

Biochemical profile of urine in guinea pigs (*Cavia porcellus*)

HANA CERNOCHOVA^{1*}, ANNA HUNDAKOVA¹, EDOARDO BARDI², ZDENEK KNOTEK¹

¹Avian and Exotic Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

²Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Milan, Milano, Italy

*Corresponding author: cernochovah@vfu.cz

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Abstract: Guinea pigs are common patients in veterinary clinics. Knowledge of the urine composition is necessary for the evaluation of their health. We, therefore, analysed the urine of fifty guinea pigs, thirty-two males, and eighteen females, aged between four months and seven years. None of these guinea pigs showed clinical signs of urinary tract diseases. The urine samples were obtained as part of a preventive check-up, during a regular dental check-up or another minor procedure. The urine was acquired by spontaneous micturition after inducing a short-term, light isoflurane anaesthesia. A macroscopic evaluation of the urine samples and a urine dipstick test were used for the preliminary examination. The average pH was determined to be 8.5 ± 0.5 , and in three animals, moderate glycosuria was detected using the urine dipsticks. No urobilinogen, ketone bodies, haemoglobin, or blood traces were present in any of the samples. Severe proteinuria was detected in all the samples. The samples were subsequently evaluated in a laboratory for the following values: specific gravity $1.024.40 \pm 1.83 \text{ kg/m}^3$, osmolality $601.14 \pm 52.28 \text{ mOsm/kg}$, total protein $290.16 \pm 34.73 \text{ mg/l}$, albumin $12.04 \pm 1.92 \text{ mg/l}$, glucose $0.77 \pm 0.20 \text{ mmol/l}$, urea $217.60 \pm 24.23 \text{ mmol/l}$, creatinine $3.98 \pm 0.48 \text{ mmol/l}$, bilirubin $9.63 \pm 1.73 \text{ }\mu\text{mol/l}$, calcium $6.14 \pm 0.40 \text{ mmol/l}$, phosphorus $4.95 \pm 1.30 \text{ mmol/l}$, magnesium $9.86 \pm 0.57 \text{ mmol/l}$, sodium $49.15 \pm 6.67 \text{ mmol/l}$, potassium $152.21 \pm 10.62 \text{ mmol/l}$, chloride $51.14 \pm 5.81 \text{ mmol/l}$, activity of gamma-glutamyltransferase $0.72 \pm 0.14 \text{ }\mu\text{kat/l}$, alkaline phosphatase $0.56 \pm 0.11 \text{ }\mu\text{kat/l}$ and lactate dehydrogenase $0.68 \pm 0.14 \text{ }\mu\text{kat/l}$. The descriptive values of the urine biochemical parameters of guinea pigs were determined for the first time in this study.

Keywords: clinical biochemistry; herbivorous mammals; urinalysis

Based on our experience, guinea pigs, alongside rabbits, are the most commonly kept exotic small mammals, which makes them frequent patients in veterinary clinics. The evaluation of the blood and urine samples is part of routine testing that complements the clinical examination of small exotic mammals (Quesenberry et al. 2012). A standard urine evaluation includes macroscopic, mi-

croscopic, and biochemical analyses (Ness 1999; Piech and Wycislo 2019). The available information about guinea pigs' urine composition (Bishop et al. 2010) is limited compared to other pet mammals (Thornton et al. 1979; Ozkan et al. 2012; DiBartola and Westropp 2014). The aim of this study is, therefore, to present the biochemical urine composition of fifty guinea pigs without any major health issues.

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MATERIAL AND METHODS

Animals

Fifty guinea pigs, thirty-two males, and eighteen females, aged between four months and seven years were included in this study. The anamnestic data like the breed, sex and age of each guinea pig are listed in the table (Table 1). The guinea pigs were fed hay, vegetables and commercial diets – pellets 58% [Brit Animals Guinea Pig Complete (Czech Republic) 24%, VerseleLagaCavia Complete (Belgium) 18%, unidentified brands 16%] and mixtures 42%. Half of the guinea pigs received additional vitamin C supplementation.

