

Evaluation of trypsin-like immunoreactivity, pancreatic lipase immunoreactivity, cobalamin, and folate in zoo-housed African painted dogs (*Lycaon pictus*) at a North American zoological facility

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OBJECTIVE

Assess markers for pancreatic function and gastrointestinal malabsorption in African painted dogs (*Lycaon pictus*), including canine trypsin-like immunoreactivity (cTLI), canine pancreatic lipase immunoreactivity (cPLI), cobalamin, and folate at one North American facility.

ANIMALS

15 healthy African painted dogs held at one institution were sampled during routine health examinations.

METHODS

Blood was collected at routine health examinations, and serum was separated and stored until testing. Serum was analyzed for cTLI, cPLI, cobalamin, and folate. The results were evaluated for correlation to sex, age, and storage time of samples.

RESULTS

All individuals had cTLI and folate levels below normal reference ranges for domestic dogs (< 5.0 µg/L and < 7.7 µg/L, respectively). Cobalamin values were within or above reported domestic dog ranges, and cPLI values were within range as well. No analytes were significantly influenced by sex or time in storage, while cTLI was positively correlated with age.

CLINICAL RELEVANCE

cTLI and folate did not fall within normal domestic canid reference ranges in this population of healthy African painted dogs. Clinical interpretation of these values based on domestic canid recommendations would indicate clinical disease, which was not apparent in this population. Analytes for pancreatic function and malabsorption or gastrointestinal indicators, including cTLI, cPLI, and folate, in African painted dogs should be interpreted with caution when using domestic dog reference ranges.

Keywords: canine trypsin-like immunoreactivity, folate, canine pancreatic lipase immunoreactivity, cobalamin, African painted dog (*Lycaon pictus*)

African painted dogs (*Lycaon pictus*) are an endangered wild African canid with a tight social structure, often kept in packs in managed care.¹ These exotic canids may be susceptible to many of the same diseases as domestic dogs, including gastrointestinal and malabsorptive diseases. In a morbidity review of African painted dogs housed in zoological institutions in the United Kingdom, the digestive system accounted for the second most commonly reported

morbidity.² In this study, digestive disease was most often associated with positive fecal parasite screens; however, causes of digestive issues independent of parasitic infections were not detailed. Significant gastrointestinal disease has been reported in other exotic canids. Clinical signs such as chronic vomiting and diarrhea, weight loss, and malaise have been seen with inflammatory bowel disease in other exotic canids, such as maned wolves (*Chrysocyon brachyurus*) and Mexican grey wolves (*Canis lupis baileyi*), as well as mortality due to gastrointestinal disease in red wolves (*Canis rufus*).^{3,4} Gastrointestinal or pancreatic disease has not been significantly reported in

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African painted dogs; however, validation of testing to detect disease is warranted to help diagnose and properly treat this species in cases of clinical gastrointestinal signs.

Gastrointestinal and pancreatic disease is more well studied in domestic canids. Once infectious causes of gastrointestinal disease and noninfectious causes, such as foreign bodies, have been ruled out, complete blood counts and serum biochemistry may show nonspecific signs of inflammation and protein loss. If maldigestive or malabsorptive disease is suspected, imaging, such as abdominal ultrasound, may show structural changes to the pancreas or intestinal walls, although biopsy would more definitively diagnose these causes of gastrointestinal distress.^{5,6} Malabsorption refers to impaired nutrient absorption wherever nutrients are absorbed, while maldigestion refers to impaired digestion within the intestinal lumen; however, since these processes are interdependent, the terms are often used interchangeably.⁷ When more invasive procedures, like biopsy, are not available, or imaging is not definitive, maldigestive disorders may be further evaluated through the evaluation of serum pancreatic and absorptive indicators, such as trypsin-like immunoreactivity (TLI), pancreatic lipase immunoreactivity (PLI), cobalamin, and folate.

