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ABSTRACT

In numerous species, low birth weight is a risk factor for neonatal mortality. In the canine species, definition of a low birth weight is complex due to the huge interbreed variability in size. To identify puppies at higher risk of neonatal death, data from 6,694 puppies were analysed. The data were collected from 75 French breeding kennels, examining 27 breeds and totaling 1,202 litters of puppies. Generalised linear mixed models allowed to identify birth weight, birth weight heterogeneity within the litter, and size of the breeding kennel as significant risk factors for neonatal mortality. Receiver Operating Characteristics (ROC) and classification and regression tree (CART) analyses were combined to define breed specific thresholds for birth weight allowing the identification of puppies at higher risk of neonatal mortality. Due to differences in birth weights between breeds, including when belonging to the same breed size, analyses were conducted at the breed level. First, ROC analysis thresholds were successfully established for 12 breeds (area under the ROC ≥ 0.70; sensitivity ≥ 75%; specificity: 45–68%) and they ranged from 162 g in the Maltese to 480 g in the Bernese Mountain dog. Secondly, CART analysis thresholds from 22 breeds ranged from 105 g in the Maltese and 436 g in the Boxer. Puppies were grouped into three categories according to birth weight: low, moderate and high risk of neonatal mortality (higher than the ROC threshold, between ROC and CART thresholds, and lower than the CART threshold respectively). In the current study, 44% of the puppies were classified as at moderate risk and 5.3% for a high risk of neonatal mortality. Thresholds defined by CART analysis (and not ROC analysis) were used to define low birth weight puppies and were sometimes quite different between breeds with similar birth weight distributions suggesting a variable relationship between birth weight reduction and neonatal death. These results allow the identification of puppies at an increased risk of neonatal death, thus requiring specific nursing to improve their chances of survival. With these high risk puppies identified, both animal welfare and kennel productivity is predicted to improve.

1. Introduction

Despite progress in veterinary medicine, mortality rate from birth to weaning remains high in the canine species with approximately one puppy out of ten live births dying before the age of 2 months (Gill, 2001; Indrebø et al., 2007; Chastant-Maillard et al., 2017a, 2017b). Most of the deaths (70–80%) occur during the three first weeks of life (i.e. during the neonatal period), and identification of the factors involved in neonatal mortality is essential.

In numerous species, including humans, porcine, and bovine, low birth weight is considered a major risk factor for neonatal mortality (Wu et al., 2006; Fix, 2010). Similarly, in dogs, low-birth-weight newborns are at a higher risk of death, with a risk of mortality twelve times higher when compared with normal-birth-weight puppies (Groppetti et al., 2015; Mila et al., 2015). Past studies conducted in dogs focused on one or two breeds and few analyses were performed at a breed size level (Nielen et al., 2001; Indrebø et al., 2007; Fiszdon and Kowalczyk, 2009; Mila et al., 2015). Selective breeding of the domestic dog (*Canis familiaris*) has caused the differentiation of 344 breeds (Fédération Cynologique Internationale, 2018) with the greatest

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Table 1
Quartile values by breed used to classify 6694 puppies from 27 breeds for four parameters: birth weight, early growth rate, litter size and litter heterogeneity.

