

**Treatment and prevention of Salmonellosis in puppies using
Lactobacillus acidophilus.**

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Summary

This study was carried out to evaluate the ability of *Lactobacillus acidophilus* to prevent and treat *Salmonella typhimurium* in puppies. In vitro antibiotic sensitivity test of *S. typhimurium* was made and the result revealed that Ciprofloxacin is the most effective. Isolation of *L. acidophilus* from the intestinal tract of the normal dogs and also revealed that all *Lactobacillus* strains were biochemically identical to standard strain. A bacterial strain that had high ability to inhibit the growth of *S. typhimurium* in vitro with high ability to adhere to intestinal epithelial cells and tolerate the low pH and bile salts was chosen for the experimental study.

Twenty puppies were divided into four groups and treated in different ways. The clinical, haematological and biochemical parameters were obtained from all animals at the period of two days before inoculation until the death of animals of the first group at the sixth day post infection. The results revealed that , puppies experimentally infected with *S.typhimurium* showed both septicemic and gastrointestinal forms of the disease accompanied with isolation of *S.typhimurium* from the blood and stool throughout the experiment. The statistical analysis of the results of all parameters among all groups revealed ,for the first time, that *L.acidophilus* plays an important role in the prevention of *S.typhimurium* infection in puppies . It also has high therapeutic effect against *S.typhimurium*, which was almost similar to that of Ciprofloxacin.

العلاج والوقاية من الخمج بالسالمونيلا تايفيميوريم في الجراء باستخدام
(*Lactobacillus acidophilus*). جراثيم العصيات اللبنية المحبة للحموضة

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الخلاصة

اجريت هذه الدراسة لمعرفة مدى كفاءة جراثيم العصيات اللبنية المحبة للحموضة في الوقاية والعلاج من احدى مسببات الاسهال الجرثومي في الكلاب وهي جراثيم سالمونيلا تايفيميوريم. تم اجراء فحص الحساسية للمضادات الحيوية لجرثومة سالمونيلا تايفيميوريم وقد أظهرت النتائج بان المضاد الحيوي السيروفلوكساسين كان اكثر المضادات الحيوية فعالية في تثبيطها خارج النسيج الحي. تم عزل جراثيم العصيات اللبنية المحبة للحموضة من القناة الهضمية للكلاب, وقد اظهرت النتائج بأن الجراثيم المعزولة كانت مطابقة من حيث الفحوصات الكيموحيوية للصفات القياسية, وقد اختيرت العزلة التي اظهرت قابلية على تثبيط نمو جراثيم السالمونيلا خارج النسيج الحي والتي لها قدرة عالية على الالتصاق في الخلايا الطلائية المبطنه للأمعاء في الكلاب بالإضافة الى قدرتها على تحمل الاس الهيدروجيني الواطيء وأملاح الصفراء. قسم عشرون جروا الى اربعة مجاميع عولجت بطرق مختلفة وتم متابعتها يوميا لملاحظة الأعراض السريرية والتغيرات الدمية والكيموحيوية لمدة يومين قبل الخمج ولغاية هلاك حيوانات المجموعة الأولى في اليوم السادس منه , وقد أظهرت النتائج ان الحيوانات المخمجة تجريبيا بجراثيم السالمونيلا اظهرت اعراض كلا الشكلين الانتاني والمعدني المعوي وأظهرت نتائج التحليل الإحصائي لجميع المعايير , ولأول مرة, إن لجراثيم العصيات اللبنية المحبة للحموضة دورا مهما في الوقاية من جراثيم السالمونيلا في الجراء, وكذلك امتلكت هذه الجراثيم تأثيرا علاجيا جيدا ضد جراثيم السالمونيلا وكان هذا التأثير مساويا للتأثير العلاجي للسيروفلوكساسين .

Introduction

Lactobacillus spp. had an important properties to be an effective probiotic organisms, these properties include the ability to adhere to cells, reduce pathogenic adherence , persist and multiply, produce acids , hydrogen peroxide and bacteriocins antagonistic to pathogen growth, be safe. non-invasive, non-carcinogenic and non pathogenic (1 and 2).

