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Authors: Cuadrado, M., Díaz-Paniagua, C., Quevedo, M. A., Aguilar, J. M., and Prescott, I. Molina

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HEMATOLOGY AND CLINICAL CHEMISTRY IN DYSTOCIC AND HEALTHY POST-REPRODUCTIVE FEMALE CHAMELEONS

M. Cuadrado,[1] C. Díaz-Paniagua,[1,3] M. A. Quevedo,[2] J. M. Aguilar,[2] and I. Molina Prescott[2]

[1] Estación Biológica de Doñana—CSIC, Pabellón del Perú, Avda. de María Luisa s/n, E-41013 Sevilla, Spain
[2] Zoológico de Jerez, Taxdirt s/n, E-11404 Jerez de la Frontera (Cádiz), Spain
[3] Corresponding author (email: paniagua@cica.es)

ABSTRACT: Prevalence of egg-laying disorders and hematology and blood chemical parameters were analyzed in free-ranging common chameleons (*Chamaeleo chamaeleon*) from southern Spain. During four reproductive seasons oviposition failures occurred only during dry years. Egg binding appeared to be an important cause of mortality. Oviductal eggs of dystocic females were longer and wider than those of females with normal oviposition. Blood cell counts, white blood cell differentials, hematocrit, and concentration of hemoglobin, total plasma protein, glucose, uric acid, aspartate aminotransferase, and creatinine phosphokinase in blood were determined for eight apparently healthy post-reproductive females and considered as reference values. These chameleons differed from other reptiles in high concentrations of glucose in blood, averaging 362 mg/dl, and in high numbers of heterophils. Females with dystocia differed from reference values in an increase in monocytes and in the high concentrations of aspartate aminotransferase probably associated with tissue trauma.

Key words: Blood chemical profiles, *Chamaeleo chamaeleon,* common chameleon, dystocia, hematology, oviposition, Spain.

INTRODUCTION

Serious reproductive difficulties are not common in reptiles, but one of the most common problems in females is failure to produce viable eggs or young (Frye, 1991). Dystocia has been reported in snakes, turtles, and lizards, mostly occurring in oviparous species (Frye, 1991; DeNardo, 1996). Dystocia may be caused by a physical inability to deliver the eggs or fetuses due to maternal abnormalities or to oversized or malformed eggs or fetuses. Other cases of nonobstrusive dystocias may occur when females and eggs (or fetuses) do not present abnormalities but retention is attributed to possibilities such as poor physical condition of females (DeNardo, 1996). A frequent problem associated with dystocia is that the eggshell may rupture into the oviduct with egg content eventually exiting to the coelomic cavity, frequently causing death (Frye, 1991).

The common chameleon, *Chamaeleo chamaeleon,* is an oviparous medium-sized lizard in which egg retention and serious difficulties at oviposition have been reported in nesting females (Blázquez et al., 2000). In order to explore the causes of these reproductive disorders, we compared hematologic and biochemical parameters of healthy post-reproductive females with those of females with difficulties at oviposition. Reference values for blood parameters of reptiles are few, and mammalian and avian profiles are commonly used (Campbell, 1998). Therefore, our second aim was to report reference values of blood chemical profiles and hematology of healthy female chameleons.

MATERIALS AND METHODS

Common chameleons lay a single clutch of 4–40 eggs per year (Blasco et al., 1985; Cuadrado and Loman, 1999; Díaz-Paniagua et al., in press). Mating season is from mid-August to mid-September and egglaying occurs from mid-September to early November. Sixteen female chameleons were captured from 20 October to 15 November 1999 for blood collection. Eight clinically healthy females were captured soon after oviposition (normal females) and the remaining eight females (dystocic females) had been observed in subsequent unsuccessful attempts of nesting. Dystocic females had eggs in oviducts which were detected by palpation or by radiography; some of them had oviposited a partial clutch. All females were captured by hand in the field (Rota, 36°37′N, 6°20′W) and transported to the veterinary department at Zoológico de Jerez de la

Frontera (approx. 30 km away from site of capture). Because ambient temperature is known to influence blood chemistry concentrations (Seidel, 1980), dystocic females were acclimated in laboratory terrarium 1–7 days prior to blood analyses. Food (grasshoppers) was provided ad libitum; water was provided 3–4 times daily by misting, and heating was provided by a light bulb. Blood analyses were performed from 26 October to 30 November 1999. Healthy females were bled within 1–2 hr after their capture and then they were released.

