Effect of Yogurt and *Bifidobacterium* on *Cryptosporidium parvum* Infection In Experiential Infected Mice

S.T. Mohammed, K. Jabur, H.A. Aja
Deparment of Biology, College of Science, University of Al-Mustanserria
Received in:1 July 2010
Accepted in:16 June 2011

Abstract

This study aimed to collected 150 feces sample from calves suffering sever diarrhea ,then there were isolation and purification of *Cryptosporidium parvum* oocyst from samples contain it. These oocysts were used to induce experimental infection in the Immunosupperessed mice, then the mice treated by Bifidium bacteria , its supernatant and local yogurt were used for bacterial isolation.

This study showed that the local yogurt was the best treatment which led to stop oocyst shedding from the mice in the 8^{th} day after treatment under sufficient treatment (89.72%) , at the same time the mice treated with bacteria stopped the oocyst shedding in the 9^{th} day with sufficient treatment (81.40 %) , while its supernatant were stopped the oocysts shedding in the 10^{th} day with sufficient treatment (43.4%).

Key words: *Cryptosporidium parvum* - *Bifidobacteria* - *Lactobacilli* - probiotic

Introduction

Cryptosporidium parvum is water -borne protozoan parasite that causes acute diarrhea in humans and domestic animals [1]. Infection occurs when oocysts in food water, or the environment are ingested,the exposure to reduce conditions or pancreatic enzyme & bile salts causes oocyst to excyst and release four sporozoites that invade the hosts intestinal epithelium and undergo both asexual and sexual multiplication cycles. The life cycle produces new oocysts that are shed in large quantities in the feces [2].Management of cryptosporidiosis , the disease caused by *C. parvum* infection, has been hampered by the look of effective drugs.

Bifidobacterium are normal inhabitants of healthy human and animal intestinal tracts .Tissier was the first to promote the therapeutic use of Bifidobacteria for treating infant diarrhea by giving them large doses of *Bifidobacteria* orally.

Probiotics are viable non pathogenic microorganism that ,when ingested have beneficial effects in the prevention and treatment of pathological condition [3] .

Lactobacilli and *Bifidobacteria* are lactic acid bacteria (LBA) commonly found in fermented dairy products such as yogurt which exhibit probiotic properties [4,5] .
Bifidobacteria produce antimicrobial compounds can inhibit growth of strains of *E.coli*,

Pseudomonas spp and *Sallmonella* [6,7] . *Bifidobacteria* have also been found to aid in anti tumor activity in the host by stimulating the hosts immune response, lowering cholesterol level, synthesize vitamins ,thiamin as well as folic acid. [8] .

The aim of this research was to study the effect of bacterial cell-free supernatants from *Bifidobacterium* and yogurt on *C. parvum* in immune deficiency mice.

Materials and Methods

Feces Samples

One hundred fifty feces samples were collected from calves suffering from severe diarrhea, from Al-Eshaki calves farm in Al-Eshaki district . Fecal samples of animal's rectum were collected directly and save it in tightened plastic cold container till arrive to the lab.

Microscopic examination

Glass slides were prepared from each fecal sample by direct smear method, the positive slide fixed by methanole and stained by modified cold Zehil Neilsen stain according to Beaver and Jung methods [9], then examined under oil immersion(X1000) to detect the presence of oocyst .

Isolation and purification of *Cryptosporidium* oocyst .

The oocyst were isolated and purified from the positive sample by using sucrose flotation method followed by Ungar *et.al*. [10] as a followed:-

-Emulsify 1gm of feces in 10ml of normal saline.

-centrifuge for 1minte.

-add 10 ml of saturated sucrose solution.

-centrifuge then collected the supernated

-washing by normal saline several times.

-. purified oocysts were kept at 4C° until use.

***Cryptosporidium* oocyst preparation** .

About 10 ml of C. oocyst suspension which was isolated as described above , were put in clean centrifuge tube and centrifuged at (700 x) for 10 min, and then the supernatant was decanted .

- The pellet of oocysts were suspended in PBS and oocysts were counted and the number were adjusted at ($1x10^4$) oocyst / ml and then inoculated orally by using stomach tube to cause infection into mice.

- **Preparation of *Bifidobacterium* and supernatants**.
  Bifidobacterium was isolated from probiotic yogurt available in local markets. Re-detected according to Wood and Holzaptel method [11].
- Then ($1x10^8$) cell/ml of *Bifidobacterium* suspension and its supernatants were prepared according to Contreras *et.al* . methods [12].
- **Animals :**
  Male white Swiss mice were obtained from medical college –Al-Nahrein university Their ages were between 11-16 weeks with weights between 22-25gm . Mice feces was examined before starting experiment to insure the intestinal vacancy of parasitic infections.

