Data-based Approaches for Healthy Companion Animal Breeding

Sylvia Keijser

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2019

Cover concept: Guido Hommel, Sylvia Keijser, Hille Fieten.
Lay-out: Sylvia Keijser. Design: GVO.
Printed: GVO drukkers & vormgevers, NL.

Keijser, S.F.A. – **Data-based Approaches for Healthy Companion Animal Breeding**
PhD thesis, Faculty of Veterinary Medicine, Utrecht University, 2019

ISBN: 978-94-6332-556-1

Publication of this thesis was financially supported by: Ministerie van Economische Zaken en Klimaat, Koninklijke Nederlandse Maatschappij voor Diergeneeskunde (KNMvD), Dactari Dierenartsencoöperatie, Sterkliniek Dierenartsen B.V., Nederlandse Herdershonden Club (NHC, rasvereniging voor de Kort-, Lang- en Ruwharige Hollandse Herdershond.), Nederlandse Labrador Vereniging (NLV), Reaal Dier & Zorg, Royal Canin Nederland B.V., IDEXX Animana, Nederlandse Databank Gezelschapsdieren (NDG).

Data-based Approaches for Healthy Companion Animal Breeding

Het gebruik van data voor het
fokken van gezonde gezelschapsdieren

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof.dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op maandag 30 september 2019
des ochtends te 10.30 uur

door

Sylvia Florence Antoinette Keijser

geboren op 11 november 1981 te Delft

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Content

Setting the scene

Disease phenotype epidemiology

Genetic testing

Summarizing discussion and conclusion

Chapter 1

General introduction, aims and scope

The dog and cat have been on man's side for a long time.

The grey wolf is the common ancestor of all dogs. Canine domestication started more than 15 thousand years ago, when the wolf began to live closer to man and experienced genetic bottlenecks in the process. What followed was an actual companionship bond and selective breeding, creating different breeds with a focus on different tasks in cooperation with man. Selective breeding excluded individuals from procreation, thereby limiting the genetic material available for the next generation. This caused increased genetic homozygosity, i.e. reduced variety in the genome and breed associated variations (1). Although some dog breeds were already described around 2,000 years ago, it took until the 19th century before dog breeds and breed groups became explicitly recognised. Nowadays, the breeding of dogs (and cats) is not limited to a kennel club or breed organisation; individual and commercial initiatives are also present. Originally, dogs were grouped based on their work abilities, but this clustering slowly shifted to include companion animals with specific phenotypic characteristics (2).

The start of feline domestication was long thought to lie in Egypt 3,600 years ago, where the cat was considered a deity. However, as shown in a timeline research (3), the cat had found its way into man's proximity almost 6,000 years earlier. The archaeological findings of a human and cat burial site on the island of Cyprus, a location where cats did not naturally live, suggested transportation and a relationship between the two species. As with the dog, modern breeds were thought to have developed in the 19th century. However, no breeding pressures existed for the cat as they did for the dog regarding, for example, herding ability. This thesis primarily focusses on canine breed health.

The variety in breed phenotypes through gradual selection is the result of just a small number of gene variants (4). In the last decade, society has become aware that certain aspects of dog breeds and the limiting of genetic material actually causes breed-specific health issues. These issues were most notably publicised by a television documentary discussing breed standards and breeding practice compromising health (5). Breed health issues are now considered to be one of the main concerns in canine welfare. Two kinds of issues can be identified. Firstly, inherited diseases, which may accidentally increase in frequency in the population due, for example, to inbreeding. Secondly, harmful breed characteristics that are related to extreme exterior features. These extreme features have been intentionally bred as they were seen as desirable by breeders and prospective owners. In a two-part literature review Asher, Summers and colleagues investigated the two types of breed-related health issues for the 50 most popular pedigree dog breeds in the United Kingdom. The investigation shows that each of the 50 breeds was thought to have at least one health issue related to physical conformation. The total number of health issues unrelated to conformation came to

more than 300 for these 50 breeds. This extensive review of breed health made the need for a reliable and population-specific quantification of the problem, both at the population level and at the individual level, clear (6,7).

Alongside the quantification of breed-related health issues at the population level, a large number of DNA tests are available which test for certain diseases or for the genetic variety within an individual or a population. For example, there are some breed-specific tests available, and although these may be useful, they are not being used. It is also possible to perform tests to check for low prevalence or non-existent disorders in a breed. A problem with many of these tests is that they need further research or require a knowledgeable interpreter. Furthermore, not every genetic disorder can be tested, despite the fact that the disorder may affect breed health (2).

As previously concluded by Asher and Summers in relation to the UK (6,7), no quantitative data was available regarding the health status of the Dutch population of companion animals. In order to prioritise and organise an effective approach to breed health, information is needed indicating which health issues occur, and their impact. Additionally, in order to help guide breeders in improving companion animal health, it would be useful to gain insight into the multitude of DNA tests available.

Aims and scope

The main aims of the research described in this thesis are to 1) to gain insight into how to quantitatively evaluate breed health issues in the Dutch companion animal population, and 2) to combine such phenotypic results with genetic data to support a sensible and substantiated breeding process.

Chapter 2 describes aspects of canine breed health as discussed at an international conference with representatives from a multitude of stakeholders, including kennel clubs, breed organisations, researchers, and private owners. Discussion groups determined the challenges and priorities of six subthemes: individualised breed-specific strategies for health and breeding, extreme conformations, education and communication, behaviour, genetic testing, and population-based evidence.

In **Chapter 3,** data from various sources (veterinary practice, two insurance companies, and a histopathological laboratory) were used to quantify Labrador retriever health and compare this to the health of mixed-breed dogs. The evaluated parameters were longevity, the number of visits to a veterinary practice, expense claims at two insurance companies, and specific diagnoses in the insurance and laboratory data.

Chapter 4 describes the performance of a qualitative query, which was followed by a referral clinic case control study and a practice-based extended cross-sectional study. A selection of potentially relevant disorders, limited to five organ systems, was examined in the cross-sectional study for each of the breeds under study: three purebred dog breed populations (Chihuahua, French bulldog, and Labrador retriever) and one purebred cat breed (Persian cat).

The implementation and application of a newly developed software tool for collecting companion animal population data is discussed in **Chapter 5**. The chapter uses preliminary data to examine the reliability of the collected data, and discusses the future potential for prioritisation of genetic studies.

Selective breeding, especially in populations of limited size, may result in a decrease in genetic diversity. **Chapter 6** focuses on genetic testing for heterogeneity and disease screening as a tool in dog breeding. The chapter discusses the application in breeding strategies, as well as the importance of investigating the clinical relevance of mutations found during screening.

The overall findings and the future perspectives of the studies considered in this thesis are summarised and discussed in **Chapter 7**.

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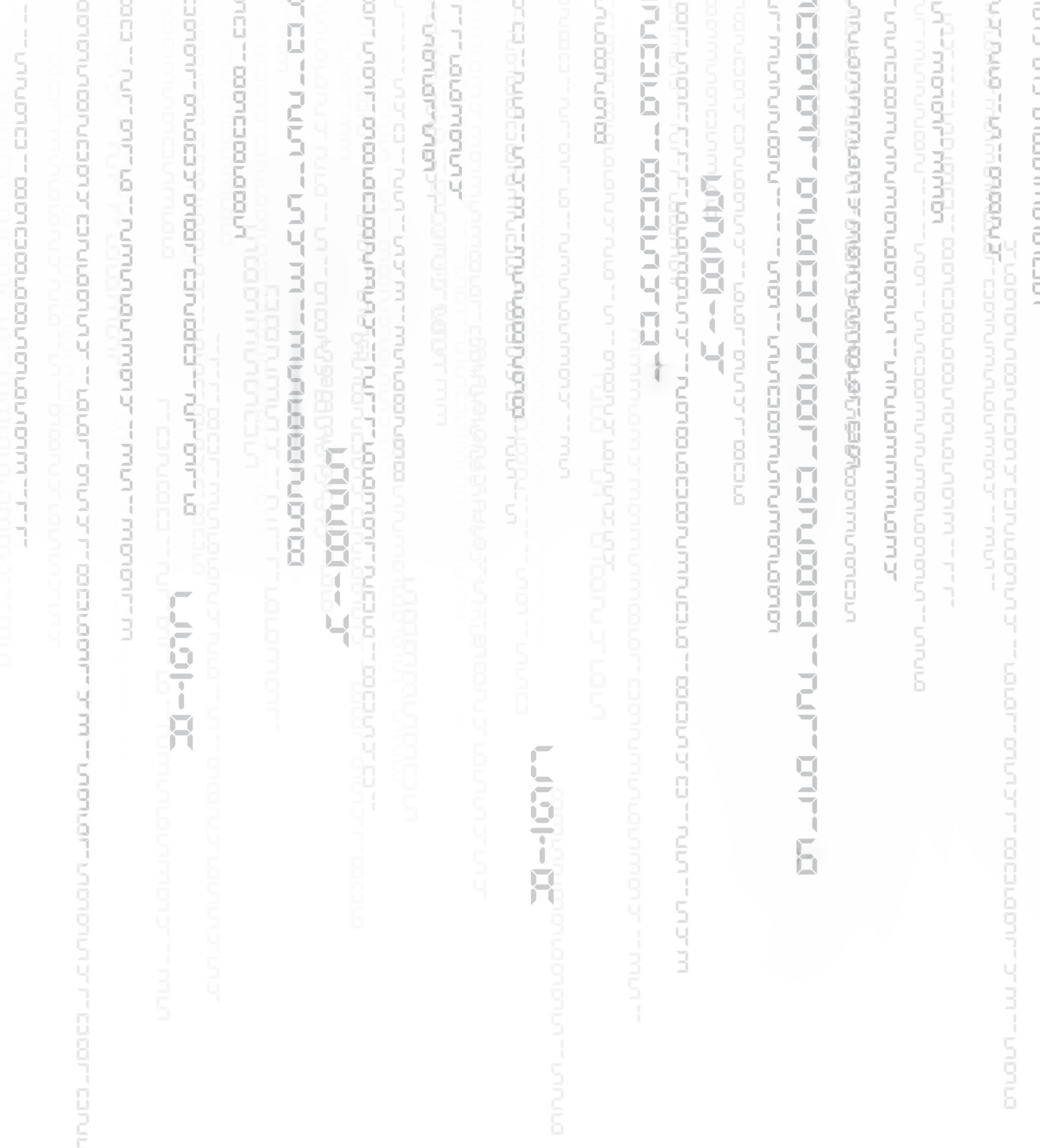

Chapter 2

Moving from information and collaboration to action

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Canine Genet Epidemiol. 2017 Dec 7;4:16.

Abstract

Breed-related health problems in dogs have received increased focus over the last decade. Responsibility for causing and/or solving these problems has been variously directed towards dog breeders and kennel clubs, the veterinary profession, welfare scientists, owners, regulators and the media. In reality, all these stakeholders are likely to share some responsibility and optimal progress on resolving these challenges requires all key stakeholders to work together. The International Partnership for Dogs (IPFD), together with an alternating host organization, holds biennial meetings called the International Dog Health Workshops (IDHW). The Société Centrale Canine (French Kennel Club) hosted the 3rd IDHW, in Paris, in April, 2017. These meetings bring together a wide range of stakeholders in dog health, science and welfare to improve international sharing of information and resources, to provide a forum for ongoing collaboration, and to identify specific needs and actions to improve health, well-being and welfare in dogs.

The workshop included 140 participants from 23 countries and was structured around six important issues facing those who work to improve dog health. These included individualised breed-specific strategies for health and breeding, extreme conformations, education and communication, behaviour, genetic testing and population-based evidence. A number of exciting actions were agreed during the meeting. These included setting up working groups to create tools to help breed clubs accelerate the implementation of breed-health strategies, review aspects of extreme conformation and share useful information on behaviour. The meeting also heralded the development of an online resource of relevant information describing quality measures for DNA testing. A demand for more and better data and evidence was a recurring message stressed across all themes.

The meeting confirmed the benefits from inclusion of a diverse range of stakeholders who all play relevant and collaborative parts to improve future canine health. Firm actiosn were set for progress towards improving breed-related welfare. The next international workshop will be in England in 2019 and will be organised by the UK Kennel Club.

Background

Breed-related health problems in dogs, especially inherited diseases in pedigreed dogs, have received increased attention in the media and veterinary literature over the last decade, and this has been followed inevitably by a public blame game [1]. Some place the responsibility for breed-related health problems firmly on dog breeders and kennel clubs by focussing on ill-advised selective-breeding decisions and lack of proactive measures for dog health [2]. Other authors have suggested that the veterinary profession could have been more proactive [3, 4], while yet other studies have addressed the role of consumer attitudes and actions [5, 6]. In reality, all these stakeholders, as well as others such as the media and celebrities who popularise certain breeds [7, 8], are likely to share some responsibility because each plays important but differing roles in promulgating various aspects of this complex issue of breed-related health problems in dogs. Efforts to understand and address health and welfare problems in dogs are complicated by issues around the sourcing of puppies. In many countries, the majority of apparently purebred dogs are thought to come from commercial breeders who are not registered with relevant kennel clubs and therefore may fall outside the normal influences, controls and regulations of such bodies (HSUS 2016 www.humanesociety.org). Clearly, this all leads to a very complex situation and optimal progress on resolving these challenges will be achieved only if key stakeholders can coordinate and work together to embrace positive and evidence-based change. A critical element required for such progression is the provision of a forum for formal and informal discussions between all relevant groups where key issues can be identified and defined, and plans can be agreed for effective actions to address them.

There are undoubtedly many important issues facing those who work to improve dog health. In this publication, we focus on six in particular. Although some over-arching concepts may apply across all dogs worldwide, individualised breed-specific strategies for health and breeding are needed and may vary by country [9, 10]. Complex conditions, such as those associated with brachycephaly and other extreme conformations, negatively impact not only the health but also the welfare of individual dogs [11, 12]. The intricacies facing stewards of well-being in dogs including kennel clubs, breeders, veterinarians, scientists and regulators are such that that collaborative, international and multi-stakeholder efforts on education and communication are needed [4, 13]. In order to breed healthier dogs, many other aspects of canine health need to be considered, as not all challenges are traceable solely to genetic influences; for example, disease, behaviour, and welfare also interact to influence dog health [9]. Great advances in the study of genetic disease have led to a growing plethora of genetic tests but the complexity of optimal usage of these tests has also caused breeders, kennel clubs and breeding advisors to struggle as they try

to reduce the burden of inherited diseases in the dog population [10, 14]. And finally, the true burden of disease within individual breeds, as well as across national populations of dogs, is poorly understood and there is little reliable population-based and generalisable evidence to quantify the prevalence of various conditions [15].

In June 2012, the first International Dog Health Workshop (1st IDHW) [16] was organized by the Swedish Kennel Club and held in Stockholm, Sweden as a satellite meeting to the 6th International Conference on Advances in Canine and Feline Genetics and Genomics. The 1st IDHW brought together representatives from many of the groups that share a responsibility for dog health. Numerous recommendations came from the workshop, including that an international platform for collaboration among stakeholders in dog health and welfare should be developed (i.e., a suggested prototype that later spawned the International Partnership for Dogs (IPFD) and DogWellNet.com) [17]. Another key recommendation was that (standardized) procedures for and validation of both DNA-tests and testing laboratories should be defined and communicated, along with recommendations for proper use of genetic testing in different populations (which has led, eventually, to the Harmonization for Genetic Testing in Dogs (HGTD) initiative, see below).

The 2nd International Dog Health Workshop (2nd IDHW) in Dortmund, Germany in February 2015 was coordinated by the IPFD and the German Kennel Club (VDH) [18]. This meeting marked the launch of DogWellNet.com [17], the internet platform of the IPFD which was registered as a non-profit organization in August 2014. The IPFD was initiated by several national Kennel Clubs (Sweden, Finland, Germany, France, Norway, the UK and the USA) and other stakeholders in dog health including The Orthopedic Foundation for Animals, USA [19] and the Agria Pet Insurance-SKC Fund, Sweden. The Fédération Cynologique Internationale [20] represents 91 national kennel clubs and is an Initiating Patron and Member. The Irish KC [21] is a partner; and, current collaborating partners also include VetCompass (UK) [22], the Australian Shepherd Health and Genetics Institute (ASHGI0 [23] as well as this journal (Canine Genetics and Epidemiology, CGE). More recent Corporate Partners include Mars Veterinary and Royal Canin while additional collaborators and sponsors are being sought from all stakeholder groups. The IPFD's mission is to facilitate collaboration and sharing of resources to enhance the health, well-being and welfare of pedigreed dogs and all dogs worldwide.

In April 21-23, 2017 the IPFD 3rd International Dog Health Workshop (3rd IDHW) [24] was hosted by the Société Centrale Canine (French Kennel Club) in Paris, France. Major sponsors were Agria Pet Insurance (Sweden, UK, France) and Royal Canin. The objective of this article is to present a summary of the structure, goals and outcomes of this meeting that can inform and engage stakeholders and act as a blueprint for progress assessment at the planned 4th IDHW in the UK.

Meeting Format

The 3rd IDHW followed a similar format to the previous meetings. Organized along a working and networking framework, the IDHWs are designed to identify and prioritize issues and challenges in breeding, health and welfare of dogs, to encourage dialog across stakeholder groups, to promote international collaboration and action, and to define and address common goals. In total, 140 participants from 23 countries attended the 3rd IDHW and comprised decision-leaders from most major stakeholder groups in dog health and welfare. The attendees were diverse and included breeders, members of breed club health committees, kennel clubs, breeding advisors, veterinarians, educators, researchers, geneticists, behavioural specialists, regulators, welfare organizations, industry, media, health campaigners, dog owners and show judges.

The meeting was formatted around the 6 key themes outlined above as issues that regularly feature as discussion points in relation to breed-related health in dogs (*table 1*). Short plenary presentations from international experts on the morning of the first day were followed by breakout sessions for each theme over the two days that were interspersed with two sharing sessions in plenum. The format was designed to maximize communication and networking while at the same time clustering recognised experts within theme hubs to encourage original thinking and solutions.

From the outset, it was emphasised to all delegates that IPFD and the 3rd IDHW aimed to provide the forum and structure to support collegiate progress in dog health but that it was neither the mandate nor the intent of IPFD to directly produce regulations or directives. The participants were provided with information relevant to their specific themes in advance of the conference in order to focus activities both during and after the meeting. Possible outcomes suggested as desirable from the themes included sharing of existing information, templates, and tools; identification and prioritisation of key actions to support breed-related health; development of collaborative strategies and community building. The strapline for the 3rd IDHW was 'from information and collaboration to action' and therefore, *a priori*, the meeting aimed to go beyond mere discussion to generate meaningful outcomes. Pre- and post-meeting resources and material for the 3rd IDHW are available on DogWellNet.com [17]. This paper summarizes the discussions, recommendations and actions identified and committed to by participants during the 3rd IDHW.

Work Themes and Outcomes

As described above, the meeting was structured around 6 key themes that were identified in advance as offering substantial opportunity for action to improve breed-related health in dogs (*table 1*). Each theme is described below with information provided on the discussions that took place and any actions proposed by participants.

Table 1. Six overall themes for the 3rd International Dog Health Workshop in 2017 in Paris, France.

Theme	Session leader(s) (number of participants)
Breed-specific health strategies: needs and opportunities; innovations, nationally and internationally.	Helena Skarp, Sweden; Ian Seath, UK; Gregoire Leroy, France. (34)
Exaggerations and extremes in dog conformation: health, welfare and breeding considerations; latest national and international efforts.	Åke Hedhammar, Sweden; Rowena Packer, UK; Kristen Prestrud, Norway (27)
Education and communication: how can international collaboration improve education and communication within and across stakeholder groups [especially between veterinarians and breeders]; using the example of antimicrobial resistance.	Gilles Chaudieu, France; Jason Stull, USA (13)
Behaviour and welfare: how can we better integrate actions to address issues in welfare, behaviour and health in breeding and raising dogs?	Nathalie Marlois, France; Patricia Olson, USA; Caroline Kisko, UK (15)
IPFD Harmonization of Genetic Testing for Dogs: an international, multi-stakeholder initiative to address selection, evaluation and application of genetic testing.	Aimee Llewellyn-Zaidi, USA; Brenda Bonnett, Canada (34)
Show me the numbers: integrating information from various sources for prevalence, risks and other population-level information; latest national and international strategies to collect data and disseminate information.	Dan O'Neill, UK; Sylvia Keijser, The Netherlands; Sofia Malm, Sweden (14)

1. Breed-specific Health Strategies

Breeding advisors, breed clubs and individual breeders frequently struggle with two main issues. First, how to define and understand the 'big picture' for their breed in terms of disease, genetics, population numbers, breeding and general management. Second, how to process all the complex inputs that affect the health and welfare of their dogs. Without access to full information, adequate evidence or effective tools to define the big picture, stakeholders tend to view challenges more narrowly and in the shorter term. This often means that they end up running after the DNA 'test

of the month' or imposing knee-jerk reactions to media storms that may lead to breeding strategies that change again as soon as the executive of the breed club changes. Optimal and selected approaches to managing health and disease at a breed level also vary widely across countries, kennel clubs, breed clubs and breeds. It is therefore important to build on experiences from these different countries and groups, in order to facilitate exchange and collaboration, harmonise health assessment and screening programmes, and propose optimal strategies and health strategies for use at the breed level. This was the background to the session on breed-specific health strategies.

The participants in the session were truly multi- and inter-disciplinary, with a liberal mixture of geneticists, veterinarians and epidemiologists, but also breeders, owners and dog-health campaigners. With 34 participants, this was the most popular stream at the workshop. Ian Seath (UK), Chairman of the UK Dachshund Breed Council [25], shared his experience based on the approach taken by his breed council and stressed the importance of applying accepted business management elements including leadership, planning, engagement and improvement.

The group agreed that effective and sustainable implementation of health strategies requires innovative solutions to many different challenges. Provision of sufficient and reliable information was agreed as critical, for both situational assessment as well as day-to-day screening of dogs. On the one hand, a diversity of survey templates for breed health assessments have been developed and are available for individual breeds [26]. On the other, veterinary screening programmes and diagnosis-based research requires harmonization across breeds for effective application in health programmes, especially at an international scale. Considering the design of health strategies, the group decided that it was important to identify and balance the major issues for each individual breed and give guidelines on how priorities could be determined for each [4], while still allowing breeders some discretion to make their own decisions within an overall framework of requirements and recommendations. The group agreed that it would be useful to develop a model to evaluate generic breed problem categories (e.g. inherited disorders with DNA test available, multi-factorial conditions with existing screening programmes) in order to define breeding strategy solutions (e.g. breeding recommendations based on DNA tests adapted to disorder prevalence, development and use of estimated breeding values (EBV)). Importantly, the group also concurred on the deleterious consequences of inappropriately removing dogs from the breeding gene pool when breeders failed to understand the conflicting influences and effects that may arise from disease control strategies versus a need for genetic diversity.

The group considered that achieving compliance by breeders and owners to recommended or required screening and breeding guidelines was a challenge for breed-based health strategies. Imposition of mandatory screening programmes and open registries of test results as a prerequisite

for kennel club registration could result in breeders choosing to breed non-registered dogs instead. However, lack of adherence to programmes and incomplete data pose significant barriers to achieving health improvement. This underlined the importance of education and communication between the different stakeholders (including breeders, owners, veterinarians, geneticists and judges) in the design and implementation of effective health strategies.

The group discussed their diversity of experiences across countries in relation to breed-specific health strategies and access to their local resources and tools that could be shared more widely. The use of the DogWellNet.com platform website as a repository for such resources was recommended. The general conclusion was that there is no "one size fits all" solution for developing breed-specific health strategies and that the most effective interventions would be adapted according to the specific context of each breed [27]. The impact of national cultures on successful approaches can be significant. For example, the Nordic countries enjoy a culture of regulation and compliance from breeders and have advanced breed-specific strategies in place. However, , a similar regulatory approach would risk driving breeders away from their kennel club's sphere of influence in other locations (e.g. the Benelux and Southern Europe regions).

The group felt that a more holistic approach to breeding was needed, with greater focus on population-specific situations and reduced emphasis on breeding decisions based solely on single diseases and DNA tests. To that extent, the group considered that it was inadvisable to conduct health strategies within individual breeds focused on single diseases independently from a more broadly focused breeding strategy. The participants agreed to set up a working group, led by Professor Jerrold Bell and including 12 other participants from the workshop, to take forward the ideas discussed and to create a set of resources and tools that could help breed clubs to accelerate the creation and more importantly, the implementation, of strategies that benefit their dogs. Subgroups from this working group will also work on the development of breeding strategies for specific breeds, such as the Bernese Mountain Dog or Dachshund.

2. Exaggerations and Extremes in Dog Conformation

As the popularity of small-sized flat-faced breeds continues to increase around the world, the health and welfare of brachycephalic breeds has become an increased priority issue. Rowena Packer (UK) outlined current understanding of health consequences from extreme brachycephaly in her plenary presentation and described mounting evidence of breathing, thermoregulation, eye, skin, spinal and birthing problems associated with this phenotype [28-32]. In consequence, this theme elected to focus exclusively on brachycephalic health, with specific emphasis on breathing

problems (brachycephalic obstructive airway syndrome; BOAS) that were considered the most severe welfare concern in brachycephalic breeds [33, 34]. However, key points of the discussion also relate more generally to other issues of exaggerations and extremes.

Discussions across the 27 participants covered both current efforts, such as the formation of brachycephalic working groups in the UK [35] and by the Nordic Kennel Union [36], whilst also debating alternative future strategies. Although kennel clubs have developed initiatives to improve brachycephalic health (e.g. 'Breed Watch' in the UK [37], 'Breed Specific Instructions' in the Nordic region [38]), significant challenges remain. It is increasingly clear that the brachycephalic issue is largely a 'human' problem, with change hindered by frequent 'blindness' to the health problems in these breeds, and 'normalisation' of their health issues [39, 40].

The group formulated 5 goals to improve brachycephalic health:

1. Kennel clubs and the FCI should further educate breeders and judges on brachycephalic health and police those who promote unhealthy practices; encourage/enforce fitness tests [41, 42] prior to breeding/showing; and review breed standards to remove features detrimental to health and increase their objectivity.
2. Show judges should be well-educated on the detrimental consequences of extreme conformation; interpret breed standards with canine health in mind; and only award prizes to less extreme dogs that are free of signs of ill-health.
3. Breeders should choose less-exaggerated breeding stock that have undergone appropriate health testing for breeding.
4. Puppy buyers should have enough knowledge to make informed choices, should not focus solely on looks and should demand increased health testing and reduced exaggeration.
5. Veterinarians should be actively involved in breed health, via e.g. breed health testing; education of puppy-buyers via pre-purchase visits; and participation in data collection (e.g. reporting of conformation-altering surgery and caesarean sections) and sharing clinical data with national epidemiological research programmes.

To achieve these goals, sub-groups were created, who will work to:

1. Document ongoing international projects on brachycephalic health to promote collaboration and share best practices.
2. Compare current methods to measure exercise tolerance with a view to validation and harmonisation.
3. Quantify brachycephaly-related disorders in registered and non-registered populations.
4. Identify phenotypic and genetic variation *within* breeds to evaluate whether this variation can be utilised as an alternative to outcrossing e.g. unregistered dogs and breed variants.

5. Review breed standards to highlight points that encourage exaggeration or allow misinterpretation.
6. Evaluate the ways in which human behaviour can be changed; including judges, breeders and puppy-buyers.
7. Influence media portrayal of brachycephalic breeds to move from promotion of extreme breeds in mainstream advertising to communication of educational messages.

