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A RETROSPECTIVE ANALYSIS OF MORBIDITY AND MORTALITY IN THE CAPTIVE LEADBEATER'S POSSUM (*GYMNOBELIDEUS LEADBEATERI*) POPULATION FROM 1970 TO 2021

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Abstract: The Leadbeater's possum (*Gymnobelideus leadbeateri*) is a critically endangered nocturnal marsupial with a restricted range in the Central Highlands of Victoria, Australia. There are two genetically distinct populations divided by location: highland and lowland. Lowland possums exist in one remnant swamp forest and entered captivity in 2012 when ~60 individuals remained. Today, with less than 20 lowland individuals remaining, any information that informs the yet-unsuccessful breeding program is critical. This study encompasses a retrospective analysis of the causes of mortality and significant histological lesions in captive highland and lowland individuals across seven institutions internationally from 1970 to 2021. During this time, 245 possums lived in captivity. Postmortem records exist for 99 animals, including 349 histopathology diagnoses from 80 reports and 264 gross necropsy diagnoses from 78 reports. Diagnoses were assigned into two categories based on the importance to the individual (causing death or morbidity to a single animal [$n = 194$]), or importance to the wider population (causing death or morbidity to more than one animal or was related to reproduction [$n = 155$]). Individual animals had multiple diagnoses, which were tallied as individual data points. Renal disease was diagnosed 57 times; the most common finding was chronic nephropathy (43/57). Cardiovascular disease was diagnosed 33 times; atherosclerosis associated with obesity was common ($n = 10/33$). Both categories suggest causal association with captive husbandry but elicit no comment on the lack of success of the breeding program. Reproductive disease was diagnosed 36 times in 24 animals (14 females and 10 males). In females, 11 cases of uterine inflammation and associated clinical signs were associated with ascending infection or neoplasia. Of the seven lowland male possums with mortality data, five were infertile (azoospermia or testicular atrophy). More investigation into the reproductive health of this population is indicated to understand the lack of success in the current breeding program.

INTRODUCTION

The Leadbeater's possum (*Gymnobelideus leadbeateri*) is an arboreal, nocturnal, marsupial found in a restricted range in the Central Highlands of Victoria, in southeastern Australia.¹³ The species comprises two genetically distinct populations, separated geographically:¹² the highland possum rediscovered in 1961, and the lowland possum discovered in 1986 in Yellingbo, Victoria and the

subject of a captive breeding program from 2012 to the present.^{14,30,38} The species was upgraded to critically endangered in 2015, with habitat diminished and under threat from logging and fire; today fewer than 20 lowland individuals remain in the wild and captivity.^{11,14,17}

Highland possums entered captivity in 1967 via a private naturalist and were kept initially by universities and researchers before colonies were donated to zoological institutions in 1971. The early captive population had some highly fecund individuals and, as a result, possums were transferred around Australia and internationally to North America and Europe. The population had 11 founding animals that bred prolifically, and numbers peaked in 1986, when captive facilities were near capacity.²⁰ In the 1990s, when the vulnerability of the wild population was prioritized, no further animals bred despite continued pairing, and the last highland possum of this cohort died in Canada in 2010.

As the conservation status of the only remnant lowland population worsened, lowland possums

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were collected from the wild between 2012 and 2016 and brought to Healesville Sanctuary (Zoos Victoria, Melbourne) for captive breeding. Possums were identified as successful breeding pairs prior to collection because of fur discoloration and pouch distention/dyscoloration (saliva staining from the courtship behavior of cloacal licking) or the presence of dependent young (Harley, pers. comm.). Despite a decade of effort, including observation of mating behavior, no successful breeding has occurred; this is the subject of ongoing review. Highland possums were initially in captivity in small numbers incidentally (post bushfire collapse of an environment), but then were purposefully collected for the captive breeding program that has pivoted to a focus on genetic rescue, pairing highland and lowland possums together as part of wider translocation efforts.^{15,35} The health of the highland Leadbeater's possum has been reported in two previous reviews; findings include high incidences of renal disease, cutaneous disease, trauma, and respiratory illness.^{3,19} A single case series investigated nutritional-based cutaneous disease and the captive diet.¹⁸ All studies linked stress and/or diet to disease incidence and made recommendations for improved diet and husbandry. There are no published data on the health of the lowland population, other than a suspected plasmoidal pneumonia, and a novel finding of a *Breinvia* sp. nematode.^{29,34} The current cohort of Leadbeater's possums in captivity are all wild born; any subclinical disease carried by these animals from the wild may be exacerbated by stressors associated with captivity. Wild-born animals subsequently living in captivity have shown reproductive abnormalities including ovotestis, feminization, and uterine cysts.³⁷