Salmonellosis is a world wide problem and considered to be one of the most important zoonotic diseases (3).

The clinical signs of Salmonellosis in dogs are septicemic and gastrointestinal forms beside a carrier state which is asymptomatic (4).

Salmonella typhimurium is the most common serotype in dogs (5 and 6).

The aims of this study were to evaluate the ability of *L.acidophilus* in treatment and prevention of experimental *S.typhimurium* infection in dogs.

Materials & Methods

Salmonella typhimurium was isolated from dog with early stage of enteritis (7), and serotyped in the central public health laboratories, ministry of health. Antibiotic sensitivity test of S.typhimurium was made (8). Lactobacillus acidophilus was isolated from intestinal contents of a dog (9).

Determination of the inhibitory effect of L.acidophilus against S.typhimurium in vitro was made (10) also determination of adherence activity of L.acidophilus with intestinal mucosa of the dog (11) beside the determination of the ability of L.acidophilus to tolerate low pH (0) and ability of L.acidophilus to tolerate the bile salts (Fuller,1975). Preparation of bacterial suspension (Baron, et al 1994). Total leukocytic count (12) was made.

Sodium, potassium and chloride were estimated by using atomic absorption, and kits from Randox company (13). Statistical analysis using ready-made statistical design (SPSS).

Experimental design: Twenty puppies from local breed aged 2-4 months and weighted between 3-4.5 Kg were used in this study. All animals were prepared to the experiment by treatment with Ciprofloxacin 20mg/kg BW daily for six days, Ivermectin 0.2mg/kg BW s/c one dose, and Niclosamid 50 mg/kg BW. The first group (control infected) 5 dogs inoculated orally with 10ml of trypticase soya broth which contain 4.8×10^9 CFU/ml S.typhimurium. the second group (5 dogs) inoculated orally with the infective dose of S.typhimurium (above) and treated after the appearance of clinical signs with 2×10^9 CFU of L.acidophilus daily for six days. The third group (5 dogs) inoculated with the same infective dose and treated after the appearance of clinical signs with Ciprofloxacin 20 mg/kg BW daily for six days. The fourth group (5 dogs) inoculated orally with 2×10^9 CFU of L.acidophilus daily for 2 weeks followed by oral inoculation with infective dose of S.typhimurium.

Daily observations : All animals were observed daily pre & post infection until the death of the control infected (1st) group as the following : general physical examination, shedding of S.typhimurium in stool & culture of blood (7).

Serum sodium, potassium and chloride were estimated (12).

Results

S.typhimurium was susceptible to ciprofloxacin, gentamycin, chloramphenicol, cephalixin, and ampicillin, but resistant to doxycycline, trimethoprim and erythromycin. *Lacidophilus* showed an inhibitory effect against *S.typhimurium* in vitro and the diameter of zone of inhibition was 36mm, and the inhibitory effect disappeared after neutralization of acidity by adding NaOH 1% solution. *L.acidophilus* showed high adherence activity to intestinal mucosa of dog and had the ability to grow in pH 3 and in bile salts.

The clinical signs in the control infected group (1st) started with vomiting which appeared 6 hours post infection, followed by diarrhea after a day, and diarrhea increased in severity with progression of the disease, dehydration, sunken eyes and loss of skin elasticity. Fever began to appear during the second day, remain high for two days, then fall until death of animals in the sixth day (Table 1). *S.typhimurium* were isolated daily from stool and blood of all dogs in this group. The means of temperature were elevated significantly during the second day post infection in the first, second and third groups compared with the fourth group ($P < 0.01$) (Table 1). However on the fourth day the means of temperature were elevated in the first and second groups compared with other groups ($P < 0.01$).

On the sixth day the mean of temperature decreased in the first group only which had highly significant difference compared with other groups ($P < 0.01$) while the temperature of the fourth group remained within normal, *S.typhimurium* were isolated daily from stool and blood of dogs in the first, second, and third groups while the fourth group showed isolates from stool only.

