Therapeutic Effect of Lactobacillus-based Probiotic on Canine Acute Diarrhea

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ABSTRACT

The present study was conducted to evaluate therapeutic effect of commercial lactobacillus-based probiotic as a single therapeutic agent against acute diarrhea in dogs. About 47 dogs were studied in the present work, included 20 apparent healthy dogs and 27 diseased dogs suffered from acute diarrhea. About 17 diarrheic dogs were treated with probiotic for 5 days. Physical and clinical examinations were performed for all dogs. Blood and serum samples were collected to evaluate hemato-biochemical status at Day 0 and Day 5 after treatment. Stool was observed and scrutinized for its consistency during treatment. Results showed a decreased recovery time of diseased cases at 2.4±0.02 days. Significant decrease of PCV and WBCs count, significant increase total protein, albumin, A/G ratio and significant decrease of elevated ALT, AST and bilirubin at Day 5 was recorded compared to diarrheic dogs at day 0. Conclusion: the data indicates an efficient treatment with lactobacillus-based probiotic as solo therapeutic agent against canine acute diarrhea, not only reduce the recovery time, but also, enhance liver functions and improve the fecal consistency.

Key words: Lactobacillus- Probiotic, Acute Diarrhea, Dogs, CBC, Biochemistry

INTRODUCTION

Acute diarrhea is a common cause of veterinarian admission for dogs worldwide. It represented as increased defecation frequencies or water content of stool and clinically manifested by anorexia, lethargy, and dehydration (Rakha et al., 2015). Antimicrobial intervention such as amoxicillin and metronidazole in treatment of acute diarrhea in dogs was a common procedure. Anti-microbial medication not only showed no effect on acute canine diarrhea (Werner 2018) and (Ortiz et al., 2018) but also, found to be associated with high prevalence of Antibiotic-Associated Gastrointestinal signs (AAGs) (Whitemore et al., 2019). (Sharkey et al., 2016) suspected the development of anti-microbial resistance and even had an acute impact on the gut Microbiome which defined as "second genome, interacting system of the host mammalian cells and the resident of the microbial community (Chandler 2015). Acute diarrhea may result in dysbiosis which defined by any change in the composition of resident commensal communities relative to the community found in the healthy individuals (Petersen and Round 2014). Microbiome modulation was identified as a potential therapeutic agent against canine gastro-intestinal disorders (Schmitz and Suchodoleski 2016) and (White et al., 2017) and the probiotic was able to normalize the Microbiome of dogs (Ziese et al., 2018).

Probiotic included live micro-organisms that achieve a health benefits to the host, when delivered in an adequate balanced amount (Hill et al., 2014). Probiotic mechanisms of action included Inhibition the growth of pathogens (Piewngam et al., 2018), enhancement the intestinal epithelial cell integrity (Ho et al., 2020), restoration of tight junction of intestinal cells, normalization of the Microbiome in dogs with acute hemorrhagic diarrhea syndrome (Ziese et al., 2018), Favorable modulation of the immune system, Competition with pathogens nutrient and adhesion site, anti-inflammatory activities and have anti-oxidant properties (Grześkowiak et al., 2015).

The aim of the present study is to evaluate the value of probiotic sole therapy without the need to use antimicrobial medication against cases of canine acute diarrhea and its effect on hemato-biochemical profile among dogs in Egypt.

MATERIALS AND METHODS

Animals and sample

This study was conducted on 47 different breeds and sexes of dogs, that admitted to the small animal clinic, Faculty of veterinary medicine, Cairo University, Egypt with ages ranged from 5 months to 7 years, of which, 27 were suffering from acute diarrhea, while 20 were apparently healthy dogs and served as control during the period between April 2019 to October 2019. Complete case
history and clinical signs were recorded at time of admission. Both physical and clinical examinations were performed. The exclusion criteria included: duration of diarrhea was less than 3 days, dogs with other known diseases, dogs had no history of antibiotic treatment at least 2 months before or during the experiment and tested negative for parvovirus infection and Toxocara infestation. Of the 27 diarrheic dogs in the present study, 17 dogs received probiotic as main treatment, and were re-admitted to the clinic for follow up.

