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2 Prevalence of ultrasound-determined cystic endometrial hyperplasia and the

3 relationship with age in dogs Rachel Moxon[a*], Helen Whiteside[a] and Gary C.W.

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13 **Abstract**

14 To investigate the potential relationship between age and diagnosis of cystic

15 endometrial hyperplasia (CEH) in the bitch, 348 ultrasound examinations from 240

16 bitches (Labradors, Golden Retrievers, German Shepherds, Flat Coated Retrievers

17 or crosses of those breeds aged between 1.6 and 7.2 years at examination) were

18 examined. A subpopulation of 32 bitches that had completed their breeding careers

19 at ≥ 6 years of age was also identified. 18.3% of bitches were diagnosed with CEH;

20 these cases were newly diagnosed when bitches were between 2.5 years and 7.3

21 years of age. The proportion of ultrasound examinations where CEH was identified

22 increased from 6.8% of examinations on two year old breeding bitches to 60.0% of

23 examinations on six year old bitches. Logistic regression identified a positive

24 correlation between mean age at examination and presence of CEH (x^2 = 30.74, DF

25 = 1, p <0.001). Within the 32 bitches that had retired from breeding the prevalence of

26 CEH was 56.3%, age at diagnosis ranged from 3.8 to 7.3 years and the proportion of

27 bitches affected with CEH increased from 6.3% at 3 years of age to 56.3% at 7 years

28 of age. Thus the data support the contentions of other authors that CEH is related to

29 age.

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32

33   **1. Introduction**

34   Hormonal stimulation during the oestrous cycle of the bitch induces changes in the

35   endometrium including glandular proliferation and secretion [1-3]. Cystic endometrial

36   hyperplasia (CEH) is an abnormality of uterine growth and repair arising from the

37   endometrial glandular epithelium where there is cystic distension of the endometrial

38   glands [4,5]. In breeding bitches, CEH seems to be an abnormal response to

39   stimulation of the uterus by ovarian hormones; progesterone and oestrogen [2,3,6-8]

40   and can also be triggered by uterine irritants and endometrial trauma [2,3,9,10]. In

41   CEH cases the number and size of endometrial glands are increased and there is

42   disparity in the number and configuration of glands causing a thickened

43   endometrium and increased secretory activity [2,11]. In bitches affected with CEH,

44   post-mating endometritis appears to impact fertility by reducing the uterine

45   vasodilatory response to mating and impairing the clearance of uterine fluid as a

46   result of decreased uterine contractions when compared to normal bitches [12]. A

47   larger PMN influx has been observed in bitches with CEH post-mating which could

48   affect the ability of spermatozoa to attach to the uterine epithelium with related

49   impacts on fertility [13]. Previous research has demonstrated poor conception rates

50   and lower litter sizes for bitches affected with CEH without treatment [12,13]. In

51   addition to reduced fertility, the degenerative changes within the tissue associated

52   with CEH can provide conditions suitable for the establishment of uterine infections

53   and in some cases pyometra can develop [2,7,14,15]. The relationship between age

54   and incidence of pyometra has been documented [22,23,24]. Age has been

55   proposed as a risk factor for a breeding bitch being affected by CEH due to the

56   repeated hormonal stimulation of successive oestrus cycles in entire bitches as they

57   age. Indeed it has been suggested that older bitches are likely to have some degree

58   of CEH [11,14,16] whilst Verstegen et al [15] suggest that all dogs will develop CEH

59   with age. However, no previous work has presented data for the prevalence of CEH

60   alone in a population of bitches by age, without associated pyometra. The seminal

61   work by Dow [11] only included bitches with disease rather than a whole population

62   and considered the cystic hyperplasia pyometra complex rather than two distinct

63   diseases. In Zoo canids and elephants, significant association between the

64   development of endometrial hyperplasia and increasing age has been established

65   and reported based on the prevalence of CEH within a population [17,19].

66   Therefore, this study aimed to examine a population of breeding bitches and to

67   report on three factors: 1) the age at which cases of CEH were diagnosed; 2) the

68   prevalence of CEH in ultrasound examinations conducted on bitches at different

69   ages and 3) the incidence of CEH in a population of retired breeding bitches which

70   had been examined throughout their breeding lives.

71

72   **2. Materials and methods**

73   Between 21 September 2012 and 20 September 2014, 240 bitches from a large,

74   relatively closed, breeding population were examined as part of routine health

75   management prior to breeding. Bitches were Labradors, Golden Retrievers, German

76   Shepherds, Flat Coated Retrievers or crosses of those breeds (Table 1), were from

77   68 unique sires and 126 unique dams and were between 1.6 and 7.2 years of age at

78   examination. One hundred and thirty-nine bitches were examined once, 94 were

79   examined twice and seven were examined three times within the two-year study

80   period.