Assays for TLI in domestic dogs reflect the amount of functional pancreatic tissue present by quantifying trypsin and trypsinogen, which are pancreas-specific enzymes.⁶ Low TLI is the most sensitive marker for exocrine pancreatic insufficiency (EPI), which is associated with maldigestion and chronic gastrointestinal signs.⁶ PLI assays measure lipase that specifically originates from the exocrine pancreas; elevations in this analyte are a sensitive indicator of pancreatitis in domestic dogs.⁶ Cobalamin (vitamin B12) and folate (vitamin B9) are both water-soluble vitamins that are acquired through diet. In domestic dogs, cobalamin is absorbed across the distal small intestine, whereas folate is absorbed across the proximal small intestine.⁵ Since complete canine diets are supplemented with these vitamins, low serum levels suggest malabsorptive disease of the small intestine.⁸ Using these analytes when evaluating gastrointestinal signs can help determine whether pancreatic insufficiency, inflammation, or malabsorption in the small intestine are contributing to clinical signs. These diagnoses would then affect treatment options, which may include supplementation with pancreatic enzymes or vitamins.

Although it is assumed that exotic canids are analogous to domestic canids, values for these 4 analytes have not been reported for nondomestic canid species nor have these assays been validated in African painted dogs. Immunoassays such as TLI and PLI are species specific and may not crossreact with all canid species. Understanding whether indicators such as TLI, PLI, cobalamin, and folate used in characterizing gastrointestinal and malabsorptive diseases of domestic species are comparable to exotic canids is important to ensure accurate diagnosis of gastrointestinal disease. These 4 analytes

were evaluated in the population of healthy African painted dogs at one institution from stored serum samples with the goal of evaluating whether values fall within normal ranges reported in healthy domestic canines. These values were also compared to several case reports of sick animals, with and without evidence of gastrointestinal and pancreatic disease. It is hypothesized that African painted dog TLI, PLI, cobalamin, and folate will fall within the ranges reported in domestic canines because of their close taxonomic relationship.

Methods

Banked serum samples collected from African painted dogs from a single North American institution were analyzed. Fifteen presumed healthy dogs were included in the study, including 7 males and 8 females, ranging in age from 1.5 to 7.5 years old. Blood had been collected at routine health examinations in which dogs were fasted for 8 to 16 hours and anesthetized. Samples were included if the individuals had a normal physical examination and normal complete blood cell counts and serum biochemistry. At the time of collection, serum was separated and stored at -80°C for up to 6 years. Diets of these individuals consisted of Nebraska brand Premium Carnivore Diet with 5% to 15% fat content (Central Nebraska Packaging Inc) as well as whole prey items, including rabbit and goat carcass, or cow and horse bones. Nebraska meat is manufactured with supplemental vitamin B 12 at 0.04 ppm and folic acid at 1.3 mg/kg on a dry matter basis. All dogs were managed as a pack; thus, social dynamics influenced the amount consumed by each individual on a daily basis.

Serum from the 15 clinically normal African painted dogs was evaluated for TLI, PLI, cobalamin, and folate. Canine TLI (cTLI), cobalamin, and folate were analyzed via IMMULITE 2000 systems (Siemens Healthineers) via a solid-phase, enzyme-labeled, chemiluminescent immunometric assay commercially available for domestic dogs. Canine pancreatic-like immunoreactivity was analyzed by ELISA via the cPL Test Kit (Idexx). All analyses were performed at the Texas A&M University Gastrointestinal Laboratory. Reference ranges for domestic dogs were provided based on analysis at the Texas A&M University Gastrointestinal Laboratory for canine PLI (cPLI), cTLI, folate, and cobalamin (**Table 1**). Validation of these tests in this species was not performed; values are based off of assays developed for domestic canines.

Statistical analyses were performed using R CRAN Statistical Software (R Core Team⁹). Because all variables of interest did not meet assumptions of normality, nonparametric alternatives were used. To test the effect of sex, an unpaired Wilcoxon test was used. Spearman's rank correlation tests were used to test whether individual age or sample storage time impacted analyte concentrations. Differences were considered statistically significant if $P < .05$. Graphs and summary tables were created in RStudio (Posit team¹⁰) using the ggplot2 package (Wickham¹¹), and dplyr package (Wickham et al¹²).

Table 1—Domestic canine reference ranges provided by Texas A&M University Gastrointestinal Laboratory, including clinical interpretation for values above and below normal values.

