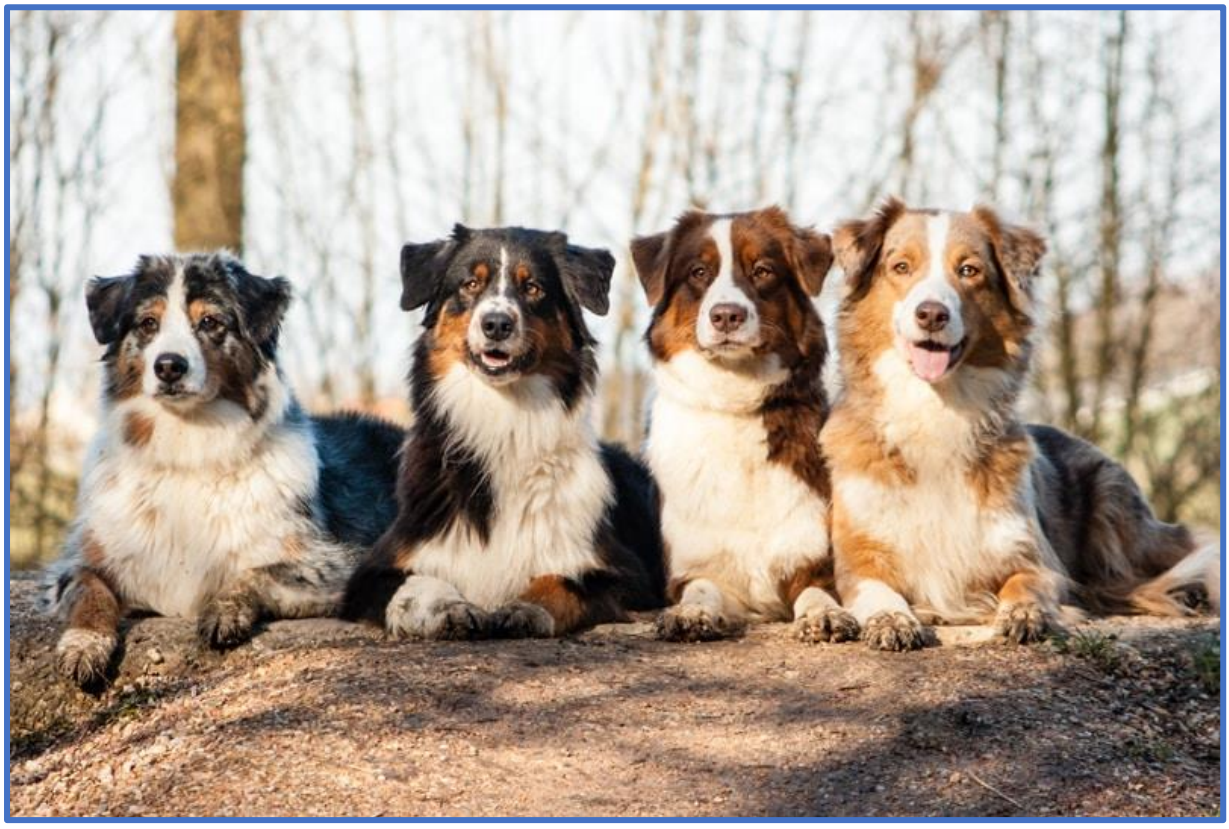
Universiteit Utrecht

AUSTRALIAN SHEPHERD CLUB NEDERLAND

Development, distribution and analysis of a health inventory questionnaire for the Australian Shepherd as an indication for inherited disorders



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Summary

The general health of a broad variety of dog breeds is a hot topic. To get a view over more generations of the breed and of disorders for which no routine screening exists, a health inventory in the form of a questionnaire for owners can give valuable information on the health status of the breed. For this research project the Dutch Australian Shepherd was used. The questionnaire was developed by consultation of veterinary specialists of several departments of the faculty of veterinary medicine of University Utrecht, the Dutch Australian Shepherd Club (ASCN) and experts in analysis of questionnaires. The final questionnaire consisted of six different sections: (1) introduction, (2) general section, (3) health section, (4) behavioral section, (5) reproduction section and (6) final questions. For the behavioral section the validated short version of the Canine Behavioral and Research Questionnaire (C-BARQ)© was used. The final questionnaire consisted of 233 questions, these were not all visible for the owners, only questions that applied to the dog filled in, were visible. The estimated fill in time was 10-20 minutes. A total of 448 questionnaires was filled in, from this number, 428 could be used for the 'medical section', 427 could be used for the 'general section' and 'reproduction section', and 426 could be used for the 'behavioral section. The results were analyzed descriptively and a multiple logistic regression model was used to investigate the correlation between the 14 C-BARQ subscales and the general characteristics of the dog (gender, neuter status, if the dog was rehomed and usage of de dog). In addition the independent variable age was analyzed (age category 1: 0 years old; age category 2: 1-3 years old; age category 3: 8-15 years old; age category 4: 4-7 years old). Since there was no normal distribution, the median was used to make the subscale scores binary: ≤ median = 0, > median = 1. The Odds Ratio and the confidence interval were calculated as a measure to establish the predictability of the independent variables with regard to the dependent variable. Finally the means of the 14 subscale scores were compared to the means of the general population.

To investigate the reliability of asking owners about screening results, a sample size of 188 dogs with a Dutch pedigree number was selected and the hip dysplasia (HD) and eye examination (ECVO) results given by the owner were compared to the results in the 'Raad van Beheer' database. The Cohen's kappa coefficient was determined as a measure of agreement.

The results show that a point of moderate concern was the prevalence of epilepsy/movement disorders, more research is recommended to discriminate between the hereditary idiopathic epilepsy and the non-idiopathic epilepsy/movement disorders. Points of mild concern were the allele frequency of the MDR1 gene mutation and cancer as the cause of death. The ASCN is already screening MDR1 gene mutations in the breeding population, by doing this the allele frequency should go down in the future. If the ASCN wants to further investigate cancer, histopathological examinations are needed to discriminate between the different forms of cancer in order to know the prevalence of the different cancer forms and maybe to set up a breeding program. No behavioral problems emerged in this questionnaire, but with behavioral problems it has to be taken into account that this is most of the time something subjective. This questionnaire also shows that asking owners for screening results, it is reliable for hip dysplasia, but unreliable for eye examinations. The reason for this seems to be that owners are not aware of the screening tests done before the dog came into their possession.

Introduction

The general health of a broad variety of dog breeds is a hot topic. Commercial breeders, breed associations and the Kennel Clubs get a lot of criticism about breeding programs that do not favor the health of the dog. The intense usage of popular sires results in inbreeding, also known as 'the popular sire effect' (1). Of certain hereditary disorders it is clear in which breed they occur, but in addition some breeds are suspected of certain hereditary disorders. Besides, these hereditary disorders can change over time, such as other diseases that may arise or the frequency to which they occur (1).
Nowadays health screening of purebred dogs is done a lot to prevent several diseases to transfer to the next generation, for example hip dysplasia and elbow dysplasia in the Labrador Retriever (2). This provides valuable information about breed health, but to get a broader view over more generations of the breed and of disorders for which no routine screening exists, a health inventory in the form of a questionnaire for owners can give valuable information on the health status of the breed. By using a similar questionnaire for different breeds, this can help to compare the incidence of diseases among dog populations. The results can be used to prioritize breeding program goals or a reason for more thorough investigation. Evaluation of the breeding program can subsequently be done by repeating the questionnaire after a certain time.
In this study, a questionnaire to evaluate dog health and behavior was developed and Australian Shepherd owners were approached to fill this in, in cooperation with the Dutch Australian Shepherd Club (ASCN).

When selecting of breeding animals, knowledge about heredity is needed. Monogenetic hereditary diseases can be caused by a dominant or a recessive gene. This can be autosomal or X-chromosomal, but in most cases it is a autosomal recessive gene. If a disease is caused by a autosomal recessive gene it is acceptable to breed with a combination of which one dog is free of the mutated gene or the pathogenic allele. It has to be taken into account that this is not the case when there is a X-chromosomal inheritance, in that case carriers of the mutant allele cannot always be used for breeding. With a dominant condition you cannot cross with a dog that is heterozygous for the mutation, because a combination between a heterozygous animal and a homozygous-free animal still gives the chance of 50% sick puppies. This must be taken into account when a specific dam and sire are combined. You do not usually want to exclude animals that are carriers of autosomal recessive disorders, because you want to preserve genetic variation. In addition, it is possible that one condition is given priority in a breeding program over another, e.g. because of the frequency or severity of the disease. Selection against diseases is therefore a trade-off between the risk of sick animals, the impact of the disease on the animal and the maintenance of genetic variation.

A number of screenings and tests are available for the Australian Shepherd and some are required in order to breed with the dog within the ASCN. These screenings and tests can be divided in DNA and non-DNA. Many DNA tests are available for the Australian Shepherd, but the most common tests done are for MDR1 (multidrug resistance gene 1), CEA (collie eye anomaly), PRA (progressive retinal atrophy), HSF4 (hereditary cataract) and DM (degenerative myelopathy). The non-DNA screenings most commonly done are for hip dysplasia (HD), elbow dysplasia (ED) and hereditary eye diseases (ECVO (European College of Veterinary Ophthalmologists)) (3).

Other hereditary diseases that seem to be common in this breed are epilepsy, lymphoma and hemangiosarcoma. Beside the inherited diseases, behavioral problems seem to occur as well. Behavior is very important for a well-functioning companion animal. Various studies show a heritability in behavior, which seems to be breed dependent (4,5). This makes it important to add it to this questionnaire. According to the ASCN the behavioral problems can be caused by the character of the Australian Shepherd, because these dogs are very active, smart, guarded and independent which is needed for the farm work where they are bred for. It is imported that they are entertained by doing

dog sports or other types of exercise, otherwise they get very easily bored and can develop behavioral problems, for example excessive barking and demolition of things like furniture (3).
The goal of this research project is to gain insight in the health and behavior of the Dutch Australian Shepherd population by making, distributing and analyzing a health inventory questionnaire for owners of this breed. It can be an indication of inherited disorders, such as epilepsy, hip dysplasia and elbow dysplasia, but also behavior, which can be hereditary, is mapped. The questionnaire has to be understandable for the owners, whereby the collected data must provide valuable information for both researchers and breeders. The health and behavioral information obtained in this research project can be the basis for the development of breeding strategies that promote the health of the population or be a reason for further investigation.

This research project focused on five subjects: 1) Developing a useful health inventory questionnaire for owners that can be used for scientific analysis, 2) get an indication of the general health and incidence estimates for disorders in the Australian Shepherd population, 3) analyze hip dysplasia in more detail, looking at the following aspects: age of appearance, purpose of use of the dog, gender, degree of hip dysplasia and the degree of hip dysplasia of the parents, 4) analyze behavior in more detail, looking at the following aspects: compare it to the general population of dogs, gender, neuter status, if the dog is rehomed and purpose of use of the dog, 5) estimate the reliability of the screening results reported by owners by comparing the tests and results by the validated 'Raad van Beheer' database.

Material and Methods

Development of the health questionnaire

The questionnaire was in Dutch, since the target audience was the Dutch population. To compose the questionnaire, the computer program Formdesk® was used (6). With this program the privacy of the owners was protected, all data was available in an excel sheet and the owners were asked for consent to use the reported data for further analysis. According to the ASCN the total population of Australian Shepherds in the Netherlands is approximately 3500. It was assumed that 10% of the total population was needed for a valid overview of the health of the population. This means a minimum of 350 questionnaires was needed.

A previously developed questionnaire for the Dalmatian Dog served as an example and was taken as a starting point for this questionnaire. In addition the questions were adjusted to get a generally usable questionnaire for different breeds, with the possibility for adding breed specific questions, in this case for the Australian Shepherd. This was done by consultation of veterinary specialists of several departments of the faculty of veterinary medicine of Utrecht University. These departments consisted of internal medicine, reproduction, behavioral science, orthopedics and ophthalmology. After this, the questionnaire was checked on the usefulness for the scientific analysis and also if it was understandable for the owners. To do so experts in analysis of questionnaires were consulted. To know if the questionnaire was understandable for owners, layman (family members, neighbors and friends who were owners of a dog) without any veterinary knowledge were asked to fill in the questionnaire and give feedback.