From 0.2–0.3 ml of blood were collected in heparinized syringes via ventral tail caudal vein puncture (see Powell and Knesel, 1992). Hematocrit was assessed by centrifuging a heparinized microhematocrit tube with the sample of blood at 12,000 × G for 5 min at room temperature. Red blood cells (RBC) and white blood cells (WBC) were counted by manual methods using Natt and Herrick solution (methyl violet 2B solution) as described by Campbell (1994). Blood smears were dried, fixed with methanol, and stained with May-Gruünwald stain (Campbell, 1994). Differential counts of white cells per 100 leukocytes were conducted by light microscopy.

Concentrations of total protein were calculated using refractometry. Concentrations of glucose, uric acid, hemoglobin, aspartate aminotransferase (AST), and creatinine phosphokinase (CK) were determined using Reflotron (Boehringer-Mannheim, Mannheim, Germany) tests for the quantitative determination of each variable on whole blood samples. These parameters provide a profile that can be used to assess health in animals (see Lewandowski et al., 1986 for a similar procedure in birds). The quantity of blood obtained from some of the dystocic females was not adequate to assess all parameters. The results of blood analyses from the eight healthy females were considered reference values for post-reproductive chameleons and were compared by analyses of variance (ANOVA).

All dystocic females died from 1–60 days after capture. All of them retained one to seven eggs in oviduct. Necropsy did not reveal macroscopic lessions. In four of these females, all oviductal eggs were extracted and measured (length and width) to the nearest 0.1 mm with calipers. In order to increase the sample size of egg dimensions from dystocic females we also measured the eggs of five wild chameleons which died after egg-retention in 1995. These data were compared with eggs from 19 females with normal oviposition from the same area. Comparisons of egg dimensions were based on mean values per female.

During the nesting seasons of 1995, 1996, 1997, and 1999, we monitored gravid females and located nests at Rota and San Fernando (36°28′N, 6°12′W), in Cádiz Province, southern Spain. Rainfall from September to August was used to classify these years as dry years: 1995 and 1999 with total annual rainfall of 218 and 235 mm respectively; and wet years: 1996 and 1997 with 862 and 758 mm total annual rainfall, respectively. We counted the number of the females which reproduced successfully and those showing reproductive difficulties. We excavated the nests once the eggs hatched. Nests without eggshells were considered to have been constructed by females with dystocia or related problems. All observed females were measured (snout-to-vent length [SVL] to the nearest 0.1 mm). Based on our experience with recaptured individuals, all females longer than 125 mm were considered older than 2 yr, while those of smaller size were considered to be young females, in which egg-binding was most likely to occur. Body sizes of young females grouped by dry or wet years were analyzed by ANOVA.

RESULTS

Females exhibiting difficulties in oviposition were detected in the two dry years. In the wet years, all monitored females successfully laid eggs (n = 59). Blázquez et al. (2000) reported 15% of 78 females died or retained eggs after oviposition in 1995. This year we found four (9%) of 47 nests in which females did not lay eggs; in 1999 we monitored 30 nesting females, of which four retained some eggs after ovipositing a partial clutch, and seven (37%) females dug and closed nest tunnels and holes without ovipositing. In addition, 10 (21.3%) of 47 nests monitored in the field did not contain eggs.

Body size of females with problems at oviposition was not different from those that successfully reproduced in 1995 (Blázquez et al., 2000) nor in 1999 ($F_{1,23}$ = 3.32, P = 0.082), although reproductive disorders mostly occurred in small females. Young female chameleons were smaller in dry than in wet years ($F_{3,82}$ = 4.16, P = 0.008; Fig. 1).