Analyte	Value	Interpretation
cPLI (µg/L)	< 200	Normal
	201-399	Equivocal, may be consistent with pancreatitis, further testing recommended
	> 400	Consistent with pancreatitis
cTLI (µg/L)	< 2.5	Diagnostic for EPI
	2.6-7.5	Highly suggestive of EPI
	7.6-10	Subnormal, further testing for EPI recommended
Cobalamin (ng/L)	10.1-50	Normal
	> 50	Clinical significance unknown
	< 250	Decreased, consistent with distal small intestinal disease, EPI or dysbiosis
	251-399	Low end of normal, may be consistent with distal small intestinal disease
	400-908	Normal
Folate (µg/L)	> 909	Unknown clinical significance
	< 7.6	Decreased, consistent with proximal small intestinal disease
	7.7-24.4	Normal
	> 24.5	Elevated, possible dysbiosis or folate supplementation

cPLI = Canine pancreatic lipase immunoreactivity. cTLI = Canine trypsin-like immunoreactivity. EPI = Exocrine pancreatic insufficiency.

Results

Fifteen African painted dog serum samples were included in this evaluation. All dogs had normal physical examinations, with normal biochemistry and complete blood counts. Several dogs (n = 4) had evidence of mild cardiomyopathy or valvular disease but no abnormal gastrointestinal signs.

The mean, median, minimum, and maximum values of cTLI, cPLI, cobalamin, and folate are provided (**Table 2**). Box plots of all sample values, divided by sex, are provided (**Figure 1**). Scatter plots of the analytes compared to age and storage time are provided (**Supplementary Figures S1 and S2**), respectively. There were no differences in analyte concentrations between sexes ($P > .33$). Individual age of an animal was positively correlated with cTLI concentrations ($P = .05$). No other analytes

Table 2—Mean, median, minimum, and maximum values of folate, cobalamin, cTLI, and cPLI measured in 15 healthy African painted dogs.

Analyte	Mean	Median	Minimum	Maximum
Folate (µg/L)	4.46	4.63	1.37	7.62
Cobalamin (ng/L)	958.47	932	561	1,849
cTLI (µg/L)	2.38	2.34	1.17	4.7
cPLI (µg/L)	50.40	40	29	137

These values are not validated as reference ranges for this species.

cPLI = Canine pancreatic lipase immunoreactivity. cTLI = Canine trypsin-like immunoreactivity.

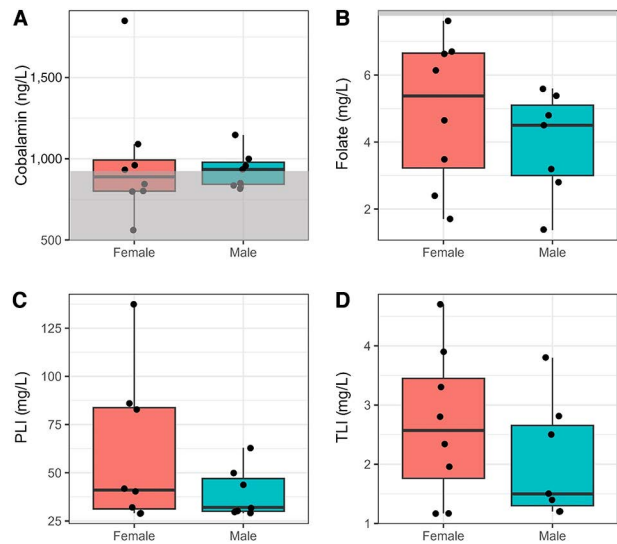


Figure 1—Box and whisker plot of (A) cobalamin, (B) folate, (C) canine pancreatic lipase immunoreactivity (cPLI), and (D) canine trypsin-like immunoreactivity (cTLI) concentrations between sexes in zoo-managed African painted dogs (*Lycaon pictus*). Gray boxes represent normal reference values in domestic dogs for cobalamin and folate. Note, all cPLI values fell within and all cTLI values fell below domestic canine reference ranges; thus, the gray box is not pictured. The upper and lower boundaries of the boxplots correspond to the 25th and 75th percentile (ie, first and third quartiles), and the solid line in the middle depicts the median value across samples (50th percentile). The length of the whiskers depicts sample concentrations that are 1.5 times above or below the interquartile range.

were significantly influenced by age, and none were impacted by storage time.

