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Evaluation of erythrocyte dysmorphism in dog urine[1]

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ABSTRACT.- Santos C.B.R., Silva L.C., Oliveira A.P., Silvestre J.A.R., Teixeira M.N. & Guimarães J.A. 2024. **Evaluation of erythrocyte dysmorphism in dog urine.** *Pesquisa Veterinária Brasileira 44:e07415, 2024.* Laboratório de Patologia Clínica Veterinária, Hospital Veterinário, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: medvetcarolinabrds@gmail.com

Glomerulopathy is an important cause of kidney disease in dogs. Diagnosis is based on anamnesis, clinical examination, and complementary tests and can be complex and costly depending on the stage of the disease. Morphological evaluation of erythrocytes observed during urinalysis is frequently conducted for humans, which may help identify kidney damage in animals with kidney disease – individuals with glomerular disease present with erythrocyte dysmorphism, particularly the presence of erythrocytes recognized as acanthocytes. Animal studies are limited, and their results are often contradictory. Thus, the objective of this study was to identify the different populations of isomorphic and dysmorphic red blood cells (RBCs) present in the urine of dogs and to compare the bright-field and phase-contrast optical microscopy techniques in identifying morphological changes in RBCs during urinalysis. A total of 40 samples were selected from dogs with microscopic or macroscopic hematuria from routine care at Federal Rural University of Pernambuco Veterinary Hospital and were sent to the hospital's Veterinary Clinical Pathology Laboratory. Physical, chemical, and sediment examinations were conducted. Subsequently, RBCs were evaluated and differentiated using bright-field and phase-contrast microscopy. In dog urine samples, it was possible to identify all populations considered isomorphic and dysmorphic that are described in humans. Among those considered isomorphic, normal RBCs, annulocytes, and echinocytes were observed, whereas stomatocytes, acanthocytes, codocytes, ghost cells, knizocytes and cells with no defined names (other) were found among the dysmorphic forms. No significant difference was observed between the two microscopic techniques used to differentiate and classify erythrocytes. Therefore, it is possible to identify and differentiate the morphological alterations found in RBCs in dogs' urine. Compared with a phase-contrast microscope, a conventional optical microscope can be used without any detrimental effects, thereby facilitating the use of this technique in the laboratory routine, as it is cost-effective. Further studies should be conducted to evaluate the sensitivity and specificity of the identification of erythrocyte dysmorphism as an early marker of glomerular lesions.

INDEX TERMS: Glomerular injury, erythrocyte dysmorphism, erythrocyte isomorphism, dogs, urinalysis.

RESUMO.- [**Avaliação do dismorfismo eritrocitário em urinas de cães.**] As glomerulopatias são importantes causas de afecções renais em cães. Seu diagnóstico é baseado na anamnese, exame clínico e exames complementares e pode se tornar complexo e oneroso dependendo da fase da doença. Uma avaliação frequentemente utilizada na medicina humana e que pode auxiliar na localização da lesão renal em animais com doença renal é a avaliação morfológica dos eritrócitos observados na urinálise. Indivíduos com doença glomerular apresentam dismorfismo eritrocitário, especialmente com a presença de eritrócitos reconhecidos como acantócitos. Estudos em animais são escassos e seus resultados muitas vezes são contraditórios. Dessa forma, objetivou-se identificar as diferentes populações de hemácias isomórficas e dismórficas presentes na urina de cães e comparar as técnicas de microscopia óptica

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de campo claro e de contraste de fase na identificação de alterações morfológicas em hemácias durante a realização da urinálise. Foram selecionadas quarenta amostras de urina de cães com hematúria microscópica ou macroscópica, oriundas da rotina de atendimento do Hospital Veterinário da UFRPE e enviadas ao Laboratório de Patologia Clínica Veterinária desta instituição. As amostras passaram por exame físico, químico e do sedimento, sendo posteriormente as hemácias avaliadas e diferenciadas utilizando-se os métodos de microscopia de campo claro e de contraste de fase. Nas amostras de urina de cães, foi possível identificar todas as populações consideradas isomórficas e dismórficas já descritas em humanos. Dentre as consideradas isomórficas foram observadas hemácias normais, anulócitos e equinócitos e dentre as formas dismórficas foram encontradas estomatócitos, acantócitos, codócitos, células fantasmas, nizócitos e células ainda sem nomenclatura definida (outros). Não foi observada diferença significativa entre as duas técnicas microscópicas empregadas na diferenciação e classificação dos eritrócitos. Conclui-se que é possível identificar e diferenciar as alterações morfológicas encontradas nas hemácias de urinas de cães e que o uso de microscópio óptico tradicional convencional, quando comparado ao microscópio de contraste de fase, pode ser empregado sem grande prejuízo para o exame, facilitando a utilização dessa técnica na rotina laboratorial por ser um equipamento mais acessível. Outros estudos devem ser realizados para avaliar a sensibilidade e especificidade do uso da identificação do dismorfismo eritrocitário como um marcador precoce de lesões glomerulares.

