

The first two days of life of puppies: crucial steps for survival

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Introduction

In the canine species, the mortality rate before weaning (between birth and 8 weeks of life) is about 20% (Table 1) (1,2,3,4). Especially the first 48 hours of life seem to be crucial for newborn puppies (5). Several factors during the first days of life, both maternal and neonatal, are known to influence the chances for a puppy to survive, such as status at birth and colostrum intake.

Reference	Number of puppies in the study	Mortality % (ni/n)				
		Stillbirth	1 st week	2-3 rd week	4-8 th week	Total
Potkay and Bacher, 1977, USA	2872	12.0 (63/524)	30.3 (159/524)	32.2 (169/524)	25.4 (133/524)	18.2 (524/2872)
Nielen et al., 1998, Netherlands	2527	31.3 (147/469)	42.4 (199/469)	14.9 (70/469)	11.3 (53/469)	18.6 (469/2527)
Gill, 2001, Australia	2574	34.7 (180/519)	57.0 (296/519)	8.3 (43/519)	*	20.2 (519/2574)
Tønnessen et al., 2012, Norway	58439	53.9 (2524/58439)	46.1 (2160/58439)	*	*	8.0 (4684/58439)
Chastant-Maillard and Mila (unpublished data), France	2288	43.1 (226/524)	27.5 (144/524)	12.2 (64/524)	17.2 (90/524)	22.9 (524/2288)

* Data not available

Table 1. Mortality in puppies at different ages.

Status at birth

Puppy health status evaluated at birth, reflecting the course of gestation and parturition, already allows to distinguish puppies at higher risk of death. Recently, an Apgar scoring system was adapted to canine neonate health evaluation (by assessment of mucous membrane colour, heart and respiratory rates, motility and irritability reflex). Apgar score below or equal 6 (scale from 0 to 10) indicated puppies at lower chances to survive the first 24 hours after birth: mortality in puppies with Apgar score ≤ 6 was 21% (6/28), while in puppies with higher score it was 1.2% (4/319) (authors' unpublished data). Low heart (<180 bpm) and respiratory rates (<6 breaths per minute), pale or cyanotic mucosa colour evidence the weakness and hypoxia of the newborn, making it unable to ingest colostrum.

Also the birth weight seems to be a factor influencing neonatal survival. Among puppies that died within the first 48 hours after birth, 81% (30/37) belonged to the 25% that were lightest at birth (6). The low birth weight was ≤ 138 g for small breeds, ≤ 179 g for medium breeds, ≤ 291 g for large breeds and ≤ 348 g for giant breeds. Puppies with low birth weight are not only more sensitive to hypothermia and hypoglycaemia, but also have a limited access to teats in case of high rivalry within a litter. Indeed, our data show that puppies from large litters have lower birth weights, meaning that indirectly they also have higher risk of death.

Also, as in some other mammal newborns, the immature immune system of puppies puts them at high risk of neonatal death. A weak response from innate immunity, together with almost null specific immunity predisposes newborn puppies to various infections and sepsis, which are often

lethal. Sixty percents (43/72) of puppies that died before three weeks of age had a positive bacterial culture from spleen tissue in our study (authors' unpublished data). *Escherichia coli*, *Staphylococcus sp.*, *Streptococcus sp.* are mostly isolated from dying neonates. Among all puppies presenting with bacterial infection in one study 36% had local infection, 36.4% had a systemic infection and 27.6% suffered from septicemia (5).

Colostrum

Due to the endotheliochorial placenta, puppies are born nearly agammaglobulinemic (immunoglobulin G (IgG) at birth 0.35 - 1.3 g/L) (7,8). At two days of age only 5-10% of circulating immunoglobulins come from transplacental transfer from dam to the foetus (IgG at 2 days of age 17 - 33.7 g/L). The vast majority of immunoglobulins are absorbed by the puppy after birth from colostrum. This special secretion is accumulated in mammary gland during the last weeks of pregnancy. Rich in many nutrients, hormones and immune cells, colostrum differs from milk mostly due to the immunoglobulin content. In mammary secretions at the first day of lactation, immunoglobulins are distributed into 80% IgG, 20% IgA and almost no IgM, whereas at Day 15 these proportions are 2.5% (IgG), 80% (IgA) and 10% (IgM) (9). Moreover, the drop of IgG concentration is sudden, with IgG decreasing from 26.5 g/L to 12.1 g/L at the second day of lactation (authors' unpublished data). IgG concentration in bitch colostrum ranges from 8.0 to 41.7 g/L, which is almost a 3-fold higher concentration than in maternal serum (10). Nevertheless, the colostrum IgG concentration is highly variable between bitches and also between mammary glands of one given dam. At the first day of lactation, the variation in IgG concentration between different bitches was 39% and 42% between different teats, respectively. A variation of 28% in energy content (mean 1831 kcal \pm SD 506kcal) in colostrum from different bitches was also evidenced (11).