Additionally, serum from 4 sick African painted dogs at the time of euthanasia or death was included, with paired histopathology of pancreas, endocrine, and gastrointestinal tissues (**Table 3**). Case 1 died with signs of vomiting and lethargy; on histopathology, this individual had islet cell vacuolar degeneration of the pancreas and moderate lymphoplasmacytic and eosinophilic enterocolitis. Islet cell degeneration in the pancreas can result in diabetes mellitus, and the enteritis is consistent with moderate colon inflammation. This individual had normal cPLI (60 µg/L), high cobalamin (2178 ng/L), low folate (3.08 µg/L), and low cTLI (2.53 µg/L). Bloodwork for case 1 6 months earlier at a routine health examination had comparable values to perimortem testing, although cobalamin was within normal range (850 ng/L). Case 2 was euthanized with perimortem signs of loose stool and weight loss, which was responsive to dietary supplementation with beef pancreas. Histopathology showed chronic interstitial necrotizing pancreatitis and moderate pyloric gastritis. Perimortem bloodwork showed normal cPLI (29 µg/L), high cobalamin (1398 ng/L), low folate (4.28 µg/L), and cTLI below the limit of detection. Case 3 died of blastomycosis with no gastrointestinal signs. Perimortem bloodwork showed normal cPLI (42 µg/L), high cobalamin (2920 ng/L),

Table 3—cTLI, cPLI, cobalamin, and folate in selected sick African painted dogs, with comparison to bloodwork from routine healthy examinations prior to death.

	cTLI ($\mu\text{g/L}$)	cPLI ($\mu\text{g/L}$)	Cobalamin (ng/L)	Folate ($\mu\text{g/L}$)	Histopathology changes
Case 1 (perimortem)	2.53	60	2178	3.08	Islet cell vacuolar degeneration of the pancreas, moderate lymphoplasmacytic and eosinophilic enterocolitis
Case 1 (healthy)	2.81	63	850	1.37	Routine health examination, 6 months prior to death
Case 2 (perimortem)	< 1.00 ^a	125	1,398	4.28	Chronic interstitial necrotizing pancreatitis, moderate pyloric gastritis
Case 2 (healthy)	1.83	29	986	4.22	Routine health examination, 20 months prior to death
Case 3 (perimortem)	1.91	42	2,920	1.65	Blastomycosis, no gastric or pancreatic involvement
Case 3 (healthy)	< 1.00 ^a	80	992	4.64	Routine health examination, 22 months prior to death
Case 4 (perimortem)	< 1.00 ^a	977	875	8.45	Gastric perforation, no significant lesions in the small intestinal tract or pancreas (pancreas autolysis obscured full evaluation)

Major histopathology findings are provided. No healthy bloodwork was available for case 4.

cPLI = Canine pancreatic lipase immunoreactivity. cTLI = Canine trypsin-like immunoreactivity.

^aIndicates values below the limit of detection of the assay.

low folate (1.65 $\mu\text{g/L}$), and low cTLI (1.91 $\mu\text{g/L}$), although histopathology indicated no abnormalities in the pancreas or gastrointestinal tract. At routine examination, bloodwork on cases 2 and 3 nearly 2 years prior to death had normal cPLI (29 and 80 $\mu\text{g/L}$, respectively), high cobalamin (986 and 992 ng/L , respectively), low folate (4.22 and 4.64 $\mu\text{g/L}$, respectively), and low cTLI (1.83 $\mu\text{g/L}$ and below the limit of detection, respectively) in each individual. Case 4 died due to a gastric perforation secondary to nonsteroidal anti-inflammatory use, with no significant lesions in the small intestinal tract or pancreas, although autolysis obscured full evaluation of the pancreas. Perimortem bloodwork showed elevated cPLI (977 $\mu\text{g/L}$), which is consistent with pancreatitis in domestic dogs (values above 400 $\mu\text{g/L}$), normal folate (8.45 $\mu\text{g/L}$) and cobalamin (875 ng/L), and cTLI below the limit of detection. Bloodwork at the time of a routine healthy examination was not available for case 4.

