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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.

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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

Fig. 1. Intra-individual coefficients of variation of colostrum IgG concentrations from different pairs of mammary glands ($n = 44$). Each bitch was identified by one unique number and each bar displays the coefficient of variation for colostrum samples from one given bitch.

dams delivered small sized litters, 34% (15/44) medium sized litters and 41% (18/44) large sized litters.

3.2. Variation in IgG concentration

In 180 pairs of mammary glands (from 3 to 5 samples per bitch) colostrum IgG concentration ranged between 0.8 and 61.4 g/L. The mean coefficient of variation between IgG concentrations from different mammary glands in a given dam was 42.0 ± 32.1% (Fig. 1).

The mean IgG concentration considered per pair of mammary gland ranged from 17.9 g/L in M1 to 21.7 g/L in M2 (Fig. 2). The IgG concentration in colostrum was not significantly different between M1, M2, M3, M4 and M5 whatever the breed size of the bitch ($p = 0.752$) but it was influenced by the dam as a random term ($p = 0.001$).

The mean IgG concentration in colostrum from anterior mammary glands ($n = 99$) was not significantly different from that from posterior mammary glands ($n = 81$), whatever the breed size of the dog (anterior 20.7 ± 12.3 g/L *vs.* posterior 20.3 ± 10.1 g/L; $p = 0.399$).

The mean IgG concentration in colostrum (value per bitch) was 20.8 ± 8.1 g/L, ranging between 8.0 and 41.7 g/L (Fig. 3). The IgG concentration in serum was 8.1 ± 4.3 g/L, ranging between 4.3 and 30.9 g/L (Fig. 3). Colostrum appeared 2.8-fold more concentrated in IgG than serum, and this ratio ranged among bitches from 0.89 to 6.3-fold. The mean IgG concentration in colostrum was not significantly associated with breed size ($p = 0.858$), litter size ($p = 0.777$), age of the bitch ($p = 0.797$) or serum IgG concentration ($p = 0.937$).

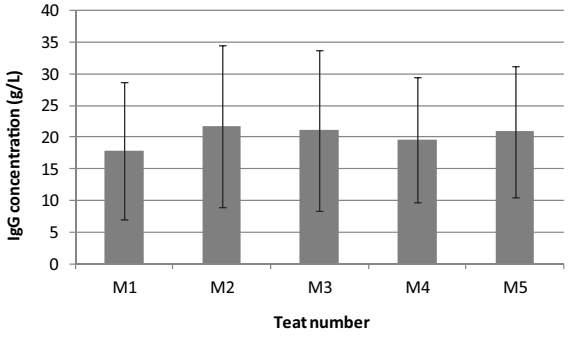

Fig. 2. Variability of colostrum IgG concentration depending on teat number (180 samples: M1 $n = 20$; M2 $n = 41$; M3 $n = 38$; M4 $n = 41$; M5 $n = 40$). Results are presented as mean ± SD.

3.3. Refractometry

The average BRIX index value was 18.2 ± 3.9% (145 colostrum samples) and refractive index value was 1.343 ± 0.003 (131 samples), with a correlation coefficient between the two indexes amounting to 0.77 ($p < 0.001$). IgG concentration was significantly, but moderately correlated with BRIX index values ($r = 0.53$; $p < 0.001$) and refractive index values ($r = 0.42$, $p < 0.001$) (Fig. 4).

4. Discussion

Colostrum plays a crucial role in the survival of canine neonates. It is indeed the only source of immunoglobulins for hypogammaglobulinemic newborn puppies (Chastant-Maillard et al., 2012). Immunoglobulin G, in this mammary secretion in the first two days *post partum*, originates almost entirely from the female bloodstream (Bourne and Curtis, 1973; Godden, 2008). During gestation, specific receptors (FcRn) develop on the alveolar epithelial cells and trap circulating IgG. These immunoglobulins are then transported into mammary secretion, making colostrum markedly more concentrated than serum. In this study, the IgG concentration was on average 2.8-fold higher in colostrum than in the serum. This ratio differs greatly from that recorded in other species (cat: 4.1-fold (Claus et al., 2006); pig: 5.4-fold (Foisnet et al., 2010); cow: 28.7-fold (Morin et al., 1997)). Similarly like in sows (Foisnet et al., 2010), but not in queens (Claus et al., 2006), no link between colostrum and serum IgG concentrations was found in the bitch in our study.