Behavioral section

For the behavioral section the validated Canine Behavioral and Research Questionnaire (C-BARQ)© was used (7). The C-BARQ is widely used as a behavioral assessment tool for dogs, has established reliability characteristics and is demonstrated to have construct validity (7,8).
The C-BARQ is freely available on the internet in two versions, the long version with 100 questions and the short version with 42 questions. In this questionnaire the short version was used in order to reduce the number of questions and fill in time as much as possible. The short version was developed by calculating the Cronbach's alfa values to determine the impact of each question on the internal reliability of the subscales. The alfa values above 0.70 were maintained wherever possible. This would have resulted in the loss of the subscale 'separation-related behavior', which was considered an important subscale so this subscale was kept in the questionnaire despite the low alfa value. In this way questionnaire items were selected and removed from the standard 100 item version of the C-BARQ and a short 42 item C-BARQ was composed (9). The C-BARQ subscales with the corresponding situations/statements are shown in *table 1*. The miscellaneous behaviors, reactions and traits are shown in *table 2.* In the study of Duffy et al. (2014) the correspondence of the newly composed short version of the C-BARQ with the original C-BARQ version was determined by using a sample of currently-owned pet dogs (n=17.307) derived from the C-BARQ database at the University of Pennsylvania. With this database the subscale scores for both the short and the original version were calculated. The subscale scores of the short version showed a strong correlation with the original version ($r_s \geq 0.80$, $P < 0.0001$ for all subscales) (9). This means the results of the short version can be compared with the original version and vice versa. In the same study they found that if shelter staff recorded aggressive behavior during intake evaluations, the dog also obtained significantly higher mini C-BARQ scores on the aggression subscales. Beside they compared euthanized and dogs offered for adoption and the last group obtained significantly lower mini C-BARQ scores on the subscale 'stranger-directed aggression'. This is the only validity they could demonstrate, but the strong correlation with the original C-BARQ who showed to have construct validity, assumes that this mini C-BARQ has also good validity (9). The subscales that has proven to have good external validity in multiple studies are marked with a star (*) in *table 1* (7).

Selection of target breed and questionnaire distribution

The ASCN has put a link to the questionnaire on their website and social media pages to reach members and nonmembers who were owners of an Australian Shepherd with a pedigree. The response time was 13 weeks. In order to have a reliable sample size, the filled in questionnaire had to meet a number of criteria: the owner had to give permission that the reported identification (chip- and pedigree number of the dog) would be used for analysis, the dog had to be an Australian Shepherd and had to have a valid chip- and/or pedigree number.

Statistical analysis

SPSS 26 was used for the statistical analysis of this study.

Statistical analysis of the general section

The age of the dog was estimated by taking 01-10-2019 as the average date when the questionnaire was filled in and deduct the date of birth from this. In order to get the age without decimals, 0.5 was deducted from this result. So that the dog was 3 years old, when he was 3 years and 11 months old.

Hip dysplasia

The statistical analysis consisted of a multiple logistic regression by using the binary dependent variable of hip dysplasia (present = 1; absent = 0) and the independent variables age, gender, purpose of use and the degree of hip dysplasia of the parents of the dog. Age was divided in the following categories: <3 years (young), 3-7 years (middle aged), 7> years (old); gender was divided in male or female; purpose of use was divided in the categories: sport or no sport; and the degree of hip dysplasia was divided in the categories: A (no signs of hip dysplasia), B (transition form, mild signs of hip dysplasia), C (slightly positive), D (positive) and E (severe positive). With the multiple logistic regression the Odds Ratio and the confidence interval were calculated as a measure of the strength of the correlation between the dependent variable and the different independent variables. The hypotheses were:

H0 = There is no association between the presence of hip dysplasia and the age of appearance, purpose of use of the dog, gender and the degree of hip dysplasia of the parents.

H1 = There is an association between the presence of hip dysplasia and the age of appearance, purpose of use of the dog, gender and the degree of hip dysplasia of the parents.

Screening result reliability

In order to say something about the reliability of the screening tests and results the owners filled in, the screening results (HD/ECVO) were compared to the 'Raad van Beheer' database (10). Because of time and efficiency reasons, a sample size from all the dogs with a Dutch pedigree number was taken. The desired precision was put on 0.05, the confidence level on 0.95 and the estimated true proportion was 0.5. The minimal sample size was calculated to be 188.

The HD and ECVO results reported by the owner and 'Raad van Beheer' were recoded (HD: 0=no test result, 1=HD A, 2= HD B, 3=HD C; ECVO: 0=no test result, 1=no abnormalities, 2=1 abnormality, 3=2 abnormalities). The reported scores by the owner and 'Raad van Beheer' were compared to each other in a crosstab and to determine the measure of agreement the Cohen's kappa coefficient was calculated. Where the agreement is regarded as (11):

- 'Poor' if $\kappa \leq 0.20$
- 'Fair' if $0.21 \leq \kappa \geq 0.40$
- 'Moderate' if $0.41 \leq \kappa \geq 0.60$
- 'Substantial' if $0.61 \leq \kappa \geq 0.80$
- 'Good' if $\kappa \geq 0.81$

DNA tests

All diseases tested are autosomal recessive and consist of two alleles, except for HSF4 which is dominant with incomplete penetration (12). For each test the phenotype frequency and minor allele frequency was calculated. The phenotype frequency was calculated by the number of free/carrier/sufferer dogs divided by the total of tested dogs. The minor allele frequency was calculated by 1*carrier number + 2*sufferer number divided by the total number of alleles (total number of dogs tested times two).

Deceased dogs

The results of the deceased dogs were analyzed descriptively, by estimating the mean longevity, percentages of the causes of death and mean longevity with the standard deviation from every cause of death.

Statistical analysis of the health section

The results of the medical health section were analyzed descriptively by estimating the percentages and mean age with the standard deviation. For every disease reported the age of onset was asked. Age reported as <1 is set to 0, age reported >13 is set to 13 and age reported as >18 months is set to 18. The frequencies of diseases were compared with reported frequencies in the general population in the literature.

Statistical analysis of the behavioral section

The subscale scores were calculated as the average of the questionnaire items that comprised each factor, shown in *table 1*. The Cronbach α values were calculated as a measure for the internal consistency of each questionnaire item and the corresponding subscale. The subscale α is considered to be reliable if the value is 0.70 or higher.

Since the subscale scores were not expected to be normally distributed, parametric statistical methods could not be used. Therefore nonparametric methods were used. In order to see if the subscale scores (dependent variable) were related to the general information of the dog (independent variables) multiple logistic regression models were used. The independent variables were: gender (male/female), neutered (no/yes), if the dog was rehomed (no/yes) and usage of the dog (practicing sports no/yes). In addition the independent variable age was analyzed: age category 1: 0 years old, age category 2: 1-3 years old, age category 3: 8-15 years old and age category 4: 4-7 years old. Since there was no normal distribution, the median was used to make the subscale scores binary: ≤ median = 0, > median = 1.

The Odds Ratio and the confidence interval were calculated as a measure to establish the predictability of the independent variables with regard to the dependent variable. In this model the last group of the dependent and independent variables served as the reference category. This means the 0 scores of the subscales were compared to the 1 scores. Females were compared to males, not neutered to neutered, not rehomed to rehomed and not practicing sports to practicing sports. The age categories were compared to age category 4 (adult dogs), since it is assumed that adult dogs show the most stable behavior. A P-value of ≤0.05 was considered significant.

In order to compare the subscale scores of the current study with the general population of dogs (obtained by the C-BARQ website (13)), the 95% confidence interval (CI) was calculated and the smallest difference between the upper of lower bound of the 95% CI was estimated. If this difference was ≥0.5 it was considered a clinical relevant finding. The same was done for the miscellaneous items, but the mean was obtained from the study of Wilson et al. 2018 (14). In this study they clustered 82 different breeds based on their C-BARQ scores. For the comparison in the current study, the Australian Shepherd cluster was used. This cluster contained 20 different breeds, mostly larger dogs, where the Australian Shepherd had average scores most similar to the cluster average (14).

Subscale	Description	Cronbach's α value
Excitability	The dog over-reacts or is excitable - Just before being taken for a walk - Just before being taken on a car trip	0.952 0.856 0.850
Stranger-directed aggression *	The dog acts aggressively - When approached directly by an unfamiliar person while being walked/exercised on a leash - When mailmen or other delivery workers approach your home - When strangers walk past your home when your dog is outside or in the yard	0.880 0.531 0.753 0.749
Owner-directed aggression *	The dog acts aggressively - When toys, bones or other objects are taken away by a household member - When approached directly by a household member while s/he (the dog) is eating - When his/her food is taken away by a household member	0.938 0.809 0.790 0.847
Dog-directed aggression *	The dog acts aggressively - When approached directly by an unfamiliar dog while being walked/exercised on a leash - When barked, growled, or lunged at by another (unfamiliar) dog	0.949 0.840 0.851
Dog rivalry	The dog acts aggressively - When approached while eating by another (familiar) household dog - When approached while playing with/chewing a favorite toy, bone, object, etc., by another (familiar) household dog	0.949 0.853 0.839
Stranger-directed fear *	The dog acts anxious or fearful - When approached directly by an unfamiliar person while away from your home - When an unfamiliar person tries to touch or pet the dog	0.959 0.871 0.875
Nonsocial fear *	The dog acts anxious or fearful - In response to sudden or loud noises (e.g. thunder, vacuum cleaner, car backfire, road drills, objects being dropped, etc.) - In response to strange or unfamiliar objects on or near the sidewalk (e.g. plastic trash bags, leaves, litter, flags flapping, etc.) - When first exposed to unfamiliar situations (e.g. first car trip, first time in elevator, first visit to veterinarian, etc.)	0.807 0.585 0.513 0.568
Dog-directed fear *	The dog acts anxious or fearful - When approached directly by an unfamiliar dog - When barked, growled, or lunged at by an unfamiliar dog	0.951 0.844 0.869
Touch sensitivity	The dog acts anxious or fearful - When having nails clipped by a household member - When groomed or bathed by a household member	0.929 0.826 0.780
Separation-related behavior *	When left alone, the dog displays - Restlessness/agitation/pacing - Barking or whining - Chewing/scratching at doors, floor, windows, curtains, etc.	0.828 0.680 0.692 0.408
Attachment and Attention-seeking *	The dog - Tends to follow you (or other members of the household) about the house, from room to room - Tends to sit close to, or in contact with, you (or others) when you are sitting down	0.909 0.771 0.723
Trainability	The dog - Obeys a 'sit' command immediately - Obeys a 'stay' command immediately - Is easily distracted by interesting sights, sounds or smells	0.412 0.325 0.216 -0.081
Chasing	The dog - Chases or would chase birds, given the chance - Chases or would chase squirrels, rabbits, etc., given the chance	0.934 0.796 0.815
Energy	The dog - Is playful, puppyish, boisterous - Is active, energetic, always on the go	0.847 0.615 0.595

Table 1: C-BARQ subscales with the corresponding situations/statements and Cronbach's α values. The subscales with a star (*) have proven to have good validity in multiple studies (7)*. Subscale Cronbach's α values below 0.70 are marked red.*

Miscellaneous behaviors, reactions and traits
Escapes or would escape from home or yard, given the chance
Chews inappropriate objects
Pulls excessively hard when on the leash
Urinates against objects/furnishings in your home
Urinates when left alone at night, or during the daytime
Defecates when left alone at night, or during the daytime
Hyperactive, restless, has trouble settling down
Chases own tail/hind end
Barks persistently when alarmed or excited

Table 2: C-BARQ miscellaneous behaviors, reactions and traits.

Statistical analysis of the reproduction section

In order to say something about the reproduction rate of the Australian Shepherd, the fertility was calculated as the number of gestations that followed out of one coverage divided by the total number of gestations. Based on specialist interpretation a fertility ≥ 80% is considered as 'good'. The other results were analyzed descriptively by estimating the percentages and means. The results were compared to in literature described fertility estimates.

Results

Developed health questionnaire

Introduction

In this part the purpose of this questionnaire was explained and the owner was told how the questionnaire was structured. Also was said that the owner needed to first get all the official papers from the dog ready and the estimated time it would take to complete the questionnaire. Finally the owner had to agree to the use of the data from the questionnaire for further analysis, this included the dogs personal data (chip- and pedigree number).

General section

The first questions were the name of the dog, his/her chip-number, pedigree number and birth date. Questions regarding longevity and cause of death showed up if the dog had passed away. This was also the case with the gender of the dog and if the dog was neutered. If the dog was a female, female related questions appeared, the same happened when the dog was a male. If the dog was neutered, reasons for neutering and age of neutering was asked. Age the dog was acquired and use of the dog (sports and therapy/assistance) were asked as well. Questions suggested by the ASCN were: coat color, markings, tail length (including kink or not), whether the dog was imported and screening tests besides hip dysplasia.

Health section

This section began with the question if the owner ever went to the vet with this dog because of health problems. If the owner answered 'no' this was the end of this section. If the answer was 'yes', questions appeared about which organ system was involved. Then for each organ system the owner said was involved, a list of clinical signs or diagnoses appeared they could choose. In every list there was also the option 'others' where the owner could fill in what was not in the list. Finally the age of onset was asked with every disease or clinical sign. The disease options were based on common known heritable diseases and very common disorders. In addition Australian Shepherd specific diseases were added.

