

Prevalence of ultrasound-determined cystic endometrial hyperplasia and the relationship with age in dogs Rachel Moxon<sup>a\*</sup>, Helen Whiteside<sup>a</sup> and Gary C.W. England<sup>b</sup>

<sup>a</sup>Guide Dogs, National Breeding Centre, Banbury Road, Bishops Tachbrook, Leamington Spa, Warwickshire, CV33 9WF, UK. [rachel.moxon@guidedogs.org.uk](mailto:rachel.moxon@guidedogs.org.uk), [helen.whiteside@guidedogs.org.uk](mailto:helen.whiteside@guidedogs.org.uk)

<sup>b</sup>School of Veterinary Medicine and Science, University of Nottingham, College Road, Sutton Bonington, Leicestershire, LE12 5RD, UK. [gary.england@nottingham.ac.uk](mailto:gary.england@nottingham.ac.uk)

\*Corresponding author:

Tel: +44 (0)845 372 7432, Fax: +44 (0)845 372 7396

Email address: [rachel.moxon@guidedogs.org.uk](mailto:rachel.moxon@guidedogs.org.uk)

## Abstract

To investigate the potential relationship between age and diagnosis of cystic endometrial hyperplasia (CEH) in the bitch, 348 ultrasound examinations from 240 bitches (Labradors, Golden Retrievers, German Shepherds, Flat Coated Retrievers or crosses of those breeds aged between 1.6 and 7.2 years at examination) were examined. A subpopulation of 32 bitches that had completed their breeding careers at  $\geq 6$  years of age was also identified. 18.3% of bitches were diagnosed with CEH; these cases were newly diagnosed when bitches were between 2.5 years and 7.3 years of age. The proportion of ultrasound examinations where CEH was identified increased from 6.8% of examinations on two year old breeding bitches to 60.0% of examinations on six year old bitches. Logistic regression identified a positive correlation between mean age at examination and presence of CEH ( $\chi^2 = 30.74$ , DF = 1,  $p < 0.001$ ). Within the 32 bitches that had retired from breeding the prevalence of CEH was 56.3%, age at diagnosis ranged from 3.8 to 7.3 years and the proportion of bitches affected with CEH increased from 6.3% at 3 years of age to 56.3% at 7 years of age. Thus the data support the contentions of other authors that CEH is related to age.

Keywords: Bitch; Endometrium; Cystic endometrial hyperplasia; Uterus; Age

## 1. Introduction

Hormonal stimulation during the oestrous cycle of the bitch induces changes in the endometrium including glandular proliferation and secretion [1-3]. Cystic endometrial hyperplasia (CEH) is an abnormality of uterine growth and repair arising from the endometrial glandular epithelium where there is cystic distension of the endometrial glands [4,5]. In breeding bitches, CEH seems to be an abnormal response to stimulation of the uterus by ovarian hormones; progesterone and oestrogen [2,3,6-8] and can also be triggered by uterine irritants and endometrial trauma [2,3,9,10]. In CEH cases the number and size of endometrial glands are increased and there is disparity in the number and configuration of glands causing a thickened endometrium and increased secretory activity [2,11]. In bitches affected with CEH, post-mating endometritis appears to impact fertility by reducing the uterine vasodilatory response to mating and impairing the clearance of uterine fluid as a result of decreased uterine contractions when compared to normal bitches [12]. A larger PMN influx has been observed in bitches with CEH post-mating which could affect the ability of spermatozoa to attach to the uterine epithelium with related impacts on fertility [13]. Previous research has demonstrated poor conception rates and lower litter sizes for bitches affected with CEH without treatment [12,13]. In addition to reduced fertility, the degenerative changes within the tissue associated with CEH can provide conditions suitable for the establishment of uterine infections and in some cases pyometra can develop [2,7,14,15]. The relationship between age and incidence of pyometra has been documented [22,23,24]. Age has been proposed as a risk factor for a breeding bitch being affected by CEH due to the repeated hormonal stimulation of successive oestrus cycles in entire bitches as they age. Indeed it has been suggested that older bitches are likely to have some degree of CEH [11,14,16] whilst Verstegen et al [15] suggest that all dogs will develop CEH with age. However, no previous work has presented data for the prevalence of CEH alone in a population of bitches by age, without associated pyometra. The seminal work by Dow [11] only included bitches with disease rather than a whole population

and considered the cystic hyperplasia pyometra complex rather than two distinct diseases. In Zoo canids and elephants, significant association between the development of endometrial hyperplasia and increasing age has been established and reported based on the prevalence of CEH within a population [17,19].

