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4	Lop-eared rabbits have more aural and
5	dental problems than erect-eared
6	rabbits: a rescue population study
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24 Abstract

This research aimed to assess whether rabbits having lop-ears is a welfare issue by
investigating the occurrence of aural and dental pathology in lop-eared compared with erecteared rabbits.

28 Thirty rabbits (15 lop-eared and 15 erect-eared) from a rabbit-only rescue shelter were

examined. An otoscope was used to visualise the ear canals and mouth. Samples were taken

30 from each ear to examine for mites, bacteria and yeast. Medical records were also examined.

Lop-eared rabbits showed statistically significantly more frequent ear canal stenosis, higher scores of cerumen and erythema, and more frequent potential pain response during ear examination, compared with erect-eared rabbits. We also found statistically significantly more frequent incisor pathology, molar overgrowth, molar sharpness, molar spurs and history of veterinary dental treatment in lop-eared compared with erect-eared rabbits. The effect sizes were often large. Age was not statistically significant between the lop- and erecteared rabbit groups.

Thus, lop-eared rabbits are at an increased risk of aural and dental pathology. This brings into debate the ethics of breeding and buying lop-eared rabbits, as they are more likely to suffer conditions that negatively impact welfare, such as pain, and potentially deafness and difficulty eating. 42 Key words: Animal welfare, Conformation; Dental pathology; Otitis; Rabbits; Veterinary43 Research

44 Introduction

Breeding animals for extreme traits is coming under scrutiny, and has thus far mostly 45 focused on $dogs^{1-3}$. However, the ethics of breeding extreme rabbit conformations, such as 46 pendulous ears and brachycephaly, is also starting to be debated^{4, 5}. Lops are among the most 47 48 common pet rabbit breed groups, representing 57% (n=58/102) and 36% (n=449/1254) of 49 pet rabbits in England according to two surveys by Mullan and Main (2006) and Rooney et 50 al. (2014), respectively. Thus, if the lop-eared phenotype is associated with suffering, it 51 could constitute a prevalent rabbit welfare issue. However, clear evidence of whether this is 52 the case is lacking.

Domestication of the wild rabbit has created at least nine breeds with a lop-eared phenotype, as it is a heritable trait^{6, 7}. The wild type has erect and mobile pinna due to three interdigitating auricular cartilages that provide a scaffold for the vertical ear canal^{8, 9}. In contrast, lop-eared breeds have 3-5mm of soft tissue between the proximal acoustic meatus cartilage ring and the tragal cartilage of the distal ear canal, causing the pinna to fold and become pendulous^{9, 10}.

59 Charles Darwin first noted how artificial selection for pendulous pinnae in rabbits was 60 associated with altered skull morphology¹¹. Cordero and Berns (2016) found supportive 61 evidence of this in natural woodrat populations that have likely undergone selection for long 62 ears, finding substantial covariance of ear length and shape of the neighbouring auditory 63 meatus ¹². This means that the lop-eared phenotype could not just affect aural function 64 directly, but also other aspects of cranial health, such as dental function.

65 Potential consequences for aural health

Lop-eared rabbits have increased stenosis (narrowing) of their ear canals^{8, 13, 14}. This, along 66 67 with ear canal occlusion by the pinna, is believed by some veterinary professionals to reduce airflow and hinder expulsion of cerumen (or 'earwax')^{9, 10, 13}. Cerumen accumulation at the 68 69 base of the ear in lop-eared rabbits has been suggested as a predisposing factor for otitis externa, caused by bacterial or yeast overgrowths^{10, 13, 15, 16}. Mite infestation with *Psoroptes* 70 *cuniculi* can also cause otitis externa, usually with extensive crusting of the pinna⁹. 71 Veterinary professionals have observed that otitis externa can cause head shaking, ear 72 scratching, pain, depression and inappetance in rabbits^{17, 18}, and if it progresses to otitis 73 media and interna then head tilts and neurological deficits can occur¹⁸. Veterinary opinion 74 75 suggests that deafness can result from excess cerumen accumulation, otitis interna, or chronic otitis externa or media, at least in cats and dogs¹⁹, so it is possible that this could 76 77 occur in rabbits also. Additionally, if the length of the lop ear is great, such as in English lops, then there could be increased risk of trauma to the pinnae⁵. 78

