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16 **Validation of an electrophoretic method to detect albuminuria in cats**

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Abstract

Objectives: The aims of this study were to validate a semi-automated high resolution electrophoretic (HRE) technique to quantify urinary albumin in healthy and diseased cats and to evaluate its diagnostic performances in cases of proteinuria and renal diseases.

Methods: Urine samples were collected from 88 cats (healthy; chronic kidney disease, CKD; lower urinary tract disease, LUTD; non-urinary tract diseases, OTHER). Urines were routinely analysed and HRE was performed. Within-assay and between-assay variability, linearity, accuracy, recovery and the lowest detectable and quantifiable bands were calculated. Receiver operating curves (ROC) analysis were also performed.

Results: All coefficients of variation were below 10%, percentage recovery was between 97% and 109% with a high linearity (r=0.99). HRE allowed the visualisation of a faint band of albumin and a diffused band between alpha and beta zones in healthy cats, while profiles from diseased cats were variable. Albumin (mg/dl) and urine albumin:creatinine ratio (UAC) were significantly (P <0.05) different between healthy and diseased cats. After ROC analysis, UAC values of 0.035 and 0.074 had a high sensitivity and high specificity, respectively, to classify proteinuria and identify borderline proteinuric cats. Moreover, an UAC of 0.017 had a high sensitivity to distinguish between healthy and diseased cats. However, UAC was not able to distinguish between renal (CKD) and non-renal diseases (LUTD/OTHER), probably

53 due to the pathophysiology of CKD in cats, which is characterised by low grade

54 proteinuria and less glomerular involvement than in dogs.

55 **Conclusions and relevance.** HRE is an accurate and precise method that could be used

56 to measure albuminuria in cats. UAC was useful to correctly classify proteinuria and to

57 discriminate between healthy and disease cats. HRE might also provide additional

58 information on urine proteins with a profile of all proteins (albumin and globulins) to

59 aid clinicians in the diagnosis of diseases characterised by proteinuria.

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Introduction

Very low concentrations of proteins are physiologically present in urine of healthy organisms, and all these proteins are collectively termed "proteinuria". The increase of proteinuria is mainly due to two different mechanisms: loss of selective glomerular filtration and impaired tubular resorption. Traces of albumin are physiologically present in urine of healthy animals. In dogs, albumin concentration greater than 30 mg/dl is defined as overt albuminuria[1,2], while microalbuminuria is defined as a concentration of albumin in urine greater than 1 mg/dl but below the limit of detection of semiquantitative screening tests (30 mg/dl). Albuminuria can also be expressed as urinary albumin:creatinine ratio (UAC) to adjust for differences in urine concentration and volume, as it is used for proteinuria (urine protein:creatinine ratio; UPC). In human medicine UAC values lower than 0.03 are considered normal, microalbuminuria is defined between 0.03 and 0.3, and values greater than 0.3 are considered as overt albuminuria.[3] Microalbuminuria is an important biomarker of glomerular damage and its role in predicting clinical end-point (cardiovascular events, renal events and mortality) and to monitor diabetic nephropathy, cardiovascular disease and hypertension is well documented in humans.[3–6]

In dogs and cats microalbuminuria has been evaluated in correlation with different pathologies and clinical conditions, such as chronic kidney disease (CKD) and renal failure[7,8], leishmaniosis[9], hypertension[10,11], hyperadrenocorticism[12,13], severe

84   inflammatory response syndrome (SIRS)[14], critically ill dogs in intensive care unit[15,16],

85   diabetes mellitus[17] and a variety of other systemic disorders.[1,18] However, only Syme et

86   al[8] reported the UAC calculated in a population of healthy cats and a reference interval

87   for cats utilising an appropriate number of individuals has not been defined so far. In

88   addition, the methods used to quantify albuminuria present some disadvantages. In

89   particular ELISA[8,19,20] is time consuming and not applicable in routine clinical

90   screening. The immunoturbidimetric assay[21,22] is based on the interaction with an anti-

91   human albumin polyclonal antibody, and the immunoassay Early Renal Damage

92   (ERD)[17,23,24] is semiquantitative.

