

RESEARCH REPORT

Evaluation of the Effectiveness of the Breeding Program on the Incidence of Congenital Cochlear Sensorineural deafness in Dalmatian Dogs in The Netherlands

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Introduction to the Report

'EVALUATION OF THE EFFECTIVENESS OF THE BREEDING PROGRAM ON THE INCIDENCE OF CONGENITAL COCHLEAR SENSORINEURAL DEAFNESS IN DALMATIANS IN THE NETHERLANDS'

At the request of the Dutch Dalmatian Club (N.C.D.H.), a study was started in 2019 to investigate the effectiveness of the club's breeding policy on the reduction of deafness. For this purpose the Health committee of the N.C.D.H., at the time consisting of Erna Kuipers, José Lagendijk, Annemiek Morgans and Lizzy Plat, contacted the Expertise Centre for Genetics of Companion Animals (ECGG) of the Faculty of Veterinary Medicine in Utrecht.

Why the desire to start this research?
The Dutch Dalmatian Club started to perform BAER-hearing tests on Dalmatians in 1995, after studies conducted by Prof. Strain in the USA had brought to the attention of the board the existence of unilateral deafness in Dalmatians. Until then, unilateral deafness was an unknown phenomenon for us.

After contact with the Faculty of Veterinary Medicine in Utrecht and the Dutch Kennel Club, also after the necessary preliminary research, we could start in 1995 with the systematic implementation of the BAER-hearing tests within our breed club. The first veterinary practice with the necessary equipment was N. Dijkshoorn in Zeist. Currently four practices on different locations are at our disposal.

The results of the hearing tests are collected by the N.C.D.H., processed in annual reports and published in the club magazine. From these overviews a gradual decline in the occurrence of deafness became apparent. After almost 25 years of collecting data the need arose for a scientific analysis of this data. In other words: we wanted to know if it could be scientifically demonstrated that our policy, excluding unilateral deaf dogs from breeding, had had any effect.

How did the data come about?
From 1995 onwards the performance of the BAER-hearing test was:

- made compulsory for all litters born to members of the N.C.D.H., stipulating that all the puppies in the litter had to be present,
- made compulsory for all breeding dogs with the stipulation that only bilateral hearing dogs were allowed for breeding. This means that from then on unilateral hearing dogs were kept out of breeding.

It should be mentioned that so far Dalmatians with visible patches and monocles are not allowed in breeding in the Netherlands because they are listed as disqualifying faults in the breed standard.

How the test is performed
The performance of the BAER-test in the Netherlands is done in the various veterinarian practices according to an established protocol, agreed in a covenant with the performing veterinarians and the Dutch Kennel Club.

It is determined that:

- the BAER-test equipment is calibrated regularly in collaboration with the Dutch Kennel Club,

- for better reliability the hearingtests will be done under light sedation,
- the hearingtests will be carried out from the age of 6 weeks,
- the results are processed and registered at the office of the Dutch Kennel Club, which then issues a certificate per puppy with the result to the breeder/owner,
- the complete results are passed on to the Dutch Dalmatian Club.

Acknowledgment
The Expertise Centre (ECGG) of the Veterinarian Faculty of Utrecht was willing to carry out the afore mentioned research. The N.C.D.H. made all its data available and then master veterinary student Vera Pleijsier took up the research under the supervision of Mr. J.C.M. Vernooij MSc, biostatistician and Dr. P.A.J. Leegwater, geneticist. In November 2019 the study was completed, in March 2020 Vera gave a presentation of the study and her findings to the members of the N.C.D.H. After that, the final report also became available. To them, and to the members of our health committee, many thanks for the fine cooperation.

We are happy to share this report with you.

June 2021,
The Dutch Dalmatian Club / Nederlandse Club voor Dalmatische Honden (N.C.D.H.)

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Evaluation of the Effectiveness of the Breeding Program on the Incidence of Congenital Cochlear Sensorineural deafness in Dalmatian Dogs in the Netherlands

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Abstract

Hereditary deafness in Dalmatian dogs has been a problem for many years, and has been studied for years. Nowadays, the hereditary deafness in Dalmatians is known to be a form of Canine Congenital Sensorineural Deafness (CCSD). Deafness in Dalmatians is not monogenic and it is not yet known how genes contribute to the onset of CCSD. Several phenotypical traits are associated with CCSD.
A breeding program for Dalmatian dogs has been set up in several countries, to prevent the development of deafness in newborn pups. In the Netherlands, the breeding program was set up by the Dutch Club for Dalmatian Dogs (NCDH). Since 1995, this program includes that only bilaterally hearing dogs can be used for breeding, meaning that every pup needs to be BAER-tested. However, the effectiveness of the breeding program on the incidence of CCSD has not yet been evaluated.
Between 1995 and 2018, 4948 BAER-tests were performed in Dutch Dalmatian pups. A logistic regression analysis has been performed to assess the strength of association between hearing status and birth year period, head spot, coat colour, blue eyes and litter size. The effects of the sire and dam were considered as random effects.
The incidence of deafness seems to decrease over the years. This effect of birth year period was proven to be significant for the periods 2001 to 2006 and 2013 to 2018. For unilateral and bilateral deafness separately, this significant decrease was also found. Females have significantly higher odds of deafness than males. Furthermore, head spot significantly decreases the odds of deafness, whereas blue eyes do not seem to have a significant effect.
This research shows that the cumulative incidence of deafness has gone down since the new breeding program in 1995. However, it might have decreased more when dogs with presence of a head spot had been allowed for breeding.

Summary for the public

Hereditary deafness has been a problem in Dalmatian dogs for years. This type of deafness is known as ‘CCSD’ (Canine Congenital Sensorineural Deafness). The cause of deafness is not exactly known, but it is known that it has a genetic background. Based on literature, the odds for this type of deafness can be linked to having blue eyes (more likely to be deaf) or a head spot (less likely to be deaf). This type of deafness occurs in Dalmatian dogs all over the world, which is why many countries set up breeding policies for Dalmatian Dogs that take this into account. In 1995, the Dutch Club for Dalmatian Dogs (NCDH) has adjusted the breeding policy so that only bilaterally hearing dogs can be used for breeding. This means that every Dalmatian pup born from then on has had to be tested for deafness in both ears, using a BAER test. This way, both unilateral and bilateral deafness can be detected and unilaterally deaf dogs can be accurately excluded from breeding.
In this study, it was found that the new breeding policy has a positive effect on the incidence of deafness among Dalmatian dogs: since the test is obligated, the number of deaf pups decreased. In the first period (1995 to 2000), 19% of the pups were born deaf in one ear or in both, whereas in the last period (2012 to 2018), this decreased to 10%. We also found that 17% of the pups without a head spot is born with hereditary deafness, but deafness occurs only in 3% of dogs with a head spot, which is a significant difference. Furthermore, female dogs seem to be more likely to have CCSD than male dogs. We could not find an association between deafness and blue eyes.
Although the incidence of deafness among Dalmatian pups has gone down since the new breeding policy, more progress could be made. DNA techniques could be used to determine how genetically different two dogs are, so that a dam and a sire can be carefully matched for breeding. Another option to decrease the incidence of deafness in Dalmatians would be to allow breeding with dogs with a certain degree of a head spot, because having a head spot decreases the odds for deafness evidently.

Introduction

Hereditary deafness in Dalmatian dogs has been a problem for many years (1-3), and has been studied for many years In 1896, a link has been found between blue eyes and congenital deafness (2). Mostly phenotypical traits were studied at that time. It has later been determined that the odds to be deaf for Dalmatian dogs with blue eyes are 2.7 times higher than for Dalmatian dogs with brown eyes (4). Nowadays, the hereditary deafness in Dalmatians is known to be a form of Canine Congenital Sensorineural Deafness (CCSD). Besides having blue eyes or not, more factors seem to be involved in the development of this disease, including other phenotypical traits as well as genetic factors (1, 4-7).

Dalmatian pups with congenital deafness show cochlear degeneration after 2 to 4 weeks of age (1, 5). Before degeneration of the cochlea, the inner ear seems to be normal in structure and function (1). It is not entirely clear what causes the strial degeneration, but the absence of melanocytes seems to be a main factor. When melanocytes do not migrate to the cochlea, they cannot differentiate into intermediate cells and perform their function in the stria vascularis, leading to a sensorineural deafness (1, 4, 5). Besides strial degeneration, defects in the hair cells and endolymph may also be found (3, 4). At 1 to 2 months of age, this degeneration is already well advanced (3).

This type of deafness in dogs generally has a genetic background and is called Canine Congenital Sensorineural Deafness (CCSD), also known as Canine Hereditary Sensorineural Deafness (CHSD) (1, 4-7). It is found in several dog breeds, but mostly in English setters, Bull terriers, Whippets, Australian cattle dogs, English Cocker spaniels, Catahoula Leopard dogs, Border collies, Jack Russel terriers and, above all, Dalmatian dogs (4-7). In 1992 in the USA, 21.6% of Dalmatian dogs showed unilateral deafness, whereas 8.1% showed bilateral deafness (overall 29.7%) (8). Verhorevoort found a prevalence of 15.9% for unilateral deafness in NCDH Dalmatian pups in the Netherlands in the period 1995-1998, whereas 4.9% of the pups showed bilateral deafness (overall 20.8%) (1). In 2004, a study performed by G.M. Strain showed a prevalence of deafness in Dalmatian dogs (unilateral and bilateral) of 29.9% in the United States, using data collected from dogs in a clinical setting or at a dog show from owners or breeders (using a total of 5333 dogs) (7, 9). In the United Kingdom, a prevalence of 18.4% was found in 1234 Dalmatians with Kennel Club pedigree data tested for deafness in 1997 (9, 10). A study in Germany using 1899 BAER-tested Dalmatian dogs from all three Dalmatian kennel clubs associated with the German Association for Dog Breeding and Husbandry (VDH) found a prevalence of 19.7% in 2003 (11).