79 Despite the above veterinary and animal welfare expertise suggesting impacts of the lop-80 eared morphology on aural health, there has been limited empirical research into the issue; 81 we found just one empirical study specifically comparing lop-eared and erect-eared rabbits, which solely investigated aural Malassezia yeast variation, and found no significant 82 difference²⁰. Thus, there is currently little evidence to argue the ethics of breeding and 83 84 buying lop-eared rabbits. Veterinary opinion is mostly formed on the basis of experience 85 with populations requiring veterinary attention, so it is unknown whether the same 86 associations exist in otherwise healthy rabbits. However, it is possible that they do, because 87 canine research has found that dogs with pendulous ears have a higher risk of otitis externa

- 88 and an increased risk of *Malassezia species* infection, compared with dogs with erect-ears²¹⁻
- 23 . Thus, we hypothesise that these findings may also extend to rabbits with lop-ears.

90 Potential consequences for dental health

91 Veterinary professionals have suggested that the altered skull morphology of lop-eared 92 rabbits can cause congenital malocclusion of the dental arcades, such as maxillary brachygnathism (relative shortening of the lower jaw)²⁴. This is also believed to occur in 93 brachycephalic and dwarf breeds including the Netherland dwarf^{24, 25}. Rabbit teeth grow 94 95 continuously, so reduced attrition caused by malocclusion (i.e. if the upper and lower teeth are mis-aligned) can cause the teeth to overgrow^{9, 26}. This has animal welfare implications 96 97 because, if the teeth are not worn down appropriately then incisor overgrowth, elongated 98 molar tooth roots and molar spurs can occur 26 . Rabbit incisors can grow at 2-2.4mm per 99 week so problems can rapidly occur and reoccur after treatment, especially if the problem is due to malocclusion and thus cannot be rectified by husbandry changes²⁷. Maxillary root 100 101 overgrowth can penetrate the nasolacrimal duct and present as epiphora (watering of the eve) and secondary dacrocystitis (infection of the lacrimal sac) $^{27-29}$. Root elongation can put 102 pressure on sensory nerves innervating the teeth causing pain²⁷. Further pain can occur if 103 104 overgrown incisors penetrate the soft tissue of the lips, and mandibular and maxillary molar spurs can lacerate the lingual and buccal mucosa respectively²⁷. 105

Dental disease is a chronic condition and the resulting oral pain and physical restrictions of
overgrown teeth can lead to inappetance, poor body condition, and if severe can cause
mortality through gut stasis^{28, 30}. Other presentations of dental disease due to oral discomfort
are hypersalivation, and a lack of grooming or caecotrophy that can lead to flystrike^{26, 28, 30}.

110 Aims and hypotheses

This research evaluated the number and type of aural and dental abnormalities in lop-eared versus erect-eared rabbit groups in a non-veterinary context: a rabbit-only rescue centre. We used two complementary sources of information: standardised direct observations of the rabbits, and medical records for the same rabbits; the latter were more heterogeneous and clinically driven than the observations, but could indicate past issues in the rabbits. We hypothesised that, if the lop-eared phenotype leads to functional impairment, there

117 would be significantly more aural pathology, such as ear canal stenosis, cerumen

accumulation and inflammation, and dental pathology, such as incisor overgrowth and molar

spurs, in lop-eared rabbits than in erect-eared rabbits.

120 Materials and Methods

121 Animals and husbandry

122

123 A convenience sample of fifteen lop-eared and fifteen erect-eared rabbits were selected for 124 examination at a rabbit rescue centre. The sample size of 30 was chosen on the basis of 125 feasibility, because the observations required completion within a relatively short time 126 period, and without disrupting the husbandry routines of the rabbits at the shelter. The rabbits 127 were selected by the rescue manager, who attempted to include a wide range of breeds and 128 ages (where ages were known), and only included rabbits amenable to handling (for animal 129 welfare, and health and safety reasons). She was not aware of the hypothesis, but she was 130 aware that we would examine the ears and teeth of the rabbits as well as general health, and 131 we did specify an equal number of lop- and erect-eared rabbits.

