Confidential: Circulating cell-free DNA (cfDNA) level measures dog health

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Abstract

Circulating cell-free DNA (cfDNA) is a promising analyte for detecting pathological processes prior to the clinical signs of a disease. In human medicine, cfDNA has revolutionised cancer diagnosis and treatment, and is used to detect a wide range of chronic illnesses. In dogs, there is a lack of cfDNA-based tests in general and tests that take into account the genetic diversity of dog breeds in particular. This study proposes a reference-based result interpretation model similar to the one used with C-reactive protein (CRP) with the added benefit of taking breed-specific information into account. Reference ranges for cfDNA concentration have been determined by analysing data from 562 dogs with cancer, 649 dogs with other illnesses, and 1,846 healthy dogs including biological replicates from a subset of 394 dogs. Results show that dog plasma cfDNA can be reliably measured with low and stable baseline levels observed in healthy individuals and increases in cfDNA level correlating with the severity of underlying pathological condition. Single measurements have been used successfully to detect cancer in asymptomatic dogs up to nine months before the onset of clinical signs. Integrating cfDNA measurements into clinical practice has the potential to detect many pathological processes at an early stage and improve the chances of treating them successfully.

Introduction

Many illnesses remain hidden for an extended period of time before they are detected, leading to significant morbidity and mortality. As an example, most cancers are diagnosed after the development of clinical signs. By then, the disease may have reached an advanced stage resulting in a poor prognosis even with aggressive treatment. Similarly, some chronic, inflammatory, and autoimmune conditions are suspected only following visible changes in a dog's movement and behavior, and this delay may have an adverse effect on the treatment success. Early detection of pathological processes is critical to improving treatment outcomes in dogs.

Medical diagnosis typically requires histopathological classification of a tissue biopsy collected from the affected tissue. Unlike tissue biopsies, liquid biopsies do not need prior information about a disease's presence and location. As liquid biopsies can detect illnesses before the onset of clinical signs, they offer an opportunity to improve prognosis by starting treatment early [1]. Their additional benefits include fast turnaround time, non-invasiveness, and the ability to cover disease heterogeneity and dynamics [2].

In human medicine, liquid biopsies have revolutionised cancer screening, diagnosis, and treatment monitoring. They are now used in hospital settings to detect cancer, guide therapeutic decisions, and monitor both treatment success and cancer recurrence [3,4,5]. Beyond cancer, liquid biopsies are increasingly used to detect early signs of inflammation, cardiovascular disease, neurodegenerative diseases, autoimmune disorders, and infections [6–10]. Their potential to detect illnesses early can also improve veterinary care, offering fast, affordable, and non-invasive diagnostics compared to tissue biopsies.

Liquid biopsies are samples collected from bodily fluids, and they are classified based on the source material, analyte, and biomarker type. While blood remains the most common source material for these tests, significant advancements have also been made in using other biofluids, such as urine, saliva, and cerebrospinal fluid [11–14]. The most commonly used analyte type in liquid biopsies is cell-free DNA (cfDNA), which is released into the bloodstream whenever cells die. The level of cfDNA is low in healthy adults and increases rapidly in response to pathological processes including cancer, inflammation, autoimmune reactions, and infections [15–22].

In this study, blood samples from 1,846 healthy dogs have been analysed to establish cfDNA reference ranges for different breed groups. Samples from 562 dogs with cancer and 649 dogs with other clinical conditions have also been analysed to determine cfDNA behaviour in each condition. Our findings show that cfDNA is found at low, stable levels in healthy individuals, and that increases in cfDNA level correlate with the severity of the underlying pathological process. The utility of cfDNA testing is highlighted for the use case of detecting cancer in dogs that do not yet present clinical signs of illness.

Materials and methods

Study population

The study was approved by the Regional State Authoritative Agency's Project Authorisation Board *(ESAVI/30126/2022)*. Blood samples were collected from 562 dogs with cancer, 649 dogs with other clinical conditions, and 1,846 clinically healthy dogs between February 2023 and February 2025. All samples were collected by veterinary professionals and with the dog owner's consent. Each dog's age, breed, sex, neutering status, diagnosed clinical conditions as well as ongoing and planned treatments were collected using standard questionnaires and entered into an internal database.