Behavioral section

For this part the 42 questions in the C-BARQ were divided in 7 different sections; excitability, aggression, fear and anxiety, separation-related behavior, attachment and attention-seeking, training and obedience, and miscellaneous problems. The questions described particular circumstances and the owner was asked to rate their dogs response by giving a number on a 5-point scale. For the sections in which responses were evaluated in terms of frequency of a particular response (sections training and obedience, separation-related behavior, attachment and attention-seeking and miscellaneous problems) the 5-point scale consisted of 0=never, 1=seldom, 2=sometimes, 3=usually, 4=always. For the sections in which the response was evaluated in terms of intensity of particular behavior (sections aggression, fear and anxiety and excitability) the 5-point scale consisted of 0=little or no signs of the particular behavior, 1-3=mild-moderate signs or the particular behavior and 4=severe signs of the particular behavior. Each section had an introduction with instructions and which signs of behavior fit with mild-moderate and extreme or serious behavior (15). The questions could eventually be divided into 14 subscales which are listed in table 1 and in table 2 the miscellaneous behaviors are listed (7,14).

Reproduction section

In case of a male dog, the first question was if the dog ever covered a female. If the answer was 'yes' questions appeared about whether the dog produced litters, with how many different females and how often the coverage did not lead to a gestation. In case of a female dog, the first question was if the female was ever offered for coverage, if the answer was 'yes' questions appeared if the female became pregnant, if there was a birth, if there were any problems with the birth and thereafter. Finally was asked how many puppies per litter the female produced.

Final questions

At the end of the questionnaire the owner was asked whether the ASCN may contact the owner and if so, the personal information could be filled in. Also was asked if the owner was a member of the ASCN and finally there was an open space for comments.

The final questionnaire consisted of a total number of 233 questions, but these were not all visible for the owners. As explained above, many questions were only shown if the owner indicated that the dog had a disease or condition. The estimated time to fill in the questionnaire was 10-20 minutes.
The full questionnaire can be requested from E.R. den Boer.

Questionnaire results

A total of 448 dogs were entered. 20 dogs were excluded for different reasons: 2 did not agree with the additional terms, 11 dogs did not have a pedigree or chip-number and 7 dogs were entered twice. The dogs who were entered twice were compared to each other and if there were different diseases they were added up to each other. One of the dogs that was entered twice, had two different owners. This dog was excluded except for the medical health part, because both entries were identical for this part. The owner of one dog indicated in the comments that the behavior part of the dog was not reliable, so this dog was excluded for that. The final number of dogs for the different parts was: 427 for 'general section' and 'reproduction section', 428 for 'health section' and 426 for 'behavioral section'.
274 (64,2%) owners were members of the ASCN and 153 (35,8%) were not.

General section

181 (42.4%) males and 246 (57.8%) females were entered in the questionnaire. Of these dogs, 340 were alive when the questionnaire was filled in. The age distribution of the living dogs is shown in *figure 1*. 49.7% of the dogs was <4 years old.

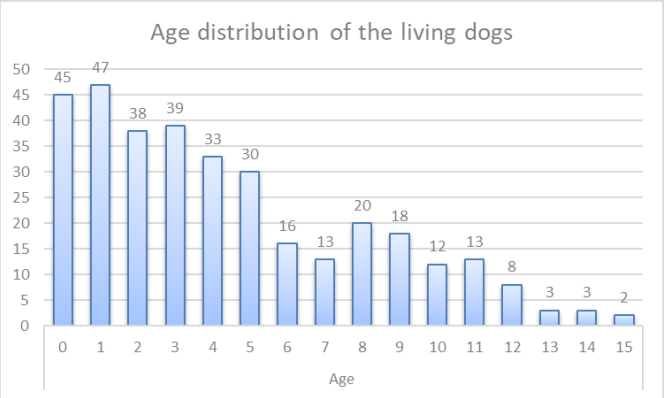

Figure 1: Age distribution of the living dogs

327 (76.6%) of the dogs were bought as a puppy from the breeder, 77 (18%) were self-bred and 23 (5.4%) were rehomed dogs. The age the dog was acquired was distributed as follows: <8 weeks: 142 (33.3%); 8-12 weeks: 233 (54.6%); 3-6 months: 25 (5.9%); 6-12 months: 6 (1.4%); 1-5 years: 18 (4.2%); and >5 years: 3 (0.7%). Most dogs were born in the Netherlands, but 66 (15.5%) dogs were imported. Most dogs were imported from the United States (30.3%), Belgium (22.7%) and Germany (19.7%). The exact countries and numbers are shown in *appendix 1, table 27*.
With 68 (15.9%) dogs no sport was practiced and with 359 (84.1%) dogs one or more sports were practiced. 243 (56.9%) owners indicated more than one sport. Agility and obedience were the most popular sports with 20.9% and 22.9%. The exact sports are shown in *appendix 1, table 28*.
Also was asked if the dog was used as a therapy or assistance dog. 35 (8.2%) dogs were used as a therapy dog and 9 (2.1%) dogs were used as an assistance dog.

External characteristics

The most common colors were: black tricolor (97 (22.7%)), red tricolor (93 (21.8%)), blue merle white & tan (113 (26.5%)) and red merle white & tan (88 (20.6%)). Some owners said the coat color did not have any white markings, but in the next question they indicated that the dog had white markings on the head. In this case the color was changed into the same color but with white. 333 (78%) dogs had white markings on the head. 329 (77.0%) dogs had a full length tail, 73 (17.1%) had a natural bobtail and 25 (5.9%) dogs were docked. 15 (3.5%) dogs had a kinked tail.

Male reproduction status

In 5 (2.8%) males both testicles had not descended: 3 had only one descended testicle and 2 had no descended testicles. In the other 176 dogs in which both testicles were descended, 119 (67.6%) had both testicles at the age of 9 weeks, 20 (11.4%) had them between 9 and 21 weeks of age and in 37 (21.0%) dogs the age of descending was unknown.

Reason	Number	Percentage	Mean age
Behavior	28	51.9%	2.1 (±1.4)
Medical: cure disease	10	18.5%	4.5 (±1.3)
Medical: prevent disease	4	7.4%	3.5 (±1.7)
Birth control	8	14.8%	2.4 (±1.7)
Practical*	4	7.4%	1.8 (±1.5)
Total	54	100	2.7 (±1.7)

*Table 3: Reason for neutering male dogs with the number of dogs, percentages of the total number of neutered male dogs (54) and mean age of neutering in years with the standard deviation between brackets. *: 'having a female dog in the same household' (2), 'easy' (1), 'practical with a boarding kennel' (1) were categorized in 'practical'.*

54 (29.8%) of the male dogs were neutered, the reasons for neutering are shown in *table 3*. 4 owners gave more than one reason for neutering their dog. 27 (50%) dogs had the age of 2 years or younger when they were neutered. For the reason 'behavior' almost all dogs were under 4 years old, the same as for 'birth control'. For the reason 'medical: cure disease' almost all dogs were older than 5 years. The mean age for neutering was 2.7 (±1.7) years.
The diseases reported for the option 'medical: cure disease' were 'perineal hernia' (2), 'benign prostate hyperplasia' (2), 'tumor' (2), 'preputial inflammation' (3) and 'urethrostomy' (1).

Female reproduction status

The mean age of the first heat was 11.5 months (±3.2) and 46 times (18.7%) 'unknown' was reported. 117 (47.6%) of the female dogs were neutered. 109 (93.2%) of these dogs had been in heat before neutering. The reasons for neutering are shown in *table 4*. 22 owners reported two or more reasons for neutering their dog. Some owners gave hip dysplasia, underbite, distichiasis or cataract as a reason to neuter, since these dogs were unfit as breeding animals, this was added to the reason 'birth control'. The diseases given for the option 'medical: cure disease' were 'endometritis' (3), 'ovarian cysts' (2), 'tumor' (1) and 'others' (4). Reasons meant by 'others' were: uterine tear during pregnancy, suffer from hormones: itch, umbilical hernia and irregular heat.

Reason	Total	Percentage
Birth control	55	47.0%
Medical: cure disease	10	8.5%
Medical: prevent disease	37	31.6%
Behavior	8	6.8%
Pseudopregnancy	12	10.3%
After litter	9	7.7%
Practical*	7	6.0%
Commissioned by the breeder or other organization	4	3.4%
Unknown	1	0.9%
Total	143	

*Table 4: Reasons for neutering female dogs with the number of dogs and percentage of the total number of neutered female dogs (117). *: 'Having an intact male go in the same household', 'doing a sport', 'other dog in the same household had issues with it' were categorized in 'practical'.*

Deceased dogs

87 (20.7%) dogs were passed away at the time the questionnaire was filled in. The reasons for passing away are shown in *table 5*.

Reason of death	Number	Percentage	Mean longevity
Old age/multiple disorders	32	36.8%	12.4 (±1.8)
Cancer	30	34.5%	9.1 (±2.6)
Neurology	7	8.1%	7.6 (±5.2)
Accident/trauma/ poisoning	4	4.6%	8.8 (±3.8)
Liver failure	1	1.1%	12*
Hart failure	1	1.1%	8*
Unknown	2	2.3%	10 (±4.2)
Others	10	11.5%	7.5 (±4.7)
Total	87	100%	10.1 (±3.5)

*Table 5: Reasons of death, numbers, percentages of the total number of deceased dogs (87) and the mean longevity in years with the standard deviation between brackets. *only one dog in this section. Categorized under 'others': deviation of neck vertebrae, aneurysm, intestinal infection, immune mediated polyarthritis, internal bleeding, enlarged liver, Weil's disease, pulmonary embolism, larynx paralysis and behavior.*

Some owners indicated 'old age/multiple disorders' as well as other diseases, this was adjusted to only old 'age/multiple disorders'. Neurological diseases such as epilepsy and brain infarction were adjusted to 'neurology'. 36 (41.4%) dogs became 13 years or older. 29 (80.6%) of these dogs passed away due to 'old age/multiple disorders'. The longevity is shown in *figure 2*. The mean longevity was 10.1 (±3.5) years.

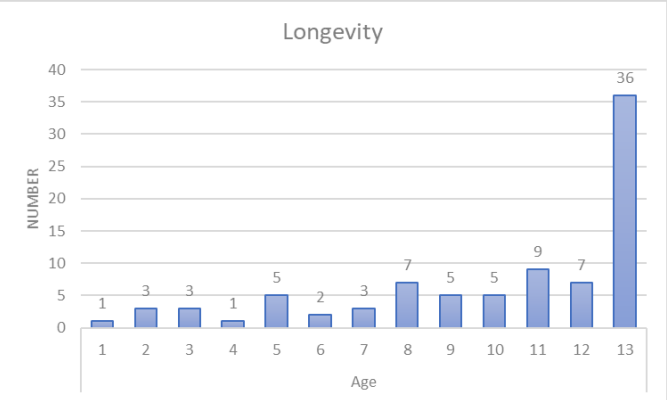

Figure 2: Longevity shown as number of dogs per age

Screening

A total of 286 (67.0%) dogs were screened for heritable diseases according to the owners and 141 (33.0%) were not screened. 203 (47.5%) were screened for HD, 140 (32.8%) were screened for ED and 241 (56.4%) were ECVO screened. 184 (90.6%) of the dogs screened for HD had a score of A or excellent/good. 138 (98.6%) of the dogs screened for ED had no signs of ED. 216 (89.6%) of the dogs ECVO screened had no eye abnormalities. The exact numbers and results are shown in *appendix 1, table 29-34*.

From 350 (82.0%) dogs the HD results of the parents was known. The HD results from the mother were as follows: 327 (76.6%) HD A, 22 (5.2%) HD B and 1 (0.2%) HD C. The HD results from the father were as follows: 303 (71%) HD A, 40 (9.4%) HD B, 6 (1.4%) HD C and 1 (0.2%) HD D. Some owners reported the result HD TC, since this was previously the score for the transitional form of HD, this was added to HD B.

The average age of screening for HD/ED was 1.9 (±1.0) years and for ECVO was 4.1 (±3.1) years.

Hip dysplasia and ECVO screening

The overall agreement of the different screening results (hip dysplasia (HD) and ECVO-test together) was correct for 103 (54.8%) dogs and incorrect for 85 (45.2%).

171 (91.0%) of the reported HD results were correct and 112 (59.6%) of the reported ECVO results were correct. The exact numbers are shown in *appendix 1, table 35 and 36*. The Kappa-value for the HD-results was estimated at 0.825 and for the ECVO-test results was estimated at 0.259. 49 out of 76 incorrect ECVO results were about the eye examination that was performed when the dog was under 8 weeks old and still in the litter at the breeder.

DNA tests

215 (50.4%) dogs were DNA tested and 212 (49.6%) was not tested according to the owners. The different DNA tests and the results are shown in *table 6*. No carriers and sufferers were found for MH (malignant hyperthermia), Vwd (Von Willebrand's disease type 1), CD (Cone Degeneration), PRA (progressive retinal atrophy) and NCL 8_2 (Neuronal Ceroid Lipofuscinosis 8-2). Some owners indicated the dog was DNA tested, but they did not know the results anymore. These are not included in *table 6*. The 'Merle' DNA test was asked too, but has been removed since the options were free/carrier/sufferer and these were not appropriate for Merle DNA tests.