Breed	Number of puppies included	Birth weight, g			Early growth rate, %			Litter size		Litter heterogeneity, %	
		Q1	Q2	Q3	Q1	Q2	Q3	Q1	Q3	Q1	Q3
Alaskan Malamute	104	500	580	631	8.1	15.1	22.0	5	8	6.7	11.1
Australian Shepherd	420	315	365.5	410	2.0	9.0	15.8	6	8	8.1	15.1
Beagle	124	280	310	342.7	4.0	13.7	22.5	5.8	7.2	6.2	11.9
Bernese Mountain dog	265	445	480	555	−4.9	3.9	9.1	5	8	6.4	11.6
Bichon Frise	107	162	183	212.5	−5.3	4.4	13.2	4.2	7.8	9.3	16.6
Boxer	123	415	470	520	−5.8	−1.2	7.2	5.5	8	6.2	10.1
Cavalier King Charles Spaniel	155	202	230	250	−5.0	1.0	7.6	3.2	6	7.4	11.6
Chihuahua	157	102	118	133	1.7	7.9	14.0	2	4	7	14.7
Cocker Spaniel	477	223	270	310	0.0	7.8	14.8	4	7	8.3	16
Coton de Tulear	159	165	190	210	4.5	10.0	16.4	4	5.2	7	14.4
Dachshund	152	163.75	184	203.5	6.7	8.3	15.0	3	5	5.3	15.6
English Bulldog	123	262	310	370	−5.2	2.3	10.0	4	6	8	15.9
French Bulldog	111	210	240	266	2.8	9.5	13.1	3.2	6	9.1	16.4
German Shepherd	197	436	500	570	−2.7	4.5	12.5	5	9	5.9	9.9
Golden Retriever	483	350	400	438.5	2.4	9.0	16.6	5.8	9	7	14
Jack Russell Terrier	122	185	205.5	225	7.4	12.4	19.0	3	5	8.9	20.8
Labrador Retriever	1,846	363.2	410	460	0.0	7.5	14.0	6	9	6.7	11.7
Leonberger	216	450	520	581.25	2.0	6.6	12.1	5.8	10	6.9	13.8
Lhasa Apso	153	165	190	216	4.8	11.8	16.8	3	6	6.8	13.3
Maltese	178	140.5	165	187	−1.3	6.3	13.8	4	6	7.8	15.5
Newfoundland	163	570	640	688	−3.7	3.2	9.2	4.2	7	6.7	14.3
Pomeranian	117	130	150	166	−7.2	2.7	8.1	2	4	6.3	15.8
Rottweiler	111	370	410	447.5	−8.8	−2.7	4.7	6	9	6	16.8
Shih Tzu	225	158	172	190	−5.7	4.1	10.6	3	6	6.3	12.3
White Swiss Shepherd	114	420	472.5	520	2.2	9.0	16.0	6	8	5.5	10.3
West Highland White Terrier	164	176.75	200	224.25	−0.3	10.7	19.0	3	5	6.3	11.9
Yorkshire Terrier	128	120	140	164.25	9.8	15.8	22.0	3.2	5.8	10.2	14.5

Q1 = first quartile value, Q2 = second quartile value (i.e. median), Q3 = third quartile value.

morphological variability within any land mammal species. Adult body weight ranges from 500 g in miniature breeds (Chihuahua) to more than 100 kg in giant breeds (Mastiff) (Boyko et al., 2010). Birth weight should be analysed according to breed because of this large variation of body weights.

The aims of this study were i) to demonstrate differences in birth weight between breeds and within breed sizes, ii) to identify risk factors of neonatal mortality in puppies, specifically the impact of birth weight in a large canine purebred population with multiple breeds, and iii) to determine breed-specific cut-off values for birth weight to identify at-risk puppies.

2. Materials and methods

2.1. Study population

This study was based on data collected through a questionnaire administered to dog breeders from 2015 to 2017. This questionnaire was completed voluntarily by breeders in France. The questionnaire included four sections in order to obtain information about the breeding kennel, dam and sire, mating and birth, and puppies of the litter. At the kennel level, the information included the number of puppies produced per year. For dam and sire, the information included the breed, the dates of birth and the parity of the dam. At the litter level, the information included the dates of the first mating and whelping, the kind of parturition (natural birth or caesarean section), the total number of puppies born alive and stillborn. For puppies, the information included the sex, the birth weight, and mortality during the first 21 days of life. The questionnaire was distributed to breeders using published ads within articles, mailings, Facebook® messages, during canine exhibitions and via various dog breed associations. Information was then anonymously transferred with the breeders' consent into a Microsoft Excel table (Microsoft Corporation, Redmond, Washington, USA) for analysis. Only breeds represented by 100 individuals or more were analysed.

2.2. Data management and analysis

Statistical analyses were performed using R software (R Core Team, 2016; version 3.3.2). Results with p-values less than 0.05 were considered to be significant. Statistical uncertainty was assessed by calculating 95% binomial confidence intervals (95%CI).

2.2.1. Breed size and breed influence on birth weight

Depending on adult weight, breeds were classified into four sizes: small (< 10 kg), medium (10–25 kg), large (26–45 kg) and giant (> 45 kg; Gandini et al., 2003; Royal Canin, 2013). The effects of breed size and breed on birth weight were tested with the Kruskal-Wallis rank sum test and pairwise Mann-Whitney *U* test with Bonferroni correction.

2.2.2. Neonatal mortality risk factors

Neonatal mortality rates included the deaths of live-born puppies within the first 21 days after birth. The neonatal period was divided in two successive periods: early neonatal period (birth: Day 0 to Day 2) and late neonatal period (Day 2 to Day 21; Chastant-Maillard et al., 2017a, 2017b; Mila, 2015).

