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Immunoglobulin G concentration in canine colostrum: Evaluation and variability

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ABSTRACT

Canine neonates are born hypogammaglobulinemic, and colostrum is their main source of immunoglobulins. The purpose of this study was to evaluate the immune quality of canine colostrum and its variability both among bitches and among mammary glands. The immune quality was estimated from immunoglobulin G (IgG) concentration (ELISA test). The correlation of IgG concentration with refractometry was evaluated. From a total of 44 bitches from 13 different breeds from a single breeding kennel, samples of colostrum and blood were collected one day after the parturition onset. Colostrum was collected separately from each pair of mammary glands (180 pairs). The mean colostrum IgG concentration in our population was 20.8 ± 8.1 g/L (ranging from 8.0 to 41.7 g/L) with no influence of breed size, litter size, age of dam or serum IgG concentration. Colostrum IgG concentration varied widely among pairs of mammary glands within one bitch (variation coefficient: 42 ± 32.1%). Nevertheless, no single pair of mammary glands was found to produce regularly a secretion of higher quality. No difference in IgG concentration was recorded between anterior and posterior pairs either. The BRIX index and the refractive index were significantly, but moderately correlated with colostrum IgG concentration ($r = 0.53$ and 0.42, respectively). This study demonstrates a great variability in immune quality of colostrum among bitches and among mammary glands within one bitch. Further studies on the suckling behavior of puppies and on determination of the minimal immune quality of colostrum are required to evaluate their impact of this high variability on neonatal mortality in dogs.

1. Introduction

The immune status of the newborn puppy depends entirely on colostrum ingestion, since canine neonates are nearly agammaglobulinemic at birth (Bouchard et al., 1992). From all circulating immunoglobulins after closure of the intestinal barrier, 90–95% originate from the colostrum (Chastant-Maillard et al., 2012). Inadequate colostrum intake leads to a deficit in the transfer of passive immunity, associated with higher mortality and morbidity rates in calves, lambs and piglets (Christley et al., 2003; Devillers et al., 2011; Virtala et al., 1999), but also in puppies (Mila et al., 2014). In large animals, apart from quantity and age at ingestion, the concentration of immunoglobulins in the colostrum is one of the limiting factors of adequate passive immune transfer to the newborn (Weaver et al., 2000). In ruminants and foals, the immune quality of colostrum is easily evaluated by refractometry before first suckling (Morrill et al., 2012; Waelchli et al., 1990). This dam-side test indicates refractive or BRIX index values, well correlated with the immunoglobulin G (IgG) concentration (Bielmann et al., 2010; Morrill et al., 2012). Colostral total proteins, of which immunoglobulins account for a large portion, refract light. This property has been used in refractometry in order to estimate the level of proteins, and thus indirectly IgG. To date, only laboratory procedures (ELISA test) allow to determine the IgG concentration in dog colostrum; however, these are time-consuming, expensive and not adapted for in-kennel application.

The amount of IgG in colostrum varies widely between females, ranging from 11.7 to 101.4 g/L in sows, and from 25.7 to 168.7 g/L in cows (Inoue et al., 1980; Quigley et al., 1995). Within one given dam, IgG may vary also between mammary glands, as described

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in pigs and cows (Farmer and Quesnel, 2009; Guatteo et al., 2013). Numerous factors, such as parity, nutrition and genetic selection are known to impact colostrum immunoglobulin concentration (Godden, 2008; Inoue et al., 1980; Quesnel, 2011). The immune quality of the colostrum in the canine species has been poorly explored. IgG concentration values were reported only in a few studies conducted on a few animals from one breed (Heddle and Rowley, 1975; Schäfer-Somi et al., 2005). Variation factors have neither been evaluated.

This study was designed to analyze the variability in IgG concentration in colostrum among bitches and among teats, and to identify some factors influencing the quality of colostrum. The value of refractometry for evaluating IgG concentration in colostrum was also investigated.

2. Materials and methods

The study protocol was reviewed and approved by the Royal Canin Internal Ethics Committee (AF/20140704).

2.1. Animals and data collection

Forty-four bitches from one breeding kennel were included in the study. Starting 2 weeks before parturition, each female was single housed and fed a dry balanced diet for growing dogs (Starter, Royal Canin, Aimargues, France) *ad libitum*. The bitches belonged to 13 different breeds: Bichon Frise ($n = 4$), Bichon Maltese ($n = 4$), Cocker Spaniel ($n = 4$), German Shepherd ($n = 1$), Golden Retriever ($n = 8$), Jack Russell Terrier ($n = 1$), Labrador Retriever ($n = 4$), Lhasa Apso ($n = 6$), Pomeranian ($n = 1$), Poodle ($n = 4$), Shih Tzu ($n = 3$), West Highland White Terrier ($n = 3$), Yorkshire Terrier ($n = 1$). The age of each bitch and the total number of puppies born (litter size) were recorded. Samples of blood and colostrum were collected once from each bitch after expulsion of the last puppy (between 8 and 24 h since the onset of parturition). Mammary glands were washed with antimicrobial soap containing chlorhexidine and dried prior to the collection. Each pair of mammary gland was collected separately after intramuscular administration of oxytocin (1–2 UI; Ocytovem®, CEVA, Libourne, France). About 1 ml colostrum samples were obtained by a gentle massage of the mammary gland and subsequently manual milking. Puppies had no access to their dams only during the duration of samples collection (15–20 min), and no anesthetics were administrated neither to bitches nor to puppies. Blood, collected from the jugular vein into a plain tube, was centrifuged (15 min, $1500 \times g$). Serum and colostrum samples were stored at −20 °C until analysis.