Therefore, this study aimed to examine a population of breeding bitches and to report on three factors: 1) the age at which cases of CEH were diagnosed; 2) the prevalence of CEH in ultrasound examinations conducted on bitches at different ages and 3) the incidence of CEH in a population of retired breeding bitches which had been examined throughout their breeding lives.

## **2. Materials and methods**

Between 21 September 2012 and 20 September 2014, 240 bitches from a large, relatively closed, breeding population were examined as part of routine health management prior to breeding. Bitches were Labradors, Golden Retrievers, German Shepherds, Flat Coated Retrievers or crosses of those breeds (Table 1), were from 68 unique sires and 126 unique dams and were between 1.6 and 7.2 years of age at examination. One hundred and thirty-nine bitches were examined once, 94 were examined twice and seven were examined three times within the two-year study period.

INSET TABLE 1 NEAR HERE

In total, 348 detailed transabdominal ultrasound examinations were conducted using a 10 MHz transducer. In every ultrasound examination, the uterine body and distal uterine horns were studied; proximal uterine horns were examined wherever possible. Bitches were allocated to control or CEH groups as previously described [12]. CEH cases were categorised as 'New case' or 'Existing case' dependent on whether the bitch was diagnosed with CEH during the current examination and had not been diagnosed at any previous examination (New case) or had been diagnosed with CEH prior to the study period (Existing case). Age at first diagnosis was recorded for all CEH affected bitches.

A subpopulation of 32 bitches out of the 240 had retired from the breeding programme at  $\geq 6$  years of age. These bitches had been examined as part of the current study but additionally had ultrasound examinations recorded prior to the study commencing. The historic data and current study data were examined to report the prevalence of CEH and to determine the proportion of CEH affected bitches at each year of age.

## *2.1 Statistical analysis*

Data were investigated using XLStat (Addinsoft, USA) and IBM SPSS Statistics 20 (USA). Age at diagnosis was described for all CEH affected bitches. The number of ultrasound examinations conducted on bitches of each age was reported along with the proportion of examinations at each age where CEH was observed.

To determine whether there was a relationship between age and presence of CEH, repeat examinations for individual bitches were excluded by calculating mean age at examination. A binary logistic regression was conducted to predict presence of CEH, using age as the predictor.

## **3. Results**

### *3.1 Number of bitches with CEH during the two year period and age at diagnosis*

There were 44/240 (18.3%) bitches that were diagnosed with CEH when examined by ultrasound; 40 New cases and four Existing cases. These cases were newly diagnosed when bitches were between 2.5 and 7.3 years of age (mean 4.9  $\pm$  0.2 years; Figure 1).

INSERT FIGURE 1 NEAR HERE

### *3.2 The prevalence of CEH in ultrasound examinations conducted on bitches at different ages*

The proportion of ultrasound examinations conducted on bitches of each year of age from 1.0 to 7.99 years where CEH was present, including new and existing cases,

increased from 6.8% of examinations on two year old breeding bitches (n=46) to 60.0% of examinations on six year old breeding bitches (n=20; Table 2).

INSERT TABLE 2 NEAR HERE

Mean ages were calculated for 101 bitches that had data for more than one ultrasound examination. Repeat examinations were  $0.91 \pm 0.03$  years apart. Logistic regression revealed a positive relationship between mean age at ultrasound examination and presence of CEH ( $\chi^2 = 30.74$ , DF = 1,  $p < 0.001$ ; Figure 2).

INSERT FIGURE 2 NEAR HERE

For breeds with >10 individuals, the highest proportion diagnosed with CEH was German Shepherds (26.3%; Table 3). The difference between breeds was not significant (Yates' Chi Square = 0.575, D.F. = 3,  $P = 0.902$ ).

INSERT TABLE 3 NEAR HERE

### *3.3 CEH in retired breeding bitches*

There were 32 bitches within the sample of 240 that had been examined each year from three years of age to retirement at  $\geq 6$  years of age and prevalence of CEH for these bitches was 56.3% (n=18). Age at diagnosis ranged from 3.8 to 7.3 years (mean =  $5.8 \pm 0.25$  years). The prevalence of hyperplasia increased each year with age so that the proportion of bitches affected with CEH increased from 6.3% at 3 years of age to 56.3% at 7 years of age (Figure 3).