93   In this context, there is a need for a fast and reliable method applicable in routine

94   clinical diagnostics. Therefore, the aims of this study were to validate a semiautomatic

95   electrophoretic method on high resolution agarose gel (HRE) to quantify albuminuria in

96   cat urine and test its clinical applications in routine analysis.

97

98   Material and methods

99   *Study design*

100   This study was performed using 88 urine samples submitted to the Veterinary

101   Clinical Pathology Service of the Veterinary Teaching Hospital of the University of

102   Bologna for a variety of clinical conditions.

103   The control group (H) comprised 22 cats considered healthy on the basis of

104 history and physical examination and without any other abnormality in serum

105 biochemistry and urinalysis. The diseased cats were assigned to one of three categories

106 of disease based on history, clinical signs, clinicopathological and imaging data as

107 follows: chronic kidney disease (CKD) n=32, lower urinary tract disease (LUTD) n=18,

108 other disease without involvement of the urinary system (OTHER) n=16

109 (inflammatory/infectious diseases n=6; orthopedic/neurologic diseases n=4;

110 gastrointestinal disease n=3; neoplastic disease n=3). Cats with clinical and cytological

111 evidence of urinary tract infection were included in the LUTD group. CKD cats were

112 diagnosed and staged following IRIS guidelines. [25]

113   All experimental procedures were approved by the Institutional Scientific

114 Ethical Committee for Animal Testing of the University of Bologna (approval number

115 8-72-2012; date of approval 01 October 2012) and the owners signed an informed

116 consent form before inclusion of their animal in the study.

117

118 *Urinalysis*

119   Complete urinalysis, including measurement of the specific gravity by a

120 refractometer and semi-quantitative dipstick test (Combur10Test, Roche Diagnostic)

121 was performed on all samples. After centrifugation at 1,500 x g for 10 minutes and

122 microscopic sediment analysis at low (100x) and high (400x) power fields, the

123 supernatants were divided into two aliquots and stored at -80°C for subsequent analysis.

124　Urine total proteins (UTP) and creatinine were determined using commercial kits

125　(Urinary/CSF Protein, OSR6170, and Creatinine OSR6178, Olympus/Beckman

126　Coulter) on an automated chemistry analyser (AU 400, Olympus/Beckman Coulter)

127　allowing the calculation of the urinary total protein-to-creatinine ratio (UPC).

128

129　*High Resolution Electrophoresis (HRE)*

130　　　All urine samples were analysed with high resolution electrophoresis (HRE) on

131　0.8% agarose gel at pH 8.6 (HydraGel HR 15, Sebia) in combination with the semi-

132　automated system Hydrasys (Sebia), according to the manufacturer instructions. The

133　gels were dried, stained with acid violet solution and band staining density acquired by

134　the Epson Perfection V700 photo scanner/densitometer. The obtained pherograms were

135　analysed by the Phoresis software (version 6.1.2). The relative percentage and absolute

136　concentration of albumin were calculated based on the density determined by the

137　densitometer. As reported by the manufacturer, the limit of detection of this technique

138　for serum albumin is 1.5-2 mg/dl (0.15 to 0.2 micrograms per band), with

139　concentrations linear up to at least 5.8 g/dl and optimal protein concentration of 200

140　mg/dl. Urine samples with higher protein concentrations were diluted in order to

141　achieve the desired optimal concentration.

142

143　*HRE validation*

144   To validate the HRE method for albumin quantification in cat urine, precision

145   (within and between assay variability), linearity, accuracy and analytical sensitivity

146   were calculated. Within-assay variability was assessed on five cat urine samples with

147   different albumin concentrations ranging from 7.4 to 67.7 mg/dl (7.4; 13.4; 23.5; 38.8;

148   67.7). For within-assay, samples were run six times on the same gel. Between-assay

149   variability was evaluated measuring 4 samples (7.4; 13.4; 23.5; 67.7 mg/dl) in duplicate

150   on five different days.