The working groups are committed to these actions, and joint coordinator Kristin Prestrud will present these plans at the WSAVA/FECAVA congress 2017 [43].

3. Education and Communication of Antimicrobial Resistance

The emergence and expansion of antimicrobial resistance (AMR) has been widely documented and challenges current antimicrobial therapy protocols. It has increased human and veterinary treatment costs and patient morbidity and mortality [44, 45]. AMR is geographically widespread and can be transmitted between humans and animals [46, 47]. AMR remains a challenge in veterinary medicine with limited and differing guidelines across countries that results in fragmented communication and education approaches.

The AMR theme subgroup reviewed selected materials covering national AMR guidelines [48-51], antimicrobial prescribing pressure in healthcare [52], and AMR transfer between people and companion animals [53] prior to the meeting. The conference plenary presentation from Jason Stull (US) further explored these topics and highlighted issues such as unnecessary/inappropriate antimicrobial prescribing in medicine, lack of studies addressing usage in breeding dogs, and stressing the importance of targeting behavioural change in antimicrobial use at multiple levels (i.e., intra-personal, inter-personal, community, institutional) [54-56].

The subgroup included 13 participants representing five countries from sectors including academia, veterinary medical associations, private practice, pet insurance, kennel clubs and foundations. An initial presentation reviewed actions taken in France to address AMR in companion animals, including development of surveillance collaboration with veterinary practitioners and laboratories (RESAPATH) [55], recent policy and law to reduce usage of critically important antimicrobials, guidelines to promote prudent antimicrobial use, and training and campaigns to create awareness. Following these efforts, a 20% reduction in antimicrobial use in animals was observed (2011 to 2015; estimated to be 10% reduction in dogs and cats) [56]. Other countries have employed similar approaches with comparably successful outcomes [57].

Challenges discussed to replicating the French model in other countries included limited stakeholder buy-in, strong lobbying groups, resistance to top-down approaches, and varying backgrounds of breeding groups across countries. Additional challenges included sustaining and enforcing prescribing requirements and antimicrobial use reporting. Lack of published antimicrobial usage and AMR data in breeding dogs and limited prudent-use guidelines for breeders and veterinarians were considered major limitations. Participants agreed that veterinarians should work collaboratively with breeders to effect change and that a multi-national educational approach aimed at breeders was needed to unify groups and drive positive change.

The group identified four main future priorities to address AMR in dogs:

1. Create a global AMR network comprising key stakeholder groups across countries including IPFD, kennel and breed clubs, veterinary medical associations, and industry.
2. The global AMR network would develop and promote (if not already in-place) antimicrobial use guidelines for breeders and veterinarians aimed at general healthcare and conditions specific to breeding (e.g., use surrounding breeding and whelping) and dog shows (e.g., gastrointestinal signs associated with stress).
3. Identify and develop funding initiatives to support research and surveillance efforts with breeding groups and provide data (antimicrobial use, resistance and perceptions) to support and provide feedback on established guidelines. Relevant studies might include literature review and data collection specific to AMR, breeding, and antimicrobial use practices; studies establishing normal and antimicrobial-induced alterations to relevant microbiomes (e.g., vaginal).
4. Development of certificate and learning modules for breeders and veterinarians in order to provide education and communicate developed guidelines. Module materials would include information on negative outcomes from imprudent antimicrobial use and alternative approaches to antimicrobials. The modules would encourage the use of storytelling to personalize the issue and target intra-personal, inter-personal and community pressures to alter behaviours.

Given international differences in culture and infrastructure, it is perhaps unsurprising that there is currently a fragmented approach to addressing AMR in dogs across country/region and stakeholder groups. The discussions of this multi-stakeholder international group highlighted the limited information currently published on this topic in breeding dogs and that a unified approach is required to capitalize on current successes and resources. This conference and resulting working groups are an excellent step toward this unified approach.

4. Behaviour and Welfare

Socialisation of puppies at appropriate ages is considered critical for optimal behavioral development of dogs to facilitate their life as pets within human homes. Dogs with appropriate behavioural responses are more likely to remain with owners or adopters, thereby strengthening the human-animal bond and promoting animal welfare and human well-being. Conversely, dogs that display undesirable behaviours may have compromised welfare driven by their underlying emotional motivations for the behaviour (e.g. anxiety) or from how owners/adopters might seek to achieve resolution (e.g. aversive techniques, relinquishment) [58-60].

A thought-provoking plenary talk from Paula Boyden (UK) entitled *The intersection of welfare and behaviour in dogs and relation to health and breeding* set the tone for the theme by focusing on socialization in puppies. Some complex interactions across this topic were highlighted including selection for physical features that may limit expression of normal behavioural communications with dogs and people, and early life experiences that impact later health and welfare and influence human-animal interactions. Examples of puppy programs that support development of positive health behaviours were also described.

The theme included 15 participants with diverse backgrounds from eight countries (France, Sweden, Switzerland, Finland, Germany, Ireland, UK, and USA). The group explored knowledge and beliefs around several aspects of puppy socialization that relate to later behaviours and animal welfare. Topics discussed included the critical sensitive period for socializing, evidence for outcomes with different socialization methods, potential breed differences, gaps in knowledge, access to international literature on the subject, existing programs that might be replicated/tested, correlations between puppy testing and future outcomes of behavior, educational needs for new owners, and educational needs for breeders and other stakeholders.

This group particularly focused on setting goals and refining specific actions to achieve these goals. Six key goals were developed during the workshop:

1. Behavioural consideration should form part of routine pre-breeding decision-making by contributing to breeding choices (e.g. temperament of bitch and sire) Good management should aim to minimize stress throughout pregnancy.
2. Improved behavioural education of breeders (novice, professional, commercial), veterinarians, veterinary students, allied health professionals, novice and experienced owners and handlers.
3. Address issues that may adversely affect ideal socialization including sourcing issues such as importation, puppy mills/farms, pet stores.

4. Develop simple and powerful public messages that promote the benefits of purchasing an appropriately socialised puppy.
5. Determine which (if any) excellent socialization programmes already exist, and replicate widely.
6. Consider that individual puppies may require adapted socialization protocols.

The participants prioritised 5 action items to be addressed by members of the group over the following 24 months.

1. Prepare public messages that will promote the acquisition of well-socialised puppies.
2. Conduct a comprehensive, international literature review to identify evidence-based socialisation/puppy testing methods.
3. Following this literature review, identify research gaps whereby academic centers might generate topics for future scientific studies of socialisation methods (e.g. longitudinal/prospective studies).
4. Identify previously unpublished but useful data that might be analyzed and published to increase the body of evidence on socialisation.
5. Survey national kennel clubs for socialisation materials/resources that could be validated and replicated internationally.

Throughout the workshop, the information and experiences shared by participants were highly instructive and led to shared goals for international collaboration. The group agreed that puppy socialisation has many important requirements, from providing excellent prenatal care, to minimising stress throughout pregnancy and minimising fear with proper housing, addressing critical times for introducing puppies to novel environments/people, and determining the evidence/outcomes for various methods utilized. While proper socialisation should not be considered the only criterion for producing healthy puppies, it was deemed a critical for developing a dog with good behavior and a chance for a good life.

5. IPFD Harmonization of Genetic Testing for Dogs

Increasing demand for genetic testing has led to a boom in led to a boom in for-profit and non-profit, commercial and academic genetic test providers (GTPs) and available tests [61]. Defining "good quality" GTPs and DNA testing, in the current absence of independent regulation, is almost impossible for dog owners, veterinary scientists, and breed/kennel clubs [62].

In parallel with an increase in breeding policies incorporating genetic testing [63], there are no standards, regulations, or quality control metrics for GTPs providing DNA testing in veterinary

medicine. Along with anecdotal experiences of poor GTPs, this brings genetic testing in dogs broadly into disrepute, and disincentivises conscientious GTPs to maintain high standards. Even in human testing, serious questions are raised about the regulation of medical testing [64].

In response to, and building on discussions at the 1st IDHW and 2nd IDHW, the IPFD has overseen the development of an online resource of relevant information from GTPs describing quality measures (QMs) for DNA testing. Further development into 2018 will include platforms for expert reviews of tests; coordinating a proficiency testing scheme, and genetic advice and education. The model depends on GTPs and multi-stakeholders participating voluntarily. An open-access prototype was developed using data provided by GTPs indicating a spectrum of initial QMs, from international accreditations to customer care. This centralized resource aims to aid kennel/breed clubs, breeding advisors and owners to make better informed decisions on GTPs and testing.

The 34 theme participants included representatives from GTPs, geneticists and researchers, kennel clubs/registration bodies, and owners/breeders. In preparation for the 3rd IDHW, theme participants were provided with a reading list including the prototype description, and recommended websites of similar systems in human/non-companion animal testing (www.dogwellnet.com , www.eurogentest.org , www.orpha.net , www.icar.org , www.acmg.net).

Objectives for the workshop were to encourage stakeholder engagement with the project and to identify experts/ participants for future development of the platform.

Following a plenary presentation from Aimée Llewellyn-Zaidi (US), the theme discussed issues including the independent evaluation of GTPs, individual DNA tests, and genetic advice. The group accepted that most genetics experts affiliate with at least one GTP and therefore may not be truly unbiased. To address this, the IPFD was identified as an independent organization capable of leading a strategy of balanced and collaborative review/assessment of GTPs.

The group felt that building a definitive list of current QMs and GTPs was paramount. An agreed action was to host this list on DogWellNet.com (expected early 2018). Concerns were raised on standardizing QMs across international boundaries and laboratory types (i.e. commercial vs. primarily research laboratories). The result was to form a working group of multi-stakeholders and laboratories to be hosted on a DogWellNet.com forum.

Future priorities included development of a proficiency testing scheme and collation of resources for genetic advice. This lead to forming working groups to address evaluation of genetic testing, advice, sustainability, and proficiency testing. Leaders for each working group are experts in relevant fields, and a balance across stakeholders was determined. External experts would be sought where relevant.

The group considered that the lack of accreditation and standardization across DNA testing is putting the health of dogs at risk. Without adequate guidelines, or external validation, consumers risk making detrimental breeding decisions based on irregular results, or fraudulent activities. Without consumer confidence in DNA testing, GTPs and researchers will struggle, and preventable inherited diseases will continue. The group agreed that the Harmonization of DNA testing for Dogs project, is a major step towards engaging with GTPs, and experts, to improve use of DNA testing.

6. Show me the Numbers: Integrating information from various sources for prevalence, risks and other population-level information

Data-deficiencies are widely acknowledged to constrain improvement in companion animal health [15]. A demand for more and better data was identified across each of the other five themes with a recurring message that actions should ideally be based on good evidence wherever possible. The Numbers theme aimed to identify opportunities to increase the availability of data in order to improve dog health. With 14 participants from six countries, the Numbers group benefitted from inclusion of leading representatives from academia, animal insurance, kennel clubs, data analysis, laboratories and business, enabling discussion on a wide range of data topics.

Participants were provided with selected pre-meeting reading material covering data limitations and opportunities (e.g. [65-69]. A plenary talk from Sofia Malm (Sweden) discussed integration of information from various sources. The first breakout session stimulated debate on the epistemological nature of information as theme leaders, Dan O'Neill (UK) and Sylvia Keijser (Netherlands), directed participants to consider why specific types of health knowledge are often unknown or ignored [70]. All 14 participants contributed enthusiastically and openly during the two-day discussions which identified four main data areas:

1. Data access and the representativeness of data. The group discussed that true representativeness requires a national dog registry and should also include designer types and non-pedigreed purebred dogs [68]. Openness in data sharing was encouraged, but with some caution because of the complexity of such data and challenges to proper interpretation including the choice of appropriate control groups [71].
2. Multifaceted roles for veterinarians. Veterinarians hold key opportunities for generating and disseminating health data in collaboration with owners/breeders.

Examples of successful veterinary data initiatives were cited including VetCompassTM in the UK and Australia [22, 72] and PETscan in the Netherlands [73].

3. Some key factors around data collection:
    a. Cultural impact: each country has its own cultural incentives and potential sources of information that need to be considered for successful data collection.
    b. Impact of funding: passive ignorance of alternative topics is risked when funding focuses on one area. For example, government funding focussed on dangerous dogs could lead to avoidance of welfare research.
    c. Stewardship: the end-users and purposes of the data should be determined in advance to ensure optimal gains.
    d. Dissemination: for real-world impact, data should affect the decisions and actions of stakeholders.
4. Prioritisation of data needs:
    a. Better demographic information was a core need.
    b. Information on prevalence/incidence, risk factors, and geographic spread, as well as, genetic background to disorders and genetic structures of populations. Capturing trends on emerging diseases, for example, could then create predictive data.
    c. Quality-of-life and end-of-life data capture was also considered very important. These data could predict breed longevity, and estimate summary measures of population health (SMPH) such as disability adjusted life years (DALY) and quality adjusted life years (QALY) [74]. The group considered that DALY and QALY data may be more relevant welfare indicators than longevity.

These four main data areas were also echoed from each of the other themes. Some additional numbers-based comments from the other themes included the value of longitudinal evaluation of breed health to assess the impact of programs and that data collection efforts (e.g. breed club health surveys, antibiotic use and AMR, behavioural assessments) need to be enhanced and coordinated. Additional actions to facilitate progress on all identified needs included publishing data results, for example in CGE; creating a meeting place for people who have data or questions regarding data on DogWellNet.com; and exploring funding for knowledge sharing and working together on an international level.

Poster Presentations

Attendees were offered the option to present a poster on topics of relevance to the themes of the 3rd IDHW. The poster presentation proved very popular and included 24 posters that represented research from breed clubs, scientists, students, veterinarians and breeders, and covered not only specific research but also educational and breed-specific programs. The posters offered the authors the chance to present their institution or work in an efficient manner to a large audience while other attendees were easily able to identify useful connections and concepts that might offer future collaborative potential. Posters were not orally presented or judged in order to remove any competitive element; instead the aim was for breadth of topics, easy access and general benefit.

Discussion

The 3rd IDHW was structured around 6 key themes. Attendees were allocated to their specific theme and stayed with this group for the duration of the meeting. In effect, each theme began in the weeks leading up to the meeting with the provision of open delegate lists and selected reading lists for each theme to encourage prior preparation and discussion. At the meeting, the tone for each theme was set by a plenary talk followed by a series of dedicated break-out sessions. To further increase productivity, each theme had at least two session leaders who had been involved for several months in its design and who provided an overview of possible discussion topics and a reference list on various work to focus the thoughts of participants. In addition, each theme was assigned a note-taker to ensure that all ideas and comments were formally recorded to assist with later dissemination. This strategy resulted in higher levels of active contribution from each individual compared with traditional conference formats that rely on mainly didactic lecture programmes and it fitted the aims of the meeting which were to generate new collaborations and actions. This structure could be recommended for future meetings that aim for high participant engagement.

The 3rd IDHW aimed *a priori* to move from information and collaboration to action. Although the precise resultant actions could not be predicted in advance, the lists of actions agreed upon during the meeting suggest that this aim was largely achieved. Within each theme, participants determined their own specific priorities and challenges, and created their own lists of opportunities and needed actions. These reflected the goals of identifying priorities, gaps and actions, as well as building on international communication and collaboration. In many cases, firm plans were drawn up during the meeting to meet these actions. Working groups with specific tasks were identified and many plan to communicate through forum communities on DogWellNet.com. Each of these outcomes are hugely welcome for their own direct value but also because they are strong evidence of the

willingness of the various stakeholders to share data and resources and to work as teams for the greater benefit of canine health. The greatest challenge will be to continue the positive momentum generated by the meeting into sustained action. At the time of publication, several groups from the conference remain very active. Another exciting development post-workshop was an IPFD veterinary student project which has assembled resources on the AMR topic (https://dogwellnet.com/content/hot-topics/antimicrobial-resistance-prudent-use-of-antibiotics/antimicrobial-resistance-resources-r488/).

The poster exhibition represented another effective communication strand from the meeting. The posters allowed participants an opportunity to share their work across the spectrum of attendees, to trigger a two-way dialogue on the work and to build new networks for the future. The presentation of activities and programs, in addition to research, allowed attendees to connect with others working on similar issues. This increased awareness of developments in canine health will underpin sustained collaborative efforts.

Diversity among the participants has been a noticeable feature of all IDHWs. The Workshops are open to all stakeholders in canine health and it was refreshing to see the spectrum of players interacting openly and with little apparent prejudice during the most recent 3-day meeting at the 3rd IDHW. With 140 participants from 23 countries, truly international views on canine health were shared and discussed. The value of this internationality was evident as groups explored the effects of differing national cultures, regulations and organisational structures on canine health programmes and prospects. Given the widespread movement of dogs and breeding material, both legally and illegally, between countries that was reported by many participants, it is clear that canine health in any one country does not exist in isolation but must be considered of substantial relevance to other countries. Participant diversity was equally underlined by the range of professional, organisational and interest-group stakeholders that attended. Organisations included kennel clubs/registration bodies, veterinary medical associations, welfare organizations, animal insurance, academia, regulators, media and foundations. The specific roles encompassed geneticists, veterinarians and epidemiologists, breeders, owners and dog-health campaigners, researchers, educators, data analysts, behavioural specialists and show judges. Many individuals have more than one affiliation and therefore carry out more than one role in relation to dogs. This eclectic mix of organisations and individuals promoted very healthy discussions and novel outcomes and actions. Exposure to opinion that was previously external to many groups was found to trigger original thoughts and solutions as well as building more cross-functional teams than those that normally tend to be assembled for canine health activities.

Despite the diversity of participants, three groups in particular could be encouraged to have greater input at future meetings. Although there were some individuals from each, these groups included the government, the media and general-public owners. First, although governments in most countries are well aware of, and often even involved in, the debate on canine health [75-77], their further engagement as collaborators towards effective solutions would be welcome. Governmental representatives could attend the 4th International Dog Health Workshop (4th IDHW) and, e.g. explain the challenges that regulators face in prioritising effective canine welfare from a legal and political perspective. The power of media as an agent for both positive as well as negative change on the public psyche in relation to dogs is immense. The media can impact awareness of breed health and health-testing, basic canine health knowledge and trends towards breed popularity phenomena [5, 6]. Greater engagement by representatives from the media at future IDHW meetings could offer an effective route towards positive change in public opinion and behaviours. Third, current and future dog owners could lend a useful 'personal' perspective on the supply and demand market within the canine industry as well as allowing an immediate 'reality check' on actions proposed by other groups.

Next meeting

Each of the three previous IDHW meetings have occurred at locations that are easily accessible which has allowed delegates from all around the world to participate in these intensive meetings. In keeping with this, the 4th IDHW will be held in England and will be organised by the UK Kennel Club. At this next workshop, the progress of the range of action plans specified within each theme at the 3rd IDHW will be presented and reviewed. It is anticipated that some of these actions may be completed and that the outcomes can be evaluated. For other actions that are still underway, the meeting will offer an opportunity to review progress and gather fresh input for potential acceleration. For actions that have yet to start or that have been deleted, the reasons for these results can be explored with a view to learning from failure as well as also searching for any opportunities to reset these goals. In addition, the 4th IDHW will allow the exploration of novel themes and the introduction of new delegates to the current worldwide collegiate from previous meetings. Efforts made to improve canine health must never stand still because new methods, knowledge and perspectives are constantly coming available and there are always fresh opportunities for progress.

Conclusions

All three International Dog Health Workshop meetings have confirmed the benefits from inclusion of a diverse range of stakeholders who all play relevant and collaborative parts to improve future canine health. The 3rd IDHW expanded the emphasis on sustainable and measurable actions and outcomes, as well as information-sharing, discussion and networking. Participants were encouraged to share not only their expertise but also to update others on their current areas of work while holding open minds to new collaborations. So far, it appears that the workshop has been successful in terms of open sharing of information and tools, increasing connectivity and prioritization of main needs in canine health improvement. A number of exciting actions have also been agreed. The 4th IDHW will determine if these actions have been realised and whether meaningful improvements in canine health and welfare have been achieved.

Abbreviations

1st IDHW = 1st International Dog Health Workshop 2012

2nd IDHW = 2nd International Dog Health Workshop 2015

3rd IDHW = 3rd International Dog Health Workshop 2017

4th IDHW = 4th International Dog Health Workshop

AMR = antimicrobial resistance

BOAS = brachycephalic obstructive airway syndrome

CGE= Canine Genetics and Epidemiology

DALY = disability adjusted life year

EBV = estimated breeding values

FCI = Fédération Cynologique Internationale

GTP = genetic test provider

IPFD = International Partnership for Dogs

KC = Kennel Club

QM = quality measure

SMPH = summary measures of population health

QALY = quality adjusted life year

VDH = German Kennel Club

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Chapter 3

Quantification of the health status of the Dutch Labrador retriever population

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Accepted Preventive Veterinary Medicine, 2019.

Abstract

Health issues in purebred dogs are currently considered one of the biggest problems in companion animal health. The Labrador retriever (LR) is one of the most popular dog breeds. The aim of this study was to quantify LR breed health in comparison with mixed-breed dogs (MB), by using four different data sources: a veterinary practice management system (appr. 35,000 unique individuals LR + MB), data from two animal insurance companies (appr. 15,500 and 4,500 individuals respectively), and a histopathological laboratory (appr. 4,000 individuals).

After extensive recoding of the data, health parameters utilised to quantify breed health were longevity, frequency of practice visits and insurance expense claims, and diagnostic codes. A Kaplan-Meier univariate and multivariable Cox proportional hazard model were used to evaluate longevity. A negative binomial model was used to analyse the frequency of visits, claims, and diagnostic codes in both sets of insurance data. Logistic regression was used to look into the categorical diagnostic codes in the laboratory data.

The median lifespan of the LR was similar (12 years, practice data) or longer (10 versus 8 years, insurance data) than MB for individuals with a known birth and death date. When including censored individuals, survival time in the LR was comparable to MB individuals up to 10 years of age. Above 10 years of age, the LR lived a similar length as MB with a medium to large body size, but shorter than all MB. The LR visited the veterinary practice more often (risk ratio (RR) 1.2, 95% confidence interval 1.2-1.3), and also showed a higher frequency of insurance expense claims (RR 2.2 (2.1-2.3) and RR 1.2 (1.1-1.3) respectively for the two insurance data sets). The largest difference in organ systems between the LR and MB in insurance claims was related to ears (RR 5.3 (4.8-5.8) and RR 2.6 (2.3-3.1)), followed by airways (RR 2.6 (2.4-2.8)), tendons & muscles (RR 2.4 (2.2-2.6) and RR 1.4 (1.1-1.7)), and joints (RR 1.7 (1.3-2.1)), without a difference in median age at diagnosis. The data from the histopathological laboratory suggested a higher disease burden related to oncology for the LR compared to MB (OR 1.2, 95% CI 1.0-1.3). Oncological diagnoses were made at a younger age in the LR (8.8 versus 9.4 years).

The disease burden was significantly higher for the LR than MB, but these results may suffer from substantial bias such as selection bias towards the database, and different behaviour of LR versus MB owners with regards to veterinary care. In the future, longer term population data can corroborate these results.

Introduction

The health of dog breeds has become an important topic in recent years (1). The two types of breed related health issues are inherited diseases and extreme conformation traits, which vary per specific breed (2,3).

The Labrador retriever (LR) is one of the most popular dog breeds. The Dutch LR breed population consists of phenotypic LR, including pedigreed and non-pedigreed dogs, with the pedigreed LR bred in a closed population. More knowledge on the inherited disease status of the LR is needed to inform dog breeders, policy makers and the public. Reported inherited diseases in the Dutch population include orthopaedic issues such as elbow dysplasia (4) and hip dysplasia (5), as well as copper-associated hepatitis (6). From other populations, oncological problems such as cutaneous mast cell tumours were reported (7-9). Recently, a genetic variant related to mast cell tumours was found in both the LR and the Golden retriever (10). Soft tissue sarcomas of mesenchymal origin are described in the Dutch population of the Golden retriever (11), which might also share a common genetic predisposition with the LR. A disease-specific search in data from veterinary practice showed only overrepresentation of orthopaedic diagnoses in the Dutch LR population (12,13). A comparative Dutch dog population needs to be defined in order to be able to quantify the health of the LR breed. In earlier studies, mixed-breed dogs (MB) were used as a reference population, representing the genetically most heterogeneous dog population (13). A MB is defined as an individual with a mixed lineage, not belonging to any particular breed, including crossbreeds and mongrels.

Our aim is to quantify the health of the LR population in the Netherlands compared to MB through the use of different sources of data.

Material and Methods

Population

The LR population in this study refers to the group of exposed individuals, who are, in effect, exposed to the risk factor of being a phenotypic LR, regardless of pedigree. The MB is considered to be unexposed to this risk factor. Data were provided from four different sources and across different time frames (*table 1*).

Table 1. Main features of data used to compare the health of the Labrador retriever and mixed-breed dog population in the Netherlands.

Data	Full source	Time frame	Breed label
Practice	Practice management system "Idexx Animana"	6 years (2012-2017)	Free text
Insurance1	Animal insurance company "Reaal Dier&Zorg"	11 years (2006-2016)	Coded
Insurance2	Animal insurance company "Petplan"	7 years (2010-2016)	Coded
Pathology	Histopathology laboratory "GD Animal health Deventer"	10 years (2006-2015)	Free text

Data management per data set

General management

Data recoding and cleaning occurred for all four data sets. Unrealistic values, such as a birth date in the future, were set to "not available". Variables with a missing percentage of 90-100% were excluded from further analysis. New variables were created based either on direct coding such as lifespan if birth and death date were known, or on proxies such as a cremation event as a proxy for death date. Pedigree was determined by the available chip code, with which the individual should be interpreted as a Dutch pedigree recognised by the Fédération Cynologique International (14). Age was recoded in birth cohorts relative to the starting point of the data set to allow adjustment for confounding bias in statistical models. Individuals were grouped in birth cohorts with cut-off points of 1, 5 and 10 years before or after the start date of the respective data sets. *Supplemental figure 1* shows the change in number of rows per data set during the data management process. Data management was performed in R for statistics (R Core Team (2016) (15), and supplemental table 3, scripts available from first author).

Veterinary practice management system

Individuals in the veterinary practice data originated from 33 veterinary practices and had a unique identification number within the set. The maximum recorded weight of an individual was selected out of multiple measurements, to limit inclusion of weight during growth. Breed names were highly variable in the original data as the field was free text, resulting in typing errors and alternative spellings. The selection steps of the over 8,000 types of entries resulted in three levels of breed: LR, MB, or other specific breeds. The definition of MB was based on the Dutch equivalents of "mongrel", or a combination of two different breed names. No diagnostic data was available in the practice data, only a date of visit.

Animal insurance companies

Individuals in both insurance data sets had a unique identification number within the set. Breed names were coded at the data source. Breed names available were: LR, MB, other specific breeds and "unknown". Individuals labelled as breed "unknown" were assumed to be MB for the current analysis.

Histopathological laboratory

Individuals in the pathology data were identified by unique information regarding breed, date of birth, sex, zip code and name. Histopathological examination was available with year and month information. Results of examinations occurring within two months of each other were combined and the first (combined) examination record used for analysis. To enable the calculation of time to event, all examinations were assumed to occur on the 15th of the month. Breed labels were produced from a free text field and recoded. If a specific breed could be deduced from the free text, it was relabelled as such. If the breed was not clear it was labelled as an unknown breed. If it was clearly MB, it was relabelled as MB in the new breed label. Similar relabelling was carried out for the LR.

