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Monitoring of the newborn dog and prediction of neonatal mortality

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ABSTRACT

Despite the high neonatal mortality rate in puppies, pertinent criteria for health evaluation of the newborns are not defined. This study was thus designed to measure and to characterize factors of variation of six health parameters in dog neonates, and to evaluate their value as predictors of neonatal mortality. A total of 347 purebred puppies under identical conditions of housing and management were examined within the first 8 h after birth and then at Day 1. The first health evaluation included Apgar score, weight, blood glucose, lactate and β-hydroxybutyrate concentration, rectal temperature and urine specific gravity (SG). The second evaluation at Day 1 included the same parameters, excluding Apgar score and weight. The mortality rate over the first 24 h and over 21 days of age was recorded. The early predictors of neonatal mortality in the dog were determined with generalized linear mixed models and receiver operating characteristic curves analyses. An Apgar score at or below 6 evaluated within the first 8 h after birth was found associated with a higher risk of death during the first 24 h. A reduced glucose concentration (≤92 mg/dl) at Day 1 was found to be associated with higher mortality between 1 and 21 days of age. Low-birth-weight puppies were characterized by both low viability (low Apgar score) and low blood glucose concentration, and thus were found indirectly at higher risk of neonatal mortality. This study promotes two low cost easy-to-use tests for health evaluation in puppies, i.e. Apgar scoring and blood glucose assay. Further investigation is necessary to establish if the strong relationship between blood glucose and neonatal survival reflects high energy requirements or other benefits from colostrum intake.

1. Introduction

The mortality rate in puppies until weaning (7–8 weeks of age) is high, estimated to account for 20% of all newborns (Gill, 2001; Lawler, 2008; Mila et al., 2014; Nielen et al., 1998; Potkay and Bacher, 1977). The majority of deaths (75–90%) occur during the first 3 weeks corresponding to neonatal mortality (Gill, 2001; Indrebø et al., 2007; Mila et al., 2012; Nielen et al., 1998; Potkay and Bacher, 1977). The adaptation from the intra to the extra uterine life at birth, as well as colostrum intake during the first 24 h are critical steps for the canine newborn, as is true for many mammals (Alonso-Spilsbury et al., 2005; Hillman et al., 2012). Despite this, no validated monitoring system exists to identify puppies at elevated risk of death and so to provide them adequate aid, as practiced in other species. In human medicine, the newborn is systematically evaluated through an Apgar scoring system 1 and 5 min after birth (Casey et al., 2001), and a complete physical examination together with blood screening tests are performed within the first free days of life in order to estimate the health status (Lumsden and Holmes, 2010). In foals, there is macroscopic examination of placenta and an evaluation of foal vitality (similar to the Apgar score) at birth, followed by the evaluation of passive immune transfer during the first day of life to identify individuals at risk of asphyxia or septicemia (Sanches and Giguère, 2012; Shepherd, 2010). In the canine neonate, Apgar score (adapted for puppies) and umbilical lactate concentration at birth, reflecting the level of oxygenation of the newborn, were found to be associated with the risk of mortality during the first 24–48 h (Groppetti et al., 2010; Veronesi et al., 2009). However, the predictive value of these parameters for canine mortality during the first three weeks after birth has never been tested. Furthermore, even when hypoglycemia and hypothermia are described as major causes of canine neonatal mortality (Johnston et al., 2001; Lawler, 2008; Münnich and Küchenmeister, 2014), the published reference values for the canine newborn vary widely (88–133 mg/dl for glucose concentration) (Grundy, 2006; Ishii et al., 2013; Rosset et al., 2012). Neither glucose concentra-

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Table 1
Breeds of puppies included in the study.

Breed size[a]	Breed	Litters (n)	Puppies born (n)	Puppies examined (n)[b]	TOTAL (n)[b]
Small (<15 kg)	Bichon Frise	2	8	8	141
	Bichon Maltese	3	20	19	(40.6%)
	Jack Russell Terrier	3	9	9	
	Lhasa Apso	6	29	28	
	Poodle	4	18	15	
	Scottish Terrier	1	1	1	
	Shih Tzu	3	13	13	
	Spitz	3	11	11	
	West Highland White Terrier	5	26	22	
	Yorkshire Terrier	2	17	15	
Medium (15–25 kg)	Cocker Spaniel	14	73	72	72 (20.7%)
Large (>25 kg)	Boxer	1	8	8	134
	German Shepherd	1	5	5	(38.6%)
	Golden Retriever	10	74	74	
	Labrador Retriever	8	55	47	
TOTAL		66	367	347	

[a] According to expected adult body weight.
[b] Puppies not stillborn and not euthanized.

tion nor rectal temperature critical thresholds, defining the at-risk population, have been established for the newborn puppy.