Presumably healthy dogs were recruited in Finland through open sample collection events and dog shows organised by breed associations. To be eligible to join the study, dogs had to be at least two years old, not pregnant, and with no clinical signs of disease. Dogs with non-cancer clinical conditions were collected the same way. Dogs that presented with an

ongoing infection, a physical injury, or a recent surgery (performed within seven days), were asked to join another sample collection event on a later date.

Dogs with cancer were recruited through animal hospitals in Finland, France, Netherlands, Poland and Slovenia. Cancer diagnosis was based on clinical examination, blood tests, imaging, and cytology or histopathology as determined necessary by the treating veterinarian.

Sample collection

For each dog, venous whole blood was collected. Plasma was separated from blood cells using a standard double centrifugation protocol within the same working day. Separated plasma was transferred into clean tubes and stored frozen until ready to be transported to the research laboratory.

Plasma samples were assessed for hemolysis, lipemia, and icterus on reception. Information about sample quality was entered into an internal database and samples were stored frozen until cfDNA extraction. Samples were centrifuged upon thawing to remove remaining cell debris and divided into aliquots. One aliquot was used for cfDNA extraction and the rest returned to a freezer for later use.

cfDNA extraction and quality control

Internal controls were added to each plasma sample in known quantities before cfDNA extraction as a quality control measure. cfDNA was extracted from thawed plasma using an appropriate kit according to the manufacturer's instructions. cfDNA was eluted into an elution buffer provided by the kit manufacturer and stored frozen until further analysis. cfDNA was analysed using capillary electrophoresis according to the manufacturer's instructions.

Test reproducibility

The test's technical reproducibility was determined in a small number of presumably healthy dogs by dividing received plasma into multiple aliquots and processing them independently on different days. Biological reproducibility was assessed by collecting multiple blood samples from a group of 394 healthy dogs on different days and processing them independently.

cfDNA reference ranges

Physiological cfDNA reference ranges were determined by measuring cfDNA concentration as above in 1,846 healthy dogs. Measurements were grouped by breed to create breed-specific reference ranges. Reference ranges were deemed reliable for those breeds where good quality blood samples (no visible hemolysis, lipemia, or icterus) were available for at least 40 healthy dogs with an even spread between sexes and age groups.

For breeds with samples from less than 40 dogs or with a biased age or sex distribution, a group reference range was created for a set of closely related breeds (as an example, data sets for Golden retrievers, Labrador retrievers, and Flat-coated retrievers were combined to

create a reference range for Curly-coated retrievers). For mixed breed dogs, the dog's weight was measured and data from a mixture of pure-bred dogs of similar weight range (dog's weight ±3kg) was used to generate a reference range.

Determination of reference range cutoffs

cfDNA reference range cutoffs were determined using the breed-specific reference ranges. Briefly, median with two standard deviations (SD) were calculated for each breed or breed group. Values within 2 SD were considered to be within the reference range, and values above the 2 SD were considered to be outside the reference range. Classifiers used to assess the severity of the underlying condition for values outside range are summarised in **Table 1**.

Table 1. Classification of cfDNA concentration values based on the number of standard deviations outside breed-specific median. SD, standard deviation.

Classifier	**Corrected cfDNA value**
Within reference range	< 2 SD
Mildly elevated	2-3 SD
Moderately elevated	4-5 SD
Significantly elevated	6-7 SD
Markedly elevated	8-10 SD
Severely elevated	> 10 SD

Test sensitivity was determined for each clinical condition as the proportion of diagnosed samples that were outside the reference range. Test specificity was assessed as the proportion of samples from healthy dogs that stayed within reference range.

Results

Study population

Group characteristics for the healthy dogs (n=1,846), dogs with cancer (562) and dogs with other clinical conditions (649) are summarised in **Table 2**. There were no marked differences in the age range or median age between groups and the sex distribution was fairly even in all three groups. However, there was a clear difference between the proportions of intact and neutered/spayed dogs in both males and females. Intact dogs formed the majority of the healthy group (76% of males and 60% of females) but only 50% of males and 25% of females in the two disease groups. The cancer group also had a substantially lower proportion of pure-bred dogs (82%) than the two other groups (98%).

The majority of differences between the three groups were due to the recruitment methods used. Healthy dogs and dogs with non-cancer clinical conditions were recruited through breed associations and were therefore overwhelmingly pure-bred. In contrast, dogs with cancer were recruited through animal hospitals and clinics, and had a higher proportion of mixed breed dogs. Differences in the neutering status could also be attributed to the recruitment methods. Dogs recruited through breed associations had a larger proportion of intact individuals, and those recruited through clinics had more neutered or spayed individuals.