Diagnostic code analysis

Codes within both sets of insurance data may refer to general veterinary consultations, diagnostics, organ systems or specific medical conditions. For the analysis, certain diagnostic codes were grouped together (*see supplemental tables 1a and 1b*). In the pathology data, most codes were highly specific diagnoses based on protocolled (histo) pathological processes. In the current study, we analysed the code for any kind of tumour, as well as three sub-diagnoses within those tumours: benign tumour (yes/no), soft tissue tumour (yes/no) and mesenchymal origin (yes/no). An overlap between the sub-diagnoses was possible. Individuals with a known age at the time of the event were selected and the presence of any, and different types of, tumours evaluated.

Statistical methods

The uncorrected difference in survival time between the LR and MB was visually evaluated using the Kaplan-Meier univariate approach, and the crude and adjusted hazard ratio (HR) was determined in the multivariable Cox proportional hazards model analysis. Individuals without a death date were assumed to be censored at the time of the last observation in the data. The frequency of practice visits and insurance expense claims in respectively the practice and insurance data were analysed in a negative binomial model to account for the large number of low counts in

the data. Diagnostic codes related to claims occurring in at least 5% of both the LR and MB in the insurance data were selected for this analysis. Logistic regression was used for categorical outcomes in the pathology data.

The Akaike Information Criteria (AIC)-based backward selection method was used to determine the best fitting models with the lowest AIC for all above models, while confounding was checked (>10% in parameter estimate (16). Data analyses were performed in R for statistics (R Core Team (2016) (15), and supplemental table 3, scripts available from first author).

Results

Baseline characteristics

The overall characteristics are shown in *table 2*. The percentage of males and females was close to 50/50 in all data sets. In the practice data, not all individuals were microchipped, while neuter status was known for most individuals.

Evaluation of the median year of birth showed that the LR were born slightly earlier than MB in the practice and insurance1 data cohorts. The year of birth suggested a younger group of the LR in the pathology and insurance2 data (*table 3*).

Results per data set

Practice management system (n LR/MB = 10,429/24,670)

The median lifespan of approximately 12 years was similar for the LR and MB when a birth and death date was available (*table 4*). The weight distribution for all individuals for whom a weight was recorded is shown in *figure 1*. The median weight (interquartile range) for the LR was 30.0 kg (25.0-35.1) and for MB 12.4 kg (7.0-23.3).

Survival analysis for all individuals in the data set (including censored individuals) violated the proportional hazard assumption (*figure 2a*). Based on figure 2a, it was decided to use two different models up to and above 10 years of age, with the proportional hazard assumption holding for both models. The adjusted (sex and neuter status) HR (CI), based on a Cox model, for individuals up to 10 years of age was 0.8 (0.7-0.9) for the LR compared to MB, indicating a lower death rate for the LR (*see also supplemental figure 2 for the uncorrected visual illustration*). Above 10 years of age, the adjusted (sex and neuter status) HR (CI), based on a Cox model, for all the LR with a registered weight compared to MB > 25 kg in weight was 1.2 (0.8-1.9), indicating similar death rates (*figure 2b*).

The frequency of practice visits for MB was approximately eight consultations within the eight-year observation period, with an adjusted RR of 1.2 for the LR (*table 5*). The frequency of practice

visits for the microchipped LR showed a significant difference between pedigreed and non-pedigreed LR versus MB, with an adjusted RR of 1.6 (1.6-1.7) and 1.4 (1.3-1.4) respectively.

Animal insurance companies

(insurance1 n LR/MB = 7,151/8,412; insurance2 n LR/MB = 3,156/1,389)

The median lifespan of the LR exceeded that of the MB by almost three years for animals with a birth and death date in the insurance1 data (10 versus 7 years, *table 4*).

Evaluation of the survival of all individuals in insurance1 showed that the proportional hazard assumption did not hold (*figure 3a*). Based on figure 3a, it was decided to use two different models up to and above 10 years of age, with the proportional hazard assumption holding for both models. The adjusted (sex) HR (CI), based on a Cox model, for individuals up to 10 years of age was 0.8 (0.7-1.0) for the LR versus MB (*see also supplemental figure 3 for the uncorrected visual representation*). The adjusted (sex) HR (CI), based on a Cox model, for LR versus MB with a survival time of at least 10 years was 1.9 (1.5-2.5) (*figure 3b*), showing an increased death rate for the LR.

The frequency of insurance expense claims in the insurance data was defined as an expense claim for any reason. The baseline frequency of claims for the MB in the insurance1 data was 5.1 claims in an 11-year time frame, with an adjusted RR of 2.2 for the LR, indicating twice the rate of insurance claims in the available time frame. In the insurance2 data, the baseline frequency of claims for MB was 5.0 claims in a seven-year time frame, with an RR of 1.2 for the LR (*table 5*).

The top three diagnostic codes in the event of an insurance expense claim in the LR compared to MB are ears, tendons & muscles, airways (insurance1), and joints (insurance2) (*table 6*). A significant RR > 1 was found for many of the diagnostic codes evaluated (*supplemental table 2*), with the diagnostic code for ears being the highest (RR = 5.3 (4.8-5.8)). The median age at the first event of the diagnostic code did not differ much between the LR and MB. The crude RR for the top three results in relation to pedigree were also evaluated, often showing a significantly higher RR for pedigreed LR than non-pedigreed LR in comparison to MB (*table 6*).

Histopathological laboratory (n LR/MB = 1,529/2,576)

The results of the logistic regression model for specific diagnostic tumour codes are shown in *table 7*. Tumours originating in mesenchymal cells showed the highest OR (CI) of 1.4 (1.2-1.7) for the LR compared to MB. The median age in years at the first event of the code was consistently lower in the LR than in MB, with the largest difference being 1.1 years.

Table 2. Baseline characteristics of the population of Labrador retrievers (LR) and mixed-breed dogs (MB), within data sets from a practice management system, two animal insurance companies, and a histopathological laboratory in the Netherlands.

	Practice (2012-2017)		Insurance1 (2006-2016)		Insurance2 (2010-2016)		Pathology (2006-2015)	
Breed	LR	MB	LR	MB	LR	MB	LR	MB
Total # individuals	10,429	24,670	7,151	8,412	3,156	1,389	1,592	2,576
Variable	n (%) [1]	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Sex								
Female	4,947 (47.4)	11,731 (47.6)	3,229 (45.1)	4,209 (50.0)	1,444 (45.8)	714 (51.4)	729 (45.8)	1,301 (50.5)
Male	5,094 (48.9)	12,153 (49.3)	3,906 (54.6)	4,174 (49.6)	1,709 (54.2)	672 (48.4)	836 (52.5)	1,228 (47.7)
Unknown	388 (3.7)	786 (3.2)	16 (0.2)	29 (0.4)	3 (0.0)	3 (0.2)	27 (1.7)	47 (1.8)
Neuter status			-		-			
Neutered	5,436 (52.1)	13,308 (53.9)					605 (38.0)	1,102 (42.8)
Intact	4,605 (44.2)	10,576 (42.9)					157 (9.9)	246 (9.5)
Unknown	388 (3.7)	786 (3.2)					830 (52.1)	1,228 (47.7)
Sex + Neuter status [2]			-		-			
Neutered female	3,143 (63.5)	7,251 (61.8)					331 (45.4)	603 (46.3)
Neutered male	2,293 (45.0)	6,057 (49.8)					274 (32.8)	498 (40.6)
Chip present [3]							-	
Unknown	4,174 (40.3)	11,701 (47.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Yes	6,255 (59.7)	12,969 (52.6)	7,151 (100.0)	8,412 (100.0)	3,156 (100.0)	1,389 (100.0)		
Dutch pedigree	2,845 (45.5) [4]	0 (0.0)	3,408 (47.7)	0 (0.0)	828 (26.2)	0 (0.0)		
No Dutch pedigree	3,410 (54.5) [4]	12,969 (100.0)	3,743 (52.3)	8,412 (100.0)	2,328 (73.8)	1,389 (100.0)		

1) *n = number per category, % for total n per breed group*
2) *% of Sex + Neuter status = neuter status per sex*
3) *Chip mandatory for insurance*
4) *% of pedigree presence calculated for individuals with chip.*

Table 3. Year of birth distribution with interquartile range (iqr) of the population of Labrador retrievers (LR) and mixed-breed dogs (MB) within a practice management system, two animal insurance companies, and a histopathological laboratory in the Netherlands.

Data source (time frame)	LR median (iqr)	MB median (iqr)
Practice (2012-2017)	2008 (2004-2012)	2009 (2004-2012)
Insurance1 (2006-2016)	2008 (2003-2012)	2009 (2003-2012)
Insurance2 (2010-2016)	2011 (2009-2012)	2010 (2009-2012)
Pathology (2006-2015)	2003 (2000-2005)	2002 (1999-2006)

Table 4. Median lifespan in years and interquartile range (iqr) from two different Dutch data sources for Labrador retrievers (LR) and mixed-breed dogs (MB) for which both birth and death date was recorded.

Data source (time frame)	n LR/MB	Median lifespan in years (iqr) LR	 MB
Practice (2012-2017)	1,782/4,009	12.2 (10.4-13.6)	13.0 (10.2-14.8)
Insurance1 (2006-2016)	399/310	10.3 (7.3-12.1)	7.6 (3.3-11.3)

Table 5. Baseline number of practice visits and insurance expense claims for a mixed-breed dogs (MB) with a Risk Ratio (RR) versus the baseline number of events for Labrador retrievers (LR) in a practice management system, and two animal insurance companies, as analysed with a negative binomial model.

CI = 95% confidence interval. Data collected in the Netherlands.

Data source (time frame)	n LR/MB	Baseline number of events MB CI)	Adjusted RR LR vs. MB (CI)
Practice (2012-2017)	10,429/24,670	8.3 (8.1-8.4)	1.2 (1.2-1.3) [a]
Insurance1 (2006-2016)	7,151/8,412	5.1 (5.0-5.2)	2.2 (2.1-2.3)
Insurance2 (2010-2016)	3,156/1,389	5.0 (4.7-5.3)	1.2 (1.1-1.3)

*a = adjusted for age, sex, neuter status and sex * neuter status, rest adjusted for age.*

Figure 1. Maximum recorded weight (kg) distribution of the population of mixed-breed dogs (control, n=3,844 of 24,670) and Labrador retrievers (n=1,478 of 10,429) with a recorded weight within a practice management system in the Netherlands.

Median weight (with interquartile range) was 30.0 kg (25.0-35.1) for Labrador retrievers and 13.0 kg (7.1-24.5) for mixed-breed dogs.

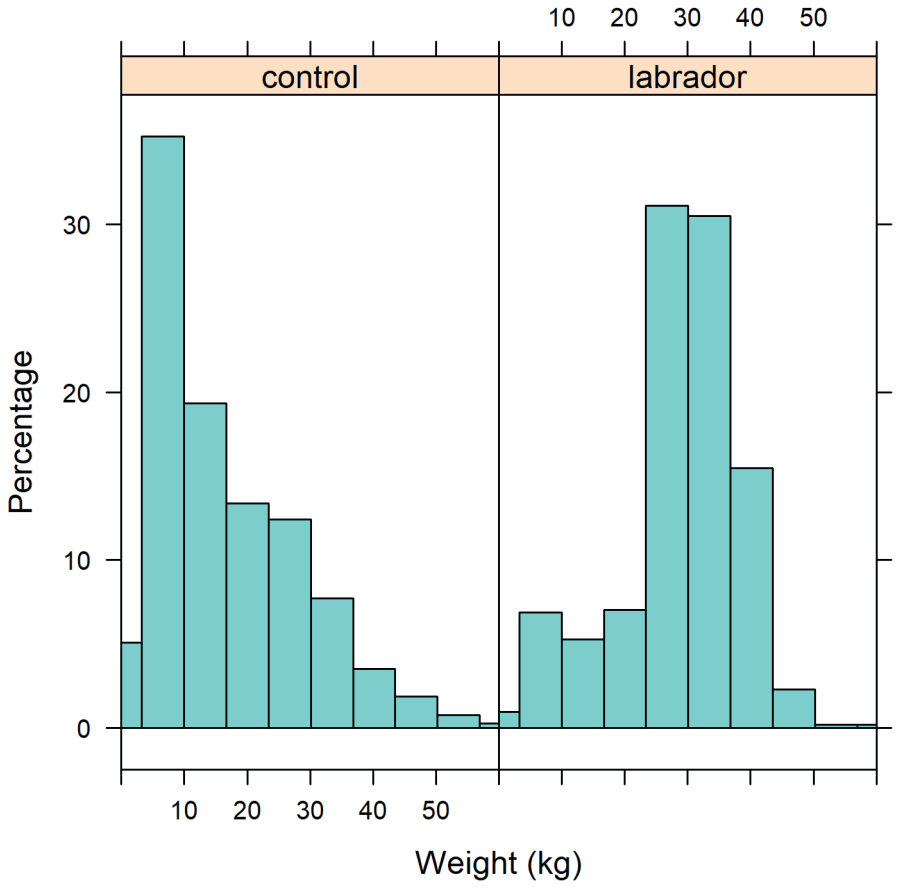

Figure 2. Survival proportion and time to event in a practice management data set in the Netherlands.

(2a) Survival in years, from birth date to death or censoring event, for all available 9,992 Labrador retrievers (labrador) and 23,490 mixed-breed dogs (control). (2b) Survival in years, starting at minimal ten years survival time, from birth date to death or censoring event,for 294 Labrador retrievers with a registered weight (labrador) and 171 mixed-breed dogs > 25 kg in body weight (control). *(See also supplemental figure 2.)*

Figure 2a.

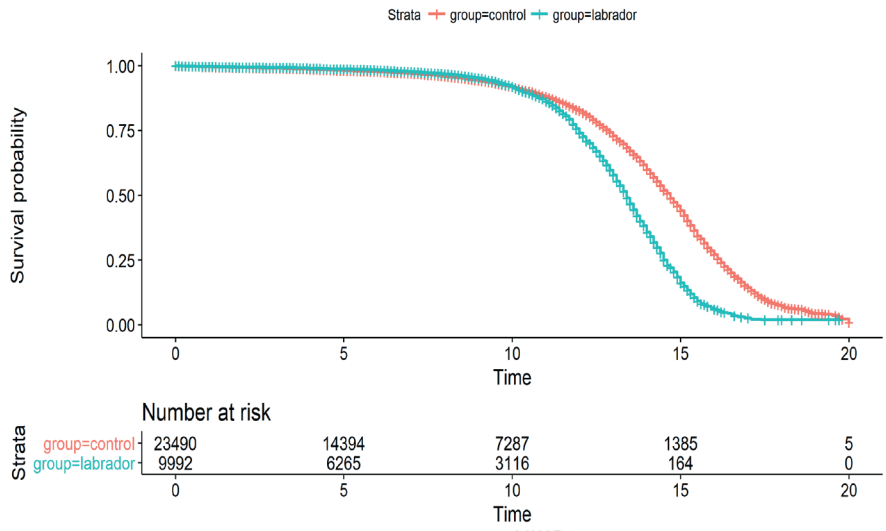

Figure 2b.

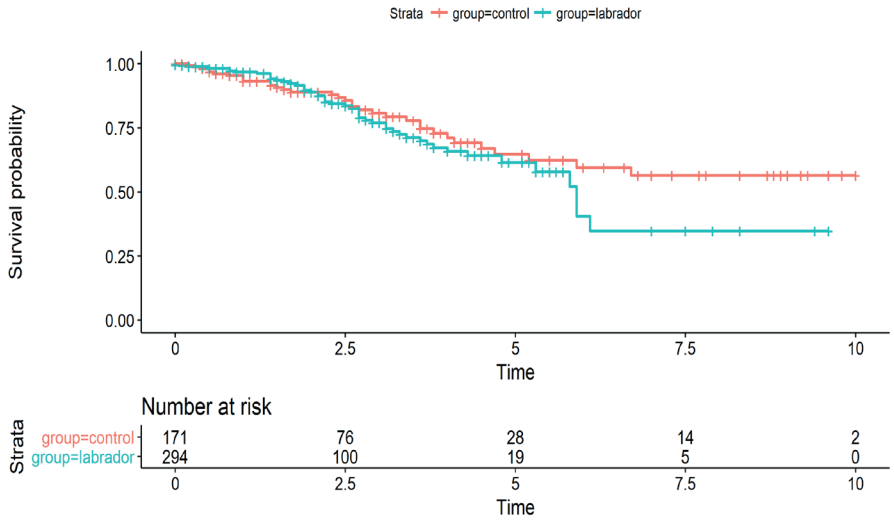

Figure 3. Survival proportion and time to event in an insurance company data set in the Netherlands.

(3a) Survival in years, from birth date to death or censoring event, for all available 4,029 Labrador retrievers (labrador) and 4,225 mixed-breed dogs (control). (3b) Survival in years, starting at minimal ten years survival time, from birth date to death or censoring event, for 462 Labrador retrievers (labrador) and 253 mixed-breed dogs (control). (*See also supplemental figure 3*.)

Figure 3a.

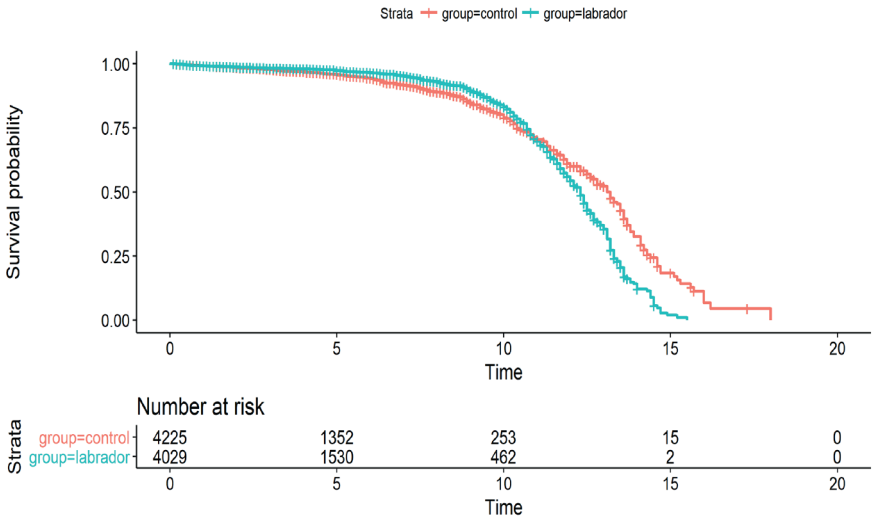

Figure 3b.

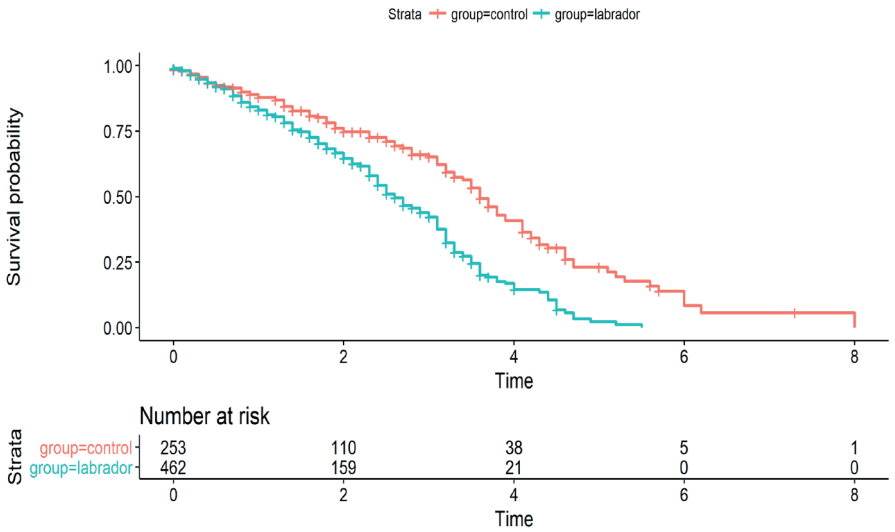

Table 6. Number of insurance expense claims, with 95% confidence interval (CI) for the top three diagnostic codes in two Dutch animal insurance data sets for the Labrador retriever (LR) compared with the baseline number of expense claims in mixed-breed dogs (MB).

Ordered from highest adjusted Risk Ratio (RR) for LR to MB to the lowest, as calculated in a negative binomial model. Diagnostic codes selected for the analysis occurred in at least 5% of both populations. The median age in years with interquartile range (iqr) at first event is shown for individuals with a known age at event. The adjusted RR (CI) for pedigreed and non-pedigreed LR versus MB are also shown. (*See also supplemental table 2.*)

Diagnostic code per Data source (time frame)	n cases LR/MB	Baseline number of insurance expense claims (CI) for MB	Adjusted RR (CI) of LR vs MB	Median age, in years at first event (iqr) (n individuals)		Adjusted RR (CI) of LR pedigree and LR no pedigree vs MB
				LR	MB	
Insurance1 (2006-2016)						
Ears	2,518/924	0.2 (0.2-0.2)	5.3 (4.8-5.8)	2.9 (1.2-5.6) (n=2,141)	2.3 (1.1-4.5) (n=804)	Ped: 6.9 (6.2-7.6) No ped: 3.9 (3.5-4.3)
Airways	2,883/1,859	0.6 (0.5-0.6)	2.6 (2.4-2.8) [a]	2.5 (1.1-5.6) (n=2236)	2.3 (1.1-4.8) (n=1495)	Ped: 3.4 (3.1-3.7) [a] No ped: 1.9 (1.7-2.1) [a]
Tendons & Muscles	1,838/1,113	0.3 (0.3-0.3)	2.4 (2.2-2.6)	4.3 (1.2-8.3) (n=1,167)	2.7 (1.1-6.2) (n=701)	Ped: 2.8 (2.5-3.1) No ped: 2.0 (1.8-2.3)
Insurance2 (2010-2016)						
Ears	982/228	0.3 (0.2-0.3)	2.6 (2.3-3.1)	2.4 (1.3-4.2) (n=982)	2.3 (1.3-4.0) (n=228)	Ped: 3.7 (3.1-4.6) No ped: 2.3 (1.9-2.7)
Joints	455/143	0.2 (0.2-0.3)	1.7 (1.3-2.1)	3.0 (1.2-5.6) (n=454)	3.8 (1.5-6.6) (n=143)	Ped: 2.1 (1.6-2.9) No ped: 1.5 (1.2-2.0)
Tendons & Muscles	272/99	0.1 (0.1-0.1)	1.4 (1.1-1.7)	3.2 (1.6-5.6) (n=272)	3.0 (1.9-5.7) (n=99)	Ped: 1.9 (1.4-2.5) No ped: 1.2 (0.9-1.5)

a = adjusted for age and sex, rest is adjusted for age.

Table 7. Adjusted Odds Ratios (OR) with 95% confidence interval (CI) of 4 logistic regression models on diagnostic tumour codes in data from a Dutch histopathological laboratory on biopsies or full animals (2006-2015), for 1,505 Labrador retriever (LR) versus 2,503 mixed-breed dogs (MB), with median age (interquartile range (iqr)) at diagnosis.

Diagnostic code outcome [1]	n (% of larger total)		Adjusted OR LR vs. MB (CI)	Median age, in years at diagnosis (iqr)	
	LR	MB		LR	MB
Total n	1,505	2,503		1,505	2,503
Any tumour	959 (63.7)	1,512 (60.4)	1.2 (1.0-1.3) [a]	8.8 (6.8-10.2)	9.4 (7.0-11.5)
Within any tumour: yes					
Benign: yes	453 (47.2)	685 (45.3)	1.1 (0.9-1.3) [a]	8.3 (6.4-9.8)	8.7 (5.7-10.8)
Soft tissue: yes	153 (10.2)	290 (11.6)	0.8 (0.6-1.0)	9.3 (7.7-10.5)	10.4 (8.2-12.0)
Mesenchymal cell origin: yes	382 (25.4)	480 (31.7)	1.4 (1.2-1.7) [a]	8.8 (7.3-10.0)	9.9 (7.8-11.7)

1 = yes versus no of diagnosis, a = adjusted for age.

Discussion and Conclusion

In this study, the health of the Dutch Labrador retriever (LR) was evaluated by comparing health parameters from four different data sources with that of mixed-breed dogs (MB). We discuss longevity, the number of practice visits and insurance expense claims, as well as specific diagnoses.

Longevity

The lifespan of approximately 12 years within the practice data was previously found for the LR in the UK (17,18). The finding of an 11-year lifespan in the LR in the insurance data is closer to what Proschowsky, Rugbjerg et al. (2003) (19) found earlier in questionnaire data in Denmark. Adams, Watson et al. (2016) (20) combined these and other studies to come to a consensus of a 12 year lifespan in general for the LR. McGreevy, Wilson et al. (2018) (21) found a similar lifespan for LR in practice data. Overall, these results suggest similarities between the LR across different subpopulations with regards to lifespan.

The effects of sex and neuter status on longevity, regardless of breed, were as reported earlier (21,22), with females living longer, and intact dogs living shorter than neutered dogs. The Kaplan-Meier plot suggested a change in HR for the LR compared to MB from approximately 10 years of age, with equal death rates for the LR and MB below 10 years of age. The difference in death rate above 10 years of age may be confounded by body size (17,23,24), as the median weight of the MB group was less than half that of the LR. The comparison in a Cox model between the LR with a registered weight and MB with a body weight of > 25 kg, showed no significant difference in death rate between LR and MB. We assumed the adult LR to have a body weight of around 30-35 kg (Dutch kennel club (Raad van Beheer) (25)), while MB with a body weight of > 25 kg excluded smaller dogs with a subsequent longer lifespan. Based on these combined results, we concluded that the LR live shorter than all MB, but similarly long compared to middle and large sized MB. However, longevity in itself is not necessarily a measure of good health because it does not indicate the health and wellbeing during life (26).

Frequency of practice visits and insurance expense claims

The frequency of practice visits in the timeframe available was 20% higher for the LR compared to MB, but the frequency of expense claims was twice as high for the LR compared to MB. Repeated visits or claims could be associated to a single disease episode, thereby overestimating the disease burden. However, this was equally overestimated for the LR and MB, and each practice visit can be seen as a burden for the dog and the owner. It may be that different types of owners visit the veterinary practice sooner and more frequently, and more vet visits lead to more insurance

events and a higher likelihood of showing up in pathology data due to samples being further diagnosed. The difference in expense claims between the LR and MB is higher than expected based on visits to primary practice, and could be the result of more claims for medication and preventive medicine in the LR (*supplemental table 2*). The possible influence of selection bias is discussed below.

Diagnostic codes

The RR for specific diagnostic codes also showed a higher insurance expense claim rate in the LR for almost all codes. The most commonly occurring code was general consultation. The top four overrepresented diagnostic codes in the LR were ears, tendons & muscles, joints and airways. Apart from airways, these results are supported by previous studies in the Dutch LR population (13,27), as well as in the UK LR population (21).

The OR for the occurrence of any tumour diagnosis in the pathology data was 1.2, suggesting a higher oncological disease burden for the LR compared to MB. Within tumours, the mesenchymal cell origin had the highest OR, suggesting higher genetic burden for these type of tumours, as was found in the Golden retriever (11). Also, the lower median age at tumour diagnosis in the LR may represent a younger age at the start of the disease. Furthermore, the higher frequency of practice visits by the owners of an LR may account for a faster diagnosis, indicating a potential for detection bias.

The ratio of the LR compared to MB in the pathology data (3 to 5) is skewed towards more LR relative to the source population in the primary practice (2 to 5), leading to an underestimation of the increased tumour risk in the LR. The diagnostic code analyses identified previously unreported health issues such as ear and airway problems in the LR. The results also support the previously reported increased disease burden in the locomotor system, as well as a higher tumour risk for the LR. The potential biases influencing the diagnostic code results is discussed below.