132 The rescue centre was capable of holding approximately 100 rabbits. All rabbits observed

133 were housed in outdoor enclosures measuring at least 8ft x 6ft, which varied but typically

134 comprised a large aviary with a hutch or shelter. Most rabbits (22/30) were housed in

opposite sex neutered pairs, whilst eight were singly housed, and environmental enrichment
included tunnels and chew toys. Water was provided *ad libitum* from bottles, and the diet
consisted of a twice daily supply of hay, pellets (BurgessTM, Pickering), and fresh leaves and
vegetables.

139 Data were collected about each rabbit, including ear type (lop or erect), breed, face shape, sex, age, weight, and body condition score³¹. Face shape was assessed by subjectively 140 141 observing the approximate length and breadth of the skull, and subsequently categorised into 142 brachycephalic (broad, short skull, particularly seen in breeds such as the Netharland Dwarf 143 and Lionhead), mesocephalic (moderately short and broad skull) and doliocephalic (narrow 144 and long skull, as seen in the wild type) based on the independent opinion of two observers. 145 Data were collected on standardised recording sheets (Supplementary material). The study 146 received ethical approval from the Royal Veterinary College's Clinical Research Ethical 147 Review Board (URN 2015 1372).

148

149 Aural health examination

150 Firstly, an observer (JCJ) stood quietly, approximately 1m from the enclosure, for 5 min to 151 record presence or absence of head shaking and ear scratching, and the number of bouts of 152 these. Then, the rabbit was gently restrained by an assistant whilst the observer performed a 153 subjective clinical examination as summarised in Table 1 (details can be seen in 154 supplementary information). Unfortunately, it was not possible to test inter-observer 155 reliability during the examinations, as the available assistants were not medically trained so 156 could not assess the ears or teeth. During direct observations, the observer could also not be 157 blind to ear shape, so to minimise bias, this was explicitly discussed before observations 158 started; we examined the possibility that lop-eared rabbits having conformational problems

could be erroneous, especially since the only scientific comparison to date had found no
cytological issues²⁰, and that science was important for debunking incorrect perceptions. We
were also aware of methods for interpreting and publishing studies where no statistical
significance was founde.g. ^{32, 33}, which minimised any incentive to find differences where
none may exist.

164 The bases of the ears were palpated for swelling and the skin checked for evidence of scratching. The external pinnae were observed for crusting and inflammation (erythema, 165 166 heat and swelling). An otoscope was used to visualise the ear canal to look for erythema, 167 stenosis, increased cerumen, abnormal masses and potential pain responses such as flinching 168 or struggling during only this part of the exam: the examination was shortened and not 169 forced if this occurred, for animal welfare reasons (if the rabbit showed this response 170 generally upon initiation of the exam then it was deemed to be due to fear, rather than pain, 171 and the rabbit was excluded from the study due to it being unable to be examined). The 172 otoscope heads were sterilised in a chlorhexidine solution between subjects.

Table 1. The descriptive criteria for each variable investigated statistically. The criteria are arranged
such that aural pathology is first described, followed by dental pathology. Full details of the data
collected are provided in the Supplementary information.

Examination	Variable for	Criteria required for each variable to be identified as 'present'	
area	analysis		
Aural	Stenotic ear canals	On examination the internal ear canal was deemed to be narrow, the	
		otoscope specula was unable to be passed down the canal, or being	
		unable to visualise down the canal due to limited space between the	
		ear canal walls	

Potential aural	On examination the rabbit appeared painful when palpating the base		
pain response	of the ear, inserting the otoscope specula into the ear canal, or		
	swabbing the ear canal		
	'Appeared painful' was defined as: flinching or struggling during		
	only this part of the exam		
Cerumen of the ear	On aural examination there was subjectively deemed to be increased		
canal	cerumen in the ear canal.		
	Categories were defined as:		
	0= normal low level of cerumen		
	1= mild amount of cerumen		
	2= moderate amount of cerumen		
	3= large amount of cerumen		
	Each ear was categorised separately, then a combined total of both		
	ears was used for the analysis		
Erythema of the	On aural examination there was subjectively deemed to be increased		
ear canal	reddening of the ear canal		
	Categories were defined as:		
	0= pale pink, normal		
	1= pink		
	2= dark pink		
	3= red		
	Each ear was categorised separately, then a combined total of both		
	ears was used for the analysis		
Scratching the ears	A bout of head shaking or ear scratching was seen during the 5 min		
	pre-examination observation;		
	Or there was fur loss, skin scaling or scabs around the ears on		
	examination		
Malassezia-like	This was categorised in multiple formats:		
yeast species	Categorisation 1:		

