

## Effect of probiotic acidophilus plus against infection with secondary hydatid disease in BALB /c mice

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### Abstract

The current study investigated the effect of acidophilus plus probiotic in the immune activities in mice to infestation with the cystic echinococcosis. Two dilutions of the probiotic bacteria  $9 \times 10^6/0.1$  ml,  $30 \times 10^3/0.1$  ml CFU were used, by intraperitoneally injection in the experimental animals, pre and post infections with protoscoleces of *Echinococcus granulosus*. Before infection remedy comprised injection by acidophilus plus twice with 72 hours interval, on the seventh day, animals were injected with protoscoleces intraperitoneally, after infection remedy implicated injection of animals with protoscoleces of *Echinococcus* protoscoleces first, next to 72 hours, probiotics were inoculated intraperitoneally. Many criteria were taken into consideration including, numbers, weights, diameters and percentage reduction of hydatid cysts of treated mice in contrast to the animals infested with only. The study showed a decline in cysts including their diameter, weighting, digit, accompanied by increasing the percentage reduction of hydatid cysts in treated mice, the highest percentage reduction was 98.03%, at both dilutions, 6 months post infection, and the minimum cysts number was 0.8 in comparison with the control group 39.4, with significant difference, in the same experiment. The minimum cysts weight was 0.0104 gm, 6 months post infection at the dilution  $9 \times 10^6/0.1$  ml CFU, compared with the control group 0.442 gm. The smallest cysts diameter was 0.057 mm in comparison with the control group 0.882 mm at dilution  $9 \times 10^6/0.1$  ml CFU, 6 months post infection. Acidophilus plus it may well be deduced that probiotic bacteria can be used as medicinal and remedial method against infection with hydatidosis.

**Keywords:** Probiotics, *Lactobacillus acidophilus*, *L. casei casei*, *L. casei rhamnosus*, Hydatid disease

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### تأثير المعزز الحيوي acidophilus plus ضد الإصابة بداء الأكياس العدرية الثانوي في الفئران

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### الخلاصة

تحررت الدراسة الحالية عن تأثير المعزز الحيوي acidophilus plus في الفعاليات المناعية للفئران تجاه الإصابة بداء المشوكات الكيسي. استخدم تخفيفان من البكتريا  $30 \times 10^3/0.1$  مل وحدة عد المستعمرات الجرثومية و  $9 \times 10^6/0.1$  مل وحدة عد المستعمرات الجرثومية بواسطة الحقن داخل البريتوني في حيوانات التجربة، قبل الإصابة وبعدها بالرويسات الأولية لدودة المشوكات الحبيبية. تضمنت المعاملة قبل الإصابة حقن المعزز الحيوي مرتين، بفترة فاصلة ٧٢ ساعة، وفي اليوم السابع، حقنت الحيوانات بالرويسات الأولية داخل البريتون. تضمنت المعالجة بعد الإصابة حقن الحيوانات بالرويسات الأولية للمشوكة الحبيبية أولاً، ثم حقنها بالمعزز الحيوي داخل البريتون بعد ٧٢ ساعة من الإصابة. أخذت معايير عدة بنظر الاعتبار متضمنة أعداد، أوزان، أقطار ونسبة اختزال الأكياس العدرية في الفئران المعاملة بالمقارنة مع الحيوانات المصابة بالرويسات الأولية فقط. أظهرت الدراسة انخفاضاً في أقطار، أوزان، وأعداد الأكياس العدرية، صاحبه زيادة في نسبة اختزال الأكياس في الفئران المعاملة. كانت أعلى نسبة اختزال  $98.03\%$  عند كلا التخفيفين بعد ٦ أشهر من الإصابة، وبلغ أقل عدد للأكياس  $0.8$  بالمقارنة مع مجموعة السيطرة  $39.4$  في نفس التجربة، وبلغ أقل وزن للأكياس  $0.0104$  غم عند تخفيف

