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Timing of the Intestinal Barrier Closure in Puppies

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Contents
As puppies are born with very low immunoglobulin concentrations, they rely on passive immune transfer from ingested colostrum to acquire a protective immunity during the first few weeks of life. The purpose of this study was to describe the timing of gut closure in canine neonates. Twenty-two Beagle puppies received 3 ml of standardized canine colostrum at 0, 4, 8, 12, 16 or 24 h after birth using a feeding tube. Blood immunoglobulins G (IgG, M and A) were assayed 0, 4 and 48 h after colostrum ingestion. IgG absorption rate was significantly affected by the time of colostrum administration, and the IgG concentrations in puppies serum 48 h after administration were significantly higher when colostrum was ingested at 0–4 h of age than at 8–12 h or 16–24 h (1.68 ± 0.4, 0.79 ± 0.07 and 0.35 ± 0.08 g/l, respectively; $p < 0.001$). In the canine species, gut closure seems thus to begin as early as 4–8 h after birth and to be complete at 16–24 h. Consequently, this phenomenon appears to occur earlier in puppies than in most other species.

Introduction

In puppies, mortality rates from birth to weaning range from 20% to 40%, with more than half of the cases occurring during the first 3 weeks after birth. Infection, especially by *E. coli*, *Bordetella bronchiseptica* and *Streptococcus sp*, is identified as the cause of at least 30% of the deaths (Nielen et al. 1998; Schäfer-Somi et al. 2005).

As puppies are nearly agammaglobulinaemic at birth (Bouchard et al. 1992), absorption of colostral antibodies is crucial for neonatal and paediatric immunity. Puppies rely on colostrum as the main source of circulating antibodies during the first 3–6 weeks of their life. In bovine, equine and porcine species, the passive immune transfer to the newborn through colostrum is one of the key elements to control morbidity and mortality rates until weaning (Levieux 1984; Besser and Gay 1994). Optimization of the passive transfer of ingested immunoglobulins requires that ingestion of colostrum occurs within the first hours after birth (24 h for calves, for example): the ability of gut to absorb ingested immunoglobulins decreases with time elapsed from birth (Stott et al. 1979a). This phenomenon, known as intestinal barrier closure (Lecce and Morgan 1962), is not conclusively timed yet in the canine species, despite its interest for puppies' management. The aim of this study was thus to describe the kinetics of the intestinal barrier closure in puppies.

Materials and Methods

Bitches

All bitches used in this study were housed in an experimental kennel. They were routinely vaccinated against distemper, adenovirus, parvovirus and parainfluenza and specifically vaccinated against canine herpesvirus 1 within 10 days after insemination and at 30 days after insemination (EURICAN HERPES; Merial, Lyon, France).

Colostrum

Mammary secretions from five Beagle bitches were manually collected 1 or 2 days after whelping. They were pooled, aliquoted (3 ml samples) and frozen (−20°C) until distribution.

Puppies

Four Beagle bitches (aged 20, 22 months, 4 and 5 years, respectively, and different from those on which colostrum was collected) were inseminated with the sperm collected from two Beagle males of proven fertility. Ovulation time was determined through regular blood progesterone assays and transabdominal ultrasound examinations. Insemination was performed with fresh sperm 48 and 72 h after ovulation. Sixty or 61 days after ovulation, 22 puppies were delivered by elective caesarean section with no sign of anoxia. Puppies were weighted at birth. Every 4 h, they were fed with artificial milk using a baby bottle (Mixol, Laboratoire Moureau, Luzarches, France) previously assayed for canine immunoglobulins (containing no detectable canine IgG, IgM or IgA following the method below), except for one meal when they were given 3 ml frozen/thawed colostrum via an orogastric tube. Depending on the experimental group, colostrum was given at birth (H0 group; n = 4), four (H4; n = 3), eight (H8; n = 3), 12 (H12; n = 4), 16 (H16; n = 3) or 24 (H24; n = 5) hours after birth. Blood (1 ml) was collected from the jugular vein into plain tubes immediately before colostrum administration and then at 4 and 48 h after it. After the second blood sampling, puppies were allowed to suck from their dam.

Immunoglobulins (Ig) assay

Canine IgG, IgM and IgA were assayed in duplicate on sera, on artificial milk and on colostrum (Dog IgG-, IgM-, IgA-Quantitation Kits; Bethyl Lab, Montgomery, AL, USA). IgG absorption rates were calculated for each group as the ratio between the amount of IgG contained in 3 ml colostrum (IgG concentration in colostrum × 3/1000 g) and the amount of IgG in the puppies bloodstream 48 h after colostrum administration (blood volume × (1−%PCV) × serum IgG concentration).

Statistical analysis

Data were pooled (H0 + H4: group H0–4 n = 7; H8 + H12: group H8–12 n = 7; H16 + H24: group H16–24 n = 8) and analysed through the non-parametric Kruskal–Wallis and Mann–Whitney tests. Results are expressed as mean ± SEM, and differences were considered significant when $p < 0.05$.