The means of total leucocytes count (Table 2) were significantly elevated ($P < 0.01$) during the second and third days post infection then decreased to normal values on the fourth day and significantly declined ($P < 0.01$) on the fifth day in all groups compared with the fourth group. On the sixth day the mean of the first group only went on decreasing.

The means of serum sodium, potassium and chloride (Tables 3,4 and 5) were decreased significantly ($P < 0.01$) during the second day post infection in all groups compared with the fourth group. However on the fifth day the means continued decreasing in the first group only ($P < 0.01$).

Table (1) Means $\pm$ SE of temperatures (C⁰) in different groups.

Days Groups	Inoculation							
	1 day pre	Day of	1 day post	2 days post	3 days post	4 days post	5 days post	6 days post
1 st	37.8 a 0.162	37.9 a 0.172	37.82 a 0.192	39.2 b 0.158	41.24 b 0.336	40.22 b 0.13	38.6 b 0.65	36.32 b 0.238
2 nd	37.87 a 1.46	38.14 a 0.2	37.9 a 0.158	39.14 b 0.23	41.2 b 0.2	40.16 b 0.24	38.24 b 0.194	38.04 a 0.433
3 rd	37.96 a 0.24	37.95 a 0.207	38.04 a 0.181	39.54 b 0.296	39.16 b 0.167	37.96 a 0.16	37.94 a 0.054	37.82 a 0.03
4 th	37.92a 0.216	37.84 a 0.279	37.66 a 0.172	37.76 a 0.114	37.72 a 0.130	37.76 a 0.08	37.72 a 0.08	37.76 a 0.167
P value	P<0.05	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

Different letters between row or column means significant differences.

Table (2) Means $\pm$ SE. of W.B.C. counts $\times 10^6$ cell/mL in different groups.

Days Groups	Inoculation							
	1 day pre	Day of	1 day post	2 days post	3 days post	4 days post	5 days post	6 days post
1 st	11760a 255.9	12620a 230.8	13210a 263.15	20630b 774.27	28450b 469.041	13370a 330.088	8550b 951.38	6380b 450.83
2 nd	11970a 626.1	11920a 231.325	12650a 395.3	20640b 430.67	27200b 223.6	12590a 338.01	9350c 430.116	11810a 389551
3 rd	11680a 461.9	12610a 294.5	12440a 366.4	21390b 412.9	27450b 348.21	14520a 201.866	8290b 263.153	8380c 381.772
4 th	12360a 198116	12320a 246.5	1220a 230.76	12180a 207.966	12280a 340.22	123190a 332.916	12010a 240.831	12050a 200
P value	P<0.05	P<0.05	P<0.05	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01

Different letters between row or column means significant differences.

Table (3) Means $\pm$ SE. of serum sodium concentration by mmol/L in different groups.

day Groups	Inoculation							
	1 day pre	Day of	1 day post	2 days post	3 days post	4 days post	5 days post	6 days post
1 st	150.4 a 1.14	150.4 a 2.297	151.2 a 3.033	118.6 b 6.268	94.9 b 4.335	80.4 b 3.05	68.8 b 3.114	56.4 b 8.502
2 nd	150.6 a 1.14	149.6 a 2.607	151.4 a 2.607	118.4 b 5.272	110.6 b 3.974	107.0 c 10.049	129.4 c 4.878	151.2 a 3.114
3 rd	150.2 a 1.48	149.8 a 1.923	152.2 a 3.114	119.6 b 5.77	110.4 b 7.344	105.8 c 6.1	87.0 d 4.847	94.4 c 3.420
4 th	149.8 a 3.033	150.4 a 2.792	151.6 a 4.335	148.8 a 2.744	151.4 a 2.073	151.4 a 2.509	150.4 a 1.516	151.2 a 2.588
P value	P<0.05	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

Different letters between row or column means significant differences.

Table (4) Means $\pm$ SE. of serum potassium concentration by mmols/L in different groups.