In the face of an unsuccessful breeding program, with translocations and genetic rescue now forming the backbone of conservation efforts, a constantly evolving and improving set of husbandry conditions, and a perceived increase in clinical cases involving reproductive disease, a review of all captive health records is timely. This study aimed to assess necropsy records to identify significant disease processes in this captive population and trends of disease that could inform future health outcomes.

MATERIALS AND METHODS

Acquisition and compiling of records

Zoos Victoria (Healesville Sanctuary and Melbourne Zoo) data were accessed via Species 360 Taxa reports from Zoo Information Management

Software (ZIMS) and a custom-made database (FileMaker Pro, Filemaker, Inc). Institutions that had also held Leadbeater's possums were identified from historic transfer records and a physically printed studbook.²¹ All institutions were contacted with a request for records. Institutions that contributed records to this study were Zoos Victoria (Melbourne Zoo and Healesville Sanctuary, Victoria Australia), Taronga Zoo (Sydney, Australia), the Australian Wildlife Health Registry (Sydney, Australia), Currumbin Wildlife Centre (Queensland, Australia), Toronto Zoo (Toronto, Canada), and Brookfield Zoo (Chicago, United States of America).

Data were received primarily in the form of electronic documents entailing individual identifiers, and birth, death, acquisition, and transfer dates. Less frequently the data included health records, weights, clinical veterinary records, postmortem records, and husbandry reports. Data for each individual was compiled in an electronic spreadsheet (Excel, 2020, Microsoft Corporation, USA) which allowed for animals that existed in multiple institutions to be identified. During compiling, records were assessed for evidence of transfer to or from previously unidentified institutions to acquire more records via snowball sampling methodology.

All available necropsy records were individually scanned as part of this study and histopathological and gross diagnoses were compiled into two individual data sets. The wide range of descriptive terminology used by different pathologists were manually assigned to 117 standardized terms to allow compilation and analysis. This methodology was co-designed by the principal investigator, a veterinary pathologist, and a senior veterinarian. Histopathology necropsy findings were assigned to 95 standardized terms using a modified method described in Eder et al⁷ (Supplementary Materials 1 and 2). These terms were grouped into 13 body systems (cardiovascular, central nervous system, endocrine, gastrointestinal, hematopoietic, hepatic, integument, musculoskeletal, ophthalmic, renal, reproductive, respiratory, and systemic) (Supplementary Table 1). The range of descriptive terminology in gross necropsy reports was assigned to 83 standardized terms and grouped by the same 13 body systems (Supplementary Table 2). For many individuals, multiple diagnoses coexisted and were treated as separate data points and tabulated by diagnosis to ensure that multiple comorbidities were represented.

The results were assigned two categories based on whether the diagnosis was important to the individual (cause of death or morbidity to a single

Table 1. Histopathology diagnoses observed from the lowland and highland Leadbeater's possum (*Gymnobelideus leadbeateri*) between 1970 and 2021, filtered by importance to the population (caused death or morbidity in >1 animal or were reproductive) and grouped by body system. Data from 70 animals (50 highland and 20 lowland) contributed 155 diagnoses. *N dx*: total number of diagnoses, *n* = number of animals, followed by number of diagnoses found in individuals that were male; female in parentheses. (%) indicates % of total diagnoses.