INSERT FIGURE 3 NEAR HERE

## **4. Discussion and conclusion**

This study examined a large population of breeding bitches that were subjected to ultrasound examinations of the uterus as part of routine breeding management. Almost one fifth of the bitches within the breeding population had CEH, which was first diagnosed between 2.5 and 7.3 years of age. Examinations on older bitches were more likely to find individuals affected with CEH (60% of examinations on bitches 6 to 7 years of age and 100% of examinations on bitches 7 to 8 years of age) than examinations on younger bitches (6.8% of examinations on bitches 2 to 3 years of age and 9.6% of examinations on bitches 3 to 4 years of age). There was a significant positive relationship between mean age at examination and presence of CEH, with more examinations having CEH present when mean age was higher. While the calculation of mean age at examination, required due to the repeat examinations for 101 of the bitches in the study, may have had a small influence on this finding, it remains consistent with the contentions of other authors. Bigliardi et al [14] suggested that bitches over five years of age in Italy, where the average age at neutering is higher than in the UK or USA, are more frequently diagnosed with CEH and Dow [11,18] suggested that cystic endometrial hyperplasia is rarely observed in bitches of <4 years of age.

In the population of retired bitches (n=32) prevalence of CEH was 56.3%. This study is unique in that it was able to examine the CEH status of a number of bitches over their lifetime. The bitches were neutered at the end of their breeding careers (approximately 8 years of age) and so it is possible that more bitches would have developed CEH later in life had they been left entire and examined regularly. Additionally, as all of the bitches studied were in a controlled breeding colony, all were mated on average once every other oestrus with high conception rates (>90%, data not presented). CEH has been reported to be more common in nulliparous mammals, including dogs, and therefore a protective effect of pregnancy proposed [11,18,19-21]. Ultrasound examination of a large population of entire nulliparous bitches throughout their lifetimes would be required to provide greater accuracy regarding true prevalence and age of appearance. In addition, examining a large number of older entire bitches with no clinical signs could provide further useful information on the prevalence in older bitches since the sample size in the present study was small (n=4).

When studying pyometra, a difference in risk between breeds has been reported with Golden Retrievers as one of the breeds demonstrating an increased risk [22,23]. Protective effects of pregnancy have been noted to vary by breed for pyometra and have been reported to be present in the Labrador but not the Golden Retriever [21,22]. Within the current study there were non-significant trends towards differences in the proportion of bitches of each breed that were diagnosed with CEH; Labradors and Golden Retriever cross Labradors had lower proportions of bitches affected with CEH than Golden Retrievers and German Shepherds. While a protective effect of pregnancy against CEH has been proposed, this is the first time that a possible difference between breeds for CEH has been reported. It is also possible that the difference may be due to a higher mean age of bitches examined within the Golden Retrievers compared to Labradors and the Golden Retriever cross Labrador Retrievers and differences may have been influenced by the degree of relatedness between bitches from this breeding programme. Future analysis of larger populations from each breed group would be useful to further investigate any relationship with breed. In addition, examination of the heritability of CEH in dogs would be useful. While the reasons for the possible breed differences remain unclear, they are worthy of note and future analysis should take into account a breed interaction [21].

It is possible that those studies of uterine disease in pet dogs that are reliant upon presentation to a veterinarian when signs of disease manifest, may underestimate the true proportion of dogs affected with CEH due to the lack of clinical signs. Within the current population, routine examination of all bitches allowed for identification of all CEH cases, including mild cases in young dogs with no history of failed conception or uterine infection.

## **5. Acknowledgments**

The authors would like to thank the Guide Dogs breeding technicians for their time in helping to collect the data for this study.