Eggs from dystocic females were longer (average = 19.0 mm, SD = 0.4 mm, range = 15.5–25.0 mm, n = 9 females) and wider (average = 11.2 mm, SD = 0.3 mm, range = 8.2–13.1 mm, n = 9) than those

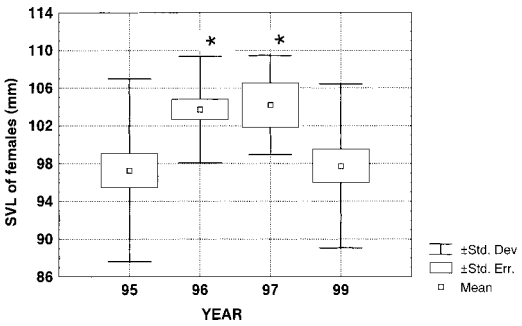

FIGURE 1. Variation in snout-to-vent length (SVL) of young (<2 yr) reproductive female chameleons during the nesting seasons in dry (1995, 1999) and wet (1996, 1997) years. Asterisks show similar years differing from those without asterisks.

of normal females (average length = 16.8, SD = 0.2, range = 13.7–21.3 mm; average width = 10.6 mm, SD = 0.1 mm, range = 8.6–11.7 mm, n = 19). Eggs of both groups differed significantly (ANOVA: $F_{1,26}$ (egg length) = 16.5, $P < 0.001$; and $F_{1,26}$ (egg width) = 6.0, $P = 0.021$).

Hematologic and biochemical blood profiles of normal females are shown in Table 1. Most blood parameters were within normal ranges reported for other lizards (Dessauer, 1970), except for the concentration of glucose, which was higher than that reported in any other species of reptile. Concentrations of hemoglobin were low and could not be determined in six of the eight females analyzed. For these apparently healthy females, we found that large individuals had lower levels of glucose ($r = -0.800$, $P = 0.017$) and higher concentrations of uric acid ($r = 0.777$, $P = 0.023$). Differential leukocyte counts are shown in Table 2.

Hematologic and biochemical parameters of dystocic females are shown in Table 1. Most dystocic females had low RBC and WBC counts. White blood cell differential counts were significantly different from normal females (Table 2). There was a considerable increase in monocytes ($F_{1,12} = 4.25$, $P = 0.062$) and decrease in the numbers of heterophils ($F_{1,12} = 6.5$, $P = 0.026$). Significant differences were present in concentrations of AST ($F_{1,10}$ =

TABLE 1. Mean, standard deviation (SD), and range of hematologic and biochemical analyses in healthy female chameleons after egg-laying and in dystocic female chameleons.

Parameters	Units	Healthy females			Dystocic females		
		n	Mean ± SD	Range	n	Mean ± SD	Range
Red blood cells	$\times 10^{12}$/l	8	0.8 ± 0.4	0.4–1.7	6	0.7 ± 0.08	0.5–0.8
White blood cells	$\times 10^{9}$/l	8	31.2 ± 15.0	12.5–56.2	6	17.1 ± 10.0	0.3–34.3
Hematocrit	%	8	24.0 ± 4.2	10.0–33.0	5	22.6 ± 6.2	14–27
Total protein	g/dl	8	4.7 ± 0.7	3.60–5.70	5	4.3 ± 0.4	3.8–6.0
Glucose	mg/dl	8	362.4 ± 105.2	202–569	6	301.5 ± 124.2	77–454
Uric acid	mg/dl	6	3.9 ± 2.4	<0.2[a]–7.0	5		<2–8.8
Hemoglobin[b]	g/dl	2	—	<5.0–10.4	4		<5–9.3
Aspartate aminotransferase	IU/l	8	218.4 ± 51.6	122–271	5	290.4 ± 43.6	239–359
Creatinine phosphokinase	IU/l	7	482 ± 106	331–>1000[c]	4	479 ± 202	338–767

[a] This minimum value was not used to calculate mean and standard deviation.
[b] Six of eight values were below the minimum registered by tests.
[c] This maximum value was not used to calculate mean and standard deviation.