Body system	Number of diagnoses in highland possums <i>n dx</i> = 96 <i>n</i> = 50 (25.25)	Number of diagnoses in lowland possums <i>n dx</i> = 59 <i>n</i> = 20 (10.10)	Total <i>n dx</i> = 155 (% total) <i>n</i> = 70 (35.35)
Renal	36	21 (<i>n</i> = 15 [7.8])	57 (36.7%) (23.28)
Reproductive	19	17 (<i>n</i> = 11 [6.5])	36 (23.2%) (10.14)
Cardiovascular	20	13 (<i>n</i> = 12 [5.7])	33 (21.3%) (12.15)
Integument	10 (<i>n</i> = 8 [4.4])	3 (<i>n</i> = 3 [2.1])	13 (8.4%) (6.5)
Gastrointestinal	5 (<i>n</i> = 5 [2.3])	4 (<i>n</i> = 4 [2.2])	9 (5.8%) (4.5)
Endocrine	5 (<i>n</i> = 5 [3.2])	0	5 (3.2%) (3.2)
Respiratory	1 (<i>n</i> = 1 [1.0])	1 (<i>n</i> = 1 [0.1])	2 (1.2%) (1.1)

animal) or had importance to the population. Population importance was defined as diagnoses relevant to the captive breeding program (such as reproductive disease) or caused morbidity or mortality in more than one individual (Supplementary Table 3). Animals were assigned to age class categories for age at death. The age class categories were defined as neonate (<90 d since pouch emergence), juvenile (90 d to 18 mon of age), adult (18 mon to <5 yr of age), aged adult (5–8 yr) and geriatric (>8 yr of age).

RESULTS

Of the 245 captive Leadbeater's possums (highland *n* = 212 and lowland *n* = 33), 99 animals were identified as having mortality records; either gross necropsy reports and/or had tissues submitted to a commercial diagnostic laboratory for histopathology. A total of 80 histology reports were available (highland *n* = 60, lowland *n* = 20), recording a total of 349 histopathological diagnoses which were assigned to 117 standardized terms. A total of 78 gross necropsy reports were available (highland *n* = 63, lowland *n* = 15), recording a total of 264 gross diagnoses that were assigned to 95 standardized terms.

Histopathological diagnoses were then categorized as “important to the population” (*n* = 155 diagnoses), and “important to the individual” (*n* = 194 diagnoses). Diagnoses important to the individual are not further discussed in this paper, but are included in the supplementary material (Supplementary Material 1 and 2).

Animals often had multiple histopathological diagnoses within body systems; diagnoses categorized as important to the population were assigned from 70 individuals; 20 lowland possums (59

diagnoses) and 50 highland possums (96 diagnoses). The most common body system assigned a diagnosis was renal (*n* = 57), followed by reproductive (*n* = 36) and cardiovascular (*n* = 33) (Table 1). All diagnoses increased with age; geriatric possums had more diagnoses (Table 2). The most common renal diagnosis was chronic nephropathy (Table 3). Reproductive disease included bacterial infections, lower urinary tract obstruction, and male infertility (Table 4). Multiorgan system pathology was noted in a range of body system diagnoses, in particular septicemia, where bacterial emboli had a heterogenous spread from a point of origin infection (e.g., gastrointestinal yersinia infection, pyometra).

Renal histopathology findings

Renal pathology was diagnosed 57 times in 51 individuals (female *n* = 28, male *n* = 23, lowland *n* = 21, highland *n* = 36). An example of comorbid renal pathology was a male lowland possum (273) who was diagnosed with both chronic nephropathy and acute urethral obstruction. Of the 51 possums, 43 were diagnosed with chronic nephropathy (Fig. 1A), which included interstitial nephritis, membranous change to glomeruli, degeneration and loss of tubules, interstitial fibrosis, and mixed interstitial inflammation (Table 3; Fig. 1B). A single case of acute renal tubular necrosis was attributed to an iatrogenic drug toxicity (aminoglycosides). Lower urinary tract obstruction (*n* = 4) was seen exclusively in lowland male animals that had reached adult age; this diagnosis was associated with hydronephrosis, mineralization and mucoid debris in ureters, and cystitis (Fig. 1C). Three of these males had been in captivity for >1 yr, but one had recently entered captivity (<1 mon) and had not left quarantine.