151   Linearity was tested by serial dilution of one sample (79 mg/dl) until reaching

152   the expected albumin concentration under the limit of detection (LOD) defined by the

153   manufacturer (1.5 mg/dl). All samples were analysed in duplicate. The analytical

154   sensitivity was obtained by the definition of the lowest detectable band (the lowest

155   concentration with weak or barely visible albumin band on the gel) and the lowest

156   quantifiable band (the lowest concentration with a quantifiable peak in the pherogram).

157   To evaluate accuracy, in the absence of a reference method for cat urine, a %

158   recovery study was made. Three solutions were prepared by adding to 100 µl of cat

159   urine, containing 24.5 mg/dl of albumin, 100 µl of saline solution (12.25 mg/dl of

160   expected albumin), (L) and 100 µl of two cat urine samples reaching 32.9 mg/dl (M)

161   and 53.8 mg/dl (H) of expected albumin. Five replicates were made for each solution.

162

163   *Statistical analysis*

164   Statistical analysis was performed with MedCalc ® 11.3.3.0. Data are reported

165 as median (range). Precision was evaluated by the calculation of the Coefficient of

166 Variation (CV) as follows: CV = (SD/mean)*100. Correlation and regression analysis

167 were performed on expected and observed values for albumin and on UPC and UAC.

168 According to IRIS[25] CKD staging guidelines , cats with UPC >0.4, 0.2-0.4, and <0.2

169 were classified as proteinuric, borderline proteinuric or non-proteinuric, respectively.

170   Receiver operator curves (ROC) were generated to evaluate sensitivity (SE),

171 specificity (SP), positive and negative predictive values (PPV; NPV) and positive and

172 negative likelihood ratios (LR+; LR-) of UAC and dipstick to classify proteinuria, using

173 as a reference cut-off UPC 0.2 (according to IRIS guidelines). ROC curves were also

174 generated to evaluate SE, SP, PPV, NPV, LR+ and LR- of UAC, UPC and dipstick to

175 classify patients as healthy or diseased.

176   Non parametric statistic (Kruskal-Wallis with post-hoc test for pairwise

177 comparison of groups) were performed to evaluate differences between groups (H,

178 CKD, LUTD and OTHER) for UPC, UAC and age. Values of albumin less than 5 mg/dl

179 (defined as lowest quantifiable band) were set at 2.5 mg/dl and UAC and albumin

180 percentage values were calculated according to this value. Significance was set at *P*

181 <0.05 unless otherwise specified.

182

183 **Results**

HRE validation and albumin quantification

185   HRE had within-assay CVs of 2.6% (67.7 mg/dl), 5.8% (38.8 mg/dl), 3.4% (23.5

186   mg/dl), 7.4% (13.4 mg/dl), and 7.6% (7.4 mg/dl) and between assay CVs of 3.5% (67.7

187   mg/dl), 1.2% (23.5 mg/dl), 7.4% (13.4 mg/dl) and 9% (7.4 mg/dl). Percentage recovery

188   of 97%, 93% and 109% for high (H), medium (M) and low (L) concentration

189   respectively were calculated and a significant correlation was found between measured

190   and expected albumin concentration (r = 0.99; *P* <0.001). The lowest detectable band of

191   1.25 mg/dl was set by visual inspection of the gels and the lowest quantifiable band of 5

192   mg/dl was determined by densitometric analysis.

193   After the validation, HRE was performed on the 88 urine samples. Nineteen out

194   of the 32 urine samples with UTP <40 mg/dl and 6/56 urine samples with UTP >40 had

195   a barely visible protein band corresponding to an albumin concentration lower than

196   lowest quantifiable band.