Table2. Sample characteristics. BMD, Bernese mountain dog; FCR, Flat-coated retriever; GD, Great Dane; GR, Golden retriever; GSD, German shepherd dog; Mixed, mixed breed; LSA, lymphoma; MCT, mast cell tumour; OSA, osteosarcoma; OA, osteoarthritis; Benign, Benign tumour.

		Healthy *(n=1,846)*	**Cancer** *(n=562)*	**Other** *(n=649)*
Age	*Median (years)*	8	10	9
	Range (years)	2-18	2-18	2-20
Sex	*Male*	746 (40%)	242 (43%)	305 (47%)
	- *intact*	569 (76%)	115 (48%)	165 (54%)
	- *neutered*	160 (21%)	104 (43%)	139 (46%)
	Female	1117 (60%)	315 (57%)	344 (53%)
	- *intact*	702 (63%)	79 (25%)	93 (27%)
	- *spayed*	383 (34%)	203 (75%)	245 (71%)
Breed	*Pure-bred*	1,816 (98%)	460 (82%)	637 (98%)
	Mixed breed	30 (2%)	102 (18%)	12 (2%)
	Top 3 breeds	GSD (122) FCR (116) GD (102)	Mixed (102) BMD (40) GR (32)	FCR (44) GR (41) GSD (40)
Health	*Top 3 clinical conditions*	n/a	LSA (73) MCT (66) OSA (28)	OA (110) Benign (65) Atopy (40)

For healthy dogs, a lower age limit of two years was chosen because a pilot collection of samples from 452 0-7 year old dogs showed that cfDNA concentration was elevated in dogs that were less than 2 years old as seen in **Table 3**.

Table 3. Age-specific cfDNA concentrations. n, number of dogs in an age group; SD, standard deviation.

Age (years)	**n (dogs)**	**Median cfDNA (mg/L)**	**SD**

0-1	62	n/a	n/a
2	78	n/a	n/a
3	78	n/a	n/a
4	78	n/a	n/a
5	78	n/a	n/a
6	78	n/a	n/a

Dogs with recent infections, physical traumas, or surgical operations were re-scheduled to donate a sample on a later date because these types of events were found to cause a temporary increase in the cfDNA concentration that could potentially mask an elevation caused by a hidden illness *(data not shown)*.

Test reproducibility

The test's biological reproducibility is summarised in **Table 4**. As cfDNA was present at very small concentrations in 394 healthy individuals, small variations in sample handling processes were found to have large effects on the test results. To minimise the effects of these preanalytical factors, our test went through a rigorous evaluation process including every step in the workflow from sample collection to data generation. The resulting customised sample processing and analysis workflow was found to reproduce cfDNA concentrations with high concordance.

Table 4. Biological reproducibility of cfDNA concentration measurement. Biological, biological replicates.

Breed	**n (dogs)**	**Median cfDNA (mg/L)**	**SD**
Belgian shepherd dog	98	n/a	n/a
Bernese mountain dog	34	n/a	n/a
Dobermann	13	n/a	n/a
Flat-coated retriever	47	n/a	n/a
German shepherd dog	41	n/a	n/a
Golden retriever	41	n/a	n/a
Great Dane	56	n/a	n/a
Irish wolfhound	32	n/a	n/a
Staffordshire bull terrier	32	n/a	n/a

The most critical confounding factors affecting cfDNA concentration were haemolysis, plasma contamination with genomic DNA (gDNA) from white blood cells (WBC), and variations in plasma handling conditions. Sources of haemolysis included the use of needles with too small gauge size, poor blood-drawing technique, use of blood collection tube types that were not fit for the purpose, and a delay between sample collection and plasma separation. Plasma contamination by gDNA was caused by accidental pipetting of WBC while transferring separated plasma into a clean tube. Temperature fluctuations during plasma storage and transport were also found to decrease cfDNA concentration. In contrast, lipemia and icterus were not found to have a significant effect on cfDNA extraction.

cfDNA reference ranges

In total, dogs from 191 breeds were included in the study and fully listed in **Table S1**. Breed-specific profiles were generated for all breeds with good quality samples from at least 40 dogs, resulting in breed-specific reference ranges for 20 dog breeds listed in **Table 5**. These were supplemented with profiles that combined cfDNA concentrations from closely related breeds. As the study population grows, reference ranges will be added for more breeds, thus improving the test reliability further.