Pedigree

Pedigree might be associated with decreased health, because the smaller the effective population size, the more likely the spread of deleterious changes in the genes (28). Our results suggested no association between pedigree and longevity in the LR (results not shown). However, the frequency of practice visits and insurance expense claims were significantly higher for the pedigreed LR than the non-pedigreed LR. Whether this indicates an increased disease burden in the pedigreed LR or merely suggests a more health conscious or worried owner remains to be elucidated. Also, the pedigree status was unknown for part of the individuals in the data sets, resulting in bias, and - as

pedigree is associated with cost - the financial means of the owner of a pedigreed dog versus a non-pedigreed dog may differ.

Data validity

The data sources explored in this study are a non-random sample of the total dog population, resulting in potential for selection bias. The obvious reasons are that not all owners visit a veterinarian, that in the Netherlands only a limited number of dogs are insured (estimates are < 10%). Also, in daily veterinary practice, even if there is an indication, biopsies are not always taken and examined nor is an autopsy always performed. Even more selection bias may be caused by the veterinarian's laboratory preference, the owner's financial means, and the owner's perception of the choices relating to the health and burden on the animal or themselves, all influencing whether or not a certain diagnostic or treatment procedure is started. There may even be a systematic difference between LR and MB owners. More knowledge about dog owner motivation to own a specific type of dog, such as pedigreed versus non pedigreed or MB, would be interesting.

We consider the practice data to be the best representation of the source population in the Netherlands, because there is only one step between the dog's health and it being present in the data. Other efforts to collect health data from primary practice are available in other countries (29,30). The ratio of the LR and MB is skewed towards the LR in the insurance and pathology data, indicating a large risk of selection bias in such data sources, as reviewed earlier (31).

Information bias was probably present in all data sets, in particular regarding exposure, i.e. breed. It is possible that individuals registered as MB were in fact crossbred dogs or even purebred dogs, reducing the aspired genetic heterogeneity of the reference population. If this is the case, it would reduce the estimated negative effect on health of being an LR in this study. However, it was difficult to ascertain MB status in the available data sources. The outcome variable may also be prone to information bias, but we assume this error to be the same in the LR and MB and thus not influencing the associations.

No confounding was found in the current study for the limited number of available variables. Other external factors such as living circumstances, husbandry and exercise were not available, but may influence health (20,32).

Conclusions

The LR live equally long as MB of a similar body size, but shorter than MB of all sizes. In their lives, LR owners visit a veterinary practice and submit a claim to an insurance company more often. Specific diagnoses are related in particular to ear and locomotion problems, while tumours

found in the LR were sent to a pathological laboratory more often. All these aspects point to an increased disease burden for the LR, but might be heavily influenced by owner behaviour and financial means. More intensive and improved data collection from veterinary practices, including diagnosis, are needed to conduct further research in the future.

Abbreviations

AIC = Akaike Information Criteria

CI = 95% confidence interval

HR = hazard ratio

LR = Labrador retriever

MB = mixed-breed dog

OR = odds ratio

RR = risk ratio

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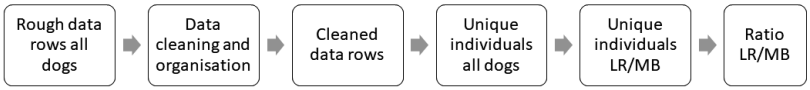
Supplemental information

Supplemental figure 1. Flow chart for number of rows in data sets.



Data set					
Practice	1,451 x 10^3	1,448 x 10^3	172 x 10^3	10,429/24,670	2/5
Pathology	22 x 10^3			1,592/2,576	3/5
Insurance1	1,884 x 10^3	285 x 10^3 (study breeds)		8,473/7,151	1/1
Insurance2	66 x 10^3 (proposed study breeds)	64 x 10^3 (study breeds)		3,156/1,389	2/1

Supplemental table 1a. Diagnostic codes in animal insurance1.

Insurance1	*Subgroups included*
Airways	
Alternative therapy	Acupuncture, Alternative, Chiropractor, Homeopathy, Orthomanual therapy, Osteopathy
Behaviour	
Cushing / Addison	
Dental	
Diabetes	
Diagnostics	Blood, Lab, Diagnostics
Diet	Diet, Supplement
Ears	
Eyes	
Epilepsy	
Gastrointestinal	
General consultation	Administrative, Blank Consultation
Heart	
Joints	
Kidneys	
Liver	
Mammary glands	
Medication	Antibiotics, Benazepril, Pain medication, Other medication*
Neurological	
Pancreas	
Physical therapy	
Preventive medicine	Vaccination, Travel*
Reproductive organs	
Surgery or Treatment	Bandage, Surgery (material), Suture line, IV line, Anesthesia, Treatment
Tendons and Mucles	
Tumor treatment	Chemotherapy, Radiation
Urinary tract	

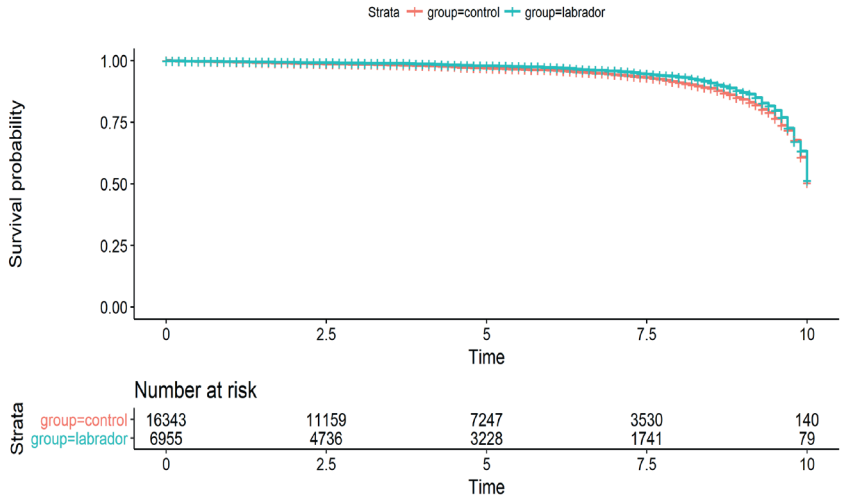
* Antiparasitic medication & Deworming codes were also available, but no conclusion can be drawn on the preventive or therapeutic reason for this, so there were excluded from both the Medication group and the Preventive medicine group.

Supplemental table 1b. Diagnostic codes in animal insurance2.

Insurance2	
Accident	
Airways	
Chemotherapy / Radiotherapy	
Dental	
Diagnostics	
Ears	
Eyes	
Gastrointestinal	
General consultation	Administrative, Blank, Consultation, Other
Heart	
Hormonal	
Immune issues	
Infectious diseases	
Joints	
Neurological	
Orthopaedics	
Preventive medicine	
Reproductive organs	
Skeleton	
Skin	
Specific treatments	
Tendons and Muscles	
Urinary tract	

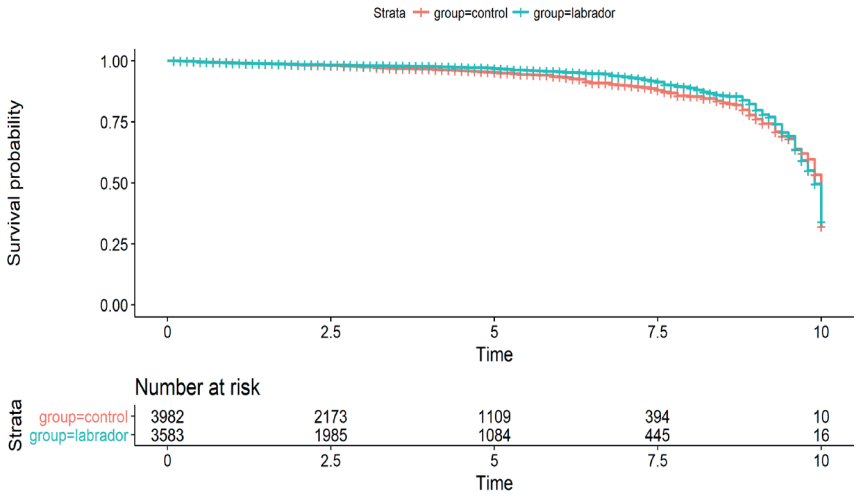
Supplemental figure 2. Survival proportion and time to event (in years, from birth date to death or censoring event) below ten years survival time in a practice management dataset in the Netherlands.

Survival for 6,955 Labrador retrievers (labrador) and 16,343 mixed-breed dogs (control).



Supplemental figure 3. Survival proportion and time to event (in years, from birth date to death or censoring event) below ten years survival time in an insurance company data set (Insurance1) in the Netherlands.

Survival for 3,982 Labrador retrievers (labrador) and 3,583 mixed-breed dogs (control).



Supplemental table 2. Number of insurance expense claims, with 95% confidence interval (CI), of diagnostic codes (other than top three) in two Dutch animal insurance data sets for the Labrador retriever (LR) compared with the baseline number of expense claims in mixed-breed dogs (MB).

Ordered from highest adjusted Risk Ratio (RR) for LR to MB to the lowest, as calculated in a negative binomial model. Diagnostic codes selected for the analysis occurred in at least 5% of both populations.

Diagnostic code per Data source (time frame)	n cases LR/MB	Baseline number of insurance expense claims (CI) for MB	Adjusted RR (CI) of LR vs MB
Insurance1 (2006-2016)			
Medication	3,449/2,561	1.1 (1.1-1.2)	2.1 (2.0-2.3)
Eyes	1,770/1,063	0.3 (0.3-0.3)	2.1 (1.9-2.3) [a]
Gastrointestinal	2,924/1,944	0.6 (0.5-0.6)	2.1 (1.9-2.2)
Preventive medicine	3,058/2,273	0.5 (0.5-0.5)	2.1 (1.9-2.2)
Surgery or treatment	2,904/2,146	0.8 (0.8-0.8)	1.9 (1.7-2.0)
General consultation	5,233/6,020	1.6 (1.6-1.7)	1.7 (1.7-1.8)
Reproductive organs	2,051/1,655	0.2 (0.2-0.2)	1.6 (1.5-1.7) [a]
Behaviour	488/434	0.1 (0.1-0.1)	1.2 (1.0-1.4)
Dental	614/725	0.1 (0.1-0.1)	1.0 (0.9-1.1)
Insurance2 (2010-2016)			
Urinary tract	293/109	0.2 (0.1-0.2)	1.4 (1.1-1.9) [a]
Eyes	608/204	0.3 (0.2-0.3)	1.3 (1.1-1.5)
Skin	1,324/507	1.0 (0.9-1.2)	1.3 (1.1-1.4) [a]
Preventive medicine	2,226/831	2.6 (2.4-2.8)	1.3 (1.1-1.4)
Diagnostics	1,177/394	0.8 (0.7-0.9)	1.3 (1.1-1.4)
General consultation	3,122/1,345	7.3 (6.9-7.7)	1.3 (1.2-1.3)
Accident	439/154	0.2 (0.1-0.2)	1.2 (1.0-1.4)
Reproductive organs	867/309	0.3 (0.3-0.3)	1.2 (1.1-1.4)
Gastrointestinal	1,152/390	0.7 (0.6-0.7)	1.2 (1.0-1.4) [a]
Airways	246/90	0.1 (0.1-0.1)	1.1 (0.9-1.5)
Skeleton	244/90	0.1 (0.1-0.1)	1.1 (0.9-1.5)
Special treatment	198/83	0.1 (0.1-0.1)	1.0 (0.7-1.3)
Immune system	242/126	0.1 (0.1-0.2)	0.8 (0.6-1.0) [a]
Nervous system	92/88	0.2 (0.1-0.2)	0.5 (0.3-0.8)
Dental	159/150	0.2 (0.1-0.2)	0.5 (0.4-0.6)

a = adjusted for age and sex, rest is adjusted for age.

Supplemental table 3. R packages that were used in this study, with references.

Dplyr	Hadley Wickham, Romain Francois, Lionel Henry and Kirill Müller (2017). dplyr: A Gram mar of Data Manipulation. R package version 0.7.4. https://CRAN.R-project.org/package =dplyr
Ggplot2	H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 200 9.
Lattice	Sarkar, Deepayan (2008) Lattice: Multivariate Data Visualization with R. Springer, New Yor k. ISBN 978-0-387-75968-5
MASS	Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0
Stringr	Hadley Wickham (2018). stringr: Simple, Consistent Wrappers for Common String Operati ons. R package version 1.3.0. https://CRAN.R-project.org/package=stringr
Survival	Therneau T (2015). _A Package for Survival Analysis in S_. version 2.38, <URL: https://C RAN.R-project.org/package=survival>. Terry M. Therneau and Patricia M. Grambsch (2000). _Modeling Survival Data: Extending the Cox Model_. Springer, New York. ISBN 0-387-98784-3. Therneau T (2015). _A Package for Survival Analysis in S_. version 2.38, <URL: https://CRAN.R-project.org/package=survival>. Terry M. Therneau and Patricia M. Grambsch (2000). _Modeling Survival Data: Extending the Cox Model_. Springer, New York. ISBN 0-387-98784-3.
Tidyr	Hadley Wickham and Lionel Henry (2018). tidyr: Easily Tidy Data with 'spread()' and 'gathe r()' Functions. R package version 0.8.1. https://CRAN.R-project.org/package=tidyr
Tidyverse	Hadley Wickham (2017). tidyverse: Easily Install and Load the 'Tidyverse'. R package versio n 1.2.1. https://CRAN.R-project.org/package=tidyverse

Chapter 4

Disease burden in four populations of dog and cat breeds compared to mixed-breed dogs and European Shorthair cats

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Prev Vet Med. 2017 May 1;140:38-44.

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, and Labrador retriever) and one purebred cat breed (Persian cat) 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.

Introduction

The number of dog and cat welfare problems associated with breed has become a hot topic (1) resulting in many studies on various diseases and breeds. Both the general public and veterinary professionals have expressed concerns about the high frequency of health problems in purebred dogs and cats. However, quantitative data to compare specific breed populations with data from the general population are rarely available.

Breed-specific health issues in dogs and cats can be classified into two categories: inherited diseases and harmful breed characteristics. A reduction of genetic variation because of inbreeding and frequent use of the same breeding stock decreases the effective population size (2-4), and leads to a greater incidence of inherited diseases: pathogenic mutations may have accidentally been co-selected with desired phenotypic variants (5-7). Breed characteristics can become harmful when they lead to an exaggerated phenotype that disturbs physiological functions (5,8,9). Although there is much public debate about harmful breed characteristics, there are no objective criteria by which to measure their frequency and thus their impact on animal wellbeing. A clear example is the Bulldog phenotype with a short snout leading to dyspnoea. If this causes clear and prolonged discomfort, we assume that the pet owner would consult a veterinarian for treatment or correction the phenotype. We therefore propose using veterinary consultation as an objective and quantifiable indicator of an intolerable reduction of wellbeing due to a breed-associated disease, which is measurable by investigating veterinary databases (10,11). The frequency of breed-associated diseases in specific breeds needs to be quantified in comparison with the general population to objectively estimate their relative impact on animal welfare (12-14). Different data sources can be used to monitor diseases, each with its own advantages and disadvantages, as reviewed by O'Neill et al. (15). The current research focuses on two data sources: referral clinic and primary practice.

The objective of this historical comparative observational study was a quantification of the burden of 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*) (16).

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 (*Supplemental information 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 (*Supplemental table 1*). 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 (17). 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 (*Supplemental table 2*). 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 primary-care companion animal practices. These practices were selected because they use protocol-led filing in the same practice management software (Viva, Corilus Veterinary BV). The files from the ten selected practices were considered to be a fair representation of the total primary care population, being geographically spread throughout the Netherlands, including rural and urban areas and different-sized practices.

Individual animals registered as one of the selected breeds, or as mixed-breed dogs or European Shorthair cats were selected from the practice's patient files over a period of two years (January 1st 2011 to November 12th 2013). The purebred animals were considered to be exposed to their genetic profile, the mixed-breeds as unexposed to such a homologous genotype.

'European Shorthair cat' is the most frequently entered breed name for a common cat in veterinary practice. This may include European or Domestic Shorthair cats or mixed-breed cats. The time frame of two years was chosen to assure large enough numbers per breed to reach statistical significance based on power calculation. Moreover, it has been shown that the general patient population will visit a veterinarian at least once every two years, on average (18).

Sample size was determined through a number of steps. With the assumption that the national breed-specific populations exceed 20,000 individuals, the exact size of the population is irrelevant to determining the sample size. The sample size was calculated using Win Episcope software (19),

with a sampling error around the estimated proportion of 5% for purebreds and 3% for the unexposed group. The higher level of precision for the mixed-breeds was because lower disease proportions were expected, which therefore demanded greater accuracy (20). For expected prevalence we used 50%, since the actual population prevalence was unknown. A total number of 400 individuals per breed and 1000 individuals for the unexposed group were found to be necessary. The number of individuals per veterinary practice was weighted to practice size for the purebred animals. Two-and-one-half times that number of non-breed animals were randomly selected per practice, which corrected for differences between practices (*table 1*).

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

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

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

Search terms were determined for each of the identified organ systems per breed (*Supplemental information 2*) and the 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 (21). 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. (8) 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 7,8).

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

Case control analysis of the University Clinic database shows that the Chihuahua was overrepresented in hepatology and neurology (OR > 1.5 and $p < 0.05$) in comparison to mixed-breed dogs (*table 2*). The median age at presentation in the neurology department in Chihuahuas was half that in mixed-breed dogs (*table 3*).

Practice-based extended cross-sectional study showed that disease proportion was significantly higher in Chihuahuas than in mixed-breed dogs for extremities, dystocia and hypoglycaemia. The organ system extremities – in effect the knee – had the highest disease proportion and proportion difference (*table 4*). The median age of presentation of Chihuahuas versus mixed-breeds at the time of research was lower for all organ systems, with a significant difference for extremities (*table 5*).

French bulldog

The French bulldog was overrepresented in the University Clinic in otorhinolaryngology and neurology (OR > 1.5 and $p < 0.05$) (*table 2.*). The median age at presentation for otorhinolaryngology consultation in the French bulldog was a third of that in the mixed-breed dogs (*table 3.*).

Analysis of primary practice patient files showed that disease proportion was significantly higher in French bulldogs versus mixed-breeds for all selected organ systems. The upper respiratory tract had the highest disease proportion and proportion difference (*table 4*). The median age at presentation of French bulldogs versus mixed-breeds was lower in all organ systems, with significant difference in spinal column problems (*table 5*).

Labrador retriever

Case control analysis of the University Clinic database showed that the Labrador retriever was overrepresented in orthopaedics, urology and reproductive medicine (OR > 1.5 and $p < 0.05$) in comparison to mixed-breed dogs. The overrepresentation in the reproductive medicine department was caused by individuals presented for the removal of retained ovarium tissue, the incidence of which was not analysed further (*table 2*). The median age at presentation in the orthopaedics department in Labradors was half that in mixed-breed dogs. The urology department also saw four times younger 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*.

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

**Significant with Fisher's exact test*

Table 3. Median age, minimum and maximum (months) for breed and non-breed at presentation in a medical discipline at the University Clinic (non-breed being mixed-breed dogs or European 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*

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

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.*

Table 5. Median age, minimum and maximum (months) for breed and non-breed at presentation with specified disease, in ten primary care practices (non-breed being mixed-breed dogs or 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

**Significant difference median tested with Mann-Whitney U test. **Dystocia evaluated in a female sample, hypoglycaemia in a juvenile sample. ***URT = Upper respiratory tract*

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. (22) and Packer et al. (23).

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 (24).

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. (25) – 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 (18,26). Underrepresentation of a breed in comparison to the control group was not part of this study, but may be interesting to analyse further to counterbalance the negative attention to breed health and welfare.

Taking these limitations into account, it is our assumption that the University clinic database can be used to indicate relations between breeds and complex diseases in various organ systems.

The use of practice-based patient files has a number of disadvantages: the pet owner may provide information that is incomplete or inaccurate, the veterinarian's interview of the owner or examination of the patient may be incomplete, and the resulting report's information may be incorrect or incomplete. In addition to these factors, a correct diagnosis is not guaranteed and depends on the complexity of the disease, the veterinarian's knowledge and experience, and the owner's wishes and perception of the animal's health. Standardisation of procedures both in veterinary practice and in data collection are essential to compensate for these effects (10,11). However, any such bias was assumed to be the same between purebred and mixed-breed individuals in each practice and would therefore not create misclassification bias in these results.

The practice-based extended cross-sectional study starts with the assumption that a patient is presented to the veterinarian in the first place. The likelihood of an owner presenting a pet to the veterinarian may be subject to bias, in that owners may have variable tolerance for clinical signs of disease. This tolerance may be breed-related – e.g. a bulldog owner might not recognise respiratory distress for what it is because of the snorting breathing pattern of the breed – but because disease can only be detected in animals presented to a veterinarian when using clinical data, it cannot be corrected for. On the other hand, owners of an expensive purebred individual might be willing to spend more on veterinary care.

Potential differences between practices, including the definition and registration of a diagnosis, the veterinarian's knowledge and experience, do need to be corrected for. This was done by using an unexposed group that was proportionally similar to the number of breed-specific individuals sampled from a particular practice. Although search terms were as broad as possible, it is possible that individuals 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 (25). 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(8) 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. (9), 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 (15). Although Egenvall et al. (27) 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.

Abbreviations

BDWIS = Breed-Disorder Welfare Impact Score

CI = 95% confidence interval

DALY = Disability-Adjusted Life Years

GISID = Generic Illness Severity Index for Dogs

OR = odds ratio

PD = proportional difference

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Supplemental information

Supplemental information 1. Standardised questionnaire for specialist veterinarians in qualitative analysis (16).

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 (*Supplemental table 2*) (Add/remove diseases from selection list)

   Go through 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?

Supplemental table 1. Medical disciplines included in the referral clinic case control study, in alphabetical order.

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

Supplemental table 2. Selection of organ systems and diseases per breed to be quantitatively analysed in a random sample of patient files from ten primary practices (16).

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.*

Supplemental information 2. Search terms used in quantitative research in randomly selected patient files, from ten primary practices (adapted from Dutch search terms (16)).

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

Chapter 5

PETscan: measuring incidence of disease phenotypes to prioritize genetic studies in companion animals.

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Anim Genet. 2018 Oct;49(5):492-495.

Abstract

Reliable incidence measurement of diseases is necessary for identification of hereditary diseases in companion animal populations.

The data collection system "PETscan" was developed to facilitate standardized registration of diagnoses in veterinary practice. In the development, we attempted to counter challenges known from other primary practice data systems.

PETscan includes a comprehensive list of potential diagnoses and supports the veterinary professional in the diagnostic process. Demographics, individual data and standardized diagnostic data are collected through the Practice Management Software in a central database for epidemiological analysis.

A preliminary data-analysis from PETscan showed specific health issues in 4 canine breeds.

As a real-time prospective monitoring tool, PETscan summaries can objectively assess the incidence of disorders in companion animal populations, and can be used to prioritize disease-gene identification studies and evaluate the effects of breeding strategies for example after implementation of a new DNA-test in the breeding strategy.

Introduction

Inbreeding and selection for specific phenotypic characteristics result in health and welfare issues in companion animal populations, initiating public debate. Inbreeding and selection for desired, but unhealthy breed standards often lead to a higher frequency of recessive defects within a population. At the same time, increased disease frequency in a genetically homogeneous population creates an opportunity for discovery of causal genes (1). This also creates the opportunity of a dog model for human diseases, as shown by the collaborative LUPA iniative (2). The first step in prioritizing genetic studies is knowledge about disease incidence in specific populations, however this is largely undocumented (3).

Material and Methods

We developed the data collection system "PETscan" to document disease phenotypes in companion animal populations via veterinary practice management software. PETscan enables prospective collection of standardized diagnostic data. The Practice Management Software (PMS) of a veterinary practice is connected to a central MySQL database, allowing information to be shared and used for epidemiological analyses (4). PETscan opens from the PMS and is organised as a branching tree, which is set up according to organ system, anatomic location and diagnosis, to mimic the medical reasoning in veterinary practice. Multiple diagnoses per individual and consultation may be entered. "No abnormalities" can be selected at a preventive consultation of a healthy animal or a repeat consultation.

PETscan information includes: demographic data (species, breed, sex, date of birth), unique identification (transponder code) and consultation information (practice code, date, weight, neuter status and diagnosis). PETscan was evaluated in a pilot study, in which practices participated that were equally distributed throughout the Netherlands. Dog breeds evaluated in the pilot phase of PETscan included the Chihuahua, French bulldog, and Labrador retriever with mixed-breed as a heterogeneous control group.

To illustrate the potential of PETscan data, we compared preliminary results of occurring organ system entries and specific diagnoses in these groups to other companion animal population studies (5-10).

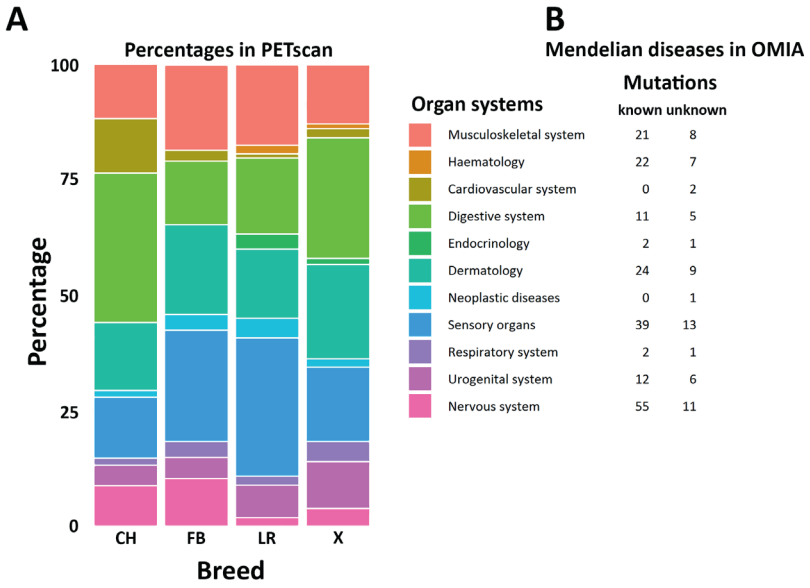
Results

Between September 1st 2015 and September 1st 2017, 6162 diagnoses were entered in PETscan. The overall data includes 3224 individual dogs, with 47.6% female dogs. The Labrador retriever (n=276), Jack Russell terrier (n=203), French bulldog (n=94) and Chihuahua (n=90) were the four most common breeds. Mixed-breed dogs comprised the largest group of dogs (n=579).

In the 4 populations, the percentage of females varied between 44 and 50% and between 50 and 65% of dogs were born after the year 2010.

Individuals with at least one entry in PETscan per unique organ system are shown (*figure 1a*). As comparison, per organ system, the number of hereditary diseases, with or without a known mutation, reported as Mendelian traits in the Online Mendelian Inheritance in Animals database ((11) OMIA, www.omia.org, 20180222) are also shown (*figure 1b*).

Figure 1. (1a) Percentage of unique organ system entries per dog population. (1b) Number of diseases per organ system from 1a reported as Mendelian traits in the Online Mendelian Inheritance in Animals database (OMIA), with known and unknown mutation.



