EVALUATION OF *LACTOBACILLUS SALIVARUS* AS A PROBIOTIC IN DOGS

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ABSTRACT

Lactobacillus salivarus has been studied extensively as a probiotic in human . However the ability of an organism to survive passage through the intestinal tract and exert beneficial effects can not be directly extrapolated between species. This study evaluated the ability of *L.salivarus* to survive gastrointestinal transit in dogs and assessed whether oral administration of *L.salivarus* is safe in order to determine whether studies evaluating the efficacy of *L.salivarus* in the treatment of canine disease are indicated. Dogs were divided into 5 groups receiving doses of 0 (control group n=8),1×10^9 (group 1, n=8), 1×10^{10} (group2 ,n=8) , 5×10^{10} (group3, n=8) and 5×10^{11} (group 4,n=8) colony forming unite per day orally for 5 days.

L.salivarus was detected in the feces of 3/8 dogs in group 1 and 2, 4/8 dogs in group 3; 8/8 dogs in group 4 and 0/8 dogs in control group. Fecal colonization was significantly greater in group 4 than in any other groups (P< 0.01). Differences between groups 1,2 and 3 were not significant. No adverse effects were noted. Fecal colonization of *L.salivarus* in dogs is somewhat variable; however clinical studies are indicated to evaluated this organism in the treatment and prevention of canine disease.

INTRODUCTION

Probiotic have been defined as live microorganisms which upon ingestion in certain numbers exert health effects beyond inherent basic nutrition (1). The concept of probiotics was first reported by Elie Metchnikoff in 1907 (2). He postulated that consumption of fermented milk products was responsible for longevity of certain ethnic groups and suggested that these products manipulated the intestinal microflora to maintain the normal balance between pathogenic and non pathogenic bacteria (2). A variety of microorganism typically lactic acid bacteria have been evaluated as potential probiotics (3). A small numbers of yeast

have also been evaluated (4, 5). Probiotic therapy is being used increasingly in human and veterinary medicine. Appealing properties of probiotic include the ability to reduce antibiotic use, the apparently high index of safety, and the publics positive perception about natural or alternative therapies. Probiotics are classified and generally regarded as safe, as opposed and antibiotics, which have a number of recognized adverse effects (6).

Commercial probiotic preparations are available for human and animal use, however little or no objective research has been done on many. Based on definition of probiotics stated above, it is clear that adequate number of viable organisms must reach to the intestinal tract. For this happen, probiotic organisms must be able to survive transit through the acidic environment of the stomach and resist digestion by bile. Organisms that survive acid and bile must posse's variety of other properties including the ability to adhere to intestinal epithelial cells colonize the intestinal tract and Produce antimicrobial factors to inhibit enteric pathogens (7, 8, 9, 10, and 11). Other properties such as immunomodulation, modulation of metabolic activity and inactivation of procarcinogens are also desirable (8, 12).An organism can only be considered to be a probiotic after these properties have been identified and positive health effect has been documented.

One of the best studied probiotic in human medicine is *Lactobacillus Spp*. Lactobacillus has been shown to survive acid and bile digestion and colonize the gastrointestinal tract of human (13, 14, 15, 16). Its also posses powerful adhesive properties , suppress bacterial enzyme activity, can displace or eliminate certain component the normal intestinal flora and produces an antimicrobial substance active against a variety of bacteria including *Escherichia coli*, *Salmonella Spp*… etc (11).

In human *L.salivarus* has been shown to be effective in the treatment of several forms of diarrhea including , antibiotic associated diarrhea in children and adult, travellars diarrhea and relapsing *Clostridium difficile* diarrhea in placebo-controlled studied (11,17,18,19,20,21,22,23,24). Recent studies using animal models have suggested that *Lactobacillus Spp* may be beneficial in the treatment of inflammatory bowel disease, pouchitis and ulcerative colitis in humans (25, 26). These results suggest that probiotics particularly *Lactobacillus Spp* might be of value in treatment of canine gastrointestinal disease.

MATERIALS AND METHODS

Fourty clinically health dogs were including in this study. Animals were housed in close proximity. Dogs were divided in to 5 groups. *L.salivarus* isolated from intestinal content

of healthy dog was administered orally at dose of 1×10^9 CFU (group 1, n=8), 1×10^{10} CFU (group 2,n=8) , 5×10^{10} CFU (group 3, n=8), 5×10^{11} CFU (group 4, n=8) and 0 CFU (control group, n=8) once daily for 4 days.