The concentration of maternally derived IgG absorbed after birth varies considerably among puppies and among litters, with 18% of neonates suffering from poor passive immune transfer (Mila et al., 2014). One of the reasons for this deficit in IgG might be the ingestion of low immune quality colostrum. In this study, the average IgG concentration in colostrum, partially reflecting its immune quality, was 20.8 g/L, with a huge variation among bitches (range from 8.0 until 41.7 g/L). Large difference in colostrum IgG concentration, recorded also in sows and cows (Inoue et al., 1980; Quesnel, 2011; Quigley et al., 1995), may put some litters or some puppies within a litter at a risk of death. None of the bitch characteristics, such as breed, age of the dam or litter size could account for this variability in our study population, although a breed effect was described in other species. Large White sows present higher colostrum IgG levels than Landrace × Large White crossbred sows (Quesnel, 2011) and beef cows present higher IgG colostrum levels than dairy cows (Guy et al., 1994).

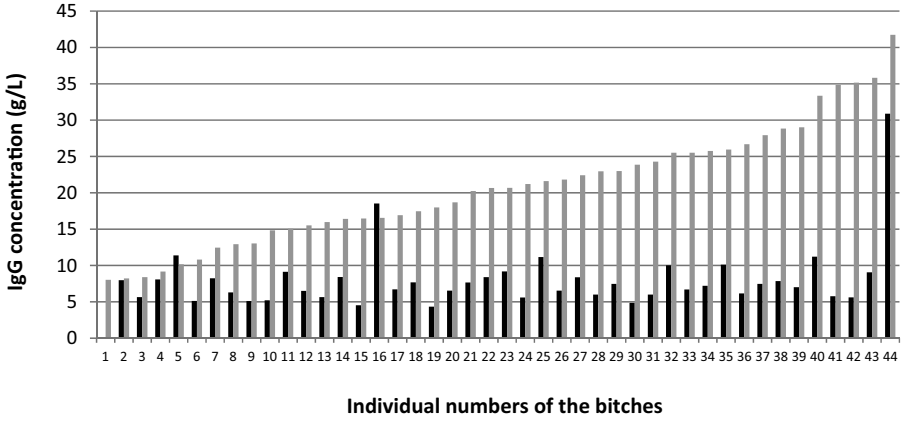

Fig. 3. Mean IgG concentrations in colostrum (gray bars, $n = 44$ bitches) and in serum (black bars, $n = 43$ bitches).

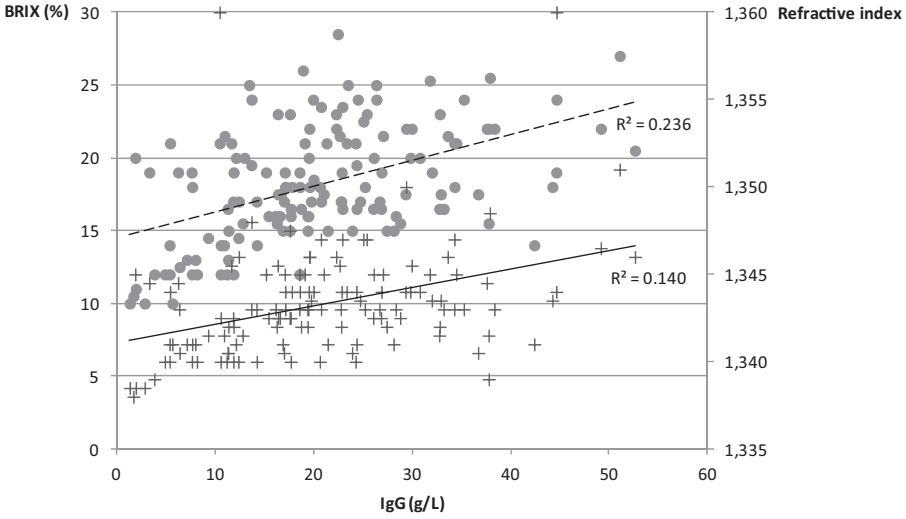

Fig. 4. Correlation between IgG concentration and BRIX index (dots and interrupted line, $n = 145$; $r = 0.53$; $p < 0.001$) and IgG concentration and refractive index (crosses and continuous line, $n = 131$; $r = 0.42$; $p < 0.001$) in colostrum samples.