Generalised linear mixed models were fitted using R package "lme4" (Bates et al., 2015), with early neonatal mortality and late neonatal mortality as binary outcome variables. Explanatory variables were introduced in the models only if missing values represented less than 15% of the data. Thus, the fixed-effects introduced in the models were birth weight, stillbirth in the litter, sex, litter size (total number of puppies born alive), litter heterogeneity and breeding kennel size. Breeding kennel and dam were introduced as random effects to deal with the non-independence of puppies sharing the same breeding kennel and the same mother. All parameters were categorical variables. Multicollinearity was assessed among the predictors using the Cramer's V coefficient which expressed the strength of the association between two categorical variables. Values upper than 0.7 were indicative of collinearity (Boukary et al., 2013). The size of breeding kennel was categorised into three groups: "Small" for breeding kennels producing

Table 2
Birth weight, litter size, sex ratio, litter heterogeneity and mortality rates by breed for 6694 purebred puppies born in France (27 breeds).

Breed	Breed size*	Number of puppies included	% of the total population	Mean birth weight, grams (± SD)	Mean litter size (± SD)	Sex ratio	Litter heterogeneity, % (IQR)	Litters with at least one stillborn (%)	Neonatal mortality (%)
Alaskan Malamute	Large	104	1.6	562.5 (± 93.3)	6.1 (± 1.9)	0.9	8.6 (6.7-11.1)	11.8	0.0
Australian Shepherd	Medium	420	6.3	363 (± 82)	7 (± 1.8)	1.0	11 (8.1-15.1)	23.3	5.7
Beagle	Medium	124	1.9	309 (± 50.4)	6.4 (± 1.6)	1.0	9.8 (6.2-11.9)	18.2	7.3
Bernese Mountain dog	Giant	265	4.0	490.1 (± 77.6)	6.7 (± 2.7)	1.0	9.1 (6.4-11.6)	34.1	9.8
Bichon Frise	Small	107	1.6	189 (± 37.5)	5.6 (± 2.2)	1.1	12 (9.3-16.6)	23.8	21.5
Boxer	Large	123	1.8	464 (± 71.7)	6.7 (± 1.5)	0.8	7.7 (6.2-10.1)	31.6	9.8
Cavalier King Charles Spaniel	Small	155	2.3	225.4 (± 39.7)	4.8 (± 2.2)	1.0	9.9 (7.4-11.6)	42.9	13.5
Chihuahua	Small	157	2.3	119.6 (± 25.6)	2.8 (± 1)	1.2	9.4 (7-14.7)	8.6	1.3
Cocker Spaniel	Medium	477	7.1	266.1 (± 64.1)	5.3 (± 2)	1.1	11 (8.3-16)	23.4	11.7
Coton de Tulear	Small	159	2.4	187.9 (± 35.5)	4.4 (± 1.6)	0.7	11.5 (7-14.4)	13.9	3.1
Dachshund	Small	152	2.3	184 (± 36.5)	3.6 (± 1.6)	0.8	9.2 (5.3-15.6)	18.6	7.2
English Bulldog	Medium	123	1.8	315.9 (± 68.1)	5.4 (± 2)	0.8	14.2 (8-15.9)	52.4	11.4
French Bulldog	Small	111	1.7	237.6 (± 42.6)	5.1 (± 2.4)	1.1	11.4 (9.1-16.4)	15.4	15.3
German Shepherd	Large	197	2.9	506.2 (± 93.8)	6.5 (± 2.7)	1.0	7.9 (5.9-9.9)	32.0	10.2
Golden Retriever	Large	483	7.2	395.4 (± 71.7)	7.2 (± 2.7)	1.0	9.4 (7-14)	39.7	8.1
Jack Russell Terrier	Small	122	1.8	202.1 (± 36.2)	3.6 (± 1.7)	1.0	11.7 (8.9-20.8)	26.3	9.8
Labrador Retriever	Large	1,846	27.6	410.2 (± 69.7)	7.3 (± 2.6)	1.0	8.6 (6.7-11.7)	34.1	6.2
Leonberger	Giant	216	3.2	516.7 (± 104.1)	7.9 (± 3.8)	0.8	10.2 (6.9-13.8)	17.9	10.2
Lhasa Apso	Small	153	2.3	187.5 (± 40)	4.5 (± 1.8)	1.2	9.2 (6.8-13.3)	20.6	12.4
Maltese	Small	178	2.7	164.7 (± 35.6)	4.9 (± 1.5)	1.1	11.8 (7.8-15.5)	27.0	13.5
Newfoundland	Giant	163	2.4	630.3 (± 112.1)	5.4 (± 2.2)	1.1	9.7 (6.7-14.3)	30.0	4.3
Pomeranian	Small	117	1.7	152.1 (± 40)	3.4 (± 1.5)	1.4	11.5 (6.3-15.8)	31.6	17.1
Rottweiler	Giant	111	1.7	403.8 (± 58.6)	7.6 (± 2)	1.4	9 (6-16.8)	37.5	18.9
Shih Tzu	Small	225	3.4	176.4 (± 27.9)	4.8 (± 2.1)	1.0	9.6 (6.3-12.3)	30.4	19.1
White Swiss Shepherd	Large	114	1.7	473.4 (± 80.7)	6.5 (± 2.4)	0.9	7.5 (5.5-10.3)	22.2	6.1
West Highland White Terrier	Small	164	2.4	196.3 (± 37.5)	4.2 (± 1.5)	1.4	8.6 (6.3-11.9)	38.7	16.5
Yorkshire Terrier	Small	128	1.9	142.3 (± 30.9)	4.3 (± 1.8)	0.9	11.3 (10.2-14.5)	22.2	7.0
Total		6,694		345.4 (± 142.1)	6.3 (± 2.7)	1.0	9.6 (6.8-14)	28.4	9.0