To determine the hearing status of pups, a BAER test (Brainstem Auditory-Evoked Response test) can be performed (3, 12), see figure 1. This test is used to classify an ear as ‘deaf’ or ‘not deaf’. In the Netherlands, this test is performed by professionals acknowledged by the Board of Management of Cynological Affairs in the Netherlands (‘Raad van Beheer’) (13). The test however is used worldwide, although it might be performed differently in each country (3, 12, 14).

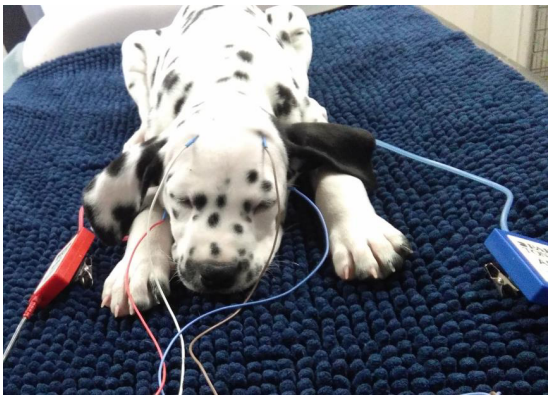


Fig. 1: Dalmatian pup undergoing a BAER test

Besides the Netherlands, there are guidelines for breeding Dalmatian dogs in various other countries. These rules are partly set to try to decrease the prevalence of deafness in Dalmatian pups, though they are not evenly strict everywhere (15-18). The effectiveness of the breeding programs on the incidence of CCSD is unclear. However, there seems to be a clear effect of allowing blue eyes: the prevalence of deafness in Dalmatian dogs in the USA, where dams and sires with

blue eyes are allowed, is relatively high (29.9% in the United States vs. 20.8% in the Netherlands in 2008) (1, 7, 9).

It is generally assumed that CCSD is a genetic disease, but which genes exactly are responsible for the development of CCSD has been unclear for a long time. The mechanisms leading to deafness have not completely been clear either, and a lot of research is still being done on this subject (4, 6). A study in 2002 found that female Dalmatians and blue-eyed Dalmatians were more often affected than other Dalmatians (6), though other studies could not find a sex effect (5). The absence of patches – a spot of dark fur that is already visible at birth – was associated with a higher prevalence of CCSD in several studies (5, 6). Generally, there seems to be a defect in genetic control of melanoblasts differentiation and/or migration, due to which the melanocytes do not reach the stria vascularis in the cochlea (5).

Several studies have found significant associations between eye colour, presence of patches, sex and parental hearing status, and deafness. These associations have mostly been studied in Dalmatian dogs (1-4, 6, 7, 19). The link between blue eyes and CCSD may be explained by the 'extreme white piebald' allele s^w (5, 6, 20). The *S* locus, associated with the distribution pattern of pigmented and white spots, has four different alleles. The associated gene is called the MITF gene (Microphthalmia-associated Transcription Factor). The *S* allele is dominant and is non-spotted, or 'self'. The s^i allele produces Irish spotting: generally, only a few white spots/areas on the thorax, face, head or feet. The s^p allele stands for piebald spotting, which gives more white spots and/or bigger white areas than the s^i allele: dogs with the s^p allele may have white spots on their limbs as well. Lastly, the s^w allele, the extreme white piebald allele, gives the greatest extent of white pigmentation, leaving only small spots on the coat pigmented (5, 7). Dalmatian dogs are most likely all homozygous for this allele, but there is much variation in expression level among Dalmatian dogs. The presence of patches has been associated with a lower expression of the s^w allele (5-7, 20).

The *M* locus, a pigmentation locus known as the 'merle' locus, is another locus that plays a role in hereditary deafness. Dogs homozygous for the recessive allele *m* have a uniform pigmentation, whereas dogs with *Mm* are dappled: they have body areas that are fully pigmented and areas that are pale or even white. Homozygous merle – *MM* – produces an almost completely white coat and is associated with deafness, blindness and sterility. Heterozygous dogs may vary in their likelihood of being deaf, depending on the present alleles for other genes associated with pigmentation (1, 6, 7). Bull terriers, Australian cattle dogs, English Cocker spaniels, Catahoula Leopard dogs, Border collies can be heterozygous and are thus more likely to be deaf that other breeds. When corrected for eye colour, presence of patches, sex and parental hearing status, the heritability for deafness has been found to be 0.21 in 1996 (19), implying that there is much genetic variation. This would mean that selective breeding could decrease the incidence of congenital deafness in the population.

Nonetheless, there are other genes beside the ones associated with the phenotypical traits that contribute to the occurrence of CCSD (6). Furthermore, coat colour does not seem to contribute to the occurrence of CCSD (5-7, 19). In some other breeds than mentioned above, such as the Doberman Pincher and the Shropshire Terrier, CCSD can be found without white pigmentation. On the other hand, albinism, in which melanocytes are sufficiently present but cannot properly produce melanin, is not generally associated with deafness (7).

A study in 2013 performed in Germany (4) has found several CCSD-associated genotypes (CCSD-associated SNPs). Four of these CCSD-loci were found close to genes that are known to influence melanocyte migration and melanosome transport. Several of the CCSD-loci that were found are similar to the loci in humans associated with hearing loss, but more research is needed to fully understand the implications of this (4). It was shown in this same study that when a dog has at least one CCSD-associated SNP, the risk of developing CCSD increases. Dalmatian dogs with blue eyes seem to have (specific) CCSD-associated SNPs more often than brown-eyed Dalmatians. Furthermore, it was found that bilaterally deaf dogs generally had more CCSD-associated SNPs than unilaterally hearing dogs (4).

Because of congenital deafness in the Dalmatian breed, Dalmatian clubs in several countries have set up a breeding policy and aim to decrease the number of Dalmatian pups with CCSD (15-18). The Dutch Club for Dalmatian Dogs (NCDH) has decreed that only bilaterally hearing dogs, without blue eyes or a head spot, can be used for breeding (1, 13). They advise breeders to euthanise bilaterally deaf pups (21).

For more information about the anatomy of the ear, melanocyte migration, congenital deafness, welfare implications, the BAER test, DNA research and breeding standards, see Appendix I.

Research goal

The purpose of this study is to evaluate the effect of the currently used breeding policy of the Dutch club for Dalmatian Dogs on the cumulative incidence of deafness amongst Dalmatian pups from 1995 to 2018. A second aim is to assess strength of association between the presence of CCSD and the phenotypical traits head spot, eye colour, coat colour and sex. This study also aims to make a simple estimation of breeding value for Dalmatian sires and dams.

Materials and methods

Data on deafness in Dalmatian pups has been collected by the NCDH. These data contain information about pups born in or after 1988. Data from 1988 to 31-12-1994 is mostly incomplete, whereas data from 1995 is mostly complete. However, not all pups in the dataset born in 1995 or later have BAER test results (see Table 1). Variables in the dataset are hearing status (bilaterally hearing, unilaterally deaf or bilaterally deaf), the phenotypical traits head spot, coat colour and blue eyes, litter size and data of the parents (registration number and nationality). The hearing status is based on the result of the BAER test. The BAER test was not obligatory before 1995 and was thus not performed consistently to all pups born. Since 1995, all pups born within NCDH ought to have been BAER tested by the Board of Management of Cynological Affairs in the Netherlands (‘Raad van Beheer’), certified experts in a standardised way, as part of a new breeding policy. However, 605 of these pups (mostly whole litters, according to the NCDH all pups that have been bred by breeders not associated with the NCDH) do not have BAER test results in the dataset (see Table 1).

Table 1: BAER test results for Dalmatian pups born within NCDH before and after 1995

	Before 1995		1995 – 2018	
BAER test result	Number	Proportion	Number	Proportion
Bilaterally hearing	157	0.84	3741	0.74
Deaf	2	0.01	166	0.03
Unilaterally hearing	27	0.15	549	0.11
Unknown	0	0.00	605	0.12

Dalmatian pups born before 1995 are not analysed, because of incomplete BAER-test data (186): only the dogs that have been used to breed after 1995 that have BAER-test results. Pups without an NHSB registration number (28) and pups with a mother without an NHSB registration number (105) were excluded from the analysis, because these pups are either not Dutch or not registered to the NCDH. There were 5247 pups in the original dataset, of which 4948 have been analysed. For the analysis containing unilateral or bilateral deafness, 4362 pups have been analysed due to missing BAER test results of 586 pups.

For the analysis the variables 'blue eyes' and 'head spot' have been recoded to 'present' or 'not present'. The same has been done for coat colour, to 'black on white', 'brown on white' and 'other'. 'Other' contains the colours lemon, tricolour and unknown colour, because there were too few dogs in each of these categories to analyse those separately in an accurate way. The variable 'birth year' was given categories: '1995 to 2000', '2001 to 2006', '2007 to 2012' and '2013 to 2018', each category containing six years (though the data of the year 2018 is not complete, as only litters born in January were present in the dataset). The variable 'litter size' was also given categories: 'less than 9', '9 to 11' and 'more than 11', with an as equal as possible number of pups in the categories. This was done because of the approximately normal distribution in litter size.