Results

Weights at birth were not significantly different between groups (mean ± SEM: 272 ± 8.7 g, n = 22). Before colostral administration, circulating immunoglobulin concentrations were low (0.3 ± 0.01 g/l IgG, 0.1 ± 0.01 g/l IgM, non-detectable IgA) and not different between groups. The colostrum fed contained 17.8 g/l IgG, 1.1 g/l IgM and 20.6 g/l IgA.

Four hours after administration, a significant increase in blood IgG concentration was observed for group H0–4 and H8–12, but not for H16–24 (Fig. 1). IgG concentrations at 4 h after administration were affected by the age at colostrum ingestion ($p < 0.001$). IgG concentrations were significantly higher in group H0–4 than in group H8–12 (1.68 ± 0.4 and 0.79 ± 0.07 g/l, respectively; $p = 0.007$), and higher in group H8–12 than in group H16–24 (0.35 ± 0.08 g/l; $p = 0.006$). Within groups, IgG concentrations were not significantly different between 4 and 48 h after administration, and the same effect of the age at colostrum administration was noticed ($p < 0.001$; Fig. 2). Similarly, the IgG absorption rate steadily decreased with age at colostral administration ($p < 0.001$), being higher in group H0–4 than in group H8–12 (29.6% ± 8.2% vs 10.7% ± 1.2%; $p = 0.01$) and higher in group H8–12 than in group H16–24 (2.1% ± 1.1%; $p = 0.001$; Table 1).

The same differences were observed between groups for IgA, both at 4 and 48 h after colostrum administration. At 4 h after administration, IgA concentrations were 0.7 ± 0.3 g/l for group H0–4, 0.4 ± 0.2 g/l for group H8–12 and 0.1 ± 0.0 g/l for group H16–24 ($p < 0.05$). Serum IgA concentrations decreased between 4 and 48 h after colostrum administration.

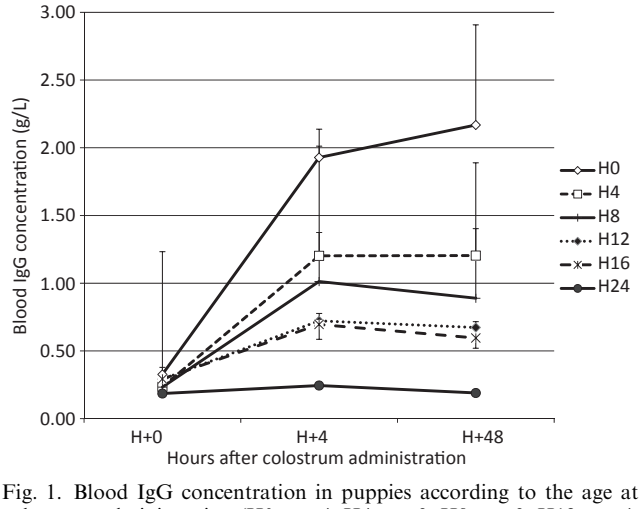

Fig. 1. Blood IgG concentration in puppies according to the age at colostrum administration (H0 n = 4; H4 n = 3; H8 n = 3; H12 n = 4; H16 n = 3; H24 n = 5). IgG were assayed 0, 4 and 48 h after administration

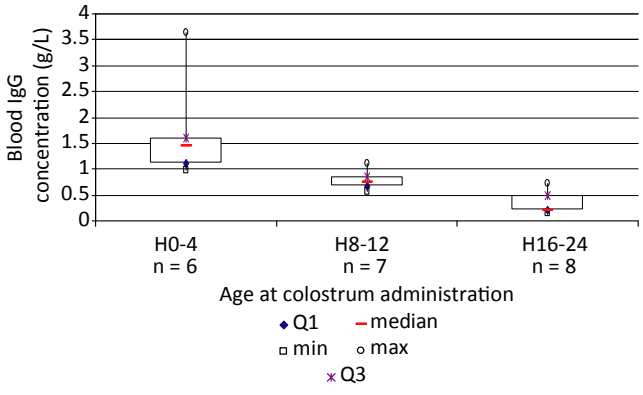

Fig. 2. IgG concentration at 48 h after administration according to the age at colostrum ingestion. Box plot analysis. Q1: upper quartile; Q3: lower quartile; min: minimum; max: maximum

Conversely, IgM concentrations increased over the same period. The time elapsed from birth and colostrum ingestion influenced IgM only at 4 h post-ingestion ($p = 0.01$) and not at 48 h.

Over the 22 puppies studied, 19 reached the age of 2 months without any morbidity.

