Proceedings of the 8th International Symposium on Canine and Feline Reproduction

ISCFR

June 22-25, 2016
Paris, France

In a joint meeting with the XIX EVSSAR Congress

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Effect of oral hyper-immune plasma administration on intestinal microbiota and growth in puppies

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The survival rate in pre-weaning puppies is low (in average 80%; between 100 and 60% among kennels) (1-2) leading to a high economic loss for dog breeders. Acquisition of maternal immunoglobulins (Ig) from colostrum is associated with chances to survive, as puppies with low serum IgG concentration are at higher risk of death (2). Adequate growth at the early stage of life, as well as completeness of intestinal microbiota reflects health status of the newborn animal. The aim of this study was thus to evaluate the effect of immunoglobulin supplementation on growth rate and intestinal microbiota diversity in pre-weaning puppies. Blood was collected from routinely vaccinated adult dogs, and the plasma was stored at -20°C. At birth and subsequently every two days until Day 56, 28 puppies (13 large and 15 small breed size puppies) were treated orally with collected plasma and 30 puppies (8 large and 22 small breed size puppies) served as controls. All puppies were allowed to suckle their dam during the entire experiment. Randomization by birth weight and breed size ensured equal distribution of individuals into studied groups. Puppies were weighed at birth and every week until postnatal day 56, and weight gain over the neonatal period (0-21 days) and pediatric period (21-56 days) was calculated (g). Linear mixed model (MIXED proc, SAS Institute Inc., Cary, NC, USA) with Scheffe adjustment was used to evaluate the effect of supplementation on weight gain. Fecal samples were collected on postnatal days 2, 21, 42, and 56. DNA was extracted using the ZR Fecal DNA Kit™ (Zymo Research Corporation, Irvine, CA). The fecal microbiota was analyzed 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. Data are presented as mean ±SD. Weight gain was found significantly associated with studied period (p<0.001), breed size (p=0.004), supplementation (p=0.026) and interaction between the three factors (p<0.001). Supplemented large breed-sized puppies gained more weight during the neonatal period than large breed-sized controls (1408 ± 217g vs. 815 ± 376g). Microbial communities were found also significantly different between supplemented and control puppies from large breeds at postnatal day 21 (p=0.030) and 42 (p=0.020), which was preceded by increased species completeness in supplemented puppies regardless of breed size compared to controls at day 2 (Observed Species; p=0.001). Neither weight gain, nor microbial communities were influenced by hyper-immune plasma supplementation in small breed-sized puppies during neonatal or pediatric period. Large breed puppies have been demonstrated to be more susceptible to diarrhea during the pre-weaning period, associated with lesser weight gain3. Decreased diversity in intestinal microbiota was described in dogs with gastrointestinal disease4. An increase in weight gain and microbial diversity, as well as modified microbial communities were demonstrated in large breed supplemented puppies during the neonatal period in this study. It could be hypothesized that the hyper-immune supplementation affects digestive and global health of puppies; however, our findings require further analyses on larger number of individuals.