

Comparative haematology and blood chemistry of endangered lizards (*Gallotia* species) in the Canary Islands

A. MARTÍNEZ SILVESTRE, M. A. RODRÍGUEZ DOMÍNGUEZ, J. A. MATEO, J. PASTOR, I. MARCO, S. LAVÍN, R. CUENCA

Blood samples were taken from the ventral coccygeal vein of 15 El Hierro giant lizards (*Gallotia simonyi*) (seven females and eight males), six La Gomera giant lizards (*Gallotia bravoana*) (four males and two females) and four Tenerife giant lizards (*Gallotia intermedia*) (two males and two females), and 31 blood parameters were measured. Among the haematological parameters there were significant differences between the three species in heterophils, azurophils and lymphocytes, but no significant differences in red blood cell count, white blood cell count, haemoglobin, packed-cell volume, monocytes, eosinophils and basophils. In terms of blood chemistry there were significant differences between the three species in cholesterol, triglycerides, glucose, sodium, chloride, urea, uric acid, total proteins, prealbumin, albumin and gamma globulins, but no significant differences in calcium, potassium, aspartate aminotransferase, alanine aminotransferase, creatine kinase, bile acids, alpha-1 and alpha-2 globulins and beta globulins.

THE lizards of the Canary Islands belong to the endemic genus *Gallotia* (Arnold 1973), and are considered endangered species by the International Union for Conservation of Nature (Pleguezuelos 2002); they are all strictly protected by national and international laws.

In the 1930s the El Hierro giant lizard (*Gallotia simonyi*) was believed to be extinct, but it was rediscovered in 1974 (Böhme and Bings 1975). The estimated total number of animals in the wild population ranges between 140 (Pérez-Mellado and others 1999) and 1613 (Rodríguez-Domínguez and others 2000). As a result of a recovery plan, and using 12 animals captured in their natural habitat, new populations have been created in some parts of the island. At present, 250 of the lizards, including adults and hatchlings, are maintained in captivity.

The Tenerife giant lizard (*Gallotia intermedia*) is medium to large in size, and lives in a number of subpopulations on the Teno and Los Gigantes cliffs on the western side of Tenerife (Hernández and others 2000); at present, there are between 250 and 500 wild animals (Rando and Valido 2000). Although there is no captive recovery plan for the species at present, four of the lizards are kept in terrariums in the Fundación Neotrópico in Tenerife, and these are the only captive animals.

The La Gomera giant lizard is clearly differentiated from the above two species but its scientific classification is unclear; it has been given the name *Gallotia bravoana* on the basis of the Code of Zoological Nomenclature (Nogales and others 2001). It occupies a small habitat in the south-west of La Gomera and has an estimated population of no more than 20 individuals. The recovery plan for the species includes a captive breeding programme; at present there are six captive lizards, and 10 hatchlings have been born in the past two years.

Haematology and serum chemistry play an important role in the diagnostic evaluation of reptiles (Campbell 1998) and to obtain basic data, legal support and permission was obtained from the Canary Government to take blood samples. The aim of this study was to determine reference ranges for haematological and blood chemistry parameters for the three *Gallotia* species. These reference ranges would be useful for physiological evaluation and for the detection of pathological changes in wild and captive Canary giant lizards. There have been some studies of the haematological and biochem-

ical parameters of herbivorous saurians such as common iguanas (*Iguana iguana*) (Frye 1991, Wagner and Wetzel 1999), prehensile-tailed skinks (*Corucia zebrata*) (Wright 1993), barbed dragons (*Pogona vitticeps*) (Ellman 1997) and El Hierro giant lizards (Martínez Silvestre and others 2002b), but no comparative haematological and biochemical reference values have previously been published for lizards of the genus *Gallotia*. This paper also provides the first descriptions for the La Gomera and Tenerife giant lizards.

