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Short communication

Inadequate passive immune transfer in puppies: definition, risk factors and prevention in a large multi-breed kennel

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ABSTRACT

The prevalence of neonatal mortality is high in the canine species and far from well-studied. In most domestic neonates, an appropriate colostrum intake is a key element of the control of neonatal mortality. The aim of this study was to evaluate the impact of passive immune transfer on puppy mortality, assessed through serum immunoglobulin G (IgG) concentration at 2 days of age. Factors impacting passive immune transfer and the value of an oral immunoglobulin supplementation to prevent it were also analyzed. A total of 149 puppies from 34 litters (12 breeds) within one breeding kennel were included. Blood samples were collected at 2 days of age and colostrum was collected from their dams 1 day after whelping to assay IgG concentration. Puppies were weighed at birth and at 2 days of age for calculation of growth rate. Mortality was recorded until 3 weeks of age. Seventy randomly assigned puppies were orally supplemented with hyper-immunized adult plasma twice within the first 8 h of life. IgG concentration at 2 days of age was significantly correlated with weight gain during the first 2 days of life. The multivariable model with litter as a random effect demonstrated that neonatal mortality was not influenced by breed size, sex, supplementation, litter size, nor colostrum IgG concentration, but by puppy IgG concentration at 2 days of age. According to the ROC curve, the minimal IgG concentration at and below which puppies were at higher risk of death was determined at 230 mg/dl. Puppy IgG concentration was significantly associated with growth rate, but not with breed size, sex, supplementation, litter size or colostrum IgG concentration in a multivariable model with litter as a random effect. This study demonstrates that neonatal mortality in puppies is related to the quality of passive immune transfer. The oral supplementation with hyper-immunized canine plasma neither decreased risk of mortality, nor improved serum IgG concentration at 2 days of age in puppies. Attention must thus be paid to early colostrum intake to control the neonatal mortality in puppies.

1. Introduction

Neonatal mortality in the canine species (within the first 3 weeks after birth) is highly prevalent ranging between 17 and 26% (Bowden et al., 1963; Nielen et al., 1998; Indrebø et al., 2007). Infectious diseases are described as the primary cause of death in puppies born alive

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(Nielen et al., 1998; Van der Beek et al., 1999) with *Escherichia coli*, *Staphylococcus* sp., *Streptococcus* sp., canine herpesvirus type 1 most frequently isolated from neonates dying within the first week after birth (Münnich, 2008; Dahlbom et al., 2009).

Puppies, as well as calves, piglets, foals and kittens, are born agammaglobulinemic or hypo-gammaglobulinemic (Bouchard et al., 1992). In many species, it has been demonstrated that adequate transfer of maternal immunity through colostrum is crucial for survival and control of infectious diseases in the newborn (Tyler et al., 1998; Christley et al., 2003; Vallet et al., 2013). Blood immunoglobulin G (IgG) concentration in neonates is the routine criteria used to evaluate the quality of passive immune transfer (Beam et al., 2009). Transfer of immunoglobulins from dam to a newborn is influenced by numerous maternal factors (age of the dam, parity, maternal behavior, quantity and quality of colostrum), together with several neonatal factors (litter size, birth weight, vitality, time and quantity of ingested colostrum). Despite the high prevalence of lethal infections in canine species, the direct link between mortality and passive immune failure has been poorly evaluated in puppies.

The economic importance of correct passive immune transfer in large animals has led to the development of numerous colostrum replacers and immunoglobulin supplements (calves: Vega et al., 2011; piglets: Yokoyama et al., 1992; foals: Franz et al., 1998). In contrast, few studies have investigated IgG supplementation in small animal neonates. In experimental conditions, adult dog serum, administrated either orally or subcutaneously to colostrum-deprived newborn puppies, proved to be an alternative source of immunoglobulins (Bouchard et al., 1992). However, there is no data in the literature regarding the improvement of passive immune transfer in puppies under natural conditions and with unlimited access to colostrum, with the aim to control mortality.