Table 5. Breeds with samples from at least 40 dogs.

Breed	**n (dogs)**
American staffordshire terrier	50
Beagle	52
Belgian shepherd dog	289
Bernese mountain dog	127
Border collie	43
Border terrier	41
Boxer	61
Bullmastiff	49
Cocker spaniel	52
Dobermann	65
Flat-coated retriever	174
German shepherd dog	171
Golden retriever	149
Great Dane	130
Irish wolfhound	79
Jack Russell terrier	45

Labrador retriever	109
Mixed breed	142
Nova Scotia duck-tolling retriever	50
Shetland sheepdog	98
Staffordshire bull terrier	79

Overall performance of cfDNA test in disease detection

In total, 120 clinical conditions (40 cancers and 80 non-cancer) were included in this study and listed in **Table S2**.

Approximately 3% of samples collected from presumably healthy dogs were outside the reference range. For these dogs, a second test was run at least a week after the first test to establish whether the increase in cfDNA concentration was temporary (possibly caused by an infection or a minor physical injury) or chronic (indicating an underlying pathological process).

Conversely, 73-96% of samples taken from dogs with diagnosed cancers were outside the reference range, depending on the clinical condition. The performance of our cfDNA test in healthy dogs and a set of cancers is summarised in **Table 6**.

Table 6. cfDNA test performance in selected cancer types and healthy dogs.

Test set	**Test results**	**Test performance**
Hemangiosarcoma (n = 14)	Outside reference range 13/14	Sensitivity 93%
Histiocytic sarcoma (n = 22)	Outside reference range 21/22	Sensitivity 96%
Lymphoma (n = 83)	Outside reference range 79/83	Sensitivity 95%
Mast cell tumour (n = 40)	Outside reference range 29/40	Sensitivity 73%
Melanoma (n = 16)	Outside reference range 14/16	Sensitivity 88%
Osteosarcoma (n = 28)	Outside reference range 25/28	Sensitivity 89%
Healthy dogs (n = 1,846)	Within reference range	Specificity 97%

Case studies: cfDNA test in cancer detection

cfDNA test performance is illustrated by two case studies.

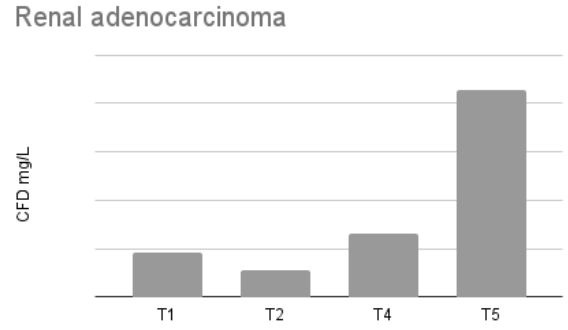
Case 1 (**Figure 1a**) was a 5-year-old female Golden retriever diagnosed with renal adenocarcinoma nine months after our baseline sample showed elevated cfDNA concentration. Note that at the time the first two samples were collected, we did not have

enough data to assess what was normal for the breed and age group. However, by the time the third sample came in, it was clear that the test value was outside the breed-specific reference range and the dog underwent further testing to confirm a cancer diagnosis.

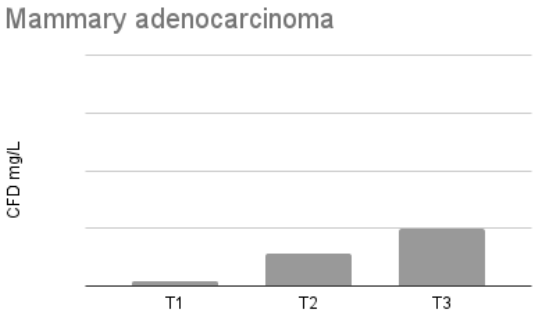
Case 2 (**Figure 1b**) was a 9-year-old female Belgian Laekenois. The dog appeared clinically healthy during both sample collection events and the first cfDNA test value was within the reference range for Belgian shepherd dogs. However, the second sample taken three months later showed a marked elevation in cfDNA value. It took another eight months before the dog was examined at a clinic, diagnosed with mammary adenocarcinoma, and treated with surgery.

Figure 1. cfDNA test in disease detection. 1a) renal adenocarcinoma; 1b) mammary adenocarcinoma. T1-T4, sample collection time points; CFD, cfDNA.

a.



b.