MATERIALS AND METHODS

Blood samples were taken from the animals kept at the recovery centres for the giant lizards at Frontera, on El Hierro, at Alajero, on La Gomera, and at the Fundación Neotrópico on Tenerife, during a clinical control carried out in the spring of 2001. There were 15 adult El Hierro lizards (eight males and seven females), six adult La Gomera lizards (four males and two females) and four adult Tenerife lizards (two males and two females). The animals were captured in rocky areas of the Canary Islands, specifically from Tibataje cliff on El Hierro, Valle Gran Rey on La Gomera, and Teno on Tenerife. All the lizards were kept in pairs and in identical conditions before the blood samples were taken. They were fed regularly on a diet of fruit, vegetables and endemic plants from their respective islands, supplemented with crickets, giant mealworms and locusts, and water was available ad libitum (Orrit and others 1999). They were maintained on a natural photoperiod of 14 hours light and 10 hours darkness. The daytime air temperatures ranged from 23 to 35°C and the terrariums were maintained at the same temperature. The lizards ate, drank and behaved normally, and were all clinically healthy at the beginning of the study.

The blood samples (0.7 ml) were taken from the ventral coccygeal vein with disposable syringes and 23 G needles, and immediately transferred to a heparinised tube; a fresh blood smear was made and air-dried. The samples were refrigerated at 4°C until they were analysed on the day they had been taken. Blood was centrifuged at 800 g for 10 minutes and the plasma was removed for the biochemical measurements.

Complete blood counts were made by using the standardised recount system in a modified Neubauer chamber,

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A. Martínez Silvestre, DVM, MSc, MCVS, Catalonian Reptile and Amphibian Rehabilitation Centre, 08783 Masquefa, Barcelona, Spain

M. A. Rodríguez Domínguez, MS, Centro de Recuperación e Investigación del Lagarto Gigante de El Hierro, El Hierro, Canary Islands, Spain

J. A. Mateo, MS, PhD, Centro de Recuperación del Lagarto Gigante de La Gomera, Valle Gran Rey, La Gomera, Canary Islands, Spain

J. Pastor, DVM, PhD, I. Marco, DVM, PhD, S. Lavín, DVM, PhD, R. Cuenca, DVM, PhD, Departament de Medicina i Cirurgia Animal, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain

Dr Marco and Dr Lavín are also at the Servicio de Ecopatología de Fauna Salvaje, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain

Dr Cuenca is also at the Servicio de Hematología Clínica Veterinaria, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain

Correspondence to Dr Cuenca

TABLE 1: Mean (sd) haematological parameters in three species of giant lizard

Parameter	<i>Gallotia bravoana</i> (n=6)	<i>Gallotia intermedia</i> (n=4)	<i>Gallotia simonyi</i> (n=9)
RBC ($\times 10^{12}$ /litre)	0.954 (0.26)	0.925 (0.17)	1.081 (0.26)
Haemoglobin (g/litre)	62.5 (9.2)	73.5 (7.3)	73.2 (12.1)
PCV (litre/litre)	0.30 (0.05)	0.34 (0.04)	0.31 (0.04)
MCV (fl)	318 (46.9)	377 (57.6) ^c	290 (40.5)
MCH (pg)	67.6 (10.9)	80.6 (8.73)	70.4 (8.76)
MCHC (g/litre)	211 (10.8) ^b	216 (27.8)	239 (19.9)
WBC ($\times 10^9$ /litre)	8.00 (2.28)	6.50 (1.73)	6.00 (2.29)
Thrombocytes ($\times 10^9$ /litre)	40.7 (2.21)	27.6 (5.84)	19.4 (4.91)
Differential leucocyte count			
Lymphocytes ($\times 10^9$ /litre)	0.68 (0.36) ^a	1.47 (0.44)	0.82 (0.47)
Monocytes ($\times 10^9$ /litre)	0.21 (0.17)	0.15 (0.09)	0.096 (0.082)
Azurophils ($\times 10^9$ /litre)	1.70 (1.15) ^a	0.39 (0.11) ^c	1.27 (0.74)
Heterophils ($\times 10^9$ /litre)	4.67 (1.88) ^b	3.26 (1.53)	2.34 (1.02)
Eosinophils ($\times 10^9$ /litre)	0.17 (0.11)	0.19 (0.17)	0.22 (0.24)
Basophils ($\times 10^9$ /litre)	0.56 (0.47)	1.03 (0.22)	1.23 (1.25)