Linear mixed effect logistic regression models (22) were used to analyse the outcome variable 'deaf'. The BAER test result was recoded in a variable 'deaf', consisting unilaterally or bilaterally deaf (coded 1) or bilaterally hearing (coded 0). The explanatory variables used in these models are date of birth (categorised in periods), sex, head spot, blue eyes, coat colour and litter size. The effects of the dam and sire on the hearing status of the pups have been taken into account as random effects. First a univariable logistic regression model was used to assess each of the respective risk factors separately by estimating the odds ratios. Secondly a univariable logistic regression model with random effects was used to estimate the odds ratios for each respective risk factor. Lastly a multivariable logistic regression model with random effects was used to estimate odds ratios adjusted for the presence of other variables in the model. Odds ratios and confidence intervals for the odds ratios have been calculated to determine the strength of the association between deafness and the respective risk factors.

In 61 dogs the sex was unknown. Of these dogs 60 were bilaterally deaf. To be able to include these dogs in the analysis, they were appointed a sex in a random way, using the ratio of deafness in female to deafness in male dogs (14:12). After this adjustment, the multivariable model containing random effects for sire and dam could not converge with all variables, hence, the variables 'coat colour' and 'litter size' have been left out as these are of minor interest.

The data have been analysed using R Version 3.4.2 (22).

Results

Of the 4362 pups analysed (Table 1), most were born in the period 1995 to 2000 (Figure 2). In general, there is a decrease in deafness over the years (Figure 3), from a proportion of deafness of 0.19 in 1995 to a proportion of 0.08 in 2017. A decrease in the cumulative incidence of deafness is also visible for unilateral deafness (Figure 4), but less clearly visible for bilateral deafness, due to a peak in bilateral deafness in 2015 (Figure 5).

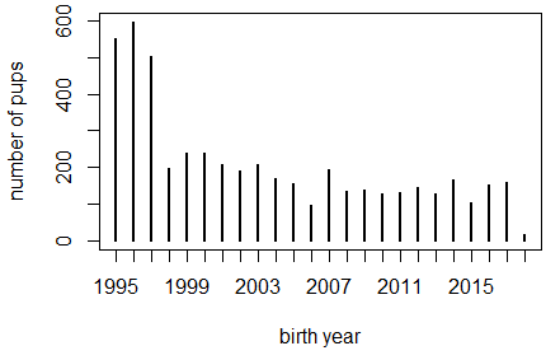


Fig. 2: Number of pups born within each birth year

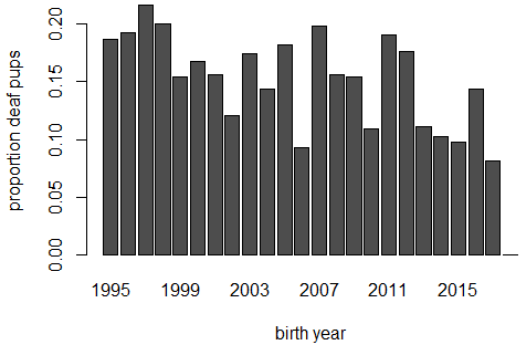


Fig. 3: Proportion of deaf pups (uni- and bilateral) within each birth year

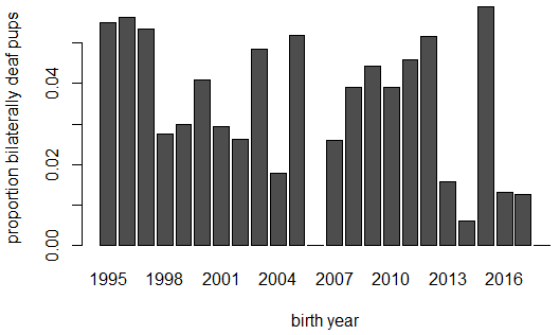


Fig. 4: Proportion of unilaterally deaf pups within birth year

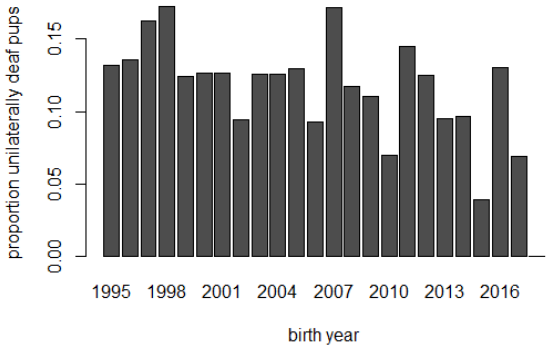


Fig. 5: Proportion of bilaterally deaf pups each within each birth year

Table 2 presents the summary of deafness in the several categories of possible risk factors. The percentage of pups unilaterally or bilaterally deaf is in the first birth period (1995 to 2000) about 19 % decreasing to 11% in the latest birth period (2013 to 2018). Deafness occurs in 16% of the female pups and in 13% of the male pups. About 4% of the pups have blue eyes and about 8% has a head spot. Most of the pups in the dataset have a Black on White coat colour (74%), Lemon and Tricolour are rare (both rounded 0%). Most pups come from a litter of 9 to 11 pups (40%).

Table 2: Cross tables and odds ratios of the univariable models associating deafness with the possible risk factors birth year period, sex, head spot, blue eyes, coat colour and litter size, using a logistic regression model.

	Percentage per category*		**Bilaterally hearing**		**Unilaterally deaf**		**Bilaterally deaf**		**Deaf (one-sided or two-sided)**		
									Odds Ratio	95% Confidence Interval	
										Lower	Upper
	%	**n**	**%**	**n**	**%**	**n**	**%**	**n**			
Birth year period											
1995 – 2000	47	(1769)	81	(1434)	14	(250)	5	(85)	1	Ref	
2001 – 2006	24	(1020)	85	(868)	12	(120)	3	(32)	**0.8**	**0.6**	**0.9**
2007 – 2012	19	(851)	83	(709)	13	(108)	4	(34)	0.8	0.7	1.1
2013 – 2018	17	(722)	89	(646)	9	(63)	2	(13)	**0.5**	**0.4**	**0.7**
Sex											
Male	52	(2248)[2]	86	(1944)[1]	11	(250)[1]	2	(54)[1]	1	Ref	
Female	47	(2053)[2]	83	(1712)	14	(291)	2	(50)	**1.3**	**1.1**	**1.5**
Unknown	1	(61)[2]	2	(1)	0	(0)	98	(60)	383.7	84.2	6793.2
Head Spot											
Not present	92	(4030)	83	(3337)	13	(530)	4	(163)	1	Ref	
Present	8	(332)	96	(320)	3	(11)	0	(1)	**0.2**	**0.1**	**0.3**
Blue eyes											
Not present	96	(4205)	84	(3532)	12	(512)	4	(161)	1	Ref	
Present	4	(157)	80	(125)	18	(29)	2	(3)	1.3	0.9	2.0
Coat Colour											
Black on White	74	(3224)	85	(2748)	13	(418)	2	(58)	1	Ref	
Brown on White	23	(1012)	86	(870)	12	(122)	2	(20)	0.9	0.8	1.2
Lemon	0	(17)	88	(15)	6	(1)	6	(1)	0.8	0.1	2.7
Tricolour	0	(1)	100	(1)	0	(0)	0	(0)	**		
Unknown	2	(108)	21	(23)	0	(0)	79	(85)	21.4	13.6	34.9
Litter size											
Less than 9	28	(1205)	82	(987)	15	(183)	3	(35)	1	Ref	
9 to 11	44	(1922)	85	(1635)	11	(217)	4	(70)	**0.8**	**0.7**	**0.96**
More than 11	28	(1234)	84	(1034)	11	(141)	5	(59)	0.9	0.7	1.1
Unknown	0	(1)	100	(1)	0	(0)	0	(0)	**		

[1] sum of percentages of deafness within a category of a risk factor adds up to 1

[2] sum of percentages per category within risk factor adds up to 1

** = proportion within factor*

Ref = Reference Category

*** = not calculated due to scarcity of data*

Results of the univariable model (Table 2) show that birth year period has a decreasing effect on the proportion of deaf pups: for the periods 2001 to 2006 (OR= 0.8, 95%CI: 0.6; 0.9) and 2013 to 2018 (OR=0.5, 95%CI: 0.4; 0.7) this is significant. Females have 1.3 times higher odds (95%CI 1.1; 1.5). to be deaf than males. Furthermore, pups with a head spot have 0.2 times the odds to be deaf than pups without a head spot, which is significantly less (OR=0.2, 95%CI: 0.1; 0.3). Blue eyes and coat colour of the pup does not seem to have a significant effect on the odds of deafness (OR=0.3, 95%CI: 0.9; 2.0). Pups from an intermediate litter size (9 to 11 pups) are less likely to be deaf than pups from small (less than 9) or big (more than 11) litter sizes. Pups from large litter sizes are equally likely to be deaf as pups from small litter sizes. The data on the litter sizes of the pups were in some cases incomplete or inaccurate (see Appendix V).

Table 3: Presence of head spot

Head spot			
Present (side unknown)	*Both sides*	*Left*	*Right*
178	21	68	66

Table 4: Presence of blue eyes

Blue eyes			
Present (side unknown)	*Both sides*	*Left*	*Right*
78	12	42	27

Table 5: Results for the univariable model associating deafness with birth year period, sex, head spot, blue eyes, coat colour and litter size respectively, including random effects for dam and sire, using a linear mixed effects logistic regression model.

