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Improvement of Intestinal Microbiota Richness in Puppies After Oral Hyperimmunized Plasma Supplementation

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The prevalence of neonatal mortality is high in the canine species and poorly studied. Microbiome, undergoing massive changes during the early stages of life, is becoming increasingly recognized as critical to understanding the immune system and metabolic function in neonates. It has been noted in many species that adequate transfer of maternal immunity through colostrum is crucial for survival. Given the highly dynamic and unique interaction between the immune system and the intestinal microbiome, strategies for conditioning and maintaining a healthy gut may be useful in prevention of neonatal mortality in puppies. This study was designed to evaluate longitudinally the fecal microbiome in puppies administered a hyperimmunized plasma supplementation since birth until weaning.

Blood was collected from routinely vaccinated adult dogs, and the plasma was stored at –20°C. At birth and subsequently every two days, 28 puppies were treated orally with hyperimmunized plasma and 30 puppies served as healthy controls. Fecal samples were collected on days 2, 21, 42, and 56 after birth. DNA was extracted using the ZR Fecal DNA Kit™ (Zymo Research Corporation, Irvine, CA). The fecal microbiota was analysed by 454-pyrosequencing of the 16S rRNA gene. Microbial communities between groups were compared using the ANOSIM function (package PRIMER 6, PRIMER-E Ltd., Plymouth, UK) to evaluate beta diversity. Observed species, Chao1, and Shannon diversity indices were used to evaluate alpha diversity.

Microbial communities were found to be significantly different between hyperimmunized and healthy control puppies at days 2, 42, and 56 (ANOSIM: $p = 0.0030$, 0.0030, and 0.0400, respectively). At day 2, the observed species metric revealed that species richness was significantly increased in hyperimmunized puppies compared to control puppies (median [range]: 191 [91–259] and 129 [89–288], respectively; $p = 0.0015$). Similarly, at day 2, the Chao1 metric estimated that true species richness was significantly increased in hyperimmunized puppies compared to control puppies (median [range]: 329 [128–549] and 203 [120–495], respectively; $p = 0.0029$). The Shannon diversity index for species richness and evenness distribution was also significantly increased in hyperimmunized puppies at day 2 compared to control puppies (median [range]: 5.4 [4.2–6.2] and 5.0 [3.6–6.7], respectively; $p = 0.0205$).

Previous studies have shown decreased diversity in dogs with gastrointestinal disease and those that receive antibiotics. Supplemented puppies were characterized by an initial increase in diversity and modified microbial communities. It could be hypothesized that the microbiome of hyperimmunized puppies offers a bolstered immune system in neonate puppies, but this conjecture warrants further research.

Disclosure

This study was sponsored by Royal Canin. H. Mila, C. Mariani, A. Feugier, and A. Grellet are Royal Canin employees.

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