# Paper



# Seasonality in oestrus and litter size in an assistance dog breeding colony in the United Kingdom

### Eleanor E Wigham, Rachel S Moxon, Gary C W England, James L N Wood, Michelle K Morters

Evidence of seasonality in oestrus in bitches within specialist breeding programmes, such as those for assistance dogs, may support colony management through tailoring the distribution of resources required for breeding throughout the year. However, at present there are conflicting data regarding seasonality in oestrus (and litter size) in domestic dogs. The primary objective of this study was to investigate seasonal variations in oestrus and litter size in a large assistance dog breeding colony in the UK in order to optimise colony management. The authors analysed the annual distribution of 3624 observations of oestrus collected from 568 brood bitches from January 2005 to June 2014. The authors also evaluated the relationship between month and litter size for 1609 litters observed during the same period. There was no evidence of regular seasonal variations in oestrus or litter size by meteorological season or month. The lack of seasonality in oestrus may be a function of dogs in the UK, particularly valuable breeding bitches, being exposed to fairly constant environmental conditions throughout the year as a consequence of artificial light and heating during the winter months. The authors' findings suggest that special consideration of the annual distribution of oestrus and litter size is unnecessary for the management of assistance dog breeding colonies similar to those in the UK.

#### Introduction

Knowledge of seasonality in oestrus and litter size of bitches in assistance dog breeding colonies may support colony management through the efficient allocation throughout the year of resources required for the breeding programme. However, while it is generally accepted that day length is the main factor regulating seasonal variations in breeding in most species,<sup>1</sup> experimental and observational studies of seasonality in oestrus, and its regulatory factors, are limited and somewhat conflicting for domesticated dogs.<sup>2–5</sup> This may reflect the diversity of the study populations, which include free-roaming, predominately mixed breed dogs in less developed communities, and purebreed or first crossbreed pet and laboratory dogs in developed communities.

Studies from less developed communities include one from Jaipur, in the temperate region of northern India, where oestrus was more commonly observed in free-roaming bitches during late autumn and winter.<sup>3</sup> By contrast, there was no evidence of seasonality in impounded bitches in the tropics of Merida, Mexico.<sup>5</sup> Likewise, seasonality in pregnancy was not observed in two

#### Veterinary Record (2017)

doi: 10.1136/vr.104217

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Provenance and peer review Not commissioned; externally peer reviewed.

Received November 17, 2016 Revised June 30, 2017 Accepted July 29, 2017 entire free-roaming populations in Bali, Indonesia, with a tropical climate and fairly constant day length all year round. However, nor were regular seasonal variations in pregnancy observed in two free-roaming dog populations in Gauteng Province, South Africa; a region with a temperate climate and marked seasonal variation in day length.<sup>4</sup>

Studies of seasonal variations of oestrus in bitches in developed communities, generally living under more regulated environmental conditions, have also generated contradictory results.<sup>6-11</sup> These studies include laboratory and pet dogs of various breeds. Regular seasonal variations in oestrus were reported in 449 purebreed dogs in England and Wales  $^{8}$  and 594 German Shepherd Dogs in Kenya in the tropics.<sup>10</sup> Similarly, a colony of laboratory Beagles in Quebec, Canada, housed indoors under constant temperature control but with exposure to natural daylight, also demonstrated a seasonal pattern in oestrus (peaking in winter and summer)<sup>7</sup>; a peak in oestrus in May was observed over a 12-month period in a Beagle laboratory population in Sweden housed outdoors without supplementary light or heating9; and, whelping varied with season over a 12-year period in purebreed pet and breeding bitches housed under artificial conditions during the winter months in Sweden.<sup>6</sup> By contrast, Labradors and Golden Retrievers kept as pets in Sweden did not show any periodicity in oestrus during a one-year period<sup>9</sup>; however, nor did purebreed dogs in the USA exposed to seasonal variations in day length through natural and artificial lighting over a three to four-year study period.<sup>11</sup> Likewise, observed variations in litter size with meteorological season are also inconsistent<sup>6 10 12</sup> and, thus, subject to debate.