The objective of this study was to develop new techniques of neonatal monitoring in the dog in order to identify puppies at higher risk of death and requiring specific medical assistance. Moreover, we focused on easy-to-use techniques, readily available to clinicians, providing immediate results, and minimally invasive for puppies. Six parameters were measured, evaluating general vitality (Apgar score), energetic metabolism (rectal temperature, glucose, β-hydroxybutyrate), oxygenation (lactate) and hydration status (urine specific gravity). After describing the evolution of monitored parameters during the first day of life, predictors of neonatal mortality were identified and their cut-off values determined.

2. Material and methods

2.1. Ethics statement

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

2.2. Animals

The study was conducted within one breeding kennel on all puppies born from 66 included bitches. Management conditions including diet, feeding system, housing conditions, hygiene protocols and veterinary prophylaxis were identical for all animals included. From one week before parturition until the end of lactation, bitches were housed in a single, heated box (under floor heating continuously plus a heat lamp during the first 3–5 days after whelping; temperature at puppy level between 28 and 31 °C). During this period, dams were fed a dry balanced diet for growing dogs (Starter, Royal Canin, Aimargues, France) *ad libitum*. The date and time of whelping, as well as the total number of puppies born per litter (litter size) were registered for each dam. Each puppy was identified by a colored woolen collar, and its sex and breed recorded. Puppies remained with their dams and were allowed to suckle freely until the end of the experiment. None of the whelpings were assisted, no cesarean section was performed and no puppies were hand reared during the experiment. Depending on expected adult body weight, puppies belonging to different breeds were classified into small (<15 kg), medium (15–25 kg) and large (>25 kg) breed dogs (Table 1).

2.3. Experimental protocol

Between ten minutes and 8 h after birth (defined as <8 h), a first evaluation of the neonate was performed including Apgar score, weight, blood glucose, lactate and β-hydroxybutyrate concentrations, rectal temperature and urine specific gravity (SG). A second evaluation, including the same parameters, with the exception of Apgar score and weight, was performed 24 h later (defined as Day 1). The mortality between birth and 21 days of age was recorded.

Puppies were weighed using a calibrated analytical scale in 1 g increments (Fisher Scientific International Inc., Hampton, USA). Apgar scoring was performed according to Veronesi et al. (Veronesi et al., 2009) including: mucous membrane color, heart rate, respiratory rate, irritability reflex and motility evaluation. For each criteria puppies were scored from 0 (the worst note) to 2 points (the best note) and the values obtained for the five criteria were summed, so that the final score ranged from 0 to 10 points. A drop of blood was obtained by pricking the marginal ear vein (Ettinger and Feldman, 2005) and the following metabolites assayed in sequence using disposable test strips and portable devices: glucose (Freestyle Optium, Abbott, Illinois, USA), lactate (Lactate Pro, Arkray, Kyoto, Japan) and β-hydroxybutyrate (Freestyle Optium). The measuring range and precision are as follow: glucose 20–500 mg/dl, coefficient of variation (CV) = 2.7–4.0%; β-hydroxybutyrate 0.07–5.2 mmol/L, CV = 3.1-3.8%; lactate 0.8-23.3 mmol/L, CV = 2.6–3.2%. Body temperature was measured via a digital rectal thermometer (Torm 10 s mt-403s, Hangzhou Sejoy Electronics & Instruments Co., Hangzhou, China) with the measuring range from 32 °C to 42.9 °C and precision from ±0.1 °C (for temperatures between 35.5 °C and 42 °C) to ±0.2 °C (for temperatures below 35.5 °C and above 42 °C). Urines were obtained by genital swabbing and urine SG was measured using a clinical refractometer (Rogosampaic, Wissous, France) with the measuring range from 1.000 to 1.050 and precision of ±0.002. All values below the device detection range were included in the calculations as the lowest detectable value, and all values above as the highest detectable value.

2.4. Data analysis

Statistical analyses were performed with the SAS version 9.3 software (SAS Institute Inc., Cary, NC, USA). The normality was evaluated with Shapiro-Wilk test.

The effect of time on each parameter evaluated <8 h and at Day 1 of life was tested with Wilcoxon signed-rank test.