DISEASE BURDEN IN FOUR POPULATIONS OF DOG AND CAT BREEDS COMPARED TO MIXED-BREED DOGS AND EUROPEAN SHORTHAIR CATS

S.F.A. Keijser[a*#], L.E. Meijndert[a*], H. Fieten[a], B.J. Carrière[c], F.G. van Steenbeek[a], P.A.J. Leegwater[a], J. Rothuizen[a], M. Nielen[b]

a) Expertise Centre Genetics of Companion Animals, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Postbus 80154, 3508 TD Utrecht, The Netherlands, ecgg@uu.nl
b) Centre of Evidence Based Veterinary Medicine, Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Postbus 80154, 3508 TD Utrecht, The Netherlands, M.Nielen@uu.nl
c) 'Sterkliniek' Veterinary Practice Ermelo, Horsterweg 66, 3851 PL Ermelo, The Netherlands, bjcarriere@dierenkliniek-ermelo.nl

* Authors contributed equally.

Corresponding author. E-mail address: s.f.a.keijser@uu.nl. Tel.: +31302534202.

ABSTRACT

Current public and professional opinion is that many dog breeds suffer from health issues related to inherited diseases or extreme phenotypes. The aim of this historical comparative observational study was to evaluate the breed-related disease burden in three purebred dog populations (Chihuahua, French bulldog, Labrador retriever) and one purebred cat breed (Persian cats) in the Netherlands by comparison to a control population of mixed-breed dogs and European Shorthair cats.

A qualitative query was performed, consisting of a literature review and collecting the expert opinions of University veterinary specialists, to gather insight into potential diseases of the study population.

Next, a referral clinic case control study of the patients referred to specific medical disciplines in the University Clinic was performed. The odds ratio (OR) was calculated to determine the likelihood of a patient referred to a particular medical discipline being a certain breed.

Together, the qualitative query and the case control study resulted in a list of potentially relevant diseases limited to five organ systems per breed. These were analysed in data from primary practices. Patient files from ten primary practices over a period of two years were manually extracted and examined. Four-hundred individual patient records per breed as well as 1,000 non-breed records were randomly selected from the 10 practices, weighted per practice size. Records were then examined and the presence or absence of certain diseases was identified. To evaluate the disease burden per breed, proportional difference (PD) was estimated, as well as the animal's age at presentation in months.

The results of the referral clinic case control study showed an overrepresentation (Odds Ratio >1.5) of the selected breeds in several medical specialties, while median age at presentation was in some cases significantly lower than in the non-breed animals.

Results of the practice-based extended cross-sectional study showed that only a few of the selected diseases contribute to the disease burden in these purebred populations, which was different from the expectations derived from the literature or expert opinion. Additional results included age difference at presentation, which may be interpreted as age of onset, and could indicate a higher disease burden for the individual animal. Also, only a small percentage of purebred dogs was registered with the national kennel club.

Our final recommendation is that population-based data mining is needed to evaluate country-specific companion animal health and welfare.

KEYWORDS

Disease burden, breed characteristics, inherited disease, companion animal health, companion animal welfare

INTRODUCTION

54 The number of dog and cat welfare problems associated with breed has become a hot topic (Higgins &
55 Nicholas, 2008) resulting in many studies on various diseases and breeds. Both the general public and
56 veterinary professionals have expressed concerns about the high frequency of health problems in
57 purebred dogs and cats. However, quantitative data to compare specific breed populations with data from
58 the general population are rarely available.
59 Breed-specific health issues in dogs and cats can be classified into two categories: inherited diseases and
60 harmful breed characteristics. A reduction of genetic variation because of inbreeding and frequent use of
61 the same breeding stock decreases the effective population size (Nielen et al., 2001; Peelman, 2009;
62 Oldenbroek & Windig, 2012), and leads to a greater incidence of inherited diseases: pathogenic mutations
63 may have accidentally been co-selected with desired phenotypic variants (Ubbink, 1998; Arman, 2007;
64 Summers et al., 2010). Breed characteristics can become harmful when they lead to an exaggerated
65 phenotype that disturbs physiological functions (Ubbink, 1998; Asher et al., 2009; Collins et al., 2011).
66 Although there is much public debate about harmful breed characteristics, there are no objective criteria
67 by which to measure their frequency and thus their impact on animal wellbeing. A clear example is the
68 Bulldog phenotype with a short snout leading to dyspnea. If this causes clear and prolonged discomfort,
69 we assume that the pet owner would consult a veterinarian for treatment or correction the phenotype. We
70 therefore propose using veterinary consultation as an objective and quantifiable indicator of an intolerable
71 reduction of wellbeing due to a breed-associated disease, which is measurable by investigating veterinary
72 databases (Thrusfield 1983; Jansen et al., 2005). The frequency of breed-associated diseases in specific
73 breeds needs to be quantified in comparison with the general population to objectively estimate their
74 relative impact on animal welfare (Bonnett et al., 2005; Egenvall et al., 2006; Bellumori et al., 2013).
75 Different data sources can be used to monitor diseases, each with its own advantages and disadvantages,
76 as reviewed by O'Neill et al. (2014). The current research focuses on two data sources: referral clinic and
77 primary practice.
78
79 The objective of this historical comparative observational study was a quantification of the burden of
80 disease associated with specific health issues in the Chihuahua, French bulldog, Labrador retriever and

Persian cats in comparison to mixed-breed dogs and cats through an estimation of the proportional difference, evaluation of age at presentation and disease severity.