٩×١٠/١،٠ مل وحدة عد المستعمرات الجرثومية بالمقارنة مع مجموعة السيطرة ٤٤٢،٠ غم بعد ٦ أشهر من الإصابة، بينما بلغ اقل قطر للأكياس ٥٧،٠ ملم وبالمقارنة مع مجموعة السيطرة ٨٨٢،٠ ملم عند التخفيف ٣٠×١٠/٣،٠ مل وحدة عد المستعمرات الجرثومية بعد ستة أشهر من الإصابة. يمكن الاستنتاج بأن المعزز الحيوي acidophilus plus يمكن أن تستخدم كطريقة طبية وعلاجية ضد الإصابة بداء العدريات.

## Introduction

Cystic echinococcosis is the larval cystic stage (called Echinococcal cysts) of a small taeniid-type tapeworm (*Echinococcus granulosus*) that may cause illness in intermediate hosts, generally herbivorous animals and people who are infected accidentally, it a zoonotic disease spreads worldwide, a common prevalent in stock raising areas, like Australia and Middle East region (1). It is neglected tropical areas disease, which is the most important reason of health deficiency and economic losses. China is account as a considerable part of the global loss (2). Cystic echinococcosis is causing by *Echinococcus* cestoda, the common species, *E. granulosus*. Humans are accidentally infected by ingesting the parasite eggs which are raised from specific hosts (e.g. wolves, dogs, foxes, and jakals) (3), by fecal-oral stream. Parasite ova ingested in the human digestive systems, hatched in duodenum, growing up as larvae and passing through the gut wall into liver with blood route. About 75% of cases affect liver (2,4,5). The taxonomy of genotypes/species, leading to cystic echinococcosis, includes the subdivided *E. granulosus sensu lato* complex groups, *E. granulosus sensu stricto*, *E. equines*, *E. ortleppi*, *E. Canadensis*, and *E. felids* (lion strain) (6,7). In medicine, it is difficulty for drugs to permeate into the multiple layers of the hydatid cyst, but chemotherapy and operation are still the most utilized methods for therapy of cystic Echinococcosis (8). Albendazole drug is ovicidal, larvicidal and vermicide, but operation is still the main treatment (3,9). So, researchers, in recent years, have developed new alternatives in an attempt for elimination of some parasitic infections. The most important is the use of probiotic bacteria, probiotics represent microbiota of different sources endogenous and exogenous, of which are most important for human and animals if administrated on adequate amount (10). The prophylactic activity of this type of bacteria may be by competition, seclusion and impedance to harmful bacteria of the intestine. Other technique involves synthesis of compounds against bacteria, includes H<sub>2</sub>O<sub>2</sub>, bacteriocin, and immunomodulation (11). Several studies proved the efficacy of probiotics for prevention against many diseases such as, cancer, allergies, inflammatory, intestinal and autoimmune disease (12). Probiotics effect on other eukaryotic pathogens, explained the efficacy of *Lactobacillus* in the prevention contra extra intestinal parasites, such as *Plasmodium*, *Babesia* (13). The effects of

useful microorganisms, probiotics became interesting for their protection or therapeutic applications against diseases including worm diseases (14) like zoonotic Schistosomiasis which is caused by genus *Schistosoma* (15). Other probiotics strains such as *L. acidophilus*, *L. plantarum* and *L. delbrueckii* revealed an obvious activity versus ancylostomiasis in normally affected canine with a disease. Elevation of white blood cells has been observed (16). Researchers found that *L. casei* is the firstly strain among other strains of *Lactobacilli* has anthelmintic effect of about 75-100% prevention, another strain is *L. plantarum* with efficacy of 90% against *Trichinella spiralis* (17).

The aim of the current study is to use probiotic bacteria, for the first time, against infection with secondary hydatid disease as an antiparasitic immunomodulator in BALB/c mice, depending on many criteria including numbers, weights, diameters, and percentage reduction of hydatid cysts in the experimental animals, in comparison with the control group.