		5 yeasts or more identified on average per 10 high power fields
		Categorisation 2 (presence of yeast species):
		1 yeast or more identified on average per 10 high power fields
		Categorisation 3 (average number of yeasts per high power field):
		Total number of yeasts for combined left and right ears analysed
	Aural veterinary	The veterinary medical history reported a treatment for aural disease
	treatment	whilst the rabbit was in the rescue's care, including ear flush, ear
		canal surgery, and ear drop prescription
Dental	Incisor pathology	Incisor malocclusion (underbite or overbite), overgrown incisors or
Dentai	neisor patiology	fractured incisors were identified on oral examination;
		Or veterinary treatment for incisor pathology was recorded in the
	Molor overerowth	veterinary record
	Molar overgrowth	Abnormally long molars or molars of different height to the other
		molars were identified on oral examination
	Sharp molars	On oral examination there was subjectively deemed to be an
		increased sharpness of the molars at the buccal and lingual points,
		but that were not severe enough to be classified as spurs
	Molar spurs	Buccal or lingual spurs were identified on oral examination;
		Or veterinary history of treatment for molar spurs (burring)
	Oral mucosal sores	Current or scarred sores were visible on the oral mucosa on oral
		examination
	Potential oral pain	The rabbit appeared painful when palpating the jaw region
	response	The rabbit appeared painful when using the otoscope to examine the
		oral cavity
		'Appeared painful' was defined as: flinching or struggling during
		only this part of the exam
	Epiphora	Excess discharge from the eye, a crusting or damp area below the

	eye, seen on gross visual examination;		
	Or excess discharge from the eye reported in the rescue's or		
	veterinary history;		
	Or a treatment for epiphora reported, such as a tear duct flush		
Dental veterinary	The veterinary medical history reported a treatment for dental		
treatment	disease whilst the rabbit was in the rescues care, such as incisor		
	trimming or removal, or molar spur burring		
Poor coat	The coat looked unkempt on examination, such as matting of the fur		
condition			
Hypersalivation	There was deemed to be a large amount of saliva at oral		
	examination;		
	Or if there was wet or crusty fur around the mouth, dewlap or front		
	paws on examination		
Lack of	Caecotrophs were present in the perineal region on examination		
caecotrophy			

176

177 Two samples were taken from each ear by gently inserting a sterile cotton swab into the 178 external part of the ear canal and rotating fully three times. The samples were labelled with 179 codes so that the observer could examine them blind to whether they originated from a 180 rabbit with lop or erect ears. One sample from each ear was put onto a glass microscope 181 slide with liquid paraffin and then covered with a cover slip. These slides were evaluated 182 microscopically the same day using low magnification (10X) and high contrast to assess for 183 mites. The other sample was rolled onto a microscope slide, allowed to dry for 10 min and then stained using Diff-quik (Rapi-Diff II stain, Atom Scientific, Cheshire) as per the 184 185 manufacturer guidelines. Cytological assessment began with 40X magnification to find a 186 representative area of interest, which was then evaluated using oil immersion and 100X

magnification, to count the average number of bacteria (cocci and rods), and yeasts across
10 microscopic high-power fields. Cocci were identified as well-defined, circular and blue
stained, and rods were identified as well-defined, rod shaped and blue stained.

190 Dental health examination

191 After conducting the aural examination, the rabbit's face was palpated externally for facial 192 swellings, bony protrusions along the mandible and maxilla, ability to close the mouth and 193 symmetry of the face. The examiner gently separated the lips manually using fingers and the 194 eight incisors were evaluated for pathology including malocclusion, overgrowth, fractures or 195 abnormal appearance such as horizontal ridging of the tooth surface. Then an otoscope was 196 used in the mouth to assess the molars for overgrowth, sharpness, spurs, fractures and 197 variation in tooth height. The lingual and buccal soft tissues were examined using the 198 otoscope for sores and scars indicating healed sores. Evidence of potential pain was 199 monitored for, such as flinching or struggling during only this part of the exam, and the 200 examination was again shortened if this occurred. Secondary signs of dental disease were 201 looked for including epiphora, poor coat condition, hypersalivation (also seen as wet or 202 crusty fur around the mouth and throat), and caecotrophs around the perineum.