Individuals may have multiple organ systems entered. Breeds shown are the Chihuahua (CH, 68 entries for 66 individuals), French bulldog (FB, 87 entries for 85 individuals), and Labrador retriever (LR, 213 entries for 200 individuals), versus mixed-breed dogs (X, 391 entries for 368 individuals), registered in the practice-based monitoring system PETscan in The Netherlands between September 1st 2015 and September 1st 2017.

Within organ system, diagnoses that were entered most frequently in PETscan for the Chihuahua, French bulldog, Labrador retriever and mixed-breed dog were compared to diagnoses reported in other pet population studies. Studies that showed similar findings are indicated (*table 1*). The percentage with code "no abnormalities" was approximately 3 times lower in the French bulldog (11%) compared to the other dog populations (28-35%) (t*able 1*). Surprisingly, only 1% of diagnoses in the French bulldog was coded as Brachycephalic Obstructive Syndrome (BOS), which seems low because the national breed population is considered genetically predisposed to upper respiratory tract disorders such as BOS amongst others (12). In a study on conformational risk factors, 89% of the 214 French bulldogs was affected by BOS (13). The current study population consisted mainly of adult individuals, so issues with BOS should have been apparent (14). Possibly Dutch veterinarians accept BOS as the 'normal' phenotype in French bulldogs and therefore did not register it as a diagnosis in PETscan (15).

Table 1. Specific diagnoses most frequently entered per breed (CH = Chihuahua; FB = French bulldog, LR = Labrador retriever) versus mixed-breed dogs (X), registered in the practice-based monitoring system PETscan in The Netherlands between September 1st 2015 and September 1st 2017. *Comparable companion animal population studies that show the same diagnoses occurring in these breeds are referenced.*

Breed	*N (individuals)*	*% entries "No abnormalities"*	*One or two most frequently entered specific diagnosis (number) (reference)*	
CH	90	28	Post formative dental issues (8) (ref #1)	Cardiac valve degeneration (8) (ref #2, 3 & 4)
FB	94	11	Skin neoplasia (6) (ref #5)	-
LR	276	30	Otitis externa (53) (ref #1)	Arthrosis/Arthritis (14) (ref #2 & 6)
X	579	35	Otitis externa (43) (ref #1)	Anal gland disease (23) (ref #1)

References:
#1 O'Neill, Church et al. 2014b
#2 Asher, Diesel et al. 2009
#3 Summers, Diesel et al. 2010
#4 Mattin, Boswood et al. 2015
#5 Mochizuki, Motsinger-Reif et al. 2017
#6 LaFond, Breur et al. 2002

Discussion

O'Neill, Church et al. (16) reviewed specific advantages and limitations of data sources for population estimates. Limitations include: questionable representativeness and excluded disorders in insurance databases, referral bias in referral clinic data, diagnostic unreliability, technical complexities, poor representativeness in cancer registries, validation issues in questionnaire data, selection bias in health schemes and under-reporting and poor generalizability in specific surveillance systems e.g. on pharmacovigilance. Specific challenges for practice data include labor-intensiveness, confidentiality, unsustainability, lack of structured coding, large volumes of data, and lack of completeness for all events. In PETscan, we counter challenges of primary practice data systems: diagnoses are organized and coded according to a clinical rationale, all events are registered including health checks and end-of-life events, diagnoses are automatically sent to the central database without owner information, and the standardized coding allows for automated analysis of large volumes of data, creating a sustainable system. Practices that participated in this pilot study of PETscan represented practices based in cities and rural areas and varied according to size of the clinic and level of care; therefore we assumed an unbiased sample of the pet population. However, variation in veterinary opinion on breed specific health issues should be included in the interpretation of the results and representativeness discussed.

The entering of a diagnosis into PETscan requires active participation of the veterinarian, which may be subject to variation caused by time and effort and considered the biggest challenge in this population data system. However, diagnoses in the breed populations evaluated in the pilot phase of PETscan are similar as reported in comparable population survey studies that did not require such participation, suggesting that PETscan data can provide a valid random sample of veterinary diagnoses in the companion animal population. Evaluation of participation levels is needed to assess whether the sample size reflects the actual number of visits in practice.

The feedback of the PETscan pilot phase has been used in an expanded version (2.0) with an elaborated diagnosis list, including infectious diseases which may be analysed geographically, and pop-up advice for every diagnosis, which provides the veterinarian with a summary of diagnostic possibilities and additional useful information such as information about availability of DNA-tests for hereditary diseases (Supplemental f*igure 1*). As a long term project, PETscan will need to be evaluated continuously, expanding the diagnostic list with any missing or new diagnoses. The intended implementation in the University Clinic will ensure the evaluation by the veterinary specialists.

Population based measurement of disease incidence can give insight in breed predisposition for disease, providing data for prioritization of genetic studies. A first breed specific screening may be done by organ system (*figure 1a/b*), followed by more detailed analyses of diagnoses. The number of mutations in the OMIA database per organ system in comparison to the PETscan entries, show discrepancies that suggest that OMIA is a representation of specific study interests and findings, mostly monogenic, and not a representation of disease frequency. We compared the number of Mendelian diseases reported in OMIA for the Chihuahua, French bulldog and Labrador retriever: 22 diseases for various organ systems are registered for the Labrador retriever. For the Chihuahua and the French bulldog this is 2 and 1 respectively. The overrepresentation of the Labrador in OMIA may be caused by the long term popularity of the breed on an international level resulting in a research and publication bias. Another cause for overrepresentation may be reduced heterogeneity of the Labrador when compared to the Chihuahua and the French bulldog, which increases the risk of Mendelian disorders. However, the median genetic diversity in the three breeds as available from a tested subset of individuals ((17) MyDogDNA, www.mydogdna.com) seemed comparable: Chihuahua 39.8%, French bulldog 34.3%, and Labrador retriever 35.5%, as measured on the genome wide screening of thousands of sites in the individual DNA. A third cause could be that the Chihuahua and the French bulldog are more prone to disorders caused by extreme conformation, which are less likely to show in the OMIA database (12).

Conclusions

PETscan offers quality assurance in the diagnostic process and standardized coding of diagnostic terminology, which allows for routine periodic data and trend analysis. As it moves from the pilot phase, PETscan allows for quantification of - genetic - disease issues in the companion animal population, thus allowing for prioritization of genetic studies, as well as evaluation of breeding strategies.

Abbreviations

CH = Chihuahua

FB = French bulldog

LR = Labrador retriever

OMIA = Online Mendelian Inheritance in Animals

PMS = practice management software

X = mixed-breed dog

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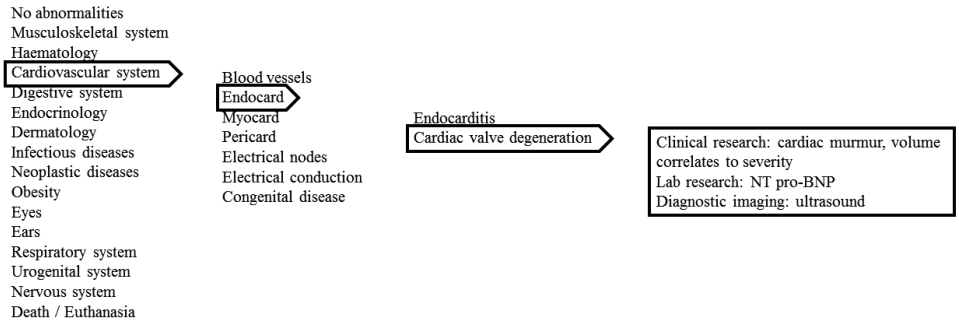
Supplemental information

Supplemental figure 1. PETscan main list with organ systems and conditions, elaborated with the pathway to the diagnosis of cardiac valve degeneration, including a diagnostic pop-up advice to support veterinary decision making as provided in the expanded version 2.0. Translated from Dutch, shown in simple format.



Chapter 6

Heterozygosity testing and multiplex DNA panel screening as a potential tool to monitor health and inbreeding in a small, closed dog population

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Canine Genet Epidemiol. 2018 Dec 28;5:12.

Abstract

Selective breeding in populations with a limited effective population size may result in a loss of genetic diversity, which can cause an increased concentration of specific disease liability genes. The Dutch Shepherd Dog (DSD) in the Netherlands is an example of such a breed with a small effective population.

To evaluate the measurement of genetic diversity and multiplex DNA panel screening for implementation in a breeding strategy for the DSD and to investigate the clinical relevance of potentially identified mutations in the multiplex DNA panel screening.

Genome-wide Single Nucleotide Polymorphism testing showed genetic isolation and reduced genetic diversity within coat variety subgroups of the DSD. Panel screening identified a Von Willebrand's Disease type I mutation (VWD-I). Although decreased Von Willebrand's Factor proteins were significantly lower in DSDs carrying the VWD-I allele compared to the wildtype, clinical follow-up did not show a significant association between the clinical phenotype and VWD-I genotype.

Genetic relationship measurement within a breed population may be a useful tool to enable breeding strategies to conserve genetic diversity. Results from a disease panel screening need to be evaluated for clinical relevance before breed selection restrictions can be considered.

Introduction

Dog breeds are known to be subject to human-induced limitations of the gene pool such as a popular sire effect and a breed barrier – a dog can only be registered as a certain breed if both parents are registered as such – resulting in reproductive isolation. Consequently, dogs from the same breed are genetically similar to each other (1), to such an extent that the breed can often be assessed by genotype alone, indicating genetic isolation between breeds (2). Demographic models have shown that a small, effective population size and genetic bottlenecks may have a major effect on the spread of genome changes through a population, where deleterious mutations may result in genetic disorders in later generations (1,3). In small dog breed populations with a limited gene pool, such as the Dutch Shepherd Dog[1] (DSD) population, an active approach to breeding healthy individuals is warranted to maintain genetic diversity for the future.

The DSD belongs to the shepherd dog type that originated in the Netherlands in the 19th century. It is grouped with e.g. the Saarloos wolfdog (4), and is a medium sized breed, measuring 55-62 cm high, and weighing between 23-28 kg. The DSD has an estimated population of approx. 2,400 individuals in the Netherlands, with an assumed life expectancy of 11 years (estimated by the Dutch Shepherd Dog Club). The DSD population size is roughly thirty times lower than that of the Labrador retriever, which is the most popular breed in the Netherlands. The DSD has three coat varieties (short, long, and wire haired), which historically were not allowed to breed, although limited crossbreeding has been allowed since October 1st 2014 (5,6): the guidelines of the Fédération Cynologique International (7) still do not allow crossbreeding between long haired and wire haired varieties as coat issues such as felting would occur. Previous information on DSD health showed no indication of an increased predisposition to any genetic diseases (8-11). However, the DSD population is thought to have limited genetic diversity, which harbours the risk of health issues related to inbreeding depression or increase of recessive disease in the future (12).

Genetic heterozygosity testing is currently routinely based on single nucleotide polymorphism (SNP) genotyping. SNP data can be used to test the genetic relationships of individuals and the genetic diversity of a population (13). Heterozygosity is associated with an increase in e.g. cognition and memory (14,15), thus shaping a population with the ability to respond to changing circumstances (12,16). A larger population size provides a greater predicted genetic diversity (17,18).

[1] See: http://www.hollandseherder.nl/details/the_dutch_shepherd/

Genome-wide SNP testing offers more accurate genetic diversity estimates than pedigree records or short-tandem repeat molecular markers (19) and the release of the canine genome sequence (20) facilitated an increase in research into genetic disorders (21,22). The development and availability of genomic tools has increased over the past two decades, allowing for more elaborate and precise testing in the future (23). One of the possible tools is the MyDogDNA™ assay[2], which includes both a canine within-individual heterozygosity test, and multiplex DNA panel screening for known inherited genetic disease variants (*Supplemental table 1*) and traits such as coat varieties. The inclusion of the panel screening offers the opportunity to explore possible predispositions or exclude known disease variants in the breeding strategy.

The aim of this paper is to evaluate the measurement of genetic diversity and multiplex DNA panel screening for implementation in a breeding strategy for the Dutch Shepherd Dog (DSD). The Von Willebrand's Disease type I (VWD-I) gene mutation c.7437G>A (p.Ser2479Ser, OMIA ref: (24)) was identified in a single long haired DSD during this study, a mutation that has so far been found in at least 20 breeds or breed variants (25). Thus, assessing the prevalence of the VWD-I mutation and the clinical consequences in the DSD population emerged as a second aim.

Material and Methods

Dogs

Members of the Dutch Shepherd Dog Club volunteered the individual DSDs tested in this study. The numbers in each of the consecutive steps were 1) MyDogDNA™ screening first testing group (10 short haired, 10 long haired, 10 wire haired); 2) Von Willebrand's Factor (VWF) type I genotype testing in the long haired DSD population through continued MyDogDNA™ testing and Sanger sequencing (14 in pedigree first identified individual, 42 long haired Dutch breeding population 2013-2015); 3) MyDogDNA™ combined results (13 short haired, 28 long haired, and 13 wire haired for the genetic relationships; 18 short haired, 46 long haired, 16 wire haired, and 25 variety crosses for the heterozygosity); and 4) Evaluation of the bleeding history and coagulation (19 individuals, of which nine wildtype, eight carriers and two homozygously affected).

Crossbreeding of coat varieties in this study took place between short haired and long haired DSDs only. Any crossbreeding between the short haired and wire haired variety is not included in this study, and crossbreeding between long haired and wire haired varieties is not allowed. The parent population of short haired and long haired DSDs were matched (in effect, mated) to produce first generation variety crosses. Matching first generation variety crosses with a parent population

[2] See: http://www.mydogdna.com

(backcross) resulted in second generation variety crosses, matching second generation variety crosses with a parent population resulted in third generation variety crosses (*figure 1A*).

MyDogDNA™ testing

MyDogDNA™ (Genoscoper Laboratories Oy, Helsinki, Finland) testing consists of two main tests: heterozygosity testing, and multiplex DNA panel screening. Heterozygosity is determined using a genome-wide single nucleotide polymorphism (SNP) test – evaluating genetic relationships and genetic diversity, respectively relating to the individual and the population (e.g. (38)). For the present study, MyDogDNA™ derived genotypes were available for 2,642 SNPs. Genetic diversity is expressed as SNP heterozygosity ratio, in effect the proportion of heterozygous SNPs out of all examined SNPs. The statistical testing of the median genetic diversity was carried out non-parametrically with a Kruskal-Wallis test ($p<0.05$) The multiplex DNA panel screening is a genotyping microarray, which, at the time of the present study, tested for 189 known disease variants and 22 traits, including coat length and colour (32) (*Supplemental table 1*). The validation and power of the panel as a research discovery tool was previously described in detail by Donner et al. (30,31).

Sequencing of the VWD-I gene

Sanger sequencing was performed as follows. DNA was isolated from Oragene Animal-400 saliva swabs using the manufacturer's instructions (DNA genotek). We performed PCR to capture the VWD-I mutation (forward primer (5'- AAATCTCCTTCATAAGCATCCC-3') and reverse primer (5'- CTGCCTTTCACCCAACCT-3')). The PCR product was treated with Exonuclease I and Shrimp Alkaline Phosphatase. Sequence reactions, performed with Big Dye Terminator Ready Reaction Mix v3.1 (Applied Biosystems), were sequenced on an ABI3500XL and analyzed in Lasergene (version 12.0 DNASTAR).

Evaluation of the bleeding history and coagulation

To evaluate the clinical significance of the VWD-I mutation, haemostasis was assessed in 19 DSDs in the following numbers - wildtype (n=9), carriers (n=8) or homozygously affected (n=2). A detailed history, VWF concentration, coagulation profile, and thrombocyte count were collected. Blood samples from the jugular vein (4 ml sodium citrate 3.8%, 4 ml EDTA) were used to determine the VWF I antigen concentration by enzyme-linked immunosorbent assay (ELISA, (39)), coagulation parameters (Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), fibrinogen), and thrombocyte count. All tests were performed in the University Veterinary

Diagnostic Laboratory (Utrecht University). The difference in VWF distribution between the three genetic groups were tested non-parametrically using the Kruskal-Wallis test. The difference between the wildtype group and the combined group of heterozygous and homozygous individuals was tested non-parametrically using the Mann-Whitney U test. Significance level was set at $p<0.05$ for both tests.

Results

Figure 1A shows the method of crossbreeding and backcrossing coat varieties between short haired and long haired DSDs, resulting in several types of variety crosses.

MyDogDNA™ testing

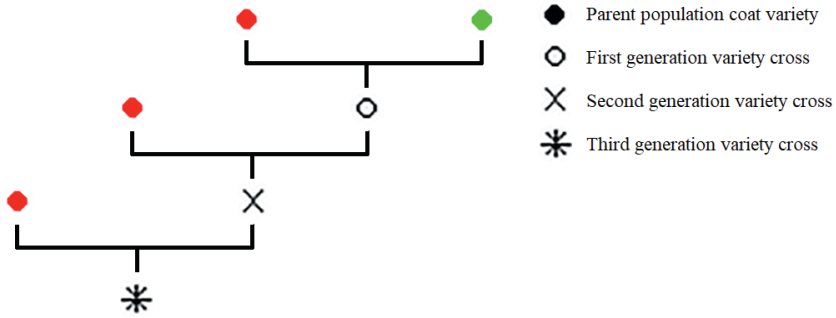
The genetic relationships are shown through a multidimensional scaling plot (*figure 1B*). The visual representation of genetic relationships shows the separation between the coat varieties of the DSDs. The progeny resulting from crossbreeding between the short haired and long haired coat varieties are included in the plot as variety crosses and shown in the colour of the genetically confirmed coat variety (trait testing MyDogDNA™). The coat genotype and phenotype agree in all cases.

The median genetic diversity of the short haired DSDs was significantly higher than that of the other two varieties (38.3% for the short haired DSD versus 25.4% and 26.7% for the long haired and the wire haired respectively ($p<0.05$)). All variety crosses of short haired x long haired together had a genetic diversity of 29.4%, which was significantly higher than the 25.4% of the long haired parent population ($p<0.05$) (*figure 1C*).

Results of the disease variant panel screening in the 30 DSDs that were tested in the first phase of this study showed one carrier for VWD-I in the long haired DSD variety, in which a c.7437G > A variant was present (*figure 2*). All dogs were clear for the remaining 188 disease-causing mutations present on the MyDogDNA™ array.

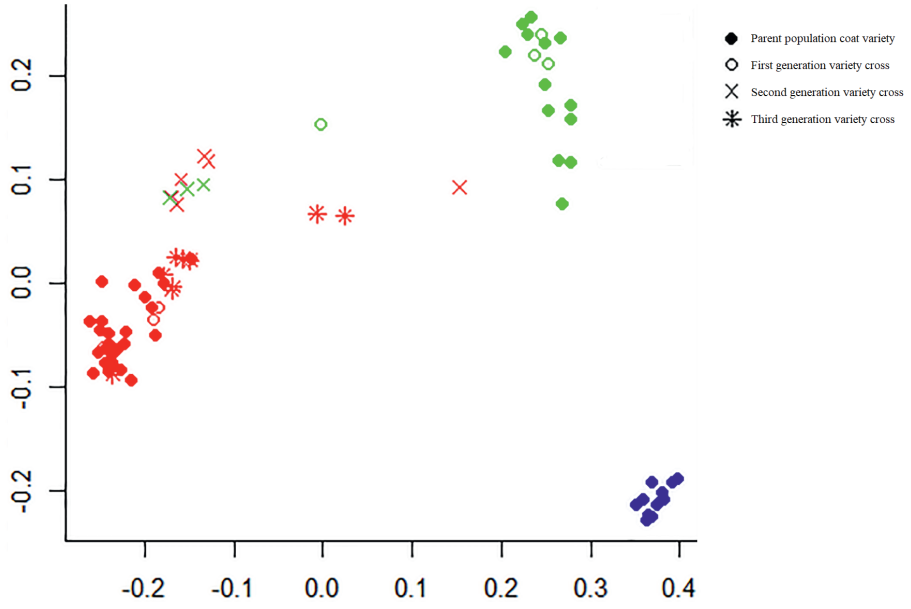
Figure 1. Genetic relationship and diversity in the Dutch Shepherd Dog.

1A. Crossbreeding of short haired and long haired Dutch Shepherd Dogs.



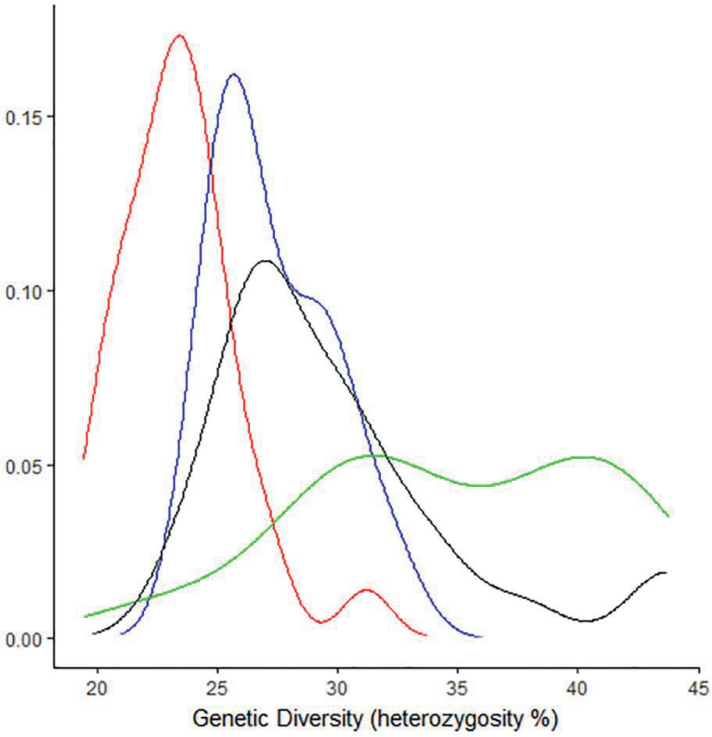
Parent populations of short haired (green) and long haired (red) dogs were matched to produce first generation variety crosses. Matching variety crosses with a parent population (backcross) resulted in the next generation of variety crosses.

1B. Genetic relationship of the tested population of Dutch Shepherd Dogs in a multidimensional scaling plot.



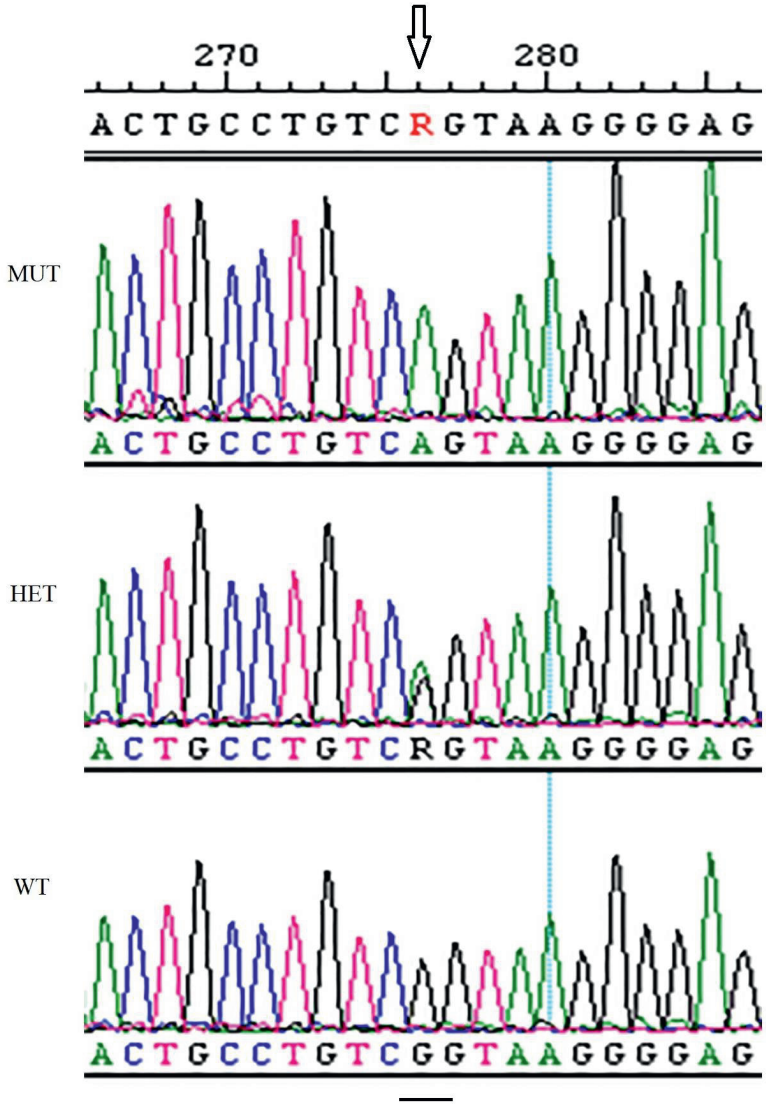
The parent populations shown are short haired (green, n= 13), long haired (red, n= 28), and wire haired (blue, n= 13) varieties. Variety crosses are shown in the colour of the genetically confirmed coat variety (trait testing MyDogDNA™).

1C. Genetic diversity of the tested population of Dutch Shepherd Dogs.



The short haired (green, n= 18), long haired (red, n= 46), and wire haired (blue, n= 16) are shown together with a combination of the variety crosses between long and short haired (black, n= 25).

Figure 2. Von Willebrand's Factor mutation analysis.



Example of chromosomal DNA containing Von Willebrand's Factor c.7437G > A. WT = wildtype, HET = heterozygous carrier, MUT = homozygous mutant.

A structural or quantitative defect in the Von Willebrand's Factor (VWF) (26) leads to a bleeding disorder called Von Willebrand's Disease (VWD) (27). VWD-I is characterized by a decrease in the concentration of plasma VWF. VWD-I is associated with mild clinical signs only (27). To assess whether the VWD-I mutation was a de novo mutation or a segregating mutation, family members of the VWD-I carrier were subsequently tested with MyDogDNA™, which identified multiple carriers, as well as two homozygous individuals (*figure 3*), indicating that it was a segregating mutation.

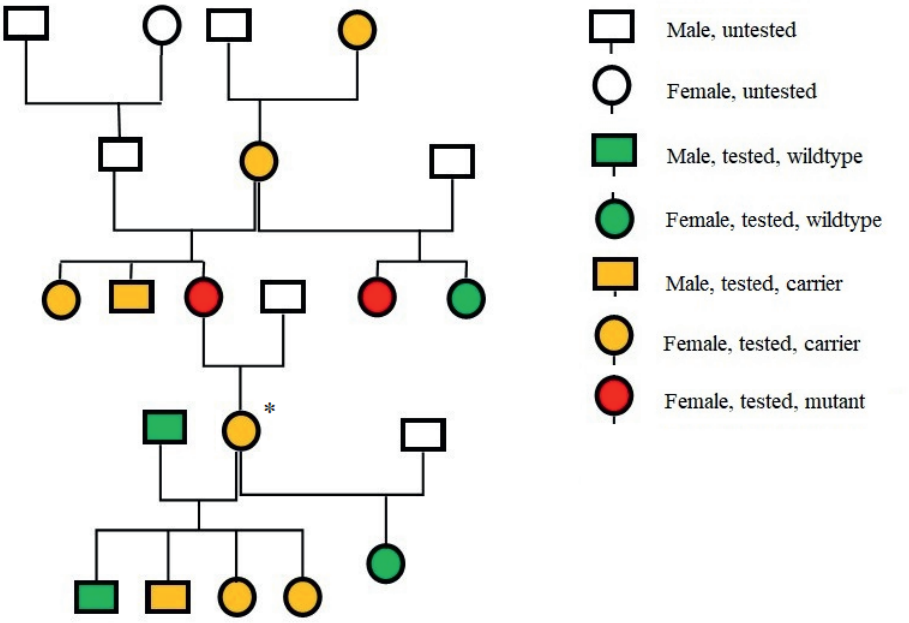
Allele frequency VWD-I gene in population

Combining the results of the panel screening with the Sanger sequencing, the cross-section of the long haired breeding population from 2013-2015 (n=42, 89% of the dogs from the Dutch population used for breeding) showed an allele frequency of <3% (2 alleles of 84 tested alleles), since two breeding individuals were carriers and no homozygously affected individuals were found (*Supplemental figure 1*).