	Odds Ratio	95% Confidence Interval	
		Lower	Upper
Birth year period			
1995 – 2000 (Ref)	1		
2001 – 2006	0.7	0.5	1.1
2007 – 2012	0.8	0.5	1.2
2013 – 2018	**0.5**	**0.3**	**0.8**
Sex			
Male (Ref)	1		
Female	**1.3**	**1.1**	**1.6**
Head spot			
Not present (Ref)	1		
Present	**0.2**	**0.1**	**0.4**
Blue eyes			
Not present (Ref)	1		
Present	1.3	0.8	2.0
Coat Colour			
Black on white (Ref)	1		
Brown on white	1.0	0.8	1.3
Other	**		
Litter size			
Less than 9 (Ref)	1		
9 to 11	0.8	0.6	1.01
More than 11	0.8	0.5	1.1

Ref = reference category
*** = not calculated due to imprecise estimate*

Of about half of the dogs that have a head spot (178 of the 332 dogs with a head spot, see Table 4 and 5), the side of the head spot is unknown. Of the dogs in which the side of the head spot is known (155), there are approximately equally many dogs with a head spot on the right as with a head spot on the left (42.6% and 43.9% respectively). A head spot on both sides occurs less often (13.5%). Of about half the dogs with blue eyes (78 of the 157 dogs with blue eyes, see Table 2 and 4), the side of the blue eyes is also unknown. Of the dogs in the side is known, a blue eye on the left side is most common (51.9%). A blue eye on the right side occurs in 33.3% of the dogs with blue eyes and on blue eyes both sides in 14.8%.

Table 5 shows the results for a univariable model that includes the random effects of the parents. In this model, only birth year period 2013 – 2018 odds of deafness were significantly decreased compared to the odds of deafness in the first birth period. Female pups are 1.3 times more likely to be affected by congenital deafness than male pups (OR=1.3, 95%CI: 1.1; 1.6) and pups with a head spot are 5 times less likely (OR=0.2, 95%CI: 0.1; 0.4) to be deaf than pups without a head spot. Blue eyes, coat colour and litter size do not have a significant effect on the odds of deafness in this model.

Table 6: Summary of results for the multivariable model associating deafness with birth year period, sex, head spot and blue eyes, including random effects of the mother and father, using a linear mixed effects logistic regression model

	Odds Ratio	**95% Confidence Interval**	
		Lower	**Upper**
Birth year period			
1995 – 2000 (Ref)	1		
2001 – 2006	0.8	0.5	1.1
2007 – 2012	0.8	0.5	1.3
2013 – 2018	**0.5**	**0.3**	**0.8**
Sex			
Male (Ref)	1		
Female	**1.3**	**1.1**	**1.5**
Head spot			
Not present (Ref)	1		
Present	**0.2**	**0.1**	**0.4**
Blue eyes			
Not present (Ref)	1		
Present	0.9	0.5	1.4

Ref = reference category

In a multivariable model that includes the random effects for parents and the recoded variable 'sex' , the last birth year period (2013 to 2018) (OR=0.5, 95%CI: 0.3; 0.8) and a present head spot (OR= 0.2, 95% CI: 0.1; 0.4) significantly decrease the odds of deafness (Table 6). Female pups are significantly more likely to be deaf than male pups (OR=1.3, 95%CI: 1.1; 1.5). Blue eyes do not have a significant effect on the odds of deafness.

The estimated random effects for father and mother from the multivariable model are presented in figure 6 and 7 respectively. The random effect gives an indication for the risk for deafness that a pup has due to the mother and/or father based on the cumulative incidence of deafness in their prodigy. A positive value indicates a higher risk than average of having deaf prodigy, a value of approximately zero indicates an average risk (mean risk for deafness in the population) and a negative value indicates a lower risk than average. A list of random effect estimates for sires and dams born since 2013 is presented in Appendix III.

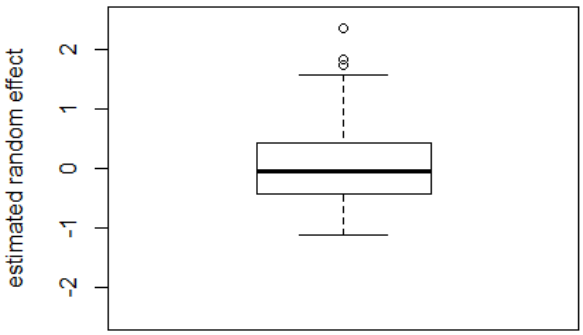


Fig. 6: Boxplot of the estimated random effects on the log odds scale for sires

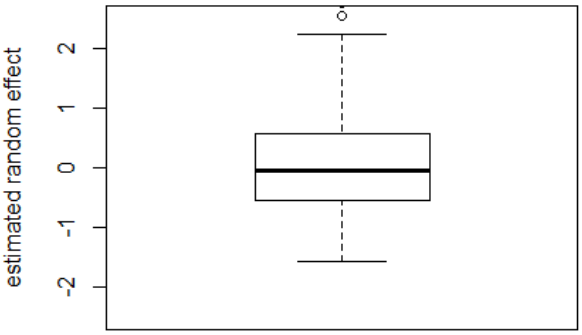


Fig. 7: Boxplot of the estimated random effects on the log odds scale for dams

Using the random effects of the dams and sires, estimations could be made for the probability of deafness of (potential) pups born from a certain combination of a sire and a dam. Pups without a head spot born from a combination of high risk parents have more than 30% risk to be deaf where as the risk for a a pup is less than 3% when both parents have low risk estimates (Appendix IV). Pups with a headspot have between 9 and 12% risk born from both high risk parents and less than 1% risk to be deaf born from both low risk parents.

Discussion

In this study, a large dataset was used to study the cumulative incidence of deafness over time and to assess the strength of association between several factors and deafness in Dalmatian dogs. Overall, there is a decrease in the cumulative incidence of deafness over the years from approximately 19% to 10%. A similar trend has been found for unilateral and bilateral deafness separately (see Appendix II). In all models, this decrease was found to be significant for the last birth year period (2013 to 2018). Female pups are more likely to be born deaf than male dogs. A head spot has a clear decreasing effect on the odds of deafness: a head spot decreases the odds to be affected by congenital deafness by approximately 80%. This effect was also found to be significant in all models. Blue eyes do not have a significant effect on the odds of deafness in any of the models.

The decrease in cumulative incidence of deafness among Dalmatian pups was expected due to the BAER test that became obligated from 1995 as part of the new breeding policy (13, 23). In this research, a decrease in cumulative incidence was found in each of the birth year periods, which would indicate a positive effect of the new breeding policy to breed only with dogs that have a BAER test result that shows that they are bilaterally hearing. However, the positive effect could have been bigger, had a head spot been allowed according to the breeding policy. According to the FCI-standard, patches on the head are 'not desirable'(24). Deafness however is seen as a 'disqualifying trait'. Allowing the presence of a certain degree of a head spot in dogs used for breeding would lead to a further decrease the cumulative incidence of CCSD in Dalmatians (the 'disqualifying trait'), which is most sought after.

Sex has been found to have a significant effect on the odds of deafness in some studies, but in others it has only been found to be a trend (not significant) (1-7, 19). In this study, the cumulative incidence in females is 3%

higher than in males. A large number of dogs was included in this study (the entire Dutch population of Dalmatian dogs registered to the NCDH), which could be the reason why it was found to be significant in this study and not in other studies that have used less dogs and may have a selection bias. The 61 dogs in this research of which the sex was unknown and that have been appointed a sex randomly, may also have had an effect on the estimated odds ratio. It could be that the sex ratio in these dogs, of which 60 were deaf, did not match the ratio found among dogs of which the sex was known (information bias). This could mean that females are even more likely to be deaf than males, but it could also mean that there is no significant sex predisposition.

Head spot and blue eyes are both known in literature to decrease the odds of deafness (1, 5-7, 19). Head spot was found to significantly decrease the odds of deafness in this research, but no significant effect was found for blue eyes. This could be due to the low prevalence of blue eyes or due to the correlation between head spot and blue eyes as presence of blue eyes occur more often in dogs without head spot (1-4, 6, 7, 19, 20). Blue eyes point to a lack of melanocytes in the region of the head, whereas a head spot points to an abundance of melanocytes in the region of the head. If there is an abundance of melanocytes in the region of the head, the chance that the melanocytes have also migrated to the inner ear is bigger than when there is a lack of melanocytes in the region of the head. The pathogenesis of congenital deafness consists of the lack of migration of melanocytes to the inner ear. This corresponds with the head spot decreasing the odds of deafness, which was found in this study.

In Australian Cattle dogs, the side of the head spot or blue eyes were found to have a significant effect on the side of deafness (in case of unilateral deafness). However, in this study, this association could not be tested due to limited data on the side of the head spot and/or blue eye(s).

In the estimation of the random effects of the dams and sires, it shows that some dams and sires are less likely (estimate lower than 0) to have deaf pups than the average parent, whereas other dams and sires are more likely (estimate larger than 0) to have deaf pups than the average parent. That parental hearing status has an effect on the hearing status of their offspring corresponds to what can be found in literature (1-4, 6, 7, 19). Because it is a relatively small breed (about 20 litters a year), there are relatively few Dutch dogs that are used for breeding. This leads to a small genetic base among dams, as not many dams are imported from abroad. Sires are often foreign, which leads to a broader genetic base. However, there is often little known about the incidence of deafness among the prodigy of foreign sires. These foreign sires may affect (decrease or increase) the risk of deafness among Dutch Dalmatian pups.

The Dutch Club for Dalmatian Dogs (NCDH) advises breeders to euthanise bilaterally deaf pups, as they claim that bilaterally deaf dogs have a substantially impaired welfare (21). Deaf dogs would have a higher chance of getting injured and get startled more easily, which would result in unwanted (anxious) behaviour. However, euthanising deaf pups can be very straining for both breeders and veterinarians, especially because in most cases, these pups are otherwise healthy. And although it is likely that the welfare of the animal is affected by bilateral deafness, there is no evidence to be found in literature on how much deafness impairs animal welfare. This underlines the importance of a breeding policy that aims to prevent deafness in Dalmatian pups.