197

198   *Diagnostic performances of HRE and clinical application*

199   Twenty-two cats were included in the study as the healthy group (Table 1;

200   Supplementary Table 1). The median age was 22.5 (6-168) months and median UPC

201   was 0.12 (0.06-0.32). HRE allowed the visualisation of a faint band of albumin and a

202   diffused band between alpha and beta zones (Figure 1a) in most samples. Only in nine

203   samples (41%) albumin concentration was quantifiable, the median concentration was

11

204 <5 mg/dl (<5-29.8) and the calculated median UAC was 0.011 (0.004-0.069). No

205 differences were found between males and females.

206   Sixty-six samples were included in the diseased group (Table 1; Supplementary

207 Table 2) with a median age of 132 (24-268) months and median UPC of 0.51 (0.07-

208 16.15). These samples had variable electrophoretic profiles (Figure 1b). In particular,

209 the albumin band was evident and quantifiable in 54 samples (82%) and the median

210 albumin concentration and UAC were 12 mg/dl (<5-962) and 0.111 (0.009-7.056),

211 respectively, both significantly higher than in healthy cats (P <0.01). Moreover, it was

212 also possible to separate and identify the alpha, beta and gamma globulins zones (Figure

213 1c).

214   *Albuminuria vs Proteinuria* - Using the IRIS guidelines, the 88 samples were

215 classified as non-proteinuric (UPC <0.2; n = 38) borderline proteinuric (UPC 0.2-0.4; n

216 = 11), and proteinuric (UPC >0.4; n = 39). UAC was significantly correlated to UPC (r

217 = 0.967; P <0.0001). ROC curves were generated to evaluate diagnostic performance of

218 UAC and dipstick (considered as positive for trace results) to correctly classify

219 proteinuria, considering as positive samples with UPC >0.2 (borderline proteinuric and

220 proteinuric). Area under curve (AUC) for UAC (0.939) was significantly greater (P

221 <0.01) than dipstick (0.537). Table 2 shows SE, SP, PPV and NPV for the best criteria

222 calculated by the ROC curves for UAC and dipstick. For UAC, two different values

223 demonstrated high sensitivity (0.035) or high specificity (0.074) for overt or borderline

224 proteinuria, while dipstick showed very low specificity due to the high number of false

225 positive results. Using these two cut-off values, as reported in Table 3, 10 non-

226 proteinuric samples had abnormal UAC values.

227   *Albuminuria vs Diseases* – According to serum creatinine concentration and

228 IRIS guidelines, CKD cats were staged as follows: stage I n = 3, stage II n = 8, stage III

229 n = 9 and stage IV n = 12. CKD cats were significantly older with a median age of 163

230 (24-252) months (P <0.01) and had significantly increased UPC (0.63; 0.10-16.15),

231 albumin concentration (11.4 mg/dl; <5-962) and UAC (0.117; 0.009-7.057) than the H

232 group (P <0.01). LUTD cats were significantly older than H group cats with a median

233 age of 132 (36-268) months (P <0.01) and had significantly increased UPC (0.7; 0.07-

234 4.59). Albumin concentration (20.2 mg/dl; <5-134.2) and UAC (0.184; 0.010-1.487) in

235 LUTD cats were significantly higher than H group (P <0.01). OTHER cats were

236 significantly older than H and younger than CKD group with a median age of 102 (24-

237 192) months (P <0.05) and had UPC (0.21; 0.09-1.52) significantly higher than the H

238 group (P <0.05). Albumin concentration (15.2 mg/dl; 6.6-147.2) and UAC (0.0532;

239 0.013-0.465) in this group were higher than in H group (P <0.05). Figure 2 shows the

240 comparison of UAC among H, CKD, LUTD and OTHER groups.