## Material and methods

### Laboratory animals

One month-aged experimental animals (Swiss mice) were utilized.

### Cysts samples

Ovine cysts containing protoscoleces were obtained under sterilized conditions, alive protoscoleces, 20 µl of protoscoleces suspension were added to 20 µl of 0.1% of eosin pigment on a slid, then tested under light microscope. Bright green protoscoleces were considered as alive because of eosin exclusion by protoscoleces membranes, whereas red protoscoleces were regarded as dead because of acceptance of the pigment gained and counted according to authenticated manner (18, 19,20).

### Bacterial strains preparation and inoculation

One capsule of acidophilus plus provides lactobacilli culture, typically providing 2 billion of *L. acidophilus*, *L. casei* casei and *L. casei rhamnosus*. These lactobacilli strains were grown in nutrient broth medium for 24 hours then the culture was diluted in normal saline to contain 9\*10<sup>6</sup> /0.1 ml CFU of acidophilus plus probiotics, the second dilution in normal saline contains 30\*10<sup>3</sup> /0.1 ml CFU of acidophilus plus probiotics (16,20).

**Experimental design**

Animals were divided into seven groups, each group comprised of five animals. Group one: 30 Mice (5 mice for each experiment as control group) were injected with 2000 protoscoleces /20 gm body weight intraperitoneally by 0.1 ml of protoscoleces diluted in phosphate buffer saline (PBS). Group two: 5 Mice were injected intraperitoneally with the probiotic acidophilus plus  $9 \times 10^6$ / 0.1 ml CFU, twice with interval 72 hours, on the 7<sup>th</sup> day mice were injected with 2000 protoscoleces. Injection with probiotics continued for 50 days. Mice were dissected 2 months post infection. Group three: 5 Mice were injected intraperitoneally with probiotic acidophilus plus  $9 \times 10^6$ / 0.1 ml CFU, twice with interval 72 hours, on the 7<sup>th</sup> day mice were injected with 2000 protoscoleces intraperitoneally. Injection with probiotics continued for 110 days. Mice were dissected 4 months post infection. Group four: 5 Mice were injected intraperitoneally with probiotic acidophilus plus  $9 \times 10^6$ /0.1 ml CFU, for 170 days with 72 hours intervals, on the 7<sup>th</sup> day mice were injected with 2000 protoscoleces intraperitoneally. Mice were dissected 6 months post infection. Group five: 5 Mice were injected intraperitoneally with 2000 protoscoleces, after 3 days the mice were injected intraperitoneally with acidophilus plus  $30 \times 10^3$ /0.1 ml CFU for 50 days, with 72 hours intervals, mice were dissected after 2 months. Group six: 5 Mice were injected intraperitoneally with 2000 protoscoleces, after 3 days mice were injected intraretinally with acidophilus plus  $30 \times 10^3$ /0.1 ml CFU for 110 days with 72 hours intervals, mice were dissected after 4 months. Group seven: 5 Mice were injected intraperitoneally with 2000 protoscoleces, after three days mice were injected with acidophilus plus  $30 \times 10^3$ /0.1 ml CFU for 170 days with 72 hours intervals, mice were dissected after 6 months.

**Statistical analysis**

For testing the variances between the periods and dilution of bacteria acidophilus plus, a Complete Randomized Design (CRD) was performed. Then, the variances between group's mean values were tested by Duncan's multiple range test. The test periods, as a quantitatively value, were compared by the Analysis of Variance (ANOVA). Two ready packages, Statistical Analysis System (SAS) and Minitab were applied (21).

**Results**

Cysts weights declined obviously in groups cured with acidophilus plus, reached the minimum 0.0104 gm at the dilution  $9 \times 10^6$ /0.1 ml (CFU) after 6 months of infestation, opposed infected animals with the parasite only 0.442 gm (Table 1, Table 5).

