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Communication

Effect of Specific Probiotic Mixture on Coronavirus (FeCoV) Viral Load in 25 Naturally-Infected, Clinically Healthy, Purebred Birman Cats

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Simple Summary: *Feline coronavirus* (FeCoV) causes enteric disease in cat worldwide by self-limiting gastroenteritis but not understood changes can lead to the development of feline infectious peritonitis (FIP). Although current evidence shows that probiotics have promise for enhancing health benefits protecting from viral infection, today there are no studies regarding the use of probiotics in reducing fecal emission of feline coronavirus in naturally infected cats. The aim of the present study was to evaluate the effect of specific multi-strain probiotics on 25 naturally-infected purebred Birman cats. All animals did not present alteration in the clinical condition after probiotic administration; seventeen out of 25 cats tested negative at the end of the study. The results of the present study suggest that high concentrations of live probiotics supplementation may potentially reduce the fecal FeCoV viral load.

Abstract: *Feline coronavirus* (FeCoV) causes enteric disease in cat worldwide. In general, FeCoV infection is characterized by self-limiting gastroenteritis but not understood changes can give rise to mutants that lead to the development of feline infectious peritonitis (FIP). In cats, FeCoV replicates in the intestines and spreads by fecal-oral transmission and persists in multi-cats households by infection and re-infection by the same or another viral strain. Although current evidence shows that probiotics have promise for enhancing health benefits protecting from viral infection, today there are no studies regarding the use of probiotics in reducing fecal emission of feline coronavirus in naturally infected cats. The aim of the present study was to evaluate the effect of specific multi-strain probiotics on FeCoV viral load in 25 naturally-infected cats. The study population consisted of purebred Birman cats, of which 14 females and 11 males, aged from 1 to 4 years, and positive for FeCoV by Real-time PCR on fecal sample. Two microcaps of probiotic formulation containing billion bacteria were administered to all cat once a day for 60 consecutive days. After probiotic administration, clinical conditions and FeCoV viral load was assessed by Real-time PCR on fecal samples. All animals did not present alteration in the clinical condition at T1 (after probiotic administration); seventeen out of 25 cats tested negative at the end of the study, while the remaining 8 cats tested positive at the end of the study. The results of the present study suggest that high concentrations of live probiotics supplementation are well-tolerated and may potentially reduce the fecal FeCoV viral load.

Keywords: FeCoV; cat; Birman; probiotic; fecal viral-load

1. Introduction

Feline coronavirus (FeCoV) is a positive-stranded RNA belonging to the species *Alphacoronavirus* of Coronaviridae family that causes enteric disease in cats worldwide [1,3]. In general, it causes asymptomatic infection or mild diarrhea, but not fully understood changes can give rise to mutants that lead to the development of feline infectious peritonitis (FIP) [2,3]. Following the infection, infected cats eliminate the virus or may develop a persistent infection increasing the chance of a virulent FIP strain to emerge in multi-environment cats [3]. FeCoV persists in domestic cats (about 40% of individuals) and multi-cat households (around 90% cases) by infection and re-infection from the same or another strain of virus [4]. In the gastrointestinal tract, FeCoV replicates in the intestine and spreads by fecal-oral transmission [4]. Feline Infectious Peritonitis is widespread worldwide and presenting a higher incidence in young animals. Different clinical forms can occur based on Th1 or Th2 immune response [5]. The presence of cavity effusion identifies “effusive”- due to a prevalence of humoral response- and “non-effusive or dry” clinical form- due to cell-mediated response- [5]. In some cases, a strong cell-mediated response would be able to counteract the virus FIPV. The “effusive form” results from vasculopathy induced by the high expression of vascular-endothelial growth factor increasing in vascular permeability involving all serosae as peritoneum, pleurae, and the pericardium [6]. The “dry form” has a chronic course than the effusive and it is associated with neurological and/or ocular or both and or lymphadenomegaly [5].

The need for a method to prevent the infection with FeCoV in cats has led many research groups to search for a therapy. Treatment with Mutian® X has been proposed with a successful elimination of fecal FCoV shedding in chronically infected cats [7]. Probiotics are defined as beneficial live microorganism which are known to improve the microbial balance in the gut when administrated in sufficient quantity [8]. Selection of potential beneficial bacterial strains appears key to the effect of treatment using probiotics. They can stimulate the immune system increasing Immunoglobulin (IgA and IgG) concentration in intestinal mucus layer, enhancing leucocytes activity, and protecting from virus infections by enhancing cytokine antiviral responses in the intestinal mucosa [9-12]. Gut hosts approximately 70% of the cells of the immune system which continuously discriminate between harmless and pathogenic antigens [10]. Multiple mechanisms by which probiotics may impart protective effects and interact with the host have been suggested. They include the displacement and suppression of the growth of pathogens, by rapid colonization of the gastrointestinal tract competitively inhibiting pro-adherence of pro-inflammatory pathogens to the Intestinal Epithelial Cells (IECs); the improvement of the mucosal barrier function, by the release of specific metabolites that interact directly with gut epithelial cells; modulation of the host immune response to pathogens, through a direct adjuvant effect on immune factors, such as cytokines [11]. Scientific evidence describes the probiotics efficacy against viral diseases by modulating the anti-inflammatory interleukins and antibodies production and the viral loads reduction [13,14]. Studies showed that probiotics also have anti-viral effects, including against coronavirus, interfering with virus colonization, and promoting its inactivation [14]. These effects are due to the ability of probiotics to modulate immune stimulatory responses upon interaction with dendritic cells (DCs). DCs recognize antigens in the intestinal lumen and transport them into the Peyer’s patches via specialized IEC, known as M cells, or via dendrites of DC that protrude between the IEC. The up-regulation of surface markers following the exposure to mucosally encountered antigens, including viruses, gives rise to the production of different cytokines with anti-viral and immune-regulatory activities. As soon as the antigen is recognized, DCs migrate to the mesenteric lymph nodes and activates T cells which migrate back to the intestinal lamina and carry out their effector functions [9]. The aim of the present study is to evaluate the effect of a specific multi-strain probiotic in modulating the viral load in coronavirus-spreading purebred Birman cats. The results suggest that long-term administration of the probiotic mixture may reduce viral excretion via fecal sample by reducing the prevalence and risk of re-infection in multi-cat house.

