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Plasma protein electrophoresis in green iguanas (*Iguana iguana*) suffering from hepatic, renal and inflammatory skin diseases

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Abstract: The goals of this study were to evaluate electrophoretograms in green iguanas suffering from hepatic, renal and inflammatory skin diseases. Plasma samples were obtained from 14 green iguana patients. The diagnosis was based on clinical examination, evaluation of biochemical and haematological profiles or histopathological examination. Agarose gel electrophoresis was performed using an automated HYDRASYS system. A decrease in the albumin/globulin ratio was observed in 11 out of 14 patients (three out of four patients with liver failure, one out of one patient with chronic renal insufficiency and suspected hepatopathy, two out of two patients with chronic interstitial nephritis, four out of five patients with chronic renal insufficiency and one out of two patients with dermatitis). The alpha globulin fraction was elevated in 1 out of 14 patients (patient with dermatitis). The fraction of beta globulins was increased in 8 out of 14 patients (two out of four patients with liver diseases, four out of eight patients with various forms of renal failure and two out of two patients with inflammatory skin diseases). Increased gamma globulin levels were observed in 4 out of 14 patients (one out of four patients with impaired function of the liver, two out of eight patients with renal diseases and one out of two patients with dermatitis). Changes in the electrophoretograms of green iguanas are not disease-specific, but in conjunction with other biochemical blood tests the electrophoresis of plasma proteins may be a useful indicator in the diagnosis of metabolic and infectious diseases and in the monitoring of disease progress.

Keywords: albumin; globulins; reptile blood analysis

Serum or plasma protein electrophoresis is used as a routine test for health assessment in human and veterinary medicine, including exotic patients. In avian medicine, it is an important laboratory tool for diagnosis of infectious diseases, various hepatopathies and chronic renal failure (Quesenberry and Moroff 1991; Cray and Tatum 1998). The main protein fractions (e.g. albumin or prealbumin, alpha, beta and gamma globulins) have been observed in reptiles (Girling 2010). Reference values have been published for a limited number of reptile species (Deem et al. 2006; Deem et al. 2009; Gimenez et al. 2010; Cooper-Bailey et al. 2011; Proverbio et al. 2012; Fiorucci et al. 2013; Andreani et al. 2014; Flower et al. 2014, Musilova et al. 2015) changes in the proportions of plasma protein fractions in reptile patients were described only in some studies (Gimenez et al. 2010; Moore et al. 2014). The aim

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Table 1. Green iguana patients