In this study, a purebred is any animal that can phenotypically be considered to belong to a certain breed, regardless of registration at a kennel club in the case of dogs. A pedigree dog is a dog registered with the Dutch national kennel club. A mixed-breed is an individual with a mixed lineage, not belonging to any particular breed.

MATERIAL AND METHODS

Breed selection

Criteria for including breeds were: population size in the Dutch national top ten, veterinary awareness of overrepresented diseases and/or harmful breed characteristics in the national breed population, and willingness of the breed club to cooperate. The breeds that were selected were the Chihuahua, French bulldog, Labrador retriever and the Persian cat. In this study 'Persian cat' also includes the Exotic Shorthair cat, since both are allowed to mix and both have the same breed requirements with the exclusion of coat length.

Qualitative analysis

First, a literature study was performed using PubMed incorporating the search terms [breed, i.e. the selected four breeds], [incidence] and [prevalence]. Relevant references from the resulting publications were consulted, as well as a number of veterinary textbooks and three reports published in The Netherlands. This information, as well as data from online databases and websites maintained by genetic laboratories, was combined to result in a long list of registered diseases per breed (*long list organised per breed and medical specialty available from author, translated*) (Meijndert et al., 2014).

Second, 15 veterinary specialists, approved by the European Board of Veterinary Specialists and employed by the Department of Clinical Sciences of Companion Animals of the Veterinary Faculty of Utrecht University were interviewed, using a standardised questionnaire (*Appendix 1*). Each of these

specialists acted as a coordinating super-specialist for a specific organ system (e.g. dermatology, neurology and endocrinology) and was asked to adapt or extend the list with common diseases per breed.

Referral clinic case control study

The database of the University Clinic for Companion Animals was analysed for the period January 2008 to January 2013 in a case control design. This time frame was chosen to ensure a sufficient number of individuals per breed were included to permit statistically reliable outcomes. Referrals for specific screening programmes were excluded. Cases included individuals that visited a specific medical specialist, either a selected breed or mixed-breed/European Shorthair cats (*Appendix 2*). The control population included animals of the same breed – and thus exposure – referred to the University Clinic for any reason other than that specific medical specialty.

Statistical analyses for the referral clinic case control study

The statistics in this study were calculated with Excel (Microsoft) and SPSS (International Business Machines Corporation).

The odds ratio (OR) was calculated and significance tested using the Fisher's exact test (www.Rproject.org). This determined the likelihood that a patient referred to a particular medical discipline would be of a specific breed versus a mixed-breed. An OR above 1.5 was considered an overrepresentation of that breed with respect to referral to that specialism. Any underrepresentation that occurred was not analysed further. Also the median, minimum and maximum age at presentation were calculated. Significance of the median age between purebred and non-breed animals was tested by a Mann-Whitney U test (p value < 0.05).

Practice-based extended cross-sectional study

The qualitative analysis and referral clinic case control study resulted in a selection of organ systems and diseases for entry in the practice-based extended cross-sectional study (*Appendix 3*). Certain specific diseases were expected to be associated with the selected organ systems and to be among the most frequently diagnosed. The selected organ systems and diseases were next evaluated in files from ten

136 primary-care companion animal practices. These practices were selected because they use protocol-led
137 filing in the same practice management software (Viva, Corilus Veterinary BV). The files from the ten
138 selected practices were considered to be a fair representation of the total primary care population, being
139 geographically spread throughout the Netherlands, including rural and urban areas and different-sized
140 practices.
141
142 Individual animals registered as one of the selected breeds, or as mixed-breed dogs or European Shorthair
143 cats were selected from the practice's patient files over a period of two years (January 1st 2011 to November
144 12th 2013). The purebred animals were considered to be exposed to their genetic profile, the mixed-breeds
145 as unexposed to such a homologous genotype.
146 'European Shorthair cat' is the most frequently entered breed name for a common cat in veterinary
147 practice. This may include European or Domestic Shorthair cats or mixed-breed cats. The time frame of
148 two years was chosen to assure large enough numbers per breed to reach statistical significance based on
149 power calculation. Moreover, it has been shown that the general patient population will visit a veterinarian
150 at least once every two years, on average (Reid-Smith, 1999).
151 Sample size was determined through a number of steps. With the assumption that the national breed-
152 specific populations exceed 20,000 individuals, the exact size of the population is irrelevant to
153 determining the sample size. The sample size was calculated using Win Episcope software
154 (www.winepi.net), with a sampling error around the estimated proportion of 5% for purebreds and 3%
155 for the unexposed group. The higher level of precision for the mixed-breeds was because lower disease
156 proportions were expected, which therefore demanded greater accuracy (Parker, 2012). For expected
157 prevalence we used 50%, since the actual population prevalence was unknown. A total number of 400
158 individuals per breed and 1000 individuals for the unexposed group were found to be necessary. The
159 number of individuals per veterinary practice was weighted to practice size for the purebred animals.
160 Two-and-one-half times that number of non-breed animals were randomly selected per practice, which
161 corrected for differences between practices (*Table 1*).