203 Medical history records

The medical history of the rabbits whilst at the shelter was checked using hard copies of veterinary records and the rescue centre's weekly health check record, especially focusing on dental and aural disease and any treatments for these. This was carried out after all rabbit examinations were completed, so that the examinations were not biased by knowledge of existing medical conditions.

209 Statistical analysis

210 Where necessary, rare categories of data were collapsed together with similar categories to 211 enable statistical analysis, with the final categories as described in Table 1. IBM SPSS 212 statistics version 24 was used to carry out binary logistic regression on binary dependent 213 variables. The predictors used in the model were ear type, sex, weight and face shape. Five 214 rabbits had unknown ages (three with erect ears and two with lop ears), so, because age was 215 not a statistically significant predictor in any of the initial models, and because mean age 216 showed no significant difference between both groups (as analysed using binary logistic 217 regression: P=0.492), it was removed from the final models, allowing data from all 30 218 rabbits to be included. The following outcomes were compared in lop- and erect-eared rabbit 219 groups using binary logistic regression: incisor pathology, molar overgrowth, sharp molars, epiphora. lack of caecotrophy, stenosis of the ear canal, potential aural pain, and the 220 221 presence of yeast.

However, no rabbits from the erect-eared group at all were affected for the following
variables: molar spurs, potential oral pain response, oral mucosal sores, poor coat condition,
hypersalivation, veterinary dental treatment, aural veterinary treatment, and scratching the
ears. This meant there were fewer than five values in one group, so a Fisher's exact
statistical test was carried out using GraphPad Prism Version 7.

For outcomes that were recorded as continuous scores, Mann-Whitney U statistical tests were carried out using GraphPad Prism Version 7. This was the case for the average number of yeasts per high power field, cerumen in the ear canal and erythema in the ear canal.

230 Results

232 Demographics

233

Rabbit signalment is shown in Table 2. The known ages of the erect-eared rabbit group
ranged from 5 months to 9 years, whilst those of the lop-eared rabbit group ranged from 8
months to 7 years 9 months. The mean age was not statistically different between the lopand erect-eared groups (P=0.492). The rabbits' body weights ranged from 1.4kg to 3.5kg in
both groups. The erect-eared breeds mainly consisted of cross breeds (n=10), followed by
Lionheads (n=3), whilst Dwarf lops (n=3) and Mini Lops (n=3) were the most common lopeared rabbits.

Signalment	Lop-eared	Erect-eared
Mean known age +/-	4.1 +/- 2.2	3.6 +/- 2.7
standard deviation		
(years)		
Sex (Females;	5; 10	7; 8
Males)		
Mean body weight	2.2 +/- 0.5	2.3 +/- 0.6
+/- standard		
deviation (kg)		
Mean body	3.00 +/- 0.27	3.03 +/- 0.35
condition score +/-	Range = 2.5-3.5	Range = 2.5-3.5
standard deviation		
Face shape	3 brachycephalic	2 brachycephalic
	6 mesaticephalic	2 mesaticephalic
	6 doliocephalic	11 doliocephalic
Breeds	Dwarf lops (x3); Mini lops (x3); Crossbreeds	Crossbreeds (x10);

(x3); Cashmere lops (x2); English lop (x1);	Lionheads (x3); Astrex
Butterfly lop (x1); Harlequin lop (x1); Frost	Rex (x1); Angora (x1)
point lop (x1)	

Table 2. Demographic details of the lop-eared and erect-eared rabbit groups.

243

245

244 Aural health results

246 Statistical results are summarised in Table 3. Lop-eared rabbits had approximately forty-

three times higher odds of having stenotic ear canals (OR=42.7; 95% CI: 4.2-434; P=0.002)

and a statistically significantly higher erythema score of the ear canal (U=50.5; N=15;

249 P=0.004; Figure 1), compared with erect-eared rabbits. They also had a statistically

250 significantly higher cerumen score in the ear canal compared with erect-eared rabbits

251 (U=24; N=30; P<0.001; Figure 2). Lop-eared rabbits had approximately fifteen times higher

252 odds of demonstrating a potential pain response during ear examination than erect-eared

253 rabbits (OR=14.8; 95% CI: 1.1-200.9; P=0.043).