Test	Free	Carrier	Sufferer	Total number of animals tested	Minor allele frequency
MDR1	111 (51.6%)	64 (29.8%)	5 (2.3%)	180	0.21
DM	50 (23.3%)	14 (6.5%)	-	64	0.11
Brachyuria	22 (10.2%)	5 (2.3%)	-	27	0.09
IGS3	25 (11.6%)	1 (0.5%)	-	26	0.02
HUU	21 (9.8%)	1 (0.5%)	-	22	0.02
HSF 4	113 (52.6%)	12 (5.6%)	1 (0.5%)	126	0.06
CEA	100 (46.5%)	5 (2.3%)	-	105	0.02
CMR 1	42 (19.5%)	2 (1.0%)	-	44	0.02
NCL 6	22 (10.2%)	1 (0.5%)	-	23	0.02

Table 6: Different DNA tests results reported. (%)=phenotype frequency in the sample size dogs that were tested (215). MDR1: Multi Drug Resistance gene 1, DM: degenerative myelopathy, Brachyuria: stumpy tail/bob tail, IGS3: Selective Vitamin B12 Malabsorption 3, HUU: Hyperuricemia, HSF4: Hereditary Cataract, CEA: Collie Eye Anomaly, CMR1: Canine Multifocal Retinopathy, NCL6: Neuronal Ceroid Lipofuscinosis 6.

BEAR test

40 (9.4%) dogs have had the BAER (Brainstem Auditory Evoked Response) test to determine deafness. In 35 (87.5%) of these dogs the test was done under the age of 1. The result was in all 40 dogs that they were hearing with both ears.

Health section

203 (47.4%) of the owners reported to have been gone to the veterinarian with their dog because of health issues and 225 (52.6%) did not went to a veterinarian. The different organ systems involved and how often they are involved are shown in *table 7*. 67 (15.7%) of the owners reported more than one organ system and 19 (4.4%) reported more than two organ systems.

Organ system	Total	Percentage
Musculoskeletal system	68	15.9%
Tumors/cancer	51	11.9%
Gastro intestinal system	40	9.3%
Skin	23	5.4%
Teeth	23	5.4%
Neurological system	20	4.7%
Kidneys and urinary tract	19	4.4%
Eyes	18	4.2%
Respiratory tract	13	3.0%
Ears	9	2.1%
Endocrinology	7	1.6%
Cardiology	5	1.2%
Hematology	2	0.5%

Table 7: Reported organsystems involved in health problems. Percentage is the percentage of the total sample size (428).

Musculoskeletal system

68 (15.9%) dogs showed musculoskeletal problems, the results are shown in *table 8*. In 14 cases more than one disease was given. Mostly 'trauma/sprain/fracture' was given as the problem in this organ system (44.1%). In this organ system also skin injury was given as the problem, but this is adjusted to the organ system 'skin'. Comments given that were appropriate for spinal problems were: deviation of the neck vertebrae, lumbosacral instability and spondylosis. Because of the small sample size (4), the proposed statistical analysis for hip dysplasia was not possible.

Disease	Total	Percentage	Mean age + SD
Trauma/sprain/fracture	38	8.9%	4.7 (±3.63)
Arthritis in one or more paws	17	4.0%	7.6 (±3.01)
Spinal problems	12	2.8%	6.2 (±3.87)
Immune mediated polyarthritis	4	0.9%	4.0 (±3.65)
Hip dysplasia	4	0.9%	5.5 (±4.51)
Torn cruciate ligament	4	0.9%	6.0 (±4.36)
Elbow dysplasia	3	0.7%	0 *
Others	4	0.9%	Unknown

*Table 8: Number of musculoskeletal diseases reported with the percentage of the total sample size (428) and age of onset in years with the standard deviation (SD) between brackets. *=all dogs were <1 years old. Categorized under 'others': 'growing pain', 'shoulder OCD (osteochondrosis dissecans)' and 'a tendon rupture'.*

Tumors/cancer

51 (11.9%) owners indicated that their dog has or had a tumor or cancer. 5 owners reported more than one location. The results are shown in *table 9*.

Location	Total	Percentage
Skin	10	2.3%
Gastro-intestinal system	8	1.9%
Systemic	6	1.4%
Mammary glands	5	1.2%
Spleen	5	1.2%
Respiratory system	5	1.2%
Reproduction organs	4	0.9%
Nose	3	0.7%
Teeth/jaw/mouth	2	0.5%
Unknown	2	0.5%
Musculoskeletal system	1	0.2%
Neurological system	1	0.2%
Others	4	0.9%

Table 9: locations of tumor/cancer reported with the numbers with the percentage of the total sample size (428). Categorized under 'others': 'cardiac', 'eyes', 'thyroid gland' and 'blood vessel in the neck'.

Diagnoses as 'lymphoma' were transformed to 'systemic', 'fat bumps in the skin' were transformed to 'skin' and 'nipples' were transformed to 'mammary gland'. Some owners gave as the cause of death cancer, but did not indicate it in this part of the questionnaire, in these cases it was added to 'tumors/cancer' and as location 'unknown' was given. The age at onset of the tumor of cancer is known for 44 (86.3%) dogs. The mean age was 8.8 (±2.24) years.

Gastro-intestinal system

40 (9.3%) owners reported that their dog has or had problems with the gastro-intestinal system. 4 owners reported more than one gastro-intestinal disease. The given diseases are shown in *table 10*.

Disease	Total	Percentage	Mean age + SD
Intestinal complaints due to parasite/infection/wrong food	24	5.6%	unknown
Colitis	4	0.9%	5.5 (±3.67)
Enteritis	4	0.9%	8.5 (±3.42)
Unknown	3	0.7%	Unknown
Food allergy	2	0.5%	<1*
Blocked bowel	2	0.5%	Unknown
Perineal hernia	2	0.5%	Unknown
Pancreatitis	1	0.2%	10,0*
Insufficient Vit. B12 intake	1	0.2%	Unknown
Irritable bowel disease (IBD)	1	0.2%	8,0*
Others	2	0.5%	Unknown

*Table 10: number of gastro-intestinal diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets. *=only one or both dogs were the same age. Categorized under 'others': 'sensitive gastro-intestinal system' and 'hepatomegaly'.*

The diseases 'colitis', 'enteritis' and 'intestinal complaints due to parasite/infection/wrong food' were mentioned together in different combinations in 6 different dogs. 24 (5.6%) dogs showed 'intestinal complaints due to parasite/infection/wrong food', also categorized under this item was poisoning.

Eyes

18 (4.2%) owners reported an eye problem with their dogs. 3 owners reported more than one eye problem. The different clinical signs/diseases that were given are shown in *table 11*.

Clinical sign/disease	Total	Percentage	Mean age + SD
Ocular discharge	4	0.9%	2.5 (±1.00)
Trauma	3	0.7%	Unknown
Reduced eyesight	2	0.5%	5.0 (±2.83)
Discoloration of the eye	2	0.5%	Unknown
Inflammation	2	0.5%	Unknown
Excessive flashing	2	0.5%	2.5 (±0.71)
Juvenile cataract	1	0.2%	Unknown
Unilateral iris hypoplasia	1	0.2%	Unknown
Others	4	0.9%	Unknown

Table 11: number of eye diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets. Categorized under 'others': 'lytic ulcer due to a fungus', 'blocked tear duct', 'anisocoria' and 'allergic reaction to eye-medication'.

The options given for eye problems were based on the clinical signs. Only 8 times one of the given options was chosen, the other times the owners filled in the disease/clinical signs as a comment. One of the dogs with reduced eyesight showed mainly complains in the evening and from the other dog it was unknown. The discoloration of the eye were red conjunctivae and discoloration of the third eyelid.

Skin

23 (5.4%) owners reported a skin problem with their dogs. 5 owners reported more than one skin disease. The different skin diseases given are shown in *table 12*.

Disease	Total	Percentage	Mean age + SD
Trauma/skin injury	7	1.6%	Unknown
Food hypersensitivity	5	1.2%	0.6 (±0.89)
Atopic dermatitis	4	0.9%	2.0 (±2.71)
Hotspot	3	0.7%	Unknown
Inflamed sebaceous gland	3	0.7%	3.5 (±0.71)
Unknown	2	0.5%	Unknown
Flee allergy	1	0.2%	2.0*
Others	4	0.9%	Unknown

*Table 12: number of skin diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets. * = only one dog reported. Categorized under 'others': 'broken toenail', 'obsessive licking', 'inflamed food pad' and 'stress rash'.*

The main cause of skin problems is 'trauma/skin injury'. The 'skin injury' from the musculoskeletal system is added to this number. One owner reported a food hypersensitivity and flee allergy, but also said this was due to a vitamin B12 deficiency (see gastro-intestinal system), therefore this information was excluded for this organ system.

Kidneys and urinary tract

19 (4.4%) of the owners reported a problem with their dog in the kidneys or urinary tract. 2 owners reported more than one disease. The diseases given are shown in *table 13*.

Disease	Total	Percentage	Mean age + SD
Recurrent cystitis	6	1.4%	3.8 (±4.22)
Bladder/kidney stones	5	1.2%	5.2 (±2.78)
Incontinence	2	0.5%	11.5 (±2.12)
Polyuria/polydipsia	2	0.5%	Unknown
Single cystitis	2	0.5%	Unknown
Chronic kidney failure	1	0.2%	8.0*
Unknown	1	0.2%	Unknown
Others	2	0.5%	Unknown

*Table 13: number of kidney and urinary tract diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets. * = only one dog reported. Categorized under 'others': 'bladder polyp' and 'dark urine due to a high bilirubin concentration'.*

2 cases of bladder/kidney stones also mentioned recurrent cystitis. The different stones reported were: cystine (1), struvite (3), calcium-oxalate in combination with struvite (1) and 1 was unknown.

Teeth

23 (5.4%) of the owners reported a problem with their dogs teeth. 2 owners reported more than one teeth problem. The different diseases/complaints are shown in *table 14*.

Disease	Total	Percentage	Mean age + SD
Gingivitis	13	3.0%	7.5 (±3.59)
Trauma/broken teeth	6	1.4%	Unknown
Underbite	2	0.5%	Unknown
Glaze deviation	1	0.2%	4.0 (±5.66)
Trouble changing teeth	1	0.2%	0*
Others	2	0.5%	Unknown

*Table 14: number of teeth diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets. * = only one dog reported. Categorized under 'others': 'epulide' and 'side effects prednisolone'.*

More than half the owners who reported a teeth problem, reported gingivitis with their dogs. One case was added to 'gingivitis' because the owner gave the comment 'rotting tooth'. In this category 72.7% of the dogs was 8 years or older.

Neurological system

20 (4.7%) of the owners reported a neurological problem with their dog. The different diseases given are shown in *table 15*.

Disease	Total	Percentage	Mean age + SD
Epilepsy/movement disorder	11	2.6%	4.7 (±4.47)
Vestibular syndrome	2	0.5%	Unknown
Dementia	1	0.2%	9.0*
Border Collie Collapse	1	0.2%	Unknown
Unknown	3	0.7%	Unknown
Others	2	0.5%	Unknown

*Table 15: number of neurological diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets. * = only one dog reported. Categorized under 'others': 'meningitis' and 'stroke at the age of 15'.*

One owner reported seizures with a unknown origin, this is added to the category 'unknown'. The age of onset of epilepsy/movement disorders was ≤5 years in 7 dogs.

Endocrinology

7 (1.6%) owners reported an endocrinologic problem with their dog. 1 owner reported more than one disease. The different diseases given are shown in *table 16*.

Disease	Total	Percentage	Mean age + SD
Endometritis	3	0.7%	Unknown
Hypothyroidism	2	0.5%	7.0 (±1.41)
Benign Prostate Hyperplasia	2	0.5%	Unknown
Ovarian cysts	1	0.2%	Unknown

Table 16: number of endocrinologic diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets.

The original options were based on endocrine diseases, reproduction organ disorders were not included. However owners did report diseases in the comments that belonged to the reproduction organ system. These diseases were included in the endocrinology organ system.

Cardiology

5 (1.2%) of the owners reported a heart disease with their dog. The different diseases given are shown in *table 17*. The cardiac arrest happened during an ovariectomy.

Disease	Number	Percentage	Mean age +SD
Heart-murmur unknown origin	3	0.7%	5.7 (±4.93)
Non-congenital heart-disease	1	0.2%	11.0*
Cardiac arrest	1	0.2%	Unknown

*Table 17: number of cardiological diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets. * = only one dog reported.*

Respiratory tract

13 (4.3%) of the owners reported a respiratory disease with their dog. The different diseases given are shown in *table 18*. One owner reported kennel cough with their dog, this was added to 'anterior airway infection'.