The first objective of this study was to evaluate the impact of passive immune transfer through puppy blood IgG concentration at 2 days of age on mortality within the first 3 weeks after birth; subsequently to determine a critical threshold below which the risk of neonatal mortality is significantly increased. The second objective was to identify factors influencing IgG concentration at 2 days of age. A third objective was to estimate the efficacy of an oral immunoglobulin supplementation within the first hours after birth on passive immune transfer.

2. Materials and methods

2.1. Animals

The experiment was conducted in a French breeding kennel from March to June 2012. All canine neonates born during this period ($n = 195$) and their dams ($n = 39$) were included in the study. All bitches in the kennel were routinely vaccinated annually (EURICAN CHPPI2, Merial, Lyon, France). Puppies were housed with their dam in a single, heated whelping box (2–4 m^2 of surface) from birth until 56 days of age and were allowed to suckle freely. Both bitches and their offspring at weaning were fed a dry balanced diet for growing dogs (Starter, Royal Canin, Aimargues, France) ad libitum. From a total of 195 puppies born in the kennel, 149 puppies from 34 litters which survived with no abnormalities until the time of blood collection were included in the study. Depending on the average adult body weight of their breed, puppies were divided into small breed dogs (body weight < 25 kg: Poodle ($n = 10$), Cocker Spaniel ($n = 16$), Bichon Frise ($n = 9$), Bichon Maltese ($n = 15$), Lhasa Apso ($n = 20$), Shih Tzu ($n = 17$), West Highland White Terrier ($n = 12$), Jack Russell Terrier ($n = 3$), Pomeranian ($n = 4$)) and large breed dogs (body weight ≥ 25 kg: Labrador Retriever ($n = 10$), Golden Retriever ($n = 28$), German Shepherd Dog ($n = 5$)).

2.2. Data collection and immunoglobulin G assay

Immediately after birth, each neonate was identified by a colored woolen collar and its breed and sex were recorded. Puppies were weighed at birth and at 2 days of age to calculate a growth rate over the first 2 days of life ((weight at 2 days – weight at birth)/weight at birth). For each litter, the total number of puppies born was recorded (litter size). Mortality over the first 3 weeks after birth was also recorded. One milliliter of blood was collected into a plain tube from each puppy at 2 days of age (between 36 and 48 h) from the jugular vein. One day after whelping onset, 0.5–1 ml of colostrum was collected from the dams. Milk and blood were stored at −20 °C until IgG assay in duplicate using a previously described and validated ELISA method (Dog IgG-Quantitation Kit, Bethyl Lab, Montgomery, USA) (Schäfer-Somi et al., 2005).

2.3. Immunoglobulin supplementation

Seventeen large breed non pregnant bitches from the same kennel, which were not included in the protocol described above, were vaccinated against canine herpesvirus type 1 (EURICAN Herpes 205, Merial) and *Bordetella bronchiseptica* (PNEUMODOG, Merial). The same vaccination was repeated 2 weeks later combined with a polyvalent vaccine (EURICAN, Merial). Two weeks after the last vaccination, blood (7.5 ml/kg body weight) was collected from each bitch into heparinized containers and centrifuged. Plasma from the 17 bitches was pooled and aliquoted before storage at −20 °C. Plasma IgG concentration was assayed as previously described. Ensuring equal distribution of individuals, in terms of birth weight and breed size, puppies were assigned to 2 different groups within each litter. In the first group (non-supplemented, $n = 79$), puppies did not receive hyper-immunized plasma. In the second group (supplemented, $n = 70$), puppies received 2 doses of hyper-immunized plasma before intestinal barrier closure: the first dose was administrated a maximum of 4 h after birth by a feeding tube (1.5 ml/100 g body weight); the second administration, using the same method and dose, was performed 4 h later, at a maximum of 8 h after birth. Both supplemented and non-supplemented puppies were allowed to suckle their dam during the entire experiment. The dose of hyper-immunized plasma administrated to puppies was chosen as the best compromise between maximal volume for administrated supplement

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