TERMOS DE INDEXAÇÃO: Lesão glomerular, dismorfismo eritrocitário, isomorfismo eritrocitário, urinálise.

INTRODUCTION

Glomerulopathy is an important cause of kidney disease in dogs and cats. Hence, an approach should be systematized to identify the cause, starting with few or no invasive tests, such as urinalysis (Crivellenti & Giovaninni 2021). The incidence of glomerular diseases in dogs has increased, partly due to greater recognition of the problem and greater demand for early diagnosis and treatment (Polzin & Cowgill 2013). According to Cavalcante et al. (2015), the frequency of glomerular disorders in dogs ranged from 43% to 90% and increased with age. In addition, glomerular diseases can cause chronic kidney disease, with glomerulonephritis accounting for more than 50% of these cases.

The main diseases affecting the glomeruli in domestic animals are glomerulonephritis and glomerular amyloidosis, with persistent and marked proteinuria associated with inactive sediment being the main characteristics of this clinical condition (Chew et al. 2012, DiBartola & Westropp 2015).

The diagnosis of glomerulopathy is based on clinical and laboratory findings, including detailed anamnesis, clinical examination, various laboratory tests that include urinalysis, urine protein testing, serum biochemistry to determine the concentrations of urea, creatinine, symmetric dimethylarginine, and electrolytes, blood pressure determination and renal biopsy (Crivellenti & Giovaninni 2021). Crivellenti & Giovaninni (2021) reported such examinations starting with the least invasive to the most invasive.

Urinalysis is a simple, quick, noninvasive, and inexpensive screening test. Its results, together with biochemical tests and clinical and imaging examinations, provide information on the function and structure of the urinary system (Kandula & Karlapudi 2015, Takahira 2015, Thrall et al. 2015).

Piech & Wycislo (2019) reported that the test performed during urinalysis stands out when evaluating urinary sediment. It provides the clinician with evidence of the presence of inflammatory, infectious, or hemorrhagic processes, renal tubular disease, metabolic and hepatic disorders, urolithiasis, and neoplasms present in the urinary tract.

An important and common finding in the evaluation of sediment is the presence of hematuria, characterized by an increase in the number of erythrocytes in the urine. Erythrocytes are small cells that have several different appearances, depending on urine concentration and the time between sample collection and examination (Cowell et al. 2021).

Stockham & Scott (2011) reported that a certain number of erythrocytes can be found in the urine of healthy animals. However, the amount considered normal may be influenced by the collection method. Hematuria was confirmed by microscopically identifying the erythrocytes. The presence of more than five erythrocytes per field (high magnification) is considered abnormal. The presence of erythrocytes in the sediment may indicate the existence of bleeding in some locations of the urinary and/or genital tract (Osborne & Finco 1995, Cowell et al. 2021, Stockham & Scott 2011, Chew et al. 2012, Thrall et al. 2015, Piech & Wycislo 2019, Crivellenti & Giovaninni 2021).

Hematuria can be classified as glomerular (nephrological origin) or non-glomerular (urological origin) (Vasconcellos et al. 2005). Moreover, some authors have recognized tubular hematuria as the source of nephrological hematuria (Nádasdy et al. 1989, Nguyen 2003). In addition, hematuria is categorized based on the intensity of bleeding observed macroscopically, when the color of the urine indicates the presence of blood, and microscopic when red blood cells (RBCs) are detected only by urine sediment analysis (Osborne & Finco 1995, Forrester 2004, Vasconcellos et al. 2005). Regarding frequency, hematuria can be permanent (constant presence of RBCs in the urine sediment), isolated (single occurrence of hematuria), or recurrent when there are periods of remission, with intervals varying from months to years. Finally, depending on the clinical repercussions, it can be categorized as symptomatic or asymptomatic (Vasconcellos et al. 2005).

In addition to the quantitative evaluation of RBCs in urine, several human studies have shown that it is possible to determine the origin of hematuria through RBC morphological analysis. Thus, it contributes to the precise diagnosis of kidney injury at an early stage at a low cost and with invasiveness (Comerlato et al. 2006). When hematuria is of non-glomerular origin, the morphology and size of RBCs are uniform, and these characteristics are referred to as erythrocyte isomorphism. When hematuria originates from a glomerular lesion, RBCs present with changes in shape, color, volume, and membrane; these characteristics are referred to as erythrocyte dysmorphism (Birch & Fairley 1979).

