Reproductive demographics of the Dutch Kooiker Dog

Consequences of selective breeding in 1990-2010
on the global population

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Contents

A note on the coverpage:

The artwork shows a Kooiker dog with a family painted by Jan Steen. The original painting is owned by the Royal Picture Gallery Mauritshuis, The Hague.

Reference: Steen J. The Artist's Family. 1665. Downloaded from: http://www.the-athenaeum.org/art/display_image.php?id=208045

Abstract

In the last two decades, von Willebrand disease (VWD) and Hereditary Necrotising Myelopathy (HNM), two genetic diseases that affect the Dutch Kooiker dog, have become a major concern within the Kooiker dog breeder communities. As a consequence, breeders implements restrictive breeding program in an effort to eliminate the diseases. It is hypothesised that the restrictive selection program resulted in an inbreeding depression due to reduced number of breeding animals. The aim of this retrospective study was to investigate the reproductive demography of the worldwide Kooiker dog population in 1990-2010 and the frequency of VWD and HNM. The reproductive characteristics investigated were: effective population, inbreeding rate and coefficients of the litters, dams and sires, litter size, the number of litters, breeding frequency of selected animals, peripartal deaths, maternal parity, maternal and paternal age at the birth of the litters. In addition, his study aimed to explore the lifespan of the Kooiker dog born in this period. Data from the Association of the Dutch Kooiker dog, comprising a total of 807 sires, 1232 dams, and 15398 puppies worldwide in 1990-2010, were analysed. Descriptive statistics were used to describe the population. The worldwide absolute effective breeding population had steadily increased since 1990. Ne was 817 animals in the five-year-interval. The mean breeding frequency for both sires and dams were approximately 4 times in every five-year-interval studied. However, the maximum breeding frequency showed some important variations. The highest rate of inbreeding (ΔF) was observed in Norway (2.03%), because of a small effective population. ΔF was lowest in the Netherlands (0.19). The number of litters increased with time. The worldwide average litter size (excluding peripartal deaths) had also increased and was 5.12 ± 2.13 pups (maximum 12, n = 169 litters) in 2010. But, more medium to medium-large sized litters (4.00 to 7.00 pups) were born, when compared with a study in 1994. The high number of bitches that were bred only once may have caused the decline in litter size. The global mean inbreeding coefficient of the litters decreased from 0.07 ± 0.05 (minimum 0.00, maximum 0.26) in 1990 to 0.02 ± 0.02 (minimum 0.00, maximum 0.12) in 2010. This decline in the mean inbreeding coefficient may have been caused by the increase in the number of breeding animals. Available data suggest that the restrictive breeding program implemented in 1990-2010 has not caused an inbreeding depression in the global Kooiker dog population.

Introduction

Selective breeding has always been associated with purebred dogs. Studies have shown that selective breeding can eventually put enormous pressure on the sustainability of a population [1–4]. Because selective breeding excludes dogs that are actually available for mating and allows only the selected few to breed, it essentially creates a group of highly related individuals. The mating of such individuals produces homozygous inbred offspring with less vigor. Thus, selective breeding ultimately leads to inbreeding. As inbreeding creates less vigorous individuals, continued inbreeding over many generations can eventually create a population bottleneck. Bottlenecks in population size augment the homozygosis of a population and so, reduce genetic diversity [5–8]. A population bottleneck usually leads to inbreeding depression [4,7], which has detrimental effects on the components of fitness associated with the reproductive capacities of an organism [1,2,6,9]. In addition, hereditary diseases and developmental defects occur more frequently by a population with a high number of homozygous individuals [9].

Despite breeders' effort to make optimal use of the selected animals, inbreeding will manifest rapidly, when there are only a limited number of foundation animals available. A similar circumstance occurred by the Dutch Kooiker dog. The Kooiker dog is a dog breed from the Netherlands, which was originally used to lure wild ducks into traps so that these could then be caught by hand. Although the origin of the Dutch Kooiker dog goes back to at least the sixteenth century [10], the current worldwide population is descended from the 15 dogs used to reestablished this breed in the early 1940s. The external appearance was the first and most important selection criterion in this early period to set consistent phenotypic features in the breed [11]. Twenty years from the start of the reestablishment, several Kooiker dogs suffered from Hereditary Necrotizing Myelopathy (HNM) in the early 1960s. The breed was confronted with type III von Willebrand disease (VWD) in the 1990s. Unfortunately, the emergence of HNM and VWD stimulated only stronger selection of breeding animals to eliminate these diseases.

Hereditary Necrotizing Myelopathy is a familial neurological disorder that affects the Kooiker dog. Affected dogs showed signs of progressive paresis starting from the hind limbs at a young age (3 – 12 months) and they died or were euthanized before reaching the age of 20 months. A segregation analysis revealed that HNM has an autosomal recessive mode of inheritance. Parents of affected dogs were non-symptomatic carriers of HNM, and both sexes were equally affected [12]. The disease became a major concern for the Kooiker dog breeders and the elimination of HNM from the breed by excluding carrier dogs emerged as one of the top selection criteria. Because of this elimination size of the breeding stock declined, the inbreeding level and its negative consequences were expected to rise. Therefore, Mandigers et al. (1994) studied the population and described the relationship between various reproductive traits in Kooiker dogs born between 1956 and 1990 in the Netherlands. The study showed that despite the necessary selection, the breed had improved. Mandigers et al. observed an increase in mean litter size and a decrease in the inbreeding level of the litters born within that period [11].

However, also in the early 1990s, excessive bleeding tendency after a trauma and epistaxis were frequently seen in Kooiker dogs. A study revealed that this susceptibility to bleeding is caused by the type III VWD, the most severe variant of VWD. As with HNM, this disease also has an autosomal recessive inheritance pattern. Both male and female dogs are equally affected. In addition, the carriers of the disease are clinically normal. After the revelation of the familial aspect of the disease, breeders were advised to exclude affected dogs and carriers in their family from breeding program [13]. When the mutated von Willebrand gene was identified and DNA analysis became available in 1998, the Association of Dutch Kooiker dog (VHNK) made the test mandatory for all dogs in the breeding program [14]. This resulted in a further drain on the number of available breeding animals.

In the last twenty years, other diseases that have or may have a genetic base started to emerge within the population; a motive for further selection. Knowing that strong selection is applied within the Kooiker dog population and that inbreeding level depends on the effective population size [3], high coefficient of inbreeding (F in equations or COI) in the present population and decreased effective population size are expected. It is hypothesized that the restrictive breeding program implemented to eliminate VWD and HNM from the breed resulted in an inbreeding depression due to reduced number of breeding animals. As mentioned above, one of the first components of fitness affected by inbreeding is fertility, such as the total number of offspring born, viability of the offspring, litter size, and the number of litters [1,2,9]. Therefore, the objective of this study was to investigate and report the reproductive demography of the worldwide Kooiker dog population in the period 1990 to 2010, the carrier and health status of these Kooiker dogs in relation to HNM and VWD. In addition, this study aimed to explore the lifespan of the Kooiker dog born in this period.

Materials and methods

Data

This retrospective study is based on the records of Kooiker dogs provided by the VHNK. The club's complete registry in document format contains records of Kooiker dogs going back to 1942 (the base population) and dogs from the Netherlands and Austria, Belgium, the Czech Republic, Estonia, Japan, Italy, the USA, the UK, Norway, Switzerland, Sweden, and Finland. The VHNK listed both registered dogs and puppies unregistered by the local kennel clubs, which were born out of registered parents. The complete registry consisted of the Kooiker dogs' pedigree registration number, their gender, birth date, parents' registration number, date and cause of death, and litter size. The records also included the dogs' status regarding (hereditary) diseases including VWD and HNM, and the clinical signs showed by the dogs. In addition, the VHNK also managed pedigree, test results for VWD and HNM, and the Wright's inbreeding coefficients [1] over five generations in Dogbase version 5.6. Information kept in Dogbase was converted into a Microsoft Excel file for this study.

Population

Only records from Kooiker dogs born from 1990-2010 were analysed in this present study. The mating of 807 sires and 1232 dams worldwide resulted in 2922 litters, consisted of 15398 puppies (7985 males, 7380 females, and 24 of unknown gender) during the studied period. These numbers exclude puppies that died during the peripartum period. Peripartal death comprised stillbirths, pups that lived only one day and pups listed as 'dead' without additional information on the time of death and cause.

Characteristics studied

The characteristics investigated in this study were: the effective population, inbreeding rates and coefficients of the litters, dams and sires, litter size, the number of litters, breeding frequency of selected animals, maternal parity, and paternal and maternal age at the birth of the litters. This study also analysed peripartal mortality, the number of carriers and dog affected by VWD or HNM born each year, and the general life expectancy of Kooiker dogs.

Statistical analysis and methods

All records were integrated and analysed in OpenOffice Calc version 3.4.0. Tables and graphs were also created in Calc. The database and data subsets were generated in MySQL. Descriptive statistics were used to analyse the characteristics and only available records were analysed.