Although the entire Dutch population of Dalmatian dogs registered to the NCDH was included in this research, some data were missing or inaccurate. Before 1995, the BAER test was not obligated yet, but after 1995 there are still BAER test results missing, mostly from entire litters in the first few years after 1995. In personal communication with the NCDH it was mentioned that this could partly be due to the contribution of breeders outside of the NCDH that did not perform a BAER test on these pups. It might also be explained by perhaps not all breeders immediately adapting to the new rule of performing a BAER test (BAER test results not passed on to the NCDH, consciously or not; seen as an unimportant test; high costs for the breeder and eventually the buyer; ignorance; etc.). Later, the NCDH mentioned that these pups, of which the BAER test results are missing, were all bred by breeders not associated with the NCDH. Striking is that most of the pups with missing BAER test results are born in the first 3 to 4 years after the BAER test became obligated as part of the breeding policy.

This specific lack of data could have led to an information bias in this study. Furthermore, the sometimes incomplete or inaccurate data on the litter sizes of the pups could be implicative for the quality of the dataset. The inaccuracy could partly be explained by how the pups are registered: the number of pups born within a nest can be higher than the number of pups that have been BAER-tested due to mortality within the first eight weeks (see Appendix V).

The last model (see Table 6) could not be extended with the variables coat colour and litter size. In the other models, a small effect of litter size on the odds of deafness was found and no effect of the coat colour was found. No evidence has been found in literature for associations between litter size and coat colour and CCSD (5-7, 19).

To further decrease the odds of deafness DNA techniques can be used to breed based on genetic diversity. DNA samples could be taken from each dog with a breeding purpose to carefully match sires and dams based on genetic variation. This could lead to more genetic variation within the population, which could lead to a further decline of the incidence of CCSD in Dalmatian pups. In the future, test based on the SNPs associated with deafness may be developed. In that case, genetic research could be used to screen dams and sires for these SNPs and match a sire and dam accordingly, depending on the number and genetic location of SNPs in the dogs' genomes.

Conclusion

Since the new breeding program of the NCDH (including for each pup to be BAER-tested), the cumulative incidence of deafness has decreased from approximately 19% to 10%. For unilateral and bilateral deafness separately, a significant decrease was also found. The breeding policy of the NCDH has shown to be effective. This effect could have been bigger had dogs with (a certain degree of) a head spot been allowed to breed.

To further decrease the cumulative incidence of deafness in the Dalmatian breed in the future, other measures could be taken. In order to choose a dam and sire that are genetically most distinct from each other, genetic tests could be used to determine the genetic variation (inbreeding coefficient) between the two. Breeding value of the parents could also be used to minimise the risk of deaf prodigy. In the future, tests based on the SNPs associated with deafness may be developed and dams and sires used for breeding could be matched based on a SNP profile. Another measure that could be taken to decrease the cumulative incidence of CCSD in Dalmatians would be to allow dogs with (a certain degree of) a head spot to breed, as dogs with head spots have significantly lower odds at deafness than dogs without.

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Appendix I: Hearing, congenital deafness and BAER testing

Hearing

The ear is divided into the external ear, middle ear and inner ear. The external ear in the dog contains the pinna and the external ear canal or auditory meatus. The shape of the pinna varies widely among dog breeds (25, 26).

The middle ear contains the tympanum (eardrum), the ossicles, the auditory (Eustachian) tube and, behind the tympanum, the tympanic cavities (see figure 8). The middle ear is lined with secretory epithelium, which creates a slight negative pressure. The tympanum is kept in place by the tensor tympani muscle, innervated by the mandibular branch of the trigeminal nerve. Directly behind the tympanum lie the ossicles – malleus, incus and stapes, inside the tympanic cavity. The auditory tube forms a connection between the middle ear and the nasopharynx, which keeps the pressure inside the middle ear approximately equal to the pressure in the atmosphere (25-27).

The inner ear contains the cochlea, the semi-circular canals and the vestibulum, embedded in tubular and spiral cavities in the petrous temporal bone, called the labyrinth (see figure 8). The cochlea is a very long organ that is coiled like a snail shell. It has two membranes in it, the basilar membrane and Reissner's membrane (vestibular membrane). These membranes both cover the length of the cochlea and divide it into scalae (see figure 9). The middle scala contains endolymph, a fluid with a unique ionic composition, containing mostly potassium (K) and sodium (Na). The outer scalae contain perilymph, which has a more negative electric potential than endolymph, as it contains more sodium than potassium. On top of the basilar membrane, which forms the floor of the middle scala, lies the organ of Corti. This is a hair cell receptor organ. On top of it lies the tectorial membrane, a gel-coated ridge that is anchored to the spiral limbus (1, 25, 26).When a sound wave – air vibration – hits the ear, it is first funnelled by the external ear into the ear canal. From there, it travels to the tympanic membrane (eardrum). There, the wave is transferred to the middle ear, where the ossicles pass on the vibration while amplifying it so that it becomes strong enough to move through the endolymph of the

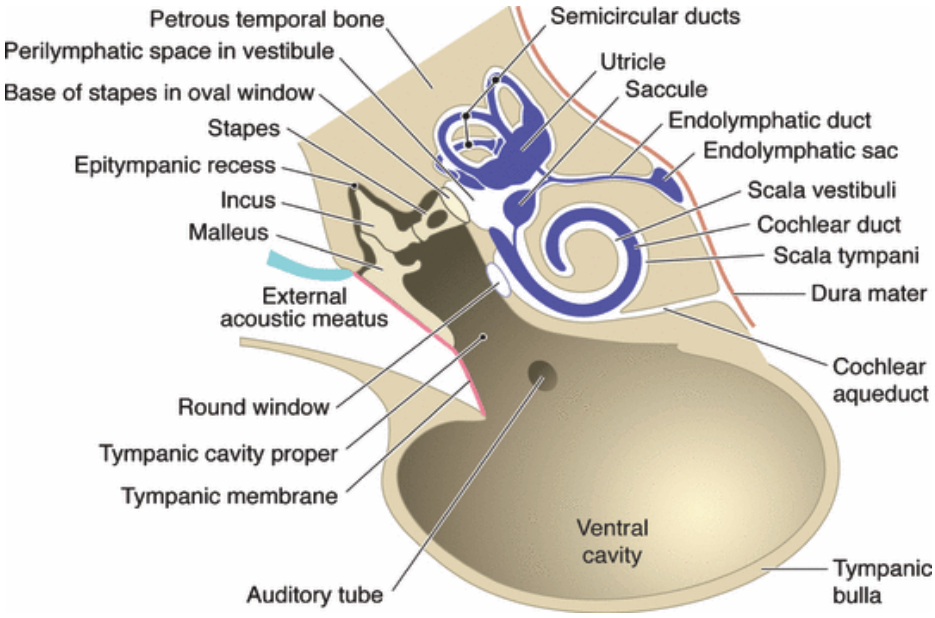


Fig. 8: The external acoustic meatus, middle ear and inner ear (31)

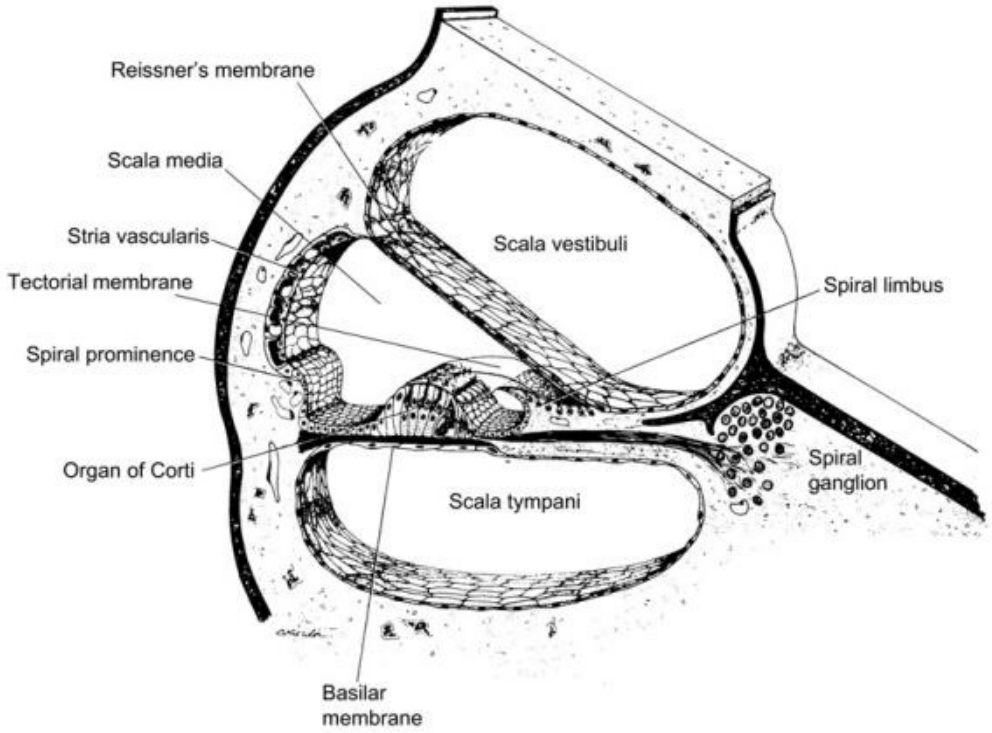


Fig. 9: Cross-section through the mammalian cochlea (30)

cochlea. Through the oval window – a membrane between the middle and inner ear, the vibration is passed on the auditory part of the inner ear: the cochlea. In there, the vibration causes the basilar membrane with the organ of Corti on it to move against the tectorial membrane. Because the tectorial membrane is anchored and cannot move, the hairs of the organ of Corti bend when they are pushed against it. Once they bend, the hair cells change their release of neurotransmitters to the auditory nerves. In their turn, these nerves change their action potential firing rates. These action potentials are passed on to the brain (1, 25, 26).