81

82   INSET TABLE 1 NEAR HERE

83

84   In total, 348 detailed transabdominal ultrasound examinations were conducted using

85   a 10 MHz transducer. In every ultrasound examination, the uterine body and distal

86   uterine horns were studied; proximal uterine horns were examined wherever

87   possible. Bitches were allocated to control or CEH groups as previously described

88   [12]. CEH cases were categorised as 'New case' or 'Existing case' dependent on

89   whether the bitch was diagnosed with CEH during the current examination and had

90   not been diagnosed at any previous examination (New case) or had been diagnosed

91   with CEH prior to the study period (Existing case). Age at first diagnosis was

92   recorded for all CEH affected bitches.

93

94 A subpopulation of 32 bitches out of the 240 had retired from the breeding

95 programme at ≥ 6 years of age. These bitches had been examined as part of the

96 current study but additionally had ultrasound examinations recorded prior to the

97 study commencing. The historic data and current study data were examined to report

98 the prevalence of CEH and to determine the proportion of CEH affected bitches at

99 each year of age.

100

101 *2.1 Statistical analysis*

102 Data were investigated using XLStat (Addinsoft, USA) and IBM SPSS Statistics 20

103 (USA). Age at diagnosis was described for all CEH affected bitches. The number of

104 ultrasound examinations conducted on bitches of each age was reported along with

105 the proportion of examinations at each age where CEH was observed.

106 To determine whether there was a relationship between age and presence of CEH,

107 repeat examinations for individual bitches were excluded by calculating mean age at

108 examination. A binary logistic regression was conducted to predict presence of CEH,

109 using age as the predictor.

110

111 **3. Results**

112 *3.1 Number of bitches with CEH during the two year period and age at diagnosis*

113 There were 44/240 (18.3%) bitches that were diagnosed with CEH when examined

114 by ultrasound; 40 New cases and four Existing cases. These cases were newly

115 diagnosed when bitches were between 2.5 and 7.3 years of age (mean 4.9 +/- 0.2

116 years; Figure 1).

117

118 INSERT FIGURE 1 NEAR HERE

119

120 *3.2 The prevalence of CEH in ultrasound examinations conducted on bitches at*

121 *different ages*

122 The proportion of ultrasound examinations conducted on bitches of each year of age

123 from 1.0 to 7.99 years where CEH was present, including new and existing cases,

124 increased from 6.8% of examinations on two year old breeding bitches (n=46) to

125 60.0% of examinations on six year old breeding bitches (n=20; Table 2).

126

127 INSERT TABLE 2 NEAR HERE

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129 Mean ages were calculated for 101 bitches that had data for more than one

130 ultrasound examination. Repeat examinations were 0.91 ± 0.03 years apart. Logistic

131 regression revealed a positive relationship between mean age at ultrasound

132 examination and presence of CEH (x^2 = 30.74, DF = 1, p <0.001; Figure 2).

133

134 INSERT FIGURE 2 NEAR HERE

135

136 For breeds with >10 individuals, the highest proportion diagnosed with CEH was

137 German Shepherds (26.3%; Table 3). The difference between breeds was not

138 significant (Yates' Chi Square = 0.575, D.F. = 3, P = 0.902).

139

140 INSERT TABLE 3 NEAR HERE

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143 *3.3 CEH in retired breeding bitches*

144 There were 32 bitches within the sample of 240 that had been examined each year

145 from three years of age to retirement at ≥ 6 years of age and prevalence of CEH for

146 these bitches was 56.3% (n=18). Age at diagnosis ranged from 3.8 to 7.3 years

147 (mean = 5.8 +/- 0.25 years). The prevalence of hyperplasia increased each year with

148 age so that the proportion of bitches affected with CEH increased from 6.3% at 3

149 years of age to 56.3% at 7 years of age (Figure 3).

150

151 INSERT FIGURE 3 NEAR HERE

152

153 **4. Discussion and conclusion**

154   This study examined a large population of breeding bitches that were subjected to

155   ultrasound examinations of the uterus as part of routine breeding management.

156   Almost one fifth of the bitches within the breeding population had CEH, which was

157   first diagnosed between 2.5 and 7.3 years of age. Examinations on older bitches

158   were more likely to find individuals affected with CEH (60% of examinations on

159   bitches 6 to 7 years of age and 100% of examinations on bitches 7 to 8 years of age)

160   than examinations on younger bitches (6.8% of examinations on bitches 2 to 3 years

161   of age and 9.6% of examinations on bitches 3 to 4 years of age). There was a

162   significant positive relationship between mean age at examination and presence of

163   CEH, with more examinations having CEH present when mean age was higher.

164   While the calculation of mean age at examination, required due to the repeat

165   examinations for 101 of the bitches in the study, may have had a small influence on

166   this finding, it remains consistent with the contentions of other authors. Bigliardi et al

167   [14] suggested that bitches over five years of age in Italy, where the average age at

168   neutering is higher than in the UK or USA, are more frequently diagnosed with CEH

169   and Dow [11,18] suggested that cystic endometrial hyperplasia is rarely observed in

170   bitches of <4 years of age.