- **Experimental Design**

Forty eight mice were Immunosupperessed by which injection with (0.1)ml\mice\day with dexamethazone according to Regh [13] .Then divided into 4 groups each containing 6 mice .

*** Group 1 (G1):**

The mice of the first group were given 0.1ml of suspension of *Bifidobacterium* which contain ($1x10^{8}$ □) cell/ml for (10) days by using stomach tube . The dose is determined

according to Alak *et.al* [14] . Then inoculated with ($1x10^{4}$) oocyst inoculation with *Bifidobacterium* continued till the end of experimental period .

***Group 2 (G2) :**

The mice of the 2nd group were inoculated with o.1ml of bacterial supernatants. for (10) days. Then each mouse inoculated with ($1x10^{4}$) oocyst. Inoculation with bacterial supernatants continued till the end of experimental period.

***Group 3 (G3) :**

Each mouse was inoculated with o.1ml of probiotic yogurt which was used previously in bacterial isolation for (10) days . Then each mouse was inoculated with ($1 x 10^{4}$) oocyst ,their feeding with yogurt continued till the end of experimental period.

*** Group 4 (G4)** :

Mice in this group were inoculated with 0.1 ml of PBS for (10) days , then inoculated with suspension C. oocyst which contain ($1 x 10^{4}$) oocyst/ml inoculation the PBS continued till the end of experimental period as a control group.

- During experiment the following data had been noticed.
  1-recording any clinical clear sign .
  2-recording in perpant period.
  3-feces examination to calculate of oocyst in one gram , according to Ryan . *et.al.* method.[15] .

4- sufficient treatment for bacterial *Bifidobacterium* supernatant and yogurt was measured according to Xiao *et.al.* method [16].

sufficient treatment %=(mean number of oocyst in 1gm)_(mean number of oocyst in 1gm)

of feces of control group of feces of treated group X100

mean number of oocyst in 1gm of feces of control group

Statistical analysis

Data are reported as mean ± . Standard deviation .

Result and Discussion

In this study we didn't record any clinical signs in infected and treated group , while prepant period recorded in all infected mice was between (3-4) days for all groups.

The results in Table -1- showed that the numbers of oocyst in G1 and G3 were similar which reach (400) oocyst /gm at first day , while different in G2 and G4 which reach (516.66) and (633.33) oocyst / gm respectively.

Re-detected Table -2-showed the sufficient treatment for three groups ,noticed that the sufficient treatment for yogurt was high which reached (89.72%),followed by Bifidium bacteria that reached (81.43%).while the supernatant sufficient treat was the lowest reaching (43.4%) only.

We should notice that the sufficient treatment calculated depended on the average shedding oocyst through the treatment period without taking in mind the time used by substance to stop shedding the oocyst totally .

In this study , we investigated the effect of administration of probiotic bacteria on the development and progression of experimental infection in mice were fed daily with ($1x10^8$) cell/ml of *Bifidium* bacteria , its supernatant and yogurt starting (10 days) before the infection until the spontaneous clearance of the parasite .

Our results showed that daily administration of yogurt and Bifidium bacteria was able to eradicate the parasite in mice , but the yogurt was more efficient than bacteria because the yogurt contains many types of bacteria like Bifidium and lactic acid bacteria (LAB)(

Lactobacillus acidophilus and *L.veuteri*) which exhibit probiotic properties [17],also Microbial balance is an important factor in the maintenance of intestinal homeostasis and

yogurt or fermented milk supplementation has been proposed to control diarrheal disease [18]

In fact , *Lactobacillus acidophilus* and *L.veuteri* have shown to reduce the duration and number of*C.parvum* oocyst shed in feces of experimentally infected mice [19,20,21]. which suggested that certain LAB may possess potential therapeutic properties against *C.parvum* [22],and that may make clear the high sufficient treatment of yogurt.

Faster *et al.* [23] found that *Lactobacilli* and *Bifidobacterium* & their supernatant effect on the viability of *C. parvum* oocysts . These bacterium may still have potential for therapeutic use against *C. parvum* as suggested by the ability undiluted *B.longum* supernatant to significantly reduce oocyst viability [24].

Administration of mixture of *Bifidobacterium spp* or *L. acidophilus* to pig lets positively affected the elimination of cryptosporidium oocyst from feces and improved systemic metabolism and composition of the intestinal micro flora [25].

The protective mechanism that these *Bifidobacterium* confer against cryptosporidium and the nature and identity of the factors involved requires further study both *invitro* and *invivo*.