Conflicts of interest: none

- 218 **6. References**[1] Barrau MD, Abel JH, Verhage HG, Tietz WJ. Development of the  
219 endometrium during the estrous cycle in the bitch. *Am J Anat* 1975;142:47-65.
- 220 [2] Feldman, EC and Nelson, RW. Chapter 23: Cystic endometrial  
221 hyperplasia/pyometra complex. In: *Canine and Feline Endocrinology and*  
222 *Reproduction*, 3rd edn., Missouri, USA: Saunders; 2004, p. 852-853.
- 223 [3] Chen YM, Lee CS, Wright PJ. The roles of progestagen and uterine irritant in the  
224 maintenance of cystic endometrial hyperplasia in the canine uterus. *Theriogenology*  
225 2006;66:1537–44.
- 226 [4] Schlafer DH, Gifford AT. Cystic endometrial hyperplasia, pseudo-placentational  
227 endometrial hyperplasia, and other cystic conditions of the canine and feline uterus.  
228 *Theriogenology* 2008;70:349-58.
- 229 [5] Schlafer DH. Diseases of the canine uterus. *Reprod Domest Anim*  
230 2012;47(s6):318-22.
- 231 [6] Okkens AC, Bevers MM, Dieleman SJ, Van Haaften B, Van Sluijs FJ. Fertility  
232 problems in the bitch. *Anim Reprod Sci* 1992; 28:379-87.
- 233 [7] De Bosschere H, Ducatelle R, Vermeirsch H, Van Den Broeck W, Coryn M.  
234 Cystic endometrial hyperplasia-pyometra complex in the bitch: should the two  
235 entities be disconnected? *Theriogenology* 2001;55:1509-19.
- 236 [8] De Bosschere H, Ducatelle R, Tshamala M, Coryn M. Changes in sex hormone  
237 receptors during administration of progesterone to prevent estrus in the bitch.  
238 *Theriogenology* 2002;58:1209-17.
- 239 [9] Hadley JC. The development of cystic endometrial hyperplasia in the bitch  
240 following serial uterine biopsies. *J Small Anim Pract* 1975;16:249-57.
- 241 [10] Noakes DE, Dhaliwal GK, England GCW. Cystic endometrial  
242 hyperplasia/pyometra in dogs: a review of the causes and pathogenesis. *J Reprod*  
243 *Fertil Suppl* 2001;57:395–406.
- 244 [11] Dow C. The cystic hyperplasia-pyometra complex in the bitch. *J Comp Pathol*  
245 *Ther* 1959;69:237-50.
- 246 [12] England GCW, Moxon R, Freeman SL. Delayed uterine fluid clearance and  
247 reduced uterine perfusion in bitches with endometrial hyperplasia and clinical  
248 management with postmating antibiotic. *Theriogenology* 2012;78:1611-17.
- 249 [13] England GCW, Burgess CM, Freeman SL. Perturbed sperm–epithelial  
250 interaction in bitches with mating-induced endometritis. *Vet J* 2012;194:314-18.



- 251 [14] Bigliardi E, Parmigiani E, Cavarani S, Luppi A, Bonati L, Corradi A.  
 252 Ultrasonography and Cystic Hyperplasia–Pyometra Complex in the Bitch. *Reprod*  
 253 *Domest Anim* 2004;39:136–40.
- 254 [15] Verstegen J, Dhaliwal G, Verstegen-Onclin K. Mucometra, cystic endometrial  
 255 hyperplasia, and pyometra in the bitch: advances in treatment and assessment of  
 256 future reproductive success. *Theriogenology* 2008;70:364–74.
- 257 [16] Wilborn RR, Maxwell HS. Clinical approaches to infertility in the bitch. *Vet Clin*  
 258 *North Am Small Anim Pract* 2012;42:457-468.
- 259 [17] Moresco A, Munson L, Gardner IA. Naturally occurring and melengestrol  
 260 acetate-associated reproductive tract lesions in zoo canids. *Vet Pathol*  
 261 2009;46:1117–28.
- 262 [18] Dow C. Experimental reproduction of cystic endometrial-pyometra complex in  
 263 the bitch. *J Pathol Bacteriol* 1959;78:267–79.
- 264 [19] Agnew DW, Munson L, Ramsay EC. Cystic endometrial hyperplasia in  
 265 elephants. *Vet Pathol* 2004;41:179-83.
- 266 [20] Davidson A. Current concepts on infertility in the bitch. *Waltham Focus*  
 267 2006;6:2.
- 268 [21] Hagman R, Lagerstedt AS, Hedhammar Å, Egenvall A. A breed-matched case-  
 269 control study of potential risk-factors for canine pyometra. *Theriogenology*  
 270 2011;75:1251-57.
- 271 [22] Egenvall A, Hagman R, Bonnett BN, Hedhammar A, Olson P, Lagerstedt AS.  
 272 Breed risk of pyometra in insured dogs in Sweden. *J Vet Intern Med* 2001;15:530-38.
- 273 [23] Niskanen M, Thrusfield MV. Associations between age, parity, hormonal therapy  
 274 and breed, and pyometra in Finnish dogs. *Vet Rec* 1998;143:493-98.
- 275 [24] Fukuda, S. Incidence of pyometra in colony-raised Beagle dogs. *Exp Anim*  
 276 2001;50:325-329.