The probiotic used in this study, Probio-Pet (Biovee animal health), at a dose of 1/tablespoon per-os twice daily. Each 100 gm contain Lactobacillus plantarum, Lactobacillus bulgaris, Lactobacillus rhamnous, Lactobacillus acidophilus (4 × 10^10 CFU) according to manufacturer pamphlet. After the treatment period (Day5), a basic physical examination was conducted and blood samples were collected for re-investigation. The minimum diagnostic evaluation in all dogs included a complete blood count (CBC) and serum biochemistry. Blood samples were obtained from both healthy, diseased and probiotic recovered dogs for determination of hemoglobin, PCV (Packed Cell Volume), RBCs, total and Differential Leukocytic counts (DLC). EDTA-blood was used for hematologic examination using veterinary hematology analyzer. Serum was separated and used for detection of total protein, albumin, globulin, bilirubin, urea and creatinine and serum activities of ALT (Alanine Aminotransferase test), AST (Aspartate Aminotransferase test) and GGT (gamma-glutamyl transferase test) according to specific test kits (spectrum diagnostics Egypt).

Statistical analysis
All the data was added to Microsoft Excel sheet and results were shown as mean ± SE (Standard Error). Diseased animals at Day 0 data were compared with control animal's data and diseased animals at Day 5 data were compared with diseased animal's at Day 0 using student T-Test through SPSS program version 16. P value ≤0.05 were considered significant.

Ethics statement
The use of dogs in this experiment was permitted by the owners of the dogs. All animal procedures were performed in accordance with the Guidelines of department of medicine, Faculty of veterinary medicine, Cairo University, Egypt.

RESULTS
The present study results showed a clinical recovery time of 2.4 ± 0.02 days in which the stool samples regained a normal consistency. Regarding hematological parameters, (Table1), diseased dogs at day 0 showed significant (P≤0.01) increase in PCV and significant increase (P≤0.05) toward WBCs count. Regarding monocytes level, it showed a significant decrease (P≤ 0.05) in comparison with control group. At day 5, probiotic treated group showed significant (P≤0.01) decrease in PCV and significant (P≤0.001) decrease in WBCs count, in comparison with diseased dogs at day 0.

In terms of serum biochemical parameters, diseased dogs at day 0 showed significant decrease in total protein (P≤0.001), albumin (P≤0.001), globulin (P≤0.05). Significant increase was recorded in ALT and AST (P≤0.01) and significant increase in total bilirubin (P≤0.05) when compared to control group. At day 5, probiotic treated cases showed significant increase in total protein (P≤0.05), albumin (P≤0.001), A/G ratio (P≤0.05) and significant decrease in ALT (P≤0.05), AST (P≤0.01) and bilirubin (P≤0.05) while compared to diseased dogs at day 0.

DISCUSSION
Results indicated that the duration of recovery of diseased dogs receiving probiotics was 2.4±0.02 days. Clinically, watery stool in dogs returned to normal consistency in relatively short time. However, several studies demonstrated shortening of diarrhea duration after probiotic use (Tláskal et al., 2007; Herstad et al., 2010; Fenimore et al., 2017; Nixon et al., 2019). Nixon et al., (2019) justified his results, that probiotic had accelerated normalization effect on gut Microbiome.

Regarding hematomal parameters, PCV in dogs with diarrhea showed significant elevation and the same results were reported by Brown et al., (2008) as a result of dehydration. One possible explanation for reduction of PCV to normal level at Day 5 after probiotic administration may be due to cessation of diarrhea and normal rehydration status. Concurrent results illustrated that total White Blood Cells is significantly increased in diseased dogs and surprisingly decreased to normal in dogs received probiotics. In line with present findings, Lee et al., (2015) found that total WBC count is normally increased by infection and the levels in both groups (Group received placebo and other group received probiotics) were decreased compared to the baseline.

Blood biochemical analysis showed at Day 0 significant decrease in Total protein, albumin, similar to the findings of Bhat et al., (2013) and suggested that might be due to marked decline in food intake, malabsorption and ongoing protein losing enteropathy. Alterations in biochemical parameters included significant increase in serum ALT and AST concentrations and these results are in the same findings of Arora et al., (2018) might be due to involvement of liver and severe protein losing enteropathy due to intestinal villi damage.

Present study revealed that total protein was increased significantly in diseased dogs at day 5 after probiotic received. Strompfová et al., (2007) found that total protein increased in most dogs with hypoproteinaemia (suffering from acute or chronic gastrointestinal disorders) was detected after 7 days of lactobacillus probiotic application; he justified his results that increased total protein might be connected with differences in the microflora abundance. Significant albumin increment at Day 5, Similar to (Strompfová, et al., 2018) found that Albumin increased in dogs received combination of lactobacillus fermentum CCM7421 combined with alginat, on the other hand, group received alginat only showed significant decrease in albumin. These results were explained by increase in protein fractions due to the presence of probiotic bacteria that increase the efficiency of digestion through secretion of probiotic bacteria-derived proteases and peptidases and increase villus height (Neis et al., 2015).