Discussion

Because of the endothelial structure of the canine placenta, circulating Ig concentrations are quite low in the neonates at birth. In this study, serum IgG concentration before colostral ingestion was approximately 0.3 g/l, that is, only 1.5% of the IgG concentration 48 h after ingestion in the optimal conditions (H0–4). In the literature, the placental transfer was found to account for 1–7% of the total Ig concentration in the canine neonate, immunoglobulins being acquired by colostrum ingestion (Poffenbarger et al. 1991; Bouchard et al. 1992). Transfer of colostral Ig from the colostrum to the blood is the result of a transient, non-selective macromolecular transport across the small intestinal absorptive epithelium, involving uptake by apical tubules and micropinocytotic vesicles and secretion at the basement membrane. The absorbed Ig molecules enter the bloodstream with the intestinal lymph via the thoracic duct. Absorption rates vary among species and are 5–25% in piglets and 8–90% in calves, depending on the calculation method (Levieux 1984). In the present study, the maximal absorption rate at birth was approximately 40%. Our calculation method may have resulted in a lower absorption rate than actual as we presumed that the circulating volume was not modified by colostrum/milk administration, and we neglected any eventual extravascular transfer of Ig.

The intestinal epithelium of the newborn retains the ability to absorb macromolecules for only a few hours. This ‘gut closure’ phenomenon, defined as ‘the cessation of absorption of macromolecules from gut to blood in neonates’ (Lecce and Morgan 1962), seems to occur earlier in puppies than in calves or in piglets. In piglets, it is described at 24–36 h of age (Lecce and Morgan 1962). In calves, the reduction in the absorption to half its efficacy at birth is observed to be between 8 and 20 h, generally approximately 12 h (Stott et al. 1979a; Levi-

Table 1. Efficacy of IgG absorption according to the time elapsed from birth. Results are expressed as mean ± SEM. Percentage of IgG absorbed is calculated at 48 h after colostrum administration

Age at colostral administration (hours after birth)	0	4	8	12	16	24
Number of puppies	3	3	3	4	3	5
IgG concentration in serum at 48 h post-colostrum administration (g/l)	2.2 ± 0.7	1.2 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.2 ± 0.0
Percentage of IgG absorption (%)	39.0 ± 14.8	20.2 ± 5.3	14.1 ± 0.8	8.7 ± 0.3	4.9 ± 1.3	0.0 ± 0.0

eux 1984); in comparison, in our study, the same reduction by 50% was obtained already at 4 h after birth in puppies (Table 1). In cats, seems to be approximately 16 h after birth, that is, also earlier than in most other domestic species (Casal et al. 1996).

In calves, mean closure time was approximately 25–26 h and similar for IgG, IgM and IgA (Stott et al. 1979a). In our experiment, as IgM concentrations continued to increase between 4 and 48 h after birth, it was not possible to conclude about the time of closure for this Ig class, but it seems to be later than 24 h after birth. This finding is similar to what was observed in kittens, in which serum IgM concentration steadily increased to plateau only at approximately day 60 of life (Casal et al. 1996). Closure for IgA occurs approximately 16–24 h after birth, as administration at that time was followed by no increase in serum IgA concentration. In puppies as in kittens (Casal et al. 1996), IgA levels peak at colostrum ingestion and gradually decline. IgA have been shown to transudate reversely from blood through the epithelium of the respiratory tract (Salmon et al. 2009).

Gut closure seems to occur earlier in puppies than in other species, except for kittens. Because of ethical considerations, puppies were not starved until colostrum administration but fed with milk devoid of canine Ig. Nevertheless, it cannot be ruled out that feeding may have hastened gut closure by a few hours, as demonstrated in piglets, lambs and calves (Stott et al. 1979a). In calves, feeding at birth shortens the Ig absorption period by 12 h compared with calves fed for the first time at 24 h (21–24 h vs 31–33 h) (Stott et al. 1979a). Therefore, our experimental design does not exactly fit the situation encountered by puppies starved from colostrum because of their mother's death, in which the gut closure may be delayed by complete starvation. The effect of mammary suckling versus feeding with baby bottle or feeding tube on absorption has also to be examined, as suckled calves have higher absorption rates (Stott et al. 1979b). Presence of dam or occurrence of stressors may also influence the timing of gut closure (Selman et al. 1971). The exact mechanism of gut closure has yet to be elucidated, but it probably reflects a combination of exhaustion of pinocytotic capability and enterocyte replacement by a mature population of epithelial cells, together with development of intestinal enzymes, increased stomach acidity and installation of digestive flora. What determines the shift from cells capable of pinocytosis to cells with microvilli and enzymes is also unknown; this may relate to a role of some hormones such as insulin, corticosteroids and thyroxine or contact of cells with glucose at the time of colostral ingestion (Levieux 1984).

Conclusion

IgG was the predominant isotype during the first days of life of puppies as reported by Bouchard et al. (1992) and Poffenbarger et al. (1991). IgG are key elements of the immune protection during the early weeks of life. Crucial for systemic protection, they also transudate reversely into the intestinal lumen and probably decrease intestinal virus replication (Salmon et al. 2009). This study demonstrates that the canine intestinal barrier remains permeable to immunoglobulins mainly during the first 12 hours after birth, but with a sharp decrease in absorption as early as after 4 h. Therefore, attention to maternal suckling has to be given very early after birth for the optimization of the passive immune transfer in puppies. Nevertheless, the minimal quantity and quality for colostrum required to limit morbidity and mortality remain to be determined in puppies.

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Conflicts of interests

All the authors disclose any financial or personal relationships with people or organisations that could have inappropriately biased or influenced this work.

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