References

1- Heine , J.j; Pohlenz , J.F ; Moon , H.W.and Woode , G.N. (1984). Enteric lesions and diarrhea in gnotobiotic calves monoinfected with *Cryptosporidium* species .J.Infect Dis.150,768-775.

2- Pereira , S.J.; Ramirez , N.E ; Xiao .L ; Word , L.A. (2002). pathogenesis of human and bovine *Cryptosporidium parvum* in gnotobiotic pigs . J.infect .D.S .186,715-718.

3-Duggan, C.; Gannon , J.; Walked , W.A. (2002). protective nutrients and functional foods for the gastrointestinal tract, Am.J. Clin. Nutrc.75, 789-808.

4-Tejada – Simon, M.V.; Lee, J.H.; Vstunal , Z.; Peska , J.J. (1990). Ingestion of yogurt containing *lactobacillus acidophilus* and *Bifidobacterium* to potential immunoglobulin are sponses of cholera toxin in mice. J.Dairy Sci .82,649-66.

5- Canganella , F.; Ovidi , M.; Paganini,S.and Trovatelli,L.D. (1998). Survival of undesirable microorganisms in fruit, yogurts during storage at different temperature. food . microbial ,15 ,71-77.

6- O'Riordan,k.; Fitzagerald , G.F. (1998). Evaluation of bifidobacteria for the production of antimicrobial compound and assessment of performance in cottage cheese at refrigeration temperature .J.Apple . Microbial.85,103-114.

7- Searle ,L.,E; Best , A. ; Nunez , A.; Johnson , L., Colley, W.A.; wood ward ,M .J .and Robert. , M. (2009). A mixture containing galactooligosaccharid , produsedby the enzymic activity of *Bifidobacterium bifidum* , reduce *Salmonella enterica* serovar Typhimurium infedtion in mice. J.Med.Microbiol

8-Seidavi,A.R.;Mirhosseini,S.Z.;Shivazad,M.;Chamani,M.andSdeghi,A.A. (2008).

Application of aduplex PCR for the specific and simultaneous detection of *Bifidobacterium spp Lactobacillus spp.*in duodenum ,jejunum,ileum and cecum of broilers.J.of Rapid Methods and Automation in Microbiology.16,100-112.

9- Beaver ,P.C. and Jung , R.C . (1985).Animal Agent and Vectors of Human Disease .5th ed. Lea and Fibiger, pp. 294.

10-Vangar ,B.L.P ; Soave , R. and Fayer , R. (1986).Enzyme immunoassay detection of immnoglobin M and G antibodies *Cryptosporidium* in immunocorpotant and immunocomprised persons. J. Infection. Dis , 153 (3) :570-578.

11- Wood,B.J.and Holzapfel,W.H.. (1995).The genera of lactic acid bacteria .The genus Bifidio bacterium in the genera of lactic acid bacteria. Edited by Wood,B.J.and Holzapfel,W.H..P440-665.

12- Contrerus,B.G.L.;Vuyst, L.; Devreese, B.;Busan ;Basman ,F Raymaekers , J . ; Yovok.; Sablon,E and Vandamme,E.J. (1997).Isolation,purification and amino acid sequence of laction A,one of bacteriocine produced by *Lactobacillus amylovorus* LMGP.1319. Appl. Environ. Microbiol.,63(1):13-20.

13- Regh, J.E. (1996). Effect of interferony in experimental *C. parvum* infection .J. Infect. Dis .,174:22 -32.

14-Alak, J.I ; Wolf , B.W.; Mdurvwa , E.G. ; Pimentel – Smith G. E.and Adeyemo . O. (1997). Effect of *lactobacillus reuteri* on intestinal resistance of *Cryptosporidium parvum* Infection in a murine model of acquired immunodeficiency syndrome .J. Infect. Dis. 175, 218-221.

15- Ryan ,M; Carol, C.; Tim ,A. and Olson , D. (1999). Duration of naturally acquired giardiosis and cryptosporidiosis in dairy calves and their association with diarrhea. J.Amer .Vet.Med.Assoc.,214(3); 391-396.

16- Xiao , L ; Saeed , K.and Rings , D . (1996). Efficacy of albendazole and Fenbendazole against *Giardia* infection in cattle. Vet. Parasitol. 61:165-170.

17- Canganella , F; Ovidi , M. ; Pagan in , S.; Vettraino , A.M. and Trovatelli , L.D. (1998). Survival of undesirable microorganisms in fruit yogurts during storage at different temperature . Food. Microbial. 15,17-77.

18-Ivan, A.C.and Watter , J.D. (2000). Validation of probiotic concept : *lactobacillus reuter* confers broad spectrum protection against disease in humans and animals. Micro. Ecolog. in Health and disease . V12, issue 4.p: 247-285.