Table 2. The three most common histopathology diagnoses, filtered by population importance (caused death or morbidity in >1 animal or were reproductive), grouped by body system and separated by age class in the lowland and highland Leadbeater's possum (*Gymnobelideus leadbeateri*). Data from 68 animals (50 highland and 20 lowland) contributed 120 diagnoses. *N dx*: total number of diagnoses, *n* = number of animals, followed by information relating to male; female in parentheses.

Histopathology diagnoses	Juvenile <i>n dx</i> = 7 (4.3) <i>n</i> = 7 (4.3)	Adult <i>n dx</i> = 18 (11.7) <i>n</i> = 11 (4.7)	Aged adult <i>n dx</i> = 34 (20.14) <i>n</i> = 16 (9.7)	Geriatric <i>n dx</i> = 67 (21.46) <i>n</i> = 32 (13.19)	Total <i>n dx</i> = 126 (56.70) <i>n</i> = 66 (30.36)
Renal	4	10	16	27	57
Highland	4	4	8	20	36
Lowland	0	6	8	7	21
Reproductive	0	4	9	23	36
Highland	0	1	1	17	19
Lowland	0	3	8	6	17
Cardiovascular	3	4	9	17	33
Highland	3	1	4	12	20
Lowland	0	3	5	5	13

Table 3. Renal histopathology diagnoses, important to the population (caused death or morbidity in >1 animal or were reproductive), in lowland and highland Leadbeater's possums (*Gymnobelideus leadbeateri*) between 1970 and 2021. *N dx*: total number of diagnoses, *n* = number of animals, followed by number of diagnoses found in individuals that were male; female in parentheses.

Histopathology diagnosis	Highland <i>n dx</i> = 36 <i>n</i> = 36 (16.20)	Lowland <i>n dx</i> = 21 <i>n</i> = 15 (7.8)	Total <i>n dx</i> = 57 <i>n</i> = 51 (23.28)
Chronic nephropathy	29	14	43
Ascending bacterial infection	3	3	6
Hematogenous bacterial infection	2	0	2
Lower urinary tract obstruction	0	4	4
Renal tubular necrosis	1	0	1
Hemoglobinuria nephrosis	1	0	1

Table 4. Reproductive diagnoses, filtered by importance to the population (caused death or morbidity in >1 animal or were reproductive), in the lowland and highland Leadbeater's possum (*Gymnobelideus leadbeateri*) between 1970 and 2021. *N dx*: total number of diagnoses, *n* = number of animals, followed by number of diagnoses found in individuals that were male; female in parentheses.

Histopathology diagnosis	Highland <i>n dx</i> = 19 <i>n</i> = 13 (4.9)	Lowland <i>n dx</i> = 17 <i>n</i> = 11 (6.5)	Total <i>n dx</i> = 36 <i>n</i> = 24 (10.14)
Female			
Metritis	6	1	7
Endometrial hyperplasia	3	1	4
Ovarian cyst	3	1	4
Neoplasia	1	2	3
Vaginal aplasia/atrophy	0	1	1
Endometrial cyst	1	0	1
Mammary gland cysts	1	0	1
Mastitis	0	1	1
Ovotestis	0	1	1
Male			
Prostatic hyperplasia	1	3	4
Testicular atrophy	1	3	4
Oligospermia/azoospermia	1	2	3
Prostatitis	1	1	2