The actual number of dams and sires in the population represented the *absolute* effective population. The effective population size (N_e) was calculated using the following equation [2,15]:

$$Ne = \frac{4 * Nm * Nf}{Nm + Nf}$$

In the above equation, N_m is the number of selected males (sires) and N_f is the number of selected females (dams). Because the generation interval of pedigreed dogs is approximately 5 years [15], the data (1990-2010) for calculating the effective population were grouped into five 5-year periods, with the period 1986-1990 forming the first period. Breeding frequency denotes how often a sire or dam produced a litter. The results were represented in number of litters. The following equation [2,16] was used to calculate the inbreeding rate (ΔF) per generation:

$$\Delta F \approx \frac{1}{8Nm} + \frac{1}{8Nf}$$

ΔF is also related to Ne and can also be calculated with this formula [3]:

$$\Delta F = \frac{1}{2Ne}$$

Peripartal deaths were excluded from litter size and mortality analysis. Peripartal mortality was analysed separately. Ages of the parents at the birth of the litter and ages at death were measured in days from the dates of birth of the dogs and divided by 365 days to represent the results in years. Ages at death of Kooiker dogs born in 1970-1989 were also calculated to compare with the mean lifespan of those born in 1990-2010. In order to study the effect of VWD and HNM on the population, test results and terminologies used in the registry to describe clinical signs were standardised and classified. Before the DNA analysis for VWD type III in Kooiker dogs became available in 2004, immunoassays for the von Willebrand factor (VWF) antigen or ELISA was used to determine the VWD-carrier status (free, obligate or possible carrier) of these dogs. ELISA is a quantitative method, where the concentration of antigens in obligate carriers and normal dogs overlaps. To analyse the data in this study, recorded immunoassay test results were categorised according to the outcome of Slappendel's investigation [17]. Kooiker dogs with a VWF antigen concentration between 1.6% and 89% were classified as (possible) carriers. When a dog underwent both DNA analysis and ELISA, the DNA test result superseded that from the immunoassay method. In contrast, the HNM gene is not yet identified. In the case of HNM, dogs were classified as carrier, affected by HNM or free. Grouping was based on the age and cause of death (confirmed with necropsy or unconfirmed), clinical signs, the HNM status of the offspring, or information from Kooiker dog owners. 'Free' dogs included dogs, which reached the age of 24 months or older and never showed any neurological signs as reported by the owners.
In analysing the subpopulations, countries that had less than 50 litters in the entire studied period were grouped together as 'other countries'. These countries were Austria, Belgium, the Czech Republic, Estonia, Italy, Japan, the UK and the USA.

Results

An overview of the total number of breeding animals and litters born in the studied period is given in table 1. The Netherlands had the largest Kooiker dog subpopulation, with the highest number of litters. Large subpopulations of Kooiker dog were also found in Denmark, Finland, and Germany. The subpopulation in Italy, the Czech Republic, and Estonia were the smallest.

	Number of				VWD		HNM	
Country	Litters	Pups	Dams	Sires	Carrier* (dogs)	Affected (dogs)	Carrier (dogs)	Affected (dogs)
Austria	11	37	5	4	-	-	ND	ND
Belgium	22	112	12	15	-	-	-	-
Czech Rep.	2	14	2	2	-	-	-	-
Denmark	247	1364	98	71	19	-	-	-
Estonia	1	6	1	1	ND	ND	ND	ND
Finland	164	749	94	86	-	-	-	-
Germany	196	1011	80	93	-	-	7	5
Italy	2	6	1	2	ND	ND	-	-
Japan	14	37	9	8	-	-	ND	ND
Netherlands	1887	10127	742	335	68	19	19	11
Norway	79	446	31	26	5	-	-	-
Sweden	136	708	77	81	2	-	-	-
Switzerland	91	467	41	54	-	-	-	-
UK	38	174	23	18	2	1	-	-
USA	32	131	16	11	-	-	ND	ND

Table 1 Overview of the Kooiker dog subpopulations (excluding peripartal deaths) and thefrequency of VWD and HNM. - = No carrier and/or diseased dogs observed. ND = No data.

Effective population

The worldwide Kooiker dog effective breeding population showed a steady annual increase. The number of breeding animals had doubled from 1990 to 2010 (Fig. 1). There were only 95 sires and 216 dams in 1986-1990 and in 2006-2010, there were 269 sires and 541 dams. N_e of the worldwide Kooiker dog population in 2006-2010 was 871 animals (table 2). Separate analysis of the absolute effective population of each country showed the same trend in most countries. Obvious changes in the size of the breeding stock occurred in the Netherlands. The number of sires in the Netherlands reached its peak in the period 1996-2000 (135 sires) and had dropped ever since. In the period 2006-2010 (92 sires), there were almost as many sires as back in 1986-1990 (88 sires). Similarly, the number of dams in the Netherlands also suffered a decline since the period 1991-1995 (Fig. 2).

The mean breeding frequency of the effective population stayed stable at approximately four times for the sires and twice for the dams in each of the five-year-periods studied. However, the maximum breeding frequency showed some extremes. One sire fathered 30 litters in 2001-2005, consisted of 102 puppies and a total of 43 litters (172 puppies) in his lifetime. In 2006-2010, three sires produced more than 20 litters per sire (378 puppies together). The high maximum breeding frequency of sires was caused by matings with dams from outside the country's border. Thus, litters from these sires were distributed among several neighbouring countries. Similarly, a few dams were bred more than five times, with a maximum breeding frequency of eight times, in almost all of the five-year-periods.

The rate of inbreeding, ΔF, was highest in Norway (2.03%) in 2006-2010, because of the low number of breeding animals available in Norway. The Dutch subpopulation had the lowest ΔF (0.19%). The Netherlands had the largest absolute effective population with a ratio between sires and dams of 2:5.

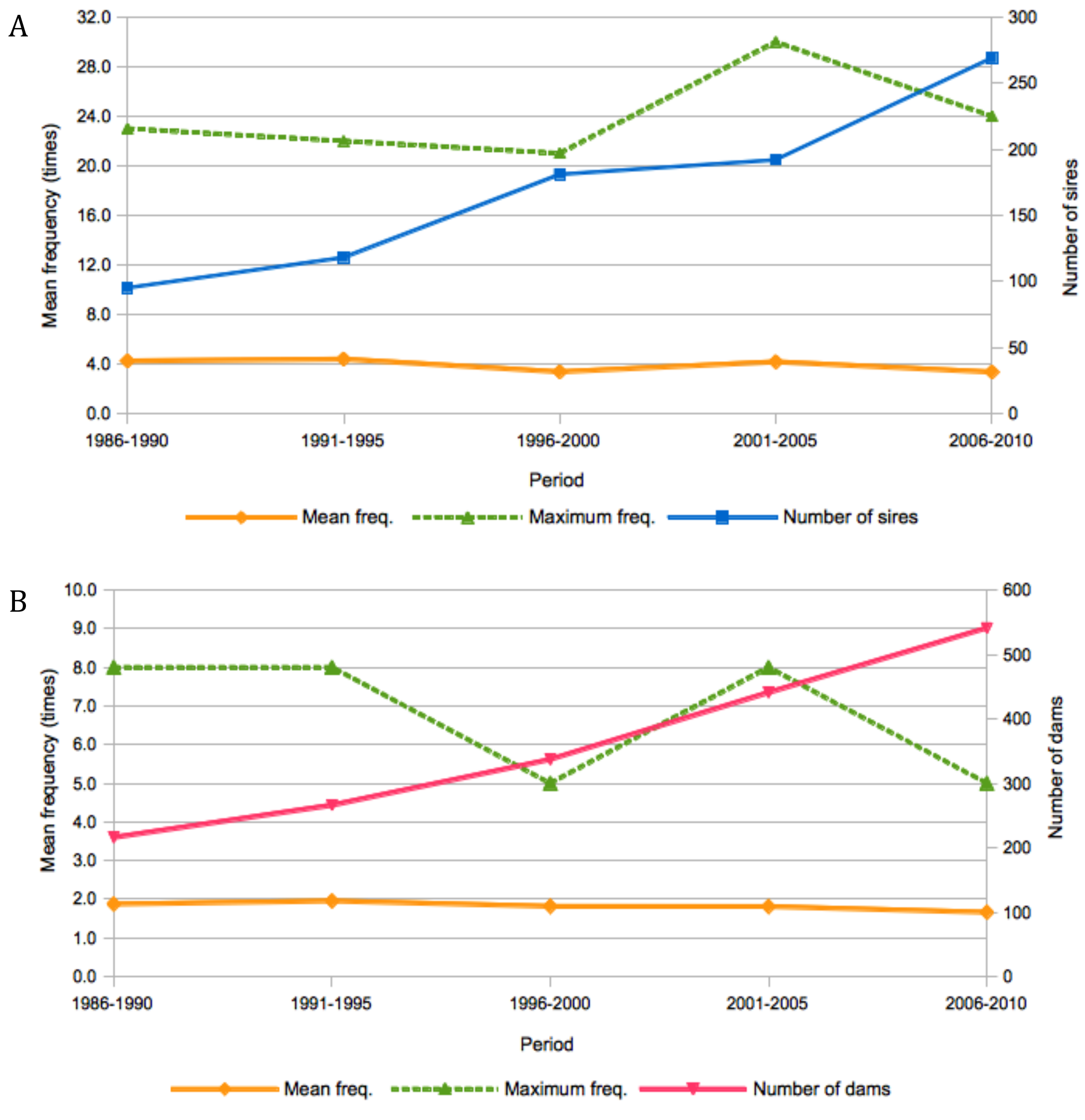

Figure 1 Absolute worldwide effective population and breeding frequency of sires (A) and dams (B) per five-year-period. The number of sires and dams are plotted on the right Y-axis. The mean and maximum breeding frequency are plotted on the left Y-axis.

	Number of			
Country	Sires	Dams	N_e (animals)	ΔF (%)
Denmark	39	43	81	0.61
Finland	56	66	121	0.41
Germany	62	67	128	0.39
Netherlands	92	220	259	0.19
Norway	10	16	24	2.03
Sweden	48	58	105	0.48
Switzerland	32	33	64	0.77
Worldwide	367	536	871	NC

Table 2 Rate of inbreeding (ΔF), absolute and estimated (N_e) effective population size per country in 2006-2010. The worldwide ΔF was not calculated (NC), because only few breeding animals mated with sires or dams from outside their own subpopulation.