None of these guinea pigs showed any clinical signs of urinary tract diseases, such as stranguria, haematuria, pollakiuria, polyuria, pyuria, perineal urine scalding, discomfort during abdominal palpation, changes in the size and shape of the kidneys, an irregular kidney surface or palpable uroliths or sludge in the bladder. In twenty guinea pigs, urine samples were obtained as part of an annual health check examination together with blood samples for haematology and biochemistry tests.

Table 1. Anamnestic data regarding the guinea pigs ($n = 50$) used for the establishment of the urine biochemical indicators

Breed	Sex	Age (years)
American ($n = 28$)	male ($n = 18$)	0.3; 0.5; 0.5; 1; 1.5; 2; 3; 3.5; 4; 4; 4; 4; 4; 4.5; 4.5; 5; 6.5
	female ($n = 10$)	1; 1.5; 2.5; 3; 5; 5; 5; 5.5; 6; 7
Abyssinian ($n = 8$)	male ($n = 5$)	4; 4; 4; 5; 7
	female ($n = 3$)	1; 4.5; 5
Skinny ($n = 5$)	male ($n = 4$)	3; 3; 3; 3.5
	female ($n = 1$)	2
White crested ($n = 3$)	male ($n = 1$)	4
	female ($n = 2$)	4; 4.5
Teddy ($n = 3$)	male ($n = 2$)	2; 3
	female ($n = 1$)	3
Coronet ($n = 2$)	male ($n = 1$)	1
	female ($n = 1$)	4.5
Peruvian ($n = 1$)	male ($n = 1$)	0.5
	female ($n = 0$)	–

All the parameters lay within the reference range. In twenty-six cases, the sample was taken from well-conditioned guinea pigs during a regular dental recheck. None of the patients suffered from anorexia. The samples from the remaining four patients were obtained while treating their mild pododermatitis. In all the cases, the pododermatitis was caused by inappropriate bedding. The affected leg was erythematous without any ulceration. The treatment included bandaging the affected leg to prevent the pododermatitis from getting worse, the additional supplementation of vitamin C and adjusting the bedding. All the animals were handled in accordance with the national and European legislation (EU council directive 86/609/EEC for the protection of animals) and with the approval of their owners.

Collection and evaluation of urine samples

All the samples were collected by spontaneous micturition after inducing a short-term, light anaesthesia using isoflurane (Aerrane, Baxter, Belgium) combined with oxygen (oxygen flow rate 1.5 l/min). The volume of each obtained sample was between 2 and 2.5 ml. A macroscopic evaluation (colour, turbidity, odour) of the samples was performed. Urine dipsticks (Hexaphan; ErbaLachema, Brno, Czech Republic) were used to determine the pH and to evaluate the presence of proteins, glucose, urobilinogen, ketone bodies, haemoglobin or blood traces in the urine, respectively. The evaluation using the urine dipsticks was undertaken on colorimetric scales.

The samples were subsequently sent to a laboratory. The time between collecting the samples and their laboratory analysis never exceeded 30 minutes. When the laboratory could not analyse the sample immediately, the sample was refrigerated in between. An HRM18-T refractometer (A. Krüss Optronic GmbH, Hamburg, Germany) was used to determine the specific gravity. The osmolality was measured using a Fiske 210 osmometer (Advanced Instruments Inc., Massachusetts, USA). All the samples were centrifuged at 1 000 g for 5 min before the biochemical analysis. The concentration of the total protein, albumin, glucose, urea, creatinine, bilirubin, calcium (Ca), phosphorus (P), magnesium (Mg) and the activity of gamma-glutamyltransferase (GMT), alkaline phosphatase (ALP),

lactate dehydrogenase (LDH) were all assessed by photometric analysis using an Abbott Architect c4000 biochemical analyser (Abbott Laboratories, Illinois, USA) and a reagent kit [Urine/CSF protein, Microalbumin, Total Bilirubin, Urea Nitrogen, Creatinine, Glucose, Calcium Next Generation, Phosphorus, Magnesium, Gamma-Glutamyl Transferase, Lactate Dehydrogenase, Alkaline Phosphatase (Abbott Laboratories, Illinois, USA)]. The concentration of the sodium (Na), potassium (K), chloride (Cl) was determined potentiometrically using the same analyser with an ICT (integrated chip technology) module (Abbott Laboratories, Illinois, USA).