The reproductive behaviour of the domestic dog is commonly described as monoestrous with a uniquely prolonged interoestrous interval<sup>13</sup>; with an average interoestrous period of approximately seven months and a range of 3.5–13 months.<sup>14 15</sup> The age of sexual maturity in the bitch may vary with breed, ranging from approximately six months to two years, with most reaching maturity

at 8–12 months.<sup>14 16</sup> As noted above, it is generally accepted that day length is the main factor regulating breeding cycles in many species, and also the Basenji breed of dog.<sup>17–21</sup> For example, in seasonal breeders such as sheep, the length of anoestrus is synchronised, generating seasonal cycles in oestrus in the population. Anoestrus is regulated through exposure of the pineal gland to light, mediating the release of melatonin and, in turn, gonadotrophin-releasing hormone. This same mechanism is known to drive annual oestrous cycles in the Basenji breed.<sup>18 21</sup> Controlled studies have demonstrated that manipulating day length using artificial light can modulate the interoestrous period and, therefore, breeding cycles in ewes.<sup>22–24</sup> Although, to the authors' knowledge, similar controlled studies have not been carried out in domesticated dogs, and despite the inconsistencies in the aforementioned observational studies, it may be reasonable to assume that exposure to artificial light, especially during the winter months, may also interfere with the regulation of reproduction in dogs, dampening any normal seasonal variations in oestrus.

Given the possible impact of seasonal breeding on the efficacy of colony management, and the existing discrepancies between meteorological season, oestrus and litter size in dogs, the authors investigated seasonal variations in oestrus and litter size in brood bitches in a large, well-managed breeding colony for assistance dogs in the UK. The results from this study may provide assistance dog charities valuable data to support or improve management of their breeding colonies by allowing the effective allocation of resources required for breeding throughout the year.

#### **Materials and methods**

Reproductive records for all bitches in the assistance dog breeding colony from January 2005 to June 2014 (inclusive) were analysed for this study. Records included oestrus, mating and whelping dates, with oestrus identified by brood bitch owners and recorded in the database at the breeding centre by the centre staff.

Generally, following recruitment into the breeding colony at about 12–15 months of age, bitches are mated approximately once a year, that is, every other oestrus. The centre staff provide owners with standardised information regarding nutrition, husbandry and identification of oestrus for their breeding bitch. The same advice was provided to owners throughout the study period.

R was used for all statistical analysis.<sup>25</sup>

#### Seasonality in oestrus

The total number of bitches present in the colony varied throughout the study period due to the continual recruitment of new bitches and the retirement of bitches after one to five years in the colony. Therefore, in order to account for variations in colony size during the study period, the proportion of the colony in oestrus, rather than the absolute number of bitches in the colony in oestrus, was evaluated by month and meteorological season. The exact date of recruitment and retirement was often not recorded; therefore, with the exception of 2014, only those bitches recorded as present in the breeding colony for that entire year were included in the analysis. Those bitches still present in the breeding colony at the end of study in June 2014 were included in the analysis for that year.

<b>TABLE 1:</b> The distribution of breeds in the study population fromJanuary 2005 to June 2014			
Labrador x Golden Retrievers (%)	41.1		
Labrador Retrievers (%)	34.3		
Golden Retrievers (%)	10.7		
German Shepherd Dogs (%)	5.7		
Others (%)	8.3		

The first day of oestrus was taken to be the day when a serosanguineous discharge was initially detected; and, month of oestrus was considered to be the month which included the first day of oestrus. For any bitches with a 'split season', the start date of the second of the two closely recorded observations of oestrus was taken as the true month of oestrus.<sup>26</sup>

The proportion of bitches in oestrus during any given meteorological season (with winter taken to be December to February; spring March to May; summer June to August; and, autumn September to November) was estimated from the total number of bitches in oestrus during the three-month period divided by the total number of bitches present in the colony during that period. Winter straddles two consecutive years, therefore the denominator was the mean number of dogs in the breeding colony for this period, given that the number of dogs in the study population in each year was similar (Table 2). Estimating the proportion of bitches in oestrus per month allowed for comparisons between years.

Variations in the distribution of oestrus by month and meteorological season were assessed by visual inspection of plots and non-parametric regression. An overall increase or decrease in the proportion of bitches in oestrus during the study period was assessed using linear regression. Finally, autocorrelation was used to detect any evidence of periodicity in the proportion of bitches in oestrus each month during the 9.5-year time series. The data by month and meteorological season were then combined (ie, all the January data for the study period, and so on; and all the summer data for the study period, and so on) and the overall or cumulative mean proportion of bitches in oestrus calculated for each month of the year and for each meteorological season.