Patient	Sex (M/ F)	Age (years)	Weight (kg)	History	Blood profile	Diagnosis
1	M	12	2.14	anorexia, muscle weakness	anaemia, leukopenia, hyperproteinaemia, hyperuricemia, hyperphosphatemia altered aspartate aminotransferase activity, altered creatine kinase activity	bacterial abscesses in different organs, acute liver necrosis*
2	M	7	3.10	lethargy, anorexia, convulsions	heterophilia (toxic granulation), azurophilia, hyperproteinaemia, hyperphosphatemia, altered aspartate aminotransferase activity, altered creatine kinase activity	liver steatosis*
3	F	9	0.84	decreased activity, loss of appetite, weight loss	leukopenia, hypoproteinaemia, hyperuricemia	liver steatosis*
4	M	9	3.90	anorexia, lethargy	anaemia, leukopenia, hyperproteinaemia, hyperuricemia, altered aspartate aminotransferase activity, altered creatine kinase activity, elevated concentration of bile acids	hepatopathy (enlarged liver with rounded edges)**
5	F	13	2.50	decreased activity, loss of appetite, convulsions	leucocytosis (heterophilia, vacuolisation of heterophils, monocytosis, lymphocytosis), hyperproteinaemia, altered aspartate aminotransferase activity, altered creatine kinase activity	chronic renal failure, hepatopathy (hepatomegaly)**
6	M	15	1.78	anorexia, decreased activity	anaemia, heterophilia with vacuolisation and toxic granulation, later leukopenia, hyperuricemia, altered alanine aminotransferase activity, altered creatine kinase activity	chronic interstitial nephritis*
7	F	15	0.97	loss of appetite, weight loss, decreased activity	leukopenia, altered aspartate aminotransferase activity, altered creatine kinase activity, hyperuricemia, hypocalcaemia	chronic interstitial nephritis, ovarian cysts, heterophile granulomatous pneumonia*
8	M	3	0.62	anorexia, poor body condition, swollen joints on legs	anaemia, leukopenia, hyperproteinaemia, hyperuricemia, hyperphosphatemia	chronic renal failure**
9	F	14	1.09	weight loss	anaemia, leukopenia, hyperuricemia, altered creatine kinase activity	chronic renal failure, gout**
10	M	10	2.50	anorexia, lethargy	anaemia, leucocytosis (heterophilia), hyperuricemia, hyperphosphatemia, hypocalcaemia, altered alanine aminotransferase activity, altered aspartate aminotransferase activity, altered creatine kinase activity	chronic degenerative changes in renal tissue*
11	M	12	3.79	anorexia, tremor	leucocytosis (heterophilia), hypocalcaemia, hyperphosphatemia, altered aspartate aminotransferase activity	chronic renal failure**
12	F	4	2.53	body distension, problems with defecation	leucocytosis (heterophilia), hypoproteinaemia, hyperuricemia	chronic renal failure, ovarian folliculitis**
13	F	2	0.71	skin lesions	leucocytosis (heterophilia, azurophilia), hyperproteinaemia	chronic dermatitis of mycotic origin*
14	M	3	2.80	skin lesions - hyperkeratosis	leucocytosis (heterophilia with toxic granulation, azurophilia), hyperproteinaemia	chronic dermatitis of mycotic origin*

* diagnosis based on histopathological examination

** diagnosis based on clinical examination, haematological and biochemical profile

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of this study was to evaluate plasma protein electrophoretograms in green iguanas suffering from hepatic, renal and inflammatory skin diseases.

MATERIAL AND METHODS

Animals and blood collection. The study included 14 green iguanas (*Iguana iguana*), patients of the Avian and Exotic Animal Clinic, suffering from hepatic, renal or inflammatory skin diseases. The diagnosis was based on clinical examination, evaluation of biochemical and haematological profiles (Divers et al. 1996) or histopathological examination of biopsies or organs post-mortem. Patients are described further in Table 1.

Blood samples from these animals were collected between May, 2008 and June, 2014. Before the blood collection, the iguanas were fasted for 48 hours; access to drinking water was unrestricted. The lizards were restrained manually and fixed in dorsal recumbence; blood samples were taken from the ventral coccygeal vein (*vena coccygea ventralis*). Plasma samples were prepared from the heparinised blood (50 i.u./ml, Heparin Léčiva inj., Zentiva, Prague, Czech Republic) by centrifugation (6440 *g* for 10 min) and kept in a freezer at –20 °C until analysis. Analysis was performed within three months, with no thawing in between.

Plasma protein electrophoresis. Haemolytic or lipaemic plasma samples were excluded from the analysis. Total protein was determined by the biu-

Table 2. Total plasma protein levels and proportions of different protein fractions in green iguana patients