Passive immune transfer

The immune status of the puppy during the first weeks of life depends almost entirely on colostrum ingestion. However, the time that immunoglobulin absorption is possible is limited, as intestinal barrier closure is completed in the canine newborn at about 16-24 hours after birth (12). The absorption rate is most intensive during the first 4 hours (60% of total IgG administered), it decreases sharply until 12 hours (23%) and it is almost null between 12 and 24 hours after birth (5%), never reaching 100%. In serum from puppies at 2 days of age, IgG is the dominant immunoglobulin (17 g/L) over 7-fold higher than IgA (2.3 g/L) and 85-fold higher than IgM (0.2 g/L) (7). The high concentration of IgG, acquired from the colostrum at early age, plays a major role in newborn protection from infectious diseases and septicemia. Puppies with a low level of passive immunity, measured through serum IgG concentration at 2 days of age, were predisposed to death within 3 weeks after birth (13). The threshold for serum IgG concentration at 2 days of age, indicating a deficit of passive immune transfer, was determined as 2.3 g/L. Eighteen percent of studied population had a deficit of passive immune transfer (IgG under or at the cut-off value). A lack of colostrum production, or its low nutritional or immune quality and insufficient or late colostrum ingestion may put some puppies or some litters at higher risk of death.

Maternally derived antibodies (MDA) absorbed from colostrum are essential not only for limiting neonatal mortality (0-21 days), but also later in life during the paediatric period (21-60 days). For example, protection against canine parvovirus type 2 (CPV2), one of the most pathogenic virus of the paediatric period, is highly dependent on the MDA amount absorbed at birth. The higher the CPV2 MDA titer at 2 days of age is, the longer the immunoprotection lasts for the weaning puppy. Seroconversion and viral secretion also occur later in puppies with high MDA titers at Day 2 (14). Bovine colostrum is often advised in case of absence of bitch colostrum. Indeed, bovine colostrum provides some interspecific hormones and growth factors. However, bovine specific antibodies present in the mammary secretion are not targeted against canine pathogens and a lifespan of bovine antibody in the puppies' bloodstream is unknown. Bovine colostrum may thus be of good

nutritional interest, but is likely to have low value for passive immune transfer in puppies. Another possibility to obtain passive immune transfer is the administration of dog adult serum. An IgG increase of 0.8 g/L (1.3 g/L at birth and 2.1 g/L at Day 2), with absorption rate of 3.6%, was achieved after subcutaneous administration of dog adult serum to colostrum deprived canine neonates (7). Instead of colostrum substitute in colostrum deprived puppies, we evaluated the effect of dog adult serum as a colostrum complement, administered to puppies suckling freely from their dam. A pooled hyper-immunized adult serum (at the dose 15ml/kg body weight) was administered twice within the first 8 hours of life to 149 puppies. Neither puppy IgG concentration in serum at 2 days of age, nor the proportion of puppies in deficit of passive immune transfer differed between supplemented and non-supplemented groups (13). Finally, as in other species, such as bovine, bitch colostrum banking could be a solution. Indeed, IgG from frozen colostrum (at -20°) administered once immediately after birth at the dose of 15ml/kg body weight was absorbed from the gut, with an absorption rate of about 40% (12).

Nutrients and energy intake

Colostrum provides not only passive immunity, but also nutrients and energy essential for neonate survival. The energy requirements in canine neonates are high, due to a specific metabolism at birth. A high body surface area/mass ratio, no shivering and vasoconstrictive reflexes predispose newborn puppies to hypothermia, and a rapid decline of hepatic glycogen, together with low ability to gluconeogenesis predispose them to hypoglycaemia. A loss of up to 10% of birth weight during the first 24 hours of life is commonly admitted for puppies. However, puppies with a positive growth rate between birth and 2 days of age are at lower risk of death until 3 weeks compared with puppies presenting negative growth rate values (OR=0.5; 95% CI=0.4–0.6; n=477) (authors' unpublished data). A low glucose level at 24 hours after birth, probably witnessing a low colostrum intake, is a risk factor for neonatal mortality. 52.3% of puppies with glucose concentration at Day 1 below 50 mg/dl died before Day 21 vs. 28.3% when glucose was between 51–100 mg/dl and 8.7% when glucose was above 100 mg/dl. The mean body temperature at Day 1 and 2 was 36.5°C (n=277) in puppies still alive at Day 21, compared to 35.8°C (n=60) in dying puppies (authors' unpublished data). Weight loss, together with low glucose concentration and low body temperature in puppies dying within the first 3 weeks in our study suggest that energy intake through colostrum is essential for canine neonates survival. Early growth rate (during the first 48h) express not only the energy intake, but also passive immunity as it was found positively correlated with the IgG concentration measured at 2 days of age (13).

The colostrum intake may be perturbed in case of high competition within a litter. In average, a puppy spends about 10 minutes suckling the dam during a 16 minute long feeding session, with the middle rows of mammary glands most frequently used (15). Although these recent findings on dog suckling behaviour show no evidence of aggressive competition between littermates, puppies use 2-3 different nipples per session. Lack of information about variability in canine colostrum or milk nutritional quality between different pairs of mammary glands does not allow to interpret if any preferences for the highest quality milk exist in puppies.

Conclusions

Colostrum, ingested by the newborn puppies during the first 2 days of life, provides them with energy and passive immunity essential for their survival. Diagnostic tests, such as the Apgar scoring system, glucose and temperature measurements at the first days of life and early growth calculation (indicative of colostrum intake and thus indirectly of passive immune transfer), may point out puppies at higher risk of neonatal death. In such cases, feeding support should be provided as quickly as possible, by administration of milk replacer via a feeding tube, ensuring safety and dose control.

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Wrocław, Poland
26th September, 2014



Editors: Sabine Schäfer-Somi, Agnieszka Partyka, Wojciech Niżański & Ragnvi Hagman