The lack of sensitive and specific markers for detecting and localizing kidney damage makes the morphological evaluation of erythrocytes in urine an important test. However, studies on domestic species are more limited than on humans, and the results are contradictory. González & Silva (2008) reported

the methodology of urinalysis in veterinary medicine and stated that the presence of erythrocyte dysmorphism in urine is indicative of nephron damage. Stockham & Scott (2011) reported that it is possible to find abnormal erythrocyte forms in humans diagnosed with glomerular hemorrhage, but the use of this method to differentiate between cases of glomerular and non-glomerular hemorrhage has not been reported in veterinary medicine. Chew et al. (2012) also reported that dysmorphic RBCs are of renal origin and can exist in humans with glomerulonephritis. However, these findings have not been reported in domestic animals.

The scarcity of relevant data in veterinary medicine suggests the need for further studies. The treatment of glomerulopathies depends on an accurate diagnosis, which requires costly tests and causes a delay in diagnosis because of serial monitoring. Thus, the addition of a low-cost, easy-to-perform test can give veterinarians greater confidence in the clinical management of cases involving the urinary system.

In view of the above, the aim of this study was to identify the different populations of dysmorphic RBCs present in dog urine and to compare the techniques of bright-field and phase-contrast microscopy in the identification of morphological alterations in RBCs during urinalysis.

MATERIALS AND METHODS

Ethical approval. The study was conducted from July 5, 2022, to November 25, 2022, using samples from the normal routine of the "Laboratório de Patologia Clínica Veterinária" (Veterinary Clinical Pathology Laboratory – LPCV) of the Veterinary Hospital (HOVET) of the "Universidade Federal Rural de Pernambuco" (UFRPE) and it was not necessary to submit the evaluation by the University's Ethics Committee on the Use of Animals (CEUA).

A total of 40 urine samples were selected from the dogs routinely treated at HOVET with hematuria detected during macroscopic or microscopic urinalysis. Urine was collected by catheterization or cystocentesis according to the veterinarian's indication and processed within 2 h of collection. Urinalysis was performed as described by Osborne & Finco (1995) in terms of physical characteristics (color, appearance, volume, and density) and chemical characteristics using Biocon11® urinalysis reagent strips (DIF CO, Gyeongsangnam-do, KR). According to this method, the urinary sediment was analyzed by transferring 5mL of the sample into a conical glass tube which was then centrifuged (Excelsa Baby II, model 206-R, FANEM®) at 1500rpm for 5 min. The supernatant was discarded, and the sediment was resuspended in 0.5mL of the remaining urine. Next, 20μL of the sediment suspension was assessed microscopically.

Microscopic evaluation of the sediment was performed using bright-field microscopy (Olympus, BX41®) at 400x magnification (Osborne & Finco 1995) to identify and quantify blood cells in the urine.

Samples with hematuria [red blood cell count >5/field (400×)] were selected for the study. One hundred erythrocytes were counted and classified as dysmorphic and isomorphic cells, and the percentage of occurrence of each alteration was calculated to determine the morphology of the RBCs in the urine. They were analyzed and differentiated using bright-field microscopy (Olympus, BX41®) at 400× magnification, and then the same procedure was performed with phase-contrast microscopy (Nikon ECLIPSE E200®). The classification of erythrocytes was based on the protocol described by Bastos et al. (1998), Vasconcellos et al. (2005) and Chu-Su et al. (2017).

Descriptive statistics were used to analyze the results. Analysis of variance was used for the non-parametric data to compare the mean values and standard deviation of the number of RBCs, according to their morphology using bright-field and phase-contrast microscopy.

RESULTS

During the study period, 307 urine samples were analyzed. Of these, 240 were from dogs, of which 40 (16.66%) had hematuria, and erythrocytes were analyzed for morphology. Hematuria was macroscopically detected in two samples (5%) and microscopically in 38 samples (95%). Regarding the method of collection, three samples were collected via catheterization (7.5%) and 37 via cystocentesis (92.5%).