SD = standard deviation; IQR = interquartile range.
* Breed sizes: Small, adult body weight < 10 kg; Medium, 10–25 kg; Large, 26–45 kg; Giant, > 45 kg.

less than 10 puppies per year; "Medium" for 10–50 puppies per year and "Large" with more than 50 puppies per year. Litter heterogeneity represented within-litter variation in birth weight and was expressed as the coefficient of variation (CV = standard deviation (SD) ÷ mean x 100; Milligan et al., 2002). Many breeds were represented to allow introduction of breed as a fixed-effect (convergence failure). Breed effect was introduced by classifying parameters influenced by breed (birth weight, litter size and litter heterogeneity; all $P < 0.001$, Kruskal-Wallis rank sum test) using breed-specific quartiles (Table 1). Each breed was divided into groups based on the calculated quartiles. For litter size and litter heterogeneity, the quartiles were calculated at the litter level and the groups were group 1 (small or low): ≤ first quartile value (Q1), group 2 (medium): between Q1 and Q3, and group 3 (large or high): > Q3 (Table 1). For birth weight, quartiles were calculated at the puppy level and the groups were group 1: ≤ Q1, group 2: between Q1 and Q2, group 3: between Q2 and Q3, and group 4: > Q3 (Table 2).

To deal with unbalanced data, the random under sampling approach described by Chan and Stolfo (Chan and Stolfo, 2001; Weiss, 2004) was used and generalised linear mixed models were fitted with all selected variables. Models were fitted on 80% of each balanced sub-datasets (training sets) and their classification performances were evaluated on the remaining 20% of each balanced sub-datasets (testing sets). Results were combined across balanced sub-datasets using the median. Then, p-values, odds ratio, and their 95%CI, were obtained for each parameter. The median area under the receiver operating characteristic curve (AUROC) was used to assess the ability of the models to differentiate puppies which die during neonatal period (0–21 days) and those that survive.

2.2.3. Birth weight thresholds

In order to determine birth cut-off values for each breed, ROC and CART procedures were carried out on the dataset using birth weight and neonatal mortality data for each breed. Analyses were performed using "pROC" (Robin et al., 2011) and "rpart" (Therneau et al., 2017) R packages. Firstly, ROC curves were used to identify optimal cut-off values for birth weight regarding neonatal mortality for each breed. The effectiveness of the parameter to discriminate between puppies dying during neonatal period and those that survive was assessed using the AUROC. If the AUROC was greater than or equal to 0.70, the cut-off value was determined for each breed on maximized Youden's J statistic ($J = Se + Sp - 1$; Greiner et al., 2000; Hajian-Tilaki, 2013). A sensitivity greater than or equal to 75% was used in order to minimize false negative result. An example of false negative result was a puppy dying before 21 days of age but who was classified as a survivor based on birth weight, and thus not specifically nursed whereas it should have been. Secondly, CART analysis, based on the recursive partitioning method, was used for the identification of puppies at high risk of neonatal mortality (for more information, see Lemon et al., 2003; Saegerman et al., 2011). To prevent the model from over-fitting the dataset, any node that as fewer than five subjects could not split and was forced to become terminal node. The decision rule of the first root node was used to defined CART threshold for each breed.

3. Results

3.1. Population characteristics

Data from a total of 6,694 live-born puppies from 27 breeds, 1,202 litters, and 75 French breeding kennels was recorded (Table 2). Litters were born between 1994 and 2017 with 82% of the litters born over the last 10 years (from 2007 to 2017). Among the 27 breeds included, 12 were in the top-twenty breeds owned in France according to the French Kennel Club (Société Centrale Canine, 2018). The median number of puppies included per breed was 155, ranging from 104 puppies for the Alaskan Malamute to 1846 puppies for the Labrador Retriever. The global mean litter size was 6.3 (SD: 2.7) puppies and 28.4% of the litters contained at least one stillborn (285/1,002). Median litter heterogeneity per breed varied from 7.5% (interquartile range, IQR: 5.5–10.3) for the White Swiss Shepherd to 14.2% (IQR: 8–15.9) for the English Bulldog. Sex ratio was calculated as 1 because there were 3,313 males and 3,314 females. Almost three quarters of the puppies were born in large breeding kennels (4,845/6,526).