Discussion

Indicators of pancreatic and gastrointestinal disease were evaluated for 15 healthy African painted dogs on banked serum samples. Values appeared consistent with normal values in domestic dogs for cPLI; however, values for cTLI, cobalamin, and folate were often outside of reference ranges provided by the testing facility. This may indicate that domestic canine reference ranges are not appropriate for determining pancreatic or gastrointestinal disease based on these assays and analytes.

TLI is one of the most common measures for pancreatic function in domestic dogs.¹³ Although there can be mild variation of TLI in healthy dogs, values below 2.5 $\mu\text{g/L}$ indicate a severe loss of pancreatic function and are diagnostic for EPI in domestic dogs.¹³ EPI is characterized by malabsorption, signs of diarrhea, and weight loss in domestic dogs and is often accompanied by cobalamin deficiency due to poor absorption in the small intestine.⁶ The clinical presentation of this disease in African painted dogs is unknown. All of the African painted dogs in this

study had cTLI values that were suspicious for or consistent with EPI based on domestic dog ranges (< 7.5 $\mu\text{g/L}$); however, these samples were taken on presumed healthy individuals without current signs of gastrointestinal disease. Few individuals had reported bouts of loose stool or vomiting associated with select prey items, but no signs consistent with EPI were noted at the time of blood collection. All individuals were in adequate body condition or overconditioned. One individual who died of non-gastrointestinal disease (blastomycosis) had a cTLI of 1.91 $\mu\text{g/L}$ at the time of euthanasia, although histopathology showed no pancreatic abnormalities. Although histopathology or pancreatic sampling was not available for all individuals sampled and the exact clinical presentation of EPI in this species is unknown, signs common in domestic dogs were not seen in this population, and EPI is unlikely for this large group of apparently healthy individuals.

Two cases were evaluated that did have pancreatic pathology, including pancreatic islet cell degeneration (case 1) and necrotizing pancreatitis (case 2). These each showed low cTLI (2.53 and < 1.0 $\mu\text{g/L}$, respectively). However, both had low cTLI at routine health examinations as well (2.81 and 1.83 $\mu\text{g/L}$, respectively). Case 3 had no evidence of pancreatic disease, although serial cTLI in this individual was below the limit of detection when healthy (2 years prior to death) and 1.91 $\mu\text{g/L}$ at the time of death. These findings further indicate that cTLI does not appear to be appropriate for determining pancreatic function in African painted dogs when using domestic dog reference ranges. All 4 clinically ill dogs were described as inappetent prior to death or euthanasia; thus, values are comparable to anesthetized samples in the healthy cohort in regards to fasting status.

Measurement of TLI in other exotic species, such as nondomestic felids, has also shown values below domestic reference ranges. Values of feline TLI in cheetahs and tigers were also reportedly low, often within the range suggestive or diagnostic for EPI in domestic felids.^{14,15} In tigers, however, additional clinical signs of EPI were present in some individuals

and were responsive to EPI treatments.¹⁵ It appears that domestic felid-based assays are not directly translatable to nondomestic species and should be interpreted once assays are validated and species-appropriate reference ranges are established. For African painted dogs, it is likely that cTLI cannot be used as an indicator of EPI at the ranges developed for domestic dogs; thus, establishing assay validation and reference ranges in accordance with the American Society of Veterinary Clinical Pathology is warranted before clinical determinations are made in this species.

This cohort of African painted dogs had cobalamin levels at or above normal ranges for domestic dogs. Cobalamin is exclusively absorbed in the distal small intestine in domestic dogs and thus is used as an indicator for malabsorption in that section of the gastrointestinal tract.⁵ The clinical significance of elevated cobalamin is unknown and likely associated with dietary supplementation and does not appear to be associated with disease in domestic dogs.⁵ Based on domestic dog references, these African painted dogs appear to have adequate or increased cobalamin levels. From the cases available, the utility of diagnosing distal small intestinal disease based on low cobalamin levels is unknown in this species. In domestic dogs, cobalamin deficiency is frequently seen in dogs with EPI.¹³ Cobalamin within normal ranges for domestic dogs further supports the conclusion that these individuals likely do not have EPI, and TLI should not be interpreted without validation in this species.