Table 1. Epidemiological data of 40 dogs with hematuria

Sex	N	Race	N	Age	Category#	No. of animals	Clinical suspicion	N
Female	21	Mixed breed	18	1 year old	Puppy	2	CVL	16
Male	19	Poodle	5	2 years old	Adult	2	CKD	9
		Yorkshire	3	3 years old		2	Cystitis	5
		Pinscher	3	4 years old		1	Urolithiasis	2
		Dachshund	2	5 years old		3	Kidney stone	1
		Schnauzer	1	6 years old		5	Renal tumor	1
		Pitbull	1	7 years old		3	HAC	1
		Shih-Tzu	1	8 years old		5	Diabetes mellitus	1
		Maltese	1	9 years old		2	Pseudocyesis	1
		Samoeida	1	10 years old	Elderly	6	Wounds	2
		Bulldog	1	12 years old		2	Breast tumor	1
		American Bully	1	13 years old		2	Tracheal collapse	1
		Dalmatian	1	15 years old		2	Perineal hernia	1
		Rottweiler	1	16 years old		2	Inconclusive	6
				Adult		1		
TOTAL	40		40			40		48*

Classification by Goldston & Hoskins (1999); CVL = canine visceral leishmaniasis, CKD = chronic kidney disease, HAC = hyperadrenocorticism; * Total number of clinical suspicions exceeds the number of animals because some had more than one diagnostic hypothesis.

The epidemiological data of the dogs whose samples were selected for the study are shown in Table 1.

Regarding epidemiological data, there was a slight predominance of hematuria in female dogs; dogs with no defined breed were the most affected; animals considered to be adults and elderly were predominant; and the most reported clinical suspicions were canine visceral leishmaniasis and chronic kidney disease.

Following erythrocyte morphologies were identified in the 40 samples selected for the study: isomorphic-annulocytes and echinocytes (Fig.1-8) and dysmorphic-stomatocytes, codocytes, ghost cells, knizocytes, acanthocytes, and cells without a defined name (Fig.9-22).

Normal-looking RBCs were found in all samples (100%) using the two analysis methods. Annulocytes were observed in two samples (5%) using bright-field microscopy and one sample (2.5%) using phase-contrast microscopy. Echinocytes were present in 39 samples (97.5%) using bright-field microscopy and in all samples (100%) using phase-contrast microscopy.

Among the dysmorphic cells, ghost cells appeared in 33 samples (82.5%) analyzed using bright-field and phase-contrast microscopy. Stomatocytes and codocytes were observed in one (2.5%) and five samples (12.5%) by bright-field and phase-contrast microscopy. Knizocytes were observed in six samples (15%) using bright-field microscopy and in five samples (12.5%) using phase-contrast microscopy. Acanthocytes were observed in seven samples (17.5%) using bright-field and phase-contrast. Cells with no defined name were observed in four samples (10%) using both bright-field and phase-contrast microscopy.

Nagahama et al. (2005) reported a specific classification for acanthocytes, classifying them into three subgroups: D1, D2, and D3. However, only D1 and D2 cells were identified in this study, as shown in Figure 23-30.

The mean and standard deviation of the distribution of the morphological types of RBCs using bright-field and phase-contrast microscopy are shown in Table 2. There was no significant difference between the two microscopy methods for evaluating the different populations of RBCs in the analyzed samples.

The data on the correlation between the presence of different isomorphic populations and urinary density is shown in Table 3. A predominance of morphologically normal RBCs was observed in the isosthenuric urine samples, while echinocytes predominated in the hypersthenuric samples, with a significant difference between the groups. There were no hyposthenuric samples, which prevented analyzing the correlation between the presence of isomorphic RBCs and low-density urine.