2. Materials and Methods

Twenty-five clinically healthy Birman cats positive for FeCoV by Real-time PCR on fecal sample were selected from the Veterinary Teaching Hospital, University of Camerino. The cats were aged between 1 and 4 years, 14 were intact female and 11 were intact male, all were non-neutered. Among the 14 females, 8 had kittens and were nursing. All cats included in the study were living in an individual environment, with single litter boxes and without contact with other animals. Before starting the treatment, all cats underwent a clinical history collection and medical examination upon enrollment. Fecal samples of three consecutive days were collected before starting the treatment (T0) and after 60 days of probiotic administration (T1) for genetic analysis and placed in sterile microtubes and stored at -80°C until Real-time PCR examination. A specific formulation of probiotics, the SLAB51®, consisting of 8 different strains of live bacteria (*S. thermophilus* DSM32245/CNCM I-5570, *L. acidophilus* DSM32241/CNCM I-5567, *L. plantarum* DSM32244 CNCM I-5569, *L. paracasei* DSM32243/CNCM I-5568, *L. helveticus* DSM32242/CNCM I-5573, *L. brevis* DSM27961/CNCM I-5566, *B. lactis* DSM32246/CNCM I-5571, *B. lactis* DSM32247/CNCM I-5572), was administered to all cats in the study. Two probiotic microcaps containing at least 200 billion bacteria/each were administered once a day for 60 consecutive days (T0 to T1). There were no dietary changes and no therapies during the study. Written informed consent was obtained from the owners before enrolling their animals in the study. For each cat, the positive condition, characterized by presence of viral RNA by fecal material in different tests, was scored 1, while the negative condition (absence of viral RNA in feces), was scored 0.

3. PCR analysis

DNA was extracted by using a commercial kit (Maxwell RSC Blood, Promega) and by employing an automated extractor (Maxwell RSC, Promega) and by following manufacturer's instructions. After extraction, DNA was PCR amplified with a nested PCR as described by literature [15].

4. Statistical analysis

Data were analyzed using GraphPad Prism 8 software (GraphPad Software, Inc., La Jolla, CA, USA). All data are presented as the means $\pm$ standard deviation (SD). Difference between the score of positive and negative patients was analyzed using a Wilcoxon matched-pairs signed rank test. The level of significance was set at $*P < 0.05$.

5. Results

Seventeen cats out of 25 included in the study, were negative at PCR for fecal FeCoV RNA after probiotic administration, while 8 were still positive. None of the cats exhibited adverse clinical signs during the treatment and the administration of probiotics was perfectly tolerated by all cats included in the study. The positive cats that were still positive after administration were lactating females.

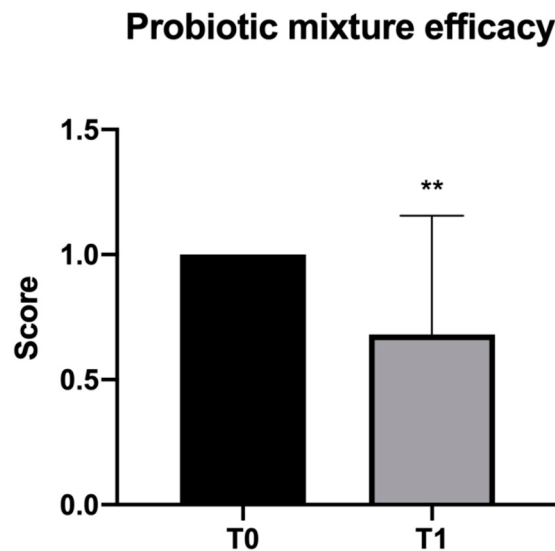


Figure 1. Schematic representation of the efficacy of probiotic mixture to induce FeCoV negativization in Birman cats.