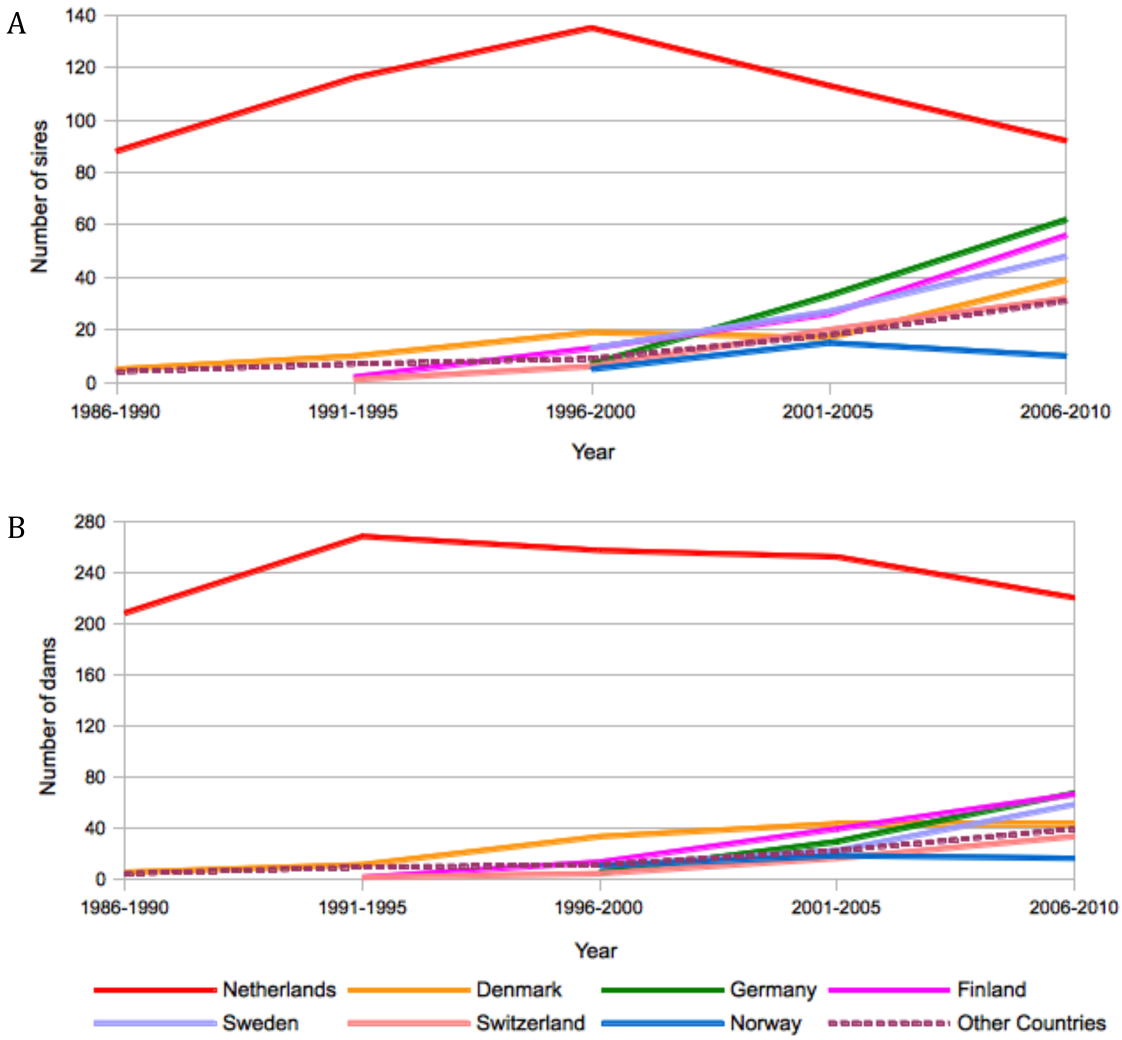

Figure 2 Number of sires (A) and dams (B) in the subpopulations per five-year-period. Note the decline in the Dutch breeding stock.

Litter size, number of litters, and the coefficient of inbreeding

The worldwide average litter size (excluding peripartal deaths) increased from 4.75 ± 2.28 pups in a litter (maximum 11, n = 95 litters) in 1990 to 5.12 ± 2.13 pups (maximum 12, n = 169 litters) in 2010. The highest mean litter size was observed in 2000 (mean 5.61 ± 2.04 pups, maximum 10, n = 125 litters) (Fig. 3). In all countries, except Switzerland, the mean litter size remained relatively stable in the medium and medium-large sized range (4.00 to 7.00 pups) in 1990-2010. Since 1997, the Swiss breeding animals had produced large litters (average > 8.00 puppies each year).
In the global population of Kooiker dog, the proportion between larger (≥ 7 pups), medium (4-6 pups), and smaller (1-3 pups) litters fluctuated from year to year. Despite these fluctuations, a subtle increase in the number of smaller and medium sized litters was observed from 2004 onward. Contrary to the worldwide mean litter size, the number of litters increased with time (Fig. 4). Subpopulation analysis showed that all countries, except the Netherlands, displayed the same trend. The number of litters in the Netherlands showed a steady decline since 2001 (111 litters). Only 57 litters were born in 2010, which was unexpectedly lower than in 1990 (91 litters).

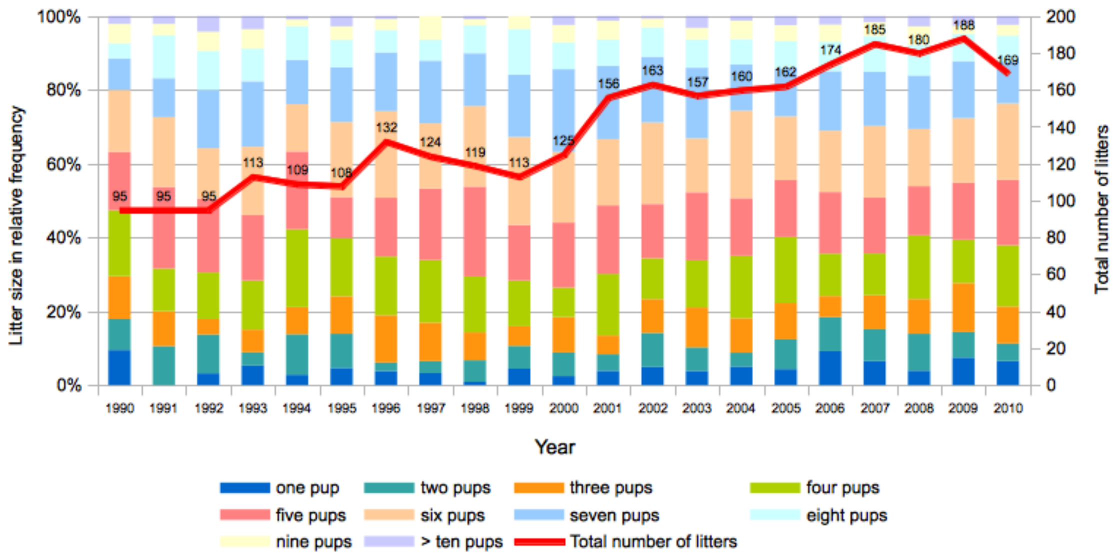

Figure 3 Variations in the annual litter size of the worldwide population and the number of litters from 1990 to 2010. Variations in litter size (excluding peripartal deaths) each year is presented in relative frequency. Increased number of small and medium sized litters was born in the last quarter of the studied period. The number of litters increased with time. Numbers over the line indicate the number of litters born each year.

Although the average litter size stayed stable, the global mean F of the litters decreased from 0.07 ± 0.05 (minimum 0.00, maximum 0.26) in 1990 to 0.02 ± 0.02 (minimum 0.00, maximum 0.12) in 2010. The mean F of the sires and dams in the studied period also declined. The mean F of the sires was 0.06 ± 0.04 (minimum 0.02, maximum 0.16) in 1990 and 0.02 ± 0.02 (minimum 0.00, maximum 0.11) in 2010. The dams' F was 0.06 ± 0.05 (minimum 0.00, maximum 0.26) in 1990 and 0.02 ± 0.02 (minimum 0.00, maximum 0.13) in 2010 (Fig. 3). Analysis of the subpopulations showed that the trend for the several country's litter mean F differed from the worldwide mean F. Decreasing trend in the litters' mean F was found in the larger subpopulations. In contrast, the mean F of the litters in Germany and Switzerland slightly increased in the fourth quarter of the studied period. Large fluctuations in the litters' mean F were apparent in the Norwegian and smaller subpopulations.

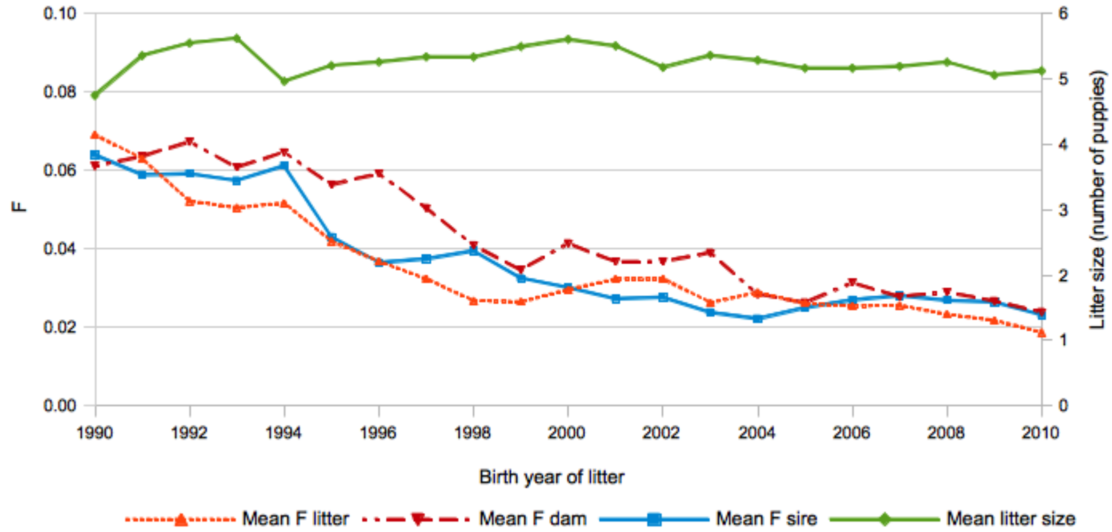

Figure 4 Mean inbreeding coefficient (F) of the litters, dams, and sires set against the mean litter size. The mean F is plotted on the left Y-axis and the mean litter size is plotted on the right Y-axis.