All African painted dogs in this study had folate below the normal range for domestic dogs (7.7 to 24.4 µg/L). Folate is absorbed primarily in the proximal small intestine; thus, decreases in folate concentrations in adequately supplemented dogs indicate malabsorptive disease.⁵ Of the cases in which histopathology was available, cases 2, 3, and 4 had normal small intestinal morphology, although folate was below 7.7 µg/L in cases 2 and 3. Case 4 folate was 8.45 µg/L, which is only minimally above the lowest domestic dog reference range. Folate was supplied in the meat diet at a standard formulation of 1.3 mg/kg dry matter, which is well above the recommended Association of American Feed Control Officials supplementation rate of 0.216 mg/kg dry matter weight for domestic dogs.¹⁶ Recommended folate and cobalamin supplementation levels are not established for African painted dogs. Individual consumption of food may vary based on pack dynamics, but all were in good body condition, so it is assumed that they had adequate consumption of diet. Folate and cobalamin can be mildly affected by fasting, but it does not result in clinically significant changes; therefore, exact fasting times are unlikely to have influenced the values reported in these individuals.¹⁷ It appears that assessing folate based on domestic dog ranges likely does not reflect malabsorptive disease of the proximal small intestine in African painted dogs, although further study is recommended.

In this study, cPLI in this species also fell within reported ranges for domestic dogs (normal range is below 200 µg/L). Pancreatic lipase originates

exclusively from the pancreas, and elevated levels are the most sensitive indicator for pancreatitis.⁶ It can also be used to confirm EPI as cPLI is often low in conjunction with low cTLI.⁶ The low cTLI with normal cPLI in this cohort of African painted dogs further supports the assumption that these dogs were not suffering from EPI but rather that cTLI is not a sensitive indicator in this species or that this assay is invalid in this species. In the few case reports evaluated, case 2 was diagnosed with necrotizing pancreatitis, although cPLI was normal (29 µg/L). However, in case 4, cPLI was elevated at 977 µg/L, which is consistent with pancreatitis in domestic dogs. cPLI is the most sensitive indicator of clinically severe pancreatitis in dogs, although a single cPLI value is not predictive of histopathological changes.⁶ Gastric perforation and peritonitis could likely lead to secondary pancreatitis, but this was not captured on histopathology, likely due to pancreatic autolysis. In this small sample size, cPLI was variably related to suspected inflammation of the pancreas when interpreted with domestic dog ranges. Because cPLI is an immunoassay that is specific for lipase that originates from the pancreas, differences in binding sites or lipase specificity between species may affect detection and results. Further research would be needed to validate this assay and confirm which types of pancreatic illness would be correlated with elevations in cPLI in this species and whether this assay is useful in the diagnosis of pancreatic disease.

There are several limitations to this study. These commercial assays have not been validated for African painted dogs. Because this species is so closely related to domestic dogs, many clinicians may utilize domestic dog testing and extrapolate the interpretation of results. However, the results of this study show that several of the analytes do not match with the clinical interpretations developed in domestic canids. The lack of correlation with clinical signs seen in domestic dogs indicates that further research is needed to validate analytes such as cTLI and folate in African painted dogs. Although values fell within clinically appropriate ranges for domestic dogs, these assays for cobalamin and cPLI should be validated in African painted dogs before relying on clinical interpretations as well. Because many tests and assays are originally designed and validated in domestic species, validation of assays in nondomestic species is commonly omitted when creating reference ranges in novel species.¹⁸ Given the lack of validation of these assays and the discrepancy of clinical interpretation of African painted dog values compared to domestic canids, the results of the analytes in this study should not be used as normal reference values for this species.