254

255 <Figures 1 and 2 about here>

256

The health records revealed that 14/15 of the lop-eared rabbits had at least one recording of excess wax during health checks by the rescue staff, compared with 3/15 of the erect-eared rabbits (Fisher's Exact $X^2 = 13.575$; DF = 1; P < 0.001). The rescue carried out repeated ear cleaning in 9/15 of the lop-eared rabbits, compared with none of the erect-eared rabbits (Fisher's Exact $X^2 = 10.159$; DF = 1; P = 0.001).

Examination	Pathology	Cases in	Cases in	Significance	

area	investigated	lop-eared	erect-eared	between lop- and
		rabbits	rabbits	erect-eared
		(n=15)	(n=15)	groups
				(P=)
Aural	Ear canal stenosis	13	2	0.002
	Potential aural pain	6	1	0.043
	response			
	Scratching the ears	2	0	0.483
	Mallasezia-like	12	8	0.174
	yeast species			
	Record of aural	4	0	0.100
	veterinary			
	treatment			
	Record of excess	14	3	< 0.001
	cerumen			
	Record of repeated	9	0	0.001
	ear cleaning			
Dental	Incisor pathology	7	1	0.015
	Molar overgrowth	13	6	0.015
	Sharp molars	13	6	0.015
	Molar spurs	11	0	0.006
	Oral mucosal	2	0	0.483
	sores/scars			

Potential oral pain	2	0	0.483
response			
Epiphora	4	1	0.095
Poor coat condition	3	0	0.224
Hypersalivation	2	0	0.483
Lack of	5	2	0.274
caecotrophy			
Record of dental	6	0	0.017
veterinary			
treatment			

Table 3. Prevalence of binary variables investigated in both lop and erect-eared rabbit groups and the significance of the difference between both groups.

265

266 No statistically significant difference was found between lop-eared and erect-eared rabbits

for evidence of scratching (fur loss or scaling) around the ears (2/15 versus 0/15,

respectively), or ear pathology requiring treatment whilst at the rescue centre (4/15 versus

 $269 \quad 0/15$, respectively). None of the rabbits had crusting of the external pinna, masses in the ear,

270 head shaking or scratching in pre-examination observation or a head tilt. No statistical

significance was found for any of the other predictors used in the binary logistic regression

272 (face shape, sex or weight) for any of the aural pathology outcomes.

273

274 Microscopy revealed the presence of yeast species in some rabbits (12/15 lop-eared rabbits

275 versus 8/15 erect-eared rabbits), which were identified as Malassezia cuniculi based on

276 morphology described in other research^{20, 23, 34}. The presence/absence, and also number of,

malassezia-like yeasts found on microscopy were not statistically significantly differentbetween lop- and erect-eared rabbits.

Only one mite was found, identified as Psoroptes cuniculi, in the ear of an erect-eared rabbit.
This rabbit had no associated clinical signs or previous history of aural problems. No rod
bacteria were identified, but cocci were found in three lop-eared rabbits and two erect-eared
rabbits.

283

285

284 Dental health results

286 Lop-eared rabbits had approximately twenty-three times higher odds of incisor pathology

compared with erect-eared rabbits (OR=23.3; 95% CI: 1.9–293.2; P=0.015). They also had

approximately twelve times higher odds of having molar overgrowth (OR=12; 95% CI: 1.6-

289 88.9; P=0.015), thirteen times higher odds of molar sharpness (OR=13.4; 95% CI: 1.7-

290 107.2; P=0.015) and were statistically significantly more likely to have molar spurs

291 (attributable risk=0.4667; P=0.006), compared with erect-eared rabbits (Table 3).

292 Veterinary records showed that 8/15 of the lop-eared rabbits had tooth abnormalities,

compared with none of the erect-eared rabbits (attributable risk= 0.53, 95% CI: 0.17-0.78;

294 P=0.002). Additionally, 6/15 of the lop ears had been for a dental whilst at the rescue,

whereas none of the erect-eared rabbits had, which was again statistically significant

296 (attributable risk=0.4; 95% CI: 0.06-0.67; P=0.017).