277

278

279

280

281

282

283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302

Table 1. The number of bitches of each breed examined prior to breeding during the two year study period.

Breed	N
Flat Coated Retriever	3
Golden Retriever	30
Golden Retriever cross German Shepherd	1
Golden Retriever cross Flat Coated Retriever	2
Golden Retriever cross Labrador Retriever	23
German Shepherd	19
Labrador Retriever	155
Labrador Retriever cross Golden Retriever	6
Labrador Retriever cross (Golden Retriever cross Labrador)	1

Table 2. The number of ultrasound examinations and number and proportion of CEH bitches examined at different ages.

Age (years)	Number of examinations	Number CEH affected	Proportion CEH affected (%)
1.0 to 1.99	54	0	0.00
2.0 to 2.99	44	3	6.82
3.0 to 3.99	83	8	9.64
4.0 to 4.99	74	12	16.22
5.0 to 5.99	69	21	30.43
6.0 to 6.99	20	12	60.00
7.0 to 7.99	4	4	100.00
Total	348	60	-

Table 3. The number of bitches of each breed/mixed breed, with >10 individuals, that were examined, and the number and proportion of CEH, age range and mean age by breed. .

Breed	Number of bitches	Number CEH affected	Proportion CEH affected (%)	Age range (years)	Mean age (years)
Golden Retriever	30	7	23.33%	1.7 to 6.7	4.2 ± 0.2
Golden Retriever cross Labrador Retriever	23	4	17.39%	1.6 to 5.7	3.5 ± 0.3
German Shepherd	19	5	26.32%	1.7 to 7.4	4.4 ± 0.3
Labrador Retriever	155	27	17.42%	1.6 to 7.2	3.9 ± 0.1
Total	227	43	-	-	-

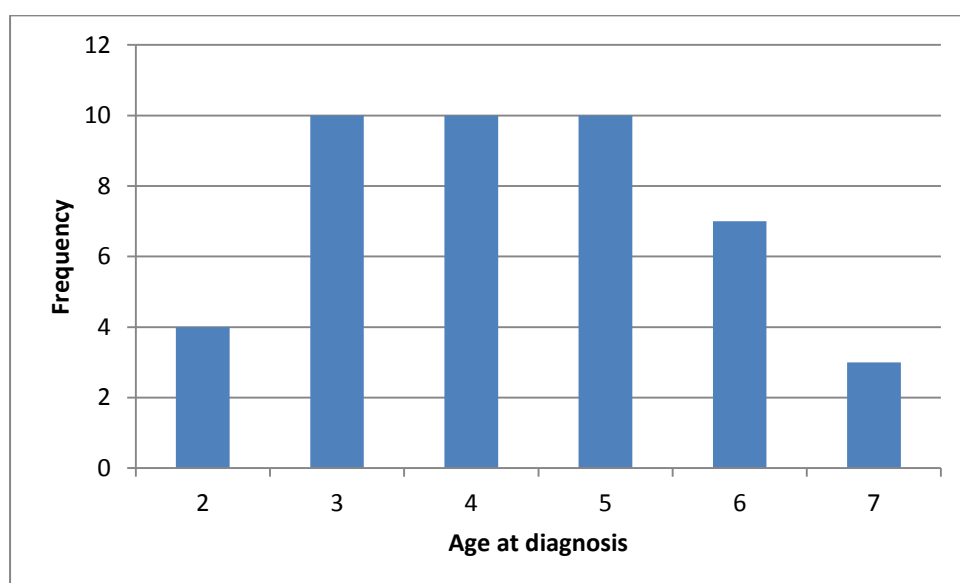
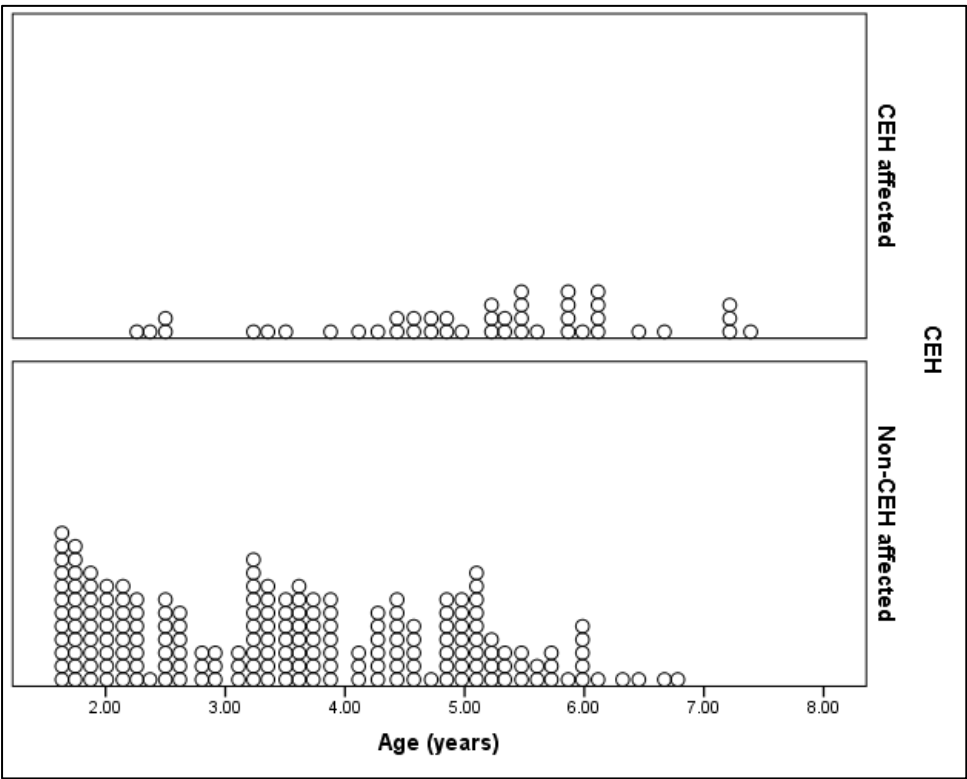


