ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

TJPS

TIKRIT JOURNAL OF PURE SCIENCE

Journal Homepage: http://main.tu-jo.com/ojs/index.php/TJPS/index

Prophylactic role of lactic acid prepared from *lactobacillus acidophilus* on cryptosporidiosis

Tawfiq I. Al-alousi , Abdullah H. Abdullah , Mayson M. Jasim

[1] *College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq*
[2] *Department of Biology , College of Education for Pure Sciences , Tikrit University , Tikrit , Iraq*

DOI: http://dx.doi.org/10.25130/tjps.23.2018.127

ARTICLE INFO.

Article history:
-Received: 27 / 2 / 2018
-Accepted: 27 / 3 / 2018
-Available online: / / 2018

Keywords: *Cryptosporidium parva*m; *Lactobacillus acidophilus*; probiotic; IgG ; IgM ; complement C3and C4

Corresponding Author:

Name: Mayson M. Jasim
E-mail: mays.mus@gmail.com
Tel:

Abstract

Cryptosporidium has considered as a prominent enteropathogen of humans and animals. Until recently, there is no totally effective therapy other than a healthy intact immune system. Probiotics reported to stimulate both innate and acquired immunity at mucosal and systemic levels. It has been suggested that probiotics inhibit infection by excretion of substances harmful to one of the parasite's developmental stages and possibly offer new therapeutic factors for the treatment of cryptosporidiosis .The aim of the present study was to detect the prophylactic effect of lactic acid prepared by *Lactobacilli acidophillus* against the infection with *Cryptosporidium* in an rabbits model, by estimating hematological and immunological criteria. The results showed that the using of probiotics induced significant reduction in parasite burden, lymphocyte numbers were increased significantly in infected and treated animals. There is a significant increase in the level of IgG ,IgM , C3 and C4 in infected animals.

Introduction

Cryptosporidiosis is one of the most important diarrheal pathogens affecting people in the worldwide [1].In the developing world it is leading to death .The mortality rate is 5-10 million deaths cases each year [2]. Although *Cryptosporidium* was first discovered in 1907, it was not until 1976 that this parasite was identified as a cause of human infection [3]. In 2004, cryptosporidiosis was added to the World Health Organization's reports as a 'Neglected Diseases Initiative', which includes diseases affecting people mainly in low-resource settings [4]. The apicomplexan protozoal parasite *Cryptosporidium,* an intracellular extra cytoplasmic protozoal parasite, has aparasitic life cycle that involves both asexual and sexual reproductive cycles, which is completed within an individual host. Mode of transmission from one host to another involves direct fecal-oral transmission often involving ingestion of oocyst-contaminated food and water [5].*Cryptosporidium* is related to direct zoonotic and this parasite infects human directly from animals [6].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 [7].

As many prevention methods for diarrhea have adverse effects, scientists are now turning to probiotics in hope of using it as a supplement to treat acute diarrhea as prophylactic agent[8]. *Lactobacillus acidophilus* wich called lactic acid bacteria LAB occurs naturally in the human and animal gastrointestinal tract and mouth. Some strains of *L. acidophilus* may be considered to have probiotic characteristics and these strains are commercially used in many dairy products [9].

The present work is to study the prophylactic efficacy of consumption of *L. acidophilus* on experimental cryptosporidiosis using male rabbits model, with estimate some hematological and immunological criteria.

I. Materials and methods

A. The parasite

Cryptosporidium parvum oocysts (isolated from fecal samples of infected calves) were purified from the feces material of infected calves by sodium chloride (NaCl) and cesium chloride (CsCl) gradient density centrifugation, then maintained in rabbits. Briefly, rabbits were placed in metabolic cages, orally

infected with 10^3 oocysts, and monitored daily for oocyst shedding by modified Ziehl Neelsen stain of prepared fecal swabs[11]. All positive feces were collected daily and concentrated by the sugar centrifugal floatation method after being stored in 2.5% potassium dichromate solution at 4°C to be used within one month [12,13].

B. Experimental animals

A total of twenty one albino rabbits (ages 10-18 months) and (weights 1000-1800 gm), and free from intestinal parasitic infections were used in this study.

