Variability of serum aldosterone concentrations in pet ferrets (*Mustela putorius furo*)

Nicola Di Girolamo DMV, PhD
Kellie Fecteau PhD
Alessandra Carnimeo DMV
Laura Bongiovanni DMV, PhD
Federico Fracassi DMV, PhD
Gloria Isani PhD
Paolo Selleri DMV, PhD

From the Clinica per Animali Esotici, Via Sandro Giovannini 53, 00137 Roma, Italy (Di Girolamo, Carnimeo, Selleri); the Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996 (Fecteau); the Faculty of Veterinary Medicine, University of Teramo, 64100 Teramo, Italy (Bongiovanni); and the Department of Veterinary Medical Sciences, University of Bologna, 40126 Bologna, Italy (Fracassi, Isani). Dr. Di Girolamo’s present address is Tai Wai Small Animal & Exotic Hospital, 69-75 Chik Shun St, Tai Wai, Sha Tin, Hong Kong.

Address correspondence to Dr. Di Girolamo (nicoladiggi@gmail.com).

OBJECTIVE
To explore sources of serum aldosterone concentration variability in a population of healthy and diseased ferrets, determine a preliminary 1-sided reference interval for serum aldosterone concentration in healthy ferrets, and identify a decision limit to differentiate healthy from diseased ferrets on the basis of serum aldosterone concentration.

DESIGN
Prospective threshold definition and diagnostic accuracy study.

ANIMALS
78 healthy (n = 56) and diseased (22) ferrets.

PROCEDURES
Serum aldosterone concentrations were measured on consecutively admitted ferrets, and an upper reference limit for aldosterone concentrations was established. Sensitivity and specificity of aldosterone concentration cutoffs to differentiate healthy from diseased ferrets were estimated with receiver operating characteristic curve analysis.

RESULTS
Measurements of serum aldosterone concentrations in the ferrets showed wide variability, with a median concentration of 4.75 pg/mL (interquartile range, 0.55 to 17.9 pg/mL; range, 0.02 to 283.9 pg/mL) and 76% (59/78) of samples having concentrations < 18 pg/mL. Ferrets that were healthy, older, or sexually inactive had significantly lower aldosterone concentrations. The upper limit of the reference interval for healthy ferrets was 13.3 pg/mL (90% confidence interval, 9.9 to 16.9 pg/mL). Analysis of receiver operating characteristic curves indicated that an aldosterone concentration cutoff value of 7.6 pg/mL differentiated healthy ferrets from diseased ferrets with a sensitivity of 72.7% and specificity of 73.2% (area under the curve, 0.79; 95% confidence interval, 0.67 to 0.91).

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that high aldosterone concentrations should not be considered diagnostic of primary hyperaldosteronism in ferrets. A need exists to develop better tests to identify primary hyperaldosteronism. (J Am Vet Med Assoc 2018;252:1372–1376)

Hyperaldosteronism (ie, high serum aldosterone concentration) is a well-described occurrence in human and veterinary medicine. Hyperaldosteronism results in excessive urinary potassium losses, sodium retention, and sometimes hypertension and metabolic alkalosis. Clinical detection of hyperaldosteronism and differentiation between primary and secondary forms of the condition are needed for prompt treatment.

Primary hyperaldosteronism, also termed low-renin hyperaldosteronism or Conn syndrome, is a disorder characterized by autonomous hypersecretion of aldosterone by the adrenal glands. In contrast, secondary hyperaldosteronism results from continued stimulation of the renin-angiotensin system (eg, as a result of cardiac, renal, or liver disease).

Ferrets (*Mustela putorius furo*) are common pets worldwide and are often affected by endocrine disorders. Two ferrets with suspect hyperaldosteronism have been described, and the clinical signs reported, including weakness and decreased activity, were nonspecific. Hypokalemia, a typical serum biochemical finding, was present in both cases. Hypertension, a common complication of hyperaldosteronism in other species, was present in one of the cases and was suspected to have caused the subject’s high-grade aortic insufficiency. To the authors’ knowledge, no published data regarding serum aldosterone concentrations in ferrets were available at the time of the present study, despite the fact that reference ranges for serum aldosterone concentration might be useful in detecting primary or secondary hyperaldosteronism in ferrets.