We demonstrated not only an important variability in colostrum IgG concentration between bitches, but also between different mammary glands within one bitch (mean CV of 42%). A few phenomena could explain such differences. As described above, IgG is not produced in the mammary gland tissue, but only stored until the end of gestation. At the beginning of lactation, milk yield is low and it increases greatly few hours after parturition. Hence, colostrum initially rich in immunoglobulins is gradually diluted in increasing mammary secretion (Levieux and Ollier, 1999; Morin et al., 2010). Drop of IgG concentration occurs shortly after the lactation onset and a decrease of 72% in goats and 32% in pigs is observed as early as 6 h later (Klobasa et al., 1987; Moreno-Indias et al., 2012). At the same time, duration of parturition may vary greatly between bitches depending on litter size or individual characteristics of the bitch. Therefore, puppies born from bitches with long delivery may experience colostrum of reduced immunoglobulin concentration. In polytocous dogs, a birth order is formulated on course of parturition and puppies born as first most probably start to suckle also as first. Thus some teats may be already drained (and of reduced IgG content), while others remain not sucked when new puppies arrive. Birth order and parturition length, associated with the quality of ingested colostrum, could be the reason for great intra and inter-individual variability in IgG concentration determined in our study.

Nevertheless, no particular pair of mammary glands appeared to be better in terms of higher IgG concentration. Posterior pair of mammary glands did not secrete colostrum of better immune quality than anterior ones, or *vice versa*. The concentration of IgG in colostrum related to mammary gland position has been studied intensively in sows, with controversial findings. From one study to another, either anterior, posterior or even middle teats have been found producing secretion of higher immunoglobulin content (Foisnet et al., 2010). Slightly higher IgG concentrations were found in colostrum from posterior quarters in dairy cows (4.7% higher than from anterior quarters) (Guatteo et al., 2013). In the absence of studies describing the early suckling behavior of puppies and measuring the immune quality of colostrum, the impact of such wide variations on passive immune transfer to the newborns remain to be established in dogs. In piglets and kittens, a teat order is early formulated, determining rather a constant position of each neonate at an occupied teat (Hudson et al., 2009; Skok and Škorjanc, 2013). To date, it is unknown whether each puppy suckles constantly a particular mammary gland or several of them between birth and closure of the intestinal barrier.

As the level of immunoglobulins in colostrum is highly variable, both among dams and among mammary glands, an easy-to-use test would be desirable for dog breeders to evaluate the immune quality of colostrum before the intestinal barrier closure. Refractometry

is routinely used in bovine and equine neonatology to evaluate the immune quality of colostrum before pooling, freezing (banking) and administration to the newborns (Bielmann et al., 2010; Cash, 1999). The present work evidenced a statistical correlation between colostrum IgG concentration and two refractometry indexes also in the canine species; although, the correlation strength was moderate. Our results remain to be confirmed on fresh colostrum samples, as in cows, freezing–thawing cycles were demonstrated to influence the relationship between IgG concentration and refractive index (Morrill et al., 2012). Rather than evaluating IgG concentration *per se*, refractometry would be expected to distinguish high from low quality colostrum. A conclusion regarding the benefit of this technique would thus require defining the minimal IgG colostrum concentration ensuring protective passive immune transfer. To date, in contrast with bovine and equine species (Bielmann et al., 2010; Pritchett et al., 1994), the cut-off value defining low and high immune quality colostrum remains to be determined in dogs.

5. Conclusions

Large variability in immunoglobulin content of colostrum among bitches and among teats of one bitch evidenced in this study could be a reason for inadequate passive immune transfer in some puppies and thus a higher risk of neonatal mortality. Defining the threshold for good colostrum immune quality would be a prerequisite for efficient colostrum banking, as performed in farm animals. Development of colostrum replacer or immunoglobulin supplement, designed for puppies, could be used to compensate for insufficient immune quality of colostrum and thus to decrease the risk for passive immune deficit in canine neonates.

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