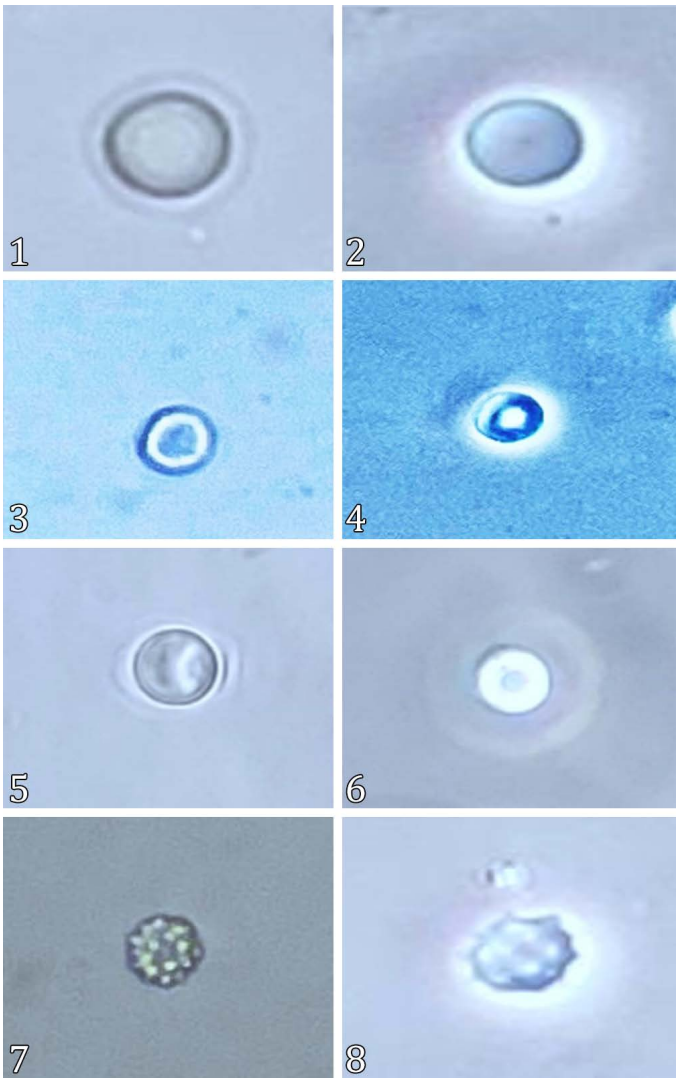

Fig.1-8. Isomorphic red blood cells (RBCs). (**1, 3, 5** and **7**) Bright-field optical microscopy. (**2, 4, 6** and **8**) Phase-contrast optical microscopy. (**1-4**) Normal RBCs. (**5** and **6**) Annulocytes. (**7** and **8**) Echinocytes.

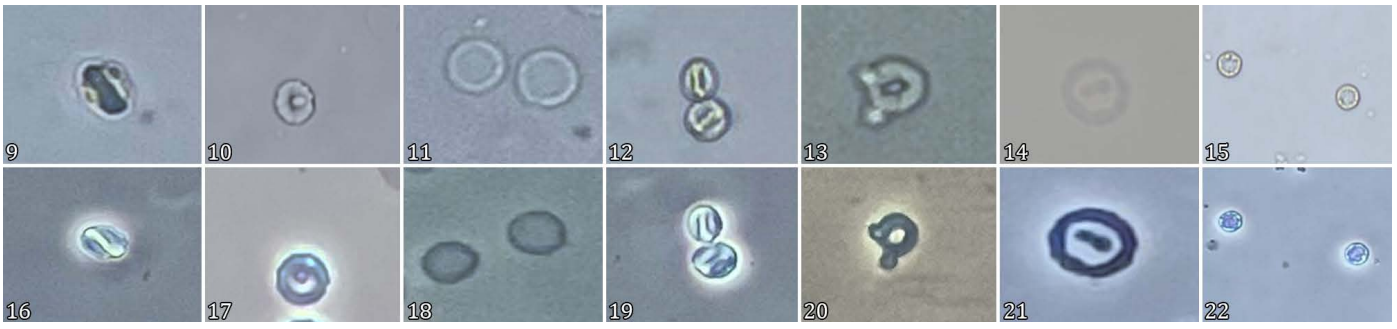

Fig.9-22. Dysmorphic red blood cells (RBCs). (**9-15**) Bright-field optical microscopy. (**16-22**) Phase-contrast optical microscopy. (**9** and **16**) Stomatocytes. (**10** and **17**) Codocytes. (**11** and **18**) Ghost RBCs. (**12** and **19**) Knizocytes. (13 and 20) Acanthocytes or G1 cells. (**14, 15, 21** and **22**) Others..

DISCUSSION

The number of hematuria cases in the case series was relatively high. Although there are studies that demonstrate the occurrence of hematuria in urine samples from dogs, in the literature consulted, no similar studies were found that cited the high casuistry of this finding. There are several explanations regarding the occurrence of hematuria. It can be pathological, i.e., associated with any disease that causes vascular alterations to the urogenital tract, such as inflammation, hemostatic disorders, drug use, idiopathic renal hematuria, tumors, and trauma, including nephroliths or urocystoliths (Forrester 2004, Crivellenti & Giovaninni 2021). The collection method can also interfere with the number of RBCs in the urine. In this study, the main collection method used by clinicians was cystocentesis, followed by catheterization. Crivellenti & Giovaninni (2021) reported that one of the disadvantages of catheterization for urine collection is the induction of hemorrhage due to the damage caused to the mucous membrane. They also reported that cystocentesis is associated with a risk of blood contamination, causing iatrogenic microscopic hematuria. Stockham & Scott (2011) also reported that samples obtained by cystocentesis and catheterization may contain blood from iatrogenic bleeding. Hüttig (2022) reported that cystocentesis can cause microhematuria because of the presence of 20 erythrocytes/field but does not lead to any other findings in urine. This is supported by the findings of Forrester (2004), who explained that the difference between pathological and iatrogenic hematuria is that the latter is expected to be mild, with no other alterations. When the test is repeated by collecting the sample through spontaneous urination, hematuria is not observed.