Iguana	TP	Albumin		*α glob*		*β glob*		*γ glob*		A/G	Month
	(g/l)	(g/l)	(%)	(g/l)	(%)	(g/l)	(%)	(g/l)	(%)	–	–
1	66.0	3.30	5.0	10.16	15.4	44.02	66.7	8.51	12.9	0.05	May
2	63.2	18.08	28.6	8.53	13.5	28.06	44.4	8.53	13.5	0.40	May
3	29.7	5.49	18.5	5.02	16.9	14.26	48.0	4.93	16.6	0.23	June
4	92.3	7.85	8.5	12.65	13.7	45.60	49.4	26.21	28.4	0.09	August
5	67.9	7.67	11.3	9.37	13.8	39.52	58.2	11.34	16.7	0.13	August
6[a]	76.3	8.70	11.4	3.89	5.1	53.41	70.0	10.30	13.5	0.13	May
6[b]	61.1	8.07	13.2	4.28	7.0	41.12	67.3	7.64	12.5	0.15	October
6[c]	56.6	7.30	12.9	3.79	6.7	39.05	69.0	6.45	11.4	0.15	December
6[d]	52.6	6.52	12.4	2.57	4.9	36.14	68.7	7.36	14.0	0.14	December
7	45.0	7.70	17.1	7.07	15.7	22.73	50.5	7.52	16.7	0.21	July
8[e]	90.7	6.26	6.9	7.07	7.8	44.72	49.3	32.65	36.0	0.07	August
8[f]	87.6	18.92	21.6	7.27	8.3	30.48	34.8	30.92	35.3	0.28	August
9[g]	41.5	7.59	18.3	6.76	16.3	18.84	45.4	8.38	20.2	0.22	August
9[h]	42.3	8.67	20.5	7.06	16.7	18.02	42.6	8.54	20.2	0.26	September
9[i]	60.8	11.00	18.1	10.46	17.2	24.08	39.6	15.26	25.1	0.22	December
9[j]	71.5	15.73	22.0	10.94	15.3	25.53	35.7	19.31	27.0	0.28	April
9[k]	53.0	13.46	25.4	8.00	15.1	17.54	33.1	13.99	26.4	0.34	June
10	38.9	7.12	18.3	4.98	12.8	19.41	49.9	7.39	19.0	0.22	October
11	64.7	12.10	18.7	10.35	16.0	34.29	53.0	7.96	12.3	0.23	October
12	29.4	7.38	25.1	3.70	12.6	12.58	42.8	5.73	19.5	0.34	March
13	70.9	17.16	24.2	9.86	13.9	36.44	51.4	7.44	10.5	0.32	August
14[l]	90.1	17.84	19.8	16.04	17.8	42.08	46.7	14.15	15.7	0.25	October
14[m]	77.8	12.21	15.7	12.60	16.2	36.02	46.3	16.96	21.8	0.19	June

interval between collection of samples a–b: 17 months; b–c: 1 month; c–d: 10 days

interval between collection of samples e–f: 12 months

interval between collection of samples g–h: 10 days; h–i: 3 months; i–j: 4 months; j–k: 2 months

interval between collection of samples l–m: 8 months

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ret method using Modular Analytics SWA (Roche Diagnostics, Tokyo, Japan). Agarose gel electrophoresis was performed using an automated HYDRASYS system and Hydragel Protein(e) 15/30 agarose gels (Sebia, Evry, France) at 20 W and 20 °C for 7 minutes. Gels were stained with Amido Black (Sebia, Evry, France). The Preference densitometer (Sebia, Evry, France) was used to scan the electrophoretograms (Musilova et al. 2015).

RESULTS

Plasma protein electrophoresis in green iguana patients

Results of the determination of total protein and protein fractions are shown in Table 2. The measured values were compared with reference values for healthy captive green iguanas measured in the same laboratory (Musilova et al. 2015).

CASE 1

At post-mortem examination, bacterial abscesses in multiple organs and acute liver necrosis was diagnosed. This patient had an increased concentration of total protein due to higher levels of beta globulin (Figure 1). Albumin concentration and the albumin/globulin (A/G) ratio were significantly decreased.

CASE 2

Liver steatosis was confirmed at post-mortem examination. There were no significant electrophoretic changes. Total protein concentration was only slightly increased in this patient due to dehydration.

CASE 3

Liver steatosis was diagnosed at post-mortem examination. A hypoproteinaemia with a decreased concentration of albumin and alpha globulins and a low A/G ratio was diagnosed.

CASE 4

Based on clinical examination and evaluation of the blood biochemistry results, a hepatopathy was diagnosed. Post-mortem examination revealed changes in liver tissue: rounded edges, hyperaemia and a hard consistency on cut section. An increased total protein concentration in blood plasma and significantly decreased A/G ratio were found. Albumin concentration was below the lower limit, whereas the concentrations of beta and gamma globulins were increased (Figure 2).