Statistical analysis

All the collected data were analysed using the IBM software SPSS v24.0. (IBM, New York, USA). The minimum and maximum obtained values, the arithmetic mean and the standard error of the mean were all determined for the quantitative values of the urine profile (specific gravity, osmolality, concentration of total protein, albumin, glucose, urea, creatinine, bilirubin, Ca, P, Mg, Na, K, Cl and activity of GMT, ALP, LD) similarly to a study dealing with such issues in rabbits written by Ozkan et al. (2012).

RESULTS

The urine colour was white to yellow in all the obtained samples. The urine was turbid without any specific odour. The specific gravity was determined to be $1\ 024.40 \pm 1.83\ \text{kg/m}^3$ with a range of $1\ 004.00 - 1\ 048.00\ \text{kg/m}^3$. The laboratory measured the same values before and after the centrifugation. The osmolality was $601.14 \pm 52.28\ \text{mOsm/kg}$ ($185.00 - 1\ 567.00\ \text{mOsm/kg}$).

The average pH value, according to the urine dipsticks, was 8.5 ± 0.5 (minimum 8, maximum 9). In three cases, the dipsticks revealed moderate glycosuria ($5.5\ \text{mmol/l}$). No urobilinogen, ketone bodies, haemoglobin, nor traces of blood were present according to the dipsticks in any sample. In contrast, the dipsticks revealed significant proteinuria in all the samples ($5\ \text{g/l}$). The urine biochemical parameters of the fifty guinea pigs received from the laboratory and statistically processed are listed in Table 2.

DISCUSSION

The aim of this study was to determine the descriptive values for the urine biochemical parameters of guinea pigs which can be used in veterinary practice where a urinalysis is a useful paraclinical examination. Samples from fifty guinea pigs were

Table 2. Values of the urine biochemical indicators in the guinea pigs without any major health issues ($n = 50$)

Values	Units	Mean \pm SEM	Range
Total protein	mg/l	290.16 ± 34.73	68.00–780.00
Albumin	mg/l	12.04 ± 1.92	5.00–57.00
Glucose	mmol/l	0.77 ± 0.20	0.06–8.91
Urea	mmol/l	217.60 ± 24.23	32.20–616.6
Creatinine	mmol/l	3.98 ± 0.48	0.43–18.43
Bilirubin	$\mu\text{mol/l}$	9.63 ± 1.73	0.90–56.80
Gamma-glutamyltransferase	$\mu\text{kat/l}$	0.72 ± 0.14	0.07–5.23
Alkaline phosphatase	$\mu\text{kat/l}$	0.56 ± 0.11	0.08–3.63
Lactate dehydrogenase	$\mu\text{kat/l}$	0.68 ± 0.14	0.16–3.98
Calcium	mmol/l	6.14 ± 0.40	0.89–13.74
Phosphorus	mmol/l	4.95 ± 1.30	1.50–60.00
Magnesium	mmol/l	9.86 ± 0.57	1.50–27.34
Sodium	mmol/l	49.15 ± 6.67	20.00–200.00
Potassium	mmol/l	152.21 ± 10.62	35.40–300.00
Chloride	mmol/l	51.14 ± 5.81	20.00–156.00

SEM = standard error of the mean

examined using a macroscopic evaluation (colour, turbidity, odour), urine dipsticks (proteins, glucose, urobilinogen, ketone bodies, haemoglobin, blood traces), refractometry, osmometry and photometric or potentiometric analysis for the biochemical parameters.

Urinalysis is a non-invasive examination method that can aid in the early diagnosis of many diseases, such as diabetes mellitus, inflammation of the urinary tract, or intravascular haemolysis of various origins in mammalian species (Parrah et al. 2013). In small exotic mammals, it is recommended to perform a urinalysis also in the case of urolithiasis, sludge, or anorexia (DeCubellis 2016).

Methods to obtain a urine sample include a spontaneous micturition, a gentle manual compression, cystocentesis, and the catheterisation of the bladder (Melillo 2007; Parrah et al. 2013). Cystocentesis is the most valuable method with respect to purity as it is possible to avoid specimen contamination. On the other hand, spontaneous micturition is the simplest and most convenient way to collect urine from the patient (Melillo 2007).