3.2. Breed size and breed influence on birth weight

Birth weights varied from 36 g (Chihuahua) to 940 g (Newfoundland). Average birth weight per breed ranged from 119.6 g (SD: 25.6) for the Chihuahua to 630.3 g (SD: 112.1) for the Newfoundland (Table 2). Mean birth weights were found significantly different between the four breed sizes (all $P < 0.001$). All small breeds (adult body weight < 10 kg) had a mean birth weight lower than the medium breeds (10–25 kg). Similarly, all medium breeds were of lower birth weight than large and giant breeds. When comparing large breeds (26–45 kg) vs giant breeds (> 45 kg) some large breeds were born at significantly higher weights than giant breeds (for example, Alaskan Malamute – Large vs. Rottweiler or Bernese Mountain – Giant). When birth weights were analysed within breed size, significant differences were apparent between birth weights of the various breeds with birth weight coefficients of variation of 26%, 26%, 20% and 22% for respectively small, medium, large and giant breed puppies.

3.3. Identification of neonatal mortality risk factors

A total of 9% (604/6,694; 95%CI: 8.3–9.7) of live-born puppies died during the neonatal period (0–21 days; Table 2).

3.3.1. Early neonatal period (0–2 days)

A total of 3.4% (221/6,473; 95%CI: 3–3.9) of live-born puppies died during the early neonatal period (0–2 days). Early neonatal mortality was influenced by birth weight ($P = 0.014$) and litter heterogeneity ($P = 0.036$) (Table 3). Early neonatal mortality rate was significantly higher in puppies with lower birth weight (from the first quartile) compared with other quartiles. Puppies born in highly heterogeneous litters also had a higher mortality rate (Table 3). Mortality rate increased further when the two risk factors, low birth weight along with a high heterogeneous litter, were combined. Mortality rates were 11.6% (95%CI: 9.2–14.2) for low birth weight puppies born in high heterogeneous litter and 2.3% (95%CI: 2–2.8) for all other types of litter heterogeneity and birth weight categories.

3.3.2. Late neonatal period (2–21 days)

A total of 6.3% (383/6,090; 95%CI: 5.7–6.9) of puppies alive at 2 days died during late neonatal period (2–21 days). Late neonatal mortality was influenced by birth weight ($P < 0.001$) and tended to be influenced by the size of breeding kennel ($P = 0.063$) (Table 3). During this period, mortality was significantly higher in puppies with lower birth weight (from the first quartile) compared with other quartiles. Additionally, late neonatal mortality tended to be higher in large breeding kennels compared to small and medium breeding kennels (Table 3). Mortality rate increased further when the two risk factors, low birth weight along with a large breeding kennel, were combined. Mortality rates were 13.4% (95%CI: 11.5–15.5) for low birth weight puppies born in large breeding kennel and 4.2% (95%CI: 3.7–4.8) for all other birth weight categories and in other breeding kennel sizes (small/medium).

The models studied presented a correct discrimination power with a median AUROC of 0.71 (range: 0.62 – 0.81) for early neonatal mortality and 0.77 (range: 0.70 – 0.84) for late neonatal mortality. Variances of random effects parameters, i.e. dam and breeding kennel, were 0.73 (SD: 0.85) and 0.46 (SD: 0.68); 1.49 (SD: 1.22) and 0.86 (SD: 0.93) for early neonatal period and late neonatal period, respectively. For both models, no multicollinearity was evidenced between predictors as estimated Cramer's V coefficients were all less than 0.2 so only weak relations existed between covariables.

3.4. Birth weight cut-off value determination

3.4.1. ROC analysis

Birth weight cut-off values regarding neonatal mortality have been identified for 12 breeds in which AUROC was greater than or equal to 0.70 (Table 4). For all breeds, the birth weight cut-off value was higher than the first quartile. In the 12 breeds, neonatal mortality rate was 14.2% (288/2,026) in puppies with a birth weight below the determined thresholds vs. 4.2% (94/2,234) for puppies with a birth weight above the determined threshold.

3.4.2. Classification tree

Birth weight thresholds, selected by the CART procedure, identified puppies at high risk of neonatal mortality for 22 breeds (Table 4). In the 22 breeds, neonatal mortality rate was 47.2% (151/320) in puppies with birth weight below determined thresholds vs. 7.5% (428/5,708) for puppies with a birth weight above the determined threshold.