2.2. Immunoglobulin G assay

IgG concentration in serum and colostrum were evaluated by a commercial ELISA test following the manufacturer's instructions (Dog IgG-Quantitation Kit, Bethyl Lab, Montgomery, USA; Mila et al., 2014). Colostrum samples were first thawed at room temperature and centrifuged (30 min, $2000 \times g$, 4 °C). Fat free whey was diluted 1:100,000; 1:400,000 and 1:600,000. Serum was thawed and diluted 1:50,000 and 1:100,000. Repeatability of the colostrum assay within one plate (intra-assay coefficient of variation) was 4.7% and 2.8% for the serum assay. Repeatability of the colostrum assay between plates (inter-assay coefficient of variation) was 5.4%. All serum samples were analyzed within a single plate.

2.3. Refractometry

The BRIX and refractive indexes were measured in thawed colostrum at room temperature (21 °C), on non-diluted samples (EkoTonick, Roubaix, France; BRIX scale: from 0 to 40%) and samples diluted 1:2 in distilled water (Rogosampaic, Wissous, France, refractive scale: from 1.333 to 1.360). The refractive index, as defined by Morrill et al. (2012), is an index of refraction of a solution measured at the wavelength of the sodium D line (589.3 nm) at 20 °C. BRIX refractometer is a modified method of refractive index evaluation. As not only proteins, but all total solids may reflect light, BRIX scale was developed to measure sugar content in no or low protein food products (jus, honey, *etc.*). In this study, BRIX refractometer was used due to larger measurement range (if converted to refractive index), and thus probability of higher precision of the measurement. The units of BRIX refractometer (%) remain not converted to refractive index in order to differentiate the two different devices used. All samples were analyzed within one session.

2.4. Statistical analyses

Statistical analyses were performed using the SAS software (version 9.3; SAS Institute Inc., Cary, NC, USA). The age of bitch was encoded as young (<3 years), middle-aged (3–6 years) and old (>6 years). The breed size was encoded as small (bitches <25 kg of body weight) or large (≥25 kg of body weight). The litter size was encoded separately for each breed size (Borge et al., 2011) as small (<4 puppies for small breed dogs; <5 puppies for large breed dogs), medium (4–5 puppies for small breeds; 5–6 puppies for large breeds) or large (>5 puppies for small breeds, >6 puppies for large breeds). The normality on colostrum IgG concentration per teat and mean colostrum IgG concentration per bitch (mean of IgG concentrations from all pairs of mammary glands within one bitch) were tested with Shapiro–Wilk test. The percentage of coefficient of variation (CV) was calculated to express the variation of the IgG concentrations among different mammary glands within one bitch. Either the average IgG concentration per pair of mammary glands or IgG concentration per bitch were used in multivariable statistical analyses and variance analyses. The effect of teat pair number, encoded respectively as M1, M2, M3, M4, M5 (with the most anterior pair as M1) on the colostrum IgG concentrations was evaluated using a linear mixed model (PROC MIXED), with a fixed effect of breed size and individual number of the bitch as a random term. Mammary glands were then classified according to the anatomical localization as anterior (three cranial pairs: M1, M2, M3) or posterior (two caudal pairs: M4, M5). The relationship between teat position (anterior or posterior) and the colostrum IgG concentration was evaluated using a linear mixed model (PROC MIXED), with a fixed effect of the breed size and individual number of the bitch as a random term. The relationship between dam serum IgG concentration, age of the bitch, breed size, litter size and mean colostrum IgG concentration were evaluated using generalized linear model (PROC GLM). Since residuals of all multivariable models were not normally distributed, non-parametric analyses were performed (rank transformation of the outcomes). The correlations between IgG concentration, BRIX index and refractive index in colostrum were evaluated by Spearman's rho correlation coefficient. The results are presented as means ± SD.

3. Results

3.1. Population

The average age of the 44 bitches included in the study was 5.1 ± 1.6 years, ranging between 2 and 8 years (4.5% young; 68.2% middle-aged; 20.5% old; 6.8% unknown); with 70.5% (31/44) of them belonging to small breed dogs. The average litter size was 5.0 ± 2.4 puppies (from 1 to 10). Twenty-five percents (11/44) of