^a Significant differences (P<0.05) between *G bravoana* and *G intermedia*

^b Significant differences (P<0.05) between *G bravoana* and *G simonyi*

^c Significant differences (P<0.05) between *G intermedia* and *G simonyi*

RBC Red blood cells, PCV Packed-cell volume, MCV Mean corpuscular volume, MCH Mean corpuscular haemoglobin, MCHC Mean corpuscular haemoglobin concentration, WBC White blood cells

using Natt-Herrick solution as the diluent (Frye 1991); the packed-cell volume (PCV) was measured by centrifuging blood in a microhaematocrit tube in a haematocrit centrifuge (Hawksley) at approximately 20,000 g for five minutes. Differential leucocyte counts were made from blood smears stained with a commercial Wright's stain (Quick Panoptic; Química Clínica Aplicada) examined under $\times 1000$ magnification; 100 leucocytes were examined on each slide. The haemoglobin concentration was determined by a semiautomatic haematological analyser (Sysmex F-800; Toa Medical Electronics) after lysates had been centrifuged. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)

were calculated from the PCV, haemoglobin concentration and red blood cell count (Campbell 1998, Watson 1998).

The biochemical analyses, except for the ions, were performed with a Clima analyser (RAL Tecnica Laboratories). The ions were determined with an ion-selective electrode (Spotchem; Kyoto Dalichi Kagaku). The activities of alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH), and the concentrations of calcium, phosphorus, triglycerides, cholesterol, uric acid, urea, bile acids, glucose, sodium, potassium and chloride were measured. Total plasma protein concentration was determined by the biuret method using a photodensitometer (Scanion; RAL). Serum proteins were electrophoresed on cellulose acetate membranes in a high-resolution buffer at 200 V and 100 mA for 80 minutes; the proteins separated were designated as prealbumin, albumin, alpha-1 globulin, alpha-2 globulin, beta globulin and gamma globulin.

The results were analysed statistically with SPSS-PC software (SPSS). The minimum significance value chosen was P<0.05. Because few animals were available, the non-parametric Kruskal-Wallis test was used to detect significant differences between the three species.

RESULTS

Tables 1 and 2 show the haematological and blood chemistry results for the three species of Canary giant lizard.

In terms of significant differences between the three species, the La Gomera giant lizard had the highest values for heterophils, azurophils, cholesterol, triglycerides, glucose, prealbumin and gamma globulins, and the lowest values for sodium, chloride and lymphocytes. The Tenerife giant lizard had the highest values for urea, lymphocytes, total proteins and albumin, and the lowest values for cholesterol and azurophils. The El Hierro giant lizard had the highest value for sodium and the lowest values for uric acid, urea, heterophils, prealbumin, albumin and gamma globulins.

TABLE 2: Mean (sd) blood chemistry parameters and protein electrophoretic fractions in three species of giant lizard