297 On the other hand, statistically significant differences between lop and erect-eared rabbits

298 were not found for oral mucosal sores, potential oral pain response, epiphora, poor coat

299 condition, hypersalivation, or lack of caecotrophy, although the trends were for all of these

300 to be slightly more likely in lop-eared rabbits (Table 3). No rabbits had an inability to close

301 the mouth or fractured teeth.

302

303 No statistical significance was found for any of the other predictors used in the binary

304 logistic regression (face shape, sex or weight) for any of the dental pathology outcomes.

305 Discussion

306 The results confirmed that lop-eared rabbits did indeed have a statistically significantly 307 higher level of aural pathology, including stenotic ear canals, potential pain response during 308 aural examination, increased levels of cerumen and erythema of the ear canal. Similarly, 309 there was also a statistically significantly higher level of dental pathology, including incisor 310 pathology, molar overgrowth, molar sharpness, molar spurs and veterinary dental treatment, 311 in lop-eared compared with erect-eared rabbits. Both the direct observations and the medical 312 records showed that the lop-eared rabbits had statistically significantly more aural and dental 313 pathologies than did erect-eared rabbits.

314 The use of a rescue population may of course not represent the general population of pet 315 rabbits. It is difficult to know if rescue rabbits would be affected more than pet rabbits by the 316 issues investigated here, or less. On one hand, rabbits in the rescue centre may be more 317 prone to health problems if they were given to the rescue because their owners could not 318 afford veterinary bills, or due to neglect and being fed an inappropriate diet. On the other 319 hand, the rescue staff were highly knowledgeable about rabbit health and carried out weekly 320 health checks, so current severe dental or aural disease was not found during the clinical 321 examination of this study. In either case, lop- and erect-eared rabbits would have been 322 affected by these factors to a similar extent. Thus, the finding that lop-eared rabbits were 323 prone to the aural and dental pathology investigated here constitutes a welfare concern

324 associated with breeding and buying lop-eared rabbits, for reasons described below in more325 detail.

326 Aural pathology

The increased erythema, ceruminous discharge and stenosis of the ear canal found in the lop-eared rabbits here, could indicate a higher frequency of otitis externa. This would concur with two canine studies that found higher prevalences of otitis externa in dogs with pendulous ears^{21, 22}. In many of the rabbits, this appeared to be a chronic condition as the medical records showed that 9/15 of the lop-eared rabbits required repeated ear cleaning whilst at the rescue.

333 Thus, the welfare consequences of a rabbit having lop-ears include pain, as indicated by statistically significantly increased pain responses during examination of lop-ears. 334 335 Additionally the higher frequency of signs consistent with otitis externa found in the lop-336 eared compared with the erect-eared rabbits, suggest potential for pain, auditory deficit or even deafness^{17, 18}, which in turn increases the vulnerability of the animal to threats and 337 could cause anxiety¹⁹. Deafness itself could not be tested in the current study, but this could 338 339 be assessed in future studies using Transient Evoked Otoacoustic Emissions testing, which 340 has been successfully used as a less invasive and relatively inexpensive alternative to brainstem auditory evoked responses in puppies³⁵. The rescue centre staff anecdotally 341 342 believed that more of the lop-eared rabbits had auditory impairment than the erect-eared 343 ones had, and unpublished research from our laboratory suggested that lop-eared rabbits showed more signs of anxiety in a novel object test than erect-eared rabbits³⁶. 344

The results here suggest that the aetiology of any potentially associated auditory deficits
would be multifactorial as it is in dogs and cats^{19, 37}, with the over-hanging immobile pinnae,

the stenotic ear canals, and the accumulation of cerumen all comprising physical barriers to
sound perception. The middle and inner structures themselves may also be more prone to
issues, such as tympanic membrane rupture and sensorineural damage, if repeated infection
occurs.