Figure 1. Age at diagnosis distribution for 44 bitches with cystic endometrial hyperplasia.

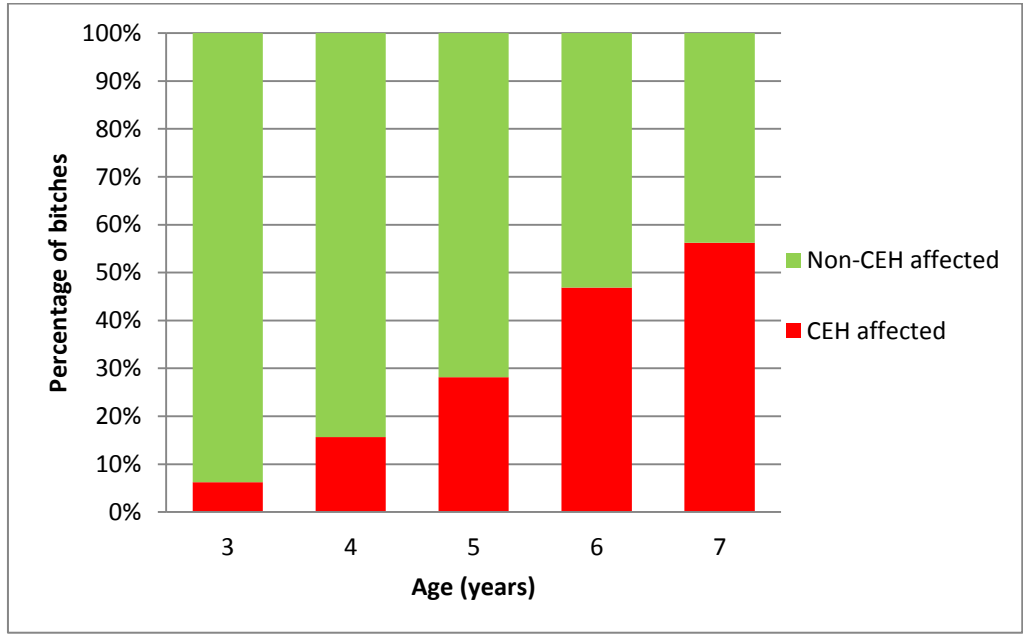
313

314



315  
316  
317  
318

Figure 2. The ultrasound examinations where bitches were found to be CEH affected and non-CEH affected by mean age at examination.



319  
320  
321  
322

Figure 3. The proportion of non-CEH affected and CEH affected bitches at each year of age throughout their breeding lives for 32 bitches that had retired from the breeding programme.

