Results of wellness examinations of 28 African hunting dog (*Lycaon pictus*) puppies at the Denver Zoological Foundation

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ABSTRACT

Since 2002 the Denver Zoological Foundation has produced 28 African hunting dog (*Lycaon pictus*) puppies in 3 litters (7, 14 and 7 pups) from the same dam and sire. Wellness examinations were performed on each puppy. The wellness examinations spanned the range of 6–14 weeks of age. During the wellness examinations, in addition to physical examinations and vaccinations, blood samples for complete blood counts and sera biochemistry were obtained. Weights, morphometric measurements, rectal cultures for enteric pathogens and dental eruption patterns were recorded. Blood samples from each age group were compared with adult values from the Denver Zoo. It was noted that animals from the 14-pup litter were 63.6 % of the mean weight of the two 7-pup litters, but size differences (in, for example, total body length) were less apparent. Two organisms were recovered from rectal cultures, namely *Yersinia enterocolitica* ($n = 2$) and *Plesiomonas shigelloides* ($n = 3$). The following deciduous eruption patterns were also noted; at 6 weeks, I1–3, i1–3, C1, c1, P1–2 and p1–2 ($n = 7$) were present, at 9–10 weeks, P3 and p3 ($n = 21$) , and finally at 12–14 weeks, P4 ($n = 28$).

Key words: African hunting dog, biochemistry, complete blood count, dental eruption pattern, *Lycaon pictus,* morphometrics, rectal culture, weight, wellness examinations.

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INTRODUCTION

Between 2002 and 2004 the Denver Zoological Foundation (Denver Zoo) had 28 African hunting dog (*Lycaon pictus*) puppies born in 3 litters to the same dam and sire. The pack size ranged from 3 to 10 adults when the 3 litters were born. In nature, wild dog puppies stay in the den with the dam for approximately the first 3 months, so this aspect of wild dog ecology has been little studied[15]. There was an opportunity to perform 3 wellness examinations on each litter with pups ranging in age from 6 to 14 weeks. In addition to complete physical examinations and administering vaccinations, blood samples were obtained for complete blood counts and serum biochemistry. Weights, morphometric measurements, rectal cultures for potential enteric pathogens were taken and dental eruption patterns were noted. Blood counts and serum biochemistry were also compared with results from the adult dogs. All 28 puppies survived at least to 24 months of age. This report details the results of the wellness examinations.

MATERIALS AND METHODS

Twenty-eight African hunting dog puppies were born in 3 litters at the Denver Zoo. There were 7 pups in the 1st litter, 14 pups in the 2nd, and 7 pups in the 3rd. The dam and sire were the same for each of the 3 litters. The dam was 22, 31 and 53.5 months old, respectively, when she had the 3 litters. Puppy sex distribution for the 3 litters was as follows: litter one, 5 females and 2 males; litter two, 7 males and 7 females, and litter three, 5 males and 2 females, resulting in a 50/50 distribution for the 28 puppies. Three wellness examinations were performed on each litter. Pups were transported to the zoo hospital for examination at ~09:00. The timing of examinations (pup age at examination) was the same for all pups within a litter but the timing varied between litters. The examination range was 6–14 weeks of age and was performed under manual restraint.

The pups were whelped in wooden crates 95 cm high, 81 cm long and 127 cm wide divided into 2 compartments with a communicating doorway. The doorway to the outside had a lip 10 cm high to keep the pups in the crate until they were walking. Pups were not offered any solid food until they were 5 weeks old. For example, at 5 weeks of age the 14-pup litter was offered ~450 g of a commercial horse meat diet (Nebraska Brand Canine Diet, Central Nebraska Packing Inc., North Platte, Nebraska, USA) in the morning. The diet was increased to 1 kg at 6 weeks and 1.6 kg at 8 weeks.