C. Bacteria strain

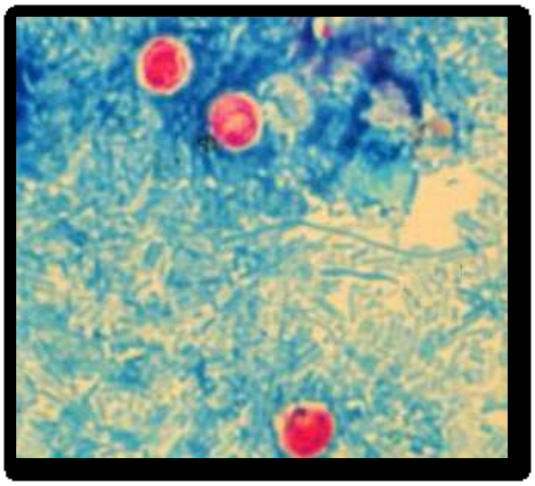
Lactobacillus acidophillus was used for this study which originally isolated from human feces. Lactobacilli (MRS) broth was used for inoculation of frozen cultures and propagation of *Lactobacillus* [14]. This lactic acid was also prepared from isolated bacteria for experimental inoculation [14] .

D. Test for adhesion of *Lactobacillus* to crop epithelial cells

An overnight culture of *Lactobacillus acidophillus* in MRS was centrifuged and the bacteria resuspended in phosphate buffered saline (PBS) at 3-7 pH . Crop epithelial cells were collected from starved rabbits by scraping the crop wall with the edge of a glass slide and suspending the scraping in PBS .The suspension were mixed to give a final ratio of 50 bacteria to one epithelial cell .After rotating (16rev./min at 37C for 30 min ، a sample was withdrawn and examined by Gram stain and the number of bacteria attached to each of ten epithelial cells was counted [15]

E. Experimental infection

Stored oocysts were washed with phosphate buffered saline (pH 7.4) by centrifugation at 1000g for 10 min just before inoculation. Infection was done by oral inoculation of 4×10^3 oocysts/ rabbit in 0.1 ml PBS. D



Oocysts of *Cryptosporidium parvum* × 100

F. Animal groups

A total of twenty one New Zealand white rabbits were used in this study (ages 10-18 months) and (weights 1000-1800gm) , divided into three groups 7 rabbits in each, group; 1(Gr.1): control group (non infected) was inoculated with 1 ml PBS S\c , group 2 (Gr.2): infection group (infected with *Cryptosporidium*) and group 3 (Gr. 3): was inoculated with prepared lactic acid and the orally inoculated daily dose / rabbit was freshly prepared and continued daily to end of experiment(14 weeks). adjusted to a concentration of 10 $x10^3$ CFU in 0.1 ml PBS twice day. This group then infected with *Cryptosporidium* (4×10^3 oocyst /ml) .

II. Assement of prophylactic efficacy of the probiotic

1. Determination of intensity of infection by Determination of *Cryptosporidium* oocysts count: fresh fecal samples from Gr 2 and Gr.3 were collected on days 3,5,7and 11 post infection (P.I.) Counting of shed oocysts in 10 microscopic fields (x400) of a modified Zeil Neelsen stained smear, calculation of the mean oocyst count and the percent reduction in each group was determined [11].

2. Hematological tests: Anticoagulated blood samples were used to determine WBCs and differential blood count [16].

3. Immunological tests: At the end of the seventh day P.I. period , exsanguinations of rabbits from the 3 groups were subjected for separation of sera. IgG and IgM levels and C3 and C4 ratio were determined by Radial Immunodiffusion Plates, commercially available (LTA / Italy).

III. Statistical analysis

Data were computerized and statistically analyzed using the arithmetic mean and standard deviation, Chi square test and one way ANOVA.

Results and discussion

This study showed that the infecting dose 4×10^3 oocyst /rabbit achieved 100% take up of infection. Clinical signs were noticed in infected animals (Gr. 2)such as diarrhea ,moderated fever and loose of appetite compared with the treated rabbits(Gr.3) that's agree with [2]

The treated rabbits(Gr.3) showed significant ($P<0.05$) decrease in the number of oocysts shed on 3,5 , 7 and 11 days in comparison with the non-treated group (table1). These results confirm the effective prophylactic role of probiotic bacteria used in the present study against Cryptosporidium and are in agreement with[20]. Probiotics are important to stimulate the proliferation of mucosal epithelial cells which are considered as the first line of defense against intestinal pathogens lik *Cryptosporidium* [21]. The infection of cryptosporidiosis is largely out of treatment especially in the immunocompromised patients ،Immunocompetent individuals can get rid of the infection because parasite eradication relies on innate and acquired immunity [17]. So, in the preset study, probiotic was used as prophylactic factor to promote individual health due to their effects on luminal microbial ecology and immune modulation. Selection of *L. acidophilus* bacteria was based on many researches that use it in a variety of fermented dairy products [18]. As well as their presence in the normal microflora of humans and their ability to resist stimulated small intestinal transit [19].