241   ROC curves were generated to evaluate and compare dipstick, UAC and UPC to

242 correctly classify cats. The performance of UPC, UAC and dipstick is summarised in

243 Table 4. ROC curves comparison for the classification of patients as healthy or diseased

244   is reported in Figure 3a. AUCs for UPC (0.909), UAC (0.877) and USG (0.865) were

245   not significantly different from each other but significantly different from AUC for

246   dipstick (0.631) (P <0.001). For UPC, the best criterion was set at 0.165, while for

247   UAC, two different values gave high sensitivity (0.017) or high specificity (0.074).

248   Dipstick showed very low specificity. ROC comparison was lastly generated to

249   discriminate cats as affected by CKD or LUTD/OTHER diseases (Figure 3b). AUCs for

250   UPC (0.665) and USG (0.740) were not significantly different from each other, but were

251   both significantly different from AUC for dipstick (0.525) and UAC (0.585) (P <0.05).

252

253   **Discussion**

254   Several studies suggest that even mild proteinuria/albuminuria can be indicative

255   of the severity of disease and predict poor outcome;[8,19,26,27] in contrast, the difficulty of

256   albumin quantification with dipsticks and the absence of a validated method for cat

257   urine highlight the importance of a sensitive, accurate and precise analytical method for

258   albumin quantification in urine. In this context, our work aimed to validate an

259   electrophoretic method for albumin quantification and to evaluate its clinical application

260   in routine urinalysis.

261

262   *Validation of HRE*

263   The electrophoretic method validated in the present study is a semiautomatic

14

264 technique and the obtained CVs were lower than 10%. As a general rule, CVs for

265 automated assays should be less than 5% and for manual assays less than 10%.[28]

266 Therefore, considering that HRE is a semiautomatic technique, the variability

267 determined in the present study can be considered acceptable. The method was linear in

268 the tested range of concentrations and the results of the recovery test can be considered

269 acceptable for routine diagnostic laboratory use.[28] Regarding the LOD, it was difficult

270 to quantify blank (saline solution, NaCl 0.9%) by densitometry. The scanner reads

271 impurities on the gel generating irregular profiles, not related to the protein content of

272 the samples; the same analytical problem was also reported by other authors.[29] As a

273 consequence, the LOD was set at 1.25 mg/dl of expected albumin, since at this

274 concentration, close to the LOD defined by the manufacturer, a faint band of the protein

275 was optically visible on the gel, though not quantifiable by the software. Furthermore,

276 the software allows the technician/clinician to reduce the interferences and

277 evaluate/validate the correspondence between visual inspection of the gel and the

278 densitometric profile. Therefore, the ease and rapidity of use, the high reproducibility

279 and accuracy and wide range of linearity, combined with the final evaluation of the

280 pherogram by the clinician/technician, allow us to conclude that HRE is a reliable

281 method for quantifying albuminuria in cats.

282

283 *Diagnostic performance of HRE and clinical application*

284   HRE was useful to highlight that urine from healthy cats is characterised by very

285   low albumin concentrations. This result is confirmatory of data reported by Ferlizza et

286   al[30], who pointed out that the most abundant proteins in urine from healthy cats were

287   cauxin (at greater concentration in entire males) and uromodulin, both produced by

288   healthy tubular cells and specifically secreted in urine. Diseased cats presented a greater

289   variability in the electrophoretic profiles with increasing concentration/density of the

290   albumin band and of alpha, beta and gamma zones. The quantification of the globulins

291   was out of the scope of the present paper and they were not correlated to the

292   presence/absence of CKD or other diseases. Nevertheless, Giori et al[29] correlated the

293   reduced percentage of alpha1-globulins to the glomerular origin of proteinuria in dogs.

294   These results, were associated with a clear difference between the pherograms from

295   healthy cats compared with the diseased ones in the present study, and allowed us to

296   hypothesize the potential usefulness of HRE to evaluate albumin, alpha, beta and

297   gamma globulins to correctly classify proteinuria in both dogs and cats.