Evaluation of the bleeding history and coagulation

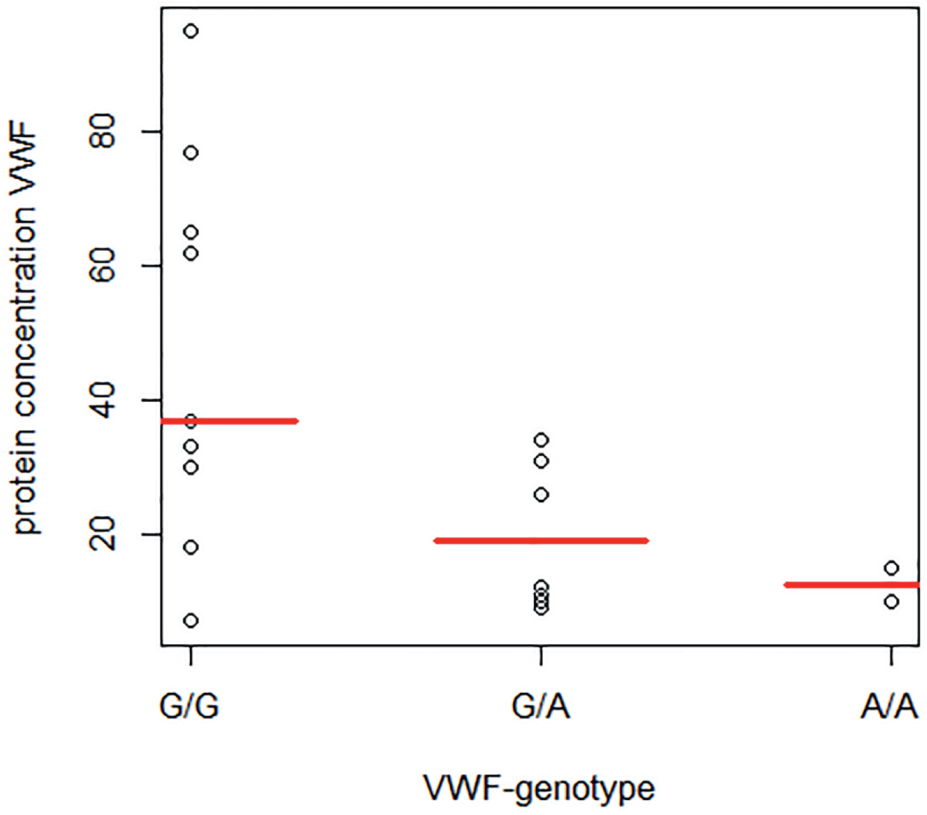
Eight out of 19 owners of the dogs included in the clinical validation experiment reported that their dog had experienced a bleeding episode (genotypes in these eight dogs were wildtype (4), heterozygous carrier (2), and homozygous mutant (2)). However, all of these episodes could be related to trauma, no excessive bleeding was reported. VWF protein concentrations ranged between 7 and 95%. No significant difference in VWF values was found when the three groups were compared (Kruskal-Wallis test, p=0.07). We found a significant difference in the Von Willebrand protein concentration when comparing the wildtype group with the other two groups combined (Mann-Whitney U test, p=0.03) (*figure 4 & table 1*). PT, aPTT, and fibrinogen were within reference range in all 19 dogs. Thrombocytes were below reference range in one dog, which was thought to be related to thrombocyte aggregates identified in the blood smear.

Figure 3. Von Willebrand's Disease segregation in the Dutch Shepherd Dog.



Dutch Shepherd Dog pedigree following first identification (*) of a carrier of Von Willebrand's Disease type I. This individual was a female long haired shepherd born in 2010.

Figure 4. Von Willebrand's Factor protein concentration and genotype correlation in the Dutch Shepherd Dog.



Correlation between Von Willebrand's Factor protein concentrations in blood (median shown in red) and Von Willebrand's Disease genotype (G/G = wildtype (n=9), G/A = heterozygous carrier (n=8), A/A = homozygous mutant (n=2)), in 19 Dutch Shepherd Dogs. No significant difference in protein concentration was found when the three groups were compared (Kruskal-Wallis test, p=0.07). Comparing the wildtype group with the other two groups combined showed a significant difference in protein concentration (Mann-Whitney U test, p=0.03).

Table 1. Individual determination of genotype and coagulation profile in the Dutch Shepherd Dog.

Dog #	*Genotype*	*VWF*	*Thrombocytes*	*Fibrinogen*
1	G/G	7	344	1,6
2	G/G	18	257	1,6
3	G/G	30	344	1,8
4	G/G	33	322	1,8
5	G/G	37	97*	4,2*
6	G/G	62	342	1,4
7	G/G	65	338	1,8
8	G/G	77	358	1,6
9	G/G	95	395	3,2
10	G/A	9	284	1,8
11	G/A	10	305	1,7
12	G/A	11	366	2
13	G/A	12	381	2,1
14	G/A	26	247	1,1
15	G/A	31	330	2
16	G/A	31	334	1,9
17	G/A	34	360	1,9
18	A/A	10	321	3,7
19	A/A	15	303	1,6

*Results for Von Willebrand's Factor protein concentrations in blood, Von Willebrand's Disease genotype (G/G = wildtype, G/A = heterozygous carrier, A/A = homozygous mutant), thrombocytes (ref. 144-603 10^9/L) and fibrinogen (ref. 1.0-2.7 g/L) in 19 Dutch Shepherd Dogs. *Many thrombocyte aggregates present.*

Discussion

The aim of this paper was to illustrate how heterozygosity testing through genome-wide SNP testing combined with multiplex DNA panel screening could be applied in sensible breeding advice. In the current study, the MyDogDNA™ assay was used because of logistics, as well as the fact that the DSD breeders had already sent samples there to assess genetic diversity of their breed. Other institutes providing similar genetic diversity testing include the University of California[3] and the University of Cornell in New York[4] (28, 29). The MyDogDNA™ assay was deemed a valid tool, as after an extensive validation and development phase on approximately 7,000 dogs representing over 230 breeds, the panel screening was shown to be instrumental in the detection of causative mutations that were previously undocumented in certain breeds (30,31), as was the case in the DSD. However, the absence of mutations does not necessarily equate to the absence of the disease allele or clinical disease, since different mutations in different dog breeds may lead to the same clinical disease. Since unidentified disease mutations may also be present, continued expansion of test panel content is paramount (30). Breeding for certain qualities and health is a multifaceted issue. Donner et al. previously discussed the applicability of the tool and its place as part of a holistic breeding strategy (30).

Individual test results should not in themselves lead to exclusion from breeding without knowledge of the pathophysiology of the disease and the connected test result. Careful interpretation of results and validation in the new population should be part of a breeding strategy including multiple tools (12).

The genetic relationships plot shows a distance between the DSD coat varieties, suggesting genetic isolation occurs not only between breeds in general, but also between subgroups of a breed if isolated populations are created. It can be seen that crossbreeding between DSD varieties bridges this genetic isolation. Allowing further crossbreeding may therefore increase the potential for choosing the best genetic diversity-increasing match within the DSD population, while conserving desired coat varieties as breed-specific trait.

The genetic diversity of two of the three DSD coat varieties is less than the median diversity of all combined purebred dogs (33.8%). The short haired DSDs have a greater level of diversity. So, in relation to purebred dogs as a whole, our observations showed that the DSD is at the lower end of the spectrum. The genetic diversity of all three DSD varieties is less than that of mixed-breed

[3] See: https://www.vgl.ucdavis.edu
[4] See: https://embarkvet.com

dogs (43.2 %) (32). Being aware of the variation in mixed-breed dogs, this last result could be expected. The short haired DSD population has the highest diversity which is most likely due to the fact that the effective population size is larger compared to the other two coat varieties. It may not, therefore, be in immediate need of crossbreeding to maintain a healthy gene pool, but it may be used to increase the diversity within the other two coat varieties. As most testing was done throughout the breadth of the DSD gene pool, we consider the genetic diversity measurements to be a fair representation of the true genetic diversity.

Breeding for heterozygosity reduces the risk of inbreeding depression, where accumulation of deleterious mutations leads to a lower individual fitness. This may lead to smaller litters, reduced lifespan and increased mortality in offspring (33). Although individual benefits are not yet apparent, breeding for heterozygosity aims at maintaining the population gene pool (12). In this study, the aim was to explore which insight on the DSD breed was provided by genetic diversity analysis. We identified an increased homozygosity within the three subpopulations of coat varieties which were previously not allowed to breed with each other. Although no obvious health issues were reported until now, continuous breeding within the subgroups and selection will likely lead to more loss of genetic diversity and carries a risk of future negative influence of recessive alleles. We would in this case advise expanding the effective population size for each coat variety; to make full use of the available gene pool whilst selecting animals with the desired characteristics for the breed. To increase the heterozygosity within the three different coat varieties, we advise to continue variety crossbreeding. It is important to note that the DSD remains a distinct dog breed in this way, but the separation between the coat varieties will decrease, decreasing the risk of accumulation of recessive alleles within coat varieties. Even with breeding between the coat varieties, one of the important desired breed characteristics (coat-length) for the DSD was maintained and future selection of dogs for breeding could be supported by using the tests for traits that are present on MyDogDNA™.

The results of the VWD-I sequencing show an allele frequency of <3% in the DSD breeding population in the years 2013-2015 in the Netherlands. This breeding population is assumed to be the parent population of the current national DSD population. In the subset of 19 dogs that were clinically evaluated, no bleeding tendency was found, although we observed a statistically significant lower VWF protein concentration in dogs hetero- or homozygous for the examined VWD-I mutation. The results of this limited sample confirm that, as in other breeds with VWD-I, the presence of a mutated allele leads to a lower VWD protein concentration but shows only

limited signs of haemorrhagic diathesis (34-36). However, the predictive value of common coagulation tests may be limited (37).This underlines the importance of assessing the phenotype associated with the mutation. Although VWD-I disease usually gives mild clinical phenotype, when additional trauma is present, the disease could lead to clinically relevant bleeding. Therefore we advise to prevent homozygous mutants arising from breeding. In the DSD population, this should be feasible without excluding breeding animals from the population, as the VWD-I allele frequency within the DSD is low.

Conclusions

Increased inbreeding of (sub) populations of a dog breed, carries the risk of inbreeding depression and increase of allele frequency of disease-causing, usually recessive alleles. Increasing heterozygosity, whilst maintaining characteristics important for the breed, and prevention the segregation of disease-causing gene mutations may be important in a sustainable, healthy breeding program.

Genetic relationship measurements can be used to match breeding couples to increase the genetic diversity in a breed population or in subpopulations within a breed. The multiplex DNA panel screening can be used to check for genetic disorders in the breed that were previously unknown and could potentially spread unintendedly in the population.

A sensible breeding programme should include application of the described genetic tools with appropriate counselling, as well as individual and population-based clinical screening for disorders with and without a known mutation.

Abbreviations

DSD = Dutch Shepherd Dog

SNP = Single Nucleotide Polymorphism

VWD-I = Von Willebrand's Disease type I

VWF = Von Willebrand's Factor

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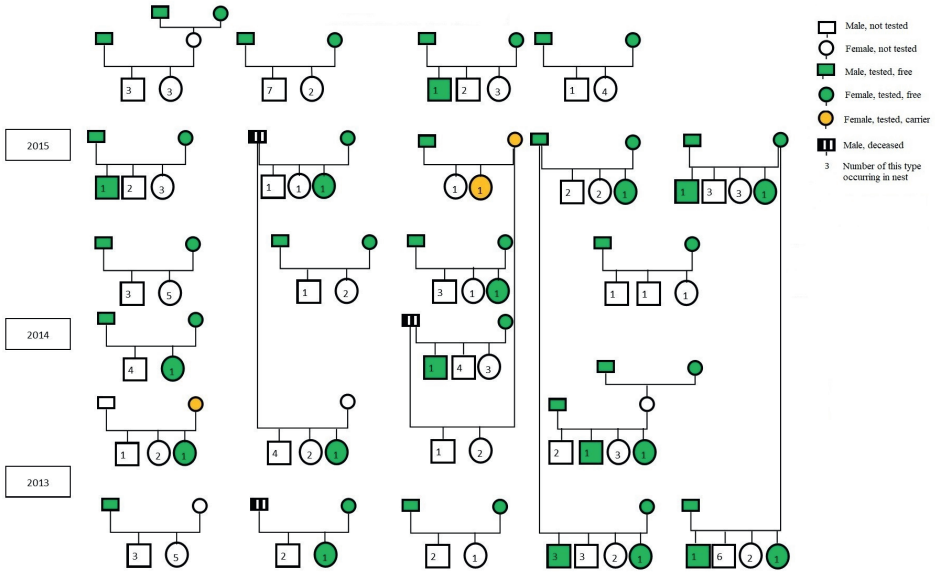
Supplemental information

Supplemental figure 1. Cross-section 2013-2015 Dutch breeding population of the long haired Dutch Shepherd Dog.



Cross-section of the long haired Dutch Shepherd Dog breeding population for the years 2013-2015 in the Netherlands, combining the results of MyDogDNA™ multiplex DNA panel screening with Sanger sequencing for the causal variant for Von Willebrand's Disease type I (89%, n=42 from 47 breeding individuals). Litters are shown by birth year, most were not related. Resulting allele frequency in the breeding population is 2%.

Supplemental table 1. Multiplex DNA panel screening.

Type of disorder	*Disorder*
Blood	Bleeding disorder due to P2RY12 defect
Blood	Canine Cyclic Neutropenia, Cyclic Hematopoiesis, Grey Collie Syndrome, (CN)
Blood	Canine Leukocyte Adhesion Deficiency (CLAD), type III
Blood	Canine Scott Syndrome, (CSS)
Blood	Factor IX Deficiency or Haemophilia B; mutation Gly379Glu
Blood	Factor IX Deficiency or Haemophilia B; mutation originally found in Airedale
Blood	Factor IX Deficiency or Haemophilia B; mutation originally found in German
Blood	Factor IX Deficiency or Haemophilia B; mutation originally found in Lhasa Apso
Blood	Factor VII Deficiency
Blood	Factor VIII Deficiency or Haemophilia A; mutation originally found in Boxer
Blood	Factor VIII Deficiency or Haemophilia A; mutation originally found in German
Blood	Factor VIII Deficiency or Haemophilia A; mutation originally found in Old English
Blood	Factor VIII Deficiency or Haemophilia A; p.Cys548Tyr mutation originally found in
Blood	Factor XI Deficiency
Blood	Glanzmann Thrombasthenia Type I, (GT); mutation originally found in mixed-breed
Blood	Glanzmann Thrombasthenia Type I, (GT); mutation originally found in Pyrenean
Blood	Hereditary Elliptocytosis
Blood	Hereditary Phosphofructokinase (PFK) Deficiency
Blood	Macrothrombocytopenia; disease-linked variant originally found in Norfolk and
Blood	May-Hegglin Anomaly (MHA)
Blood	Prekallikrein Deficiency
Blood	Pyruvate Kinase Deficiency; mutation originally found in Basenji
Blood	Pyruvate Kinase Deficiency; mutation originally found in Beagle
Blood	Pyruvate Kinase Deficiency; mutation originally found in Pug
Blood	Pyruvate Kinase Deficiency; mutation originally found in West Highland White
Blood	Thrombopathia; mutation originally found in Basset Hound
Blood	Thrombopathia; mutation originally found in Eskimo Spitz
Blood	Thrombopathia; mutation originally found in Landseer
Blood	Trapped Neutrophil Syndrome, (TNS)
Blood	Von Willebrand's Disease (vWD) Type I
Blood	Von Willebrand's Disease (vWD) Type III; mutation originally found in
Blood	Von Willebrand's Disease (vWD) Type III; mutation originally found in Scottish
Blood	Von Willebrand's Disease (vWD) Type III; mutation originally found in Shetland
Cardiac	Dilated Cardiomyopathy, (DCM); mutation originally found in Schnauzer
Cardiac	Long QT Syndrome
Dermal	Dystrophic Epidermolysis Bullosa; mutation originally found in Central Asian
Dermal	Dystrophic Epidermolysis Bullosa; mutation originally found in Golden Retriever
Dermal	Epidermolytic Hyperkeratosis
Dermal	Focal Non-Epidermolytic Palmoplantar Keratoderma, (FNEPPK); mutation
Dermal	Hereditary Footpad Hyperkeratosis, (HFH)
Dermal	Ichthyosis; mutation originally found in American Bulldog
Dermal	Ichthyosis; mutation originally found in Great Dane
Dermal	Lamellar Ichthyosis, (LI)

Dermal	Ligneous Membranitis
Dermal	Musladin-Lueke syndrome, (MLS)
Dermal	X-Linked Ectodermal Dysplasia, (XHED)
Endocrine	Congenital Hypothyroidism; mutation originally found in Tenterfield Terrier
Endocrine	Congenital Hypothyroidism; mutation originally found in Toy Fox and Rat Terrier
Immunological	Autosomal Recessive Severe Combined Immunodeficiency, (ARSCID)
Immunological	Complement 3 (C3) Deficiency
Immunological	Myeloperoxidase Deficiency
Immunological	Severe Combined Immunodeficiency in Frisian Water Dogs, (SCID)
Immunological	X-Linked Severe Combined Immunodeficiency (XSCID); mutation originally found
Immunological	X-Linked Severe Combined Immunodeficiency (XSCID); mutation originally found
Metabolic	Glycogen Storage Disease Type Ia, (GSD Ia)
Metabolic	Glycogen Storage Disease Type II or Pompe's Disease, (GSD II)
Metabolic	Glycogen Storage Disease Type IIIa, (GSD IIIa)
Metabolic	Hypocatalasia or Acatalasemia
Metabolic	Intestinal Cobalamin Malabsorption or Imerslund-Gräsbeck Syndrome, (IGS);
Metabolic	Intestinal Cobalamin Malabsorption or Imerslund-Gräsbeck Syndrome, (IGS);
Metabolic	Mucopolysaccharidosis Type IIIA, (MPS IIIA); mutation originally found in
Metabolic	Mucopolysaccharidosis Type IIIA, (MPS IIIA); mutation originally found in New
Metabolic	Mucopolysaccharidosis Type VII, (MPS VII); mutation originally found in Brazilian
Metabolic	Mucopolysaccharidosis Type VII, (MPS VII); mutation originally found in German
Metabolic	Pyruvate Dehydrogenase Phosphatase 1 (PDP1) Deficiency
Muscular	Cavalier King Charles Spaniel Muscular Dystrophy, (CKCS-MD)
Muscular	Centronuclear Myopathy, (CNM); mutation originally found in Great Dane
Muscular	Centronuclear Myopathy, (CNM); mutation originally found in Labrador Retriever
Muscular	Duchenne or Dystrophin Muscular Dystrophy, (DMD); mutation originally found in
Muscular	Duchenne or Dystrophin Muscular Dystrophy, (DMD); mutation originally found in
Muscular	Muscular Dystrophy, Ullrich-type; mutation originally found in Landseer
Muscular	Myostatin deficiency (Double Muscling, "Bully")
Muscular	Myotonia Congenita; mutation originally found in Australian Cattle Dog
Muscular	Myotonia Congenita; mutation originally found in Miniature Schnauzer
Muscular	Myotubular Myopathy; mutation originally found in Rottweiler
Muscular	Nemaline Myopathy; mutation originally found in American Bulldog
Muscular	X-Linked Myotubular Myopathy
Neurological	Acral Mutilation Syndrome, (AMS)
Neurological	Alaskan Husky Encephalopathy, (AHE)
Neurological	Alexander Disease (AxD); mutation originally found in Labrador Retriever
Neurological	Bandera's Neonatal Ataxia, (BNAt)
Neurological	Benign Familial Juvenile Epilepsy or Remitting Focal Epilepsy
Neurological	Cerebellar Cortical Degeneration, (CCD); mutation originally found in Vizsla
Neurological	Cerebral Dysfunction; mutation originally found in Friesian Stabyhoun
Neurological	Dandy-Walker-Like Malformation (DWLM); mutation originally found in Eurasier
Neurological	Early-Onset Progressive Polyneuropathy; mutation originally found in Alaskan
Neurological	Fetal Onset Neuroaxonal Dystrophy, (FNAD)
Neurological	Hereditary Ataxia or Cerebellar Ataxia; mutation originally found in Old English
Neurological	Hyperekplexia or Startle Disease

Neurological	Hypomyelination; mutation originally found in Weimaraner
Neurological	Juvenile Myoclonic Epilepsy, (JME); mutation originally found in Rhodesian
Neurological	L-2-Hydroxyglutaric aciduria, (L2HGA); mutation originally found in Staffordshire
Neurological	L-2-Hydroxyglutaric aciduria, (L2HGA); mutation originally found in West Highland
Neurological	Lagotto Storage Disease, (LSD)
Neurological	Neonatal Cerebellar Cortical Degeneration or Cerebellar Abiotrophy, (NCCD)
Neurological	Neonatal Encephalopathy with Seizures, (NEWS)
Neurological	Neuroaxonal Dystrophy (NAD); mutation originally found in Spanish Water Dog
Neurological	Neuronal Ceroid Lipofuscinosis 1, (NCL1); mutation originally found in Dachshund
Neurological	Neuronal Ceroid Lipofuscinosis 10, (NCL10); mutation originally found in American
Neurological	Neuronal Ceroid Lipofuscinosis 5, (NCL5); mutation originally found in Border
Neurological	Neuronal Ceroid Lipofuscinosis 8, (NCL8); mutation originally found in Alpine
Neurological	Neuronal Ceroid Lipofuscinosis 8, (NCL8); mutation originally found in Australian
Neurological	Neuronal Ceroid Lipofuscinosis 8, (NCL8); mutation originally found in English
Neurological	Neuronal Ceroid Lipofuscinosis, (NCL7); mutation originally found in Chinese
Neurological	Polyneuropathy with ocular abnormalities and neuronal vacuolation, (POANV);
Neurological	Progressive Early-Onset Cerebellar Ataxia; mutation originally found in Finnish
Neurological	Sensory Neuropathy; mutation originally found in Border Collie
Neurological	Spinal Dysraphism
Neurological	Spinocerebellar Ataxia with Myokymia and/or Seizures (SCA)
Neurological	Spinocerebellar Ataxia/ Late-Onset Ataxia (SCA, LOA)
Neurological	Spongy degeneration with cerebellar ataxia, (SDCA1); mutation originally found in
Neurological	X-Linked Tremors; mutation originally found in English Springer Spaniel
Neuromuscular	Congenital Myasthenic Syndrome (CMS); mutation originally found in Labrador
Neuromuscular	Congenital Myasthenic Syndrome, (CMS); mutation originally found in Jack Russell
Neuromuscular	Congenital Myasthenic Syndrome, (CMS); mutation originally found in Old Danish
Neuromuscular	Globoid Cell Leukodystrophy or Krabbe Disease, (GLD); mutation originally found
Neuromuscular	Globoid Cell Leukodystrophy or Krabbe Disease, (GLD); mutation originally found
Neuromuscular	GM1 Gangliosidosis; mutation originally found in Alaskan Husky
Neuromuscular	GM1 Gangliosidosis; mutation originally found in Portuguese Water Dog
Neuromuscular	GM1 Gangliosidosis; mutation originally found in Shiba Dog
Neuromuscular	GM2 Gangliosidosis, mutation originally found in Japanese Chin
Neuromuscular	GM2 Gangliosidosis; mutation originally found in Toy Poodle
Neuromuscular	Paroxysmal Dyskinesia, (PxD); mutation originally found in Irish Soft Coated
Ocular	Canine Multifocal Retinopathy 1, (CMR1); mutation originally found in Mastiff-
Ocular	Canine Multifocal Retinopathy 2, (CMR2); mutation originally found in Coton de
Ocular	Canine Multifocal Retinopathy 3, (CMR3); mutation originally found in Lapponian
Ocular	Cone Degeneration, (CD) or Achromatopsia; mutation originally found in Alaskan
Ocular	Cone Degeneration, (CD) or Achromatopsia; mutation originally found in German
Ocular	Cone Degeneration, (CD) or Achromatopsia; mutation originally found in German
Ocular	Cone-Rod Dystrophy 1, (crd1); mutation originally found in American Staffordshire
Ocular	Cone-Rod Dystrophy 2, (crd2); mutation originally found in American Pit Bull
Ocular	Cone-Rod Dystrophy, (cord1-PRA / crd4)
Ocular	Cone-Rod Dystrophy, Standard Wirehaired Dachshund, (crd SWD)
Ocular	Congenital Stationary Night Blindness (CSNB)
Ocular	Dominant Progressive Retinal Atrophy, (DPRA)

Ocular	Generalised Progressive Retinal Atrophy
Ocular	Golden Retriever Progressive Retinal Atrophy 1, (GR_PRA 1)
Ocular	Primary Hereditary Cataract, (PHC); mutation originally found in Australian
Ocular	Primary lens luxation (PLL) and glaucoma; mutation originally found in Shar Pei
Ocular	Primary Lens Luxation, (PLL)
Ocular	Primary Open Angle Glaucoma, (POAG); mutation originally found in Basset Fauve
Ocular	Primary Open Angle Glaucoma, (POAG); mutation originally found in Beagle
Ocular	Primary Open Angle Glaucoma, (POAG); mutation originally found in Norwegian
Ocular	Primary Open Angle Glaucoma, (POAG); mutation originally found in Petit Basset
Ocular	Progressive Retinal Atrophy Type III, (PRA type III); mutation originally found in
Ocular	Progressive Retinal Atrophy, (CNGA1-PRA); mutation originally found in Shetland
Ocular	Progressive Retinal Atrophy, (PAP1_PRA); mutation originally found in Papillon
Ocular	Progressive Retinal Atrophy, (PRA); mutation originally found in Basenji
Ocular	Progressive Retinal Atrophy, (PRA); mutation originally found in Swedish Vallhund
Ocular	Rod-Cone Dysplasia 1, (rcd1); mutation originally found in Irish Setter
Ocular	Rod-Cone Dysplasia 1a, (rdc1a); mutation originally found in Sloughi
Ocular	Rod-Cone Dysplasia 3, (rcd3)
Ocular	X-Linked Progressive Retinal Atrophy 1, (XLPRA1)
Ocular	X-Linked Progressive Retinal Atrophy 2, (XLPRA2)
Other	Acute Respiratory Distress Syndrome, (ARDS); mutation originally found in
Other	Amelogenesis Imperfecta, (AI)
Other	Congenital Keratoconjunctivitis Sicca and Ichthyosiform Dermatosis, (CKCSID)
Other	Dental Hypomineralisation; mutation originally found in Border Collie
Other	Narcolepsy; mutation originally found in Dachshund
Other	Narcolepsy; mutation originally found in Doberman Pinscher
Other	Narcolepsy; mutation originally found in Labrador Retriever
Other	Persistent Müllerian Duct Syndrome, (PMDS); mutation originally found in
Other	Primary Ciliary Dyskinesia, (PCD)
Renal	Cystinuria Type I-A; mutation originally found in Newfoundland Dog
Renal	Cystinuria Type II-A; mutation originally found in Australian Cattle Dog
Renal	Cystinuria, Type II-B; mutation originally found in Miniature Pinscher
Renal	Fanconi Syndrome
Renal	Hyperuricosuria, (HUU)
Renal	Polycystic Kidney Disease in Bull Terriers, (BTPKD)
Renal	Primary Hyperoxaluria, (PH); mutation originally found in Coton de Tulear
Renal	Protein Losing Nephropathy, (PLN); NPHS1 gene variant
Renal	Renal Cystadenocarcinoma and Nodular Dermatofibrosis, (RCND)
Renal	X-Linked Hereditary Nephropathy, (XLHN)
Renal	X-Linked Hereditary Nephropathy, (XLHN); mutation originally found in Navasota
Renal	Xanthinuria, Type 1a; mutation originally found in mixed-breed dogs
Renal	Xanthinuria, Type 2a; mutation originally found in Toy Manchester Terrier
Renal	Xanthinuria, Type 2b; mutation originally found in Cavalier King Charles Spaniel
Skeletal	Chondrodysplasia; mutation originally found in Norwegian Elkhound and Karelian
Skeletal	Cleft Palate; Cleft Lip and Palate with Syndactyly; ADAMTS20 gene mutation
Skeletal	Cleft Palate; DLX6 gene mutation originally found in Nova Scotia Duck Tolling
Skeletal	Craniomandibular Osteopathy, (CMO); mutation associated with terrier breeds

Skeletal	Hereditary Vitamin D-Resistant Rickets, (HVDRR)
Skeletal	Oculoskeletal Dysplasia 2 or Dwarfism-Retinal Dysplasia 2, (OSD2)
Skeletal	Osteochondrodysplasia; mutation originally found in Miniature Poodle
Skeletal	Osteogenesis Imperfecta, (OI); mutation originally found in Beagle
Skeletal	Osteogenesis Imperfecta, (OI); mutation originally found in Dachshund
Skeletal	Skeletal Dysplasia 2, (SD2)
Skeletal	Spondylocostal Dysostosis
Skeletal	Van den Ende-Gupta Syndrome, (VDEGS)

Tested disorders within the multiplex DNA panel screening by MyDogDNA™, organised by type of disorder.