Discussion

We have established a robust test protocol to measure circulating cfDNA concentration in dogs reliably and reproducibly. As always, there are some limitations that will be covered in this section.

The clinical utility of cfDNA-based diagnostics has been constrained by the high variability introduced during preanalytical processing steps, such as sample collection, handling, storage, transport and processing prior to molecular analysis. This variability has undermined reliability and reproducibility of cfDNA concentration measurements [23–26].

We have resolved this issue by rigorously testing preanalytical steps and optimising processes that have produced the most consistent results. Our test results are highly reliable and reproducible in a set of hundreds of healthy dogs, and we have used the test successfully to detect cancer in presumably healthy dogs up to nine months before the onset of clinical signs.

It should be noted that cfDNA concentration alone does not distinguish between specific illnesses. In this sense, cfDNA is a similar analyte to the c-reactive protein (CRP) that has been shown to correlate with the presence of pathological conditions including infections, systemic inflammation, and some cancers [27–29]. CRP belongs to a group of acute phase

proteins that the liver produces in response to cellular stress and this makes it a rather blunt tool for diagnostics.

In contrast, cfDNA is released by all tissue types in response to cell death and correlates directly with the extent of tissue or organ damage. The wide dynamic range of cfDNA gives some indication about the underlying pathological process, especially in the higher end of the spectrum. Low levels are seen in healthy dogs. Concentrations just outside the breed-specific reference range are common in inflammatory conditions, especially when these are under control. On the other end of the spectrum, very high concentrations are seen in haematological cancers and after major physical injuries or recent surgeries. Most chronic conditions and cancers fall in between the two ends with a direct correlation between cfDNA concentration and cancer staging.

Another advantage of analysing cfDNA compared to CRP include its rich informativeness. cfDNA carries information about the tissue of origin and the pathological processes that have caused its release into the bloodstream [30–33]. This information is in the form of genetic and epigenetic changes that can be readily analysed after cfDNA extraction and concentration measurement using sequencing and PCR-based technologies. We regard CRP and cfDNA as complementary biomarkers of health that should be used as part of routine health checks as well as whenever a disease is suspected.

A major difference between this and previous studies was that the majority of our samples originated from purebred dogs. This difference was largely driven by dogs collected in Finland where 86% of dogs were purebred. The large number of purebred dogs across a set of diverse breeds gave us a unique opportunity to verify the hypothesis that cfDNA concentrations in healthy dogs vary significantly between dog breeds. This was indeed the case, and the implication was that tests using a single cutoff threshold value for cfDNA concentration to estimate a dog's health would work poorly in some cases. However, it also meant that it would be possible to improve the test performance by comparing the cfDNA concentration of a new sample against a reference range collected from clinically healthy dogs of the same breed.

Due to the breed-specific variation in cfDNA concentrations, it is hard to give one cutoff threshold that would be meaningful across all breeds. We have addressed this issue by creating breed-specific reference ranges. It is possible that the breed-specific variation in cfDNA concentration reflects differences in the level of low-grade inflammation, pre-cancerous states, and other pathological processes but further research is needed to answer this question.

The concentration of circulating cfDNA changes rapidly when cells are damaged. Most often this is due to a pathological process but sometimes the damage is due to vigorous exercise. In humans, cfDNA concentrations increase in response to both endurance and high-intensity sports with the magnitude of change correlating to exercise intensity, duration, and immune activation [34–38].

Based on the results in humans, we speculated that an individual cfDNA measurement outside the reference range may be due to extended physical exercise. We confirmed this by running an event for sample collection on the last day of a working dog summer camp. A

high proportion of dogs had cfDNA concentrations outside the breed-specific reference ranges. We repeated tests a few weeks later when the dogs were back with their families, and this time the vast majority of dogs had cfDNA values within the reference range. We therefore recommend a second test to be taken at least a week after the first one to confirm the source of the result outside the reference range.

Conclusions

We have developed a fast, affordable, and non-invasive test to measure the overall health of dogs. It is important to remember that while cfDNA concentration correlates directly with the extent of cellular damage, a single test cannot distinguish between specific illnesses.

Our findings demonstrate that circulating cfDNA concentration falls within narrow, breed-specific reference ranges in healthy dogs, similar to how CRP levels are used in routine veterinary practice. The major advantage of using cfDNA in this manner is its ability to capture a broader spectrum of pathological processes, its wider dynamic range that enables more nuanced interpretation of the results, and the informativeness encoded in its sequence that allows additional analyses to reveal the tissue of origin and ongoing pathological changes.