Peripartal mortality

The number of peripartal deaths steadily increased from 0 in 1990 to 44 deaths in 2010; a 5.08 percent increase. Stillbirths (excluding other deaths of one-day-old puppies) comprised 47.6 percent of the peripartal deaths in 2010. This signified a 2.5 percent increase in stillbirths from 1990-2010 (Fig. 5).

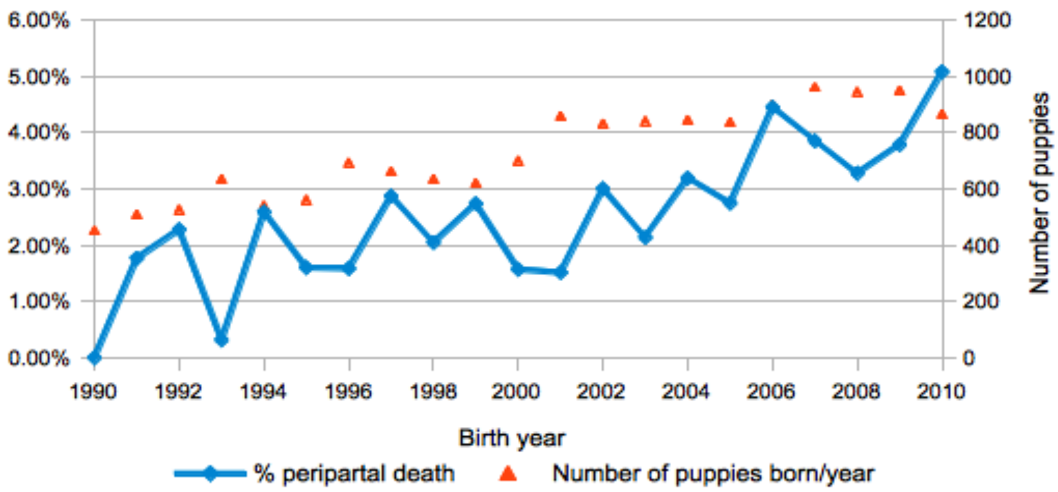

Figure 5 Annual percentages of peripartal deaths. The number of peripartal deaths increased with time. Peripartal mortality is displayed as percent of the number of puppies born each year and plotted on the left Y-axis. The number of puppies born each year is plotted on the right Y-axis.

Parity & age

The mean litter size increased with increasing parity. However, the mean litter size started to decrease gradually after the fourth parity of the dams. The maximum litter size decreased sharply with higher parity. Fifty dams were used more than four times in the studied period. The mean age of the bitches at the birth of different litter sizes was 4.3 ± 1.95 years (minimum 1, maximum 11), while the mean age of the sires was 4.2 ± 2.16 years (minimum 1, maximum 13). The average age of the dams remained stable when set against various litter sizes, but the decline in the maximum age of the dams at whelping was evident. Four to six-year-old dams produced the largest litters with 12 pups. The mean and maximum age of the sires showed similar patterns.

Frequency of VWD and HNM

An overview of the number of VWD and HNM carriers and affected dogs is given in table 1. Information on the VWD-status came from Denmark, Finland, Germany, the Netherlands, the UK, Norway, and Sweden. From 284 records available, 7.0 percent of the cases was identified as affected, 33.8 percent as obligate carriers, and 56.3 percent was classified as possible carriers. The remaining dogs were free of VWD. The Netherlands and Denmark had the highest number of both obligate and possible carriers (Fig. 6). In contrast, affected Kooiker dogs were only observed in the Dutch subpopulation and in the UK. However, no affected dogs were born after 1994 and the UK had only one case of VWD in Kooiker dog back in 1997. The majority of obligate carrier dogs were born before 2000, but the last recorded VWD carriers were born in 2005 in the Netherlands.

Reports (n = 1702) regarding HNM originated from Belgium, the Czech Republic, Denmark, Finland, Germany, the Netherlands, Norway, Sweden, Switzerland, and the UK. HNM affected 1.53% of these dogs and 0.94% was carriers. However, all carriers and affected dogs were found only the Netherlands and Germany (Fig. 7).

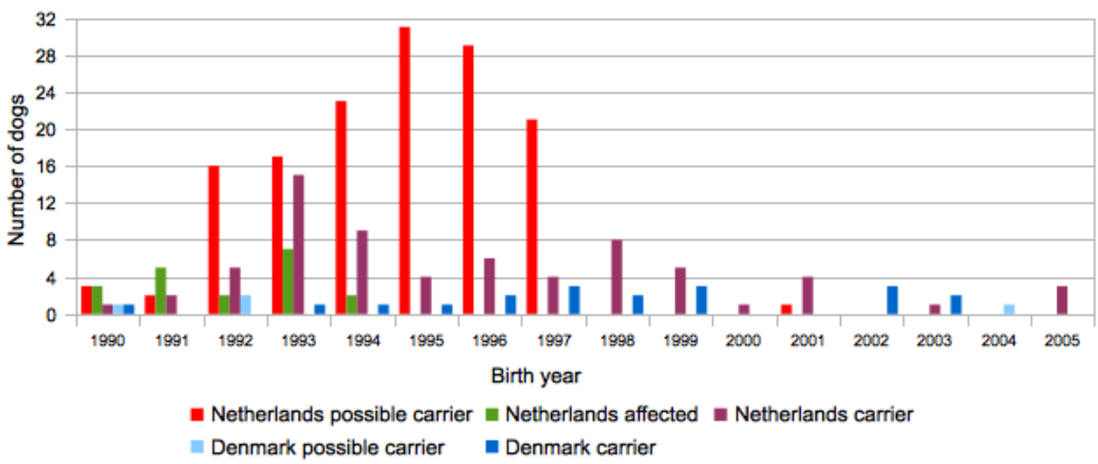

Figure 6 Frequency of von Willebrand disease in Kooiker dogs in the Netherlands and Denmark based on results of DNA analysis and immunoassays (ELISA). The last dog affected by VWD was born in 1994 in the Netherlands and no obligate carriers were born after 2005. Possible carriers are dogs with plasma von Willbrand factor antigen concentration between 1.6% and 89%. ELISA cannot differentiate a carrier from a normal dog if its vWF antigen concentration falls within this range.

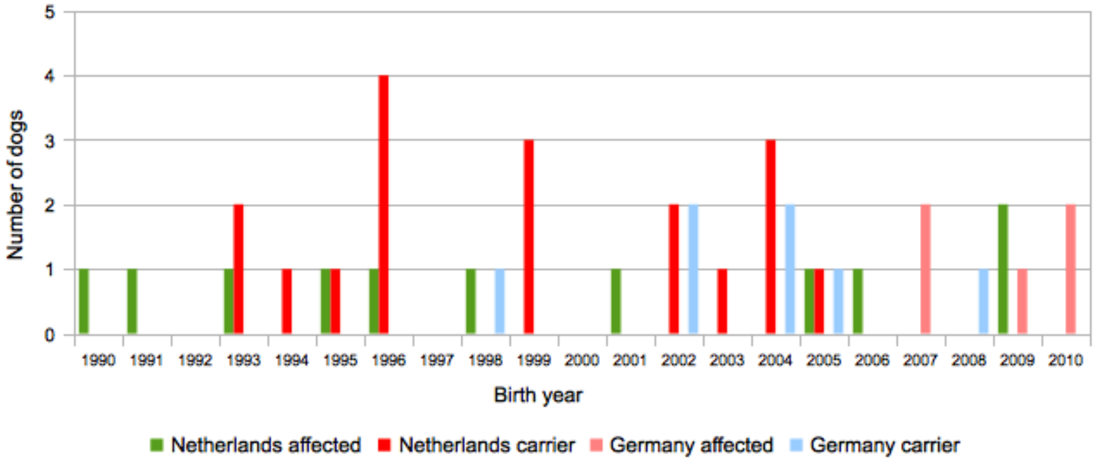

Figure 7 Frequency of Hereditary Necrotising Myelopathy in Kooiker dogs in the Netherlands and Germany. The number of animals affected by HNM remained low over the years.

Life expectancy

A total of 1215 dates of death from 1970-1989 and 1677 records from 1990-2010 were available for analysis. The average life expectancy of Kooiker dogs born in 1990 was 10.95 ± 5.02 years (maximum 19, n = 115 dogs) and had decreased ever since (Fig. 8b). A gradual decline was observed from 1990-1996, followed by a sharp fall. The mean age at death in 2000 was 6.00 ± 3.62 years (maximum 11, n = 52 dogs). By comparison, Kooiker dogs born in 1970-1989 seemed to live longer (Fig. 8a).