The frequency of the soundwave determines where in the cochlea the basilar membrane moves: each part of the cochlea represents a specific pitch of sound. At the spot where the basilar membrane moves and hair cells bend, specific auditory nerves are affected and change their action potential firing rate. In the brain, this is translated into the pitch of the sound. The loudness of the sound is determined by the amplitude of the sound wave. A higher amplitude causes the basilar membrane to move more fiercely, which leads to a bigger change in action potential firing rate of the concerned auditory nerves. This is translated to a louder sound in the brain (26).

Melanocytes

Melanocytes are cells that can produce melanin. They are commonly known as pigment cells in the skin, hair follicles and eyes, but are also present in other parts of the body, including the cochlea (5, 28).

During early embryological development, a neural plate is formed on the dorsal side of the embryo. This plate consists of ectoderm. This plate gives rise to the neural folds, which elevate and close dorsally to form the neural tube. While this happens, cells of the lateral border or neural crest (also called the crest of the neuroepithelium) actively migrate from the neurectoderm to the underlying mesoderm. These neural crest cells migrate to a specific place depending on their region of origin. Cells from the trunk neural crest, which extends from the sixth somite to the most posterior somites, migrate in three main ways. One of these ways is

the dorsolateral pathway, in which the cells migrate between the ectoderm and the somites. The migrating cells enter this space as melanoblasts, later they differentiate to melanocytes (5, 27). From there, they migrate further to their final destinations.

One of the destinations that melanocytes migrate to, is the cochlea. There they play a role as intermediate cells in the stria vascularis (see figure 9), which occupies a part of the lateral wall of the cochlear duct. The strial intermediate cells are important for normal cochlear function, as they help maintain the right ionic milieu by using their Na^+/K^+-ATPase and potassium channels. The stria vascularis secretes endolymph, giving the middle scala of the cochlea a potential of +90mV in mammals. This potential is called the endocochlear potential, or EP. This potential is necessary for the cochlea to work properly and thus for the animal to hear (5, 28).

Congenital deafness in Dalmatians

Dalmatian pups with congenital deafness show cochlear degeneration after 2 to 4 weeks of age (1, 5). Before degeneration of the cochlea, the inner ear seems to be normal in structure and function (1). It is not entirely clear what causes the strial degeneration, but the absence of melanocytes seems to be a main factor. When melanocytes do not migrate to the cochlea, they cannot differentiate into intermediate cells and perform their function in the stria vascularis. The right ionic milieu that is needed in order for the cochlea to function cannot be maintained when there are no intermediate cells, thus the stria vascularis degenerates. In some cases, a lack of melanocytes or disturbed migration is visible externally in fur and eye colour, but a disturbed migration of melanocytes to the cochlea does not necessarily mean a disturbed migration to other places (1, 4, 5).

In the process of cochlear degeneration, an initial destruction of the lateral wall of the middle scala (stria vascularis) of the cochlea has been found: cochlea-saccular degeneration of type Scheibe. This is followed by degeneration of the organ of Corti, which starts with vacuolisation of the external sulcus cells (3). After the degeneration of the organ of Corti, Reissner's membrane collapses and finally the cochlear duct collapses as well (1, 3, 4). At 1 to 2 months of age, this degeneration is already well advanced. The hair cells, supporting cells and lastly the pillar cells in the cochlea degenerate and eventually disappear. The number of spiral (cochlear) ganglion cells decreases with age: after 36 months, the number of cells has decreased by 50% (3).

The degeneration and disappearance of these structures in the inner ear lead to a perception deafness: sound waves transferred to the cochlea can no longer be translated into neural activity. This type of deafness in dogs generally has a genetic background and is called Canine Congenital Sensorineural Deafness (CCSD), also known as Canine Hereditary Sensorineural Deafness (CHSD) (1, 4-7). It is found in several dog breeds, but mostly in English setters, Bull terriers, Whippets, Australian cattle dogs, English Cocker spaniels, Catahoula Leopard dogs, Border collies, Jack Russel terriers and above all Dalmatian dogs (4-7). In 1992 in the USA, 21.6% of Dalmatian dogs showed unilateral deafness, whereas 8.1% showed bilateral deafness (overall 29.7%) (8). Verhorevoort found that in the Netherlands in the period 1995-1998, of the NDHC Dalmatian pups, 15.9% showed unilateral deafness and 4.9% bilateral deafness (overall 20.8%) (1). In 2008, the prevalence of deafness in Dalmatian dogs (unilateral and bilateral) in the USA was 29.9%, in Switzerland 16.5% and in the Netherlands 16.8% (20).

It is generally assumed that CCSD is a genetic disease, but which genes exactly are responsible for the development of CCSD has been unclear for a long time. The mechanisms leading to deafness have not completely been clear either, and a lot of research is still being done on this subject (4, 6). A study in 2002 found that female Dalmatians and blue-eyed Dalmatians were more often affected than other Dalmatians (6), though other studies could not find a sex effect (5). The absence of patches – a spot of dark fur that is already visible at birth – was associated with a higher prevalence of CCSD in several studies (5, 6). Generally, there seems to be a defect in genetic control of melanoblasts differentiation and/or migration, due to which the melanocytes do not reach the stria vascularis in the cochlea (5).

Several studies have found significant associations between eye colour, presence of patches, sex and parental hearing status, and deafness. These associations have mostly been studied in Dalmatian dogs (1-4, 6, 7, 19).

The link between blue eyes and CCSD may be explained by the 'extreme white piebald' allele s^w (5, 6, 20). The *S* locus, associated with the distribution pattern of pigmented and white spots, has four different alleles. The associated gene is called the MITF gene (Microphthalmia-associated Transcription Factor). The *S* allele is dominant and is non-spotted, or 'self'. The s^i allele produces Irish spotting: generally, only a few white spots/areas on the thorax, face, head or feet. The s^p allele stands for piebald spotting, which gives more white spots and/or bigger white areas than the s^i allele: dogs with the s^p allele may have white spots on their limbs as well. Lastly, the s^w allele, the extreme white piebald allele, gives the greatest extent of white pigmentation, leaving only small spots on the coat pigmented (5, 7). Dalmatian dogs are most likely all homozygous for this allele, but there is much variation in expression level among Dalmatian dogs. The presence of patches has been associated with a lower expression of the s^w allele (5-7, 20).

The *M* locus, a pigmentation locus known as the 'merle' locus, is another locus that plays a role in hereditary deafness. Dogs homozygous for the recessive allele *m* have a uniform pigmentation, whereas dogs with *Mm* are dappled: they have body areas that are fully pigmented and areas that are pale or even white. Homozygous merle – *MM* – produces an almost completely white coat and is associated with deafness, blindness and sterility. Heterozygous dogs may vary in their likelihood of being deaf, depending on the present alleles for other genes associated with pigmentation (1, 6, 7). Bull terriers, Australian cattle dogs, English Cocker spaniels, Catahoula Leopard dogs, Border collies can be heterozygous and are thus more likely to be deaf that other breeds. When corrected for eye colour, presence of patches, sex and parental hearing status, the heritability for deafness has been found to be 0.21 in 1996 (19), implying that there is much genetic variation. This would mean that selective breeding could have negative effect on the incidence of congenital deafness in the population.

Nonetheless, there are other genes beside the ones associated with the phenotypical traits that contribute to the occurrence of CCSD (6). Furthermore, coat colour does not seem to contribute to the occurrence of CCSD (5-7, 19). In some other breeds than mentioned above, such as the Doberman Pincher and the Shropshire Terrier, CCSD can be found without white pigmentation. On the other hand, albinism, in which melanocytes are sufficiently present but cannot properly produce melanin, is not generally associated with deafness (7).

A study in 2013 performed in Germany (4) has found several CCSD-associated genotypes (CCSD-associated SNPs). Four of these CCSD-loci were found close to genes that are known to influence melanocyte migration and melanosome transport. Several of the CCSD-loci that were found are similar to the loci in humans associated with hearing loss, but more research is needed to fully understand the implications of this (4). It was shown in this same study that when a dog has at least one CCSD-associated SNP, the risk of developing CCSD increases. Dalmatian dogs with blue eyes seem to have (specific) CCSD-associated SNPs more often than brown-eyed Dalmatians. Furthermore, it was found that bilaterally deaf dogs generally had more CCSD-associated SNPs than unilaterally hearing dogs (4).

Several studies have found significant associations between eye colour, presence of patches, sex and parental hearing status and deafness. These associations have mostly been studied in Dalmatian dogs (1-4, 6, 7, 19), but have been found in the other breeds susceptible to CCSD as well (1, 6, 7). A study on CCSD in Australian Cattle dogs found higher odds of females to have CCSD (1.6-1.9 times higher than for males), an association between hearing status of the parents and hearing status of the pups and an association between the presence of patches and the development of CCSD (for all $p < 0,05$). In unilaterally deaf dogs with a head spot, a significant link between the side of the head spot and the side of deafness has not been found (5).

There seem to be some differences between the different breeds in which factors play the most in important role in the development in CCSD, but more research is needed to verify this and, if this is indeed the case, which factors are most important for which breed.

