

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

Mila H^a, Guard BC^b, Mariani C^c, Feugier A^c, Grellet A^{a,b}, Steiner JM^b, Suchodolski J^b, Chastant-Maillard S^a

^aReproduction, Toulouse National Veterinary School, UMR INRA/ENVIT 1225 IHAP, Université de Toulouse, INP-ENVIT, Toulouse, France; ^bGastrointestinal Laboratory, Texas A&M University, USA; ^cRoyal Canin, France
h.mila@envt.fr

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 ± 217 g vs. 815 ± 376 g). 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.

[1] Gill MA. PhD thesis, University of Sidney 2001. [2] Mila H, et al. Prev. Vet. Med. 2014;116:209-213. [3] Grellet A, et al. Prev. Vet. Med. 2012;106:315-323. [4] Panagiotis GX, et al. FEMS Microbiol Ecol 2008;66: 579-589.

Effect of the hyper-immune egg yolk supplementation on weight gain in neonate puppies

Mila H^a, Oliver C^a, Feugier A^b, Mariani C^b, Grellet A^{a,b}, Chastant-Maillard S^a

^aReproduction, Toulouse National Veterinary School, UMR INRA/ENVIT 1225 IHAP, Université de Toulouse, INP-ENVIT, Toulouse, France; ^bRoyal Canin, Aimargues, France.
h.mila@envt.fr

Colostrum provides puppies with most of their passive immune transfer, as in dogs only 5% of immunoglobulin G (IgG) is acquired via transplacental transfer¹. Inadequate colostrum intake during the first day of life will deprive puppies not only of immunoglobulins, but also of many hormones, growth factors and nutrients, increasing their risk for neonatal morbidity and mortality. Supplementation during the first hours of life with canine serum or plasma increased blood IgG concentration² in colostrum deprived puppies as well as improved their growth during the entire neonatal period (0-3 weeks)³. This study aimed to evaluate the effect of exogenous specific antibodies administrated via egg yolk before the intestinal barrier closure (i.e., <8h after birth) on growth in pre-weaning puppies. Specific antibodies against canine parvovirus type 2 and *E.coli* were obtained in eggs from hens vaccinated against mentioned agents⁴. Hyper-immune solution was then prepared by mixing egg powder with a commercial milk replacer (Babydog Milk, Royal Canin, Aimargues, France; 1g of egg hyper-immunized egg powder with 12ml of reconstituted milk). A total of 334 puppies from 16 different breeds, enrolled in one breeding kennel, were included in the study. Depending on the expected adult body weight, puppies were classified into small breed dogs (S; adult weight < 25 kg), and large breed dogs (L; > 25 kg). Within each litter and taking into account the birth weight, puppies were randomly assigned into supplemented or control group, both allowed to suckle their mother freely. Each puppy from the supplemented group received orally 1.5 ml/100g bw of hyper-immune solution at once within the first 8 hours after birth. Puppies from the control group received at the same dose (1.5 ml / 100g) and time the milk replacer only. All puppies were weighed at birth and at 7, 14, and 21 days of life. Linear mixed models with litter modeled as a random effect were performed to determine the variables affecting birth weight and weight gain during the neonatal period: breed size, age, supplementation. L represent 38.3% (128/334) of the included puppies. Among L and S, 65 (50.8%) and 104 (50.5%) were supplemented, respectively. Weight gain during the neonatal period was influenced by time ($p < 0.001$), supplementation ($p = 0.031$) and the interactions between the breed size and supplementation ($p = 0.027$) and time and breed size ($p = 0.001$). L gained 176g [67; 294g] during the 1st week, 223g [158; 324g] during the 2nd week and 260g [160; 382g] during the 3rd week of life, with significantly greater weight gain compared with S only during the 1st week (S=116g [66; 172g]; $p = 0.001$). Whatever the period concerned, supplemented L gained more weight during the entire neonatal period than the controls (841g [485; 1087g] vs. 623g [436; 858g]; $p = 0.048$). No difference was evidenced between supplemented and control S ($p = 1$). In our study large breed puppies supplemented at birth with the hyper-immune egg yolk had greater weight gain during the entire neonatal period. In one study, large breed puppies presenting digestive disorders gained less weight than healthy ones⁵. Thus it could be hypothesized that better growth in supplemented puppies reflects a better health. Nevertheless, further studies are needed in order to confirm our findings in other breeding kennels.

¹ Chastant-Maillard S et al. Timing of the intestinal barrier closure in puppies. Reprod Dom Anim. 2012;47:190-193; ²Poffenbarger EM et al. Use of adult dog serum as a substitute for colostrum in the neonatal dog. Am J Vet Res. 1991; 52(8):1221-4. ³Canine health product containing antibodies against canine parvovirus type 2" WO2015004181 A1.; ⁴Nguyen S et al. Passive protection of dogs against clinical disease due to canine parvovirus-2 by specific antibody from chicken egg yolk. Can J Vet Res 2006;70:62-64. ⁵Grellet A et al. Validation of a fecal scoring scale in puppies during the weaning period. Prev. Vet. Med. 2012;106:315-323.

ABSTRACT BOOK

ISCFR 2016

8th International Symposium
on Canine and Feline Reproduction with
XIX EVSSAR Congress
Paris, France



22-25th June 2016

Maisons-Alfort, France