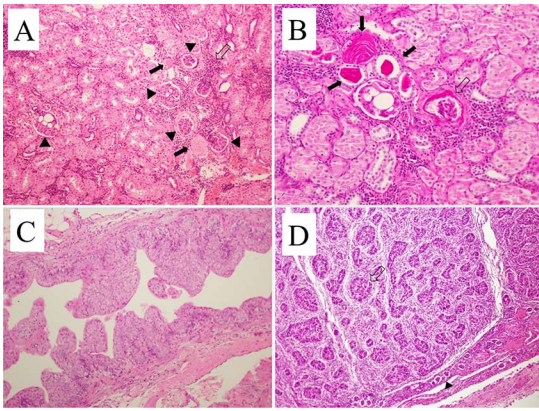


Figure 1. A. Leadbeater's possum, *Gymnobelideus leadbeateri*, kidney, H&E, $\times 100$ magnification. Chronic nephropathy. Glomerular changes include vacuolation of the glomerular tuft, thickening of the glomerular basement membrane, and periglomerular fibrosis (arrow heads). Tubules are dilated with luminal protein and some debris (solid arrows). The interstitium has moderate chronic inflammation (open arrow). B. Leadbeater's possum, *Gymnobelideus leadbeateri*, kidney, PAS (Periodic Acid Schiff), $\times 200$ magnification. The PAS stain highlights the thickened and irregular basement membrane (open arrow) and protein accumulation in the lumen of tubules (closed arrow). C. Male Leadbeater's possum, *Gymnobelideus leadbeateri*, urethra, H & E, $\times 200$. Segments of the mucosa are hyperplastic with the epithelial proliferation creating irregular projections into the lumen. D. Leadbeater's possum, *Gymnobelideus leadbeateri*, ovotestis, H&E, $\times 100$. Most of the tissue has structures consistent with seminiferous tubules (open arrow). Remaining primordial follicles (arrowheads).

Reproductive diagnoses

All reproductive diagnoses were considered important to the population because of the failure of the captive breeding program. Reproductive diagnoses were identified 36 times in 24 animals (female $n = 14$, male $n = 10$, highland $n = 13$, lowland $n = 11$). Six individual animals had two diagnoses, and two individuals had three diagnoses. For example, one animal was diagnosed with endometrial hyperplasia, acute inflammation of the cervix, and paraovarian cysts. Pathology was most seen in older animals, (see Table 2).

Uterine inflammation and pyometra on gross necropsy were diagnosed seven times, with only three animals having findings captured on both necropsy and histopathology. Eleven cases of uterine inflammation were seen in total, and in

seven cases these occurred concurrently with either endometrial hyperplasia ($n = 2$), ovarian cysts ($n = 3$), or both ($n = 2$). Multiple uterine adenomas were seen in a female possum with endometrial hyperplasia, ovarian cysts, and a gross diagnosis of pyometra, and one case of a vaginal squamous cell carcinoma was seen with a diagnosis of pyometra and metritis. The other case of neoplasia was a phenotypically female lowland possum, with no ovarian tissue found and a large sex cord stromal tumor. Another phenotypically female lowland animal was diagnosed with ovotestis and vaginal atrophy (Fig. 1D).

Eleven males were found to have histopathology reproductive diagnoses; of these, four were diagnosed with testicular atrophy of no identifiable cause and three males had reduced ($n = 1$) or absent ($n = 2$) sperm (Table 4). Seven males had gross pathology diagnoses of testicular atrophy with three of these diagnoses confirmed histologically. An additional four cases were noted at gross necropsy and not described histologically. When both modalities are considered, 11 animals had testicular atrophy (11/44, or 25% of male possums with necropsy records). Importantly, of the seven lowland males in this study, when one (fertile) wild male lowland who died of natural causes in the field is excluded, five of the six deceased male lowland possums had histopathology or gross necropsy findings that indicated infertility, despite suspicion based on cloacal staining, or colony structure (seen with presumed dependent young) that some of these animals bred prior to collection into captivity.

Cardiovascular disease

Cardiovascular disease was diagnosed 33 times in 27 animals (female $n = 15$, male $n = 12$, lowland $n = 12$, highland $n = 15$) (7 animals had 2 diagnoses). Older animals were more represented, juvenile ($n = 3$, n diagnoses = 3), adult ($n = 4$, n diagnoses = 4), aged adult ($n = 7$, n diagnoses = 9), or geriatric ($n = 16$, n diagnoses = 17) (Table 2). The most common diagnosis was cardiac necrosis and cardiomyopathy. Two cases of cardiac necrosis and one of epicarditis were associated with septicemia. The third most common findings of arteriosclerosis and atherosclerosis (accumulation of lipid plaques within blood vessels) were observed more often in older animals (Table 5). A single case of filaroid nematode parasitism seen histologically in the heart muscle, testicle, and spleen of a possum found dead in the wild was identified as *Breinlia* sp.³⁴

Table 5. Cardiovascular diagnoses, filtered for population importance (caused death or morbidity in >1 animal or were reproductive), in the lowland and highland Leadbeater's possum (*Gymnobelideus leadbeateri*) between 1970 and 2021. *N* dx: total number of diagnoses, *n* = number of animals, followed by number of diagnoses found in individuals that were male; female in parentheses.