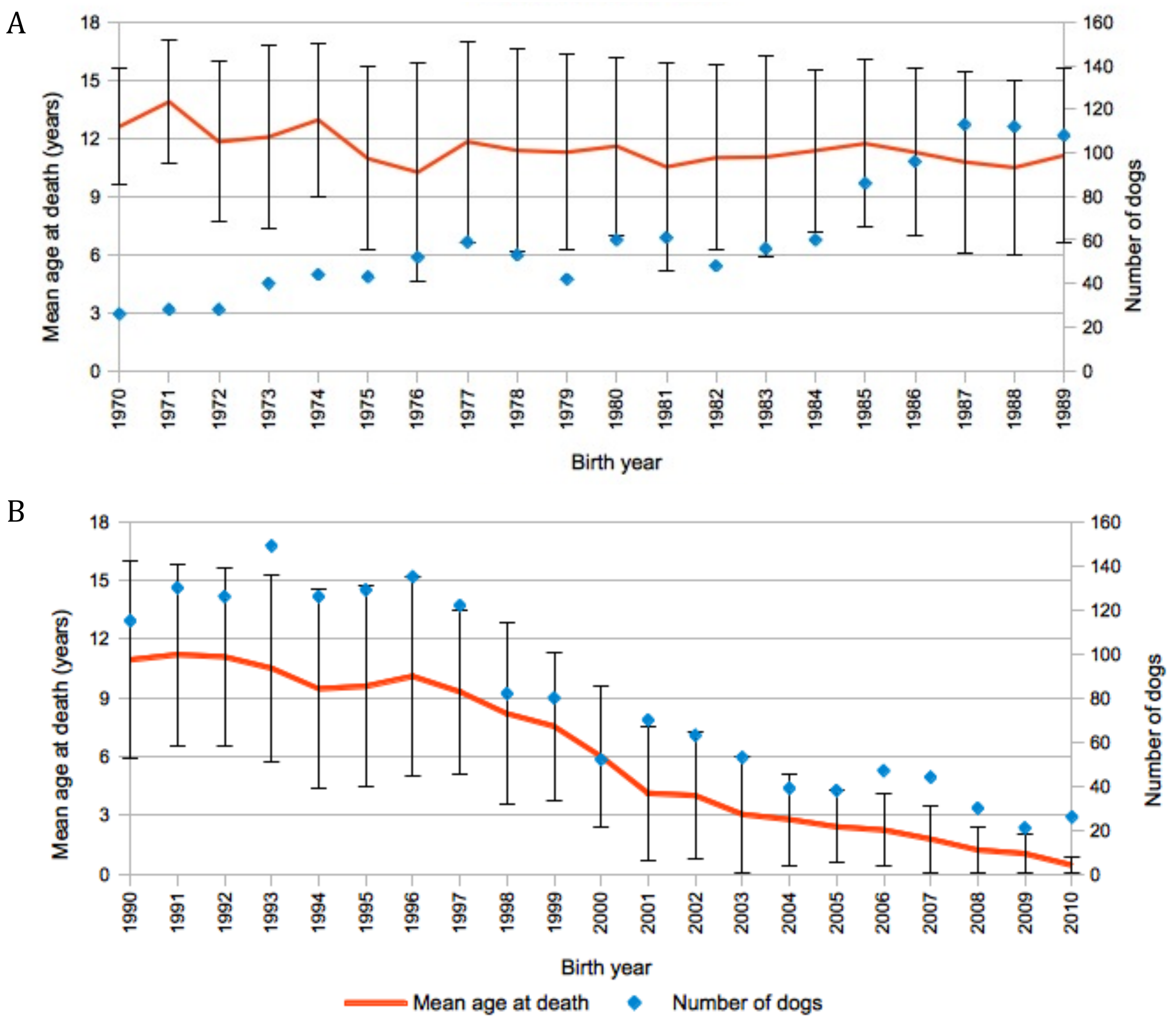

Figure 8 Mean life expectancy of Kooiker dogs born in 1970-1989 (A) and in 1990-2010 (B). Mean life expectancy is plotted on the left Y-axis. The right Y-axis represents the quantity of death records available displayed as number of dogs. Error bars represent standard deviations.

Discussion

The present study used descriptive statistics to describe the worldwide Kooiker dogs born in 1990-2010 and the results illustrated the consequences of the global Kooiker dog breeding programs. This study also took the approach of using convenience samples. One of the disadvantages of this type of samples is that the data subsets may be skewed. The quality or skewedness of the data (subset) can be influenced by the quantity of the available records. Obviously, Kooiker dog owner response rates to questionnaires put together by the VHNK, the input from other countries, and record keeping determined the number of available records. The present study did not evaluate the skewedness of the data subsets and did not investigate the significance of the findings and relationships between the variables, because of the large amount of data in combination with time constraints. The author has chosen to report the mean, standard deviation, minimum and maximum values of the variables as part of the descriptive statistics.

Effective population

Although the size of the worldwide breeding stock showed an increasing trend, there was a striking decline in the Netherlands. The decline started in the period 1991-1995 for the dams and 1996-2000 for the sires. In the same periods, studies revealed that VWD and HNM in Kooiker dog both had genetic basis with autosomal recessive pattern [12,17]. Moreover, breeders were motivated to identify carriers and select potential dams and sires more carefully [17]. This marked the start of restrictive breeding program, which can partially explain the fall in the size of the Dutch breeding stock. When researchers identified the VWD type III gene in Kooiker dog and DNA analysis became available, the mandatory test required by the VHNK might have allowed the fall to persist. Selection against other hereditary diseases exists in the breed, such as cataract and patellar luxation, intensified selection pressure and contributed to the decreased number of breeding animals.

To conserve a population with adequate fitness, the estimated effective population size (N_e) should be more than 50 animals (25 sires and 25 dams), but a safe limit is at least 100 animals (50 sires and 50 dams) for the population to be sustainable [7,15,16]. The N_e also depends on the ratio of dogs (N_m) to bitches (N_f). The optimum ratio is 1:1. Hence, if there are fewer sires, a greater number of dams is necessary to obtain N_e >100 [15]. Moreover, the N_e value is related to the rate of inbreeding (ΔF), which is a measure of the increase in inbreeding over generations [2,7] or how fast heterozygosis in the population is lost. Research found that a ΔF of 0.5% ($N_e = 100$) per generation does not have any negative consequences for the population, at least for one generation [15,16]. Therefore, a smaller effective population and a less than optimum ratio between sires and dams may accelerate inbreeding, which will reduce fitness of the population.

Increase selection pressure related to VWD and HNM may have had no visible consequences in the global population, but a detectable negative effect on litter size and the Kooiker dog effective population in the Netherlands did occur. Despite the decline in breeding stock, the estimated effective population in the Netherlands for the period 2006-2010 was well above the safe limit, with 5 dams for every 2 sires. So far, Dutch breeders have managed to maintain an optimum size of effective breeding population and control the rate of inbreeding. To assure that the Kooiker dog effective population size in the Netherlands does not continue to decrease and accelerate the loss of genetic diversity continued monitoring of the number of breeding animals is necessary. As for recessive disease, a less restrictive breeding program is a positive step toward maintaining and improving a population's genetic diversity. In this program [18], a combination between a carrier dog and a free dog will not produce a diseased offspring and should be allowed.

Contrary to the Netherlands, the N_e of Denmark, Norway, and Switzerland were lower than 100 and ΔF in these subpopulations is higher than recommended. The lower number of breeding animals in these countries suggests that the loss of diversity in these countries may occur at a relatively high rate. To slow down the decrease in heterozygosity in the breed, breeders and the Kooiker dog clubs in these countries should attempt to increase the number of breeding animals and at the same time, realize an optimum male to female ratio. For example, there should be at least 33 Kooiker dog dams for 10 sires or 44 sires for 16 dams in the Norwegian subpopulation to obtain a N_e of 100.

Beside the size of the effective population, the usage of breeding animals and the breeding frequency is also an important component in determining the rate of inbreeding. The worldwide average of the breeding frequency showed a constant and relatively low mean of breeding frequency in 1990-2010 and most of the dams and sire bred only once. However, the maximum breeding frequency remained quite high. These extreme values revealed that overuse of certain sires (popular-sire syndrome) occur from time to time, particularly in the Netherlands and Denmark. More important, these sires also mated and produced many litters across the border. Despite a large effective population, inequality in the use of breeding animals is problematic in breeding practices and highly undesirable for the health of the breed. Extensive use of certain breeding animals will not only sharply increase the rate of loss of genetic diversity in the population, but can also cause the dissemination of genetic diseases, specifically recessive disorders [5,7,8]. As carriers of recessive hereditary diseases show no clinical signs, a stud dog, who is a carrier of such disease and used frequently, will pass this on to his numerous offspring and the problem will disseminate very quickly in the population [8]. Moreover, overuse of certain sires (popular-sire syndrome) reduces the effective population size and the high rate of loss of genetic diversity is likely to cause inbreeding depression [5–7] and cause further reduction of fitness. Because all Kooiker dogs in the global population are essentially related, such practice should be avoided and breeders should exercise prudence in the use of breeding animals to sustain a healthy Kooiker dog population.

Litter size, number of litters, and coefficient of inbreeding

When compared with the results of Mandigers et al. (1994), the number of litters born per year grew steadily from 1990 to 2010. The same trend can be found in every country, except the Netherlands, where the number of litters dropped 50 percent in 2010 from that in 2000. The surge in the global number of litters can be explained by the increase in the size of the global effective population. Similarly, the decreased number of breeding animals in the Netherlands may cause the decline in the number of litters in this country.

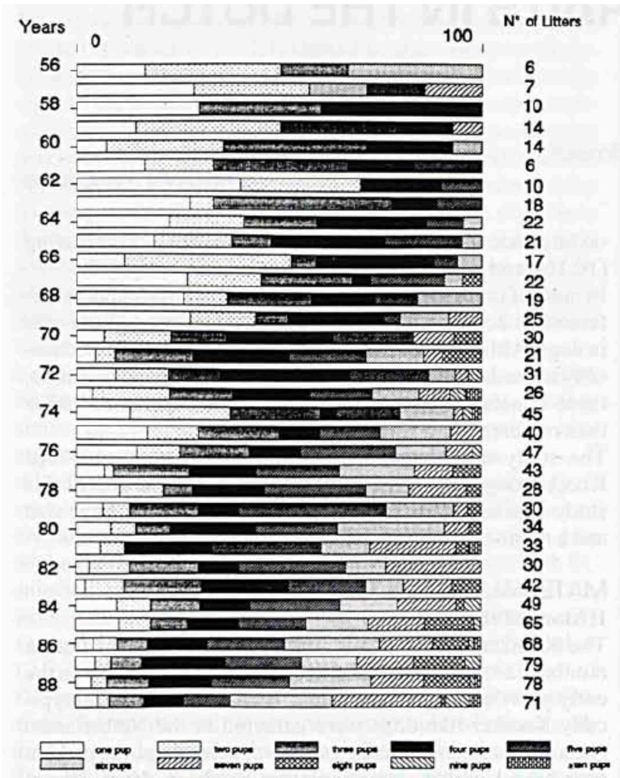

Figure 9 Litter size (excluding stillbirths) displayed as a relative frequency. Results from the study of Mandigers et al. (1994)

In contrast, a shift to smaller litter sizes was observed in litters born in 1990-2010. Mandigers et al. (1994) observed a trend in larger litter sizes (≥ 7 pups) from 1970 to 1989. Also, the mean litter size in 1990-2010 remained steady, but lower than 1989 (Fig. 9 and 10) This shift in litter size may have been caused by the increased number of dams and the majority of the dams whelped only once [19]. Studies have shown that there is a relationship between parity and litter size (see also discussion on parity & age). Primiparous bitches usually produce smaller litters and litter size increases with increasing parity [19–21].