Results revealed that significant decrease of ALT at Day 5 similar to findings of (Strompfová et al., 2007).
Table 1: Hematological parameters of diarrheic dogs at Day 0 compared with apparently healthy (control) dogs and hematological parameter in dogs at Day 5 as compared with diarrheic dogs at Day 0.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control dogs</th>
<th>Diseased dogs Day 0</th>
<th>Diseased dogs Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>39.97±0.74</td>
<td>40.61±1.08**</td>
<td>38.09±0.77**</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>14.68±0.42</td>
<td>13.63±0.47</td>
<td>13.75±0.49</td>
</tr>
<tr>
<td>RBCs (×106/µl)</td>
<td>5.67±0.16</td>
<td>4.99±0.23</td>
<td>5.30±0.21</td>
</tr>
<tr>
<td>WBCs (×103/µl)</td>
<td>11.16±0.46</td>
<td>19.49±2.38*</td>
<td>10.25±1.13***</td>
</tr>
<tr>
<td>D.L.C</td>
<td>62.79±1.82</td>
<td>60.88±2.02</td>
<td>62.19±1.27</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>3.95±0.52</td>
<td>2.73±0.43</td>
<td>3.25±0.32</td>
</tr>
<tr>
<td>Banded cells (%)</td>
<td>25.21±1.66</td>
<td>28.25±1.69</td>
<td>28.34±1.19</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>5.63±0.68</td>
<td>4.71±0.28*</td>
<td>4.44±0.71</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2.42±0.22</td>
<td>3.40±0.60</td>
<td>3.31±0.44</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0±0</td>
<td>0.04±0.04</td>
<td>0±0</td>
</tr>
</tbody>
</table>

***P<0.001; **P<0.01; *P<0.05.

Table 2: Serum biochemical parameters in dogs suffered from diarrhea at Day 0 compared with apparently healthy (control) dogs and serum biochemical parameters in diseased dogs at Day 0 as compared with dogs at Day 5.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control dogs</th>
<th>Diseased dogs Day 0</th>
<th>Diseased dogs Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proteins (g/dl)</td>
<td>5.94±0.15</td>
<td>4.51±0.16**</td>
<td>5.20±0.30*</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.09±0.09</td>
<td>2.10±0.07***</td>
<td>2.82±0.19***</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>2.85±0.12</td>
<td>2.41±0.15*</td>
<td>2.39±0.25</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.12±0.07</td>
<td>0.97±0.07</td>
<td>1.33±0.21*</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>24±1.36</td>
<td>49.37±5.46**</td>
<td>29.4±1.38*</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>25.82±2.42</td>
<td>49.8±5.73**</td>
<td>17.9±0.75**</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>2.98±0.71</td>
<td>4.83±0.75</td>
<td>3.33±0.29</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.88±0.10</td>
<td>2.45±0.89*</td>
<td>0.55±0.06*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.78±0.05</td>
<td>1.03±0.14</td>
<td>0.94±0.07</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>19.38±1.33</td>
<td>20.67±1.89</td>
<td>23.4±1.78</td>
</tr>
</tbody>
</table>

***P<0.001; **P<0.01; *P<0.05.

Also, (Adawi et al., 2001) recorded the same finding and justified this reduction by reduced pathogenic bacterial in an acute liver injury rat model after administration of different lactobacilli.

Present study found that at day 5, there was a significant decrease in bilirubin compared with diseased dogs at day 0. Results were similar to the findings of Kirpich et al. (2008), who found that in human patients suffered from liver injury treated with probiotics, there was a significant decrease in total bilirubin when compared to diseased group. Also, he reported that after 5 days of probiotic therapy, all liver enzymes were reduced to the baseline, but only AST reached significant decrease.

Conclusions

In conclusion, the present work indicates that 5 days application of lactobacillus based probiotic to dogs with acute diarrhea positively enhance a recovery time of 2.4 ±0.02 days. Increased total protein in dogs with diarrhea, as well albumin and decreased elevated ALT, AST and total bilirubin toward the normal level was achieved after treatment. Results suggest the usage of lactobacillus based probiotic as a solo therapy for acute diarrhea in dogs.

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