At each examination the pups were administered 1 mℓ of DA_2PPv vaccine (Galaxy® modified live distemper-adenovirus type 2-parainfluenza-parvovirus vaccine, Schering-Plough Animal Health Corp., Omaha, Nebraska, USA) subcutaneously (s.c.). In addition, during the 3rd examination the pups were administered 1 mℓ of rabies vaccine (Imrab® 3 killed rabies vaccine, Merial Inc., Athens, Georgia, USA) s.c. At each evaluation the pups were also prophylactically dewormed with 6.6 mg/kg pyrantel pamoate (Strongid® T, Pfizer Animal Health, Exton, Pennsylvania, USA) *per os.*

Blood samples for complete blood counts were collected in 500 $\mu\ell$ tubes (Capiject™T-MQK, Terumo® Medical Corp., Elkton, Maryland, USA). Sera for biochemistry were collected in red top tubes (Becton, Dickinson Co., Franklin Lakes, New Jersey, USA). Blood samples were processed the same day at the Denver Zoo Animal Health Center laboratory. Blood was collected on 63 of 84 opportunities for complete blood counts. Serum biochemistry evaluations were analysed using a dry chemistry analyser (VetScan®, Abaxis Inc., Union City, California, USA). The chemistry analyses performed were: total protein (TP), albumin (Alb), globulin (Glob), alanine aminotransferase (ALT), alkaline phosphatase (Alk Phos), blood urea nitrogen (BUN), creatinine (Creat), glucose, total bilirubin (TB), calcium (Ca), phosphorus (Phos), sodium (Na), potassium (K) and amylase. The number of specific biochemistries performed ranged from 42 to 63.

A mean and standard error was calculated for complete blood counts, biochem-

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Table 1: **Complete blood count values obtained from African hunting dog puppies during wellness examinations expressed as mean ± standard error. All puppies received 3 wellness examinations but they were not conducted on exactly the same schedule for each litter. ANOVA was used to compare means.**

	Pup age (weeks)				
	6 $n = 25$	**9–10** $n = 17$	**12** $n = 7$	**14** $n = 14$	**Adult** $n = 27$
Packed cell volume (%) $F_{4,84} = 40.95$, $P < 0.001$, $R^2 = 0.65$	33.9 ± 1.0	39.57 ± 0.40[a]	38.64 ± 0.32[a]	40.6 ± 0.30[a]	46.0 ± 0.80
White blood cells ($\times 10^9/\ell$) $F_{4,85} = 1.01$, $P = 0.41$, $R^2 = 0.05$	9.92 ± 0.41	11.45 ± 0.47	11.49 ± 0.91	13.43 ± 1.26	9.86 ± 0.50
Neutrophils ($\times 10^9/\ell$) $F_{4,85} = 0.77$, $P < 0.55$, $R^2 = 0.04$	7.09 ± 0.42[a]	7.91 ± 0.47[a]	8.83 ± 0.82[a]	8.86 ± 0.91[a]	7.26 ± 0.48[a]
Lymphocytes ($\times 10^9/\ell$) $F_{4,85} = 0.72$, $P < 0.58$, $R^2 = 0.03$	1.58 ± 0.12[a]	2.14 ± 0.18[a]	1.18 ± 0.19[a]	2.79 ± 0.28[a]	1.62 ± 0.17[a]
Monocytes ($\times 10^9/\ell$) $F_{4,82} = 0.98$, $P < 0.43$, $R^2 = 0.05$	0.51 ± 0.06[a]	0.59 ± 0.06[a]	0.50 ± 0.11[a]	0.84 ± 0.19[a]	0.47 ± 0.06[a]
Eosinophils ($\times 10^9/\ell$) $F_{4,82} = 5.83$, $P < 0.001$, $R^2 = 0.22$	0.53 ± 0.08[a]	0.81 ± 0.13[a,b,c]	0.98 ± 0.20[b,c]	1.02 ± 0.12[c]	0.55 ± 0.07[a,b]

[a,b,c] Pairwise comparisons with the same letter superscript were not significantly different using a Bonferroni's *post hoc* test.

istry values, weights and morphometric measurements for each age group. In addition, means were compared with ANOVA and Bonferroni's *post hoc* test used for pairwise comparisons. Data were checked for normality and homogeneity of variances using a Bartlett's test. Non-normally distributed data were transformed using either sine or log. Outliers were removed from the analysis. All pups were weighed (kg) at each examination. Morphometric measurements (cm) were not taken on all pups at each examination. Morphometric measurements performed were; total body length (TBL, the tip of the nose to the base of the tail), girth (thorax just caudal to front legs), height (top of scapula to bottom of foot placed flat), tail length and neck circumference (midpoint).