298   In the present study patients were arbitrarily classified according to IRIS

299   guidelines for proteinuria. Although these guidelines have been created to classify CKD

300   patients, the authors considered the categories proposed as useful to classify proteinuria

301   in cats regardless of the presence of CKD, since cats frequently present low grade

302   proteinuria in the clinical practice.[2,16,17] To evaluate the ability of HRE to correctly

303   classify proteinuria, a comparison was made with dipstick, using an UPC value of 0.2 as

16

304 reference. Dipstick showed high sensitivity, but very low specificity, due to the high

305 number of false positive results. However, as suggested by Zatelli et al[31] for dogs, the

306 dipsticks should always be interpreted in light of the USG and, in the present study,

307 most of the false positive samples also had high USG values (>1.035). Moreover,

308 healthy entire male cats secrete cauxin into urine and, that has also been demonstrated

309 to be a cause of false positive proteinuria.[32] The lower UAC value, 0.035, was close to

310 the cut-off used for human microalbuminuria (0.03) and using this criterion nine non-

311 proteinuric samples (UPC<0.2) were classified as microalbuminuric. The second one,

312 0.074, is similar to data reported presented by Syme et al[8] who reported an UAC of 0.08

313 as the upper limit of the reference interval in healthy cats, and lower than the value used

314 in human medicine for overt albuminuria (0.3). The population of healthy cats included

315 in the present study was not age-matched with those in the CKD and non-CKD groups

316 differently to what reported by Syme et al.[8] However, the similarity between the data

317 reported by Syme et al[8] and the cut-off reported in the present study, in spite of the

318 different age of the healthy cats (median 12 vs 1.9 years) might be confirmatory of the

319 clinical reliability of the analysis and suggests that UAC is not influenced by age alone.

320 As regards the difference between cats and humans, and also between cats and dogs,

321 could be explained by cats presenting with lower quantities of proteinuria/albuminuria

322 due to a different pathophysiology in cases of renal diseases. These preliminary results,

323 even if obtained on a limited number of cats, suggest that an UAC of 0.035 as a cut-off

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324 of microalbuminuria and an UAC of 0.074 as cut-off for overt albuminuria, if confirmed

325 by future studies with greater numbers of healthy cats, could be considered

326 complementary to UPC to estimate and identify pathologic low grade proteinuria.

327   As regards the comparison among groups, both UPC and UAC were able to

328 discriminate healthy cats from the diseased ones. Interestingly, though the renal and

329 urinary tract involvement, CKD and LUTD cats did not show different values of

330 proteinuria and albuminuria than the OTHER group. In our study, diseases apparently

331 not involving the kidney or the urinary tract were characterized by albuminuria higher

332 than healthy cats. The pathogenesis of increased urine albumin concentration in these

333 diseases has not been investigated in this study, however different conditions as

334 hypertension, previous treatments or even subclinical renal involvement could be

335 hypothesised. As previously suggested, proteinuria and albuminuria should be classified

336 as renal or pre/post renal and LUTD is considered a cause of post-renal albuminuria in

337 cats.[2,33] To the authors knowledge no data are present in the literature on albuminuria in

338 cats affected by LUTD and these are the first UAC values reported. The best criteria

339 calculated with ROC curve analysis to distinguish between healthy and diseased were

340 for UPC 0.165, close to the well-established value of 0.2, and for UAC 0.017, lower

341 than the previous calculated value of 0.035. However, after the subsequent ROC

342 analysis, UAC was not able to distinguish between CKD and non-CKD diseases. It is

343 well known that cats affected by CKD present with lower values of proteinuria and

344 albuminuria than dogs[34,35] and that even low UPC and UAC ratios are correlated with

345 poor outcome.[8] Using the calculated cut-offs (UPC 0.165 and UAC 0.017) within the

346 different groups of diseases, the sensitivity to detect low levels of

347 albuminuria/proteinuria is increased suggesting that these values (UPC <0.165; UAC

348 <0.017) could be reliable for early confirmation of disease with possible renal

349 involvement. The low level of albuminuria/proteinuria in cats with CKD seems to be

350 related to chronic tubulo-interstitial nephropathy.[30,36] Nevertheless, 8/32 CKD samples

351 had an UPC>2 considered as indicative of prevalent glomerular diseases that is not the

352 typical but a possible cause of CKD even in cats. Our results therefore suggest that in

353 these patients histopathologic characterisation of CKD should be performed to correlate

354 urinary electrophoresis with histologic findings.