Table of Contents

Chapter 7

Summarising discussion and conclusion

Over the past decades, the awareness among the general public of breed-related health issues has increased. In canine and feline purebred populations these health issues entail both inherited diseases and harmful breed characteristics, the latter originating from extreme phenotypic features that were deemed desirable by breeders or companion animal owners. Breed-related health issues form part of the companion animal welfare debate at both a national and international level. The main subject of discussion is how to tackle such breed-related health problems in a responsible manner in order to arrive at healthy, sustainable breed populations. This involves many stakeholders from various backgrounds, and the complexity of the problem requires everyone's willingness to take responsibility and work together to find solutions. In order to further develop a breeding policy, it is necessary to collect more data on the nature and extent of the different problems in the different breeds. In parallel, the further development and application of genetic testing plays an essential role in controlling specific health issues in populations.

The studies described in this thesis investigated the available sources of companion animal population health data. The investigators developed a software system which collects the information required to determine and monitor health issues in companion animal populations via veterinary practices. In the near future, this population data can be used to support optimal use of DNA testing possibilities and contribute to responsible and evidence-based breeding.

Setting the scene

Chapter 2 reports on an international conference on welfare and breed-related problems in dogs in particular. A wide range of international stakeholders came together to improve the sharing of information and resources, to strengthen mutual cooperation, and to identify and initiate necessary actions. Participants took part in discussion groups on various subthemes: individualised breed-specific strategies for health and breeding, extreme conformations, education and communication, behaviour, quality measures for genetic testing, and the need for population-based evidence. Within each theme, the participants identified priorities and opportunities, if possible alongside an immediate plan of action. For example, working groups were set up to help implement breed-health strategies at a breed club level; and an evaluation of the quality of DNA tests and the laboratories performing them led to the development of an online resource enabling better informed application of genetic tools.

A need for better data on canine health was evident in all themes. Chapter 2 also noted that every country has its own population(s) as well as approach to this issue, which is why delegates felt it was desirable for specific actions to be carried out at a national level, at least initially.

A generally shared challenge that emerged from the international discussion, was the need to continue to share information and to connect all stakeholders with a professional or personal relationship to canine health, most of whom were well represented at the conference. Unfortunately, policy makers, media and companion animal owners were only present in small numbers. Their involvement will be important in the future, because the discussion about the well-being of the dog is deliberately moving in the direction of legal and social measures.

Disease phenotype epidemiology

The second part of the thesis focused on the epidemiology of disease phenotypes.

In **chapter 3** available data sources were explored to see how and how much information on population health could be derived for the Dutch Labrador retriever; data came from a veterinary practice software system, two insurance companies for companion animals, and a pathology laboratory. The available data allowed for rough estimates on longevity, frequency of veterinary visits, and the relative chances of specific diagnostic coding occurring (at a certain age) in a breed. It suggested that the Labrador retriever population under study had a similar or longer lifespan than mixed-breed dogs of the same body size.

The frequency of veterinary practice visits and the overall frequency of insurance expense claims was higher for the Labrador retriever than for mixed-breed dogs, with insurance claims relating to ears, airways, tendons and muscles, and joints showing the largest significant difference. Age at diagnosis was similar for the two groups. The data from the pathology laboratory indicated increased tumour pathology in the Labrador retriever although the insurance and pathology data were subject to a strong selection bias, as not all individuals were insured (with one of the two companies supplying the data) and samples were not always taken and sent to the laboratory participating in the research. In addition, owner behaviour may have influenced results, leading to a detection bias towards the Labrador retriever. Information bias regarding the exposure, i.e. being a Labrador retriever or mixed-breed dog, probably reduced the estimated effect on health. The differences found could have been caused by an actual difference in population health between the Labrador retriever and mixed-breed dogs, but a bias caused by owner behaviour may also have heavily influenced this outcome. Errors in disease status registration were expected to be unbiased with regards to the comparison.

It is important to note that disease at an individual level versus a high frequency at population level can result in different priorities. A disease may be rare in the population, but the burden for the individual may be high. If such a disease can be easily tested, prevented or treated, the gain at an individual level is significant and it would be a waste not to put effort into this.

In a historical comparative observational study (**chapter 4)** the breed-related disease burden in three purebred dog populations and one purebred cat breed were evaluated by comparing them with a control population of mixed-breed dogs and European Shorthair cats. The first part of this observational study was a qualitative query, carried out by conducting a literature review and asking the opinion of experts in veterinary specialist fields. Results were verified in a case control study in the University Clinic (referral centre). The results suggested an overrepresentation of purebred populations in certain specialist disciplines and diseases. For example, the French bulldog is overrepresented in the discipline of otorhinolaryngology and the Labrador retriever in orthopaedics. This query produced a list of potentially relevant diseases limited to five organ systems per breed which were then evaluated in a primary veterinary practice-based extended cross-sectional study. Results from this practice-based study showed that not all of the selected diseases contribute to the disease burden, which is in contrast to what was expected from the qualitative query. This is an important finding, as the breed-health discussion often only takes into account those aspects that come from qualitative sources, focussing on diseases that may occur, rather than asking whether this is really relevant for the population in question. However, harmful breed characteristics such as upper respiratory tract issues related to brachycephalic phenotype were in fact mostly confirmed in the data from the veterinary practice. The conclusion is that breed-health as seen in primary practice may sometimes differ from what is suggested by the literature or experts, although certain disease predispositions were confirmed. A remarkable finding from the practice-based data was that most breed individuals were registered as not having a pedigree. This shows once again the need to involve all stakeholders in a successful breeding strategy and not only the Kennel Club and pedigree breed organisations.

Manually searching through patient files is a laborious task and not a suitable method for collecting and analysing data on a large scale to quantify population health. Therefore, a prospective nationwide automatic data collection system was developed, which allowed for a standardised recording of diagnoses in veterinary practices. In the future, this system will allow researchers to objectively determine disease incidence and the effect of intervention measures.

The development and implementation of this system – called PETscan – is described in **chapter 5**. This system was designed to standardise how diagnoses in companion animal practices are made. Information collected from participating primary practices included demographic data, unique identification (based on transponder code), and consultation information (e.g. date and diagnosis). Some preliminary data were used to explore the system's potential for population health monitoring and the prioritisation of genetic research. A higher incidence of a disease in a specific breed than in mixed-breed dogs is suggestive of a heritable component. If such a finding is

followed up by genetic research, the genetic background can be elucidated and a DNA test may be developed. The development and implementation of PETscan took into account, as much as possible, the experiences gained from comparable systems in order to arrive at an optimal system. The most critical factors that may hamper the widespread adoption of any system is the contribution of veterinarians in practice, and privacy regulations regarding owner-related information. As an incentive to veterinarians to join PETscan, diagnostic advisory texts have been included with each diagnosis. This supplies the veterinarian with an up-to-date summary of diagnostic possibilities and other important information. These texts support the veterinarian in the diagnostic process by providing a quick overview of the required steps to be able to diagnose the patient with a specific condition, and provide other important information. A mandatory diagnosis registration in PETscan, with the veterinarian being unable to exit the patient file without entering a diagnosis, was deemed undesirable as it would negatively influence participation. In terms of privacy, no owner information was included in the collected data and the transponder code (which may lead to the owner) was anonymised.

When analysing data from PETscan, it was also important to be able to rely on the diagnosing veterinarian's conclusion. Although an exact case definition cannot be based on the diagnosis alone, uniformity is stimulated through the support of the diagnostic process. The preliminary results suggest that PETscan is able to reliably distil the necessary population health information from the collected data. Long term data collection in veterinary practices with PETscan will ensure the further standardisation of diagnostic terminology and thereby the quality of veterinary care. Large scale application of a system such as PETscan as a real-time prospective monitoring tool will provide insights into the incidence of disorders, breed predisposition, the evaluation of breeding strategies, and will allow for the prioritisation of genetic studies in companion animals.

Genetic testing

Chapter 6 describes a study which evaluated the measurement of genetic diversity and multiplex DNA panel screening for implementation in a breeding strategy for the Dutch Shepherd Dog. The study also investigated the clinical relevance of potentially identified mutations. Chapter 6 shows how DNA tools can be used for sensible breeding, in the absence of quantitative data on population health, and as part of a breeding programme including counselling and clinical screening. The multiplex DNA panel screening uses genome-wide SNPs to define individual heterozygosity and detects mutations related to specific diseases which may have been previously unknown in a breed. The genome-wide SNP testing provides information both on genetic diversity in general, and on genetic relationships within the breed tested and in comparison to other breeds

or mixed-breed dogs. The function of panel screening in a breeding programme is to check for genetic disorders and prevent unintended spread in the population. Depending on the pathophysiology of the disorder, clinical relevance and mode of inheritance, the general goal could be to keep the allele frequency low and prevent homozygous mutants from arising. This was the case in the example of the Dutch Shepherd Dog, in which the panel screening identified a Von Willebrand's Disease type I mutation.

Breeding for maximum heterozygosity reduces the risk of inbreeding depression and frequency increase of deleterious alleles by maintaining the population's gene pool. The genetic relationship measurement can be used to match breeding couples in a way that bridges genetic isolation and increases genetic diversity within and between subpopulations.

Conclusions and future perspectives

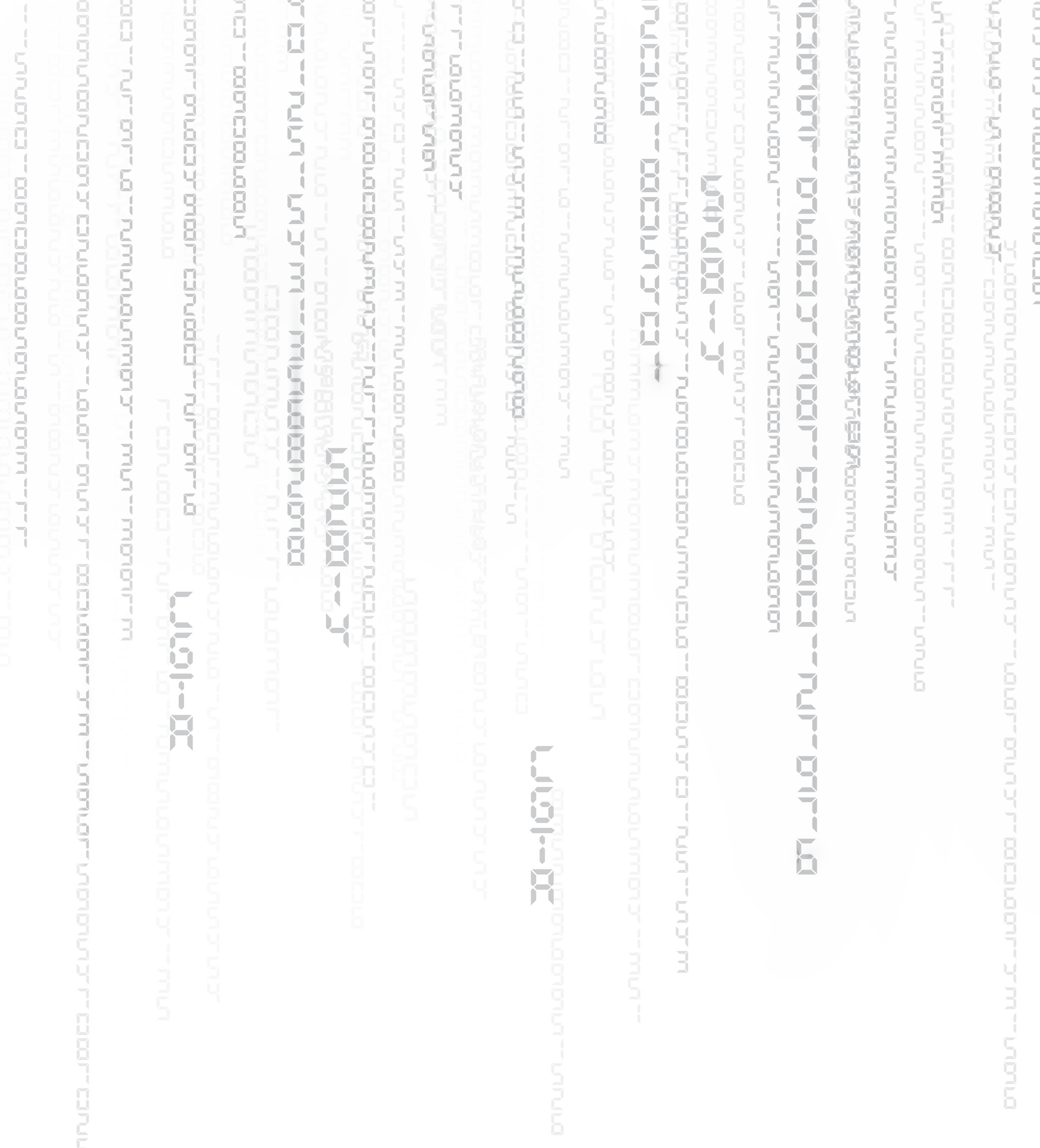
The increased awareness of pet animal welfare over the last few decades has given rise to many opportunities to increase the health and welfare of companion animals. In order to be able to implement a sound and responsible breeding policy based on current knowledge, the availability of quantitative data at both the individual and population level is essential. Not only is data collection from veterinary practices needed for a nationwide baseline measurement, to continue monitoring, and to prioritise genetic studies. There is also a major role for organisations such as the Kennel Club and breed-specific organisations, as well as for individual pedigree or non-pedigree breeders. In order to achieve the common goal, accurate and standardised data collection is essential, and it is up to all of the stakeholders to ensure further collaboration between all parties, with clear agreements on everyone's responsibility. Ideally, all animal health data should be recorded at a central point where the quality and completeness of the data can be guaranteed. Only such databases can ensure unprejudiced evaluation of population health.

When population data are available, the next challenge is to prioritise issues, both within breeds and for all breeds combined. It is logical that breeders, breed-specific organisations and owners will choose issues related to their own breed as a top priority.

Policy makers who are less directly involved in a specific breed merely want the apparent "breed-health issue" to be solved in general. However, determining the burden of disease at an individual level, breed level and population level requires both highly focused breed or gene specific research, as well as broad population level research. In the attempt to improve companion animal health and welfare, it is not always possible to remove a health issue from a population. In addition, selecting against one issue may give rise to another, as yet unknown issue, and random partner selection also gives no guarantee that the offspring will be healthy. Even maximally informed

sensible breeding is never perfect. Sometimes however, the solutions do not need to be preceded by advanced research. As most harmful breed characteristics have been bred in by human intervention, removing these harmful traits from the population should only require an opposite selection process, with the focus on more healthy exterior features of the breed, including outcrossing.

The ideal future perspective is a breed specific breeding programme in which all available information can be combined and the owner/breeder can make a fully informed and evidence-based decision on which dogs to match as a breeding couple. This decision could then be based on, amongst other factors, the phenotypes of the individuals themselves, phenotypic disease frequencies in the population, multiplex DNA panel screening for genetic disorders, heterozygosity measurements, genetic relationships, and individual screening for specific diseases. Although this full overview of phenotypes and genotypes is not yet available for each breed, a breeder already has several tools available to make a well-founded and sensible breeding decision. Researchers and policy makers should embrace breeder organisations which recognise this and support initiatives such as that of the Dutch Shepherd Dog Club (chapter 6). Continued research may include the discovery of population-specific occurrences of diseases (or issues within organ systems) in the PETscan data. In collaboration with veterinarians, breed organisations and owners, this information may even be used to perform genetic mapping on selected groups of individuals in search of the causative genes or related markers to the benefit of all companion animals.



Chapter 8

Nederlandse samenvatting

In de afgelopen decennia is het besef van rasgebonden gezondheidsproblemen toegenomen. In raspopulaties van honden en katten bestaan deze problemen uit erfelijke aandoeningen en problemen door schadelijke raskenmerken. De schadelijke raskenmerken zijn ontstaan door fokkerij gericht op een extreem uiterlijk. Rasgebonden gezondheidsproblemen zijn onderdeel van de landelijke en internationale discussie over welzijn van gezelschapsdieren. Focus van die discussie is hoe deze problemen op verantwoorde wijze aan te pakken, met als doel blijvend gezonde raspopulaties. Hierbij zijn veel verschillende partijen betrokken, en de complexiteit van het probleem vraagt ook dat elke partij bereid is verantwoordelijkheid te dragen en samen te werken aan een oplossing. Om een toekomstig fokbeleid te ontwikkelen zijn populatiegegevens nodig over welke problemen er in welke mate spelen in de verschillende rassen. Parallel hieraan zijn de ontwikkelingen op het gebied van genetische testen essentieel in het managen van specifieke gezondheidsproblemen in deze rassen.

De studies in deze thesis onderzoeken de beschikbare bronnen van populatiegegevens van gezelschapsdieren. De onderzoekers ontwikkelden een softwaresysteem om de informatie uit de veterinaire praktijk te verzamelen die noodzakelijk is voor het bepalen en monitoren van gezondheidsproblemen in gezelschapsdieren. In de nabije toekomst kunnen zulke populatie gegevens benut worden om DNA testen optimaal in te zetten en op die manier bij te dragen aan een verantwoord fokbeleid.

De situatie

Hoofdstuk 2 doet verslag van een internationaal congres over welzijn en rasgebonden problemen bij honden. De diverse betrokken partijen kwamen bijeen om informatie te delen, mogelijkheden tot samenwerking te bespreken en de belangrijkste actiepunten te identificeren. Deelnemers werden verdeeld over discussiegroepen op de subthema's: geïndividualiseerde rasstrategieën voor de fokkerij, extreme exterieur, educatie en communicatie, gedrag, kwaliteitsborging van genetische testen, en de benodigde populatiegegevens. Binnen elk thema werden prioriteiten en mogelijkheden bepaald, indien mogelijk met een actieplan voor de korte termijn. Zo werden bijvoorbeeld werkgroepen opgesteld om rasverenigingen te ondersteunen en leidde een evaluatie van DNA testen en uitvoerende laboratoria tot een online informatiebron aangaande de toepasbaarheid van genetische testen.

De behoefte aan meer uitgebreide gegevens aangaande hondengezondheid was over de hele linie duidelijk. Tevens werd geconstateerd dat elk land niet alleen haar eigen raspopulaties heeft maar ook haar eigen benadering aangaande rasgezondheid. Dit vraagt (initieel) om een plan van aanpak op nationaal niveau.

Een uitdaging over alle subthema's heen was de noodzaak voor het delen van informatie en de betrokkenheid van alle partijen die op professioneel of persoonlijk vlak iets te maken hebben met hondengezondheid. De meeste partijen waren ruim vertegenwoordigd op het congres, behalve beleidsmakers, media en diereigenaren. De betrokkenheid van deze minder vertegenwoordigde partijen is van belang voor toekomstige wettelijke maatregelen en het sociale draagvlak dat daarvoor wenselijk is.

Aanwezigheid van ziektes in de populatie

Het tweede deel van dit proefschrift beschrijft de aanwezigheid van ziektes in de populatie.

In **hoofdstuk 3** werden beschikbare databronnen bestudeerd om te onderzoeken hoe en welke informatie daaruit gehaald kon worden voor de Nederlandse Labrador retriever populatie. Databronnen waren een veterinaire praktijk software systeem, twee databases van dierziektekostenverzekeringen, en een database van een pathologisch laboratorium. De beschikbare data bood de mogelijkheid tot ruwe schattingen van levensduur, frequentie van bezoek aan de dierenarts, en de relatieve kansen voor het voorkomen van specifieke diagnostische codes (op een bepaalde leeftijd). De Labrador retriever populatie in deze studie leek een gelijke of langere levensduur te hebben in vergelijking met kruisingen van een vergelijkbare lichaamsgrootte. De frequentie van dierenartsbezoeken en de algemene frequentie van verzekeringsclaims waren hoger voor de Labrador retriever dan voor de kruisingen, en de verzekeringsclaims op gebied van oren, luchtwegen en pezen & spieren toonden het grootste significante verschil. De leeftijd bij diagnose was gelijk voor beide groepen. De gegevens uit het pathologisch laboratorium suggereerden een verhoogde tumor-belasting in de Labrador retriever. Echter, zowel de verzekerings- als de laboratoriumdata zijn een gevolg van selectie, aangezien niet elk dier verzekerd was (bij een van de twee bedrijven in deze studie) en er niet altijd een monster wordt genomen en opgestuurd naar dit laboratorium. Gedrag van de eigenaar kan de resultaten bovendien ook hebben beïnvloed, met als resultaat een mogelijk verschil in detectie tussen kruisingen en Labrador retrievers. Verkeerde informatie over tot welk ras de hond behoort (Labrador retriever of kruising) kan hebben geleid tot een onderschatting van het probleem in de Labrador retriever. De verschillen die in deze data gevonden zijn kunnen veroorzaakt worden door een werkelijk verschil in gezondheid tussen beide groepen, of kan sterk beïnvloed zijn door het gedrag van de eigenaar. Fouten in de registratie van een ziekte waren waarschijnlijk gelijk tussen beide groepen en daardoor niet van invloed op het geschatte verschil.

Belangrijk om op te merken is dat een ziekte op individueel niveau kan leiden tot andere prioriteiten voor een ras dan een hoge frequentie op populatieniveau. Een ziekte die zeldzaam is

in een ras, kan op individueel niveau grote impact hebben. Als een dergelijke ziekte eenvoudig getest, voorkomen of behandeld kan worden valt er op individueel niveau veel welzijn te winnen. In een historische vergelijkende observationele studie (**hoofdstuk 4**) is rasgebonden ziektelast geëvalueerd in drie rashondenpopulaties en in een raskattenpopulatie, in vergelijking met een controlepopulatie van kruisingen en Europese korthaar-katten. Het eerste deel van deze observationele studie betrof een kwalitatief onderzoek middels literatuuronderzoek en het interviewen van veterinair specialisten. Resultaten werden vervolgens geverifieerd in een case control studie aan de (tweedelijns) Universiteitskliniek. De resultaten suggereerden een over representatie van raspopulaties in specifieke disciplines en bij specifieke ziektes. De Franse bulldog was bijvoorbeeld over gerepresenteerd bij de discipline keel-neus-oorziektes (KNO), en de Labrador retriever bij orthopedie. De voorgaande stappen resulteerden in een lijst van aandoeningen in vijf orgaansystemen welke mogelijk van belang zouden zijn in deze populaties. Deze werden handmatig nader onderzocht in een doorsnede van de populaties in de veterinaire praktijk. Resultaten van deze praktijkdoorsnede toonden dat niet alle verwachte aandoeningen gevonden werden, in tegenstelling tot het voorafgaande kwalitatieve onderzoek. Een belangrijke bevinding aangezien in de discussie over rasgezondheid vaak alleen kwalitatieve bronnen worden meegenomen in plaats van te onderzoeken of een aandoening relevant is in de onderzochte populatie. Wat wel werd bevestigd in deze doorsnede was het voorkomen van aandoeningen door schadelijke raskenmerken zoals luchtwegproblemen in kortsnuitige dieren. Uit bovenstaande werd geconcludeerd dat de gezondheid van rashonden en - katten zoals gesuggereerd door de literatuur of experts niet per sé gezien wordt in de dagelijkse praktijk. Een andere bevinding was dat de meeste individuele dieren geen stamboom hadden, wat wederom het belang aantoont van de betrokkenheid van alle partijen en niet alleen de kennelclub en rasverenigingen.

Het handmatig doorzoeken van praktijkgegevens is tijdrovend en ongeschikt voor het op grote schaal verzamelen van gegevens en analyseren van populatiegezondheid. Om die reden is een softwareprogramma ontwikkeld dat landelijk ingezet kan worden om langdurig en gestandaardiseerd diagnoses te verzamelen in de veterinaire praktijk. In de toekomst kan hierdoor een beter beeld verkregen worden van de aanwezigheid van ziektes in de populatie en kan het effect van maatregelen objectief vastgesteld worden.

De ontwikkeling en toepassing van dit programma - PETscan - wordt beschreven in **hoofdstuk 5**. PETscan is ontwikkeld om het stellen van diagnoses in de praktijk te standaardiseren. Informatie die verzameld wordt bij deelnemende praktijken bevat gegevens over het individu (zoals geslacht, leeftijd), unieke identificatie (gebaseerd op chipcode), en informatie van het betreffende consult (zoals datum en diagnose). Verzamelde gegevens uit de eerste fase van de looptijd zijn gebruikt

om vast te stellen of het systeem de gezondheid van een populatie inderdaad kan evalueren, en daarmee ook richting geven aan verder genetisch onderzoek. Als een aandoening vaker voorkomt in een bepaald ras dan in kruisingen suggereert dit een erfelijke component. Als een dergelijke bevinding gevolgd wordt door genetisch onderzoek kan er meer duidelijkheid komen over de erfelijke achtergrond en kan er mogelijk een DNA test worden ontwikkeld. In de ontwikkeling van PETscan is zoveel mogelijk rekening gehouden met ervaringen bij soortgelijke systemen in de wereld. De meest kritische succesfactoren voor dergelijke systemen zijn de bijdrage van dierenartsen in de praktijk en de privacyregels aangaande eigenaargebonden informatie. Om deelnemende dierenartsen te ondersteunen in het diagnostisch proces is een adviestekst bij elke diagnose gevoegd. Dit biedt de dierenarts een actueel overzicht van diagnostische mogelijkheden en andere belangrijke informatie en ondersteunt op die manier het stellen van een diagnose. Het verplicht stellen van een diagnoseregistratie voordat een patiëntendossier verlaten kan worden werd als onwenselijk gezien, omdat het een negatieve invloed zou kunnen hebben op deelname. De privacy van de eigenaar wordt beschermd door geen eigenaarinformatie te verzamelen en de chipcode (die naar een eigenaar kan leiden) te anonimiseren. In het analyseren van de verzamelde gegevens moeten we kunnen vertrouwen op de diagnose van de behandelend dierenarts. Hoewel een exacte definitie van de gemaakte diagnose ontbreekt, zal de adviestekst eenduidigheid stimuleren.