CASE 5

Chronic renal failure was diagnosed on the basis of clinical examination and haematological and biochemical analysis. Due to the presence of dense urinary sand an exploratory coeliotomy and cystotomy were performed, revealing an enlarged liver with rounded edges, suggestive of hepatopathy. Hyperproteinaemia associated with elevated concentrations of beta globulins was found (Figure 3). Albumin concentration and the A/G ratio were decreased.

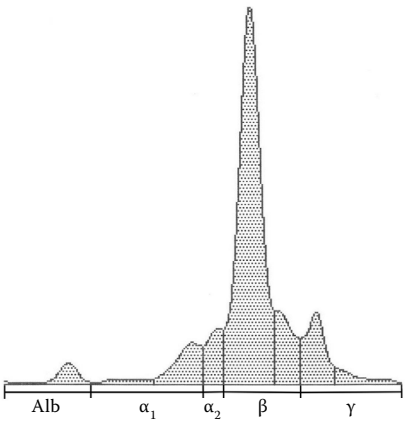

Figure 1. Plasma protein electrophoretogram – case No. 1

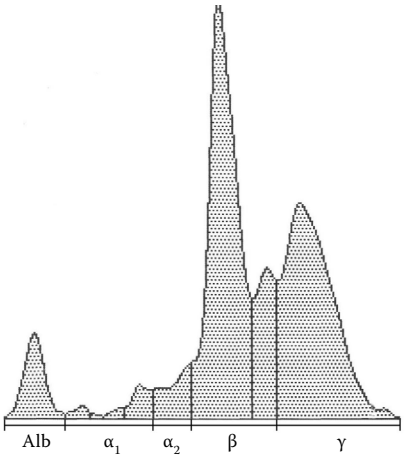

Figure 2. Plasma protein electrophoretogram – case No. 4

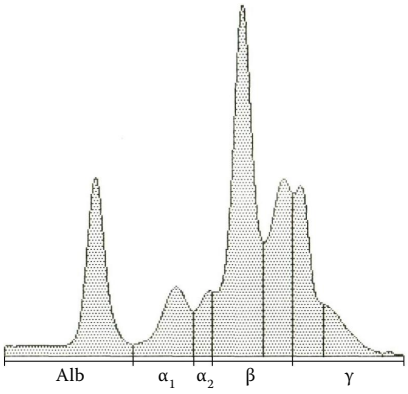

Figure 3. Plasma protein electrophoretogram – case No. 5

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CASE 6

The iguana died due to chronic interstitial nephritis. During the progress of the disease, only minor electrophoretic changes were observed. The first pattern showed an increased concentration of total protein that was later normalised by rehydration therapy. Albumin concentration and the A/G ratio were decreased throughout the whole study. A similar pattern was observed in the alpha globulin fractions. Beta globulin levels decreased slightly during the progress of the disease, but were still above the normal range (Figure 4).

CASE 7

Post-mortem examination and histopathological analysis revealed a chronic interstitial nephritis as well as a heterophilic granulomatous pneumonia, most probably of bacterial aetiology, and ovarian cysts. The only visible changes on the electrophoretogram were a reduced albumin concentration with a decreased A/G ratio.

CASE 8

Chronic renal insufficiency and gout was diagnosed in this iguana. At both blood samplings, the total protein levels were increased due to dehydration and increased levels of beta and gamma globulins (Figure 5). The most marked differences were found in albumin levels and the A/G ratio. Although these values were significantly decreased at the beginning, they returned to normal at the time of the follow-up sampling. Gammaglobulinaemia was found at the follow-up sampling.

CASE 9

Chronic renal insufficiency and gout were diagnosed in this iguana in blood samplings performed over ten months of therapy. Plasma protein electrophoresis revealed a decrease in the concentration of albumin in the first two samplings; in the other three samplings albumin concentrations were within the reference range; however, elevated levels of gamma globulins were observed (Figure 6).

CASE 10

Post-mortem histopathology in this iguana revealed chronic degenerative changes of the renal tissue. Total protein and albumin levels were probably decreased due to the protein loss via the kidneys.

CASE 11

This iguana suffered from chronic renal insufficiency. Long-term therapy resulted in some clinical improvement. Electrophoresis of plasma proteins indicated no significant changes, except for increased levels of beta globulin.