Deafness and welfare

Deafness in dogs is taken as a serious welfare issue by Dalmatian Dog Clubs internationally (13, 15-18). Deaf dogs have a higher chance of getting injured, for example by cars that they cannot hear coming, and get startled more easily, which can result in unwanted (anxious) behaviour. They need specialized training from an expert and can sometimes become quite noisy, as they cannot hear themselves. However, there is no evidence that a deaf dog's welfare is significantly impaired. Bilaterally deaf Dalmatian pups are euthanised as part of the policy of several Dalmatian clubs, which is based on welfare implications (21, 23).

However, it can be very straining for a breeder to have to put down their deaf pups that are otherwise healthy (21). It can also be difficult for the veterinarian involved. Veterinarians can refuse a request for euthanasia, but will in many cases comply to the owners wishes, regarding deaf Dalmatian pups: as the breeder, they are often concerned about what might happen to the animal otherwise (29).

BAER test

A BAER test (Brainstem Auditory-Evoked Response test) can be performed to assess hearing in dogs (3, 12) (see figure 10). This test can determine unilateral or bilateral deafness (qualifies an ear as 'deaf' or 'not deaf'). This test is relatively easy and more reliable than a test based on behaviour, which can be influenced by visionary stimuli, vibratory stimuli and mental state. In the BAER test, repetitive acoustic stimuli are given. Activity in the cranial nerve VIII (Vestibulocochlear nerve) and the auditory part of the brainstem is measured at the same time to assess the reaction of the brain to the acoustic stimuli (3, 12, 25). Three small needles are subcutaneously inserted into the dog's head to measure response: a recording electrode, a reference electrode and a ground electrode. Headphones or ear inserts can be used to give the acoustic stimulations. The stimulations are given at various frequencies, intensities and rates of delivery (number of stimulations per second).

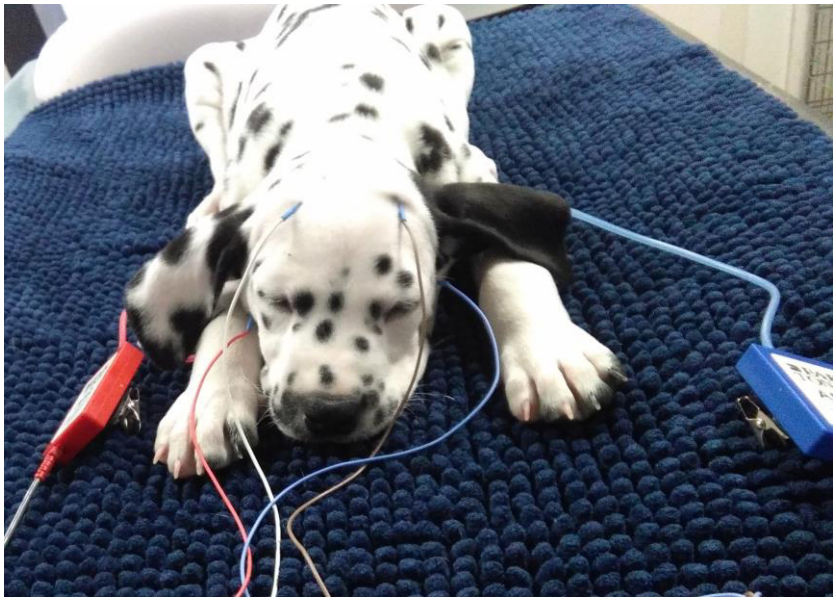


Fig. 10: Dalmatian pup undergoing a BAER test

Several areas in the brainstem respond to the auditory stimuli, which is made visible by the various amplitudes in time in the BAER test result (a line graph that shows the amount of activity measured versus time). However, when a dog is deaf due to cochlea abnormalities, recognisable amplitudes are completely absent (25).

Although the BAER test is used in dogs, its clinical relevance has not been validated: the sensitivity and specificity of this test in dogs have not yet been determined. Furthermore, a masking noise should be used to prevent a cross-over effect (14, 25), but the precise effective levels of masking noise in dogs are unclear. This is especially important in dogs with unilateral deafness (12, 14). Gonçalves et. al. (2008) found that using a contralateral masking noise of 30 dB could largely prevent the cross-over effect (14).

This test is used by the NCDH to determine the hearing status of pups. In the Netherlands, this test is performed by professionals acknowledged by the Board of Management of Cynological Affairs in the Netherlands (Raad van Beheer) (13). The test however is used worldwide, although performed differently in each of these countries (3, 12, 14).

DNA research

The genealogy of a pup can give an indication of the chance whether or not that pup is deaf (1). However, more accurate results can be retrieved from using DNA techniques such as PCR and electrophoresis, which could determine the presence of genes that lead to deafness (1). At this point this could prove difficult, because not everything regarding the genetic background of hereditary deafness is clear yet. CCSD-associated SNPs may be used in the future to determine the presence of CCSD (unilaterally or bilaterally) at an early stage of the pup's life, but further research is needed before this can be brought into practice (4). However, DNA techniques can already be used on the sire and dam: choosing a sire and dam based on the most genetic variation between them may lead to decreased odds at deafness in the pups.

Breeding standards

In the Netherlands, the NCDH has set rules for breeding Dalmatian pups regarding genetic diversity and prevention of hearing problems (1, 13, 23). A bitch cannot be bred with her grandfather, father, brother, son or grandson. Furthermore, a certain parent combination can only be used twice. The stud dog must be at least 12 months old and can be bred successfully a maximum of 5 times per calendar year. Both parents must have participated in exposition regulated by the Board of Management of Cynological Affairs in the Netherlands (Raad van Beheer) and/or the International Cynological Federation (Fédération Cynologique Internationale; FCI). Foreign stud dogs can be used, but must also comply to the rules and standards set for Dutch Dalmatian breeding dogs or rules and standards similar to these (23).

Only bilaterally hearing dogs without (partially) blue eyes or a headspot may be used to breed. A small coloured edge on the ear however is allowed (1, 13, 23). Other disqualifying traits are a coat type other than shorthair and a coat colour other than black on white or brown on white (23). Since 1995, the BAER test has become a standard test for each Dalmatian pup born in the Netherlands registered by the NCDH. This test is performed at 8 weeks of age and is performed in a standardised method. This test was needed in order to exclude unilaterally hearing dogs from the breeding programme. The effect of the consistent use of this test is still unclear (1, 13). Bilaterally deaf pups are euthanised. The latest version of the Dutch breeding regulations, containing the regulations mentioned above, has been effective since the first of Januari 2014 (23).

Besides the Netherlands, there are guidelines for breeding Dalmatian dogs in various other countries. These rules are partly set to try to decrease the prevalence of deafness in Dalmatian pups, though they are not evenly strict everywhere (15-18). The effectiveness of the breeding programs on the incidence of CCSD is unclear. However, there seems to be a clear effect of allowing blue eyes: the prevalence of deafness in Dalmatian dogs in the USA, where blue eyes are allowed, is relatively high (29.9% in the USA vs. 16.8% in the Netherlands in 2008) (20).

Appendix II: Unilateral and bilateral deafness

Unilateral deafness

Table 7: Summary of results for the univariable model associating unilateral deafness with birth year period, sex, head spot, blue eyes, coat colour and litter size, including random effects of the mother and father, using a linear mixed effects logistic regression model

	Odds Ratio	95% Confidence interval	
		Lower	Upper
Birth year period			
1995 – 2000 (Ref)*	1		
2001 – 2006	0.6	0.4	0.9
2007 – 2012	0.8	0.5	1.2
2013 – 2018	0.3	0.1	0.6
Sex			
Male (Ref)	1		
Female	1.4	1.1	1.7
Head spot			
Not present (Ref)	1		
Present	0.3	0.1	0.5
Blue eyes			
Not present (Ref)	1		
Present	1.2	0.7	1.9
Coat Colour			
Black on white (Ref)	1		
Brown on white	1.0	0.8	1.3
Other	0.2	0.0	1.8
Litter size			
Less than 9 (Ref)	1		
9 to 11	0.7	0.6	0.9
More than 11	0.7	0.6	0.9

** Ref = reference category*

Table 8: Summary of results for the multivariable model associating unilateral deafness with birth year period, sex, head spot and blue eyes, including random effects of the mother and father, using a linear mixed effects logistic regression model

	Odds Ratio	95% Confidence interval	
		Lower	Upper
Birth year period			
1995 – 2000 (Ref)*	1		
2001 – 2006	0.8	0.5	1.2
2007 – 2012	0.9	0.6	1.3
2013 – 2018	0.6	0.4	0.95
Sex			
Male (Ref)	1		
Female	1.4	1.1	1.7
Head spot			
Not present (Ref)	1		
Present	0.3	0.1	0.5
Blue eyes			
Not present (Ref)	1		
Present	1.3	0.8	2.0

** Ref = reference category*

Bilateral deafness

Table 9: Summary of results for the univariable model associating bilateral deafness with birth year period, sex, head spot, blue eyes, coat colour and litter size, including random effects of the mother and father, using a linear mixed effects logistic regression model

	Odds Ratio	95% Confidence interval	
		Lower	Upper
Birth year period			
1995 – 2000 (Ref)*	1		
2001 – 2006	0.6	0.4	0.9
2007 – 2012	0.8	0.5	1.2
2013 – 2018	0.3	0.2	0.6
Sex			
Male (Ref)	1		
Female	1.1	0.8	1.6
Head spot			
Not present (Ref)	1		
Present	0.1	0.0	0.3
Blue eyes			
Not present (Ref)	1		
Present	0.5	0.1	1.4
Coat Colour			
Black on white (Ref)	1		
Brown on white	1.1	0.7	1.8
*Other***	**		
Litter size			
Less than 9 (Ref)	1		
9 to 11	1.2	0.8	1.8
More than 11	1.6	1.1	2.5