The study shows that regular measurement of a dog's cfDNA level can aid the treating veterinarian to detect pathological processes at an early stage, with the associated increases in the chances of successful treatment. We propose that cfDNA concentration measurements are included as a standard part of wellness checks for middle aged to old dogs, especially for those breeds that have an increased risk of certain cancers or specific diseases with systemic inflammation.

Our research will now focus on developing affordable and disease-specific follow up tests using an ultra-sensitive new technology. We will also test other biofluids to determine which ones can be used for rapid testing of certain disease types, and will continue to collect samples from healthy dogs to expand the set of breed-specific reference ranges available.

Liquid biopsies have the potential to significantly enhance the management of canine diseases by enabling earlier and more accurate detection, as well as more effective monitoring of disease progression and response to therapy.

Author contributions

KJK and WP designed the study with input from JK and HL. PJ collected blood samples and associated signalment from healthy controls and from dogs with non-cancer clinical conditions. UL, ZV, LB, AR, NT, and JB collected blood samples and associated signalment from dogs with cancer and provided expert advice on canine cancers. BB, EH, GE, KJK and MT performed cfDNA extraction, QC, and data analysis. All authors contributed to the manuscript and approved the submitted version.

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extraction methods and QC methods were tested at the Institute for Molecular Medicine Finland FIMM HiPrep Unit. This study was funded by Business Finland (8371/31/2022) and DeepScan Diagnostics.

Conflict of interest

EH, GE, HL, JK, KJK, MT, PJ, and WP are employed by or affiliated with DeepScan. BB is a former employee of DeepScan. WP, JK, HL and hold vested or unvested equity in DeepScan. HL is a founding partner in two other canine diagnostics companies. GE, JK, KJK, MT, PJ, and WP are inventors of a patent application covering some of the technologies used here.

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Supplementary data

Table S1. Dog breeds (n=191) included in the study.

Afghan Hound	Catalan Sheepdog	Icelandic Sheepdog	Pug
Airedale Terrier	Cavalier King Charles Spaniel	Irish Setter	Puli
Akita	Chihuahua	Irish Terrier	Pumi
Alaskan Malamute	Chinese Crested dog	Irish Water Spaniel	Pyrenean Mastiff
American Bull Terrier	Cirneco Dell'etna	Irish Wolfhound	Pyrenean Mountain dog
American Bulldog	Cocker Spaniel	Istrian Short-haired Hound	Pyrenean Sheepdog
American Bully	Coton de Tulear	Italian Greyhound	Rat Terrier
American Cocker Spaniel	Croatian Sheepdog	Italian Pointing dog	Rhodesian Ridgeback
American Hairless Terrier	Curly-coated Retriever	Jack Russell Terrier	Romagna Water dog
American Pit Bull terrier	Czechoslovakian Wolfdog	Jämthund	Rottweiler
American Staffordshire Terrier	Dachshund	Japanese Spitz	Rough Collie
Andalusian Terrier	Dalmatian	Kangal Shepherd dog	Saint Hubert
Appenzell Mountain dog	Dandie Dinmont Terrier	Karelian Bear dog	Saluki
Australian Kelpie	Dobermann	Keeshond	Samoyed
Australian Labradoodle	Dogo Argentino	Kooikerhondje	Schapendoes
Australian Shepherd	Dogue de Bordeaux	Labrador Retriever	Schipperke
Australian Silky Terrier	Drever	Lakeland Terrier	Schnauzer
Australian Terrier	Dutch Shepherd dog	Lapponian Herder	Shar pei
Basenji	English Cocker Spaniel	Leonberger	Shetland Sheepdog
Basset Hound	English Setter	Lhasa Apso	Shiba Inu
Bavarian Mountain Hound	English Springer Spaniel	Löwchen	Shih tzu
Beagle	English toy Terrier	Maltese	Siberian Husky
Beauceron	Entlebucher Mountain Dog	Medimurje dog	Skye Terrier
Belgian Groenendael	Eurasier	Medium Poodle	Smooth Collie
Belgian Laekenois	Field Spaniel	Miniature American Shepherd	Smooth Fox Terrier
Belgian Malinois	Finnish Hound	Miniature Bull Terrier	Soft Coated Wheaten Terrier
Belgian Shepherd dog	Finnish Lapphund	Miniature Pinscher	Spanish Greyhound