CASE 12

Chronic renal failure and ovarian folliculitis was diagnosed in this iguana. Total plasma protein levels in this patient were decreased, with a con-

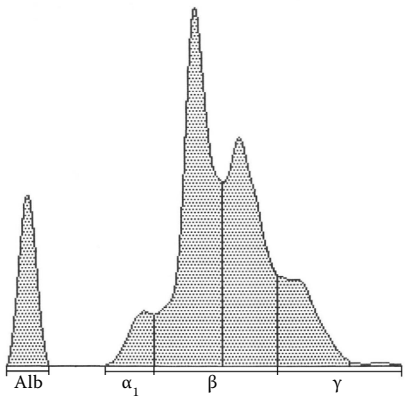

Figure 4. Plasma protein electrophoretogram – case No. 6

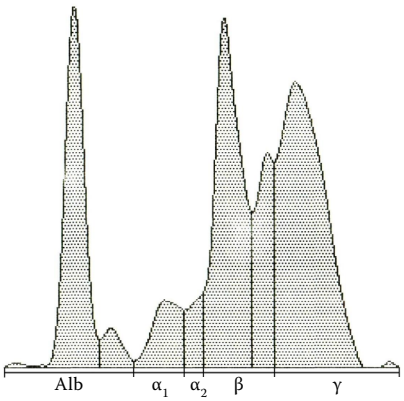

Figure 5. Plasma protein electrophoretogram – case No. 8

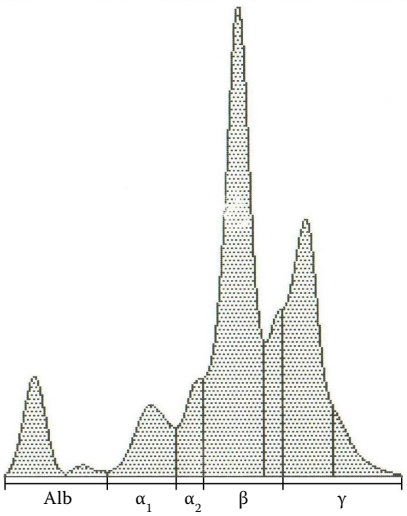

Figure 6. Plasma protein electrophoretogram – case No. 9

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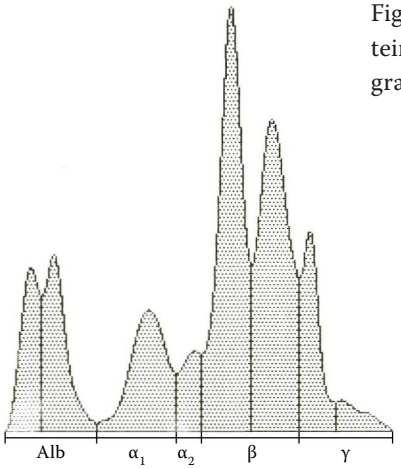

Figure 7. Plasma protein electrophoretogram – case No. 13

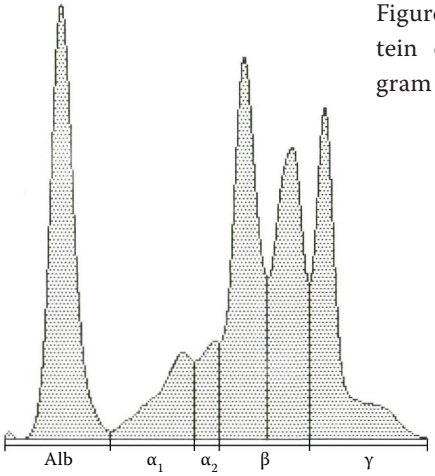

Figure 8. Plasma protein electrophoretogram – case No. 14

comitant decrease in albumin and beta globulin fractions. Alpha globulin levels were at the lower limit of the reference range.

CASE 13

This iguana suffered from chronic mycotic dermatitis with histopathology of skin biopsies revealing fungal hyphae. Plasma protein electrophoresis revealed hyperproteinaemia associated with elevated levels of beta globulins (Figure 7).