Previous studies on the relationship between litter size and the inbreeding coefficient of the litter, sires, and dams showed conflicting results. Reduction in the reproductive capacities is one of the first consequences observed in high-inbred population [1,2]. Experiments with guinea pigs demonstrated that fertility declined by increasing COI, which can be observed in a fall in the litter size [1]. However, Mandigers et al. (1994) found that litter size was not influenced by the litter's and maternal inbreeding coefficient in Kooiker dog. Urfer's (2009) study on Irish Wolfhound population demonstrated similar results. Likewise, both studies showed that paternal inbreeding did not have negative effect on litter size [4,11]. On the contrary, paternal COI had positive effect on litter size in Kooiker dog population previously studied. As for Irish Wolfhound, inbreeding and fertility do not have a strong relationship [4].

Although the paternal, maternal, and litter's COI over five generations decreased from 1990 to 2010 in this study, the global mean litter size remained relatively steady. This is inconsistent with the theory that lower inbreeding coefficient may lead to improved fertility that results in larger litter sizes. There are several arguments that may explain this circumstance. A possible cause of the shift to smaller litter sizes has been mentioned above. Likewise, the rise in the number of breeding animals within the worldwide Kooiker dog population may also have caused the decline in the mean inbreeding coefficient. Moreover, intensive, but careful selective breeding due to hereditary diseases in the twenty-one years studied may explain the decrease in COI. The mean inbreeding coefficient of the litters, dams, and sires in this study were also lower than those calculated by Mandigers et al. (1994). Thus, the combination of all of these suggests that inbreeding may have no influence on litter size. However, note that in 1994 the Wright's inbreeding coefficient was calculated back to the base

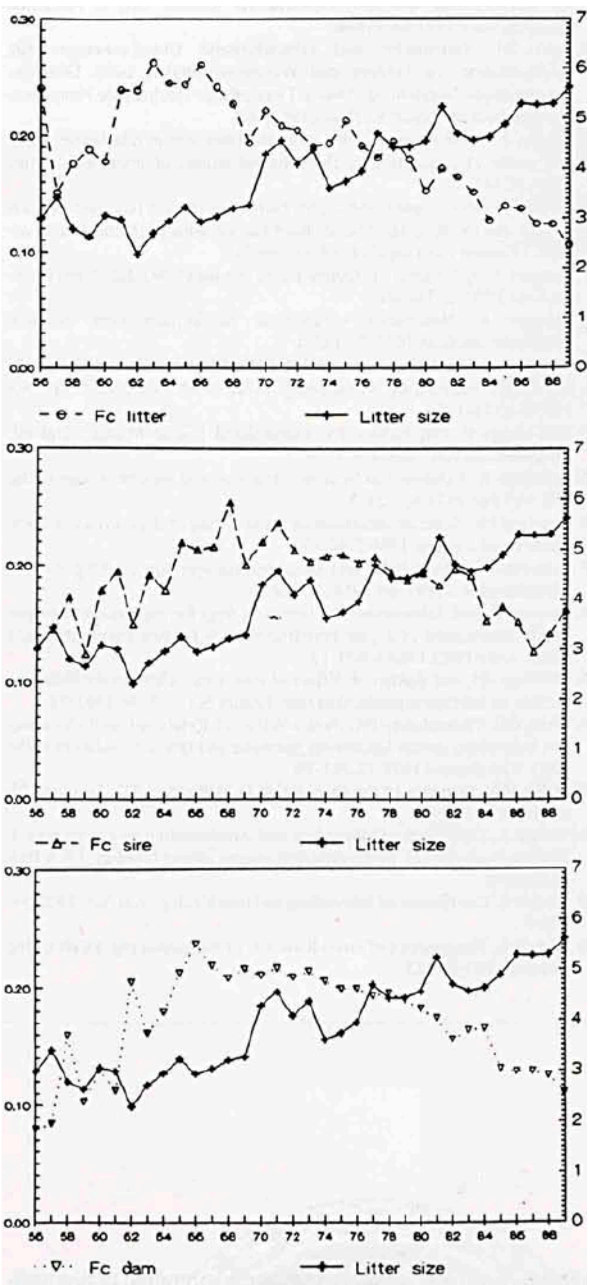

Figure 10 Litter size and the inbreeding coefficient of the litters, dams, and sires according to Mandigers et al (1994). Left Y-axis represents the mean F. Litter size is plotted on the right Y-axis and displayed as number of puppies.

population and that in the present study was only over five generations. The inbreeding coefficient will increase when calculated over more generations or back to the base population; that is back to the 15 dogs used to create the breed [1,4]. Consequently, the inbreeding coefficient of the Kooiker dogs born in 1990-2010 will be even higher than the results of the present study. This suggests that the worldwide Kooiker dog population may be more inbred than expected, which may partially explain the change in litter size and that there may be a relationship between inbreeding and litter size in the studied population. Nevertheless, further investigations of the significant of these findings and the relationship between various properties are recommended.

Mating success, viability of the offspring [2], congenital abnormalities, survival and growth rate [9] are other components of fitness that can be reduced by inbreeding. The viability of the offspring can be influenced by the maternal performance, such as maternal behavior and the bitch's milk production, which are also affected by reduced fitness. In addition, litter size can be influenced by environment factors, such as temperature and humidity [19]. No information was available for the majority of the parameters mentioned above. However, an assessment of these factors and their relationship with COI in Kooiker dog should be considered for future research. Perinatal mortality and longevity in the Kooiker dog born 1990-2010 are discussed below.

Peripartal mortality

The viability of the offspring, which is a component of fitness, is associated with the number of stillbirths {Falconer, 1989}. The viability of the offspring may decrease in a closely inbred population. Yet, the mean inbreeding coefficients of the population in this study have decreased since 1990. In the case of humans, an increased in homozygosity does not always have a negative effect on the number of offspring, but it can have an adverse effect on health. Although an inbred human individual could have more children, researchers observed increased number of perinatal death and congenital deformities in babies by such individuals [9]. This phenomenon may explain the rise in the number of stillbirths, despite the Kooiker dog population growth, because all Kooiker dogs descended from few common ancestors. Strong selection, in the light of VWD and HNM, may have strengthened this effect. However, a comparison with peripartal mortality in the earlier Kooiker dog population is not possible, because it was never investigated. Also, correlations between peripartal mortality and other characteristics in this study should first be investigated before a conclusion can be made.

Parity & age

Several studies have shown that the parity of the dam affects litter size. Litter size increases in the first few parities and then decreases after the third parity in Irish Wolfhound [19], fourth in Beagle [20], and fifth in German Shepherd [21]. Earlier investigation on Kooiker dogs found a positive relationship between increasing parity and litter size, but failed to demonstrate a decreasing pattern in litter size after certain parity due to the small sample size [11]. In the present study, fourth-parity dams produced the largest litter sizes and litter size decreased afterwards. Other studies also agreed that litter size increased with the increasing age of the dams, then decreased by dams older than 5 years [19–21]. Litter size peaked, when the dams were approximately three years old [20]. In contrast, research on the effect of paternal age on litter size showed conflicting results [4,11]. From 1990 to 2010, the mean paternal and maternal age at the birth of the litter stayed steady, while the maximum age of the dams and sires showed a distinct decline as litter size increased. This suggests that the parents' age may not influence litter size. One possible explanation for the consistent mean paternal and maternal age is the number certain litter sizes. There were a high number of small to medium litter sizes, which decreased the mean. In contrast, there were few large to very large litter sizes in 1990-2010. Breeding frequency of the dams and sires may also influence the number of litters of certain litter sizes. Many breeders breed their bitches and dogs only once or twice after they reach maturity, which gives smaller litter sizes.

Frequency of VWD and HNM

The low frequency of VWD and HNM in the studied population may suggest that restrictive selection of breeding stock by breeders, since DNA analysis for VWD in Kooiker dog and more knowledge on HNM became available [12–14], had been fruitful. However, the results should be interpreted carefully, because of the low number of records in the subset. Considering the size of the worldwide breeding stock and the number of puppies born in 1990-2010, an underrepresentation of VWD and HNM cases worldwide, particularly outside the Netherlands, is a possibility that may explain the low frequency of these diseases.

Life expectancy

Canine longevity has been the focus of many studies in the past [22–26]. These studies included many dog breeds as well as mixed-breed, because of the variation in size between different breeds of dogs. Previous observations have shown that there is a relationship between body size and lifespan in dogs. Larger breeds tend to have shorter lifespan [22,24–26]. However, many studies have sampling biases because the data came from a veterinary teaching hospital [22], animal-insurance databases [23,24], and a survey of members of the Danish Kennel Club [25]. In addition, they primarily addressed American or British breeds. Only Adams et al. (2010) did a research on the mortality of various dog breeds in the general population, which includes the Kooiker dog.

The Kooiker dog is a medium sized breed. The median age at death varied by breed, but according to Adams et al. (2010), the overall median age at death of dogs was 11 years and 3 months. As for Kooiker dogs in Adams' study, the median age at death was 3.92 years (minimum 0.5, maximum 13.92), a much lower value than the overall median age at death. It is also lower when compared with the median age of death of other breeds in the same weight group, such as the Nova Scotia duck tolling retriever (8 years), Keeshond (12.21 years) and Border collie (12.25 years). However, the Kooiker dog owners response rate to their questionnaires was very low and the number of deaths in their analysis was even lower (7 dogs) [26].