#### Seasonality in litter size

The number of bitches whelping each month varied, therefore the median litter size per month was analysed rather than the mean, given that the mean litter size varied with the number of bitches whelping per month and is sensitive to outliers. Litter size included stillbirths. Analysis for variations in litter size by month included visual inspection of plots, non-parametric and linear regression and autocorrelation, as described for the evaluation of 'Seasonality in oestrus' in the Materials and methods section.

## The effect of meteorological season and pregnancy on the interoestrous interval

To gauge the effect of meteorological season and pregnancy on the length of the interoestrous period, an arbitrary sample size of 200 bitches was randomly selected from those bitches that had at least two observations of oestrus while in the breeding colony. Bitches were categorised by the meteorological season in which the first of their two successive observations of oestrus fell and pregnancy status during the interoestrous period. Twenty-five bitches were randomly selected from each category, then for each

<b>TABLE 2:</b> The number of bitches present in the colony for theentire year			
Year	Number of bitches		
2005	148		
2006	226		
2007	233		
2008	233		
2009	261		
2010	278		
2011	280		
2012	273		
2013	259		
2014	204		

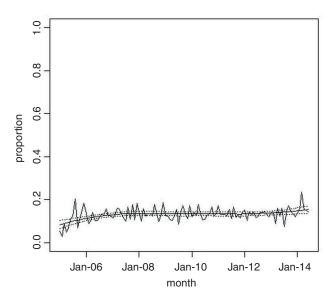


FIG 1: The number of bitches in oestrus each month as a proportion of the total number of bitches in the breeding population for that entire year. A loess model was fitted to the data, shown by the smooth non-parametric regression lines; the central non-parametric regression line shows the mean variation in the proportion of bitches in oestrus, and the dashed outer lines show the 5% and 95% Cls for the mean.

of these bitches one of their interoestrous periods was randomly selected. The length of the interoestrous period was calculated using the recorded start date of each of the two successive observations of oestrus. Analysis of variance was used to investigate the relationship between the length of the interoestrous period and pregnancy status during the interoestrous period; with the final model checked for violation of constant variance and normal error distribution assumptions.

Ethics committee approval was not required to analyse data already routinely collected.

#### Results

#### **Description of the study population**

The age of the dogs at the start of an oestrus, for the bitches included in the analysis, ranged from 5 to 101 months. The most common breeds were Labradors crossed with Golden Retrievers (41.1 per cent) and Labrador Retrievers (34.3 per cent) (Table 1).

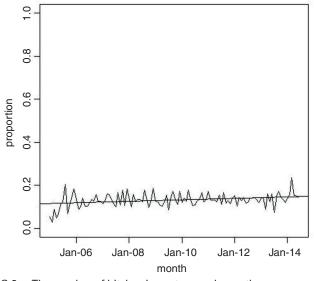


FIG 2: The number of bitches in oestrus each month as a proportion of the total number of bitches in the breeding population for that entire year. A linear regression model was fitted to the data, shown by the straight overlaid line (P=0.001).

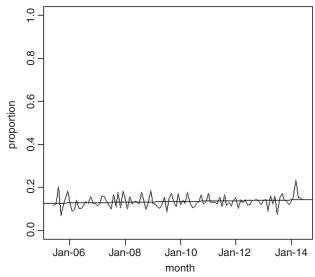


FIG 3: The number of bitches in oestrus each month as a proportion of the total number of bitches in the breeding population for that year excluding the first five data points. A linear regression model was fitted to the data, shown by the straight overlaid line (P=0.11).

#### Seasonality in oestrus

A total of 3624 observations of oestrus collected from 568 bitches were included in the analysis. A median of 246 brood bitches were included in the analysis each year, ranging from 148 to 280 (Table 2).

The results provide no clear evidence of regular seasonal variations in oestrus. There was considerable month-to-month variation in the proportion of bitches in oestrus, without regular annual or biannual peaks and troughs in oestrus, or regular cycles of intervals longer than 12 months (Fig 1). This is supported by autocorrelation, which produced no evidence of periodicity in the time series. There was a significant increase (P=0.001) in the proportion of bitches in oestrus each month during the study period (Fig 2). However, exclusion of the first five data points resulted in no significant increase or decrease in the proportion of bitches in oestrus each month with time (P=0.11) (Fig 3).

Approximately 80 per cent of the same bitches were present in the study population between successive years (Table 3).