CASE 14

Chronic mycotic dermatitis was diagnosed in this iguana. At the first visit, hyperproteinaemia with increased concentrations of alpha, beta and gamma globulins was found. After eight months of treatment, hyperproteinaemia persisted with increased concentrations of alpha, beta and gamma globulins (Figure 8).

DISCUSSION

The determination of electrophoretic values for plasma proteins in commonly kept reptiles is dependent on the electrophoretic system that is used. Several recent studies deal with the acute phase proteins in reptiles. In the Chinese soft-shelled turtle (*Trionyx sinensis*), serum amyloid A has been identified as a major acute phase protein in bacterial infections; also, a marked increase in fibrinogen transcripts and a decreased albumin concentration were noted (Zhou et al. 2011). Moore et al. (2014) investigated the effect of ranavirus infection on plasma proteins as indicators of inflammatory disease in red-eared sliders (*Trachemys scripta elegans*) and described a decrease in albumin concentrations and an increase in globulin fractions.

Cray et al. (1995) recommended using electrophoresis in clinical practice in exotic birds, particularly to diagnose psittacosis, chronic active hepatitis, systemic mycotic diseases, egg yolk peritonitis and acute nephritis. In most cases of aspergillosis of parrots, beta globulin concentrations were increased – these changes, however, were not pathognomic (Cray et al. 2009a). The results of electrophoresis can be used in combination with the results of other tests to monitor the progress of inflammatory processes associated with the disease (Ivey 2000; Cray et al. 2009b). Electrophoresis is considered a useful diagnostic and prognostic tool in birds of prey, but always in combination with other tests (Tatum et al. 2000). Briscoe et al. (2010) identified electrophoretograms as abnormal only in 25 out of 144 diseased birds (17.4%), but it should be said that these birds were classified as "diseased" only on the basis of anamnestic data obtained from the birds' owners. In contrast, Tappin et al. (2011) found changes in the electrophoretograms of 140 out of 147 dogs; 79 of them had an infectious or inflammatory disease. Changes of concentrations of plasma proteins and their proportions is characteristic for many systemic diseases in reptiles. As compared with mammals or birds, electrophoresis in reptiles is used rarely in clinical practice. This can be partly due to the limited availability of reliable data on the physiological composition of individual fractions in reptiles. Ceron et al. (2011) reported that the electrophoretic values obtained from individual laboratories can vary. The reference values are in a way dependent on the diagnostic system used by a given laboratory (Cray et al. 2011).

https://doi.org/10.17221/5/2019-VETMED

In our study, deviations from the expected A/G ratio were observed in 11 out of 14 cases (those with liver failure, chronic renal insufficiency and suspected hepatopathy, chronic interstitial nephritis, chronic renal insufficiency and dermatitis). Most frequently (in 6 out of 11 patients, cases 1, 4, 5, 6, 9, 11) the decline in the A/G ratio was caused both by a decrease in the albumin fraction and an increase in the globulin fraction. Three cases (cases 3, 7, 10) with decreased A/G ratios were associated with a decrease in albumin concentration alone, which may result from insufficient production by the liver or from renal losses. Only in two out of 11 patients with a low A/G ratio was albumin concentration normal, with changes caused by increased concentration of some of the globulins – case 8 with chronic renal insufficiency, and case 14 with chronic dermatitis of mycotic origin.

None of the green iguanas investigated in the present study showed a significant increase in albumin fraction of plasma proteins. A decreased albumin concentration was found in 10 out of 14 cases.

Among the iguanas monitored in the present study, one with chronic dermatitis of mycotic origin (case 14) showed an increase in the alpha globulin fraction, and 8 out of 14 showed an increase in the beta globulin fraction.

An increased concentration of gamma globulins is usually associated with acute or chronic inflammation, infection, chronic hepatitis or immune-mediated disease (Hochleithner 1994). In our study, an increase in gamma globulin levels was noted in four out of 14 iguanas. The other iguanas did not show a significant increase in gamma globulins, although they were suffering from a variety of severe conditions including bacterial abscesses in multiple organs, acute necrosis of the liver, liver steatosis, chronic interstitial nephritis, chronic degenerative changes of renal tissues and chronic dermatitis of mycotic origin.