355   Although the present study validated a fast and reliable technique to quantify

356 albuminuria in cats producing preliminary results of UAC, our study has a few

357 limitations. First, the limited number of healthy cats, since to construct a true reference

358 interval a greater number of samples is needed. Second, HRE was less sensitive to

359 detect low concentrations of albumin (<5mg/dl) than an ELISA (10-200 ng/ml), but fast

360 and easy to use in the routine clinic. Third, UAC was not able to discriminate between

361 CKD and other diseases characterized by proteinuria suggesting its use as an unspecific

362 marker of disease with possible renal involvement rather than a specific marker of CKD

363 as glomerular filtration rate could not be measured and used as the gold standard in this

19

364 study. HRE was unable to separate albumin from cauxin, in particular in entire male

365 healthy cats.

366

367 **Conclusions**

368 The electrophoretic method validated in this work is precise and accurate for measuring

369 albuminuria in cats. UAC was useful to correctly classify proteinuria and to

370 discriminate among healthy and diseased cats. Values for UAC of 0.035 and 0.074 are

371 suggested as the thresholds of microalbuminuria and overt albuminuria respectively in

372 cats, while a value of >0.017 could be indicative of disease with possible renal

373 involvement. Moreover, we reported the first data on albuminuria in cats affected by

374 LUTD. In addition, HRE can provide additional information on urine proteins and the

375 profile (including albumin and globulins) should be further analysed to aid clinicians in

376 the diagnosis of kidney diseases.

377

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382

383 **Conflict of interest**

20

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484

Table 1 Age and results of urine specific gravity (USG), proteinuria and albuminuria for

the samples from healthy and diseased cats. Data are reported as median (min-max)

	AGE	sCREA	sUREA	USG	UTP	uCREA	UPC	ALB	ALB	UAC
	Months	mg/dl	mg/dl		mg/dl	mg/dl		%	mg/dl	
RI		0.8-1.8	15-60	>1.035			<0.2			
HEALTHY n = 22	22.5 (6-168)	1.5 (1.03-1.8)	48.4 (32-86)	1.071 (1.036-1.090)	35.0 (16-159.6)	344 (162-766)	0.12 (0.06-0.32)	9.8 (5.5-42)	2.5 (2.5-29.8)	0.011 (0.004-0.069)
CKD n = 32	163 (24-252)	3.55 (1.33-12.7)	169 (57.8-501.7)	1.017 (1.006-1.068)	75.4 (12.8-1956.3)	92 (23-583)	0.63 (0.10-16.15)	22.3 (3.9-55.3)	11.4 (2.5-962.0)	0.117 (0.009-7.057)
LUTD n = 18	132 (36-268)	1.75 (1.37-10.78)	108.9 (41.1-479.5)	1.029 (1.008-1.072)	68.3 (30.6-280.2)	162 (91-672)	0.70 (0.07-4.59)	20.7 (5.3-59.1)	20.2 (2.5-134.2)	0.184 (0.010-1.487)
OTHER n = 16	102 (24-192)	1.59 (0.96-3.07)	53.2 (32.3-199.2)	1.058 (1.017-1.080)	74.9 (27.1-881.8)	338 (94-813)	0.21 (0.09-1.52)	20.7 (11.3-44.9)	15.2 (6.6-147.2)	0.052 (0.013 -0.465)