The smallest overall or cumulative mean proportion of bitches in oestrus by month was in July (0.107) and the largest in August (0.157) (Fig 4).

There was no clear regular variation in the proportion of bitches in oestrus by meteorological season (Fig 5). The overall or cumulative mean and median proportions were similar between meteorological seasons, with the highest median proportion of bitches in oestrus in the winter (0.411) and lowest in the summer (0.384) (Table 4).

#### Seasonality in litter size

TABLE 3:         Percentage of bitches in the stud           across successive years	y population present
Years	0/0
2005-2006	65.0
2006-2007	82.0
2007–2008	85.2
2008–2009	72.0
2009–2010	82.7
2010-2011	75.9
2011-2012	74.7
2012-2013	86.3
2013-2014	96.3

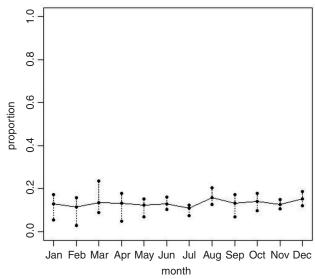


FIG 4: The overall or cumulative mean proportion of bitches in oestrus by month. The dashed vertical lines show the range in the proportion of bitches in oestrus by month.

A total of 1609 litters were analysed. The results show no clear evidence of regular seasonal variations in median litter size (Fig 6). This is supported by autocorrelation, which produced no evidence of periodicity in the time series. Linear regression demonstrated no significant increase or decrease in median litter size with time (P=0.22). The overall or cumulative median litter size was 8 for each month and meteorological season.

## The effect of meteorological season and pregnancy on the interoestrous interval

The random sample of 200 interoestrous periods was selected from 1646 interoestrous periods from 401 bitches that were pregnant during the interoestrous period and 1515 interoestrous periods from 410 bitches that were not pregnant during the interoestrous period. The overall (n=200) mean and median interoestrous period was 188 days and 185 days, respectively. The results show no clear evidence of seasonal differences in the length of the interoestrous period (Table 5); however, pregnancy significantly (P<0.001) increased the length of the interoestrous period when comparing the combined data for dogs pregnant during the period (n=100, mean=200 days, median=195 days) with dogs not pregnant during the period (n=100, mean=176 days, median=169 days).

TABLE 4:	The overall or cumulative mean and median proportion	
of bitches	in oestrus for each meteorological season	

	Winter (n=10)*	Spring (n=10)	Summer (n=9)†	Autumn (n=9)
Mean	0.393	0.389	0.392	0.395
Median	0.411	0.400	0.384	0.408
Range	0.212-0.439	0.204-0.539	0.341-0.442	0.327-0.450

\*Includes December 2004; excluding December 2004 to February 2005 n=9, mean=0.413, median=0.414, range=0.365–0.439.

†The results are the same when June 2014 is included.

#### Discussion

This study provides the most recent and comprehensive evaluation of seasonal variations in reproductive parameters in breeding bitches living under controlled environmental conditions, thus supporting the management of comparable specialised breeding colonies. The authors found no evidence of regular seasonal variations in oestrus in the purebreed and first crossbreed bitches in a large assistance dog breeding colony in the UK. Although at an individual level, longitudinal data regarding housing conditions were not available for this study, all bitches within the breeding colony are housed within home environments for their entire lives, therefore are likely to have been exposed to a fairly constant photoperiod throughout the year from artificial lighting. The results of the present study are comparable to those of a smaller study of pet bitches in Sweden, probably housed under similar conditions9; and, free-roaming dogs in the tropics exposed to fairly constant natural day length.<sup>4 5</sup> By contrast, similar populations of breeding bitches housed in controlled environments in the UK<sup>8</sup> and Sweden<sup>6</sup> reported seasonal peaks in oestrus. Despite these disparities, the most plausible explanation for the absence of seasonality in the present study population may be the effect of valuable breeding bitches being kept indoors and exposed to artificial lighting and heating, particularly during the winter months. However, given the importance of efficient colony management to generate sufficient numbers of assistance dogs for disabled citizens, more detailed and controlled studies of the effect of photoperiod on reproductive parameters in breeding bitches are warranted.