Table (1) :Cryptosporidium oocycts count among rabbits treated with probiotics versus untreated control group

oocysts account / Groups	P.I. days			
	third day	fifth day	seventh day	eleventh day
untreated group Gr.2	8620 a	8860 a	4820 a	7020 a
treated group Gr.3	680 b	360 b	140 b	40 b

(a, b): means bearing different letter within the same day are significantly different

The hematological findings between the groups are presented in table(2). The result shows no significant differences in WBCs count between the three groups. lymphocyte numbers were increased significantly in infected Gr.2 and treated Gr.3 animal groups (4.69×10^9/L and 3.39×10^9 /L)respectively comparing with Gr.1. Our results confirm previous reports that some strains of probiotic LAB can enhance several aspects of myeloid and lymphoid cell function in vivo [22], and shows that *L. acidophilus* is able to enhance immune function at both the systemic and local (intestinal) level[23]. Significantly, the level of each of these immune responses was inversely related to the degree of pathogen translocation to the liver and spleen and others, with the exception of serum antibodies responses. Since T/B cell function, phagocytosis and local (intestinal) pathogen-specific antibody production have each been demonstrate the important in immune-mediated protection against *Cryptosporidium* [24]. Granulocyte numbers decreased in treated rabbits (3.25×10^9 /L) comparing with the other two groups, while there are no significant differences between the animal groups according to the monocyte numbers .

Table 2: Hematological parameters Level among rabbits treated with probiotics versus untreated control group

heamatological criteria / groups	Total account of W.B.C $\times 10^9$ / L		differential W.B.C					
			Lymphocyte $\times 10^9$/L		granulocyte$\times 10^9$/L		Monocyte$\times 10^9$/L	
	mean	SD	mean	SD	mean	SD	mean	SD
Gr. 1	8.174 a	±0.531	3.393 b	±0.370	4.059 a	±0.402	1.344 a	±0.115
Gr. 2	7.174 b	±0.556	1.914 d	±0.205	3.907 a	±0.639	0.888 a	±0.545
Gr. 3	7.964 a	±0.734	4.461 a	±0.503	3.307 b	±0.584	1.091 a	±0.216

(a, b): means bearing different letter are significantly different

Table 3 shows the prophylactic effect of probiotic on IgG and IgM in infected rabbits with cryptosporidiosis. Results show no significant differences between Gr. 3 and Gr.1 while there is a significant increase in the level of IgG in Gr.2 . The same results were recorded for IgM level.

Sera of treated and control rabbits (Gr.3 Gr.1) showed insignificant ($P>0.05$) increase in the level of C3 and C4 in comparison to the recorded level in sera of the infected nontreated rabbits (Gr. 2) table (4).

The findings of specific IgG and IgM and the complements C3 and C4 in the sera of non-treated , oocyst excreting rabbits comparing with treated animals were expected, and agreement with Riggs[25] who found that protection against this parasite has been largely associated with production of immunoglobulins and complements; a major player not only in cell-mediated immunity, but in early innate immune responses as well. In vitro studies have demonstrated that C3 and C4 directly prevents the parasite from invading host cells [26].

On the other hands, these results show the prophylactic role of probiotic. Many studies mentioned that probiotics induce modulation of the intestinal environment by having the capacity to control the proliferation of surrounding microorganisms [27]. Wollowski et al [28] reported that probiotic bacteria increase the mechanisms of innate immunity and the activation of B cells for mucosal immunity important for protection against many pathogens, prevention of the penetration by foreign antigens, and maintenance of mucosal homeostasis.