Table 1: Impact of acidophilus plus  $9 \times 10^6$ /0.1 ml,  $30 \times 10^3$ /0.1 ml (CFU) on means of larval stages weights for animals infested with in contrast to the untreated set after 2, 4, 6 months

Dilution of bacteria	Months			Means of bacterial treatment
	Two (gm)	Four (gm)	Six (gm)	
$10^6$	0.0197 <sup>b</sup>	0.0123 <sup>b</sup>	0.0104 <sup>b</sup>	0.0284 <sup>b</sup>
$10^3$	0.00419 <sup>b</sup>	0.037 <sup>b</sup>	0.0144 <sup>b</sup>	0.018 <sup>b</sup>
Control	0.0626 <sup>b</sup>	0.316 <sup>a</sup>	0.442 <sup>a</sup>	0.273 <sup>a</sup>
Mean Periods	0.0288 <sup>b</sup>	0.1217 <sup>a</sup>	0.1558 <sup>a</sup>	0.1016

Similar letters indicate no significant differences, while different letters indicate significant differences.

Cysts numbers minimized meaningfully among all treated albino animals by acidophilus plus, reached the minimum 0.8 cyst at the dilutions  $9 \times 10^6$ /0.1 ml and  $30 \times 10^3$ /0.1 ml (CFU) 6 months post infection in comparison with the control group 39.4 cyst (Table 2, Table 5, Figures 1-3).

Table 2: Effect of acidophilus plus  $9 \times 10^6$ /0.1 ml and  $30 \times 10^3$ /0.1 ml (CFU) on means of larval stages numbers of treated animals affected of the disease opposed the unprocessed collection through 2, 4, 6 months

Dilution of bacteria	Months			Means of bacterial treatment
	Two (gm)	Four (gm)	Six (gm)	
$10^6$	2.6 <sup>cb</sup>	1.4 <sup>c</sup>	0.8 <sup>c</sup>	1.60 <sup>b</sup>
$10^3$	3.6 <sup>cb</sup>	1.6 <sup>c</sup>	0.8 <sup>c</sup>	2.00 <sup>b</sup>
Control	6.6 <sup>cb</sup>	8.2 <sup>b</sup>	39.4 <sup>a</sup>	18.06 <sup>a</sup>
Mean Periods	4.3 <sup>b</sup>	3.73 <sup>b</sup>	13.6 <sup>a</sup>	6.90

Similar letters indicate no significant differences, while different letters indicate significant differences.



Figure 1: Mouse infected with protoscoleces 4 months postinfection (control).



Figure 2: Mouse infected with protoscoleces treated by acidophilus plus  $30 \times 10^3 / 0.1$  ml (CFU) 4 months postinfection.

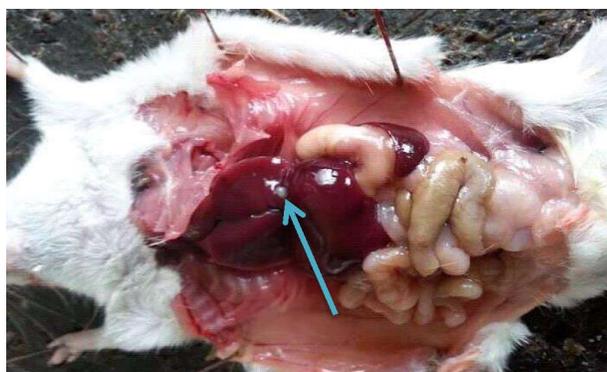


Figure 3: Mouse infected with protoscoleces treated by acidophilus plus  $9 \times 10^6 / 0.1$  ml (CFU)- 4 months post infection.

Cysts diameters diminished clearly in all handled animals by acidophilus plus, reached the minimum 0.057 mm at the dilution  $9 \times 10^6 / 0.1$  ml (CFU) after 6 months of invasion compared to uncured association 0.882mm (Table 3, Table 5, Figures 4 and 5).