Disease	Number	Percentage	Mean age + SD
Larynx paralysis	5	1.2%	10.3 (2.50)
Anterior airway infection	4	0.9%	6.0 (3.61)
Trachea collapse	1	0.2%	1.0*
Others	3	0.7%	Unknown

*Table 18: number of respiratory diseases reported with the percentage of the total sample size (428) and the mean age of onset in years with the standard deviation (SD) between brackets. * = only one dog reported. Categorized under 'others': 'prolonged cough', 'got something in the nostril' and 'fluid in the lungs due to a heart murmur'.*

Ears

9 (2.1%) of the owners reported an ear problem with their dog. The only problem reported was an otitis. The mean age of onset was 2.6 years (±4.25), the youngest was under 1 year of age and the oldest was above 13 years of age.

Hematology

Only 2 (0.5%) owners reported a hematologic problem with their dog. One dog had immune mediated hemolytic anemia at the age of 7. The other dog had an internal bleeding, the cause of the bleeding was not mentioned.

Behavioral section

The response rate for all the C-BARQ questions was 100%, except for the questions about dog rivalry (89.7%) and how the dog responds when having the nails clipped (96.7%).
The Chronbach's α values are shown in *table 1*. All α values were above 0.70 except for the subscale 'Trainability' (0.412) (see table 1). Since the 'Trainability' items 'obeys a 'sit' command and a 'stay' command immediately' are based on the higher the score, the better, but the item 'the dog is easily distracted by interesting sights, sounds or smells' is based on the lower the score, the better, the scores were transformed; score 4=0, 3=1, 2=2, 1=3, 0=4. The Cronbach α of the item 'the dog is easily distracted by interesting sights, sound or smells' raised from -0.081 to 0.081 and the α of the subscale 'trainability' raised from 0.412 to 0.542. So the question 'the dog is easily distracted by interesting sights, sounds or smells' was excluded for further analysis. The new Cronbach α value of the subscale 'Trainability' raised to 0.935.
The boxplots of the subscales, divided in the seven different sections, are shown in *figure 3*. These boxplots show that there was no normal distribution as hypothesized above.

The Australian Shepherd scored with a difference (between the upper or lower bound of the 95% CI and the mean subscale score of the population) greater than 0.5 higher on the subscales: 'attachment-attention seeking' and 'trainability'; and lower on the subscales: 'excitability' and 'chasing'. The exact values are shown in *table 19*.
The Australian Shepherd scores had no greater than 0.5 difference between the upper of lower bound of the 95% CI compared to the mean scores of the Australian Shepherd cluster in the study of Wilson et al. (2018) (14). The exact values are shown in *table 20*.

The binary logistic regression of the subscales and the dogs characteristics is shown in *table 21*. Five values were significant, where higher OR means a score ≤ the median subscale score. Female dogs showed higher odds in subscale chasing (OR: 1.6 (1.1-2.4)) than male dogs. Not neutered dogs showed higher odds in the subscales stranger-directed aggression (OR: 1.5 (1.0-2.3)), dog-directed aggression (OR: 2.1 (1.4-3.2)) and trainability (OR: 1.8 (1.2-2.8)) than neutered dogs. Dogs with which no sports were practiced showed higher odds in subscale dog-directed aggression (OR: 1.9 (1.1-3.3)) than dogs with which sports were practiced. The bar charts and crosstabs are shown in *appendix 2, figure 4*.

The multinomial logistic regression of the subscales and age categories are shown in *table 22*. The results showed twelve significant differences when compared to age category 4, where higher OR means a score ≤ the median subscale score and a lower OR a score > the median subscale score. Age category 1 showed higher odds in subscales dog-directed aggression (OR: 5.2 (2.2-12.0)), dog rivalry (OR: 2.8 (1.2-6.8)) and trainability (OR: 3.6 (1.4-9.5)); and lower odds in subscales dog-directed fear (OR: 0.4 (0.2-0.8)) and separation-related behavior (OR: 0.3 (0.2-0.7)). Age category 2 showed higher odds in subscales stranger-directed aggression (OR: 1.9 (1.1-3.4)) and dog-directed aggression (OR: 1.8 (1.0-3.1)); and lower odds in subscale separation-related behavior (OR: 0.4 (0.2-0.7)). Age category 3 showed higher odds in subscale touch sensitivity (OR: 2.3 (1.1-4.9)) and lower odds in subscale trainability (OR: 0.5 (0.3-1.0)). The bar charts and crosstabs are shown in *appendix 2, figure 5*.

Subscale	Mean Australian Shepherd	CI (95%)	Mean general population	Difference*
Excitability	1.4	1.3-1.5	2.0	**0.5**
Stranger-directed aggression	0.8	0.7-0.8	0.6	0.1
Owner-directed aggression	0.1	0.1-0.2	0.2	-
Dog-directed aggression	1.3	1.2-1.4	1.0	0.2
Dog rivalry	0.5	0.4-0.6	0.6	-
Stranger-directed fear	0.5	0.4-0.6	0.6	-
Nonsocial fear	0.7	0.6-0.7	0.8	0.1
Dog-directed fear	0.9	0.8-1.0	0.7	0.1
Touch sensitivity	0.3	0.2-0.3	0.7	0.4
Separation-related behavior	0.3	0.3-0.4	0.6	0.2
Attachment/attention seeking	2.7	2.6-2.7	1.9	**0.7**
Trainability	3.3	3.2-3.4	2.6	**0.6**
Chasing	1.5	1.4-1.6	2.1	**0.5**
Energy	2.2	2.1-2.2	2.0	0.1

Table 19: Mean subscale scores of the Australian Shepherd with the 95% confidence intervals (CI) and the mean subscale scores of the general population dogs (obtained by the C-BARQ website). The difference is the smallest difference between the upper of lower bound of the 95% CI and the mean of the population. Differences ≥0.5 are in bold.

Miscellaneous item	Mean Australian Shepherd	CI (95%)	Australian shepherd cluster (Wilson et al. 2018)	Difference*
Escapes or would escape from home or yard, given the chance	0.5	0.4-0.6	1.0	0.4
Chews inappropriate objects	0.5	0.4-0.6	0.9	0.3
Pulls excessively hard on the leash	1.2	1.1-1.3	1.2	0.1
Urinates against objects/furnishings in your home	0.03	0.01-0.04	0.2	0.16
Urinates when left alone at night, or during the daytime	0.04	0.01-0.06	0.2	0.14
Defecates when left alone at night, or during the daytime	0.02	0.003-0.04	0.2	0.16
Hyperactive, restless, has trouble settling down	0.7	0.6-0.8	0.8	-
Chases own tail/hind end	0.2	0.2-0.3	0.3	-
Barks persistently when alarmed or excited	1.4	1.3-1.5	1.4	0.14

Table 20: Mean miscellaneous items scores with the 95% confidence interval (CI) and the means from Wilson et al. (2018) (11). The difference is the smallest difference between the upper or lower bound of the 95% CI and the mean of the Australian Shepherd cluster from Wilson et al. (2018) (11).

Figure 3: boxplots of the mean subscale scores. a: section 1: excitability; b: section 2: aggression; c: section 3: fear and anxiety; d: section 4: separation-related behavior; e: section 5: attachment and attention-seeking; f: section 6: training and obedience; g: section 7: miscellaneous items

Subscale (0/1)	Male/female (1/2)		Neutered no/yes (0/1)		Rehomed no/yes (0/1)		Sports no/yes (0/1)	
	P	OR(95% CI)	P	OR(95% CI)	P	OR(95%)	P	OR(95%)
1:Excitability	0.414	0.8 (0.6-1.3)	0.376	1.2 (0.8-1.8)	0.621	0.8 (0.3-1.9)	0.734	1.1 (0.6-1.9)
2:Stranger-directed aggression	0.331	0.8 (0.5-1.2)	**0.048**	**1.5 (1.0-2.3)**	0.697	1.2 (0.5-2.8)	0.063	1.7 (1.0-3.1)
3:Owner-directed aggression	0.699	0.9 (0.5-1.5)	0.501	1.2 (0.7-2.1)	0.998	0.0 *	0.830	1.2 (0.5-2.3)
4:Dog-directed aggression	0.760	1.1 (0.7-1.6)	**0.000**	**2.1 (1.4-3.2)**	0.566	0.8 (0.3-1.8)	**0.026**	**1.9 (1.1-3.3)**
5:Dog rivalry	0.238	1.3 (0.8-2.0)	0.090	1.4 (0.9-2.2)	0.767	0.9 (0.3-2.2)	0.149	1.6 (0.9-2.9)
6:Stranger-directed fear	0.678	1.1 (0.7-1.6)	0.482	1.2 (0.8-1.7)	0.250	0.6 (0.2-1.5)	0.659	0.9 (0.5-1.5)
7:Nonsocial fear	0.589	1.1 (0.7-1.7)	0.830	1.0 (0.7-1.6)	0.679	0.8 (0.3-2.0)	0.799	1.1 (0.6-1.9)
8:Dog-directed fear	0.526	0.9 (0.6-1.3)	0.091	1.4 (0.9-2.2)	0.082	0.3 (0.1-1.1)	0.789	0.9 (0.5-1.6)
9:Touch sensitivity	0.347	0.8 (0.5-1.3)	0.245	1.3 (0.8-2.1)	0.322	0.6 (0.2-1.7)	0.280	1.5 (0.7-2.9)
10:Separation-related behavior	0.403	0.8 (0.6-1.3)	0.283	0.8 (0.5-1.2)	0.311	1.6 (0.7-3.6)	0.669	1.1 (0.7-1.9)
11:Attachment/attention seeking	0.189	0.7 (0.5-1.2)	0.812	0.9 (0.6-1.5)	0.060	2.3 (1.0-5.6)	0.060	0.6 (0.3-1.0)
12:Trainability	0.352	0.8 (0.5-1.2)	**0.004**	**1.8 (1.2-2.8)**	0.752	1.1 (0.5-2.7)	0.488	1.2 (0.7-2.1)
13:Chasing	**0.020**	**1.6 (1.1-2.4)**	0.587	1.1 (0.7-1.7)	0.810	0.9 (0.4-2.1)	0.371	1.3 (0.7-2.2)
14:Energy	0.381	1.2 (0.8-1.8)	0.811	1.1 (0.7-1.6)	0.741	1.2 (0.5-2.7)	0.574	1.2 (0.7-2.0)

*Table 21: Binary logistic regression of the dependent variables (the subscales) and the different independent variables (gender, neutering, rehomed, sports) (P=p-value, OR=odds ratio). The significant values are in bold. The last value was used as reference: value 1 for the subscales, neutered, rehomed and sports, for gender it was female (2). *=this group was too small, no reliable OR could be estimated.*

Subscale (0/1)	Age category 1 (0 years)		Age category 2 (1-3 years)		Age category 3 (8-15 years)	
	P	OR(95% CI)	P	OR(95% CI)	P	OR(95% CI)
1:Excitability	0.809	0.9 (0.4-1.9)	0.457	0.8 (0.5-1.4)	0.786	1.1 (0.6-2.0)
2:Stranger-directed aggression	0.065	2.0 (1.0-4.3)	**0.021**	**1.9 (1.1-3.4)**	0.645	1.2 (0.6-2.1)
3:Owner-directed aggression	0.257	0.6 (0.3-1.4)	0.972	1.0 (0.5-2.1)	0.242	1.7 (0.7-4.3)
4:Dog-directed aggression	**0.000**	**5.2 (2.2-12.0)**	**0.035**	**1.8 (1.0-3.1)**	0.466	0.8 (0.4-1.5)
5:Dog rivalry	**0.021**	**2.8 (1.2-6.8)**	0.505	1.2 (0.7-2.2)	0.555	1.2 (0.6-2.3)
6:Stranger-directed fear	0.805	1.1 (0.5-2.2)	0.368	1.3 (0.7-2.2)	0.053	1.8 (1.0-3.4)
7:Nonsocial fear	0.452	0.7 (0.3-1.7)	0.274	0.7 (0.4-1.3)	0.093	1.7 (0.9-3.3)
8:Dog-directed fear	**0.016**	**0.4 (0.2-0.8)**	0.114	0.6 (0.3-1.1)	0.862	1.1 (0.5-2.2)
9:Touch sensitivity	0.871	1.1 (0.5-2.4)	0.931	1.0 (0.5-1.7)	**0.025**	**2.3 (1.1-4.9)**
10:Seperation-related behavior	**0.002**	**0.3 (0.2-0.7)**	**0.001**	**0.4 (0.2-0.7)**	0.761	0.9 (0.5-1.7)
11:Attachment/attention seeking	0.942	1,0 (0,4-2,2)	0.751	1.1 (0.6-2.1)	0.486	1.3 (0.6-2.6)
12:Trainability	**0.008**	**3.6 (1.4-9.5)**	0.668	1.1 (0.6-2.0)	**0.036**	**0.5 (0.3-1.0)**
13:Chasing	0.820	0.9 (0.5-1.9)	0.449	0.8 (0.5-1.4)	0.449	1.3 (0.7-2.4)
14:Energy	**0.001**	**0.3 (0.1-0.6)**	**0.001**	**0.4 (0.2-0.7)**	0.507	0.8 (0.4-1.5)

Table 22: Multinomial logistic regression of the dependent variables (the subscales) and the independent variable age. Age is divided in four categories: 1=0 years of age, 2=1-3 years of age, 3=8-15 years of age and 4=4-7 years of age (P=p-value, OR=odds ratio). The reference category is age category 4 and 1 for the subscales. The significant values are in bold.