3.4.3. Risk categories

Both thresholds, selected by the ROC and CART analyses were different from first quartile value of even first decile value of birth weight calculated for each breed (Table 4). When combining the ROC and CART analyses, three risk categories were defined: low (birth weight ≥ ROC threshold), moderate (birth weight between CART and ROC thresholds) and high risk (birth weight < CART threshold). This combination was obtained for 12 breeds and among these 12 breeds, 52.4%, 44.3% and 5.3% of the puppies were at low, moderate and high risk of neonatal mortality respectively. Neonatal mortality rates were 4.2% for puppies considered to be low risk (94/2,234), 10.7% for moderate risk puppies (202/1,886), and 61.4% for high risk puppies (86/140).

4. Discussion

4.1. Puppy neonatal mortality

The neonatal mortality rate in the present study was found to be 9% (604/6,694). This mortality rate was calculated based on the first three weeks of life and excluded puppies that were stillborn. Similar studies have calculated mortality rates within this range. For example, in a large French canine purebred population, including 248 breeds with a total of 204,537 puppies, the postnatal mortality rate was of 6.5% (Chastant-Maillard et al., 2017a, 2017b). In Norway, over the postnatal period, a mortality rate of 6.9% was described (Indrebø et al., 2007; n = 744). Both of these mortality rates, from France and Norway, were calculated on the first two months of life and excluded puppies that were stillborn. In a cohort study including 58,439 puppies, in Norway, first-week mortality rate was 3.7% (Tønnessen et al., 2012). In a study conducted in Australia by Gill (2001), 13.2% of the puppies that were born alive, died during the first six weeks of life (n = 2,574). The current study focused on an early period (0–21 days) with results within the range of previous mortality rates reported.

4.2. Effect of breed on birth weight

A large variation in birth weight was observed between breeds with

Table 3
Predictive factors for neonatal mortality in 6694 purebred puppies in France using generalised linear mixed-models. Dam and breeding kennel were included as random effects into the two models.

Factors included in the models	Early neonatal mortality			Late neonatal mortality		
	P-value	Odds ratio (95%CI)	Mortality rate, % (95%CI)	P-value	Odds ratio (95%CI)	Mortality rate, % (95%CI)
Size of breeding kennel	0.133			0.062		
Small		1 (Ref.)	2.1 (1.4-3.1)		1 (Ref.)	3 (2.1-4.1)
Medium		0.24 (0.02-3.62)	1.1 (0.4-2.6)		0.12 (0.01-1.63)	1.1 (04-2.6)
Large		2.25 (0.64-7.31)	4.1 (3.5-4.7)		2.56 (0.66-9.38)	7.1 (6.4-7.9)
Presence of stillborn in the litter	0.588			0.65		
No		1 (Ref.)	3.2 (2.7-3.8)		1 (Ref.)	5.7 (5-6.5)
Yes		0.93 (0.45-1.87)	3.4 (2.6-4.4)		1.02 (0.51-1.95)	6.6 (5.4-8)
Litter size	0.652			0.626		
< Q1		1 (Ref.)	3.4 (2.4-4.7)		1 (Ref.)	6.8 (5.4-8.5)
[Q1-Q3]		0.81 (0.35-1.71)	3 (2.4-3.6)		1.17 (0.59-2.32)	5.5 (4.7-6.4)
> Q3		0.65 (0.22-1.96)	3.9 (2.9-5.1)		1.05 (0.41-2.53)	6.2 (4.9-7.8)
Birth weight	0.014			< 0.001		
Q1		1 (Ref.)	7.3 (6.1-8.6)		1 (Ref.)	11.3 (9.8-13)
Q2		0.37 (0.15-0.9)	2.4 (1.7-3.2)		0.34 (0.17-0.72)	6.2 (5.1-7.5)
Q3		0.26 (0.1-0.71)	1.9 (1.3-2.7)		0.18 (0.08-0.4)	3.7 (2.8-4.7)
Q4		0.22 (0.07-0.66)	1.4 (0.9-2.1)		0.06 (0.02-0.18)	2.3 (1.6-3.2)
Sex	0.457			0.524		
Female		1 (Ref.)	3.2 (2.6-3.9)		1 (Ref.)	6 (5.2-6.9)
Male		1.19 (0.63-2.25)	3.5 (2.9-4.2)		1.15 (0.66-2.01)	6.6 (5.7-7.5)
Litter heterogeneity	0.036			0.181		
< Q1		1 (Ref.)	2 (1.3-2.8)		1 (Ref.)	4.3 (3.3-5.5)
[Q1-Q3]		1.12 (0.47-3.08)	2.4 (2-3)		1.18 (0.56-2.78)	5.6 (4.9-6.5)
> Q3		2.82 (0.98-7.58)	6.4 (5.2-7.8)		1.77 (0.69-4.83)	9.4 (7.9-11.1)

Table 4
Comparison of different birth weight thresholds for the identification of puppies at higher risk of neonatal mortality for 27 canine breeds.