351 The lack of a statistically significant difference in the presence of Malassezia cuniculi 352 between lop and erect-eared rabbits could of course be due to the fairly small sample size here, but it agrees with the findings of Quinton et al. (2014), who reported no statistically 353 354 significant difference due to ear type in 146 clinically healthy domestic pet rabbits. This 355 could be explained by *Malassezia* being a normal coloniser of the rabbit ear canal, as in other species such as dogs and cats^{16, 20}. In future, culture could offer a more sensitive 356 method for quantifying *Malassezia* colonisation than cytology³⁸. Campbell et al. {, 2010 357 358 #4640} found a positive correlation between the amount of ceruminous discharge and the 359 culture of *Malassezia*. However, although the present study found higher levels of cerumen in lop-eared rabbits, the lack of difference in Malassezia colonisation suggests that this 360 361 increased cerumen was due to difficulties in expulsion, potentially due to the anatomy of the lop-ear, rather than a *Malassezia* overgrowth⁹. 362

363 Dental pathology

The increased presence of dental pathology found in lop-eared rabbits in this study partially supports results from Mullan and Main³⁹, who found that dwarf lops were reported by their owners to have statistically significantly more dental abnormalities compared with other breeds (including some other lops), but only when diet and age were excluded from the model. However, the present results disagree with a Finnish study⁴⁰ of 167 pet rabbits, which found no associations between lop-eared breeds and dental disease, although they did

find statistically significantly more dental pathology in Lionhead rabbits, which are erecteared, but brachycephalic. A possible explanation for the disagreement is that the study by
Mäkitaipale et al.⁴⁰ was carried out on healthy pet rabbits whose owners voluntarily signed
up for the study, so those with known current dental problems may have been excluded from
that study.

375 The rabbits in our study represent a convenience sample of a rescue population, but if our 376 results can be replicated more widely, the increased risk of dental pathology for lop-eared 377 rabbit welfare is concerning for several reasons. Rabbit dental pathology can lead to lesions 378 of the mouth, pain, difficulty chewing food e.g. avoidance of, or prolonged chewing of, hard foods as seen in bears with dental pathology⁴¹, and possibly gastrointestinal problems 379 following inadequately chewed food as found in humans^{42, 43}, among other issues described 380 381 earlier. The rescue staff caring for rabbits in the current study were more knowledgeable of 382 and attentive to rabbit health issues than many owners, and health records indicated that they 383 had already identified more dental issues requiring veterinary attention in lop-eared than 384 erect-eared rabbits, before our study started. Indeed, the rabbits at the rescue centre 385 underwent weekly health checks by experienced rescue staff with the use of an otoscope. 386 Despite this enhanced care and monitoring of dental pathology, we observed statistically 387 significantly more abnormalities in both incisors and molars in the lop-eared rabbits. The potentially associated secondary issues, such as trauma to the mouth and lips, 388 389 hypersalivation, and pain responses, were too rare to reach statistical significance, but all

390 showed trends towards being slightly more common in lop-eared rabbits.

The dental abnormalities here are unlikely to be due to diet, because there is currently no a priori reason to believe that lop-eared rabbits would have been fed poorer diets than erecteared rabbits. Thus, they are likely to be caused at least partly by the skull and jaw

394 conformations associated with the lop-eared phenotype. Future studies could help confirm

this by observing random sample populations, and by utilising radiology to look at oral

396 conformations, alongside oral examination if $possible^{28, 30, 40}$.

397 Conclusions

398

The results from this research support the hypothesis that lop-eared rabbits have more dental and aural pathology than erect-eared rabbits. This brings into debate the ethics of breeding and buying lop-eared rabbits, as they may be more likely to suffer from these conditions, which can be painful and often chronic or recurrent.

403

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522

523 Figure 1. Frequency distribution of erythema scores in the ear canals of lop-eared and erect-

524 eared rabbits. Black bars indicate lop-eared rabbits; white bars indicate erect-eared rabbits.

525 Each ear was scored as follows: 0= pale pink, 1= pink, 2= dark pink, 3= red, and the added

- 526 total for both ears per rabbit is shown.
- 527

528 Figure 2. Frequency distribution of cerumen scores lop-eared and erect-eared rabbits. Black

529 bars indicate lop-eared rabbits; white bars indicate erect-eared rabbits. Each ear was scored as

530 follows: 0= normal low amount of cerumen, 1= mild amount of cerumen, 2= moderate

amount of cerumen, 3= large amount of cerumen, and the added total for both ears per rabbit

532 is shown.