.V. (1985). Techniques for recovery and identification of cryptosporidium oocysts from stool specimens. J. Clin. Microbiol. 18:185-190.

Iseki, M. (1986). Two species of cryptosporidium naturally infecting house rats, *Rattus norvegicus*. Jpn. J. Parasitol. 35:521-526.

Luna L G (1986). Manual of HISTOLOGIC STAINING METHODS of the Armed Forces Institute of Pathology. Third edition. The Blakistom Division, Mc Graw-Hill Book Company, USA.

Mead, J.R., Arrowood, M.J., Sidwell, R.W. and Healey, M.C (1991) Chronic *Cryptosporidium parvum* infection in congenitally immunodeficient SCID and Nude mice. J. Inf. Dis. 163:1297-1303.

Ozkul, L.A. and Aydin, Y. (1994) Natural *Cryptospondium muris* infection of stomach in laboratory mice. Vet. Parasitol. 55:129-132.

Tarazona R., Blewett D .A. and Dommguea, M. (1998). *Cryptosporidium parvum* infection in experimentally infected mice, infection dynamics and effect of immunosuppression. Folia Parasitol. 45:101-107

Tyzzer, E.E. (1907). A sporozoan found in the gastric glands of the commoon mouse. Proc. Soc. Exp, Boil. Med. 5:12-13.

Tyzzer, E.E. (1910). An extracellular coccidium *Cryptosporidrum muris* (*gen Et sp Nov.*) of the gastric glands of the common mouse. J. Med. Res. 23:487-509.

Tyzzer, E.E. (1912). *Cryptosporidium parvum* (*sp. Nov.*) a coccidium found in small intestine of the common mouse. Arch. Protistenkd. 26:394-412.

Ungureanu E.M. and Dontu, G.E. (1992). A new staining technique for the identification of cryptosporidium oocysts in fecal smears. Trans. Royal Soc. Trop. Med. Hygiene. 88:638.

Upton, S.J. and Current, W.L. (1985). The species of *Cryptosporidium* (Apicomplex: Cryptosporididae) infecting mammals. J. Parasitol. 71:625-629