Regarding bleeding, most cases were microscopic in nature. The color of the urine is not macroscopically altered by this type of bleeding; it is characterized by a red blood cell count >5 per high power field (Osborne & Finco 1995). Crivellenti

Table 2. Comparison of the mean values and standard deviation of the number of red blood cells (RBCs) identified according to their morphology in 40 dog urine samples using bright-field and phase-contrast microscopy

	RBC morphology*	Bright-field	Phase-contrast
Isomorphic	Normal	25.68 ± 23.40 [a]	21.48 ± 24.50 [a]
	Annulocytes	0.20 ± 0.99 [a]	0.63 ± 3.64 [a]
	Echinocytes	52.45 ± 32.31 [a]	48.68 ± 32.42 [a]
Dysmorphic	Ghost cells	16.38 ± 21.99 [a]	23.85 ± 25.04 [a]
	Stomatocytes	0.05 ± 0.32 [a]	0.03 ± 0.16 [a]
	Codocytes	0.43 ± 1.28 [a]	0.55 ± 1.99 [a]
	Acanthocytes	1.43 ± 4.21 [a]	1.35 ± 3.68 [a]
	Knizocytes	1.55 ± 6.64 [a]	1.75 ± 6.62 [a]
	Other	1.85 ± 9.51 [a]	1.70 ± 9.80 [a]

* According to Bastos et al. (1998), Vasconcellos et al. (2005), and Chu-Su et al. (2017); [a] Different lowercase letters in the same row indicate a difference at 5% probability.

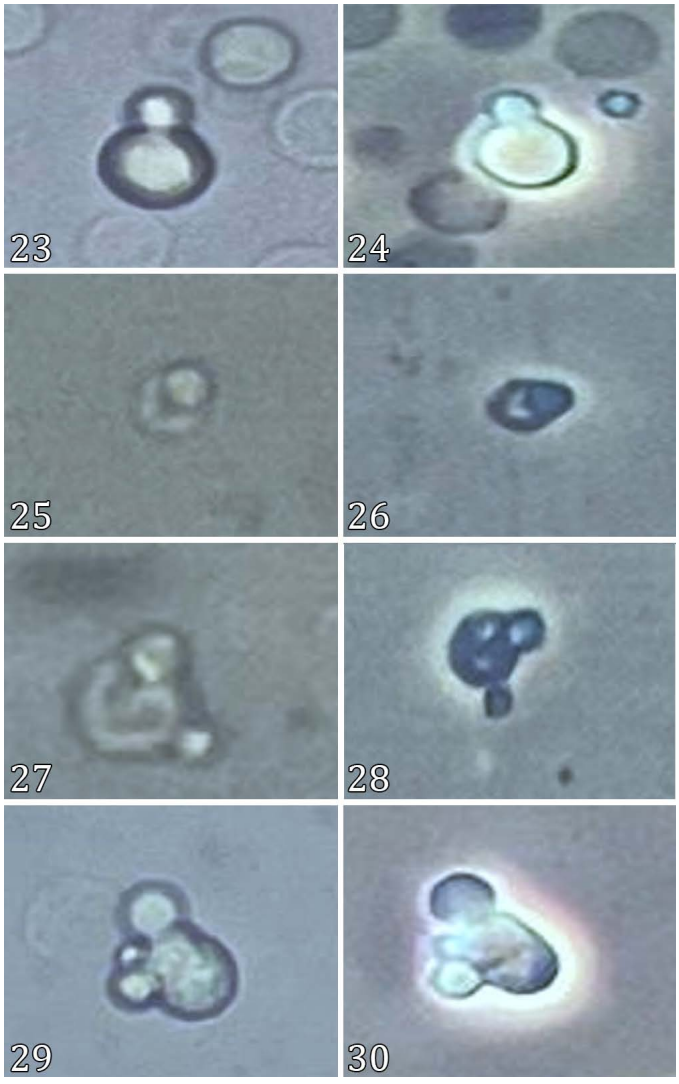

Fig.23-30. Acanthocytes – new classification. (**23, 25, 27**, and **29**) Bright-field optical microscopy. (**24, 26, 28**, and **30**) Phase-contrast optical microscopy. (**23-26**) D1. (**27-30**) D2.