The mean life expectancy of Kooiker dogs born from 1990 to 2010 observed in the present study was the highest in dogs born in 1991 (11.21±4.64 years, maximum 10 years). By comparison, the mean life expectancy from the present study is slightly lower than the median age of death in Adams' study [26], but well within the range of that for medium breeds. Interestingly, the mean age of death in the present study had been gradually decreasing when compared with the lifespan of dogs born in the 1970s. The relatively higher average lifespan of dogs born in the 1970s can probably be explained by the low number of death records of these dogs when compared with records from dogs born in 1980s and early 1990s. But, the sharp decline in life span since 1996 is surprising. Despite overall population growth, the number of death records of dogs born in 1996 and onward was approximately as low as that from the 1970s. There are several possible explanations to this phenomenon. Firstly, many dogs are presumably alive today. Secondly, many deaths went unreported. Thirdly, digital communication and record keeping in the last one and a half decade had helped owners to make the death of their dogs promptly known to the VHNK and data collection. But, the last implies that there were indeed an increasing number of Kooiker dogs born in 1996-2010 that died at a young age. The outcome of the present study also showed an increasing trend in the number of peripartal death (see peripartal mortality). Therefore, further research in the life expectancy and cause of death in Kooiker dog can be useful to confirm whether the lifespan in this breed is indeed decreasing and the cause of the decline.

In short, the results of both this study and Adams' study [26] suggest a reduced life expectancy in Kooiker dog. Nevertheless, the VHNK has not posed any concerns about Kooiker dog's lifespan. Hence, the author questions the fall in the lifespan of Kooiker dogs and presumes that the quality of the data, which are influenced by the response rate to questionnaires, may partially explain the reduced life expectancy in this study.

Conclusions

The outcome of this study does not support the hypothesis that there is an inbreeding depression in the global Kooiker dog population due to restrictive breeding program applied in 1990-2010. However, further data and statistical analysis are necessary to validate the outcome of this study. To improve data quality for future research, better collaboration between the international Kooiker dog clubs in data collection and record keeping is recommended.

Acknowledgements

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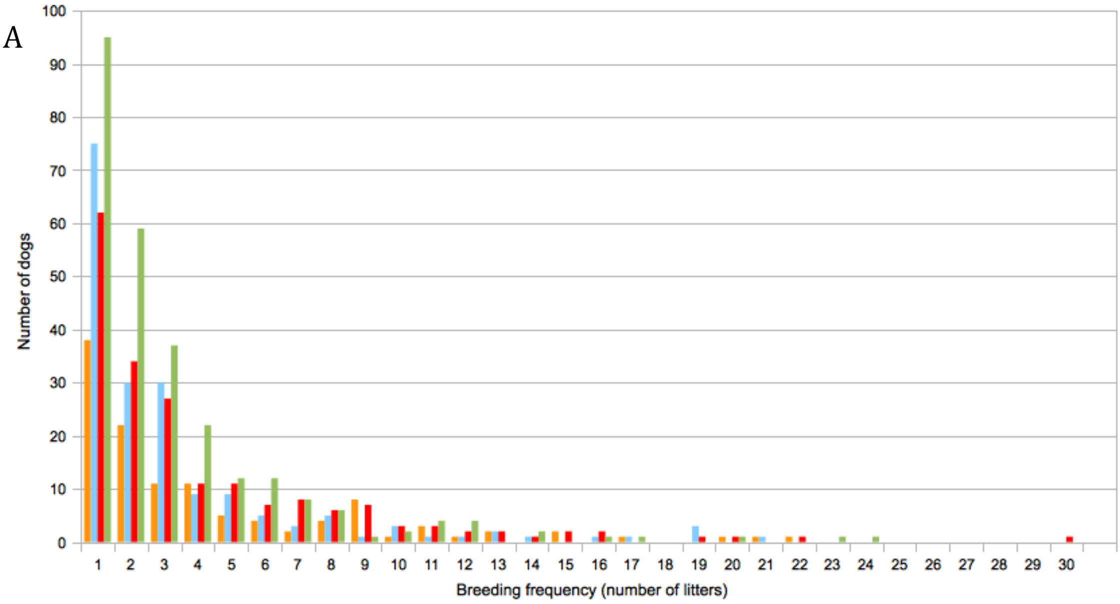
Appendix

A



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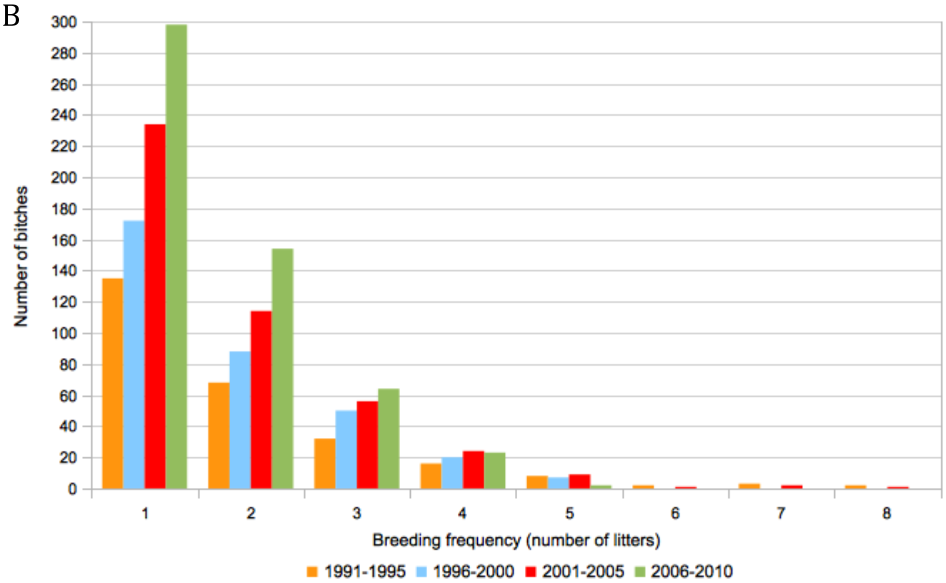
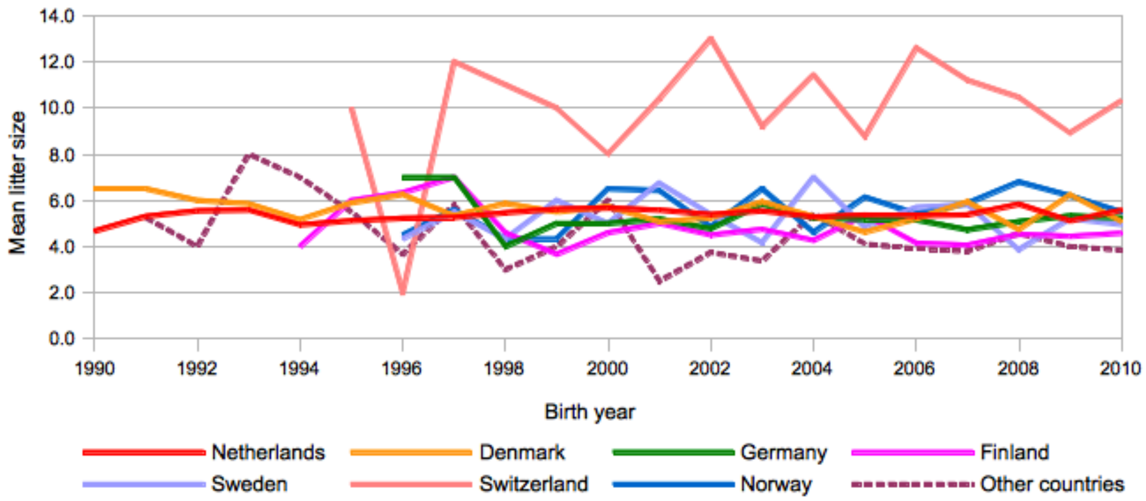
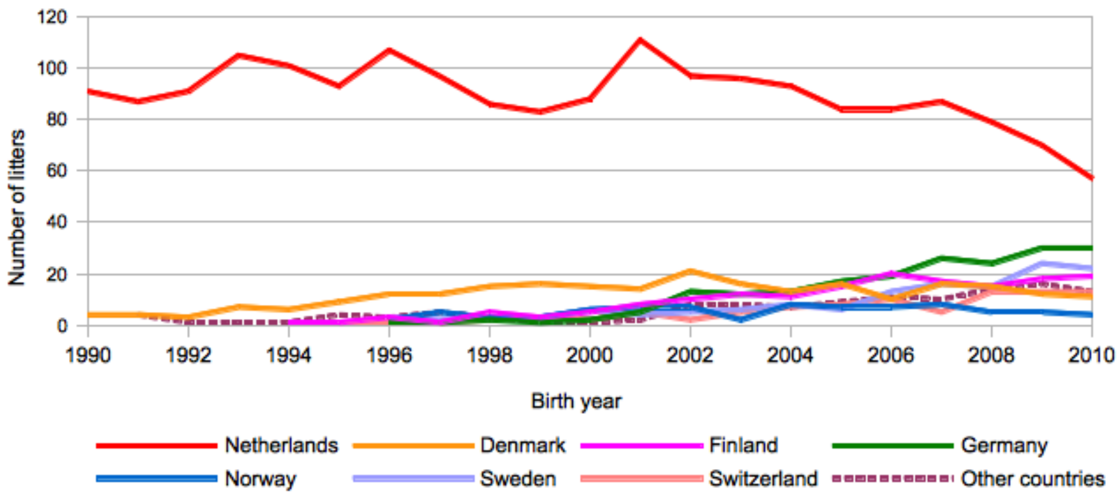