Day group	Inoculation							
	1 day pre	Day of	1 day post	2 days post	3 days post	4 days post	5 days post	6 days post
1 st	4.52 a 0.08	4.44 a 0.55	4.33 a 0.664	2.19 b 0.204	1.776 b 0.114	1.48 b 0.056	1.26 b 0.03	0.82 b 0.286
2 nd	4.57 a 0.729	4.43 a 0.494	4.3 a 0.637	2.22 b 0.228	2.5 b 0.375	2.97c 0.207	3.41 c 0.081	3.841 a 0.55
3 rd	4.36 a 0.56	4.42 a 0.549	4.31 a 0.616	2.16 b 0.288	1.79 b 0.124	1.454 b 0.074	1.234 b 0.088	1.12 b 0.135
4 th	4.53 a 0.744	4.50 a 0.421	4.48 a 0.414	4.54 a 0.403	4.72 a 0.576	4.72 a 0.454	4.73 a 0.491	4.54 a 0.493
P value	P<0.05	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

Different letters between row or column means significant differences.

Table (5) Means $\pm$ SE. of serum chloride concentration by mmol/L in different groups.

Day group	Inoculation							
	1 day pre	Day of	1 day post	2 days post	3 days post	4 days post	5 days post	6 days post
1 st	111.2 a 6.48	113.8 a 4.969	110.8 a 5.805	96.0 b 3.972	61.2 b 2.387	72.6 b 2.701	61.8 b 5.941	56.4 b 3.633
2 nd	111.8 a 6.61	113.2a 5.167	111.2a 6.18	95.2 b 3.114	91.4 b 8.08	102.6 c 2.88	112.6 a 11.013	118.0 a 9.121
3 rd	113.6 a 7.635	113.2 a 6.172	112.0 a 7.348	95.8 b 3.564	82.6 b 3.646	74.8 b 1.14	83.8 c 9.864	89.2 c 4.183
4 th	114.6 a 4.393	116.6 a 3.209	112.8 a 3.346	113.0 a 3.162	114.0 a 4.123	119.2 a 2.04	117.0 a 8.899	118.0 a 9.471
P value	P<0.05	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

Different letters between row or column means significant differences.

Discussion

The isolated strains of *L.acidophilus* had inhibitory effect against *S.typhimurum* in vitro and the diameter of zone of inhibition was 36mm, This result was in agreement with (14). The absence of inhibitory effect after neutralization of acidity with NaOH indicate that the inhibitory effect of *Lactobacillus* in vitro mainly due to high acidity resulted from primary metabolic product of carbohydrate fermentation (15).

The follicle associated epithelium and peyer patches of the mucus associated lymphoid tissues are important sites for pathogen entry as well as for immune stimulation (16), therefore the adherence of *L. acidophilus* to intestinal epithelial cells may prevent the entry of pathogenic bacteria by blocking their receptors (17 and 18) .

All infected dogs showed the two forms of the disease (septicemic and gastrointestinal) supported with isolation of bacteria from blood and stool throughout the experiment. This result was in agreement with(19). While the fourth group did not show any clinical signs and the bacteria isolated from the stool only.

The temperature was elevated at first and then decreased and this result was in agreement with (20), this decrease in temperature may be due to circulatory disturbance and diarrhea (3).

The absence of clinical signs in the fourth group can be explained by the ability of *L.acidophilus* to prevent the adhesion and colonization of *S. typhimurum* to intestinal mucosa by blocking the adhesion receptor sites in the intestinal mucosa (21).

The recovery of all animals in the second and third groups indicate the therapeutic effect of *L.acidophilus* and Ciprofloxacin (22). The death of all dogs in the infected group indicate the virulence of *S.typhimurum*, this death may be attributed to two causes hypovolemic shock and endotoxic shock (3). The results of total leukocytic count can be divided into two stages, the first stage characterized by increased total WBCs count during the second and third days post infection, while the second stage characterized by decrease in total WBCs count . These results in agreement with (23).

The gradual decrease in concentration of sodium, potassium and chloride in serum of experimentally infected animals may be attributed to the loss of these elements due to vomiting and diarrhea (Show and Ihle,1997). In the second and third groups these elements increased in the fourth day post infection to the end of the experiment, and indicate the therapeutic effect of *L.acidophilus* and Ciprofloxacin. *L.acidophilus* had the ability to improve the absorption of minerals and electrolytes from the intestinal mucosa (22).

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