Table 3: IgG and IgM Levels among rabbits treated with probiotics versus untreated control

immunological criteria / groups	IgG level (mg/dl)		IgM level (mg/dl)	
	mean	SD	mean	SD
Gr. 1	2.187 b	±0.283	2.187 b	±0.283
Gr. 2	4.073 a	±0.652	4.846 a	±0.209
Gr. 3	2.078 b	±0.421	2.044 b	±0.264

(a, b): means bearing different letter are significantly different

Table 4: C3 and C4 Levels among rabbits treated with probiotics versus untreated control group

complements / groups	C3 (mg/dc)		C4 (mg/dc)	
	mean	SD	mean	SD
Gr. 1	76.24 b	±0.211	18.89 b	±0.372
Gr. 2	91.66 a	±0.642	39.02 a	±0.201
Gr. 3	77.20 b	±0.241	19.48 b	±0.205

(a, b): means bearing different letter are significantly different

References

[1] Checkley, W.; White, A.C.; Jaganath, D.; Arrowood, M.J.; Chalmers, R.M. and Chen, X, (2015). A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. *Lancet Infect Dis*;15: 85–94.

[2] Shirley, D.A.; Moonah, S.N.; Kotloff, K.L. (2012). Burden of disease from cryptosporidiosis. *Curr Opin Infect Dis*: 25(5):555–563.

[3] Nemes, Z.(2009). Diarrhea from the infectologist's point of view. *Orv Hetil* : 150(8):353–361

[4] Speich, B.; Croll, D.; Furst, T. ; Utzinger, J. and Keiser, J.(2016) Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis. *Lancet Infect Dis*; 16:87–99.

[5] Ehsan, A.M.; Geurden, T.; Casaert, S.; Parvin, S.M.; Islam, T.M.; Ahmed, U.M.;Levecke, B.; Vercruysse, J. and Claerebout, E. (2015). Assessment of zoonotic transmission of Giardia and *Cryptosporidium* between cattle and humans in rural villagesin Bangladesh. *PLoS One*, 10(2), 1–11.

[6] Yang, Y.; Zhou, y. Xiao, P. and Shi, Y.(2017). Prevalence of and risk factors associated with *Cryptosporidium* infection in an underdeveloped rural community of southwest China, Yang et al. *Infectious Diseases of Poverty*, DOI 10.1186/s40249-016-0223-9.

[7] Bowman, D.D., Lynn, R.C. and Eberhard, M.L. (2003). Georgis parasitology for veterinarians. 8th. ed. Saunders, St. Louis, pp: 98-100.

[8] Maria, J.; Saez, L.; Carolina G.; Julio P. and Angel, G. (2015). "The Role of Probiotic lactic acid bacteria and Bifidobacteria in the Prevention and Treatment of Inflammatory Bowel Disease and Other Related Diseases: A Systematic Review of Randomized Human Clinical Trials". Biomed Res Int (Systematic review). : 15. doi:10.1155/2015/505878. PMC 4352483PMID 25793197

[9] Singhi, S.C. and Kumar, S. (2016). "Probiotics in critically ill children.". *F1000 Res* (Review). 5:407. doi:10.12688/f1000research.7630.1. PMC 4813632. PMID 27081478

[10] Henriksen, S.A. and Pohlenz, J.F.J. (1981). Staining of *Cryptosporidia* by a modified Ziehl-Neelsen Technique. Acta Vet. Scand. 22: 594-596.

[11] Anderson, B.C. (1981). Petterns of shedding of *cryptosporidial* oocyst in Indah calves. *J. Am. Vet .Med. Assoc*:178 (9):982-984.

[12] Arrowood, M.J. and sterling,C.R.(1987). Isolation of *Cryptosporidium* oocyst and Sporozoite using discontinuous sucrose and isopoycnic percoll gradients .*J.parasitol*.,73:314-319.

[13] Baron, E.J.; Peterson, L.R. and Finegold, S. M. (1994): Bailey and Scotts diagnostic Microbiology. 8th ed. Mosby Year book. Inc. America. 792.

[14] Savage, D.C. (1984). Adherence of the normal flora in: attachment of organism to the gut mucosa. voll. Boedeker, E.C.; Bocaraton crc press.pp. 3-10.

[15] Fuller, R. (1975). Nature of the determenat responsible for adhesion of *Lactobacillus* to chicken crop epithelial cell. *J .microbial*.: 87(2):245-150.

[16] Henry L. and Davidson, I. (1974). Clinical diagnosis by lab. Methods, Saunders Company. Landon.

[17] Laxer, M.A.; Alcantara, A. K.; Javato-Laxer, M.; Menorca, D.M.; Fernado, M.T. and Ronga, C.P. (1990). Immune response to cryptosporidiosis in philippine children. *Am. J. Trop. Med. Hyg*.: 42: 131-139.