162 Search terms were determined for each of the identified organ systems per breed (*Appendix 4*) and the
163 randomly selected patient files were scanned for the presence of these terms in the two-year period. The

correlating patient files were read by one veterinary researcher (LM) to determine whether the selection for that particular organ system was confirmed. A diagnosis was considered to be confirmed when the relevant combination of patient info, clinical symptoms, results of a physical exam and, if available, additional diagnostic information such as blood values or radiographs was present in the patient file. Co-authors were consulted when confirmation was not straightforward. Surgical referral records and records of a tumour in the specified organ system were excluded.

Health issues concerning pregnancy and parturition were considered in two separate categories: dystocia and juvenile hypoglycaemia. For dystocia (in the Chihuahua, French bulldog and Persian cat) a separate sample was taken of female purebred animals that were searched for either non-elective Caesarean section or administration of oxytocin because of dystocia. For hypoglycaemia (in the Chihuahua) a separate sample was taken of dogs younger than six months at any time during the two-year observation period. Two separate groups of unexposed individuals were selected for those analyses as well (*Table 1*).

Data collected from all patient files were: consultation date, species, selected breed, gender, weight, date of birth and microchip number. The microchip number was used to confirm registration with the Dutch kennel club, for the phenotypically designated breed type. For cats this was not possible, since identification is not mandatory and there is no governing organisation (Kurushima et al., 2013). The kennel club has a list of the transponder numbers of the pedigree dogs present in the Netherlands. Any other transponder number indicates a dog that was bred outside the kennel club. When an individual is registered at a veterinary practice, or when any official document such as a passport or vaccination certificate is signed, the transponder number is checked. Any dog without a transponder is by definition not a pedigree dog from the kennel club. The date of birth and the consultation data combine to yield age at presentation, which was interpreted as age at disease onset.

Statistical analyses for the practice-based extended cross-sectional study

The statistics in this study were calculated with Excel (Microsoft) and SPSS (International Business Machines Corporation).

The proportion of diseased individuals per organ system, per 100 unique presented animals of the particular breed, was calculated for the two-year sample period. The difference between specific breed and mixed-breed study populations was evaluated with a Fisher's exact test.

Proportion difference, which is the proportion of disease in the exposed population minus the proportion of disease in the unexposed group, gives us information on the disease burden of the breed population as a whole. Relative risk is a parameter to quantify the risk of disease at an individual level. As in the case control study, for both groups the median, minimum and maximum age of presentation were estimated. All tests were considered significant for $p<0.05$.

Disease severity assessment

One possible method for objectively determining the severity of a disease is the Generic Illness Severity Index for Dogs (GISID). Asher et al. (2009) describe the development of this system. Briefly, it scores four aspects of a disease – prognosis, treatment, complications and behaviour – on a five-point scale from 0-4, with 0 being the least severe and 4 the most severe. For example, treatment can vary from none required to prolonged treatment or major surgery. The scores of the four aspects are added up to come to a total of a minimum of 0 and a maximum of 16 points. A higher score indicates decreased health and welfare, which can vary for each disease. In this study, we evaluated the GISID score for those diseases that were found to be significant in the practice-based extended cross-sectional study of the selected breed populations (GISID-scores from Asher et al., 2009; Summers et al., 2010).

QUANTITATIVE RESULTS

The results for the four researched breeds are combined in four tables. Table 2 shows the odds ratio (>1) in the referral clinic case control study. Table 3 presents the median age at presentation in the referral clinic. Table 4 shows the disease proportion in the practice-based extended cross-sectional study. Table 5 presents the median age at presentation in primary practice.

Chihuahua

217 Case control analysis of the University Clinic database shows that the Chihuahua was overrepresented in
218 hepatology and neurology (OR > 1.5 and $p < 0.05$) in comparison to mixed-breed dogs (*Table 2*). The
219 median age at presentation in the neurology department in Chihuahuas was half that in mixed-breed dogs
220 (*Table 3*).
221 Practice-based extended cross-sectional study showed that disease proportion was significantly higher in
222 Chihuahuas than in mixed-breed dogs for extremities, dystocia and hypoglycaemia. The organ system
223 extremities – in effect the knee – had the highest disease proportion and proportion difference (*Table 4*).
224 The median age of presentation of Chihuahuas versus mixed-breeds at the time of research was lower for
225 all organ systems, with a significant difference for extremities (*Table 5*).
226
227 *French bulldog*
228 The French bulldog was overrepresented in the University Clinic in otorhinolaryngology and neurology
229 (OR > 1.5 and $p < 0.05$) (*Table 2.*). The median age at presentation for otorhinolaryngology consultation
230 in the French bulldog was a third of that in the mixed-breed dogs (*Table 3.*).
231 Analysis of primary practice patient files showed that disease proportion was significantly higher in
232 French bulldogs versus mixed-breeds for all selected organ systems. The upper respiratory tract had the
233 highest disease proportion and proportion difference (*Table 4*). The median age at presentation of French
234 bulldogs versus mixed-breeds was lower in all organ systems, with significant difference in spinal column
235 problems (*Table 5*).
236
237 *Labrador retriever*
238 Case control analysis of the University Clinic database showed that the Labrador retriever was
239 overrepresented in orthopaedics, urology and reproductive medicine (OR > 1.5 and $p < 0.05$) in
240 comparison to mixed-breed dogs. The overrepresentation in the reproductive medicine department was
241 caused by individuals presented for the removal of retained ovarium tissue, the incidence of which was
242 not analysed further (*Table 2*). The median age at presentation in the orthopaedics department in
243 Labradors was half that in mixed-breed dogs. The urology department also saw four times younger
244 Labrador retrievers than mixed-breed dogs (*Table 3*).