19-Alak , J.I. ; Wolf , B.W. ; Malurvwa E.G. ; Kolavala , S.; Abdelrahman , H. and suppiramanian , V. (1999). Supplementation with *Lactobacillus reuteri* or *L.acidophilus*

reduced intestinal shedding of *Cryptosporidium parvum* oocysts in immunodeficient C57BL/6 mice .Cell.Mol.Biol .45, 855-863.

20- Foster , J.C.; Glass ,M., D.; Courtney. P.D.and Wood , L.A. (2003).Effect of *lactobacillus* and *Bifidobacterium* on *Cryptosporidium parvum* Oocyst . Viability . Food .Microbial 20(3) :351-357

21-Heyman, M. (2000). Effect of lactic acid bacteria on diarrheal disease .J.Am. Coll. Nutr.19(2suppl) .1375-1465.

22- Isolauri , E . (2001).Probiotics in human disease. Am. J.Clin .Nutr 73:11425-11465.

23-Laura , E.J; Angus,B.; Alejandro, N.; Francisc , J.S. and Roberto , M.L. (2009). Amixture containing galactoSaccharid , produced by the enzumic activity of *Bifidobacterium bifidium* reduces *Salmonella enterica* servor Typhimurium infection in mice. J.Med. Microbial .58,37- 84.

24- Rotkiewicz, T.; Rotkiewicz,z; Depta , A ; and Kander , M. (2001). Effect of *Lactobacillus acidophillus* and *Bifidobacterium* sp. On the course of *Cryptosporidium parvum* invasion in new – born piglets, Bull.Vet.Inst.Pulawy .45,187-195.

Table (1): Number of *C. parvum* oocystes in treatment & control groups ± stander deviation

Day after treatment / groups	1	2	3	4	5	6	7	8	9	10
Bacteria *Bifidium* G1	400 ± 63.24	1166.66 ± 103.27	2500 ± 63.24	3050 ± 83.66	1933.33 ± 103.27	1350 ± 83.66	850 ± 83.66	166.66 ± 51.63	0	0
Supernatant G2	5166 ± 103.27	1500 ± 63.24	4033.33 ± 186.18	4950 ± 187.08	5516.66 ± 116.90	4166.66 ± 163.29	3000 ± 141.42	5800 ± 141.42	5150 ± 104.88	
Yogurt G3	400 ± 63.24	850 ± 104.88	1433.33 ± 103.27	1500	885.166 ± 440.22	733.33 ± 103.27	516.66 ± 75.27	0	0	-
Control G4	633.33 ± 98.31	2516.66 ± 116.90	5616.66 ± 285.77	6500 ± 141.42	8233.33 ± 186.18	51800 ± 8633.33	55700 ± 9283.33	9750 ± 164.31	10433.33 ± 250.331	

Table (2): Sufficient treatment for treatment groups

Type of treatment	Used Dose	Percent of treatment effeciency (%)
***Bifidium* bacteria**	(1×10^8) cell/ml	81.43
Supernatant	0.1 ml	43.40
yogurt	0.1 ml	89.72

تاثير اللبن المحلي و بكتريا Bifidobacterium في طفيلي الابواغ الخبيئة *Cryptosporidium parvum* في الفئران المصابة تجريبيا

سبأ طاهر محمد ، خولة جبر ، حمزية علي عجة
قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية

استلم البحث في:1 تموز 2010
قبل البحث في: 16 حزيران 2011

الخلاصة

تضمنت الدراسة جمع 150 عينة براز من عجول مصابة بالإسهال ،ثم تم عزل وتنقية أكياس بيض طفيلي*Cryptosporidium parvum* من عينات البراز الحاوية عليها، واستعملت أكياس البيض لإحداث إصابة في الفئران المثبطة مناعيا. عولجت الفئران المصابة ببكتريا Bifidium وروا شحها واللبن المحلي [yogurt] الذي عزلت منه البكتريا.

أظهرت هذه الدراسة أن اللبن المحلي هو الأفضل في المعالجة، إذ أدى إلى توقف الفئران عن طرح أكياس البيض في اليوم الثامن بعد العلاج وبكفاية علاجية (89.72%)، بينما توقفت الفئران المعالجة بخلايا البكتريا Bifidium في اليوم التاسع وبكفاية علاجية (81.43%) . أما الفئران المعالجة بروا شح البكتريا عن طرح أكياس البيض فتوقفت في اليوم العاشر وبكفاية علاجية (43.40%).

الكلمات المفتاحية : - *Cryptosporidium parvum* - *Lactobacilli* - probiotic - *Bifidobacteria* -