Diet was not controlled in this study. All dogs were fed the same base diet of Nebraska brand Premium Carnivore Diet, which is fortified with cobalamin and folate. The folate and cobalamin levels were well above the minimum requirements for domestic dogs, although recommendations for African painted dogs are not established. In addition, due to the pack management of this species, all dogs were fed

as a group, so the exact amount consumed by each dog is unknown; thus, conclusions about cobalamin and folate should be interpreted with caution. Each dog was in adequate body condition or overconditioned, indicating that each consumed adequate amounts of the diet to maintain a healthy weight or more, and are assumed to have received at least the minimal nutrient requirements for folate and cobalamin recommended for domestic dogs. The diet was supplemented with intermittent carcass feedings as frequently as once weekly; nutrient analysis for these was not available. Both cobalamin and folate are influenced by diet; however, low levels in adequately supplemented domestic dogs is indicative of poor small intestinal absorption. Consistently low levels in this cohort of healthy African painted dogs as well as the cases with available histopathology indicates that these domestic ranges are not appropriate for determination of malabsorption in this species.

The majority of these African painted dogs shared close heredity. Thirteen of these painted dogs were born from 2 females who shared direct parentage; thus, they were closely genetically related. The remaining 2 were one of the parents from these litters (dam and sire). Genetic influence on overall values cannot be ruled out; however, no consistent functional changes were noted between litters or parents. Case 2 is the mother of 8 of the healthy tested individuals, and case 3 is one of her offspring. Case 2's pancreatic histopathology changes do not appear to have been inherited in case 3. Additional sampling in nonrelated African painted dogs is warranted to determine if these trends are persistent throughout the species.

Additionally, many samples were stored for long periods of time, up to 6 years. Time in storage was not correlated with any of the analytes; however, the stability of these analytes has not been established beyond a year or within this species. Cobalamin and cPLI have been showed to be stable in human and feline serum (respectively) for up to 4 years; however, assays differed and may not be translatable to the assays in this study.^{19,20} Storage may lead to degradation of the serum and thus analyte levels. Lab recommendations in this study indicated that serum samples in domestic animals stored up to 1 year in storage at -80°C were unlikely to have clinically significant changes; however, longer periods of storage have not been evaluated. Regardless, even in fresh serum samples on adult African painted dogs, cTLI values were below normal reference ranges for domestic dogs and within the range of diagnosis for EPI, so clinical interpretation did not change. cTLI was positively correlated with age; this correlation has not been reported in domestic dogs, and the clinical significance is unknown. Samples were not reported to have significant lipemia or hemolysis and thus were unlikely to influence values. Small sample size may have skewed this population. Further validation of the assay and evaluation of African painted dogs of various ages is warranted to determine the significance of this trend.

Common indicators of pancreatic and malabsorptive disease were evaluated in 15 healthy

African painted dogs, including cTLI, cPLI, cobalamin, and folate. In addition, a case series of African painted dogs with available perimortem sampling and histopathology of the pancreas (3 individuals) and the gastrointestinal tract (4 individuals) were also evaluated. Folate and cTLI in healthy African painted dogs were below the ranges established for domestic dogs and should be interpreted with caution. cPLI fell within normal ranges reported in domestic dogs, although some individuals with pancreatic disease did not have corresponding elevations in cPLI. Cobalamin values appeared to be within or increased compared to normal reference ranges in dogs. Further research is warranted to establish markers of pancreatic and malabsorptive disease in African painted dogs and validate assays used in domestic canids.