TABLE 2. Differential leucocyte counts of post reproductive female chameleons.

		Mean	SD	Range	*n*
Healthy females	Lymphocytes (%)	25	3.0	22–31	8
	Heterophils (%)	66	6.2	55–72	8
	Eosinophils (%)	0	0	—	8
	Basophils (%)	0.5	0.7	0–2	8
	Monocytes (%)	9	4.3	5–18	8
Dystocic females	Lymphocytes (%)	25	3.7	20–30	6
	Heterophils (%)	58	7.8	47–64	6
	Eosinophils (%)	0	0	—	6
	Basophils (%)	0.3	0.5	0–1	6
	Monocytes (%)	17	7.3	7–27	6

6.524, $P = 0.029$) which was higher in dystocic females. The activity of AST was related to an increase in CK ($r = 0.970$, $P = 0.030$) in the four individuals recorded. Mean value of uric acid was higher in dystocic females as compared to healthy females.

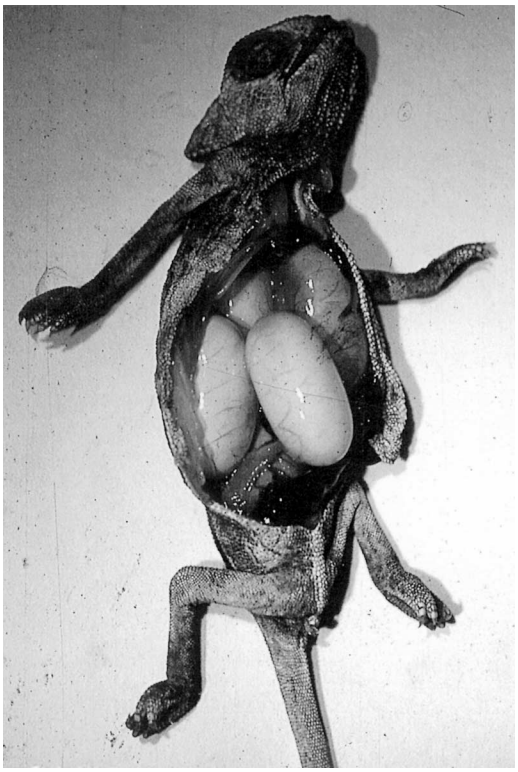

FIGURE 2. Large oviductal eggs in a dystocic female chameleon.

DISCUSSION

Problems related to oviposition in female chameleons are associated with dry years, when individuals have smaller body size and are presumably in poorer physical condition. In dry years, many gravid females made unsuccessful nesting attempts which did not conclude in oviposition, resulting in egg retention. Some females completed oviposition after several unsuccessful attempts, but most of them died prior to oviposition (Blázquez et al., 2000). Eggs take up water within the oviduct, within the physical constraint of the female's abdominal cavity (Qualls and Andrews, 1999); egg retention in oviducts might contribute to increased egg size and volume which in chameleons could result in difficult oviposition and cause egg-binding or dystocia. Egg resorption has never been observed in reptiles (Blackburn, 1998); the expulsion of retained eggs was not observed in female chameleons and all dystocic females died. Egg-binding has been reported in captive chameleons with death associated with egg yolk peritonitis (Castle, 1990). In our study, the death of some individuals was associated with obstructive dystocia, occurring in small females with one or more large eggs in the oviduct (Fig. 2). Nonobstructive dystocia was diagnosed in other very weak females which were unable to lay eggs, but oviductal eggs could be manually extruded through oviduct and cloaca. We assumed

that their effort in subsequent attempts of egg-laying probably caused them muscular injuries. Necropsy of females with oviposition difficulties did not reveal macroscopic lesions or evidence of peritonitis.

Only glucose and uric acid concentrations have been reported for a single common chameleon (Dessauer, 1970). Hematologic parameters were reported for flapneck chameleons (*C. dylepis*; Duguy, 1970) and the morphology of erythrocytes described for african chameleons (*C. africanus*) (Saint Girons, 1970). Most recently, reference values for hematology and some biochemical parameters were published for panther chameleons (*C. pardalis*) (Jones et al., 1996; International Species Information System, 1999). Although our sample is not large enough to represent a chameleon population, our data on healthy females serve as reference values for comparison with dystocic females. However, because of the small number of healthy females analyzed these values should be used with caution.