Dogs were monitored daily for change in clinical condition, vital parameters, appetite and fecal consistency. Freshly passed fecal samples were collected on days 0,1,3,5,6,7,9 and 11. Fecal sample were refrigerated for hours until being processed.

One gram of feces was serially diluted in phosphate buffered saline (pH=7.2). Aliquots of the serial dilution were inoculated onto de Man, Rogosa, Sharp (MRS) agar, a culture medium for isolation of lactic acid bacteria, and incubated in microaerophilic condition at 37 C$^{\circ}$ for 72 hours. Colonies were identified as *L.salivarus* based on colonial morphology, gram staining and biochemical test according to (27). Randomly selected isolates were confirmed as *L.salivarus* by using (API 50) CHL from Bio, Merieux. Overall growth on MRS agar on day zero also recorded.

A general linear model produced with contrasts of the overall mean *L.salivarus* level was used to compare the area under the curve for *L.salivarus* over days among groups. Univariate analysis on the residuals of the $\log_{10}$ *L.salivarus* level was run.

Linear regression was used to evaluate the association between day zero MRS growth and *L.salivarus* colonization on each sampling day. A statistical software package was used and a P <0.05 was considered significant for all comparisons.

RESULTS

L.salivarus was not detected in the feces of any dogs prior to administration. All dogs in group 1-3 readily consumed food containing probiotic. One dog in group 4 was slow to consume the food containing probiotic but all was consumed eventually. No adverse effects were noted. *L.salivarus* was not present in the feces of control group at any point during the study. Detectable level of *L.salivarus* were present in the feces of 3/8 dogs in group 1 and 2, 4/8 in group 3 and 8/8 in group 4 (Table 1). The mean number of positive samples per dog was 0.65 in group1 (range 0-2), 0.8 in group 2 (range 0-3), 1.8 in group3 (range 0-4) and 4 in group 4 (range 3-5). The *L.salivarus* was detected in feces 24 hrs after cessation of administration in 1/8 dogs of group 1, 2/8 in group 2, 4/8 dogs in group 3 and 8/8 in group 4. Forty eight hours after cessation of administration, *L.salivarus* was still present in the feces of 1/8 dogs in each group 2 and 3 and 6/8 dogs in group 4. After 72 hours *L.salivarus* was present in the feces of only two dogs in group 4.

Fecal level of *L.salivarus* in group (4) were significantly higher than in group 1,2 and 3 (P< 0.001, 0.001 and 0.004) respectively. Differences between groups 1,2 and 3 were not statistically significant (P > 0.08).

The mean growth on MRS agar at day 0 was $\log_{10} 6.5 \pm 1.4$ with range of $\log_{10} 4.6$-$\log_{10} 9.7$. There was no significant intergroup differences in dogs zero MRS growth (mean $\log_{10} 7.3$-7.8). There was no association between the level of MRS growth on day zero and fecal *L.salivarus* levels for any day of the study (P=0.16-0.98).

DISCUSSION

This study has demonstrated that, *L.salivarus* can survive gastrointestinal transit in dogs and do so without causing any clinically evident adverse effect. Fecal colonization of *L.salivarus* in dogs appears to be less efficient than in humans. Means fecal levels of 10^5-10^7 CFU 1gr were reported following PO administration to human at dose of $1X10^{10}$ CFU/day (16, 27). This level was achieved only in group 4 which received higher oral dose (5×10^{11} CFU/day) of *L.salivarus*. This significant difference in fecal *L.salivarus* level between group 4 and other groups can not be attributed simply to a higher oral dose moving passively through the intestinal tract. The difference in dose between groups (1) and (2) was only 2.5 $\log_{10}$ while differences between mean fecal levels during the administration period were 5.5-7.2 $\log_{10}$. This suggested that intestinal adhesion and colonization was responsible for the difference. Differentiation of delayed gastrointestinal transit from true intestinal colonization can be difficult, and intestinal biopsies would be required for confirmation that intestinal colonization had actually occurred. The reason that *L.salivarus* was detected in relatively high levels in the feces of some dogs; while it was infrequently or never detected in other dogs administered the same dose is unclear. Differences in the gastrointestinal microflora between dogs could play a role in the variation that was seen in this study. Dogs with high preexisting colonization by lactic acid bacteria may be more resistant to colonization with pathogenic enteric bacteria. Bacterial species may be able to limit colonization of similar organism through stable occupation of certain environmental or nutritional niches or through the production of specific antibacterial products. Many lactobacilli can produce specific antibacterial products. Many lactobacilli can produce bacteriocins, bactericidal substances that are only effective against lactobacilli or closely related species (28). In this study, however, there was no association between day – 0 MRS growth and colonization. Specific identification of resident lactic acid bacteria was not preformed so it is possible that