Reproduction section

Male dogs

42 (23.2%) of the male dogs did cover a female once or more times in their lives. 40 (95.2%) of them produced a litter of puppies. *Table 23* shows with how many different females the males produced a litter.

Number of females	Number of males	Percentage of the males that produced litters (42)
1	18	45%
2	6	15%
3	3	7.5%
4	4	10%
5	2	5%
>5	7	17.5%
Total	40	100%

Table 23: Number of different females the males produced a litter with. The percentage is from the total of males that produced litters (40).

The next question was with how many females the coverage did not lead to a gestation. The results are shown in *table 24*. 26 (65%) out of the 40 males never had a coverage without gestation. Calculation of the fertility was not possible, since the questions provided insufficient information.

Number of females no gestation									
Number of females		**0**	**1**	**2**	**3**	**4**	**5**	**>5**	**Total**
	1	14	3	1	-	-	-	-	18
	2	4	2	-	-	-	-	-	6
	3	2	-	-	1	-	-	-	3
	4	2	-	1	-	1	-	-	4
	5	1	-	-	-	-	1	-	2
	>5	3	1	-	-	-	-	3	7
	Total	26	6	2	1	1	1	3	40

Table 24: Crosstab with the number of females and the number of females where the coverage did not lead to a gestation.

Female dogs

86 (35.0%) of the females were presented for a coverage once or more times. The next question was how many heats the owner has had the female covered and how often the coverage lead to a gestation. The results are shown in *table 25*.

Number of gestations							
Number of heats presented for coverage		**0**	**1**	**2**	**3**	**>3**	**Total**
	1	1	24	-	-	-	25
	2	-	5	19	-	-	24
	3	-	2	8	14	-	24
	4	-	2	3	-	-	5
	5	-	2	1	1	1	5
	6	-	1	1	-	-	2
	>6	-	1	-	-	-	1
	Total	1	37	32	15	1	86

Table 25: Crosstab with the number of heats the female was presented for coverage and the number of gestations. The numbers in red are the females that had as many gestations as coverages.

A total of 57 dogs had the same number of heats presented for coverage as number of gestations (marked red in the crosstab above). Just as with the male dogs, it was also not possible to calculate the fertility in the female dogs, since the questions provided insufficient information.
In one dog the gestation did not lead to a birth, this dog was two times pregnant and had one birth.

In 7 (8.2%) out of 85 dogs the owners reported an abnormal birth, in one dog two abnormal births were reported. The reasons given are shown in *table 26*.

Reason	Number of female dogs	Percentage of the females that produced litters (85)
Oxytocin/calcium administration	3	3.5%
Cesarean section	2	2.4%
Two pups at ones	1	1.2%
Manuel assistance	1	1.2%

Table 26: Reported complications during birth. The percentage is the percentage from the total of females that produced a litter (85).

In the dog with two abnormal births, at the second birth oxytocin/calcium was administered and a Cesarean section was performed.
10 (11.8%) out of the 85 dogs who have had a birth had complications post-partum. 4 dogs (4.7%) had an endometritis, 3 dogs (3.5%) a mastitis, 2 dogs (2.4%) hypocalcemia and 1 dog (1.2%) had an herpes virus. A total of 1039 puppies were reported in 146 litters. The mean number of puppies born per litter was 7.1. In two dogs the number of puppies per litter was unknown, these were not included in the calculation.

Discussion

The minimal sample size estimated was 350 dogs. With 426 dogs used for the analysis, this was achieved. For all interpretations it should be taken into account that this questionnaire is filled in by owners and not veterinarians, which means the diagnoses that are reported could be wrong and it could be that diagnoses are missed. Nonetheless this is a health inventory, so further research will be needed to eliminate this bias. Also it is tempting for an owner to fill in a dog that has or has had something, this may cause a higher incidence then in the general population.

General section

The male/female distribution was 181 males versus 246 females. This does not reflect the population, since the expected male/female ratio is 1:1. Because of this overrepresentation of female dogs, it is assumed that there is also an overrepresentation of breeding dogs in this study. This has to be taken into account when the results are interpreted. 49.7% of the dogs was 0-3 years old, this is what is expected in a dog population (16).

With 84.1% of the dogs one or more sports were practiced, indicating that Australian Shepherd owners are very active with their dog and practice a wide variety of sports. The current study shows that the Australian Shepherd is not often used as a therapy (8.2%) or assistance (2.1%) dog.

Most dogs were bought as a puppy or self-bred, which correlates with the age of ownership. In the current study 23 (5,4%) dogs were rehomed. Rehoming is not desired, but sometimes inevitable. By informing people, who are interested in this breed, about the character and care of the Australian Shepherd, the degree of rehoming could be reduced. The ASCN is already doing this extensively, which is a good thing.

External characteristics

The results in the current study show that most dogs are tricolors. Since the not recognized colors are no concern in relation to health, this is not further analyzed. The Australian Shepherd Health and Genetics Institute (ASGHI) also conducted a health survey in 2009-2010. They found a prevalence of 2-3% kinked tails, in the current study a prevalence of 3.5% was found. This suggests kinked tails are not more common in the Dutch Australian Shepherd population, but it is also not preferred. Most dogs had a full length tail (77%).

Male reproduction status

A dog is called cryptorchid, when one or both testicles are not located in the scrotum at the age of six months. Then the location of the testis is abdominal, inguinal or ectopic. Normally the testis are descended about 35-40 days after birth. If the testis descent at a later point of time, this is called delayed descent (17). The prevalence of cryptorchid male dogs in the overall population is estimated at 2.1% and seems to have higher prevalence in specific dog breeds, which assumes it is hereditary (18). The 2.8% in the current study does not show a big deviation from the general population, but since the assumed heredity breeding with cryptorchid dogs should be discouraged, which is already been done by the ASCN by excluding cryptorchid dogs from the breeding population.

The prevalence of neutering in Western Europe is estimated at 54% in the United Kingdom and 47% in Ireland (19). In the current study this prevalence is 40.0% (male and female together). This assumes that Australian Shepherd are less likely to be neutered than the population dogs in Europe, but since it is assumed there is an overrepresentation of breeding dogs in the current study, no conclusions can be drawn from this.

There are multiple reasons for neutering a dog. Mostly behavior, population control and preventing or curing diseases were mentioned. 54 (29.8%) males were neutered in the current study. 51.9% of them because of behavior. This is 15.5% of all male dogs, so it seems to be quite common that male Australian Shepherds are neutered because of behavior. It would be interesting to know what type of behavior and if the neutering had any effect.

Neutering in order to prevent diseases in male dogs give inconsistent beneficial effects, yet 7.4% of the male neutered dogs was neutered because of this reason (19). Another reported reason was 'medical: cure disease' (18.5%). Two dogs had a perineal hernia, which is 1.1% of the males and 0.5% of the total sample. Both dogs were 5 years old, which is quite young because most dogs with a perineal hernia are between 7 and 9 years old (20). In a Spanish study they found a prevalence of 0.96% (21). This assumes perineal hernia's are no point of concern in the Australian Shepherd.

Female reproduction status

Normally female dogs have their first heat around 6-14 months of age, with a positive correlation on breed size (22). In the current study the mean age of the first heat was 11.5 months (±3.2), but since the options were 5 until 18 months and >18 months, from a few dogs no exact age of first heat is known, so the real mean will be higher. This mean correlates with the normal age of first heat found in the dog population.

117 (47.6%) of the females were neutered. The two most reported reasons for neutering female dogs were 'birth control' and 'medical: prevent disease'. In the current study is seen that the reason 'birth control' is often given together with other reasons, this makes it hard to interpret. Many studies do confirm the reduce of the risk of diseases related to the female reproduction organs, such as mammary tumors and pyometra/endometritis (19). The diseases given for the option 'medical: cure disease' consisted of diseases related to the female reproduction organs. In the current study the age of neutering was only asked for the male dogs, in subsequent studies this question should be added. No abnormal trends were found compared to the general dog population.

Deceased dogs

In the current study, 34.5% of the deceased dogs died of cancer. Other studies found a prevalence of 18%-23.6% (23,24). This suggests that the percentage of causes of death due to cancer is on the high side in the current sample of the Dutch Australian Shepherd population. In the ASGHI health survey they found a mean longevity of dogs deceased by cancer of 10.16 (±3.0) years, which is more than 10% higher than the longevity found in the current study of 9.1 (±2.6) years. Since an overrepresentation of dogs deceased by cancer is possible, it is questionable if further research is needed in the Australian Shepherd.

The mean longevity was 10.1 (±3.5) years. This might be higher, since the highest age option was older than 13 years and this was set to 13 in order to analyze it. In the study of Greer et al. (2007) they found a mean age of 12.88 (±1.01) in the FCI herding group, where the Australian Shepherd belongs to (25). In order to draw a conclusion on the longevity, the exact age must be asked in a subsequent questionnaire.

In the ASGHI health survey was a prevalence of 31.34% found of dogs that died of cancer and 28.10% that died of old age. Unfortunately no materials and methods are described, this makes it hard to say how reliable the results are, but the percentages are about the same as in the current study. The mean longevity found in that study was 10.74 (±3.90) years, which highly corresponds to the mean longevity found in the current study.

Interestingly out of the 7 dogs that died due to neurological problems, 2 were 2 years old and 1 was 3 years old. No exact diagnoses were asked, but this could be interesting since congenital epilepsy occurs in this breed. To draw a conclusion from this, this should be further investigated in a future study.

Screening

67% of the dogs were screened for heritable diseases (HD/ED/ECVO). To check the validity of the given data, the kappa value, as a measure of agreement, for HD and ECVO was estimated. For the HD-results the kappa was ≥ 0.81, which is regarded as 'good'. For the ECVO-results the kappa was $0.21 \leq \kappa \geq 0.40$ which is regarded as 'fair'. These results strongly suggest if an owner is asked for the HD results, the given answer has a good reliability as for the ECVO-results the given answer has a fair reliability and is not recommended to be used. When looked at which particular ECVO results were wrongly given by

the owner, 49 out of 76 incorrect results was about the eye examination that was performed with the litter. This suggests that most owners are not aware of the tests performed with their dog before they were the owner. When looked at the literature, the prevalence of HD in herding dogs in the United States and Canada is 15.23% (26) and in sheepdogs in the Netherlands 11.8%. The prevalence of ED in the Netherlands in sheepdog is 5.8% (27). This assumes that the prevalence of HD and ED in the current Australian Shepherd sample is no point of concern, but since not all dogs are screened and according to specialist thoughts the dogs with HD or ED on X-rays are most of time not send in for official screening, this result has to be interpret with care.
This result has also be taken into account when looked at the DNA-test results. In the current questionnaire was asked about the DNA-tests performed in the dog about which the questionnaire was completed. In the Australian Shepherd it is common to use the DNA-test results of the parents and grandparents, so if they were both free for a particular disease, the offspring will be too. The idea was to investigate the DNA-results of the particular dog itself and not from the parents or grandparents. It looks like many owner did fill in the test results of the parents. This makes it hard to interpret the results reported. Also this is information given by the breeder to the buyer of the puppy and when we look at the reliability of the ECVO litter results given by the owner, this strongly suggests these DNA-test results should be interpret with care. In addition sometimes carriers are not included in the breeding population, which could cause a lower reported allele frequency than in reality.
Only MDR1 and HSF4 showed sufferers in the current study. A mutation in the HSF4 gene is dominant with incomplete penetration. 40-50% of the heterozygote dogs showed cataract in both eyes in the study of Mellersh et al. (2006) (12). Presumable multiple genes are associated with the development of congenital cataract in the Australian Shepherd (28). Therefore the influence of the minor allele in the HSF4 mutation seems to be of moderate importance. The minor allele frequency in the current study was 0.06, this suggests selection of free breeding dogs is possible without making the breeding population to small. The minor allele frequency of the MDR1 gene was 0.21. In the study of Geyer et al. (2005) they found a frequency of 0.20 in the Australian Shepherd, 6.9% was sufferer and 25.2% was carrier (29). The percentage carriers was 29.8% and sufferers was 2.3% in the current study. This shows there is no substantial difference found in the current study. MDR1 mutation is autosomal recessive, so only sufferers will have clinical signs (29). The current sufferer frequency is not higher than in other studies, but it must be taken into account when selecting animals for breeding, as is already being done.
No dogs showed deafness on the BEAR test, but only 40 (9.4%) were tested, so no hard conclusions can be drawn from this result.