Although beta-gamma bridging can be seen in certain hepatic diseases, it has been shown that it cannot be considered as pathognomic (Camus et al. 2010). In the monitored green iguana patients, no case of beta-gamma bridging was observed.

In human medicine, patients with liver failure are reported to have normal beta globulin levels and, at the same time, significantly increased levels of gamma globulins (Vavricka et al. 2009). In the present study, such an increase in gamma globulins was not found in any patient with impaired hepatic function and was observed only in one patient with chronic renal insufficiency and gout.

Sevelius and Andersson (1995) considered the electrophoresis of plasma proteins in dogs as a significant prognostic tool to assess chronic hepatic diseases: a decreased albumin concentration together with normal levels of alpha globulins denotes a good prognosis, whereas decreased levels of alpha globulins with low albumin are usually seen in the terminal stages of liver failure. Out of the iguanas monitored in the present study, decreasing concentrations of alpha globulins were found in one iguana with chronic interstitial nephritis (case 6), in an iguana with hepatic steatosis (case 3) and in an iguana with chronic degenerative changes in the renal tissue (case 10). One patient suffering from chronic renal insufficiency and ovarian folliculitis (case 12) and with a decreased alpha globulin concentration survived due to the treatment.

Cray et al. (1995) described the advantage of electrophoresis in monitoring disease progress and the response of the patient to therapy. When the response to treatment is positive, albumin levels and globulin levels decrease, and the A/G ratio starts to normalise. Among the iguanas observed in this study, repeated blood sampling was performed with four patients for plasma protein electrophoresis (cases 6, 8, 9 and 14). One of them was a patient with chronic interstitial nephritis (case 6), in which the health status progressively worsened despite long-term therapy until the patient had to be euthanised. Deteriorating health corresponded to a progressive decrease in albumin concentration, alpha globulin concentration and the A/G ratio. The concentration of beta globulins slightly decreased during treatment, but in all samplings exceeded the reference range. Although the patient suffered from chronic inflammatory disease, there was no increase in the gamma globulin concentration. The second of these iguanas (case 8) had chronic renal insufficiency. Two blood samplings were performed within the space of a year, during which the overall health condition improved due to the supportive therapy. Improvement was also observed in the electrophoresis results; hyperproteinaemia persisted, but the A/G ratio and albumin concentration were within the reference ranges. Increased concentrations of beta and gamma globulins persisted in both samplings, but for each of the fractions there was a slight decline in values. The third patient that we followed over

https://doi.org/10.17221/5/2019-VETMED

a long period of time was the iguana (case 9) with chronic renal failure and gout. The health status of the animal gradually stabilised during the nine months of therapy, as manifested in a gradual increase in albumin concentration and a normalising of the A/G ratio. In contrast, the concentration of gamma globulins increased steadily during treatment until the last blood sampling, where a decreasing trend appeared. The last case (case number 14) with multiple blood samplings was the iguana with chronic dermatitis of mycotic origin, which was controlled after eight months of therapy, during which the animal's health status did not change significantly. The concentration of gamma globulins, which exceededthe reference values at first examination, further increased. Simultaneously, the albumin concentration decreased and the A/G ratio fell below the lower limit of the reference range.

The most commonly observed changes in the iguanas with liver and renal diseases were decreases in albumin concentrations with simultaneous reductions of the A/G ratio and increases in the concentration of beta globulins. Gimenez et al. (2010) described the same changes in a green iguana suffering from metabolic bone disease and renal failure. However, in clinical practice it is possible to come into contact with patients suffering from chronic renal failure or hepatopathies (e.g. liver steatosis), but showing no significant changes in plasma protein levels. Keeping this in mind, we suggest that electrophoresis of plasma proteins can help in the differential diagnosis of health disorders in reptiles. This diagnostic tool should be therefore used more often as part of the patient's comprehensive medical examination. Considering the variety of reptile species and the often very specific physiological processes in individual reptilian taxonomic groups, future studies will certainly advance our understanding of the use of plasma proteins in disease diagnosis.

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