487

488 RI = laboratory reference intervals; sCREA = serum creatinine; sUREA = serum urea;

489 UTP = urine total protein; uCREA = urine creatinine; UPC = urine protein:creatinine

490 ratio; ALB = albumin; UAC = urine albumin:creatinine ratio; CKD = chronic kidney

491 disease; LUTD = lower urinary tract disease; OTHER = other diseases not involving the

492 urinary system

493

494 **Table 2** Sensitivity, specificity, positive and negative predictive values (PPV; NPV) and

495 positive and negative likelihood ratios (LR+; LR-) for the best criteria calculated for

496 UAC (0.035 and 0.07) and dipstick (30 mg/dl, lowest level of positivity) using UPC 0.2

497 as reference cut-off

498

	UAC 0.035	UAC 0.074	Dipstick 30
Sensitivity (%)	94.00	76	82.00
Specificity (%)	76.30	100	23.70
PPV (%)	82.50	97.4	58.60
NPV (%)	90.30	74.0	50.00
LR+	3.97	--	1.07
LR-	0.079	0.26	0.76

499

500 UPC = urine protein:creatinine ratio; UAC = urine albumin:creatinine ratio;

501

502 **Table 3** Sample distribution in the different classes of proteinuria, according to IRIS,

503 and albuminuria, according to the values determined in the present study

504

		NA UAC<0.035	MA 0.035<UAC<0.074	A UAC>0.074	TOT
NP	UPC<0.2	28	9	1	38
BP	0.2<UPC<0.4	2	4	5	11
P	UPC>0.4	1	6	32	39
	TOT	31	19	38	88

505

506 NA = non-albuminuric; MA = microalbuminuric; A = Overt albuminuric; NP = non-

507 proteinuric; BP = borderline proteinuric; P = proteinuric; UPC = urine protein:creatinine

508 ratio; UAC = urine albumin:creatinine ratio.

509

510   **Table 4** Sensitivity, specificity, positive and negative predictive values (PPV; NPV) and

511   positive and negative likelihood ratios (LR+; LR-) for the best criteria calculated for

512   UAC (0.027 and 0.07), UPC (0.165 and 0.2) and dipstick (30 mg/dl, lowest level of

513   positivity) to distinguish between healthy and diseased cats

514

	UPC>0.165	UPC>0.2	UAC>0.017	UAC>0.074	Dipstick>30
Sensitivity (%)	84.8	72.7	87.9	59.1	87.9
Specificity (%)	86.4	90.9	63.6	100.0	22.7
PPV (%)	96.6	96.0	87.9	100.0	70.0
NPV (%)	66.7	52.6	63.6	44	5.6
LR+	6.2	16.0	2.42	-	1.14
LR-	0.18	0.29	0.19	0.041	0.53

515

516   UPC = urine protein:creatinine ratio; UAC = urine albumin:creatinine ratio;

517

518 **Figure captions**

519 **Figure 1** Representative HRE gels (a, b) and pherogram (c) of urine samples from

520 healthy (a) and diseased (b, c) cats. a) HRE of healthy samples; b) HRE of diseased

521 samples (CKD lanes 3,7; LUTD lanes 5, 9-14; OTHER 1, 2, 4, 6; H lane 8); c) as an

522 example, pherogram of lane 3 (CKD)

523 **Figure 2** UAC values for healthy and diseased groups. Different lower cases indicate

524 significant difference (P <0.05). UAC values greater than 2 were excluded from graphic

525 visualisation. UAC = urine albumin:creatinine ratio; CKD = chronic kidney disease; H

526 = healthy; LUTD = lower urinary tract disease; OTHER = other diseases not involving

527 the urinary system

528 **Figure 3** ROC curve comparison for UPC, UAC, dipstick and USG to classify cat (a)

529 as healthy or diseased and (b) as renal (CKD) or non-renal (LUTD/OTHER). UPC =

530 urine protein:creatinine ratio; UAC = urine albumin:creatinine ratio; STICK = dipstick;

531 USG = urine specific gravity

532