Medical section

The diseases reported ≥10 times were: trauma/sprain/fracture (8.9%), arthritis in one or more paws (4.0%), spinal problems (2.8%), skin tumors (2.3%), intestinal complaints due to parasite/infection/wrong food (5.6%), gingivitis (3.0%) and epilepsy/movement disorders (2.6%). When compared to the study of O'Neill et al. (2014), where they listed the prevalence of disorders recorded in dogs attending primary-care veterinary practices in England, the prevalence of traumatic injury was 5.5%, degenerative joint disease was 6.6%, skin mass was 2.8%, diarrhea was 6.4% and periodontal disease 9.3%. Epilepsy was not listed (30). Trauma/sprain/fractures occur more in the current study, which could be explained by the large amount of dogs with which sports are practiced and have a higher risk of injury. It is not said which diseases are mentioned by degenerative joint disease, because spinal problems could be covered by this. If so, the results match well. The results of diarrhea are also not very different. The current study shows less periodontal disease. The disease with the highest prevalence in the study of O'Neill et al. (2014) was otitis externa (10.2%), which is contrary to the prevalence in the current study (2.1%) (30).
Epilepsy/movement disorders in dogs can be divided in idiopathic and non-idiopathic. The hereditary form of epilepsy is also known as idiopathic epilepsy, because no other cause can be found. The first seizures occur when the dog is ≤ 5 years old. The prevalence of idiopathic epilepsy in the general dog

population has been estimated to be 0.6-0.75% (31). In the current study a prevalence of 2.6% was found, of which 7 dogs were ≤ 5 years old. This is 1.6% of the total sample size. According to specialists interpretation, a prevalence higher than the general dog population is considered to be a problem in the breed and if the prevalence is >1% further research is needed. Since Epilepsy can have many different causes and often these are not investigated in the dog, it would be interesting to know if these dogs really have idiopathic epilepsy or not. Further research is advised.
The results of the eye diseases have to be interpret with care, since the given options were based on clinical signs and not on eye diagnoses. Some of the owners gave specific eye diagnoses themselves in the comments. It is assumed that some owners did not know exactly their dogs condition and the given options did not fit well enough, which could have resulted in not filling in the question, so a wrong representation of the eye problems is possible.
Another reported common (≥10%) disease in the ASHGI report were autoimmune diseases. In the current study this percentage is 5.3% (based on: 'immune mediated polyarthritis' (0.9%), 'colitis' (0.9%), 'enteritis' (0.9%), 'food allergy' (0.5%), 'atopic dermatitis' (0.9%), 'food hypersensitivity' (1.2%)). This percentage might be lower since it could be a dog was reported with multiple autoimmune diseases. These results show that autoimmune diseases are less common in the Dutch Australian Shepherd population than in the American Australian Shepherd population.
The rest of the results show no indication for concern.

Behavioral section

The subscale reliability of the C-BARQ short version in this study had high α values. When compared to other studies that used this C-BARQ short version, the α values were higher on all the different subscales (9,32). In the study of Duffy et al. (2014) they used the C-BARQ on dogs relinquished to shelters and in the study of Waulthier et al. (2018) they used the C-BARQ on puppies from puppy farms. The difference with the current study is that in this study only one specific breed is used. Also only from a few dogs in the current study the owner indicaded that there were behavioral problems, all dogs had a pedigree and came from approved breeders. The homogeneous sample of the current study could explain why the α values were higher than in the other studies. The α value from the subscale 'Trainability' was low (0.412). Transformation of the 'Trainability' subscale questions to make the scores based on the same 5 point scale did not have enough effect on the internal consistency, so it was right to remove the item 'the dog is easily distracted by interesting sights, sounds or smells'. In the study of Hsu et al. (2010) this specific item was also excluded because of low internal consistency.

The Australian Shepherd in the current study scored on the different aggression subscales highest on the 'dog-directed aggression' subscale, followed by the 'stranger-directed' subscale and lowest on the 'owner-directed' subscale. The same order is seen in other breed studies: Golden Retriever (33), Labrador Retriever and English Springer Spaniel (34). Also in non-breed specific studies this order is seen (15). In the study of Hsu et al. (2010) this is explained by the fact that dog owners probably do not tolerate their dog to be aggressive towards themselves, but have a higher tolerance for aggression towards unfamiliar dogs. The aggression towards strangers is explained by the fact that those dogs are mostly used for guarding (15). These explanations seem to be suitable for the Australian Shepherd, although they are mostly not specifically used for guarding, the Australian Shepherd wants to protect their family and are reserved to strangers which results in higher scores in the subscale 'stranger-directed aggression'.

Comparison to the literature

Subscale scores

On the subscales 'attachment-attention seeking' and 'trainability' the Australian Shepherd scored more than 0.5 points higher compared to the general population. According to the ASCN the Australian Shepherd is not a dog that loves everyone, they are mostly reserved to strangers and will protect their

pack (the family). The items from the subscale 'attachment-attention seeking' are about following their owner through the house, this can be explained by the fact that they want to be with their family. They are also really smart and since they are bred to work on the ranch all day, good trainability is required. This correlates with the results in the current study. In the study of Eken Asp et al. (2015) they determined the C-BARQ differences between 'working' and 'non-working' breeds, the Australian Shepherd was classified in the 'working' group. The results from the current study partly correlate with their results. In addition to the aforementioned subscales, they found for more subscales significant differences (35).
The Australian Shepherd scored more than 0.5 points lower on the subscales 'excitability' and 'touch sensitivity' compared to the general population. These results could be explained by the fact that these dogs are bred for working and have to be tough and will score lower on 'touch sensitivity'. Since these dogs can work independent and, if they get enough exercise, will be calm when they do not have to work, it is no surprise that the subscale score for 'excitability' is more than 0.5 points lower than the general populations. When compared to the results from the study of Eken Asp et al. (2015), only 'touch sensitivity' corresponded with the current study. The 'working' dogs in their study scored significantly higher on the subscale 'excitability' (35).
It has to be taken into account that of the aforementioned subscales, only 'attachment/attention seeking' has proven to have good validity and no hard conclusions can be drawn since no confidence interval was known for the population means.

Miscellaneous items

The Australian Shepherd scores showed no more than 0.5 difference on the miscellaneous items when compared to the study of Wilson et al. (2018) (14). According to the C-BARQ website (13), the miscellaneous items are less reliable as indicators of problems than the main subscales. The reason they give for this is that individual owners vary enormously in their ability or willingness to tolerate and manage these kinds of miscellaneous behaviors. This will greatly influence how to interpret the scores. The only scores in the current study who are above 1 (=seldom) are 'pulls on the leash' and 'barks excessively'. This could be explained by the high energy levels of the Australian Shepherd. There are at least no items that scored really high and could be a point of concern.

Relation between subscale scores and general information

In the current study is found that male dogs score significantly more often ≤ median on the subscale 'chasing' than female dogs. This assumes that female dogs show more chasing behavior than male dogs. This cannot be explained by the literature, so no explanation could be given for this result.
Also is found that not neutered dogs score significantly more often ≤ median on the subscale 'stranger-directed aggression', 'dog-directed aggression' and 'trainability' than neutered dogs. This assumes that not neutered dogs show less stranger- and dog-directed aggression than neutered dogs and are less trainable than neutered dogs. Although it is still commonly thought that male dogs show more aggressive behavior than female dogs, there is no significant evidence for this (15). Also it is thought that neutering dogs results in a reduction of aggression, but the scientific results are varying, namely: reduction of aggression in both male and female, but also a rise in aggression in female dogs (36); only a reduction in male dog-directed aggression, the rest of the results showed inconsistency (37); a rise in aggression in neutered male and female dogs (38). Also the aggression could be explained by the fact that mostly dogs neutered due to behavioral issues are more aggressive and this behavior does not change with neutering. The trainability could be less in not neutered dogs due to more hormonal distraction, but these arguments are not based on scientific results.
In the study of Hsu et al. (2010) they found that if the owners spent more time with their dog, they scored lower on the aggression subscales (15). In the current study is found that dogs with which no sport were practiced scored significantly more often ≤ median on the subscale 'dog-directed aggression' than dogs with which sports were practiced. This assumes that dogs with which no sport is practiced show less dog-directed aggression than dogs with which sports are practiced. This is

contrary to the result from the study of Hsu et al. (2010). It could be that the Australian Shepherds that are used in sports have higher energy levels and are more easily excited, although there is no significant result or literature that supports this assumption.

Relation between subscale scores and age

The behavioral development of dogs can be divided in different stages. These stages are based on socialization, puberty and maturity (39). In the current study dogs under the age of 1 scored significantly lower on dog-directed aggression, trainability and dog rivalry. They scored significantly higher on dog-directed fear and separation-related behavior than adult dogs (4-7 years old). These results can be explained by pre-pubertal development of dogs behavior. These dogs are most of the time low in ranking, which explains less dog-directed aggression, dog rivalry and higher dog-directed fear. The trainability was based on listening to commands and young dogs still have to learn this. Also they have to learn to be alone, which explains the higher scores on separation-related behavior.
Dogs between 1 and 3 years of age scored significantly lower on stranger-directed aggression and dog-directed aggression and higher on separation-related behavior than adult dogs. The reasons mentioned above can be the same for this age category. Interestingly this age category scored higher on stranger-directed aggression, this could be explained by the behavioral changes due to hormonal influences in this stage of development.
Finally the dogs between 8 and 15 years old scored significantly lower on touch sensitivity and higher on trainability than adult dogs. When looked at the questions/situations this is not surprising since older dogs are mostly more fine with touching and listen better to commands.

It would be interesting to add more questions to this C-BARQ questionnaire to discriminate between different forms of aggression, because now it is unknown if the dog is showing fear related aggression or dominance related aggression. Also the way the C-BARQ is statistically analyzed is different in the studies used. A protocol should be made for the analysis in order to make this questionnaire more reliable and comparable.

So the Australian Shepherd dog is a dog with high energy levels, good trainability, shows little or no aggression towards familiar people and dogs, are tough and like to be around their family. They show mild to moderate aggression towards unfamiliar dogs and mild aggression toward strangers and are not easily frightened. No real concerns came forward from this study based on the behavior, but it has to be taken into account that the extent to which the owner sees behavior as a problem is something subjective.

Reproduction section

In order to say something about the reproduction of the Australian Shepherd, calculation of the fertility was needed. Based on specialist interpretation, the fertility should be ≥ 80%. In the current study the questions did not give the right information to calculate the fertility. Also the assumption raised that the questions in the male reproduction section were not clear enough, since some owners reported their male had produced litters, but also reported that he had the same number of different females covered as the number of coverages that did not lead to a gestation. These results could be possible since it is not asked how often the male covered females, independent of the number of different females, but it suggests that the question was not understood well. An explanation for this could be that there was a denial in the question, this should be avoided in a subsequent questionnaire. Because these results are not reliable no conclusions can be drawn from the reproduction results.

Conclusion

With the established health questionnaire a health inventory of the Dutch Australian Shepherd population could be made. Hereditary diseases that seem to be common in this breed were epilepsy, lymphoma and hemangiosarcoma. Besides screening of elbow and hip dysplasia, eyes and several DNA tests are already been done by the ASCN and are mandatory with the dogs that are used within the ASCN for breeding. The results of the current study show that the mean longevity was at least 10.1 (±3.5) years which was comparable to earlier found longevity in the ASGHI health survey. A point of moderate concern was the prevalence of epilepsy/movement disorders, more research is recommended to discriminate between the hereditary idiopathic epilepsy and the non-idiopathic epilepsy/movement disorders. Points of mild concern were the allele frequency of the MDR1 gene mutation and cancer as the cause of death. The ASCN is already screening MDR1 gene mutations in the breeding population and thereby selecting dogs for breeding. By doing this the allele frequency should go down in the future. If the ASCN wants to further investigate cancer, histopathological examinations are needed to discriminate between the different forms of cancer in order to know the prevalence of the different cancer forms and maybe to set up a breeding program. No behavioral problems emerged in this questionnaire, but with behavioral problems it has to be taken into account that this is most of the time something subjective. This questionnaire also shows that asking the owner for screening results is reliable for hip dysplasia, but unreliable for eye examinations. The reason for this seems to be that the owners are not aware of the screening tests done before the dog was acquired by the owner. Overall the general health of the Dutch Australian Shepherd population shows, beside the aforementioned diseases, no points of concern.

Recommendations

New questionnaire

The amount of questions did not seem to be a problem, since the response rate was good. It would be better to ask for exact numbers, e.g. longevity and age of first heat (female dogs), in order to give a representable mean and standard deviation. The questions regarding eye problems should not be based on clinical signs, but be more specific about diagnoses. Also the reproduction questions should not contain a denial and must be composed in such a way that they give the right data to calculate the fertility rate, since that was not possible in the current questionnaire.

Dutch Australian Shepherd Club

Regarding to the results presented in this study, the points of concern are the following and are advised to be further investigated: epilepsy/movement disorders, MDR1 gene mutation and cancer as cause of death.