Parameter	<i>Gallotia bravoana</i> (n=6)	<i>Gallotia intermedia</i> (n=4)	<i>Gallotia simonyi</i> (n=9)
Glucose (mmol/litre)	9.93 (1.94) ^b	10.7 (1.67) ^c	6.67 (2.04) (n=15)
Uric acid (μ mol/litre)	161.8 (62.4)	209 (15.5) ^c	117.8 (50.6) (n=15)
Urea (mmol/litre)	1.11 (0.14) ^a	2.03 (0.86) ^c	0.97 (0.41) (n=7)
Cholesterol (mmol/litre)	10.9 (2.76) ^{a,b}	3.99 (0.78) ^c (n=3)	6.29 (1.65) (n=15)
Triglycerides (mmol/litre)	2.79 (1.90) ^{a,b}	0.82 (0.40) (n=3)	0.49 (0.47) (n=13)
Bile acids (μ mol/litre)	26.3 (18.2)	27.7 (9.29) (n=3)	42.0 (48.7) (n=14)
ALT (u/litre)	11.8 (2.04) ^a	15.8 (2.22)	18.0 (9.27) (n=7)
AST (u/litre)	40.3 (4.03)	47.0 (6.32)	39.7 (26.4) (n=15)
LDH (u/litre)	512 (212)	–	720 (242) (n=11)
CK (u/litre)	4389 (4315)	–	4048 (3904) (n=15)
AP (u/litre)	30.5 (48.8)	–	39.7 (22.9) (n=3)
Calcium (mmol/litre)	2.95 (0.33)	2.57 (0.39)	2.80 (0.23) (n=15)
Phosphorus (mmol/litre)	2.81 (0.40) ^b	2.14 (0.42)	1.68 (0.45) (n=15)
Sodium (mmol/litre)	164.2 (4.17) ^{a,b}	173.5 (3.70) ^c	182.9 (8.75) (n=7)
Potassium (mmol/litre)	2.60 (4.13)	2.12 (0.84) ^c	1.01 (0.27) (n=7)
Chloride (mmol/litre)	137.7 (5.24) ^{a,b}	163.2 (18.8)	150.4 (13.6) (n=7)
Total proteins (g/litre)	54.2 (7.1) ^a	66.9 (6.9) ^c	58.4 (77.2) (n=15)
Prealbumin (g/litre)	9.9 (2.0) ^{a,b}	6.6 (0.4) ^c	7.71 (1.2)
Albumin (g/litre)	26.3 (3.0) ^{a,b}	34.6 (3.0) ^c	24.9 (4.1)
Globulins			
Alpha-1 globulins (g/litre)	3.0 (1.2)	4.9 (1.6)	4.93 (1.1)
Alpha-2 globulins (g/litre)	2.0 (0.9) ^b	2.2 (0.8)	3.48 (1.3)
Beta globulins (g/litre)	7.6 (6.9)	11.8 (1.6)	11.1 (3.1)
Gamma globulins (g/litre)	5.4 (2.0) ^b	6.8 (3.4) ^c	6.03 (0.8)
Albumin:globulin ratio	24.7 (8.1) ^b	17.0 (5.3)	12.8 (3.5)

^a Significant differences (P<0.05) between *G bravoana* and *G intermedia*

^b Significant differences (P<0.05) between *G bravoana* and *G simonyi*

^c Significant differences (P<0.05) between *G intermedia* and *G simonyi*

ALT Alanine aminotransferase, AST Aspartate aminotransferase, LDH Lactate dehydrogenase, CK Creatine kinase, AP Alkaline phosphatase

DISCUSSION

Samples were collected from the entire adult captive population of the three species, but the small number of individuals in each group made it impossible to apply comparative statistics with conclusive results. Furthermore, the existence of three isolated populations on different islands, even though they were maintained under identical conditions, may affect the interpretation of the results.

It is important to establish reference ranges for haematological and biochemical data for these captive giant lizards to aid in the diagnosis or prevention of disease (Martínez Silvestre and others 2002b), in health monitoring, and in the detection of any ecological and geographical differences between the species. The results obtained were similar to the reference ranges for other herbivorous or omnivorous lizards such as the common iguana (Wetzel 1998, Wagner and Wetzel 1999) and bearded dragon (Ellman 1997). However, the values for haemoglobin, PCV and RBC were lower than those reported for the prehensile-tailed skink (Wright 1993), common iguana (Frye 1991) and other genera of the Lacertidae family, such as *Lacerta* species (Dessauer 1970). In tortoises, similar differences can be related to different thermal or climatic conditions (Anderson and others 1997), but there are too few data on the effects of temperature or climate on the haematology of lizards to establish whether there might be a similar relationship.

