# Treatment and prevention of Salomellosis 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 بأستخدام جراثيم العصيات اللبنية المحبة للحموضة (*Lactobacillus acidophilus*).

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**الخلاصة**

اجريت هذه الدراسة لمعرفة مدى كفاءة جرائيم العصيات اللبنية المحبة للحموضة في الوقاية والعلاج من احدى مسببات الاسهال الجرثومي في الكلاب وهى جراثيم سالمونيلا تايفيميوريم. تم أجراء فحص الحساسية للمضادات الحياتية لجرثومة سالمونيلا تايفيميوريم وقد أظهرت النتائج بان المضاد الحيوي السيروفلوكساسين كان اكثر المضادات الحياتية 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, وفد اختيرت العزلة التي اظهرت قابلية على تثبيط نمو جراثيم السالمونيلا خارج النسيج الحي والتي لها قدرة عالية على الالتصاق في الخلايا الطلائية المبطنة للأمعاء في الكلاب يالاضافة الى قدرتها على تحمل الاس الهيدرجيني الواطىء وأملاح الصفراء .قسم عشرون جروااالى 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 والمعدي المعوي وأظهرت نتائج التحليل الإحصائي لجميع المعايير ، ولأول مرة، إن لجراثيم العصيات اللبتيه 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 labortartories, 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.acidophlius 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 $4.8x10^9$ 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 $2x10^9$ 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 $2x10^9$ 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 ($1^{st}$ )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 ciprofloxain, gentamycim, chloramphenicol, cephalexin,and ampicillin, but resistant to doxycycilne, trimethoprim and erthromycin. 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 !% solution. L.acidophilns showed high adherence activity to intestlinal mucosa of dog and had the ability to grow in pH 3 and in bile salts.

The clinical signs in the control infected group ($1^{st}$ ) 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 ±_SE of temperatures ($C^0$) 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** |
| 1st | 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 |
| 2nd | 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 |
| 3rd | 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 |
| 4th | 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 ±SE. of W.B.C. counts x $10^6$ 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** |
| 1st | 11760a 255.9 | 12620a 230.8 | 13210a 263.15 | 20630b 774.27 | 28450b 469.041 | 13370a 330.088 | 8550b 951.38 | 6380b 450.83 |
| 2nd | 11970a 626.1 | 11920a 231.325 | 12650a 395.3 | 20640b 430.67 | 27200b 223.6 | 12590a 338.01 | 9350c 430.116 | 11810a 389551 |
| 3rd | 11680a 461.9 | 12610a 294.5 | 12440a 366.4 | 21390b 412.9 | 27450b 348.21 | 14520a 201.866 | 8290b 263.153 | 8380c 381.772 |
| 4th | 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 ±SE.of serum sodium concentration bymmol/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** |
| 1st | 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 |
| 2nd | 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 |
| 3rd | 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 |
| 4th | 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 ±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** |
| 1st | 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 |
| 2nd | 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 |
| 3rd | 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.o74 | 1.234 b 0.088 | 1.12 b o.135 |
| 4th | 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 ±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** |
| 1st | 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 |
| 2nd | 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 |
| 3rd | 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 |
| 4th | 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.typhimurun 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 Cirprofloxacin. *L.acidophilus* had the ability to improve the absorption of minerals and electrolytes from the intestinal mucosa (22).

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