The practice-based extended cross-sectional study showed that the difference between the proportions of disease of the extremities in Labrador retrievers versus mixed-breed was significant (*Table 4*). No significant difference was found for the other organ systems or for the median age at presentation (*Table 5*).

Persian cat

The Persian cat was overrepresented in the University Clinic in ophthalmology (OR > 1.5 and $p < 0.05$) (*Table 2*). The median age at presentation for ophthalmology consultation in the Persian cat was two thirds of that in the European Shorthair cat (*Table 3*).

An analysis of primary practice patient files showed a significantly higher proportion of diseases in Persian cats versus European Shorthair cats for all organ systems investigated, with the exception of dystocia. Birth problems were not observed in either cat population. The eyes were the organ system with the highest disease proportion and proportion difference (*Table 4*). No significant median age difference was found (*Table 5*).

Disease severity assessment

The GISID-score was assessed for the results of the practice-based extended cross-sectional study, together with the proportion. Assessment of the patient files resulted in a list of specific diseases belonging with the selected organ systems detected. Where disease proportion was significantly different, the GISID score was included in *Table 4*.

DISCUSSION

The referral clinic case control study shows that each of the analysed purebred populations is overrepresented in consultations with veterinary specialists compared to mixed-breed dogs or European Shorthair cats. Not all reported or suspected breed-associated diseases appeared in the practice-based extended cross-sectional study. The Chihuahua and the Persian cat were shown to be affected by three out of five selected diseases significantly more often than the mixed-breed dogs and European Shorthair cats. The French bulldog has a higher risk for all selected diseases compared to the mixed-breed dogs. In

the case of patellar luxation and brachycephalic obstructive syndrome, this was also suggested in more recent work by O'Neill et al. (2016) and Packer et al. (2015). Of the long list of potential diseases, the Labrador retriever was found to have a significantly higher risk for only one inherited disease.

Only a small fraction (6.4-20.5%) of the dog breed populations had a pedigree from the Dutch kennel club. Although healthy breeding is generally considered the responsibility of the kennel clubs, in the Netherlands the overwhelming number of dogs from these three breed populations come from non-associated breeders.

It is not well known whether the subpopulations of dogs with and without a pedigree are genetically very different. The present data were not sufficient to find possible differences in the presence of disease or harmful characteristics between these subpopulations. However, this finding does stress the importance of collaboration by all breeding organisations, not just the national kennel club, in addressing breed-related health issues. This may differ between countries (Leroy, 2011).

The case control study of patients referred to the University Clinic has two challenges. First, a referral bias must be considered. Factors influencing whether or not an animal gets referred include the professional view of the referring veterinarian, the type of disease and the prognosis. Referral bias could account for the significant overrepresentation of Labrador retrievers in urology in the University Clinic, which does not show up in primary practice patient files. A breed's popularity may be considered here as well, potentially resulting in a breed bias in referral behaviour. In addition, the pet owner's financial status, willingness to travel to a referral clinic – as also suggested by Bartlett et al. (2010) – and concept of animal well-being influence referral behaviour, and a breed's association with a relatively more or less affluent population of pet owners can create a clear bias in the data. Part of this referral bias may be suggested by the within-breed differences in age at presentation.

Second, cases that are easily resolved are less likely to require a referral clinic at all. Therefore, although the diagnosis is more precise, particular diseases may be severely under- or overrepresented (Lund et al. 1999; Reid-Smith, 1999). Underrepresentation of a breed in comparison to the control group was not part