The concentrations of phosphorus, sodium, chloride, glucose and cholesterol, and the activity of AST, were higher than

those reported for the prehensile-tailed skink (Wright 1993) and *Lacerta* species (Dessauer 1970), but the concentrations of calcium and potassium were lower than in other species with similar dietary habits, such as the prehensile-tailed skink (Wright 1993). The activities of ALT and LDH, and the concentrations of albumin, sodium and uric acid were higher in the giant lizards than in the common iguana (Wagner and Wetzel 1999) and bearded dragon (Ellman 1997). The variations in albumin, sodium and uric acid suggest that the giant lizards may be specially adapted to conditions of water deprivation in the Canary Islands, where their access to water and food is more restricted than that of tropical or rainforest lizards. The *Gallotia* species occupy a specialised habitat, formed by rocky cliffs with very low vegetation and without ponds or readily accessible water. As a result, for effective homeostasis they require physiological and biochemical modifications that allow them to withstand prolonged water shortages, dry winters and light-intensive, hot summers, conditions very similar to those experienced by desert tortoises (*Gopherus agassizii*) (Christopher and others 1999, Dickinson and others 2002). Variations in sodium, uric acid and albumin have been suggested as a sign of different degrees of water or nutrient deprivation in desert tortoises (Dickinson and others 2002). However, in species adapted to dry climates, these variations cannot be interpreted as pathological and should be accepted as physiological values (Christopher 1999, Gibbons 2001, Klaphake 2001).

There were significantly more azurophils in the blood of the La Gomera giant lizards than in the Tenerife giant lizards, and more than in the El Hierro giant lizards. This difference may be related to the presence of haemoparasites of *Karyolysus* species (Martínez Silvestre and others 2001). Similar increases in azurophils have been described in king cobras (*Ophiophagus hannah*) parasitised by *Hepatozoon* species protozoans (Salakij and others 2002).

There were also significant differences between the three species in renal markers such as sodium, chloride, urea, uric acid and phosphorus. The differences suggest that the renal function of each species may be specially adapted to the different degrees of dehydration resulting from the particular ecological conditions of each island in the rocky, marine and salty areas where the lizards live.

The high values of cholesterol, triglycerides and glucose observed in the La Gomera giant lizards may be related to some degree of stress (Guillette and others 1995). This species was the last to have been discovered in the Canary Islands (Nogales and others 2001) and was therefore the most recently housed in terrariums. However, the El Hierro giant lizards were first housed in terrariums 25 years ago and the Tenerife giant lizards were housed four years ago, and time in captivity is a known stress factor in wild reptiles captured and installed in terrariums (Chiszar and others 1995). In other herbivorous reptiles such as Gopher's tortoise (*Gopherus poliphemus*) or the Mediterranean tortoise (*Testudo hermanni*), high concentrations of calcium and cholesterol have been associated with vitellogenesis (Dickinson and others 2002). The significantly lower values observed in the Tenerife giant lizard may be related to the fact that this species is not in a reproductive programme, and the captive population has only two adult females, neither showing signs of oestrus.

Very high activities of CK were recorded in the La Gomera and El Hierro giant lizards. However, those high activities may be related to the sampling method and may not always indicate muscle pathology or clinical disease. However, the activities of CK were also high in these species when they were sampled under anaesthesia (Hernandez-Divers and others 2003). Similar results have been observed in other species of the Squamata order, under the same conditions of sampling (Wagner and Wetzel 1999, Mader 2000, Martínez Silvestre and

others 2002a). In iguanas, high activities of CK may be attributable to age, capture, physical restraint or bleeding techniques (Wagner and Wetzel 1999). As in this study, the results suggest that high CK activity may not always indicate clinically apparent muscle damage.

Although most of the captive lizards on each island have been sampled, the small numbers in some populations limit the interpretation of the results and further validation is needed. As the reproductive programmes increase the number of living animals, more research will be necessary to determine the effects of climate, habitat conditions and possible seasonal fluctuations on the lizards' haematological and biochemical parameters.

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