The method of obtaining urine by spontaneous micturition after inducing a short-term, light anaesthesia was preferred in the present study. The selected approach was convenient as all the guinea pigs needed anaesthesia for other reasons such as the blood collection from the *vena cava cranialis*, a dental re-check or a pododermatitis treatment. Spontaneous micturition also prevented the samples from getting contaminated by blood traces from the manual compression of the bladder or cystocentesis.

To our knowledge, no study regarding the complete biochemical analysis of urine in guinea pigs has been published to date. The current published data incorporated only the basic urine evaluation (predominantly using urine dipsticks), colour determination, specific gravity, and urinary sediment composition (Wesche 2009; Bishop et al. 2010).

The urine of healthy guinea pigs is a relatively dense, transparent, or turbid fluid that is white or yellow in colour and rich in crystals (Quesenberry et al. 2012). The presence of porphyrins can change the colour of the urine (Riggs 2009), which can interfere with the colorimetric analysis of some parameters (Piech and Wycislo 2019). The specific gravity and turbidity of the urine in guinea pigs is affected by a high concentration of excreted calcium, similar to rabbits (Reavill and Lennox 2020).

In our set of samples, the specific gravity values before and after centrifugation were the same, thus, they were not affected by the excreted calcium crystals. The same observation regarding specific gravity value was described in chinchillas by Doss et al. (2016) and in rabbits by Ardiaca et al. (2013). The alkaline pH in herbivorous exotic small mammals and a high concentration of cations (mostly calcium and magnesium) together with anions (mostly carbonates and phosphates) leads to the formation of urine crystals and may predispose them to a urolith formation (Hesse and Neiger 2009).

Calcium-carbonate crystals were determined, in the study performed by Bishop et al. (2010), as the most common ones in the urine sediment of guinea pigs, followed by amorphous crystals, phosphate crystals and calcium-oxalate crystals. The colour of the urine samples obtained from all fifty guinea pigs ranged from white to yellow. The urine was turbid without any specific odour. Our findings correspond to the published data (Quesenberry et al. 2012).

The reported average value of the specific gravity was stated to be lower than $1\ 050\ \text{kg/m}^3$ (Wesche 2009). In our study, it was determined to be $1\ 024.50 \pm 1.83\ \text{kg/m}^3$, and it generally corresponded to the value ($1\ 030\ \text{kg/m}^3$) determined by Bishop et al. (2010). Quesenberry et al. (2012) stated the specific gravity of urine obtained from forty-four patients suffering from urolithiasis to be $1\ 015 \pm 8\ \text{kg/m}^3$ (range $1\ 004\text{--}1\ 046\ \text{kg/m}^3$).

The evaluation of the pH using Hexaphan urine dipsticks revealed a value of 8–9, which matches the findings in healthy guinea pigs (Wesche 2009). The presence of urobilinogen, ketone bodies, haemoglobin, or traces of blood was not observed in any urine samples and it corresponds to the findings in healthy guinea pigs (Bishop et al. 2010).

The intensity of proteinuria can be evaluated by assessing the amount of excreted protein relative to the specific urine gravity (Melillo 2007) or by using the protein creatinine ratio (UP/UC). Creatinine is a waste product of muscle metabolism. The urine creatinine concentration was determined to be $3.98 \pm 0.48\ \text{mmol/l}$. There is a lack of data in the available literature regarding a reference range for healthy guinea pigs. Creatinine excretion through urine is quite stable (Fisher 2006), and the UP/UC ratio helps to quantify the proteinuria. The average UP/UC ratio in healthy guinea pigs is 1.6 (Rueloekke et al. 2016). In our set of samples, the average UP/UC ratio was

lower – 0.82 ± 0.10 (0.12–3.98). The total protein values determined by the laboratory methods were significantly lower – 290.16 ± 34.73 mg/l than those evaluated using the urine dipsticks – 5 g/l. The appearance of protein in the dipstick urinalysis is assessed as a false-positive in guinea pigs together with other herbivorous mammals with a naturally alkaline urine (Wesche 2009). According to Nabity (2011), the specific gravity in dogs may influence the quantification of proteinuria using urine dipsticks, a specific gravity lower than 1.012 kg/m^3 can lead to false negative results, while a specific gravity higher than 1.030 kg/m^3 can lead to false positive results. For guinea pigs, there is no available literature on this topic yet. The urine dipsticks revealed the same proteinuria intensity in all the samples without any respect to the specific gravity of the urine. The presence of proteins in the urine may be affected by any faecal contamination in the voided urine (Bishop et al. 2010). In the present study, this was ruled out by cleaning the perineal area and collecting the sample right after it was voided.