Rectal cultures were performed on 17 occasions from 14 puppies (3 pups were cultured twice) looking for potential enteric pathogens (non-lactose fermenters). Rectal cultures were collected using a BBL™ CultureSwab™ (Beckton, Dickinson and Co.) and then (1) streaked to trypticase™ soy agar with 5 % sheep blood (Beckton, Dickinson and Co.); (2) streaked to Hektoen enteric agar (Beckton, Dickinson and Co.) and (3) transferred to a GN broth which selects for Gram-negative organisms. Cultures were evaluated after 24 and 48 hours for growth. The GN broth was streaked to Hektoen enteric agar after 24 hours to evaluate for non-lactose fermenting organisms (Beckton, Dickinson and Co.). Isolated organisms were identified using the BBL™ crystal identification system (Beckton, Dickinson and Co.).

The oral cavity was periodically inspected for erupted deciduous teeth during each examination.

RESULTS

Results from complete blood counts and sera biochemistry are given in Tables 1 and 2. It was noted that the packed cell volume (PCV) of the pups was low compared to adults and gradually increased over the course of the neonatal evaluations. White cell counts (specifically neutrophils and lymphocytes) for domestic puppies are reported to be high at birth (compared with adults), declining during the 1st month, increasing in the 2nd month, and then slowly declining as they mature[2,3,4]. White cell counts in the wild dog puppies at the Denver Zoo were approximately at adult levels at 6 week of age, rose at 10–14 weeks, and then decreased to adult values. Statistically a consistent trend for values in both Tables 1 and 2, with few exceptions, was noted. There were no significant differences at ages 10, 12 and 14 weeks. Packed cell volume, TP (primarily composed of Alb and Glob), Alt, BUN, Creat and Na had a general tendency to increase over time to adulthood. This was in contrast to Alk Phos, Phos, K and amylase, which had a general tendency to decline over time to adulthood. Calcium values remained steady within the study groups. There did not appear to be any discernible pattern for glucose and TB in this study.

Weights and morphometric measurements are reported in Table 3. Six-week-old pups from the 14-pup litter were 63.6 % of the mean weight of pups from the two 7-pup litters. There was less of a difference for TBL and girth body measurements from 7-pup litters compared to the single 14-pup litter at 6 weeks (smaller by 95.8 % and 96.2 % for TBL and girth, respectively). Results at 10 weeks of age followed a similar trend. Pups from the 14-pup litter were 66.3 % of the weight and 92.7 % and 91.2 % smaller for TBL and girth compared to pups from 7-pup litters at 10 weeks of age. The pups had a 4-fold increase in weight, 1.5-fold increases in TBL and height, 1.35-fold increases in tail length and 1.2-fold increases in girth and neck dimensions from 6 to 14 weeks of age.

At 6 weeks of age, all deciduous upper and lower incisors and canines (I1–3, i1–3, C1, c1) were present. At the same age there were also 2 upper and lower premolars (P1–2, p1–2) present in 7 pups and the 3rd lower premolar (p3) was present in 21 pups. At 9–10 weeks of age, a 3rd upper premolar (P3) was present in all pups. Finally, at 12–14 weeks of age a 4th lower premolar (p4) was present in all pups.

Non-lactose fermenting bacteria were successfully cultured from 5 of 14 cultures (3 of the pups were cultured twice). Organisms recovered were *Yersinia enterocolitica* on 2 occasions and *Plesiomonas shigelloides* on 3 occasions.