300 of this study, but may be interesting to analyse further to counterbalance the negative attention to breed
301 health and welfare.
302 Taking these limitations into account, it is our assumption that the University clinic database can be used
303 to indicate relations between breeds and complex diseases in various organ systems.
304
305 The use of practice-based patient files has a number of disadvantages: the pet owner may provide
306 information that is incomplete or inaccurate, the veterinarian's interview of the owner or examination of
307 the patient may be incomplete, and the resulting report's information may be incorrect or incomplete. In
308 addition to these factors, a correct diagnosis is not guaranteed and depends on the complexity of the
309 disease, the veterinarian's knowledge and experience, and the owner's wishes and perception of the
310 animal's health. Standardisation of procedures both in veterinary practice and in data collection are
311 essential to compensate for these effects (Thrusfield, 1982; Jansen et al., 2005). However, any such bias
312 was assumed to be the same between purebred and mixed-breed individuals in each practice and would
313 therefore not create misclassification bias in these results.
314 The practice-based extended cross-sectional study starts with the assumption that a patient is presented to
315 the veterinarian in the first place. The likelihood of an owner presenting a pet to the veterinarian may be
316 subject to bias, in that owners may have variable tolerance for clinical signs of disease. This tolerance may
317 be breed-related – e.g. a bulldog owner might not recognise respiratory distress for what it is because of
318 the snorting breathing pattern of the breed – but because disease can only be detected in animals
319 presented to a veterinarian when using clinical data, it cannot be corrected for. On the other hand,
320 owners of an expensive purebred individual might be willing to spend more on veterinary care.
321 Potential differences between practices, including the definition and registration of a diagnosis, the
322 veterinarian's knowledge and experience, do need to be corrected for. This was done by using an
323 unexposed group that was proportionally similar to the number of breed-specific individuals sampled
324 from a particular practice. Although search terms were as broad as possible, it is possible that individuals
325 with specific health issues were missed.

Tumour records were excluded because neoplastic disease did not come through the selection as an aim in the primary practice analysis. Also, tumour occurrence can be an indication of a disease that may occur in several organ systems at once.

Manually collecting data in primary veterinary care practices poses several challenges.
First, sample size was limited by the manual analysis and may underrepresent the actual number of health issues in the population. Rare diseases in particular are less likely to come up in a small sample, even if they are very breed-specific. Automated sample taking could easily increase the sample size in the future. Also, manual data collection has obvious practical issues. It is time consuming in itself, and the software for primary veterinary practice is not designed for research.
Second, the unexposed group for dogs is defined as mixed-breed, but this may differ from practice to practice. However, this is not considered to be a problem because the unexposed individuals need to be heterogenic. A specific breed is considered to be entirely non-heterogenic, with a homologous genotype.
Third, the true incidence of disease in a population is defined as the number of new disease cases in a certain period, divided by the population 'at risk' (the total number of years that all animals together were at risk of becoming sick during the research period) and differs per disease. Prevalence is given as the total number of cases present in a population at a given time.
The practice-based extended cross-sectional study most likely measured a combination of initial incident cases, repeated incident and prevalent cases. Because it was not feasible to determine this exactly within this study, we chose to calculate the disease proportion in the study population: the number of cases mentioned per 100 individuals presenting to the practice. Alternatively, this may be defined as a period prevalence, showing the proportion of a population that is diagnosed in the specified time period (Bartlett et al., 2010). Another approach might have been to perform a survival analysis where an event is defined as the first diagnosis and a hazard ratio is estimated. For ease of interpretation we have chosen to specify disease proportion, with proportion difference and relative risk.

It is tempting to label a breed according to the number of breed-related diseases that *may* occur. However, other factors need to be considered, such as the number of years of good health lost due to the disease –

known as Disability-Adjusted Life Years or DALYs, the severity and type of disease in a GISID score (Asher et al., 2009) and the incidence of similar diseases in the general population.

The earlier age at presentation for certain diseases in the Chihuahua and the French bulldog versus mixed-breeds is suggestive that these are heritable. In this study, a lower age at presentation, interpreted as age of onset, would indicate a higher disease burden for the individual dog. The life expectancy between selected breeds and mixed-breeds differs, but in general early onset of non-curable disease may lead to a greater disease burden. The calculation of DALYs could be used to correct for life span.

The GISID score is a method to assess the individual burden of disease within a breed. If this severity index is combined with information on the age at onset and the proportion of the population affected, the disease burden can be assessed at a population level. A detailed calculation of, for example, the Breed-Disorder Welfare Impact Scores as introduced by Collins et al. (2011), where BDWIS = prevalence x severity x proportion of life affected, would enable disease to be ranked across breed populations.

Different data sources are available for study on the national dog and cat population. Each data source has a number of advantages and limitations, ranging from referral bias in cancer registries to poor representation in referral clinic (O'Neill et al., 2014). Although Egenvall et al. (1998) validated agreement between animal insurance data and primary practice data in Sweden, the low number of insured animals in the Netherlands is not very representative of the population. The current study suffers from diagnostic uncertainty for the practice data. However, the estimated proportions between breed and non-breed animals are considered to be a fair representation of health differences.

Following from this study, nationwide automatic data collection from Practice Management Systems is currently being implemented to analyse disease burden on a much larger scale, in a prospective manner. Population-based data from primary practice will provide much-needed quantitative evidence to inform policy makers such as breeders and organisations as well as future pet owners and their veterinarians. The effects of intervention measures can be monitored through continued data collection in the population.