colonization by *L.salivarus* was inhibited by specific unidentified component of the bacterial microflora in some dogs. Our understanding of the interactions between components of the intestinal microflora is poor, so critical assessment is difficult. It is possible that *L.salivarus* being of human origin better adapted to colonize the human gastrointestinal tract in a lower dose than is required in dogs. This may relate to inherent differences in the bacterial microflora among species or it may be due to a variable ability to adhere to intestinal epithelial cells of different species.

Persistence of *L.salivarus* in dog is shorter than that reported in humans. Goldin *et al* (13) reported that 87% of humans execrated *L.salivarus* in feces for 4 days following cessation, while 33% shed *L.salivarus* after 7 days, while *L.salivarus* persist better in some humans than in others , it is accept that daily administration of high doses is required to maintain high fecal levels. Clinically, persistence should be less important than colonization during administration.

Lactobacillus salivarus can not be termed a canine probiotic research involving this organism in canine disease. Because this study demonstrated that *L.salivarus* could be safety administered to dogs that can survive gastrointestinal transit, it would seem logical to pursue further studies regarding this organism.

Efficacy studies are indicated to determine whether *L.salivarus* has a role in the prevention or treatment of canine disease.

It is also possible *that L.salivarus* would colonize better in dogs with diarrhea because of disruption of the normal protective intestinal microflora.

Table (1) Detectable level of *L.salivarus* in fecal samples of different groups

Groups	Total No	Positive	%
1	8	3	37.5%
2	8	3	37.5
3	8	4	50
4	8	8	100
Control	8	0	0

تقييم العصيات اللبنية اللعابية كمعزز حيوي في الكلاب

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الخــلاصــة

درست العصيات اللبنية اللعابية كمعزز حيوي في الإنسان بصورة مفصلة وعلى آية حال فأن قدرة هذه الجراثيم على مقاومة المرور خلال القناة الهضمية وبالتالي إعطاءها إعطاء تأثيرها الفعال تختلف بين الحيوانات لذلك فقد أجريت هذه الدراسة لتقييم قدرة العصيات اللبنية اللعابية على البقاء والانتقال عبر القناة الهضمية للكلاب عند إعطاءها عن طريق الفم وكذلك لمعرفة ما إذا كان استعمال هذه الجراثيم امين عند إعطائها عن طريق الفم وبالتالي للتوصية باستخدامها في علاج أمراض القناة الهضمية في الكلاب. استعمل في هذه الدراسة (40) كلب بالغ قسمت إلى خمسة مجاميع ضمت كل مجموعة ثمان حيوانات وأعطيت هذه المجاميع صفر (مجموعة السيطرة) ، 1×10^9(المجموعة الأولى) ، 1×10^{10}(المجموعة الثانية) ، 5×10^{10} (المجموعة الثالثة) و 5×10^{11}(المجموعة الرابعة) جرثومة /يومياً عن طريق الفم لمدة خمسة أيام .

عزلت العصيات اللبنية اللعابية من ثلاث من اصل ثمان حيوانات لكل من المجاميع الأولى والثانية ومن   4 **من** 8 حيوانات في المجموعة الثالثة ومن جميع الحيوانات المجموعة الرابعة ولم تعزل من أي حيوان من حيوانات مجموعة السيطرة. أن أعداد الجراثيم المتواجدة في البراز كانت أعلى بصورة معنوية في حيوانات المجموعة الرابعة مقارنة مع المجاميع الأولى والثانية والثالثة

$(P < 0.01)$ ولم تلاحظ فروقات معنوية بين المجاميع الثلاث الأولى. أن عدد هذه الجراثيم في البراز يختلف من حيوان لأخر لذا نقترح أجراء دراسة سريرية لتقييم استخدام هذه الجراثيم في العلاج والوقاية من أمراض القناة الهضمية في الكلاب.

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