Histopathology diagnosis	Highland <i>n</i> dx = 20 <i>n</i> = 15 (7.8)	Lowland <i>n</i> dx = 13 <i>n</i> = 12 (5.7)	Total <i>n</i> dx = 33 <i>n</i> = 27 (12.15)
Endocarditis	0	2	2
Cardiac necrosis	5	5	10
Cardiomyopathy	6	2	8
Arteriosclerosis	1	2	3
Atherosclerosis	5	2	7
Hemorrhage	1	0	1
Epicarditis	1	0	1
Parasitism	1	0	1

DISCUSSION

This study is the largest compilation of mortality records from the Leadbeater's possum. Renal pathology was identified as the most frequent clinically significant finding, followed by cardiovascular disease. An investigation of abnormal reproductive pathology was undertaken with a view to the animal's critical conservation status and role in breeding programs.

The most common causes of death in this population are challenging to extract. It appears that a range of chronic morbidity states result in debilitation, causing susceptibility to septicemia and death. Chronic renal or cardiovascular changes become overlaid by acute depletion of remaining residual function or by an additional disease state (neoplasia, pyometra, bacterial infection). Greater understanding of these underlying disease processes can inform clinicians about expected progression and prognosis and lead to more focused supportive care.

The most common renal diagnosis was chronic nephropathy. The etiology appears to be idiopathic, although association with chronic changes to water consumption or nutritional intake of protein or minerals in captivity cannot be dismissed. Leadbeater's possums are thought to have a primarily exudative diet in the wild, supplemented by invertebrates, which is impossible to reproduce in captivity.^{5,31} Prior to 2018, captive diets across the different zoos were broadly similar, with a highly soluble carbohydrate and protein intake and minimal fiber or insoluble carbohydrates. In their review of nutrition and the environment of captive Leadbeater's possum, McWilliams and Atkinson identified a 35.9% incidence of renal disease in this species and proposed that accelerated renal failure could be attributed to an excess of

dietary phosphorus.¹⁹ There is no record of dietary phosphorus being reduced; however, the diet of the captive population fluctuated over time, with eggshell (a source of phosphorus) being omitted in some variants.

In other species, such as domestic felids, shortened telomere length-associated cellular senescence is linked to chronic kidney disease and has been associated with inbreeding.²⁴ Given the limited genetic variability in Leadbeater's possum,¹² this could be a contributing factor to their development of chronic renal disease. Renal changes were observed in older animals, suggesting that subclinical disease progression causes chronic morbidity preceding diagnosis at death. It is unknown if other husbandry practices or environmental factors influence this disease process (e.g., unknown water consumption, electrolyte imbalances, stressors causing chronic glucocorticoid changes), but the high incidence, despite a range of institutions and time periods, make it more likely a species predilection. Clinicians are encouraged to consider renal disease as an underlying morbidity in this species.

Atherosclerosis was identified in multiple older possums in this study. It is often seen with an increased body weight, and historically, in all institutions, Leadbeater's Possums have been dramatically heavier than their 140-g wild counterparts, with some individuals weighing 250 g and above.¹⁸ Lynch analyzed the diet of the Leadbeater in captivity in response to a case of cutaneous xanthoma, finding that ongoing and extreme cases of obesity correlated with high cholesterol blood levels.¹⁸ A previous review of feeding and husbandry practices of Leadbeater's possums across eight zoos identified excess energy intake, variable protein and saturated fat levels, unbalanced fatty acid ratios, and a lack of complex

carbohydrates.³ Captive animals have historically been fed double to triple their caloric requirements, while spending up to 75% of normal nocturnal activity time in nest boxes.^{19,33} Postmortem reports in previous studies correlated with findings in this study, including diseases associated with fat metabolism (xanthogranuloma, hepatic lipidosis, renal lipidosis, and coronary atherosclerosis). Fatty acid imbalances were previously presumed to contribute to the 44% prevalence of chronic inflammatory conditions;¹⁹ supplementation with fatty acids have been linked to some improvements in chronic renal pathologies in humans.^{2,4}