Table 3. Correlation between the mean values and standard deviation of red blood cells identified as isomorphic using bright-field microscopy and urine density in urine samples from 40 dogs with micro- or macroscopic hematuria

	Isosthenuria (N = 3) UD of 1008–1012	Hypersthenuria (N = 47) UD of >1012	Hyposthenuria (N = 0) UD of <1008
Normal red blood cells	48.67 ± 38.70 [a]	19.27 ± 22.38 [b]	*
Echinocytes	10.67 ± 7.51 [a]	51.76 ± 31.71 [b]	*
Annulocytes	*	*	*

UD = urine density; [a, b] Different lowercase letters in the same row indicate differences at 5% probability; * None of the 40 samples analyzed showed hyposthenuria, thereby preventing the analysis of the correlation between urine density and observed annulocytes and other isomorphic cells in hyposthenuric urine; annulocytes were observed in only three urine samples, all with densities between 1012 and 1016.

& Giovaninni (2021) described that in animals, bleeding of renal origin is similar to bladder bleeding and that it is not possible to differentiate between them by evaluating the color of the urine alone, but that the site of the hemorrhage can be speculated based on when, during urination, the reddish color becomes more evident, and also on the presence or absence of clots. These findings corroborate Takahira (2015), who emphasized that an increase in intact RBCs can originate from any segment of the urinary tract. Peres (2010) reported that macroscopic hematuria is more associated with post-renal diseases, such as inflammatory or infectious bladder processes, bladder tumors, urothelial tumors, and benign prostatic hyperplasia in humans, whereas microscopic hematuria is mainly caused by glomerular diseases. In this study, it was not possible to correlate the findings because only a clinical suspicion was considered rather than a definitive diagnosis.

Regarding epidemiological data, no correlations were found in the literature between these variables and the occurrence of hematuria. The causes of hematuria are diverse; therefore, it is not possible to relate the findings to a single epidemiological finding. However, some diseases of the urinary tract that lead to hematuria may be related to sex, such as urolithiasis, which affects males more often, with cases of cystitis predominating in females (Bailey 1981). Certain breeds have a higher occurrence of urinary tract diseases, such as Cocker Spaniel dogs, which are prone to developing urolithiasis. However, this study found a predominance of SRD dogs, which may be explained by the characteristics of the population treated at HOVET-UFRPE and not by a natural predisposition of dogs with no defined breed (NDB). Adult-to-elderly dogs are prone to developing kidney diseases such as chronic kidney disease (CKD) and glomerulopathies (Cavalcante et al. 2015), which is in line with the findings of this study. In terms of clinical suspicion, visceral leishmaniasis was highly prevalent among animals that presented with hematuria. Although immunologically mediated glomerulonephritis is common in cases of leishmaniasis in dogs (Soares et al. 2009), this high number of cases may also be related to the fact that this was an outpatient clinic specialized in treating dogs with canine leishmaniasis at HOVET.

Regarding morphology, all forms of RBCs described in humans by Vasconcellos et al. (2005) and Chu-Su et al. (2017) were observed. The occurrence of these morphological changes in RBCs is still being studied; however, three mechanisms have been described: mechanical damage during the passage of RBCs through the glomerular basement membrane to the tubular system, exposure of RBCs to different osmotic and pH environments, and exposure to different enzymatic situations. These mechanisms cause degradation of RBC surface proteins, loss of membrane skeleton proteins, and hemolysis, resulting in glomerular hematuria with distorted red cells (Schramek et al. 1989, Haber et al. 2010).

Considering the mechanism of RBC formation, the time taken to perform the test and the collection method do not cause the formation of dysmorphic RBCs but are more related to isomorphic alterations. However, Rath et al. (1992) observed in an *in vitro* study that the formation of ghost RBCs occurs when cells are exposed to solutions with a low sodium concentration, suggesting that this alteration can occur in hyposthenuric urine. In contrast, in a similar study, Kitamoto et al. (1992) did not observe the formation of ghost RBCs after treating urine samples with different osmotic gradients. Köhler et al. (1991) reported that dysmorphic cells (acanthocytes and/or G1 cells) are irreversible and practically exclusive to glomerular disease and are not induced by changes in osmolarity, pH, or investigative methods. This finding aligns with Rizzoni et al. (1983) and Mohammad et al. (1993), who reported no dysmorphic changes in RBCs due to the analysis time. Stapleton et al. (1987) and Vasconcellos et al. (2005) demonstrated that the test should be performed within 60 min and not for more than 2 to 4 h to avoid changes caused by alkalinization, bacterial proliferation, or other factors that alter RBC morphology. Cowell et al. (2021) described that freshly collected samples are preferred for cytological evaluation because cell morphology is altered according to the time the cells are in contact with urine and RBCs have different appearances depending on the interval between collection and analysis. Hüttig (2022) reported that the delay between collection and analysis should be minimized to avoid *in vitro* alterations.