DISCUSSION

African wild dogs are medium-sized, extremely social carnivores of the family Canidae, subfamily Simocyoninae, and are the only representative of the genus and species, *Lycaon pictus*[11,15]. Wild dogs are readily identifiable by their variegated pelage. The alpha male and female typically dominate reproduction[5]. Females are reported to be sexually mature at ~23 months[13]. Gestation length as determined by steroid analysis is 73–79 days[13]. Litter sizes can range from 2 to 19, but 7–10 are more typical[11]. For 36 litters in the Selous Game Reserve the mean litter size reported was 7.9 ± 0.5 SD[6]. The mean litter size at the Denver Zoo for 3 litters was 9.3 ± 4.0 SD pups. In 3 studies it has been

Table 2: **Sera biochemistry values obtained from African hunting dog puppies during 3 wellness examinations expressed as mean ± standard error. All puppies received 3 wellness examinations but the examinations for each litter were not conducted on exactly the same schedule. ANOVA was used to compare means.**

	Pup age (weeks)				
	6	9–10	12	14	Adult
TP (gm/ℓ) $F_{4,83}$ = 200.43, P < 0.001, R^2 = 0.91	45.4 ± 0.4 *n* = 25	50.4 ± 0.4[a] *n* = 17	53.0 ± 0.4[a] *n* = 7	51.6 ± 0.6[a] *n* = 14	62.7 ± 0.8 *n* = 27
Alb (gm/ℓ) $F_{4,83}$ = 11.07, P < 0.001, R^2 = 0.30	32.6 ± 0.6[b] *n* = 25	34.8 ± 0.8[a,b] *n* = 16	33.1 ± 0.3[a,b] *n* = 7	35.6 ± 0.5[a,c] *n* = 14	37.2 ± 0.5[c] *n* = 26
Glob (gm/ℓ) $F_{4,78}$ = 47.03, P < 0.001, R^2 = 0.71	14.1 ± 0.6[a] *n* = 18	16.0 ± 0.8[a] *n* = 17	20.0 ± 0.3[b] *n* = 7	16.3 ± 0.8[a,b] *n* = 14	25.3 ± 0.7 *n* = 27
ALT (U/ℓ) $F_{4,81}$ = 22.13, P < 0.001, R^2 = 0.52	25.7 ± 1.3 *n* = 23	31.7 ± 1.2[a] *n* = 16	35.1 ± 2.2[a,b] *n* = 7	36.3 ± 2.3[a] *n* = 14	50.3 ± 4.5[b] *n* = 27
Alk Phos (U/ℓ) $F_{4,83}$ = 111.64, P < 0.001, R^2 = 0.84	258.7 ± 9.8 *n* = 25	154.3 ± 6.6[a] *n* = 17	145.0 ± 6.5[a] *n* = 7	157.7 ± 5.3[a] *n* = 14	70.9 ± 4.5 *n* = 26
BUN (nmol/ℓ) $F_{4,85}$ = 24.50, P < 0.001, R^2 = 0.54	4.4 ± 0.3[a] *n* = 25	5.3 ± 0.7[a] *n* = 17	4.8 ± 0.4[a] *n* = 7	5.5 ± 0.3[a] *n* = 14	9.0 ± 0.3 *n* = 27
Creat (µmol/ℓ) $F_{4,84}$ = 29.01, P < 0.001, R^2 = 0.58	42.4 ± 4.4[a] *n* = 24	39.8 ± 4.4[a] *n* = 17	38.0 ± 5.3[a] *n* = 7	51.3 ± 5.3[a] *n* = 14	95.1 ± 5.1 *n* = 27
Glucose (mmol/ℓ) $F_{4,85}$ = 6.62, P < 0.001, R^2 = 0.24	6.6 ± 0.4[b] *n* = 25	8.4 ± 0.4[a] *n* = 17	8.3 ± 0.2[a] *n* = 7	8.6 ± 0.1[a] *n* = 14	7.7 ± 0.2[a,b] *n* = 27
TB (µmol/ℓ) $F_{4,64}$ = 22.13, P < 0.001, R^2 = 0.58	7.7 ± 1.4[a] *n* = 18	5.0 ± 0.5[a] *n* = 11	4.1 ± 0.3[b] *n* = 7	2.4 ± 0.3 *n* = 9	5.1 ± 0.2[a,b] *n* = 27
Ca (mmol/ℓ) $F_{4,83}$ = 1.83, P = 0.13, R^2 = 0.08	2.9 ± 0.05[a] *n* = 25	2.6 ± 0.04[a] *n* = 17	2.9 ± 0.03[a] *n* = 6	2.7 ± 0.06[a] *n* = 13	2.7 ± 0.04[a] *n* = 22
Phos (mmol/ℓ) $F_{4,83}$ = 41.18, P < 0.001, R^2 = 0.67	2.9 ± 0.05[a] *n* = 25	2.9 ± 0.09[a] *n* = 17	3.0 ± 0.08[a] *n* = 6	3.0 ± 0.05[a] *n* = 13	1.8 ± 0.10 *n* = 27
Na (mmol/ℓ) $F_{4,60}$ = 14.21, P < 0.001, R^2 = 0.49	139.2 ± 0.5[a] *n* = 18	138.6 ± 0.8[a] *n* = 10	142.1 ± 0.7[a,b] *n* = 7	141.9 ± 0.7[a,b] *n* = 7	145.0 ± 0.8[b] *n* = 24
K (mmol/ℓ) $F_{4,84}$ = 59.83, P < 0.001, R^2 = 0.74	5.1 ± 0.10[a] *n* = 25	4.9 ± 0.11[a] *n* = 17	4.2 ± 0.12 *n* = 7	4.9 ± 0.06[a] *n* = 14	3.7 ± 0.3 *n* = 27
Amylase (U/ℓ) $F_{4,83}$ = 37.80, P < 0.001, R^2 = 0.65	499.5 ± 11.7 *n* = 24	418.3 ± 13.7[a] *n* = 17	386.4 ± 12.1[a,b] *n* = 7	396.4 ± 13.2[a] *n* = 14	319.8 ± 10.3[b] *n* = 27