CONCLUSIONS AND GENERAL RECOMMENDATIONS

1. The proportion of diseases in national dog and cat breed populations as reflected in clinical data may be different from what is stated in the international literature or by experts.
2. The reduction of breed-related diseases cannot be solely the responsibility of the national kennel club, but also of the non-pedigree breeders.
3. Large-scale, automated and standardised recording of diagnoses is recommended to enable a detailed analysis of many different breed populations and to follow them over time.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX

Supplementary data associated with this article can be found, in the online version, at {{web link}}

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482 TABLES

483

484 ***Table 1. Sample sizes, randomly selected from patient files from ten primary practices.***

Breed	Total	Microchip		Pedigree		Female	Juvenile	Unexposed sample*
		#	%	#	%			
Chihuahua	405	175	43.2%	26	6.4 %	405	405	1013
French bulldog	405	127	31.4%	50	12.4%	405		1013 (for dystocia 846)**
Labrador retriever	404	172	42.6%	83	20.5%			1010
Persian cat	404	93	23.0%	-	-	404		1010

485 Total number of individuals per practice rounded up, leading to totals just over the required minimum of 400. For the
486 unexposed group of mixed-breed dogs or European Shorthair cats this was multiplied by 2.5. ** Separate samples of females and
487 of juveniles (<6mo) were taken to evaluate dystocia and juvenile hypoglycaemia. Because one practice had a higher number of
488 French bulldogs on file compared to the number of mixed-breeds, the unexposed sample for dystocia of these unexposed mixed-
489 breed dogs did not reach 1000 individuals.

490
491

492 ***Table 2. The odds ratio (OR) > 1 that a patient referred to a University Clinic specialist will be a***

493 ***certain breed, in comparison to mixed-breed dogs or European Shorthair cats.***

Breed	Medical discipline	OR (CI 95%)	p value
Chihuahua	Neurology	2.36 (1.50-3.64)	< 0.01*
	Hepatology	2.11 (1.12-3.79)	< 0.05*
French bulldog	Neurology	2.65 (1.87-3.74)	< 0.01*
	Otorhinolaryngology	2.48 (1.75-3.48)	< 0.01*
	Ophthalmology	1.29 (0.96-1.71)	0.082
	Dermatology	1.14 (0.72-1.76)	0.506
Labrador retriever	Urology	2.76 (1.73 - 4.49)	< 0.01*
	Reproductive medicine	2.04 (1.32 - 3.20)	< 0.01*
	Orthopaedics – neurosurgery	1.74 (1.43 - 2.11)	< 0.01*
	Gastroenterology	1.41 (0.87 - 2.30)	0.155
	Dermatology	1.19 (0.89 - 1.59)	0.247
	Hepatology	1.09 (0.72 - 1.64)	0.689
Persian cat	Ophthalmology	5.82 (3.87 - 8.65)	< 0.01*
	Nephrology	1.72 (0.34 - 5.50)	0.426
	Haematology	1.26 (0.03 - 8.04)	0.561
	Otorhinolaryngology	1.12 (0.59 - 1.99)	0.652

494 *Significant with Fisher's exact test

495

496

497 ***Table 3. Median age, minimum and maximum (months) for breed and non-breed at presentation***

498 ***in a medical discipline at the University Clinic (non-breed being mixed-breed dogs or European***

499 ***Shorthair cats).***

Breed	**Medical discipline**	**Median (min-max)**		**p value**
		Breed	**Non-breed**	
Chihuahua	Neurology	32.4 (2.4-124.8)	68.4 (3.6-147.6)	< 0.01*
	Hepatology	24 (3.6-153.6)	54 (2.4-180)	0.158
French bulldog	Neurology	42 (6-130.8)	68.4 (3.6-147.6)	0.075
	Otorhinolaryngology	34.8 (0.6-115.2)	100.8 (2.4-194.4)	< 0.01*
Labrador retriever	Orthopaedics	30 (2.4-141.6)	58.5 (2.4-184.8)	< 0.01*
	Urology	27.6 (1.2-141.6)	103.2 (6-154.8)	< 0.05*
Persian cat	Ophthalmology	78 (3.6-201.6)	120 (1.2-236.4)	< 0.05*

500 *Significant difference median tested with Mann-Whitney U test.

501

Table 4. Proportion of diseased individuals presented in ten primary care practices, per organ system, in breed and non-breed (non-breed being mixed-breed dogs or European Shorthair cats). Exact numbers underlying the proportions differed slightly and are shown in table 1.