** Ref = reference category*
*** = not calculated due to imprecise estimate*

Table 10: Summary of results for the multivariable model associating bilateral deafness with birth year period, sex, head spot and blue eyes, including random effects of the mother and father, using a linear mixed effects logistic regression model

	Odds Ratio	95% Confidence interval	
		Lower	Upper
Birth year period			
1995 – 2000 (Ref)*	1		
2001 – 2006	0.6	0.3	1.2
2007 – 2012	0.8	0.4	1.7
2013 – 2018	0.3	0.1	0.7
Sex			
Male (Ref)	1		
Female	1.2	0.8	1.7
Head spot			
Not present (Ref)	1		
Present	0.1	0.0	0.7
Blue eyes			
Not present (Ref)	1		
Present	0.2	0.1	0.8

**Ref = reference category*

Summary

The cummulative incidence of both unilateral and bilateral deafness decreases over the years, significantly for the periods 2001 to 2006 and 2013 to 2018. Females are 1.4 times for unilateral deafness or 1.1-1.2 times for bilateral deafness more likely to be deaf then males. Blue eyes do not seem to have a significant effect on unilateral or bilater deafness. Having a head spot significantly decreases the odds of for unilateral and bilateral deafness. The litter size does not have a significant effect effect on the incidence of bilateral deafness, but it does have a significant effect on unilateral deafness: a higher number of pups in the litter seems to decrease the odds of unilateral deafness.

Appendix III: Estimated random effects of parents of pups born after year 2012

In Tables 11 and 12, the random effects for the dams and sires that have had pups after year 2012 are shown. A positive value indicates a higher risk than average of having deaf prodigy, a value of approximately zero indicates an average risk and a negative value indicates a lower risk than average. In this case, the more negative the value, the more beneficial regarding the risk of having deaf prodigy.

Table 11: Estimated random effects of mothers of pups born after year 2012

Registration number					**Mothers**
Estimated random effect					
NHSB25	**NHSB25**	**NHSB26**	**NHSB26**	**NHSB26**	**NHSB26**
0.53284840	1.13715924	-0.57136545	0.38159955	0.35759594	0.33327828
NHSB27	**NHSB27**	**NHSB27**	**NHSB27**	**NHSB27**	**NHSB27**
1.74570437	0.24533048	-1.06946406	-0.19263673	0.81136020	-0.95571025
NHSB27	**NHSB27**	**NHSB27**	**NHSB27**	**NHSB27**	**NHSB27**
0.84585793	-0.78429624	-0.43986116	-0.27354224	0.57725075	0.91092572
NHSB27	**NHSB27**	**NHSB27**	**NHSB27**	**NHSB27**	**NHSB27**
-0.19363335	-0.33891393	-0.71434439	-1.60675286	-0.11142761	-0.55426114
NHSB27	**NHSB27**	**NHSB27**	**NHSB27**	**NHSB28**	**NHSB28**
-0.84109603	0.21612478	0.56187341	0.32472605	-0.37802975	-1.33434217
NHSB28	**NHSB28**	**NHSB28**	**NHSB28**	**NHSB28**	**NHSB28**
-0.13316289	-0.55166730	-0.43704579	0.34241658	0.13480442	-0.48131778
NHSB28	**NHSB28**	**NHSB28**	**NHSB28**	**NHSB28**	**NHSB28**
0.33440686	-0.66413519	-0.74554196	1.07953711	-0.56744624	0.46385336
NHSB28	**NHSB29**	**NHSB29**	**NHSB29**	**NHSB29**	**NHSB29**
-0.47361078	0.11940568	-0.36812582	-0.55903540	-0.78196116	-0.05973976
NHSB29	**NHSB29**	**NHSB29**	**NHSB29**	**NHSB29**	**NHSB29**
-0.77358950	0.09000131	0.20467992	-0.42231459	0.87157489	0.52957162
NHSB29	**NHSB29**	**NHSB29**	**NHSB29**	**NHSB29**	**NHSB29**
-0.47379810	-0.23052326	-0.56050879	-0.51538855	-0.55209559	-0.32422763
NHSB30					
0.68985589					

Table 12: Estimated random effects of fathers of pups born after year 2012

Registration number				**Fathers**
Estimated random effect				
AKCNM89	**CLP/DA**	**CLP/DA**	**DK111**	**DKK60**
-0.085734842	0.086050910	-0.141418827	0.074285059	-0.391829971
DKK81	**DZB28**	**DZB29**	**HR120**	**KCRAQ03**
-0.256234049	-0.105281453	-0.141791551	-0.212397169	-0.125027249
LOSH11	**N0359**	**N0373**	**NHSB25**	**NHSB26**
-0.112532815	0.084309193	0.136762203	0.141516566	-0.033686131
NHSB26	**NHSB27**	**NHSB27**	**NHSB27**	**NHSB27**
0.054682667	0.008708271	0.083680524	-0.149652327	-0.062467721
HSB279	**NHSB28**	**NHSB28**	**NHSB28**	**NHSB28**
0.087881362	0.084968801	0.302317124	-0.230028927	0.097578674
NHSB28	**NHSB28**	**NHSB28**	**NHSB28**	**NHSB28**
-0.002059000	-0.446183154	-0.363433678	-0.084582676	0.515446701
NHSB29	**NHSB29**	**NHSB29**	**NHSB30**	**RKF305**
-0.376124622	0.112515080	-0.137175634	-0.106832652	0.003116062
S44942	**SE22343**	**SE48214**	**VDH/CDF3**	**VDH/DV**
0.146026754	-0.167487327	-0.132389947	-0.110265114	-0.095629945
VDH/DV	**VDH/DZ**	**VDH/DZB**	**VDH/DZB**	
0.390174100	-0.107916823	0.193339304	-0.064912765	

Appendix IV: Probabilities of deafness for certain parent combinations

By using the estimated random effects for the dams and sires, the probabilities of deafness of the pups of certain dam and sire combinations has been calculated (see Table 13). Two parents with both a high risk (both having a positive random effect), two parents with an average risk (both having a random effect of approximately 0) and two parents with a low risk of having deaf prodigy (both having a negative random effect) have been used. Table 13 shows that the lower the risk of the parents of having deaf prodigy – or the lower the random effects of the parents, the lower the probability that their pups are born deaf. A male pup has a smaller probability to be born deaf than a female pup and a pup with a head spot has a considerably smaller probability to be born dead than a pup without a head spot. This is consistent with what was found in de univariable and multivariable mixed effects models (see results section).

Table 13: Probabilities of deafness with or without the risk factors 'head spot' and 'blue eyes' for certain parent combinations

Head spot	**Father**	**Mother**	**Probability of deafness**	
			Male	**Female**
Not present	VDH/DV (high risk)	NHSB25 (high risk)	30.5%	36.7%
Not present	NHSB28 (average risk)	NHSB27 (average risk)	9.0%	11.7%
Not present	NHSB28 (low risk)	NHSB27 (low risk)	1.8%	2.4%
Present	VDH/DV (high risk)	NHSB25 (high risk)	9.3%	12.0%
Present	NHSB28 (average risk)	NHSB27 (average risk)	2.3%	3.0%
Present	NHSB28 (low risk)	NHSB27 (low risk)	0.4%	0.6%

Appendix V: Litter size according to the dataset vs. frequency of Nest IDs

In the original dataset, there is a column indicating the litter size for each pup. To check if the litter size according to the dataset matches the number of pups of that litter in the dataset, each nest has been appointed a Nest ID, containing the registration number of the mother, the registration number of the father and the date of birth. Figure 11 shows the correlation between the litter size according to the dataset and the litter size according to the frequency of Nest IDs and indicates that for some litters, the litter size mentioned in the dataset does not match the frequency of the Nest ID. There are some litters that contain more pups than the litter size mentioned in the dataset and some litters that have a bigger litter size according to the dataset than there are pups with that Nest ID in the dataset. This could partly be explained by how the pups are registered: the number of pups born within a nest can be higher than the number of pups that have been BAER-tested due to mortality within the first eight weeks.

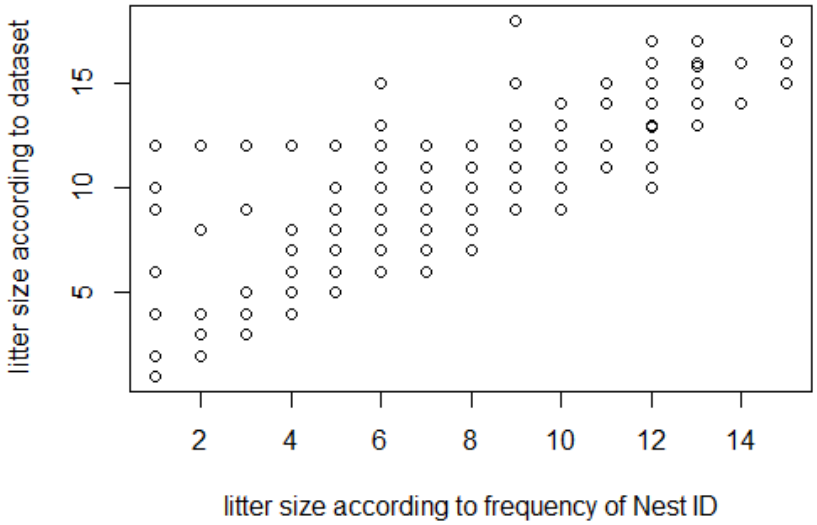


Fig. 11: The litter size according to the dataset plotted against the calculated litter size based on the frequency of Nest IDs (registration number sire, registration number dam, birth date of the litter)

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