6. Discussion

No studies have been carried out to date on the possible role of probiotics in the reduction of FeCoV viral load in cats' fecal samples. Animal coronavirus diseases are emerging or reemerging challenges worldwide. Veterinarians and researchers are focused on a better understanding of prevention and control strategies to manage the disease in multicat household. The most promising therapeutic approaches against FIP are those aimed to reduce viral loads and attenuate inflammatory/cytokine storms. Probiotics, specifically lactobacilli and bifidobacteria, have been extensively studied for their protective effects toward viral infection showing a protective role against influenza virus, rhinovirus, respiratory syncytial virus, adenovirus, and pneumovirus [1,16]. Some probiotic strains have been shown to protect against viral infection by modulating the immune stimulatory response of the host, as demonstrated in a number of clinical trials [16-18]. The innate and adaptive immune compartments play a key role in both the initiation and the development of virus-mediated inflammation. Meta-analyses suggested that oral probiotics may have a role in respiratory infections and may influence lung homeostasis via the gut-lung axis [19-20]. Evidence show that the positive effects of probiotics are due to their ability to interact with dendritic cells (DCs) inducing a rapid and strong transient up-regulation of IFN- β , which in turn stimulates transcription of a high number of genes involved in viral defence [21]. *L. acidophilus* showed to be capable to stimulate a pro-inflammatory and antiviral response by a TLR-2-dependent mechanism [21]. *Bifidobacterium bifidum* was demonstrated to modulate humoral and cellular immune responses and induce balanced Th1/Th2 immune responses against influenza infection [22]. In addition, Charrel-Dennis et al. found out that only live bacteria stimulated a stronger induction of IFN- β compared with heat-killed bacteria [23]. As it is for the human coronavirus, FeCoV replicates in the intestine and spreads by fecal-oral transmission, with potential recurrence and transmission of the virus. In this context, oral bacteriotherapy with Slab51® has shown a statistically significant impact on the clinical conditions of COVID-19 patients thanks to its biochemical and immunological profile that might trigger several protective biological functions [24]. The bacterial strains present in the product showed to enhance the production of two molecules with a significant antiviral activity through a reduction of oxidative stress [24]. Furthermore, previous studies have shown that probiotics favor an increase in the concentration of IgA and, indirectly, IgG in the intestinal mucus. Intestinal mucus is a fundamental element for the integrity of the intestinal barrier, and among its properties there is also that of containing immunoglobulins capable of neutralizing and agglutinating the viral particles that pass through the intestine. Secretory IgA in the intestine is the most important

protective humoral immune factor at this mucosal site [26]. Benyacoub and colleagues demonstrated for the first time that a dietary probiotic LAB enhance specific immune functions in young dogs. Specifically, data report that *Enterococcus faecium* (SF68) progressively increased total fecal IgA levels in orally administered puppies. This result argues in favor of a mucosal adjuvant effect of some specific probiotics, supporting that the bacteria directly triggered and stimulated the immune system underlying the intestinal mucosa [25]. This mechanism could be relevant for improving protective immune responses against various infections, stressful events or during particular stage in life, such as weaning, and may have a strong effect on the overall health of the host throughout its life, in particular on the development of the immune system. In this context, a previous study with Slab51 in healthy dogs demonstrated that the different bacterial strains in the probiotic formulation directly triggered and stimulated the local immune system associated with the intestinal mucosa. Indeed, Slab51 showed to significantly enhance the total fecal IgA, and the IgG humoral and systemic immune response following the probiotic supplementation. An increase in the intestinal IgA rate can be beneficial to prevent the entry or colonization of enteropathogens through a mechanism of immune exclusion that inhibits viruses and other noxae adherence, colonization and penetration, and can be at the basis of the antiviral activity of the probiotic formulation [12]. We believe that this mechanism also contributed to the viral clearance of cats negative after probiotic treatment. Since there are no studies regarding the use of probiotics in reducing fecal emission of feline coronavirus in naturally infected cats, with the present study was aimed to evaluate the effect of the specific multi-strain probiotics Slab51® on FeCoV viral load in a population of naturally infected Birman cats. Interestingly, 17 out of 25 cats tested negative at the end of the study by Real-time PCR on fecal samples, suggesting a possible role of a specific oral bacteriotherapy as complementary therapeutic strategy to avoid the progression of the virus, especially in multicat households. The main weakness of the study is the absence of a control/untreated group but considering that we are talking of clinically healthy animals positive for FeCoV by Real-time PCR on fecal sample, it is not expected a disappearance of fecal viral shedding in the absence of any therapy.

7. Conclusions

In accordance with author's knowledge, this communication shows the first results about the possible role of probiotics in the reduction of FeCoV viral load in fecal samples of 25 purebred Birman cats naturally-infected. Seventeen cats out of 25 were negative for fecal FeCoV RNA after probiotic administration, while 8 were still positive. None of the cats exhibited adverse clinical signs during the treatment. The results achieved in the present study bring the support for the clinical improvement of viral infection that was observed in human consuming specific probiotic strains, showing a decrease in viral load/shedding of FeCoV positive cats after probiotic administration. Additional trials are needed to confirm our results and to further explore the mechanism at the basis of the management of the infection to alleviate the clinical severity of FeCoV infection and transmission and improve health outcomes.

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