Breed	Disease	Proportion		PD (95%CI)	RR (95%CI)	p value PD	GISID**
		Breed	Non-breed				
Chihuahua	Dystocia	4.9	0	4.9 (2.8-7.0)	-	<0.01*	2-6
	Extremities	10.4	4.3	6.1 (2.9-9.3)	2.4 (2.0-2.8)	<0.01*	6-9
	Hypoglycaemia	1.5	0	1.5 (0.3-2.7)	-	<0.01*	5-12
	Liver	.2	0.4	-0.2 (-0.8-0.4)	0.6 (0-2.8)	1	
	Spinal column	2.5	2.9	-0.4 (-2.2-1.4)	0.9 (0.2-1.6)	0.857	
French bulldog	Dystocia	4.0	0	4.0 (2.1-5.9)	-	<0.01*	2-6
	Ears	10.6	6.2	4.4 (1.1-7.7)	1.7 (1.3-2.1)	<0.01*	4-11
	Eyes	9.1	4.3	4.8 (1.7-7.9)	2.1 (1.7-2.5)	<0.01*	2-8
	Spinal column	8.1	2.9	5.2 (2.3-8.1)	2.8 (2.3-3.3)	<0.01*	5-12
	URT	13.1	1.6	11.5 (8.1-14.9)	8.3 (7.8-8.8)	<0.01*	6-15
Labrador retriever	Extremities	15.6	7.8	7.8 (3.9-11.7)	2.0 (1.7-2.3)	< 0.01*	4-6/5-10
	Liver	1.2	0.5	0.7 (-0.5-1.9)	2.5 (1.3-3.7)	0.160	
	Skin and coat	11.1	9.5	1.6 (-2.0-5.2)	1.2 (0.9-1.5)	0.377	
	Spinal column	3.7	4.0	-0.3 (-2.6-2.3)	0.9 (0.3-1.5)	0.880	
	Urinary tract	2.0	2.2	-0.2 (-1.8-1.4)	0.9 (0.1-1.7)	1.000	
Persian cat	Dystocia	0	0	0 (0)	-	-	
	Eyes	11.6	3.7	7.9 (4.6-11.2)	3.2 (2.8-3.6)	<0.01*	2-8
	Kidneys	6.4	2.5	3.9 (1.3-6.5)	2.6 (2.1-3.1)	<0.01*	3-13
	Skin and coat	1.0	0.1	0.9 (-0.1-1.9)	10.0 (7.8-12.2)	<0.05*	unknown

PD = proportional difference: breed minus non-breed; RR = relative risk: disease proportion breed divided by mixed-breed; 95%CI = 95% confidence interval; Dystocia evaluated in female sample, hypoglycaemia in a juvenile sample. * Significant with Fisher's exact test. ** GISID = Generic Illness Severity Index for Dogs (extracted from Asher et al., 2009; Summers et al., 2010) scores four aspects of a disease – prognosis, treatment, complications and behaviour – with a total range of 0-16 points, with a higher score indicating decreased health and welfare. For the Chihuahua the GISID score covers dystocia, patellar luxation and juvenile hypoglycaemia. For the French bulldog the GISID score covers dystocia, otitis externa, corneal ulceration, hernia nucleus pulposis type 1 and brachycephalic obstructive syndrome. For the Labrador retriever the GISID score covers elbow dysplasia and hip dysplasia, respectively. For the Persian cat the GISID score covers for corneal ulceration and polycystic kidney disease. For dermatophytosis this was unknown.

516 ***Table 5. Median age, minimum and maximum (months) for breed and non-breed at presentation***

517 ***with specified disease, in ten primary care practices (non-breed being mixed-breed dogs or***

518 ***European Shorthair cats).***

Breed	Disease	Median (min-max)		p value
		Breed	**Non-breed**	
Chihuahua	Dystocia**	31.2 (13.2-67.2)	-	-
	Extremities	20.4 (2.4-108)	67.2 (4.8-183.6)	<0.01*
	Hypoglycaemia**	2.4 (2.4-3.6)	-	-
	Liver	-	115.2 (30-133.2)	1
	Spinal column	42 (24-122.4)	102 (9.6-183.6)	0.412
French bulldog	Dystocia**	52.8 (12-70.8)	-	-
	Ears	39.6 (2.4-142.8)	61.2 (3.6-194.4)	0.419
	Eyes	62.4 (1.2-148.8)	63.6 (1.2-199.2)	0.822
	Spinal column	44.4 (10.8-133.2)	100.8 (2.4-177.6)	<0.01*
	URT***	27.6 (0.24-104.4)	43.2 (2.4-163.2)	0.537
Labrador retriever	Extremities	75.6 (4.8-178.8)	85.2 (2.4-188.4)	0.664
	Liver	146.4 (98.4-154.8)	120 (14.4-154.8)	0.206
	Skin and coat	74.4 (2.4-178.8)	72 (2.4-85.2)	0.810
	Spinal column	117.6 (44.4-178.8)	109.2 (16.8-178.8)	0.756
	Urinary tract	93.6 (34.8-172.8)	109.2 (2.4-174)	0.682
Persian cat	Dystocia	-	-	-
	Eyes	105.6 (3.6-198)	60 (1.2-183.6)	0.22
	Kidneys	158.4 (61.2-195.6)	140.4 (8.4-200.4)	0.572
	Skin and coat	55.2 (24-72)	-	1

519 *Significant difference median tested with Mann-Whitney U test. **Dystocia evaluated in a female sample, hypoglycaemia in a

520 juvenile sample. ***URT = Upper respiratory tract

521

522

APPENDICES

APPENDIX 1

Standardised questionnaire for specialist veterinarians in qualitative analysis (Meijndert et al., 2014).

1. How often are you consulted for this breed within your specialty? (never – occasionally – frequently – often)
2. What is the estimated percentage of this breed among your patients?
3. What is the most common diagnosis? (if more, put the first three in order)
4. Is this the same as the diagnoses listed (*Appendix 3*) (Add/remove diseases from selection list)

    Go through appendix list and discuss per disease:

    -Frequency of occurrence in this breed (never – occasionally – frequently – often)

    -Clinical symptoms at presentation

    -General age at presentation with this disease

    -Sex of patients with this disease

    -Minimal diagnostic measures for this disease

    -Known connection to breeding standards or suggested heritability.
5. Are you under the impression that there is a difference in the occurrence of disease in dogs with a pedigree and the so-called 'look-alikes' without a pedigree?
6. Do you have any additional comments or questions about the discussed breeds with respect to your veterinary specialty?