Our animals had a wide variation in RBC which was similar to other lizard species, with generally higher numbers than snakes and turtles (Duguy, 1970). The RBC count may be influenced by reproductive condition, and the wide range obtained in this study may be explained by differences in female conditions during the recovery phase after reproduction. Reference values from our healthy females were similar to ranges reported in other chameleons (Jones et al., 1996; International Species Information System, 1999). Differential leukocyte counts of *C. chamaeleon* had a predominance of heterophils, while in most reptiles lymphocytes are the most numerous cell type (Duguy, 1970). Panther chameleons have more lymphocytes than heterophils (Jones et al., 1996; International Species Information System, 1999). The higher proportion of heterophils in our sample could be due to the post-reproductive stage of females; an increase in these cells has been reported for other gravid reptiles (Duguy, 1970). Hematocrit and total proteins were within the ranges for reptiles (Campbell, 1998), but concentrations of hemoglobin in our chameleons were low when compared with the values reported by Dessauer (1970). Uric acid concentration did not differ from values in other reptiles (Dessauer, 1970; Campbell, 1998). Post-reproductive female chameleons had high concentrations of glucose. This component normally ranges from 60–100 mg/dl in reptiles (Campbell, 1998). Dessauer (1970) reported higher values for several lizards species, but all below 200 mg/dl. Levels found in post-reproductive chameleons resembled those of birds, which are also much higher than in mammals (Amand, 1986). During periods of high energy demand or under suboptimal temperature, aerobic animals, as reptiles, may require anaerobic glycolysis to produce energy (Bennett and Dawson, 1976). The capacity of chameleons to sustain an autumnal reproduction (Blasco et al., 1985; Díaz-Paniagua et al., in press) and winter activity, when the other coexisting reptile species are inactive, is probably supported by anaerobic metabolism, which may be associated with high levels of glucose. Concentrations of glucose over 200 mg/dl were also reported for panther chameleons (Jones et al., 1996; International Species Information System, 1999), which suggests that high levels of glucose distinguish chameleons from other reptiles. Reference values for AST fall in the normal range described for birds and mammals, however levels of CK were far above the normal range in birds.

Dystocic chameleon females differed from healthy females in high levels of AST, and positive correlation with CK may indicate the occurrence of muscular injuries. This could be related to efforts that females may have made during attempts at nesting, either during excavation of the long nest tunnel or during unsuccessful attempts at egg expulsion. High levels of uric acid occured in some of these females, which is remarkable considering

the small body size of most dystocic females, because we detected that uric acid levels were correlated with body size in healthy females. High uric acid concentrations are normally used to diagnose renal disease, although this is not a specific test (Campbell, 1998). It may also indicate massive tissue trauma (Lewandowski et al., 1986), which in this case is in accordance with the detected high levels of AST and CK. Monocytosis may also be associated with tissue necrosis as reported for birds (Campbell, 1994).

There was a tendency toward lower numbers of RBCs and WBCs in dystocic chameleons. Erythrocyte counts in reptiles are influenced by reproductive condition (Duguy, 1970) and the tendency to lower numbers may be associated with the fact that they still bore eggs in contrast with normal females. Although in this study we did not obtain a wide sample of dystocic females, blood profiles supported the diagnosis of muscular injuries, probably caused by the effort in subsequent attempts of egg-laying. This could have weakened the females until they died.

In summary, dystocia is a frequent problem for young gravid chameleons in dry years. Dystocic females showed high levels of AST and CK, suggesting muscular injuries. Results of this study show the peculiarity of blood profiles in chameleons. Differences compared to other reptiles, especially in glucose concentrations and in differential lymphocyte counts, justify the necessity of conducting further studies to provide information about variation in blood parameters with sex, age, and different physiological conditions.

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