Reproductive disease was varied in etiology in females, suggesting there is no one simple way to mitigate disease prevalence in future populations. This study found 11 female possums with reproductive diagnoses. Metritis was common, and in seven cases it occurred concurrently with endometrial hyperplasia or ovarian cysts or both. Endometrial hyperplasia is thought to be caused by chronic exposure to estrogens, in progesterone-deficient states, both of which may be related to nulliparity, age, and obesity in humans, some forms are thought to be a neoplastic precursor.²⁷ Uterine adenoma was seen in one case with concurrent endometrial hyperplasia in this study. In dogs, cystic endometrial hyperplasia is considered a precursor to pyometra/metritis, with a heightened susceptibility to bacterial infection (most commonly *Escherichia coli*) infection during certain stages of estrous.⁸ Reproductive disease in female Leadbeater's possums has previously been attributed to stress, and females are potentially more territorially aggressive, which may lead to more stress and subsequently increased immunosuppression in captivity.³² A previous study suggested that female highland possums appeared to be overrepresented in immune-compromised or stress-related health problems, with a ratio of 61:94.¹⁹ Stress can be associated with lower urinary tract syndromes, including ascending infection or urinary tract obstruction, in other species, including domestic cats.¹⁰ It is possible that the stress of disruptive environmental conditions (sound, handling, and transport), nulliparity and reproductive cycle disturbance could predispose the female Leadbeater's possums to reproductive disease.

A phenotypically female possum in this study, initially brought to captivity with her mate and presumed offspring, was later found to have no ovarian tissue, and a large sex cord stromal tumor with possible testicular structures; although this was not assessed in this individual, potential

causes for this anatomical feature could be a translocation or an XXY chromosomal karyotype.²⁶ Translocations or fusions between autosomes and the sex chromosomes are widespread amongst marsupials.⁶ Anecdotal reports in captive sugar gliders (*Petaurus breviceps*) indicate that XXY chromosomes, associated with phenotypic intersex gliders, have produced offspring, with similar observations in other marsupials.²²

Reproductive disease in the male Leadbeater's possums has a more direct impact on fertility. Seven male lowland animals had testicular atrophy and/or very low/absent sperm production on histopathology and/or gross pathology, which is a significant contributing factor in the lack of successful breeding in the current lowland captive colony. Stress (resulting in inhibition of luteinizing hormone secretion via corticosteroids), low dietary zinc, and age-related senescence can cause testicular degeneration.^{9,23} As some of the male and female possums entering the breeding program were thought to have bred prior to collection, there may be some component of captivity responsible for male infertility (such as suboptimal diet or inadequate zinc levels).^{8,39}

This study is limited to the existence of, and access to, medical and necropsy records. Histopathology findings are the focus of this study; however, the large number of animals that did not undergo a full necropsy could have resulted in an artificial inflation or deflation of incidence described. This limitation of the present study precluded a more comprehensive analysis of relative risk and odds ratios and more than broad speculative comparisons between categories of animals, resulting in a descriptive study. There is broad consistency with previous investigations; however, future studies could encompass more records if they can be obtained or focus more on morbidity and clinical review of disease, which could provide a broader picture.

It is important to acknowledge that no body system operates independently, and that all are part of a synergistic system. Atherosclerosis can cause hypertension, which can play into chronic renal disease;³⁶ stressors and subsequent increases in cortisol can impact reproductive hormones,^{1,25} and the growing body of research in gut microbiota suggests it has an influence over multiple components of the body.^{16,28} What seems overall apparent from this study is that there is a species predilection towards chronic renal pathology, that captive animals prone to obesity are vulnerable to cardiovascular disease, and that more prospective research

is needed into reproductive health, especially in males in this species if the breeding program is to succeed.

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