[a,b,c]Pairwise comparisons with the same superscript letter were not significantly different using a Bonferroni's *post hoc* test.

reported that there was a bias in sex distribution, being skewed towards males (53.7 % to 60 % male puppies)[13]. This may be due to a female bias in leaving the natal pack and subsequent high dispersal mortality[5]. For the 3 litters born at the Denver Zoo the sex distribution was even. For free-ranging wild dogs reproduction is seasonal with births typically timed to coincide with the region's dry season[6]. The hypothesis is that dens are located close to permanent water which serves to concentrate thirsty ungulates during the dry season[6]. Denver Zoo pups were born in November, January and February. It is believed there is not a reproductive seasonality preference for captive hunting dogs.

Many newborn animals experience a neonatal or physiological anaemia[10]. At birth, domestic puppies (*Canis familiaris*) from research colonies have adult values for packed cell volume[2–4]. The PCV begins to decrease when the puppy begins nursing and continues to decline for the 1st month[2]. Suggested factors for this physiological anaemia include increased plasma volume from colostrum diluting red cell mass, decreased red cell production, shortened red cell life-span, decreased erythropoietin production, rapid growth expanding vascular space without a concomitant increase in red cell mass and the replacement of foetal red blood cells with the adult line[2–4,10]. This same pattern was also present in the Denver Zoo wild dog puppies. The range for red cell mass for pups 6–14 weeks of age

Table 3: **Mean ± standard error for body weight, total body length, height, girth, neck and tail** (weight in kg, other measurements in cm) **collected from 28 neonatal African hunting dog puppies at the Denver Zoo.**

	Pup age (weeks)			
	6	10	12	14
Weight	1.89 ± 0.09 *n* = 28	3.46 ± 0.17 *n* = 21	5.17 ± 0.07 *n* = 14	7.39 ± 0.11 *n* = 14
TBL	43.59 ± 0.32 *n* = 21	55.44 ± 0.64 *n* = 21	61.51 ± 1.03 *n* = 14	68.93 ± 0.38 *n* = 14
Height	18.64 ± 0.18 *n* = 7	25.36 ± 0.18 *n* = 7	ND	25.36 ± 0.18 *n* = 7
Girth	25.70 ± 0.30 *n* = 21	31.19 ± 0.46 *n* = 21	36.14 ± 0.32 *n* = 14	31.19 ± 0.46 *n* = 21
Neck	16.50 ± 1.02 *n* = 7	19.57 ± 0.30 *n* = 7	ND	19.57 ± 0.30 *n* = 7
Tail	14.64 ± 0.46 *n* = 7	19.64 ± 0.36 *n* = 7	ND	19.64 ± 0.36 *n* = 7

ND = not done.