548 **APPENDIX 2**

549

550 **Medical disciplines included in the referral clinic case control study, in alphabetical order.**

551

Cardiology – pulmonology	Nephrology
Dermatology	Reproductive medicine
Endocrinology	Oncology
Gastroenterology	Ophthalmology
Haematology	Orthopaedics – neurosurgery
Hepatology	Otorhinolaryngology
Neurology	Urology

552

553

554 **APPENDIX 3**

555

556 **Selection of organ systems and diseases per breed to be quantitatively analysed in a random**
557 **sample of patient files from ten primary practices (Meijndert et al., 2014).**

558

Breed	Organ system	Disease	Source
Chihuahua	Extremities	Patellar luxation	lit,exp
	Liver	Extrahepatic portocaval shunt	lit,exp,clinic
	Pregnancy and parturition	*Dystocia caused by obstruction and contraction*	lit,exp
		Hypoglycaemia in puppies and lactating bitch	lit,exp
	Spinal column	HNP type 1 - cervical, atlanto-axial	lit,exp
French bulldog	Ears	Otitis externa	lit,exp,clinic
	Eyes	Cataract	lit,exp,clinic
		Cornea ulcera	lit,exp,clinic
		Cherry eye	lit,exp,clinic
		Entropion	lit,exp
	Pregnancy and parturition	*Dystocia by obstruction*	lit,exp
	Spinal column	Hernia Nucleus Pulposus type 1	lit,exp,clinic
	Upper respiratory tract	*Brachycephalic Obstructive Syndrome*	lit,exp,clinic
Labrador retriever	Extremities	Elbow dysplasia	lit,exp,clinic
		Enostosis	lit,exp
		Hip dysplasia	lit,exp
		Sesamoid bone fracture	exp
		Tendovaginitis biceps	exp
	Liver	Copper-associated hepatitis	lit,exp
		Idiopathic hepatitis	lit,exp
		Intrahepatic portocaval shunt	lit,exp
	Skin and coat	Atopic dermatitis	lit,exp
		Food hypersensitivity	lit
		Licking granulomas	lit
		Nasal parakeratosis	lit
		Pododermatitis	lit,exp
		Primary seborrhea	lit
	Spinal column	Lumbosacral stenosis	lit,exp
	Urinary tract	Ectopic ureter	lit,clinic
		Juvenile cystitis	exp
		Sphincter incontinence	exp
Persian cat	Eyes	*Corneal ulceration/sequester*	lit,exp,clinic
		Teary eyes	lit,exp,clinic
	Kidneys	Polycystic Kidney Disease	lit,exp
	Pregnancy and parturition	*Dystocia by obstruction*	lit*

Skin and coat	Dermatofytosis	lit,exp

Italic – connection to breed standards assumed on biological and pathophysiological grounds; sources are lit=literature, exp=expert opinion, clinic=referral clinic case control study; * added by authors for practice-based extended cross-sectional study because of anatomic analogy with brachycephalic dog breeds.

APPENDIX 4

Search terms used in quantitative research in randomly selected patient files, from ten primary practices (adapted from Dutch search terms (Meijndert et al., 2014)).

Chihuahua

1. Liver

Hepat-, shunt, icterus, liver-, HE, yellow

2. Spinal column

Paresis, paralysis, -failure, back-, hernia, HNP, atlanto-, atlas, neck-

3. Extremities

Limp, patella-, knee-, lux-, PL

4. Pregnancy and parturition

Partus, labour, dystocia, C-section, sectio, hypoc-, weakness, hypogl-, nausea, vomiting, born

French bulldog

1. Spinal column

Paresis, paralysis, neurological deficit, back-, hernia, HNP

2. Upper respiratory tract

Snor-, stridor, dyspn, dyspn-, BOS, palat-, nose-

3. Ears

Otit-, ear-

4. Eyes

Cornea-, ulcus, eye-, cherry, entropion, cataract, FL+, suture nicti-

5. Pregnancy and parturition

Partus, labour, dystocia, C-section, sectio, nausea, vomiting, born-

Labrador retriever

1. Liver

Hepat-, shunt, icterus, liver-, HE, yellow

2. Spinal column

Back-, lumb-, LS

3. Extremities

Limp, hip, elbow-, grow-, HD, ED, enosto-

4. Urinary tract

Cystitis, bladder, inconti-, sphincter, ureter-, urine loss

5. Skin and coat

Itch, pruritus, alopecia, allerg-, bald-, atopi-, flake, scale, sebor-, hair loss, planum

Persian cat

1. Eyes

Cornea-, ulcus, eye

2. Kidneys

Kidney-, PKD, CIN

3. Skin and coat

Dermatophyt-

4. Pregnancy and parturition

Partus, labour dystocia, C-section, sectio, nausea, vomiting, born