Belgian Tervueren	Finnish Spitz	Miniature Poodle	Spanish Mastiff
Bernese Mountain dog	Flat-coated Retriever	Miniature Schnauzer	Spanish Water dog
Bichon Frise	French Bulldog	Mixed breed	St. Bernard
Boerboel	French Water dog	Mudi	Staffordshire Bull Terrier
Bohemian Shepherd Dog	German Pinscher	Newfoundland	Standard Poodle
Border Collie	German Shepherd dog	Norfolk Terrier	Swedish Vallhund
Border Terrier	German Shorthaired Pointer	Norwegian Elkhound Grey	Thai Ridgeback dog
Borzoi	German Spitz	Norwich Terrier	Tibetan Mastiff
Boston Terrier	German Wire-haired Pointer	Nova Scotia Duck Tolling Retriever	Tibetan Spaniel
Bouvier des Flandres	Giant Schnauzer	Old English Sheepdog	Tibetan Terrier
Boxer	Glen of Imaal Terrier	Papillon	Toy Poodle
Brazilian Terrier	Golden Retriever	Parson Russell Terrier	Weimaraner
Briard	Great Dane	Pekingese	Welsh Corgi
Brittany Basset	Great Swiss Mountain dog	Phalène	Welsh Springer Spaniel
Brittany Spaniel	Greyhound	Pharaoh Hound	West Highland White Terrier
Broholmer	Griffon Belge	Pomeranian	Whippet
Bull Terrier	Griffon Bruxellois	Poodle	White Swiss Shepherd dog
Bulldog	Hällefors Elkhound	Portuguese Podengo	Wire Fox Terrier
Bullmastiff	Havanese	Portuguese Water dog	Xoloitzcuintle
Cairn Terrier	Hovawart	Prague Ratter	Yorkshire Terrier
Cane Corso	Hungarian Vizsla	Presa Canario	

Table S2. Clinical conditions (n=120) included in the study.

Adenocarcinoma	Eye cancer	Leishmaniasis	Pyoderma
Adrenal gland cancer	Eye infection	Leukemia	Pyogranulomatotic panniculitis
Allergy	Furunculosis	Liver cancer	Pyogranulomatous inflammation
Anal sac gland cancer	Gallbladder symptoms	Liver inflammation	Pyometra
Anaplasmose	Gastric cancer	Lung cancer	Reflux
Anemia	Gastric dilatation volvulus	Lymphoma	Renal cancer

Asthma	Gastritis	Mammary carcinoma	Rhabdomyosarcoma
Atopic dermatitis	Gastroenteritis	Mast cell tumour	Sarcoma
Atopy	GI symptoms	Melanoma	Sebaceous adenitis
Benign tumour	Gingivostomatitis	Meningioma	Seizure disorder
Bladder cancer	Glaucoma	meningioma	Skin tumour
Borreliosis	Heart base tumour	Mesothelioma	SLE
Brain cancer	Heart disease	Mucocele	SLO
Cancer	Heart failure	Nasal cancer	Soft tissue sarcoma
Cataract	Heart murmur	Osteoarthritis	Spinal disc hernia
Chondrosarcoma	Heart palpitations	Osteochondrosis	Spondylosis
Chronic bladder infection	Heart valve disease	Osteosarcoma	Squamous cell carcinoma
Chronic bronchitis	Hemangiosarcoma	Ovarian cysts	SRMA
Congenical kidney disease	Hip dysplasia	Ovarian tumour	Syringomyelia
Cryptorchidism	Histiocytic sarcoma	Pancreatic cancer	Teeth issues
CUPS autoimmune disease	Histiocytoma	Pancreatitis	Testicular tumour
Cushing's disease	Histiocytoma	Pannus	Thymoma
DCM	Hypothyroidism	Paroxysmal dyskinesis	Thyroid cancer
Distichiasis	IBD	Periodontitis	Transitional cell carcinoma
Elbow dysplasia	Ichtyosis	Piloleiomyosarcoma	Urinary stones
Endometritis	Incontinence	Plasmacytoma	UTIs
Epilepsy	Intestinal symptoms	Prostate carcinoma	Vaginal cancer
Epileptoid cramping syndrome	Joint inflammation	Prostate symptoms	Vestibulary syndrome
Epithelial carcinoma	Kidney disease	Prostatic cyst	Womb tumour
Epithelioma	Larynx paralysis	Pulmonary artery disease	Yeast infection