Breed	Number of puppies included	AUROC (95%CI)	BW ROC analysis threshold, g	BW CART analysis threshold, g	BW first quartile value, g	BW first decile value, g
Alaskan Malamute	104				500	422
Australian Shepherd	420	0.72 (0.58-0.85)	375 (75;45)	166.5	315	260
Beagle	124			260.5	280	245
Bernese Mountain dog	265	0.75 (0.65-0.84)	480 (100;50)	387.5	445	397
Bichon Frise	107			172.5	162	146
Boxer	123			435.5	415	375
Cavalier King Charles Spaniel	155			181	202	177
Chihuahua	157				102	91
Cocker Spaniel	477	0.73 (0.65-0.81)	280 (84;45)	142.5	223	180
Coton de Tulear	159			121.5	165	140
Dachshund	152			119.5	164	137
English Bulldog	123			216	262	240
French Bulldog	111	0.71 (0.57-0.85)	230 (82;60)	179	210	180
German Shepherd	197	0.75 (0.62-0.88)	480 (75;59)	318	436	409
Golden Retriever	483			188	350	312
Jack Russell Terrier	122	0.71 (0.54-0.88)	202 (83;59)	116.5	185	147
Labrador Retriever	1,846	0.75 (0.70-0.79)	406 (77;56)	247	363	320
Leonberger	216	0.75 (0.65-0.85)	480 (77;68)	320	450	380
Lhasa Apso	153	0.81 (0.7-0.91)	184 (84;60)	127.5	165	132
Maltese	178	0.76 (0.64-0.88)	162 (75;57)	105	141	115
Newfoundland	163				570	492
Pomeranian	117			136	130	107
Rottweiler	111	0.7 (0.58-0.83)	410 (81;52)	345	370	330
Shih Tzu	225			128.5	158	145
White Swiss Shepherd	114				420	380
West Highland White Terrier	164	0.7 (0.58-0.82)	209 (85;41)	129	177	146
Yorkshire Terrier	128				120	103

AUROC = Area Under the ROC Curve (with 95% confidence interval), BW = birth weight, Se = sensitivity, Sp = specificity, g = grams, CART = classification and regression tree, ROC = receiver operating characteristics.

values ranging from 119.6 g in the Chihuahua to 630.3 g in the Newfoundland. The influence of breed on birth weight has already been reported in canine species (Čechová, 2006; Chatdarong et al., 2007; Fiszdon and Kowalczyk, 2009; Groppetti et al., 2017). In many studies, breeds were grouped by size (small, medium, large and giant). This study highlights the importance of breed-specific analysis because birth weight was significantly different between puppies from different breeds inside the same breed size. For example, the German Shepherd, the Boxer and the Golden Retriever are in the large size category with adult body weight ranging from about 25 to 30 kg (Helmink et al., 2000; Hawthorne et al., 2004; Trangerud et al., 2007; Posada et al., 2014). However, even though they are in the same size category, their average birth weights were significantly different (506, 464 and 395 g for respectively German Shepherd, Boxer and Golden Retriever; Table 2). Analysis at the breed size level are not sufficient and must be conducted within a given breed. More studies are needed to investigate other parameters influencing canine birth weight, especially those related to dam management. Morphology of canine breeds is continually changing due to artificial selection and birth weights may vary between different genetic lineages within the same breed (e.g. between two different countries). Further studies are needed to collect and compare data on canine birth weight of same breeds born into different countries.

4.3. Neonatal mortality risk factors

As previously discussed, birth weight belonging to the 25% lighter weights for the breed was strongly associated with neonatal mortality (Lawler, 2008; Mila et al., 2015). There is an increased risk of hypoglycaemia and hypothermia in low birth weight puppies which could explain a higher mortality rate (Mila et al., 2017).

For the first time, factors such as the size of the breeding kennel and heterogeneity of birth weight within the litter were examined in relation to neonatal mortality.