171   In the population of retired bitches (n=32) prevalence of CEH was 56.3%. This study

172   is unique in that it was able to examine the CEH status of a number of bitches over

173   their lifetime. The bitches were neutered at the end of their breeding careers

174   (approximately 8 years of age) and so it is possible that more bitches would have

175   developed CEH later in life had they been left entire and examined regularly.

176   Additionally, as all of the bitches studied were in a controlled breeding colony, all

177   were mated on average once every other oestrus with high conception rates (>90%,

178   data not presented). CEH has been reported to be more common in nulliparous

179   mammals, including dogs, and therefore a protective effect of pregnancy proposed

180   [11,18,19-21]. Ultrasound examination of a large population of entire nulliparous

181   bitches throughout their lifetimes would be required to provide greater accuracy

182   regarding true prevalence and age of appearance. In addition, examining a large

183   number of older entire bitches with no clinical signs could provide further useful

184   information on the prevalence in older bitches since the sample size in the present

185   study was small (n=4).

186 When studying pyometra, a difference in risk between breeds has been reported with
187 Golden Retrievers as one of the breeds demonstrating an increased risk [22,23].
188 Protective effects of pregnancy have been noted to vary by breed for pyometra and
189 have been reported to be present in the Labrador but not the Golden Retriever
190 [21,22]. Within the current study there were non-significant trends towards
191 differences in the proportion of bitches of each breed that were diagnosed with CEH;
192 Labradors and Golden Retriever cross Labradors had lower proportions of bitches
193 affected with CEH than Golden Retrievers and German Shepherds. While a
194 protective effect of pregnancy against CEH has been proposed, this is the first time
195 that a possible difference between breeds for CEH has been reported. It is also
196 possible that the difference may be due to a higher mean age of bitches examined
197 within the Golden Retrievers compared to Labradors and the Golden Retriever cross
198 Labrador Retrievers and differences may have been influenced by the degree of
199 relatedness between bitches from this breeding programme. Future analysis of
200 larger populations from each breed group would be useful to further investigate any
201 relationship with breed. In addition, examination of the heritability of CEH in dogs
202 would be useful. While the reasons for the possible breed differences remain
203 unclear, they are worthy of note and future analysis should take into account a breed
204 interaction [21].

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

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212 **5. Acknowledgments**

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216 Conflicts of interest: none

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297 Table 1. The number of bitches of each breed examined prior to breeding
298 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

299

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

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305 Table 3. The number of bitches of each breed/mixed breed, with >10 individuals, that were
306 examined, and the number and proportion of CEH, age range and mean age by breed. .
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308