In three patients, moderate glycosuria was observed using the urine dipsticks. The laboratory analysis validated our results with values of 4.06, 3.71, and 8.91 mmol/l, respectively. These glucose values were significantly higher when compared to the rest of the group – 0.77 ± 0.20 mmol/l. The presence of glucose in the urine samples may be related to the patients' stress caused by their manipulation during the clinical examination (Jenkins 2010). In the present study, the urine was obtained by spontaneous micturition after a light isoflurane anaesthesia in all the guinea pigs. The manipulation and the anaesthesia induction were as gentle as possible, but it might be a cause of stress followed by glycosuria in these cases. The significant glycosuria in guinea pigs may be associated with diabetes mellitus, anorexia, hepatic lipidosis and ketosis (Wesche 2009). None of the three guinea pigs with moderate glycosuria had any clinical symptoms indicating they were suffering from diabetes mellitus because they did not show any symptoms like chronic weight loss, cachexia, polydipsia and polyuria (Williams 2012); the subsequent blood tests revealed that the glycaemia and fructosamine level lay within the reference range in all three patients.

Urea is the primary waste product of protein metabolism, the renal tubules reabsorb it, and the excess is primarily excreted in the urine. The amount of blood and, subsequently, the urine urea corre-

spond to the amount of protein in the diet and can be influenced by its particular type. The urea concentration in our set of samples was 9.63 ± 1.73 $\mu\text{mol/l}$. There is a lack of data in the available literature regarding normal values in guinea pigs. The amount of urine urea seems to be influenced by the caecal microflora activity too (Kawasaki et al. 2015). In rabbits, bilirubinuria might be caused by the destruction of haem from the red blood cells or muscles and can be associated with nephritis or cystitis (Jenkins 2010). The bilirubin level determined in the present study was 9.63 ± 1.73 $\mu\text{mol/l}$. There is a lack of data concerning normal values in guinea pigs and further studies regarding the association of the bilirubinuria intensity with possible liver diseases, erythrocyte lysis, nephritis or cystitis should be conducted.

An elevated GMT activity can be used to detect renal tubular damage at an early stage. A reference value was determined in clinically healthy rabbits to be 0.045 – 1.640 $5 \mu\text{kat/l}$ (Mancinelli et al. 2012). For guinea pigs, there is a lack of data in the available literature. In our set of guinea pigs' samples, the obtained value was mostly lower – 0.72 ± 0.14 $\mu\text{kat/l}$ in comparison to rabbits.

For the rest of the parameters, the validated data are still not available as well, and, therefore, our determined values of osmolality (601.14 ± 52.28 mOsm/kg), albumin (12.04 ± 1.92 mg/l), Ca (6.14 ± 0.40 mmol/l), P (4.95 ± 1.30 mmol/l), Mg (9.86 ± 0.57 mmol/l), Na (49.15 ± 6.67 mmol/l), K (152.21 ± 10.62 mmol/l), Cl (51.14 ± 5.81 mmol/l), ALP (0.56 ± 0.11 $\mu\text{kat/l}$) and LDH (0.68 ± 0.14 $\mu\text{kat/l}$) must be assessed with caution.

The intensity of the proteinuria should always be evaluated using laboratory methods instead of urine dipsticks, as the results are significantly different. Stress caused by the manipulation and the anaesthesia induction may lead to exceeding the renal threshold in some of the guinea pigs and might imitate signs of diabetes mellitus. Future research could continue to validate our data, especially the intensity of the bilirubinuria and the activity of the GMT in the urine in connection with liver diseases and early stages of kidney damage, respectively.

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Conflict of interest

The authors declare no conflict of interest.

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