The purposes of the study reported here were to explore sources of serum aldosterone concentration variability in a population of healthy and diseased ferrets, determine a preliminary 1-sided reference in-
terval for serum aldosterone concentration in healthy ferrets, and identify a decision limit to differentiate healthy from diseased ferrets on the basis of serum aldosterone concentration.

**Materials and Methods**

**Study design**

A prospective diagnostic accuracy study was planned. Ferrets consecutively admitted to the Clinica per Animali Esotici, Rome, from March 2013 through October 2013 were included in the study. Data collection was planned before the start of the study.

**Eligibility criteria**

Ferrets were considered ineligible for the study if harvesting the minimum quantity of blood required for analysis (0.8 mL) was considered unsafe by attending clinicians or if blood sample collection was not possible with the patient conscious because of uncooperative behaviors. All other ferrets admitted during the study period were eligible for inclusion. Owners of each of the ferrets signed an informed consent form for inclusion of their animals in the study.

**Data collection**

Variables recorded for each ferret included age (months), sex (male or female), sexual status (sexually active or inactive [ie, surgically or chemically neutered]), number of reproductive events, hour at which blood was obtained, and health status (healthy or diseased). To avoid data dredging and consequent spurious results, all variables recorded in the study were specified a priori.

**Blood sample collection**

Blood samples were obtained by means of the protocol currently in use at the study institution (ie, cranial vena cava or proximal jugular venipuncture without chemical restraint). For each sample obtained, an assistant manually restrained the ferret by the scruff of its neck. A second individual then located the soft tissue area delimited by the first sternabra medially and the first rib caudally, inserted a 25-gauge needle attached to a 1-mL syringe in the area, advanced the needle craniodaually toward the opposite hind limb for approximately 6 to 8 mm and at a 30° angle of inclination relative to the skin,7 and applied minimal negative pressure (drawing the syringe plunger back slowly to avoid a vacuum higher than that generated by a 0.1-mL void in the syringe during the procedure) to the syringe as the needle was advanced and slowly retracted. The blood sample was harvested during advancement or retraction of the needle. With this technique, the 0.8 mL of blood needed for analyses was easily obtained.

To obtain serum, each of the blood samples was immediately transferred from the collection syringe to a 1-mL plain plastic tube. After 20 minutes, tubes were centrifuged at 1,300 g for 10 minutes. Serum was then harvested and stored at –20°C for a maximum of 7 days before shipping to the Endocrinology Service of the University of Tennessee for aldosterone analysis. For shipment, samples were individually labeled, sealed, and inserted into a plastic box, which was then packed along with ice in an insulated container made of extruded polystyrene foam. The polystyrene foam box was then sealed and inserted in a cardboard box for shipment.

**Sample analysis**

Serum aldosterone concentrations were measured by use of a commercially available 125I solid-phase competitive radioimmunoassay kit that had previously been validated for use in dogs and cats by the Endocrinology Service of the University of Tennessee. Performance characteristics for the aldosterone assay were determined with pooled serum samples from ferrets submitted to the Endocrinology Service for analysis. Intra-assay and interassay coefficients of variation for the pooled serum were 11.6% and 10.9%, respectively. Mean recovery of known concentrations of aldosterone standards added to the pooled sera was 96.0%. When pooled ferret serum was serially diluted, results were 89.4%, 106.8%, 107.3%, and 106.5% of the expected values. Analytic sensitivity of the assay was 11 pg/mL on the basis of information provided by the manufacturer. Laboratory personnel were not aware of the ferrets’ condition before analysis of samples. Technical and analytic issues (missing data) that limited credibility of the samples were listed, and the affected samples were not included in the study.