Table 3: Effect of acidophilus plus  $9 \times 10^6 / 0.1$  ml and  $30 \times 10^3 / 0.1$  ml (CFU) on means of cystic larvae diameters of treated animals occupied by protoscoleces rapprochement to the versus groups during 2, 4, 6 months

Dilution of bacteria	Months			Means of bacterial treatment
	Two (mm)	Four (mm)	Six (mm)	
$10^6$	0.375 <sup>cb</sup>	0.106 <sup>cd</sup>	0.057 <sup>d</sup>	0.179 <sup>b</sup>
$10^3$	0.089 <sup>cb</sup>	0.131 <sup>cd</sup>	0.071 <sup>d</sup>	0.097 <sup>b</sup>
Control	0.497 <sup>b</sup>	0.500 <sup>b</sup>	0.882 <sup>a</sup>	0.794 <sup>a</sup>
Mean Periods	0.320 <sup>ba</sup>	0.245 <sup>b</sup>	0.336 <sup>a</sup>	0.432

Similar letters indicate no significant differences, while different letters indicate significant differences.

Table 4: Effect of acidophilus plus  $9 \times 10^6 / 0.1$  ml and  $30 \times 10^3 / 0.1$  ml (CFU) on lowering proportion in larvae cysts of the treated occupied association with the parasite parallel to the surveillance animals within 2, 4, 6 months

Treatment	Months	Mice No.	Hydatid cysts treated	Reduction %
$10^6$	2	5	2.6 <sup>cb</sup>	60
$10^3$	2	5	3.6 <sup>cb</sup>	45
Control	2	5	6.6 <sup>cb</sup>	
$10^6$	4	5	1.4 <sup>c</sup>	82.9
$10^3$	4	5	1.6 <sup>c</sup>	80.5
Control	4	5	8.2 <sup>b</sup>	
$10^6$	6	5	0.8 <sup>c</sup>	98.03
$10^3$	6	5	0.8 <sup>c</sup>	98.03
Control	6	5	39.4 <sup>a</sup>	

Similar letters indicate no significant differences, while different letters indicate significant differences.



Figure 4: Mouse infected with protoscoleces 6 months postinfection (control).



Figure 5: Mouse infected with protoscoleces and treated by acidophilus  $9 \times 10^6 / 0.1$  ml (CFU) 6 months.

The percentage reduction decreased in all treated mice, reached the maximum 98.03% at the dilution  $9 \times 10^6 / 0.1$  ml and  $30 \times 10^3 / 0.1$  ml (CFU) post infection 6 months (Table 4).

Table 5: ANOVA analysis explain the effect of acidophilus plus dilutions  $9 \times 10^6/0.1$  ml and  $30 \times 10^3/0.1$  ml (CFU) on weights, Diameters, numbers means of larval stages of invaded band with the differentiated with the monitoring set

Treatment	Animals No.	df	Weight (gm)	Cysts No.	Cyst Wt. (gm)	Cyst diameter (mm)
Bacteria	5	2	68.00**	1176.1**	0.115*	1.157*
$10^6, 10^3$ verse control	5	1	107.80**	2351.1**	0.219**	2.289**
Durations	5	2	327.18**	382.4**	0.068	0.6117
Linear	5	1	584.32**	537.0**	0.137*	0.4222
Square	5	1	70.04*	227.2*	0.00007	0.8013
Bacteria in periods	5	4	41.46*	528.2**	0.0429	0.2712
Empirical error	5	36	8.06	34.7	0.03	0.306

\* refers to significant at 5%, \*\* refers to Significant at 1%, df mean degree of freedom.