Although it is generally understood that photoperiod is the main factor that synchronises oestrus in many species, seasonality in oestrus has also been attributed to other regulatory factors, such as ambient temperature<sup>25</sup> and breed.<sup>11</sup> In this study, the authors did not investigate the relationship between regular variations in oestrus and these other factors, particularly given the

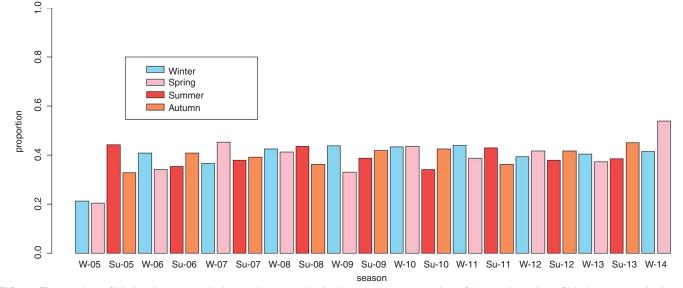


FIG 5: The number of bitches in oestrus during each meteorological season as a proportion of the total number of bitches present in that year.

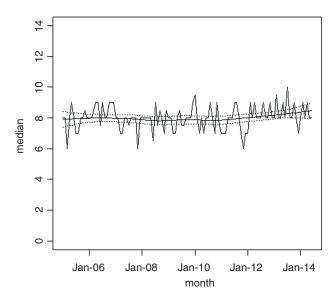


FIG 6: Median litter size by month. A loess model was fitted shown by the smooth non-parametric regression lines; the central non-parametric regression line shows the average distribution of litter size and the dashed outer lines show the 5% and 95% CIs for the mean.

limited range of breeds in this assistance dog colony. Pregnancy and parity may also influence the length of the interoestrous interval and, thus, subsequent oestrous start dates.<sup>9–11</sup> While the authors found evidence supporting these prior observations, evaluation of the effect of these factors on seasonal variations in oestrus was precluded for this study because the duration that each bitch was in the colony was limited and mating was systematic, that is, attempted every other oestrus and was generally successful for most bitches. Ideally, the authors would have also studied hormone profiles in individual bitches but this was not within the scope of the present study.

The authors also found no significant increase or decrease in the proportion of bitches in oestrus over the entire study period (Fig 3), which suggests that colony composition and management remained fairly constant throughout. This is consistent with the standardised advice provided to the owners of the brood bitches in the colony by the centre staff regarding the best possible environmental conditions and nutrition for each individual bitch.

Finally, there was no evidence of regular seasonal variations in litter size in this assistance dog breeding colony, consistent with a larger (of 10,810 litters from 224 breeds), although shorter, study undertaken in Norway.<sup>12</sup> An association between litter size and several other factors, such as age, general health and parity, has also been reported.<sup>27</sup> However, an association between litter size and these other factors was not investigated here for the reasons mentioned above, and because all of the bitches in the

Table 5: periods	Summary of the random sample of 200 interoestrous				
		Winter (days)	Spring (days)	Summer (days)	Autumn (days)
Pregnant	Mean	198	193	205	202
	Median	187	193	202	195
	Range	143-267	119-279	119-267	114-270
Not pregnant	Mean	185	173	170	174
	Median	185	167	167	167
	Range	115-251	101-248	104-279	94-285
Combined pregnancy status	Mean	192	184	188	188
	Median	186	182	188	181
	Range	115-267	101-279	104-279	94-285

present study were healthy at the time of mating. Therefore, in light of the authors' observations overall, in well-managed breeding colonies, where the majority of dogs are housed indoors and environmental factors such as light and heat are controlled, it may be anticipated that oestrus and, thus whelping, occur fairly constantly throughout the year. Thus, resources that support successful breeding, such as staff and kennel space in the breeding facility, and the distribution of puppies for training as assistance dogs can be anticipated to be allocated evenly throughout the year.

#### Conclusion

The present study has generated valuable data that support specialised breeding facilities in controlled environments such as that for assistance dogs in the UK. The authors found no evidence of seasonality in oestrus or litter size, with bitches coming into season, on average, every six months. This may be a function of the bitches in this study being exposed to artificial light and heating leading to fairly consistent environmental conditions throughout the year. Overall, the authors' findings suggest that special measures to vary the allocation of resources required to support breeding throughout the year are not necessary.

#### Acknowledgements

The authors gratefully acknowledge the data provided by The Guide Dogs for the Blind Association, UK, for this study.

#### Competing interests None declared.

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