**Statistical analysis**

Summary statistics were compiled, and the Shapiro-Wilk W test was used to test whether continuous variables were normally distributed. The potential correlation between age and serum aldosterone concentration was assessed with the Spearman ρ coefficient. Differences in aldosterone concentrations attributable to sex, sexual status, and health status were assessed with the Mann-Whitney U test. Aldosterone concentration reference intervals for healthy ferrets were calculated with a robust method as indicated by American Society of Veterinary Clinical Pathology guidelines. A lower limit of the reference interval was not calculated because it was not considered clinically relevant. Sensitivity and specificity of various serum aldosterone concentration cutoffs to differentiate healthy from diseased ferrets were estimated with ROC curve analysis. Analyses were performed with commercial software. For all analyses, values of P < 0.05 were considered significant.

**Results**

**Population summary**

Eighty-six ferrets that met eligibility criteria were examined during the study period. However, blood samples from 8 ferrets were not analyzed because of technical problems (quantity not sufficient, n = 5; sample not received, 3). The remaining 78 ferrets were included in the study.
Of the 78 samples included in the study, 43 (55%) were from female and 35 (45%) were from male ferrets. Thirty-two (41%) ferrets were sexually inactive (ie, surgically or chemically neutered) at the time of sample collection. Twenty-eight of the female ferrets were sexually active (65%) versus 18 (51%) of the male ferrets. Median age of the ferrets was 24 months (IQR, 14 to 48 months; range, 5 to 72 months), and age was not normally distributed (P < 0.001). Fifty-six (72%) of the animals were considered healthy, and 22 (28%) had 1 or more clinical abnormalities, including weight loss of undetermined cause (n = 4), chronic kidney disease (2), severe periodontitis (2), weight loss of undetermined cause and tail alopecia (2), insulinoma (2), splenic lymphoma (1), monoclonal gammopathy of undetermined clinical importance (1), cataract (1), dystocia (1), hydrourter (1), megaesophagus (1), mastitis (1), eosinophilic gastroenteritis (1), dehydration (1), and cataract with hypoglycemia and azotemia (1).

**Aldosterone concentrations**

For the entire population of ferrets, serum aldosterone concentration had wide variability and a nonnormal (P < 0.001), positively skewed distribution with a long right tail (skewness, 2.9; Figure 1). Median aldosterone concentration was 4.75 pg/mL (IQR, 0.55 to 17.9 pg/mL; range, 0.02 to 283.9 pg/mL), and 76% (59/78) of the samples had a concentration < 18 pg/mL. Healthy ferrets had a median aldosterone concentration of 1.9 pg/mL (IQR, 0.3 to 8.6 pg/mL; range, 0.02 to 156.7 pg/mL), and diseased ferrets had a median aldosterone concentration of 43.9 pg/mL (IQR, 4.1 to 86.8 pg/mL; range, 0.05 to 283.9 pg/mL). Aldosterone concentration was significantly (P < 0.001) higher in diseased than in healthy ferrets.

**Factors associated with serum aldosterone concentrations**

Ferrets that were older or sexually inactive had significantly lower aldosterone concentrations. Specificaly, aldosterone concentration decreased as age increased (Spearman ρ = -0.24; P = 0.033; Figure 2), and sexually active ferrets had a median aldosterone concentration (8.1 pg/mL; IQR, 1.3 to 33.5 pg/mL; range, 0.14 to 239.5 pg/mL) significantly (P = 0.005) higher than that for sexually inactive ferrets (0.9 pg/mL; IQR, 0.1 to 5.5 pg/mL; range, 0.02 to 283.9 pg/mL; Figure 3). Serum aldosterone concent-

**Figure 1**—Distribution of serum aldosterone concentrations in 56 healthy (striped bars) and 22 diseased (solid bars) ferrets (*Mustela putorius furo*). Notice that the distribution is positively skewed with a long right tail. The dotted line represents the normal distribution curve.

**Figure 2**—Scatterplot of serum aldosterone concentration versus age for the 56 healthy (white circles) and 22 diseased (gray circles) ferrets in Figure 1. There was a significant and weak negative correlation between aldosterone concentration and age (Spearman ρ = -0.24; P = 0.033).