## Discussion

In the recent years, the efficiency of useful probiotics to the body rely on the mechanization, through which they do their action by numerous processes implicating manufacture of microbe killer materials, modification the mucosa of the defense order, exchange gut microflora, boosting action of enzymes (22). Both dilution of bacteria probiotic (pre and post infection) exhibited significant effect on the infection in treated animals in comparison with the control group, and the effect is directly fit with period of treatment because treatment with bacteria continued nearly all the period of infection (2,6,8) months, respectively. In general, pre-infection treatment with bacteria  $9 \times 10^6/0.1$  ml (CFU) was stronger in effect that post infection treatment, this due to the stimulation of immune response of the treated experimental animals. Present study revealed antiparasitic effect of probiotic acidophilus plus (for both dilution), explained by significant decrease, of larval stage digit, weightiness, diameter, in addition increase in reduction proportion in hydatid cysts through the experiments period.

The primary route of bacterial activity to parasitic hitting possibly through boosting gut hurdle (23-26). Helpful bacteria could also increase useful microbiota numbering as *Lactobacillus*, that suppress harmful bacteria development via contesting on connection region on the gut endothelial layer. Another mechanics might imply excretion of some micro killer materials, mostly produced from lactic acid bacteria and could be hold potency against parasitic larvae (27). Moreover, bacterial output holds anthelmintic influence that might decrease maliciousness of numerous protozoa and helminthic illness (28). These results are similar to the study of Mohamed *et al* (29) and Zowail *et al* (30) who proved the protective and curative effects of useful bacteria in mice models against infection of *Schistosoma mansoni* (29-31). Many strains of probiotics, like *Zymomonas mobilis*  $1 \times 10^9$  CFU/ml, when administrated by mouth provoked minor defense reaction, in mice against *S. mansoni* by providing prevention in

treated animals (31,32). When *Lactobacillus sporogenes* used orally at a certain dose, for a long time, since the beginning of the infestation with *S. mansoni*, minimized impairment of DNA, improves liver and gut deterioration, therefore weakened adult together with ova numbers (29).

Results of the present study are also consistent with the Bsaldo *et al* (33) who assessed the protection effects of probiotic bacteria versus Toxocariasis within experimental animals. They provoked considerable shorthand in worm number in empirical animals processed with *Enterococcus faecalis*, further, *E. faecalis* in variable dosage revealed effect against larvae outside and inside the body (25). Likewise, another study on *Tricuris muris* infection in mice model, explained that oral feeding for alive *L. rhamnosus* potentially stimulated larval expulsion in *Trichuris muris* resistant C57BL/6 mice (34). A suspension of *Lactobacillus acidophilus*, *L. delbrückii* revealed potential leverage against canine ancylostomiasis (16). Researchers observed that *L. casei* has anthelmintic 75-100% prophylactic effect, and *Lactobacillus plantarum P164* (17) revealed 90% protection against *T. spiralis*, ensured that these strains are safe as protective and curative probiotics (17,23,35,36). A suspension for different probiotics revealed an increased worthy preservation 90% against *Anchylostoma caninum* infection in dogs (16), otherwise, *Bifidobacterium animalis 04450B* dose  $9 \times 10^2$  CFU showed less protection 33% decrease of adult worms and 21% decrease of egg production *Strongyloids venesuelensis* infection in mice (37). First of all, we used orally administration with probiotic bacteria in experimental animals, but we didn't see significant or obvious results that ensure the effect of bacteria as stimulative, when compared with the control group, so we used intraperitoneally injection because larval stages (hydatid cysts) are extraintestinal, so that the effect of intraperitoneal injection was very effectively obvious than oral administration. When we compared our study with others, we saw that little studies only used the intraperitoneal injection with bacteria like Babesia, plasmodium (protozoa) (13). Differences in the effect of probiotics strains, may be due the variable study design,

experimental animals, dependent doses, and manner of inoculation (14).

## Conclusion

It may well be concluded that acidophilus plus probiotics possess have an obvious stimulative and curative effect against infection with secondary hydatid disease in mice, and may be applied as an alternative strategy against different parasitic infection.

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