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Appendix 1: general section

Countries of origin other than the Netherlands

Country	Number of dogs	Percentage of total number of imported dogs (66)
United States	20	30.3%
Belgium	15	22.7%
Germany	13	19.7%
Hungary	5	7.6%
France	4	6.1%
Canada	3	4.6%
Sweden	2	3.0%
Denmark	1	1.5%
Norway	1	1.5%
Italy	1	1.5%
United kingdom	1	1.5%
Total	66	100%

Table 27: Number of dogs imported from different countries with the percentage of the total number of imported dogs (66)

Sports practiced with the dogs

Sport	Total reported number	Percentage of the total sample size (427)
Agility	183	42.3%
Obedience	200	46.8%
Hoopers	60	14.1%
Flyball	26	6.1%
Cattle driving	51	11.9%
Dog dance	40	9.4%
Dog Frisbee	87	20.4%
Horse and dog trail	10	2.3%
Mantrailing	46	10.8%
Treibball	15	3.5%
Tracing	83	19.4%
Other	74	17.3%

Table 28: Reported different sports practiced with the total of dogs with which the sport was practiced and the percentage of the total sample size (427).

Screening results

Screening	Number of dogs	Percentage of total sample size (427)
HD RvB	185	43.3%
ED RvB	132	30.9%
HD OFA	18	4.2%
ED OFA	8	1.9%
ECVO	241	56.4%

Table 29: Number of dogs reported for the different screenings. HD RvB=hip dysplasia by 'Raad van Beheer', ED RvB=elbow dysplasia by 'Raad van Beheer', HD OFA=hip dysplasia by 'Orthopedic Foundation for Animals', ED OFA=elbow dysplasia by 'Orthopedic Foundation for Animals', ECVO= European College of Veterinary Ophthalmologists. The percentage is of the total sample size (427).

Result RvB	Number of dogs	Percentage of total screened dogs by the RvB (185)
HD A	169	91.4%
HD B	12	6.5%
HD C	3	1.6%
HD D	1	0.5%
Total	185	100%

Table 30: Hip dysplasia screening results by the 'Raad van Beheer' (RvB) reported. Percentage is of the total screened dogs by the 'Raad van Beheer' (185).

Result RvB	Number of dogs	Percentage of total screened dogs by the RvB (132)
ED free	130	98.5%
Arthritis grade 1	1	0.75%
Arthritis grade 2	1	0.75%
Total	132	100%

Table 31: Elbow dysplasia screening results by the 'Raad van Beheer' reported. Percentage is of the total screened dogs by the 'Raad van Beheer' (132).

Result OFA	Number of dogs	Percentage of total screened dogs by the OFA (18)
Excellent	8	44.4%
Good	7	38.9%
Fair	2	11.1%
Moderate	1	5.6%
Total	18	100%

Table 32: Hip dysplasia screening results by the 'Orthopedic Foundation for Animals' (OFA) reported. Percentage is of the total screened dogs by the 'Orthopedic Foundation for Animals' (18).

Result OFA	Number of dogs	Percentage of total screened dogs by the OFA (8)
Grade I ED	8	100%

Table 33: Elbow dysplasia screening results by the 'Orthopedic Foundation for Animals' reported. Percentage is of the total screened dogs by the 'Orthopedic Foundation for Animals' (8).

ECVO result	Number of dogs	Percentage of total ECVO screened dogs (241)
Free	216	89.6%
Cataract (congenital)	2	0.9%
Cataract (non-congenital) cortical	3	1.2%
Cataract (non-congenital) post. Pol.	1	0.4%
Collie Eye Anomaly: coloboma	1	0.4%
Progressive retinal degeneration	1	0.4%
Distichiasis/ectopic cilië	6	2.5%
Entropion/trichiasis	1	0.4%
Irishypoplasia	2	0.9%
Membrana pupillaris persistens	7	2.9%
Traumatic cataract	1	0.4%
Total	241	100%

Table 34: ECVO results reported. Percentage is of the total ECVO screened dogs (241).

		HD owner				
		No test result	**HD A**	**HD B**	**HD C**	**Total**
HD 'Raad van Beheer'	**No test result**	100	16	-	-	116
	HD A	1	65	-	-	66
	HD B	-	-	4	-	4
	HD C	-	-	-	2	2
	Total	101	81	4	2	188

Table 35: Crosstab with the hip dysplasia results according to the 'Raad van Beheer' database and the results given by the owner. The corresponding numbers are marked red.

		ECVO Owner				
		No test result	**No abnormalities**	**1 abnormality**	**2 abnormalities**	**Total**
ECVO 'Raad van Beheer'	**No test result**	20	5	-	-	25
	No abnormalities	54	87	2	-	143
	1 abnormality	7	5	5	-	17
	2 abnormalities	1	-	2	-	3
	Total	82	97	9	-	188

Table 36: Crosstab with the EVCO results according to the 'Raad van Beheer' database and the results given by the owner. The corresponding numbers are marked red.

Appendix 2: Behavioral section

Bar charts and crosstabs dogs characteristics

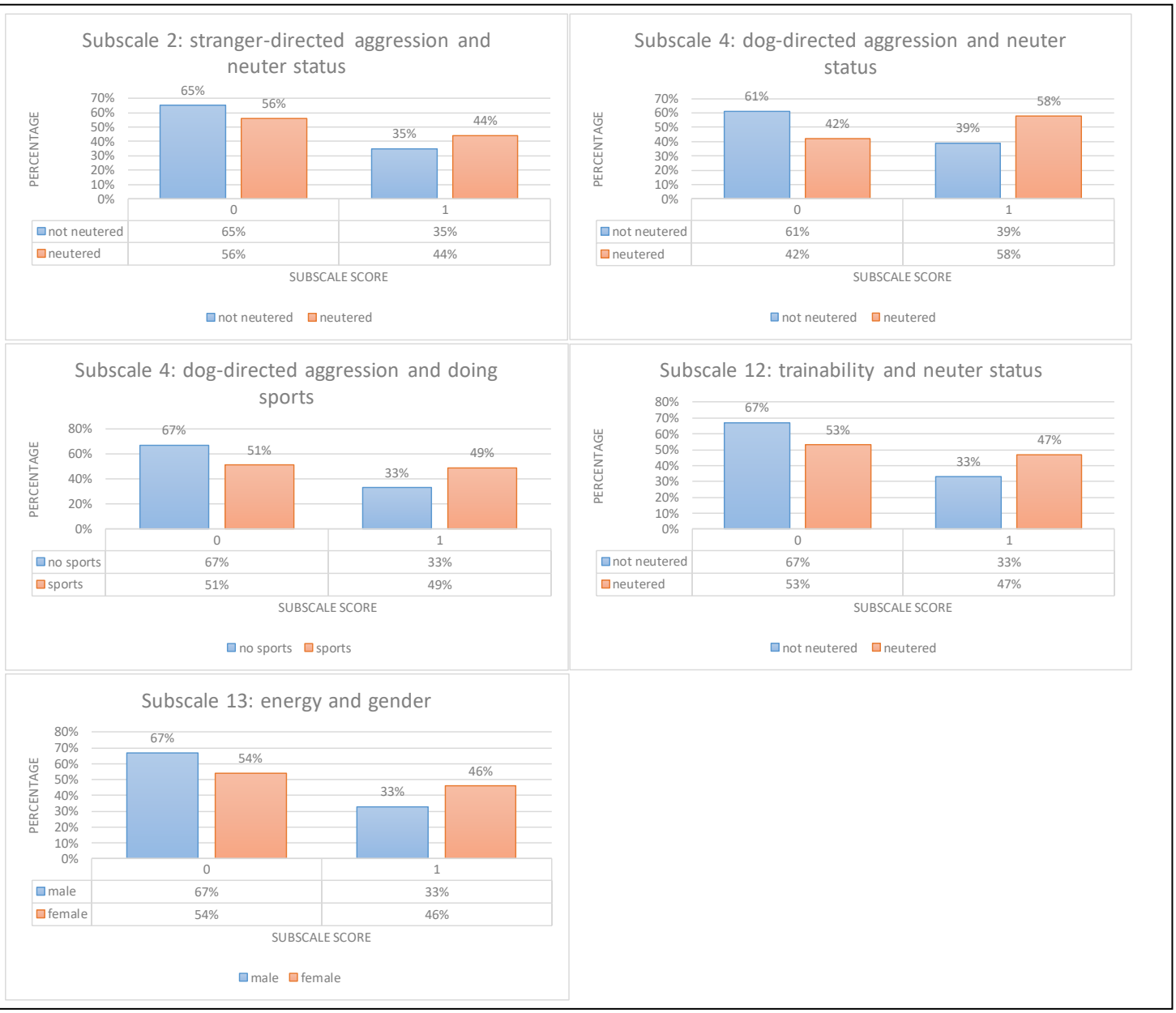

Figure 4: Bar charts and crosstabs from the five significant differences between the subscales given and the dogs characteristics.

Bar charts and crosstabs age categories

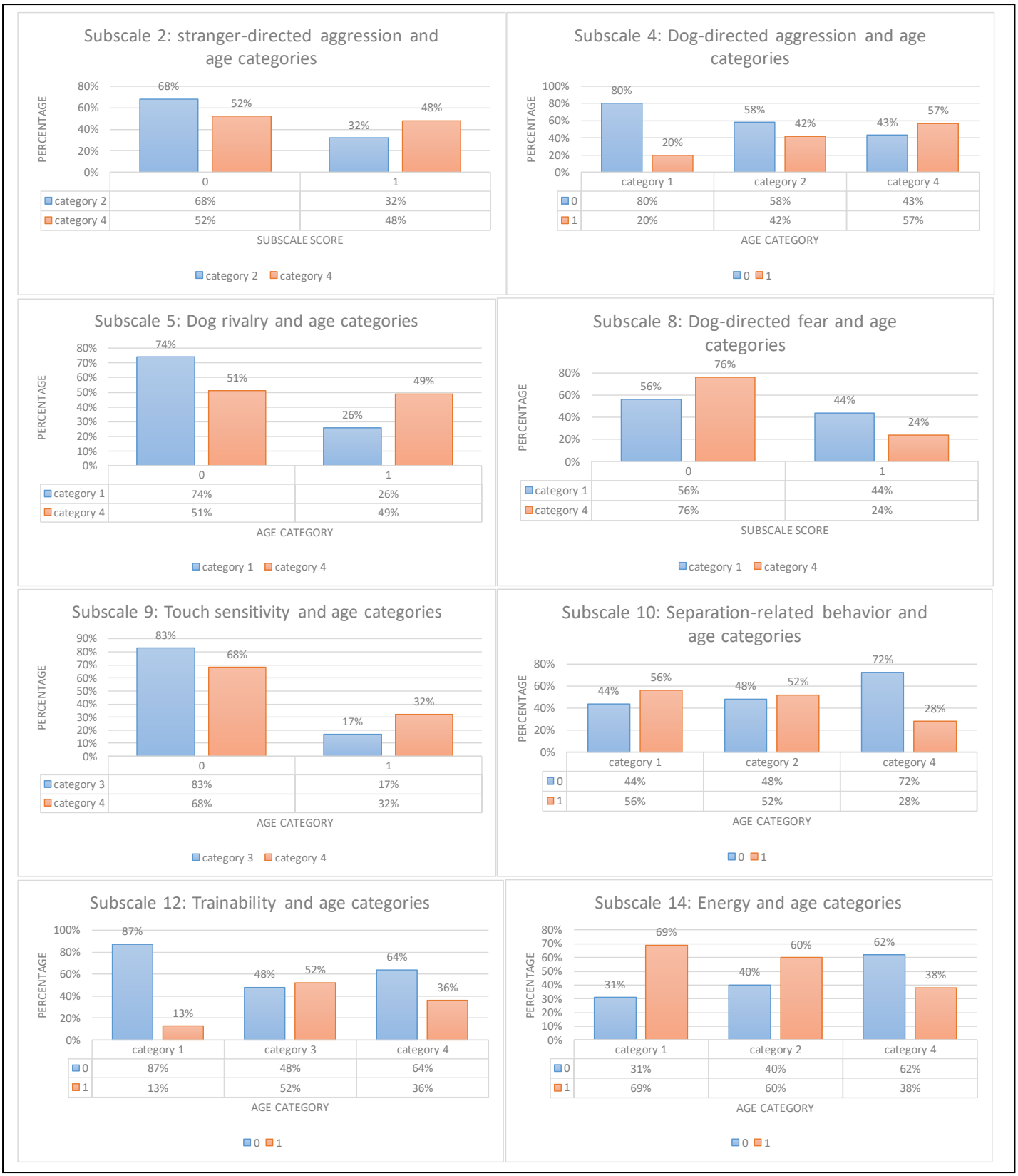

Figure 5: Bar charts and crosstabs of the twelve significant differences between the subscales given and the age categories.