Late neonatal mortality rates differed depending on the size of the breeding kennel. In large and small breeding kennels there was an increase in late neonatal mortality. The higher probability of neonatal loss in large breeding kennels could be easily explained by an increased infection rate and a lower ability to monitor of all the animals. Indeed, a significantly higher prevalence of enteropathogens during the weaning period was described in large breeding kennels compared with smaller facilities (Grellet et al., 2014). Higher mortality rate in small breeding kennels is more surprising and could be due to a lower technical level of the breeder, who typically have a less professional approach. When compared to late neonatal mortality, the size of the breeding kennel had no influence on early neonatal mortality. During the early neonatal period, puppy mortality was only influenced by puppy birth weight and litter heterogeneity.

Litter heterogeneity was associated with the risk of early neonatal mortality: 6.4% in highly heterogeneous litters vs. 2% in medium-low heterogeneous litters. This is the first time that litter heterogeneity parameter is used when discussing puppy neonatal mortality. This parameter is often used with porcine (Straw et al., 1998; Milligan et al., 2002; Alexopoulos et al., 2018) and is being adapted to discuss canine litters. In porcine, many sows have the same parturition date and cross-fostering can be practiced, unlike in canines. Fostering of piglets is a common practice to overcome litter heterogeneity and increase survival. This decreases competition with piglets as they are now in a litter with similar birth weights (Devillers et al., 2011; Ogbu et al., 2016). More competition in a highly heterogeneous litter may cause an increase in mortality rate. Parturition dates in canine litters are often far apart, thus fostering is not applied in dog breeding kennels.

We collected information on a large sample of puppies but a selection bias could not be excluded as breeders were recruited on a voluntary basis. All of the parameters that can influence neonatal mortality cannot be discussed due to lack of data. Further investigation and collection of new data on other potential risk factors of neonatal mortality described in other species (e.g. parity, age of dam) are warranted.

4.4. Birth weight thresholds

Puppies at higher risk of neonatal mortality because of a low birth weight need to be identified as early as possible to administer timely appropriate care and to improve their chance of survival. Birth weights were significantly different between breeds and cut-off values have to be defined differentially for each breed. Birth weight was previously arbitrarily considered low when belonging to the 25% lighter weights for the breed (Mila et al., 2015). In this study, critical thresholds were determined objectively within each breed based on an increased risk of neonatal death through ROC and CART analyses. Specific ROC analysis thresholds could be determined for 12 breeds and 48% of the puppies were below these thresholds (2,026 puppies of 4,260 had birth weight lower than the ROC cut-off). These results suggest that puppies susceptible to neonatal death are not only the ones belonging to the lowest 25% of birth weight as previously reported. ROC analysis thresholds were selected based on the maximisation of Youden's J statistic. A sensitivity greater than or equal to 75% was selected in order to minimize false negative rate. The cost of specific management is minimal when compared to the decrease in revenue of a deceased puppy. The specific care of almost half of the puppies (48%) would be time consuming for breeders and, the evaluation of neonatal mortality was further refined to focus on the most critical population. CART thresholds were obtained in 22 breeds and only 5.3% of the puppies were considered at high risk for neonatal mortality. As shown in Table 4, the thresholds obtained by CART analysis were close to the first decile value (lowest 10% of birth weight) for each breed, which is a threshold commonly used in human species to define low birth weight infants (McIntire et al., 1999; Xu et al., 2010). Thus, CART thresholds could be used to define low birth weight puppies. CART thresholds for breeds with similar birth weight distributions also varied. For example, the Beagle and the English Bulldog were not statistically different in birth weight but their CART thresholds were 261 and 216 g, respectively. When comparing the German Shepherd and the Bernese Mountain dog, the average birth weights were not statistically different. However, the CART thresholds were 318 g for the German Shepherd and 387 g for the Bernese Mountain dog. The data support that the relationship between low birth weight and neonatal death being different between breeds. More studies are needed to further clarify this relationship and to describe the differences between breeds. Building a larger database through automatic data collection with varying characteristics of puppies, and follow-up until being sold (at the minimum), could lead to earlier identification of at-risk neonates.

5. Conclusion

Evaluation of weight at birth is an easy-to-use tool in the field, requiring a simple and low cost instrument (scale). The results are immediately available and without any invasive manipulation. Studies on canine birth weight should be conducted within a breed due to significant differences between breeds including within the same size (small, medium, large and giant). Three parameters related with weight monitoring that influenced puppy neonatal mortality were birth weight, litter heterogeneity and early growth rate. Critical thresholds presented in the last part of this study would allow the identification of puppies with a higher risk of neonatal death and provide them with appropriate care. Further research is needed to define birth weight thresholds for the numerous remaining canine breeds, and for all breeds, across different countries. Research in pregnancy physiology would also be required to decrease the prevalence of low birth weight puppies and to reduce the heterogeneity of fetal growth within littermates.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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