De gegevens uit de eerste fase suggereren dat PETscan op een betrouwbare manier gegevens over populatiegezondheid oplevert. Het langdurig verzamelen van deze gegevens bevordert de standaardisering van diagnostische terminologie en daarmee de kwaliteit van veterinaire zorg. Grootschalige toepassing van PETscan als actuele monitoring kan inzicht geven in de aanwezigheid van ziektes bij specifieke rassen, het effect van fokstrategieën, en kan richting geven aan genetisch onderzoek in gezelschapsdieren.

Genetische testen

Hoofdstuk 6 beschrijft de meting van genetische diversiteit en een multiplex DNA test en evalueert de toepassing in het fokbeleid van de Hollandse herder. Ook werd de klinische relevantie van eventueel gevonden DNA mutaties bekeken. Hoofdstuk 6 toont hoe genetische testen kunnen worden ingezet bij verstandig fokken, ook wanneer de populatiegegevens op het gebied van gezondheid beperkt zijn, als onderdeel van een fokprogramma met begeleiding en klinische screening. De DNA testen gebruiken SNPs in het hele genoom van een hond om de individuele heterozygositeit te bepalen en een multiplex PCR om mutaties te vinden die samenhangen met bepaalde ziekten, mogelijk nog onbekend in een ras. De SNP mutaties geven informatie over de

genetische diversiteit in het geheel en over het genetische verwantschap tussen individuen van hetzelfde ras en in vergelijking met andere rassen of kruisingen. De functie van de multiplex DNA test in een fokprogramma is nagaan of er genetische aandoeningen aanwezig zijn, en onbedoelde verspreiding daarvan in de populatie te vermijden. Afhankelijk van het type aandoening, de klinische relevantie en de manier waarop de aandoening overerft, kan het doel zijn de allelfrequentie laag te houden en te vermijden dat er homozygoot mutant dieren geboren worden. Dit was het geval bij de Hollandse herder, waar de mutatie voor Van Willebrand ziekte type I werd ontdekt. Fokkerij die gericht is op het maximaliseren van de heterozygositeit verlaagt het risico op inteeltdepressie en een frequentietoename van mutaties doordat de genenpoel van de populatie behouden blijft. Het meten van genetische verwantschap kan ingezet worden om reuen en teven zo te combineren dat genetische isolatie vermindert en de genetische diversiteit binnen of tussen subpopulaties toeneemt.

Conclusies en toekomstperspectieven

Het toegenomen bewustzijn aangaande gezelschapsdierenwelzijn in de afgelopen decennia heeft veel kansen gecreëerd om de gezondheid en het welzijn van gezelschapsdieren te vergroten. Om een duurzaam en verantwoord fokbeleid te implementeren dat gebaseerd is op actuele kennis, is kwantitatieve data op individueel en populatieniveau essentieel. Er moet data verzameld worden in de veterinaire praktijk voor een landelijke nulmeting, voortdurende monitoring en het prioriteren van genetische studies. Tevens is er een grote rol weggelegd voor organisaties als de Raad van Beheer en rasverenigingen, alsook voor individuele fokkers van rashonden met of zonder stamboom. Om het gezamenlijke doel te kunnen behalen is accurate, gestandaardiseerde dataverzameling essentieel, waarbij alle betrokkenen de samenwerking stimuleren en goede afspraken bestaan over ieders verantwoordelijkheid. Idealiter zou alle diergezondheidsdata op een centrale plek verzameld moeten worden, waar kwaliteit en volledigheid van de data gegarandeerd kunnen worden. Analyse van data uit een dergelijke database is de enige weg naar onbevooroordeelde evaluatie van populatiegezondheid.

Wanneer populatiegegevens beschikbaar zijn, is de volgende uitdaging het prioriteren van problemen, zowel binnen rassen als over alle rassen heen. Logischerwijs zullen fokkers, rasverenigingen en eigenaren problemen binnen hun eigen ras de hoogste prioriteit geven. Beleidsmakers die niet direct betrokken zijn bij een specifiek ras zullen het meer algemene "rashondenprobleem" opgelost willen zien. Echter, de ziektelast op individueel, ras- en populatieniveau vereist zowel onderzoek met de focus op een ras of gen, als populatiebreed onderzoek. Het is niet altijd mogelijk om een gezondheidsprobleem uit een populatie te krijgen.

Bovendien kan selectie tegen het ene probleem een ander, tot nog toe onbekend, probleem de kop doen opsteken. Geheel gerandomiseerde partnerselectie in de fokkerij geeft ook geen garanties op een gezonde volgende generatie. Zelfs een fokbeleid gebaseerd op alle beschikbare informatie is niet perfect. Soms is echter de oplossing dichtbij en hoeft geen geavanceerd onderzoek gedaan te worden. De meeste schadelijke raskenmerken zijn doelbewust gefokt door de mens, wat suggereert dat deze eruit te fokken zijn door een omgekeerd selectieproces, inclusief uitkruisen, met de focus op een gezonder exterieur.

Het ideale toekomstperspectief is een rasspecifiek fokprogramma waarin alle beschikbare informatie gecombineerd kan worden en de eigenaar/fokker een volledig geïnformeerde en op feiten gebaseerde beslissing kan nemen over een goede combinatie reu en teef. Die beslissing kan dan onder andere gebaseerd zijn op het individuele fenotype, ziektefrequenties in de populatie, een multiplex DNA test, gemeten heterozygositeit, genetisch verwantschap, en individuele klinische screening. Hoewel het gecombineerde overzicht van fenotype en genotype nog niet beschikbaar is, heeft de fokker wel al diverse middelen tot zijn beschikking om een degelijke en verstandige keuze te maken. Onderzoekers en beleidsmakers zouden rasverenigingen die dit al doen, zoals de Hollandse Herder Club, moeten toejuichen (hoofdstuk 6). Voortgaand onderzoek kan leiden tot de ontdekking van specifieke aandoeningen (of orgaanproblemen) binnen een raspopulatie in de PETscan data. In samenwerking met dierenartsen, rasverenigingen en eigenaren zou dergelijke informatie zelfs kunnen leiden tot het genetisch in kaart brengen van oorzakelijke genen of gerelateerde markers in groepen of individuen, waar alle gezelschapsdieren uiteindelijk van kunnen profiteren.

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Acknowledgements (Dankwoord)

"A rose may be a rose by any other name, but when you call a dog a poodle it becomes a very different animal than if you call it a bulldog." ~ Heidi Parker, National Institutes of Health, Bethesda, USA.

Terugkijkend verwonder ik me over alles wat ik geleerd heb in de afgelopen vier jaar. Dat was niet mogelijk geweest zonder de betrokkenheid van begeleiders, collega's, en alle andere mensen, verenigingen en bedrijven. Ik wil graag mijn dankbaarheid uitspreken aan iedereen die een grote of kleine bijdrage heeft geleverd aan de totstandkoming van dit proefschrift.

Promotoren en copromotoren

Geachte prof. dr. Hesselink, Beste Jan Willem, je doelgerichtheid tijdens overleg heeft dit promotietraject op het rechte pad gehouden, stap voor stap werden lopende projecten afgevinkt. In je rol als promotor ben je actief betrokken geweest bij publicaties over het onderzoek, waar je ook de grote lijnen niet uit het oog verloor. Dank voor je geduldig blijven scheppen van overzicht, zodat ik het leerdoel voor ogen kon houden en er nu een proefschrift ligt.

Geachte prof. dr. Nielen, Beste Mirjam, ooit werd je gevraagd "of je wel aardig voor me zou zijn". Toen antwoordde je dat dat niet tot je takenpakket hoorde. Maar in de afgelopen vier jaar ben je me aardig tot steun bent geweest met je enorme epidemiologische kennis, je praktische instelling, en je handgeschreven feedback op manuscripten (want altijd maar achter de computer is inderdaad niet verstandig). Jouw ervaring met datasets was van onschatbare waarde tijdens dit project en de actieve rol die je op je nam heb ik erg gewaardeerd.

Mijn eerste copromotor Frank van Steenbeek. Wat had ik zonder je moeten beginnen. Je hield op een belangrijk moment in het traject een vinger aan de pols hoe het ging. Met betrokkenheid en gevoel voor humor zorgde je ervoor dat ik geconcentreerd bleef op het werk. Zowel op professioneel als persoonlijk vlak heb ik dat zeer gewaardeerd. Met name bij het Hollandse herder project hebben we intensief samengewerkt, van denktanks met heel veel post-its tot kleurrijke illustraties, het is gelukt. Heel erg bedankt en ik hoop nog veel gekke foto's van je langs te zien komen.

Hille Fieten, toen je je examen had gehaald kreeg je de kans om coördinator van het ExpertiseCentrum Genetica Gezelschapsdieren (ECGG) te worden, en mijn tweede copromotor. Een goed voorbeeld voor mij, want wat een passie heb jij voor je werk. Dank voor je positieve energie, je kritische blik op mijn teksten en de zeer efficiënte vergaderingen over logistieke zaken.

Jouw plannen om de gezondheid van rashonden en –katten in Nederland te verbeteren staan als een huis, ik ben benieuwd wat je met jouw tomeloze enthousiasme allemaal voor elkaar gaat krijgen de komende jaren.

Paranimfen

Barbara Vreede, hoe bijzonder dat we contact hebben gehouden na dat ene jaar van de studie Biologie in Leiden. Ik stapte over naar Diergeneeskunde, jij bleef daar en promoveerde daarna in Portugal. Ik waardeer je vriendschap, je altijd weer heldere kijk op zaken en de goede gesprekken die we hebben. En natuurlijk je ervaring met onderzoek en het dataprogramma R, die onontbeerlijk bleken voor dit proefschrift. Dank je wel voor alles.

Femke van der Klip, het is pas zeven jaar geleden, maar het voelt het als veel langer door onze lange gesprekken te paard in het bos, met een kop thee of iets sterkers in een bar, over alle mogelijke onderwerpen, inclusief werk. Ik ben blij dat ik je heb leren kennen en we zulke goede vriendinnen zijn geworden. Ik hoop dat we nog veel mooie ritjes kunnen maken met onze senioren en ik jullie kleine Fienou kan zien opgroeien. Dank dat je aan mijn zijde wil staan bij mijn promotie.

Externe begeleidingscommissie

Geachte prof. dr. Heesterbeek en prof. dr. Rutten, Beste Hans en Victor, dank voor de brainstormsessies vooraf aan en tijdens dit promotietraject. Wij hebben al sinds mijn studietijd contact over het doen van onderzoek en ik vind het altijd fijn even van gedachten te wisselen, nu met dit proefschrift resultaat. Hartelijk dank aan beiden.

Collega's binnen de Faculteit Diergeneeskunde

Geachte prof. dr. Rothuizen, Beste Jan, je kwam terug van emeritaat en begon als visionair aan het ECGG. Je naam en vele jaren ervaring openden deuren en mogelijkheden voor de gezondheid van rashonden en –katten in Nederland. Samen hebben we PETscan uitgebreid, wat een klus was dat. Toen Hille het coördinatorschap kon overnemen mocht je met welverdiend pensioen, maar jij was het punt waar de enorme ontwikkelingen op gebied van rasgezondheid zijn begonnen. Dank daarvoor.

Hans Vernooij, dank voor je essentiële ondersteuning in het data management en je geduld met mijn eerste pogingen met dataprogramma R. Zonder jou had ik niet bij de data kunnen komen, en telkens weer nam je de tijd in je overvolle rooster om een antwoord te formuleren, een script met voorbeelden te mailen, of een nuttige link door te sturen. Je kennis van de statistiek is indrukwekkend en ik ben blij dat je hebt willen meedenken.

Dr. Peter Leegwater, onze werkgebieden hadden niet veel overlap, maar als er vragen over DNA testen en erfelijkheid binnenkwamen op de mail van het ECGG kon ik altijd bij je terecht voor een helder antwoord. Dank ook voor je hulp bij het maken van een overzicht van zinnige DNA testen, inclusief literatuurverwijzingen, daar heb ik veel van geleerd.

Veel collega's hebben geholpen met de inhoudelijke ontwikkeling van PETscan. Het volledig krijgen van de mogelijke diagnoses, en het opstellen van adviesteksten aangaande de diagnostiek voor elk van die diagnoses was niet mogelijk geweest zonder Bjorn Meij, Hans Kooistra, Viktor Szatmari, Yvette Schlotter, Maurice Zandvliet, Ronald Jan Corbee, Sylvia Djajadiningrat, Michael Boevé, Astrid van Dongen, Paul Mandigers, Hille Fieten en Christine Piek. Dank voor jullie bereidheid om met Jan en mij ervoor te gaan zitten om de veterinaire kwaliteit van PETscan te waarborgen. Christine, jij in het bijzonder bedankt voor je advies aangaande het Hollandse herder project, je analyse van de uitslagen en je betrokkenheid bij het publiceren van het werk. Je input als hematoloog was precies wat we nodig hadden.

Daarnaast ook een bijzonder woord van dank aan collega Marloes van Dijk van het Veterinair Microscopisch Diagnostisch Centrum (VMDC). Je uitgebreide revisie van de diagnoselijst, met de nodige toevoegingen over bijvoorbeeld zoönotisch risico en diagnostiek, zijn onmisbaar geweest voor de ontwikkeling van PETscan. Het gaat weliswaar over rasspecifieke problemen, maar met het in kaart brengen van alle aandoeningen én infectieziekten is het beeld dat we krijgen van de populatie meer compleet.

Dank ook aan collega Manon Vos-Loohuis, voor je analyses van de Hollandse herder, en student Manon Sloof, voor het hercoderen van de pathologie data.

Liesbeth Meijndert, mijn voorganger bij het ECGG. Jouw literatuuronderzoek, interviews met specialisten en geduldig handmatig uitpluizen van dossiers uit de veterinaire praktijk en de specialistenkliniek van de Faculteit Diergeneeskunde: wat een werk is dat geweest, hulde. Nog zeer regelmatig kon ik teruggrijpen op de verslagen van alle studenten die je hebt begeleid.

Else den Boer, mijn opvolger binnen het ECGG. Met frisse energie en veel enthousiasme ben je begonnen eind 2018. Ik ben blij dat ik PETscan en de verdere logistiek aan jou heb kunnen overdragen. Ik heb het volste vertrouwen dat jij de rasgezondheid weer een stap verder brengt en een succesvolle promotie zal doorlopen.

Stephan van Meulebrouck, bedankt voor de plezierige overleggen over de website en andere PR zaken. Altijd handig om een afspraak op jouw kantoor te maken, dan had ik een wandeling door de klinieken gezelschapsdieren en paard. Het was erg fijn om jou als aanspreekpunt bij de afdeling Communicatie te hebben.

Kantoorgenoten Ruth van der Leij en Nicole Willems, op de meest diverse kamer qua werkzaamheden: dank voor de theepauzes en fijne gesprekken ter ondersteuning, stimulans en voor de gezelligheid. Al heb ik het laatste jaar veel thuis opgesloten gezeten achter de computer, het is altijd weer goed om jullie te spreken en te horen hoe eenieder vaart.

International colleagues

Dan O'Neill and David Broadbelt, from the Royal Veterinary Society in London, how happy am I to have been introduced to you on the conference of the Society for Epidemiology and Preventive Medicine. Thank you for the time you both took to meet with me, and discuss data collection in companion animals. Dan, your enthusiasm for this type of research has given the companion animal epidemiology momentum, great work. Dave, thank you for kindly accepting a place in the committee at my PhD thesis defense.

Thank you to the International Partnership for Dogs, the Société Centrale Canine (French Kennel Club), sponsors, participating organisations and individual attendees for making the 3rd International Dog Health Workshop in Paris a success. A special thank you to Brenda Bonnett, who is the mastermind of these workshops, and to all the co-authors of the resulting paper: Dan O'Neill, Åke Hedhammar, Caroline Kisko, Gregoire Leroy, Aimee Llewellyn-Zaidi, Sofia Malm, Patricia Olson, Rowena Packer, Jean Francois Rousselot, Ian Seath, and Jason Stull.

Colleagues at MyDogDNA, Finland, Heidi Anderson and Jonas Donner, thank you for the pleasant cooperation on the Dutch Shepherd Dog research. I hope the health of many more canine breed populations can be improved in the future with the tool you provide.

Marnie Brennan from Nottingham University, thank you for taking me into the pet population discussion group, which allowed for interesting teleconference talks on the challenges and importance of the research we do.

Bedrijven en praktijken

Verschillende bedrijven en personen daarbinnen zijn actief betrokken bij het onderzoek naar rasgezondheid.

Milo van der Zee, van Vetsware, niet alleen heb je een eindeloos enthousiasme voor het project, jouw capaciteit om de computertechnische opzet achter PETscan uit te leggen in woorden die ik kon begrijpen heb ik erg gewaardeerd. Je combineert computerkennis met een kennis over de veterinaire praktijk en daarmee was je direct de aangewezen persoon om PETscan verder te ontwikkelen. Dank voor de goede vergaderingen bij je thuis en je aanhoudende betrokkenheid. Met actieve groet.

Corilus Veterinary, onderdeel van DGB Group N.V. (De Groene Belangenbehartiger), stond samen met Vetsware aan de oorsprong van PETscan, in de persoon van Aldert de Vries. Een echte computerman met kennis van zaken en samen met Milo een topteam om te sparren over hoe PETscan het beste kan functioneren in de praktijk. Bedankt!

Sterkliniek dierenartsen, en in het bijzonder Bob Carrière van Sterkliniek Ermelo: het handmatig zoeken in praktijkdata dat mijn voorganger collega Liesbeth Meijndert heeft gedaan was niet mogelijk geweest zonder de welwillendheid van de Sterklinieken. Dank voor jullie bereidheid om inzage te geven in jullie dossiers zodat het ECGG van start kon en een eerste rapportage en publicatie mogelijk waren.

Dank aan Evert van Garderen van de Gezondheidsdienst voor Dieren, Peter van Rooijen van Petplan Nederland, Walter Boer van Reaal Dier & Zorg, en Carolina Berazay van IDEXX Animana voor het aanleveren van data voor de verschillende studies en het meedenken over de interpretatie daarvan.

Rasverenigingen

Dank aan de Raad van Beheer, in het bijzonder aan Laura Roest voor het plezierige contact de afgelopen jaren. Dank aan de Nederlandse Herdershonden Club, de Nederlandse Chihuahua Club, de Hollandse Bulldoggen Club, de Nederlandse Labrador Vereniging, de Labrador Kring Nederland en de Federatie Nederlandse Kattenverenigingen voor hun bereidheid het onderzoek van het ECGG te ondersteunen. De betrokkenheid van de rasverenigingen en diereigenaren is van groot belang en de liefde voor hun ras prachtig om te zien. Een bijzonder woord van dank aan Ineke Scholten van de Nederlandse Herdershonden Club, voor haar inzet om de huidige gezondheid van de Hollandse herder in kaart te brengen en een degelijk toekomstplan te maken.

Vrienden en familie

Dank aan al mijn vrienden en familie voor spontane uitstapjes, spelletjesavonden, etentjes, bioscoopbezoeken, concerten, promotietips en andere momenten deze jaren die voor wat afleiding zorgden. Behalve mijn paranimfen Femke en Barbara die al eerder genoemd zijn, een specifiek dankwoord voor enkelen.

Guido Hommel, dank voor al vele jaren muziekplezier en een bijzondere vriendschap. Je geduld als ik weer een deadline had en in hoog tempo uitlegde waar ik mee bezig was wordt nu hopelijk beloond doordat jouw idee de inspiratie voor het kaftontwerp van dit proefschrift is geworden. Altijd weer goed om te "connecten" en bij te praten in restaurant, bar, of op het (dak)terras.

Tagrid Ali Dinar, you are the strongest woman I know, and you combine a sense of humour with immense friendliness. I am very lucky to have met you during the MSc in Epidemiology and I hope you will be able to continue working in research. Stay calm and be fabulous.
Leo de Haan, dank je wel voor je vriendschap en het bieden van een zonovergoten rustpunt in te drukke tijden. Fijn dat we volgend jaar weer samen muziek kunnen maken.
Mariëtte van der Zande, dank voor de gezellige momenten op stal, de etentjes, en niet te vergeten de dansfeestjes op het juiste moment het afgelopen jaar. Dat er maar vele mogen volgen.
Iaira Boissevain, fijn dat je jouw viervoeters aan mij toevertrouwde, zodat ik even achter de computer weg kon. De wandelingen en knuffels met jouw Rushonden Makar en Banti, en de andere Wereldbeesten die ik telkens mocht leren kennen, waren een welkome afwisseling in mijn dagen.
Willem-jan Kitslaar, 14 jaar mijn reismaatje en collega. Wat hebben we een avonturen beleefd samen in binnen- en vooral in buitenland. In Namibië en Costa Rica vergat ik even wat ik nog allemaal moest doen om dit project tot een goed einde te brengen, dank je wel. Duizenden kilometers road trip, met een muziekselectie voor onderweg, door woestijn en jungle, ik had het niet willen missen.
Labrador Saar, en baasjes Mariëlle en Pien: bedankt voor het model staan en de mooie foto die ik mocht gebruiken voor de kaft.
Mariska en Bart-Jan, samen opgroeien, elkaar kennen en accepteren: ik ben blij met jullie als zus en broer. Hoe druk we alle drie ook zijn, toch blijven we op de hoogte van de ontwikkelingen in elkaars leven. Ook hoe jullie beiden aan de weg timmeren met Bart, Josje, kleine Mats en zijn broertje op komst. Dank voor jullie steun en ik wens jullie alle geluk van de wereld.
Pap en mam, dank jullie wel voor altijd weer een luisterend oor, of het nou om een woord van advies gaat of een klassiek concert. Jullie zijn mijn steun en toeverlaat bij klusprojecten, bij paardentransport, en nog veel meer. Ik herken in mezelf veel van jullie beiden, ook de reislust!, en prijs mezelf gelukkig met mijn roots. Op de vraag van mensen waar mijn onderzoek nou eigenlijk over ging konden jullie een verbazingwekkend gedetailleerd antwoord formuleren. Dank jullie wel voor jullie betrokkenheid bij mijn werk en leven.
Mijn vier kinderen met vacht, mijn viervoeters al zo lang bij me: katten Nala en Harrie, en paarden Pim en Phyrelli. Mijn lieve meisje Nala, ik mis je nog elke dag. Jullie hebben allemaal zo je eigen manier om mij te laten weten wat goed voor me is en laten me telkens weer voelen dat uiteindelijk alles relatief is.

About the author

Curriculum Vitae

Born in Delft, The Netherlands, on November 11th 1981, Sylvia Keijser graduated from the Cardinal Alfrink College in 2000. She started her studies in Veterinary Medicine at Utrecht University in 2003. During her studies, she did a short research project on the movement patterns of the Tasmanian Devil (*Sarcophilus harrisii*) to understand the spread of Devil Facial Tumour Disease, at the University of Tasmania – Australia.

After obtaining her DVM degree, she worked as a teacher and primary care veterinarian for four years. In 2015 she started her PhD project on the epidemiology of inherited diseases and harmful breed characteristics of companion animals, under supervision of Prof. dr. Jan Willem Hesselink and Prof. dr. Mirjam Nielen. As part of her PhD project, she obtained a Master of Science degree in "Veterinary Epidemiology" from the Graduate School of Life Sciences in Utrecht – The Netherlands.

Conference attendance and presentations

- 2nd Symposium of Kynology – 2015, Utrecht, The Netherlands – oral presentation "Incidence of inherited diseases and harmful breed characteristics in dog breeds, with and without pedigree"
- Dutch Shepherd Dog breed association, annual meeting – 2016, Woudenberg, The Netherlands – oral presentation "A first look at the health questionnaire"
- Society for Veterinary Epidemiology and Preventive Medicine, annual conference meeting – 2016, Elsinore, Denmark – poster presentation "Breed-related disorders in three dog breeds quantified in primary practice data"
- Dutch Society for Veterinary Epidemiology and Economics, annual symposium – 2016, Lelystad, The Netherlands
- Najaarsdag Conference, Groep Geneeskunde Gezelschapsdieren – 2016, Utrecht, The Netherlands – oral presentation "Primary practice data: first incidence analysis in dog breeds by PETscan"
- Weekly Interdisciplinary Meeting of the Department of Clinical Sciences of Companion Animals – 2016, Utrecht, The Netherlands – oral presentation "Expertise Centre Genetics of Companion Animals, what do we (want to) do?"
- Seminar PEGD educational programme – 2016, Utrecht, The Netherlands – oral presentations "Who's who in inherited disease policy", "PETscan" and "Interactive case discussion"
- Teachers Day 4Groen – 2017, Almelo, The Netherlands – oral presentation "Inherited Diseases, PETscan and other stories"
- Society for Veterinary Epidemiology and Preventive Medicine, annual conference meeting – 2017, Inverness, Scotland
- International Partnership For Dogs, 3rd International Dog Health Workshop – 2017, Paris, France – coordinator workshop Data collection on population level
- Dutch Shepherd Dog breed association, annual meeting – 2017, Woudenberg, The Netherlands – oral presentation "Results and discussion of the health and behaviour questionnaire"
- European Veterinary Conference Voorjaarsdagen – 2018, The Hague, The Netherlands – oral presentation "Where IT meets medicine: PETscan, where are we now?"
- Breeders meeting Dachshund association, annual meeting – 2018, Woudenberg, The Netherlands – oral presentation "The work of the Expertise Centre Genetics of Companion Animals and the Dachshund"

- Weekly Interdisciplinary Meeting of the Department of Clinical Sciences of Companion Animals – 2018, Utrecht, The Netherlands – oral presentation "Heterogeneity testing and DNA panel screening in breed health"
- Society for Veterinary Epidemiology and Preventive Medicine, annual conference meeting – 2018, Talinn, Estonia – poster "When numbers are unavailable: How to approach health issues in a small closed dog population"
- Society for Veterinary Epidemiology and Preventive Medicine, annual conference meeting – 2019, Zeist, The Netherlands

Postgraduate Master Epidemiology

Course	**ECTS**
Advanced Veterinary Epidemiology	6.0
Applied Economic Modelling for the Veterinary Sciences	3.0
Challenges in Global Health	1.5
Classical Methods in Data Analysis	6.0
Clinical Epidemiology	1.5
Economic Principles and Concepts for the Veterinary Sciences	2.5
Epidemiology of Infectious Diseases	1.5
Ethology and Welfare	6.0
Genetic Epidemiology	1.5
Introduction to Epidemiology	3.0
Introduction to Statistics and SPSS	1.5
Mathematical Modelling	3.0
Missing Data	1.0
Modern Methods in Data Analysis	4.5
Molecular Epidemiology of Infectious Diseases	3.0
Presentation and Writing Research Proposal	2.0
Research Ethics and Society	1.0
Risk Assessment and Risk Management	3.0
Study Design in Etiologic Research	3.0
Survival Analysis	1.5
Primate Culture, Empathy and Morality	1.0
Zoo Conservation Biology	6.0
Main research project	*56.0*
TOTAL	**119**