(Table 1) was 33.9 % to 40.6 % (n = 63) compared with a mean of 47.3 ± 0.7 % SE for 22 blood samples from our adult African wild dogs (adult dog age ≥1.3 ± 0.2 SE years).

Results of sera biochemistry analyses are reported in Table 2. Many of the differences between puppies and adult domestic dogs are due to growth processes[3]. Total protein primarily consists of albumin and globulin. In domestic puppies there is an initial decrease in total protein followed by an increase to adult values between 2 and 3 months of age[10]. Total protein values in the wild dog puppies were significantly lower than adult values at 6 weeks of age but continued to rise to 14 weeks. The serum albumin values for domestic puppies are equal to adult values while the globulin values are lower, being dependent on antigenic stimulation[3,4] An increase was noted in the wild dog puppies for both albumin and globulin over time (6 to 14 weeks of age). Alkaline phosphatase is an enzyme that can be found in several tissues but in young, growing animals the origin is most likely bone[10]. Domestic puppies have serum Alk Phos up to 20 times adult values in the 1st few days of life from ingesting Alk Phos-rich colostrum and then decrease to 2–3 times higher than adult values[3]. It was also noted that a high Alk Phos activity at 4 times adult levels at 6 weeks, decreasing to 2.5 times adult values by week 14. The liver-specific cytosolic enzyme Alt was 50 % of the adult enzyme activity at 6 weeks of age and continued to rise toward adult enzyme activity by week 14. Creatinine values are lower in domestic puppies because it is dependent on muscle creatine, which in turn depends on muscle to body mass ratio being lower in puppies[3]. Blood urea nitrogen values can be markedly influenced by diet[1]. Glucose levels were initially lower than in adult hunting dogs at 6 weeks of age and then were higher in puppies that were 10, 12 and 14 weeks old. Glucose values can be affected by recent meals and excitement[1,12]. Total bilirubin values in the puppies were significantly higher than adult values at 6 weeks and were 50 % of adult values at week 14. With regard to electrolytes, sodium, values were slightly lower than adult values and potassium values were higher. Serum calcium values were approximately the same as adult values but phosphorus values were approximately twice the adult values, presumably due to bone growth. Elevations in serum amylase are typically associated with acute pancreatitis and can also be elevated in chronic renal failure[10]. Amylase activity was initially high in wild dog puppies and declined to adult activity by week 14.

Weights and morphometric measurements reported in Table 3 show larger litter size (14 *vs* 7) did not appear to adversely affect the adult weight for the puppy. The mean weight for 14 adults at the Denver Zoo was 23.7 ± 0.4 SE kg (male 24.4 ± 1.0 kg, female 23.3 ± 0.3 kg). The mean adult weight for 6 pups from a 7-pup litter was 23.2 ± 0.4 kg compared to 24.5 ± 0.7 kg for 6 pups from a 14-pup litter. The larger litter was 1.3 kg heavier as adults.

Deciduous dental eruption (1st appearance of a tooth through the oral mucosa) for domestic puppies has been given for incisors at 3–4-weeks, canines at 3 weeks, and premolars at 4–12 weeks of age[8,14]. The deciduous eruption pattern and timing for wild dog puppies appeared to be similar to domestic dogs. Since the pups were not evaluated daily, it is only known which teeth were present during a specific examination; not the exact eruption date.

Plesiomonas shigelloides is a member of the family *Vibrionaceae* and *Yersinia enterocolitica* is a member of the family *Enterobacteriaceae*[7,9]. Both *P. shigelloides* and *Y. enterocolitica* can cause gastrointestinal disease (primarily diarrhoea) in humans[7,9]. Both can be found in water such as reservoirs and lakes but can also be found in animal reservoirs such as dogs[7,9]. The puppies were asymptomatic when cultured it is believed that they probably contracted the organisms from standing water or from the adults. They appear to be able to harbour these bacteria without necessarily developing overt clinical signs. Since these organisms do cause diseases in humans, they would remain a zoonotic consideration for caregivers.

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