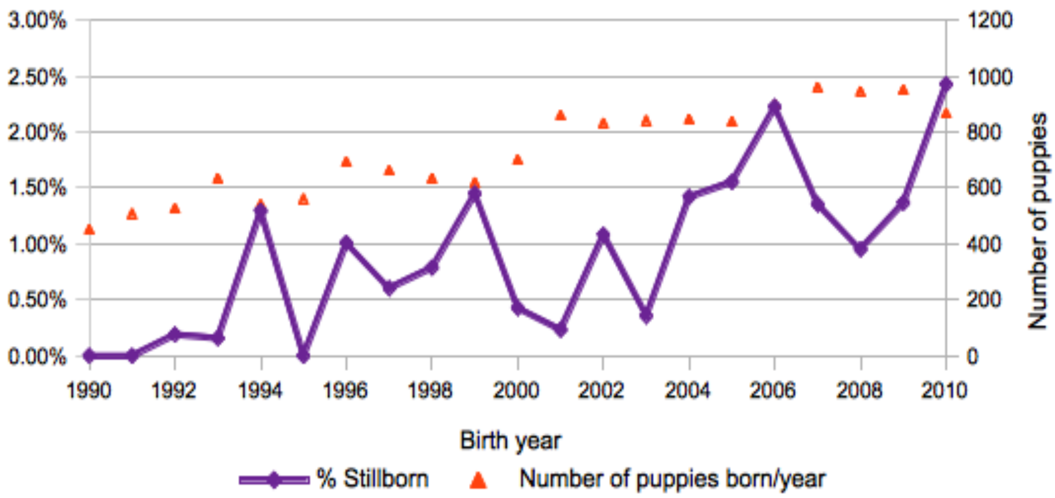
Appendix - Figure 1 Breeding frequency of sires (A) and dams (B) worldwide every five year. Breeding frequency is displayed as the number of litters. Most sires and dams were bred only once or twice. There were some important variations. One sire fathered 30 litters and two dams whelped 8 litters in five year.



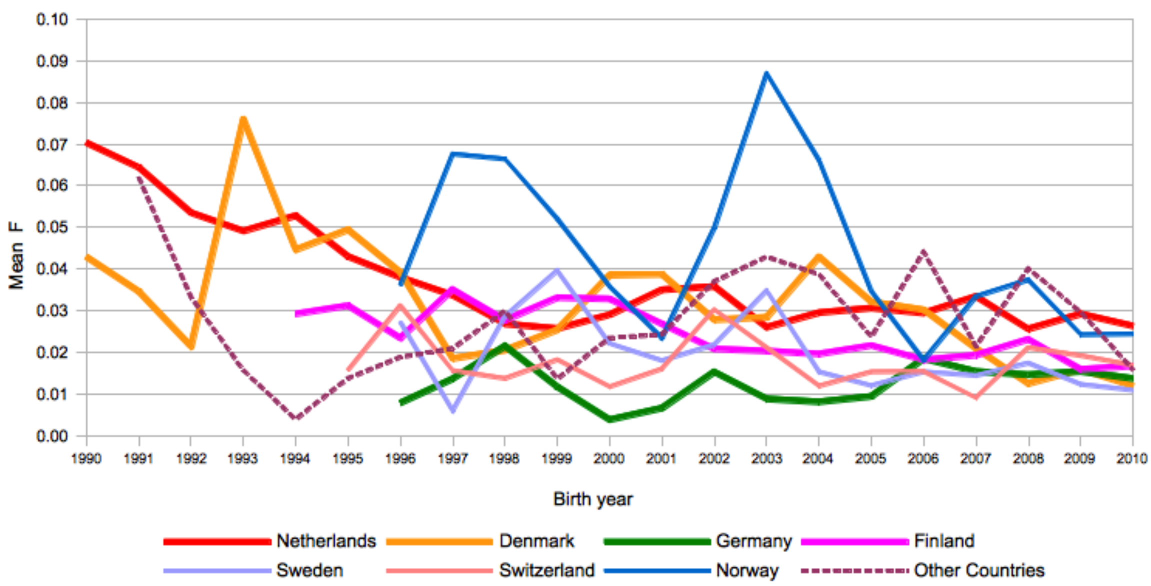
Appendix - Figure 2 Mean litter size in different countries from 1990 to 2010. Mean litter size in most countries remained steady at approximately 5 pups per litter over the studied period. Highest litter sizes were observed in Switzerland.



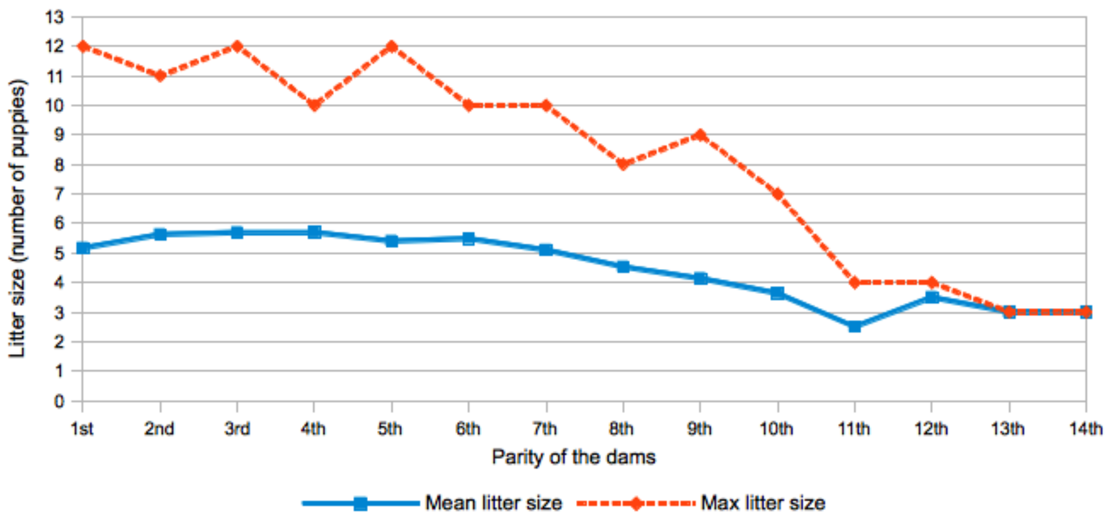
Appendix - Figure 3 Number of litters in different countries from 1990 to 2010. The number of litters decreased in the Netherlands and increased in other countries.



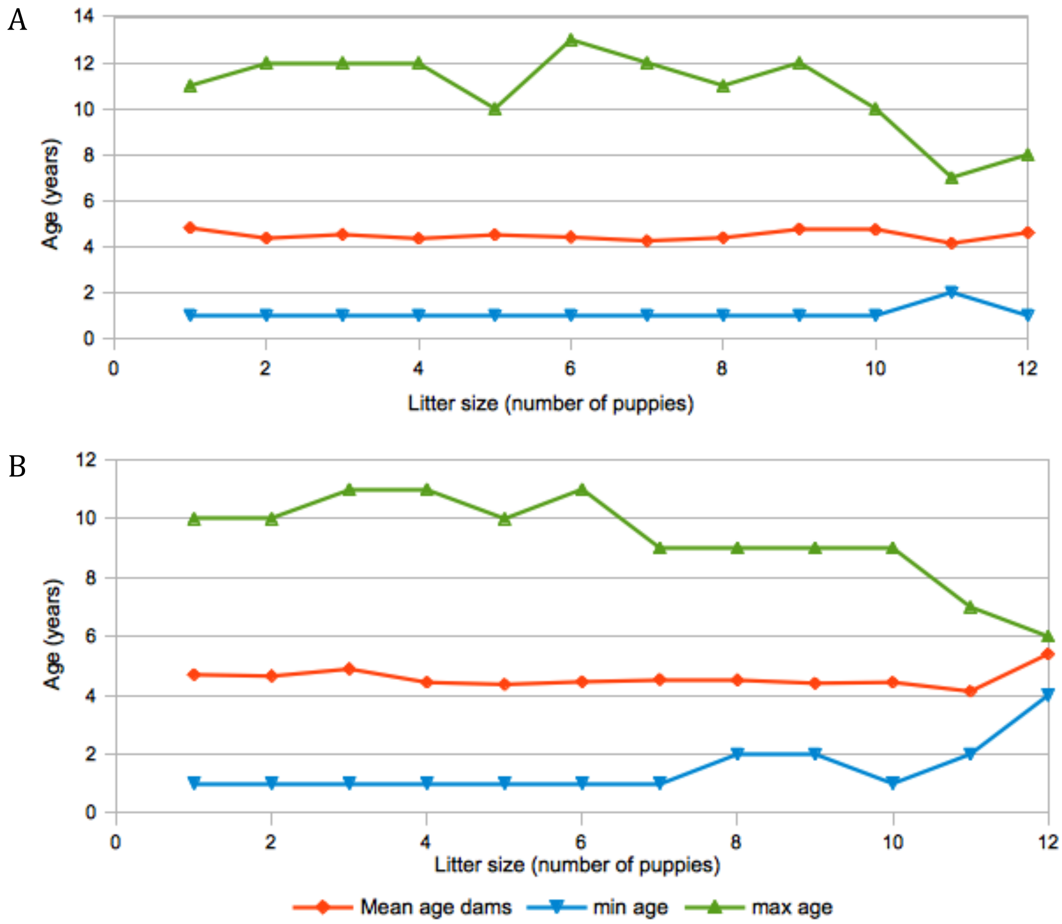
Appendix - Figure 4 Annual percentages of stillbirths. The number of stillbirths (left Y-axis) is displayed as percent of the number of puppies born each year (right Y-axis).



Appendix - Figure 5 Mean inbreeding coefficient (F) of the litters in different countries. Mean F litter in most countries showed a decreasing trend.



Appendix - Figure 6 Parity of the dams and litter size. Error bars represent standard deviations. Mean litter size declined after the fourth parity.



Appendix - Figure 7 Litter size, paternal (A) and maternal (B) ages at the birth of their litters. Mean age of both dams and sires remained stable over various litter sizes. Obvious declined in the maximum paternal and maternal ages was observed as litter size increased.