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	-	-	-

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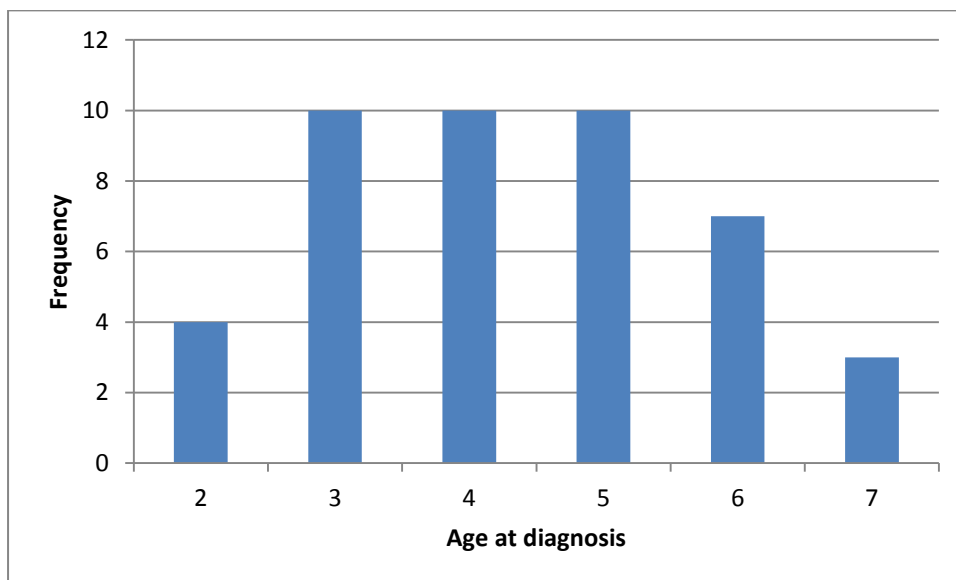
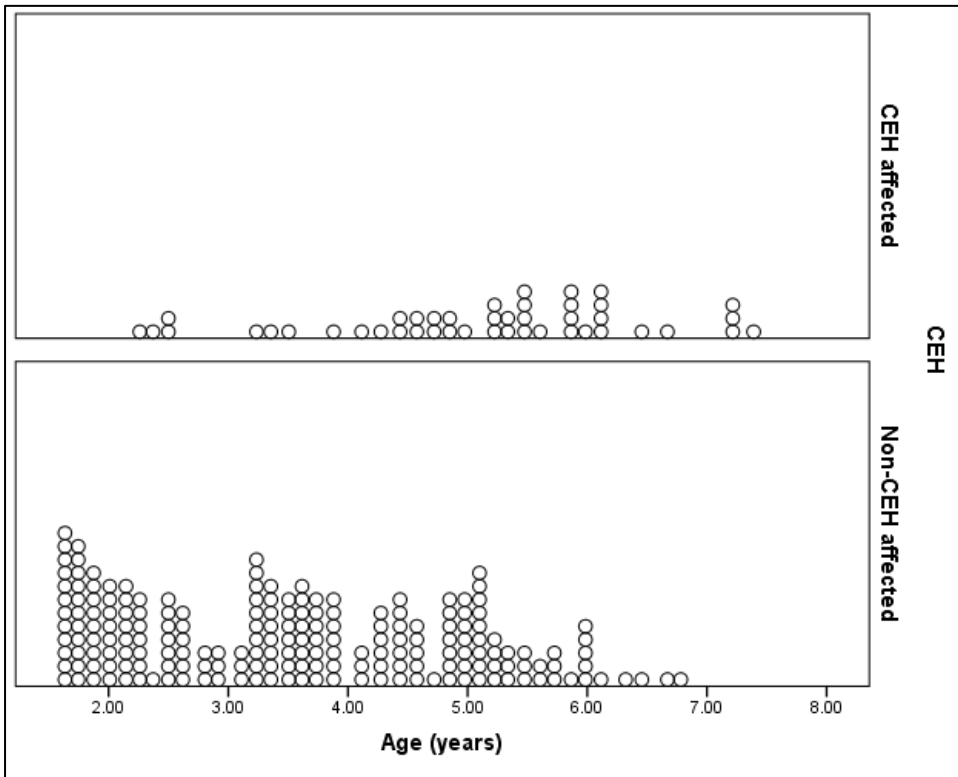311
312 Figure 1. Age at diagnosis distribution for 44 bitches with cystic endometrial hyperplasia.

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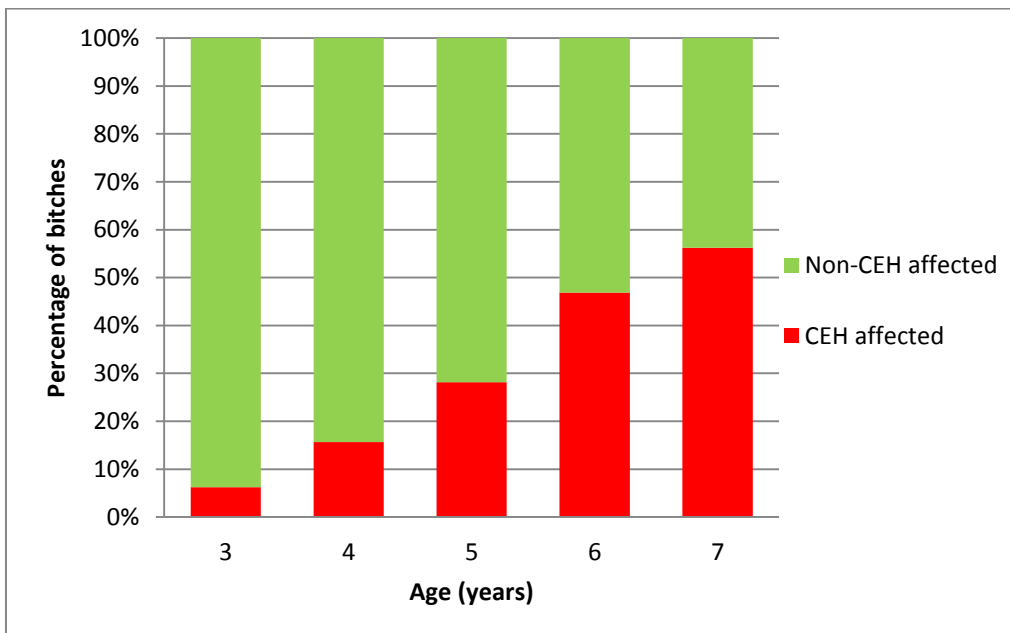
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Figure 2. The ultrasound examinations where bitches were found to be CEH affected and non-CEH affected by mean age at examination.

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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.

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