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References

1. Woodroffe R, Sillero-Zubiri C. *Lycaon pictus* (amended version of 2012 assessment). *The IUCN Red List of Threatened Species*. 2020. Accessed February 12, 2024. https://www.cms.int/sites/default/files/document/cites-cms_aci2_inf.11_iucn-african-wilddog_e.pdf
2. Cock HR, Spiro S, Stidworthy MF, Denk D, Guthrie A. Retrospective study of morbidity and mortality of captive African wild dogs (*Lycaon pictus*) held within united kingdom zoological institutions between 2000 and 2020. *J Zoo Wildl Med*. 2023;54(3):498-510. 10.1638/2022-0026
3. Padilla LR, Hilton CD. Canidae. In: Miller ER, Fowler ME, eds. *Fowler's Zoo and Wild Animal Medicine*. Vol 8. Elsevier; 2015:457.
4. Seeley KE, Garner MM, Waddell WT, Wolf KN. A survey of diseases in captive red wolves (*canis rufus*), 1997-2012. *J Zoo Wildl Med*. 2016;47(1):83-90. 10.1638/2014-0198.1
5. Heilmann RM, Steiner JM. Clinical utility of currently available biomarkers in inflammatory enteropathies of dogs. *J Vet Intern Med*. 2018;32(5):1495-1508. 10.1111/jvim.15247
6. Heilmann RM, Steiner JM. Laboratory testing for the exocrine pancreas. In: Bonagura JD, Twedt DC, eds. *Kirk's Current Veterinary Therapy XV*. Elsevier Health Sciences; 2014:554-557.
7. Belletieri C, Zuarox T. Malabsorption syndromes. Accessed March 3, 2024. <https://www.ncbi.nlm.nih.gov/books/NBK553106/>
8. Simpson KW, Worhunsky PA. Cobalamin deficiency in cats. In: Bonagura JD, Twedt DC, eds. *Kirk's Current Veterinary Therapy XV*. Elsevier Health Sciences; 2014:522-524.
9. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2022. <https://www.R-project.org/>

10. Posit team. RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. 2023. <http://www.posit.co/>
11. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag; 2016. <https://ggplot2.tidyverse.org>
12. Wickham H, François R, Henry L, Müller K, Vaughan D. dplyr: A grammar of data manipulation. R package version 1.1.4. 2023. <https://github.com/tidyverse/dplyr>, <https://dplyr.tidyverse.org>
13. Wiberg M. Exocrine pancreatic insufficiency in dogs. In: Bonagura JD, Twedt DC, eds. *Kirk's Current Veterinary Therapy XV*. Elsevier Health Sciences; 2014: 558–560.
14. Fox L, Haefele H, Uelmen J, et al. Biomarkers of gastrointestinal disease in cheetahs (*acinonyx jubatus*). *J Zoo Wildl Med*. 2021;52(3):886–892. 10.1638/2021-0012
15. Mulreany LM, Ramsay EC, Cushing AC, Suchodolski JS, Lidbury J, Steiner JM. Exocrine pancreatic insufficiency-like syndrome in four captive tigers (*panthera tigris*). *J Zoo Wildl Med*. 2021;52(3):1079–1083. 10.1638/2019-0195
16. Association of American Feed Control Officials. AAFCO methods for substantiating nutritional adequacy of dog and cat foods. 2014. Accessed February 12, 2024. https://www.aafco.org/wpcontent/uploads/2023/01/Model_Bills_and_Regulations_Agenda_Midyear_2015_Final_Attachment_A_Proposed_revisions_to_AAFCO_Nutrient_Profiles_PFC_Final_070214.pdf
17. Saver AT, Steiner JM, Hetzel SJ, Lidbury JA, Suchodolski JS, Pritchard JC. Effect of withholding food on serum concentrations of cobalamin, folate, trypsin-like immunoreactivity, and pancreatic lipase immunoreactivity in healthy dogs. *Am J Vet Res*. 2021;82(5):367–373. 10.2460/ajvr.82.5.367
18. Moore AR, Camus MS, Harr K, et al. Systematic evaluation of 106 laboratory reference data articles from non-domestic species published from 2014 to 2016: assessing compliance with reference interval guidelines. *J Zoo Wildl Med*. 2020;51(3):469–477. 10.1638/2019-0186
19. Goodband EL, Serrano G, Constantino-Casas F, Archer J, Watson PJ, Williams TL. Validation of a commercial 1, 2-o-dilauryl-rac-glycero glutaric acid-(6'-methylresorufin) ester lipase assay for diagnosis of canine pancreatitis. *Vet Rec Open*. 2018;5(1):e000270. 10.1136/vetreco-2017-000270
20. Paladugula N, Fazili Z, Sternberg MR, Gabey G, Pfeiffer CM. Serum folate forms are stable during repeated analysis in the presence of ascorbic acid and during frozen sample storage. *J Appl Lab Med*. 2019;3(6):993–1002. 10.1373/jalm.2018.027102

Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.