[18] Nawaf, M.G. (2005). The use of Lactobacillus acidophilus as a probiotic for the prevention and treatment of experimental rat enteritis.Msc.th. College of medicine, Tikrit University.

[19] Muhsen, R.K. (2007). The use of *Lactobacillus acidophilus* as a probiotic in the prevention and treatment of *Salmonella typhimurium* infection in puppies' .PH.D. Thesis. College of Veterinary Medicine. University of Baghdad

[20] Alak, J. I.; Wolf, B.W.; Mdurvwa, E.G.; Pimentel-Smith, G.E.; Kolavala, S.; Abdelrahman, H.and Suppiramaniam,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(6):855-863.

[21] Jens, W.r (2015). Ecological Role of Lactobacilli in the Gastrointestinal Tract: Implications for Fundamental and Biomedical Research. Applied and Enviromental Microbiology: l(81), Issue(23).

[22] Schiffrin, E.J., Rochat, F., Link-Amster,H., Aeschlimann, J. M., and Donnet-H. (1995). Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *J. Dairy Sci*.78: 491-497.

[23] Isolauri E.; Sütas Y.; Kankaanpää, P.; Arvilommi, H. and Salminen. *S. (2001). Probiotics: effects on immunity. Am J Clin Nutr.73 (2):444S-*450S.
[24] Haghighi, H.R.; Gong, J.; Gyles, C.L.; Hayes, M.A.; Sanei, B. Parvizi, P.; Gisavi, H.; Chambers, J.R. and Sharif. S. (2005). Modulation of antibody-mediated immune response by probiotics in chickens. *Clin. Diagn. Lab. Immunol*. 12: 1387-1392.
[25] Riggs, M. W. (2002). Recent advances in cryptosporidiosis: The immune response. *Microbes and Infection*. 4(10), 1067-1080
[26] McDonald V.; Korbel, D.S.; Barakat F.M.; Choudhry N. and Petry F. (2013). Innate immune responses against *Cryptosporidium parvum* infection. *Parasite Immunol*., 35 (2): 55–64.
[27] Gupta V. and R. Garg (2009). Probiotics. *Ind J Med Microbiol*. 27(3):202–209
[28] Wollowski, I.; Rechkemmer, G. and B. L. Pool-Zobel. (2001). Protective role of probiotics and prebiotics in colon cancer. *Am. J. Clin. Nutr.* 73: 451S - 455S

الدور الوقائي لحامض اللاكتيك المحضر من بكتريا *Lactobacillus acidophilus* ضد الاصابة بداء البويغيات الخبيئة

توفيق ابراهيم الالوسي[1] ، عبد الله حسين عبد الله[2] ، ميسون مصطفى جاسم[2]

[1] *كلية الطب البيطري ، جامعة تكريت ، تكريت ، العراق*

[2] *قسم علوم الحياة ، كلية التربية للعلوم الصرفة ، جامعة تكريت ، تكريت ، العراق*

الملخص

يعتبر طفيلي البويغيات الخبيئة من مسببات الامراض المعوية في الانسان والحيوان. الى الوقت الحاضر، لم يتم التوصل الى علاج فعال لداء البويغيات الخبيئة وما زال الاعتماد على دور الجهاز المناعي للشفاء منه. تعتبر المعززات الحيوية وعلى مدى واسع، من محفزات المناعة الطبيعية والمكتسبة، كما انها تقوم بتثبيط العدوى الطفيلية من خلال انتاج مواد توقف احدى اطوار الطفيلي، واقترح استخدامها كعلاج بديل للتخلص من الاصابة بداء البويغيات الخبيئة. تهدف الدراسة الحالية الى الكشف عن الدور الوقائي لحامض اللاكتيك المحضر من بكتريا *Lactobacillus acidophilus* للحماية من الاصابة بطفيلي البويغيات الخبيئة من خلال قياس بعض المعايير الدموية والمناعية. اظهرت النتائج ان استخدام المعزز الحيوي ادى الى انخفاض عدد اكياس بيض الطفيلي المطروحة، كما ارتفعت اعداد الخلايا اللمفية في مجاميع الحيوانات المصابة والمعالجة. كان هناك ارتفاع معنوي في مستويات الكلوبيولينات المناعية IgG و IgM والمتممات C3 و C4 في المجاميع المصابة فقط في حين لم يظهر اي تغير في مستوياتها في المجاميع المعالجة.