**Figure 3**—Box plots of serum aldosterone concentrations in 46 sexually active and 32 sexually inactive (surgically or chemically neutered) ferrets. For each plot, the box represents the IQR, the horizontal line represents the median, and the whiskers represent the range.
tion of ferrets tested had a positively skewed distribu-
tion, similar to that observed in cats. This distribu-
tion influenced the determination of the reference in-
terval, possibly resulting in a biased estimate. Thus,
for clinical purposes, we suggest that the decision
limits obtained with the ROC curve analysis be used
to interpret serum aldosterone concentrations, rather
than the upper limit of the aldosterone concentration
reference interval calculated for healthy ferrets (13.3
pg/mL). Currently, this is a standard approach also
used in human medicine.

In cats, primary hyperaldosteronism is suspected
on the basis of clinical signs (eg, weakness and hyper-
tension), laboratory abnormalities (eg, hypokalemia),
and diagnostic imaging results (ie, unilateral adrenal
gland enlargement). In ferrets, adrenal gland en-
largement is extremely common following gonadec-
tomy and usually results in high sexual hormone con-
centrations. Therefore, the utility of diagnostic
imaging for diagnosing primary hyperaldosteronism
in ferrets is limited, making the condition more dif-
ficult to diagnose in ferrets than in other species.

The high concentrations of aldosterone observed
in the present study were unlikely to have been a re-
sult of confounding factors. All samples were obtained
from conscious animals because anesthesia may alter
serum aldosterone concentrations. Samples were col-
lected either in the morning or the afternoon because,
as in cats, a circadian rhythm of aldosterone has not
been observed. Finally, none of the ferrets were receiv-
ing medications known to affect aldosterone concen-
trations (eg, antihypertensive drugs).

One limitation of the present study was that the
radioimmunoassay kit was developed to test human
samples. However, efforts were made to validate the
kit for use in ferrets, with calculation of intra-assay
and interassay coefficients of variation from pooled
ferret samples and determination of recovery after
samples were spiked or diluted. Another limitation of
the study was that analyses were not performed at a
point-of-care level, potentially increasing preanalytic
errors. However, because few laboratories perform
the needed analyses at a practice level, the pragmatic
design of our study reflected the types of errors ex-
pected to occur in clinical practice.

Because of the number of ferrets in the present
study with high aldosterone concentrations, there is
a need to develop further tests to identify primary hy-
peraldosteronism. In cats, a high plasma aldosterone-
to-renin ratio and a lack of decrease in aldosterone
concentration during a fludrocortisone suppression
test are currently considered the reference standard
diagnosis of hyperaldosteronism. In the future,
these tests could be validated for diagnosing primary
hyperaldosteronism in ferrets.

Acknowledgments
No third-party funding or support was received in connec-
tion with this study or the writing or publication of the manu-
script. The authors did not have any financial interests with com-
panies that manufactured products that were the subject of the

Discussion

Results of the present study indicated that serum
aldosterone concentrations ranged widely in ferrets,
especially unhealthy ferrets, and that high serum aldo-
sterone concentrations were common in client-owned
ferrets, with 24% (19/78) of ferrets in the study having
a serum aldosterone concentration > 18 pg/mL. Thus,
we concluded that, as in other mammals, a high aldo-
sterone concentration should not be considered diag-
nostic of primary hyperaldosteronism in ferrets.

Serum aldosterone concentrations in the popula-
tion of ferrets tested had a positively skewed distribu-

Figure 4

Receiver operating characteristic curve of serum
aldosterone concentrations in 56 healthy and 22 diseased fer-
rets (area under the curve, 0.79; 95% CI, 0.67 to 0.91). The
cutoff values that best optimize both sensitivity and specificity
(1), that best optimize sensitivity (2), and that best optimize
specificity (3) are shown.
present research or with companies that manufactured competing products.

Footnotes
b. SPSS, version 22.0, IBM Corp, Chicago, Ill.
c. MedCalc, MedCalc Software, Mariakerke, Belgium.

References