The results obtained in the present study demonstrate the possibility of identifying isomorphic and dysmorphic RBCs during urinalysis in dogs, including the identification of acanthocytes, which, in human medicine, are most associated with glomerular diseases (Köhler et al. 1991, López & Fábregas-Brouard 2002, Vasconcellos et al. 2005). The literature on veterinary medicine is scarce, and similar studies have been conducted by Akamatsu & Mathias (2006) and Scarpa et al. (2012), who obtained similar results and observed a positive correlation between the presence of erythrocyte dysmorphism and the occurrence of renal diseases in dogs, especially the presence of acanthocytes. González & Silva (2008), Stockham & Scott (2011), and Chew et al. (2012) described the use of erythrocyte dysmorphism as a marker of glomerular injury in humans but did not report its use in domestic animals.

It was possible to classify the different populations of isomorphic and dysmorphic RBCs in dog urine using the classifications of Gonçalves (1985), Köhler et al. (1991), Bastos et al. (1998), Vasconcellos et al. (2005), Peres (2010), Chu-Su et al. (2017), and Saha et al. (2022) for humans.

These authors classified isomorphic RBCs as follows: normal RBCs, which have an intact morphology in the form of a biconcave disk with a central, rounded pallor; annulocytes, which are isomorphic RBCs that swell up to form spheres in which the central pallor is not seen; and echinocytes, which are smaller and have projections on the membrane. All forms were observed using bright-field and phase-contrast microscopy.

On the other hand, dysmorphic RBCs can be classified according to their central pallor into stomatocytes, when they have a pale slit-like area in the center; codocytes, when they have a bell or shooting target shape; knizocytes, when they have a triconcave shape; and ghost RBCs, when they have intense hypochromia and thinning of the membrane. All alterations were identified in urine samples using both microscopy techniques.

Regarding membrane alterations, dysmorphic RBCs are identified as acanthocytes when they are ring-shaped with vesicle-shaped cytoplasmic protrusions and as G1 cells when they are rod-shaped with one or more cytoplasmic projections. These are considered to be the specific morphological alterations in glomerular lesions in humans (Vasconcellos et al. 2005). Köhler et al. (1991) also observed that acanthocytes were

the erythrocytes mainly indicative of glomerular bleeding. In studies conducted with dogs, Akamatsu & Mathias (2006) and Scarpa et al. (2012) obtained similar results, observing the presence of acanthocytes in the urine of most dogs with glomerular disease.

The characteristics of acanthocytes and G1 cells differ among authors. Some researchers have reported that acanthocytes and G1 cells are considered to be the same dysmorphic population (Kitamoto et al. 1992, López & Fábregas-Brouard 2002, Nagahama et al. 2005, Fogazzi et al. 2008), whereas Dinda et al. (1997) and Vasconcellos et al. (2005) reported them as different types of dysmorphia.

Recent studies have reported new classifications for acanthocytes. Nagahama et al. (2005) classified these cells as dysmorphic cells (D cells) and divided them into three subgroups (D1, D2, and D3). D1 are ring-shaped RBCs with severe loss of cytoplasmic color and membrane blebs. D2 are walnut-shaped cells with moderate loss of cytoplasmic color and membrane blebs. These cells are considered G1 cells. The last subgroup of dysmorphic cells were the D3 cells, which had a rod-like morphology with a slight loss of cytoplasmic color and blebs in the membrane. According to the morphology described by Nagahama et al. (2005), it was possible to identify D1 and D2 RBCs in this study, but D3 cells were not observed.

The results obtained when comparing the microscopy techniques used to identify and classify morphological changes in RBCs in urine corroborate those reported by Akamatsu & Mathias (2006) in a study with dogs and by Silva et al. (2010), Martinez et al. (2014), and Chu-Su et al. (2017) in studies with humans. All studies reported no significant difference between the two microscopy methods, and the use of the bright-field microscope was acceptable for identifying erythrocyte dysmorphism. However, Birch & Fairley (1979), Kouri et al. (2000), Vasconcellos et al. (2005), Fogazzi & Delanghe (2018), SBPC & ML (2017), and Saha et al. (2022) reported the difference between using a traditional optical microscope and a phase-contrast microscope, with the latter being the recommended and superior method for detecting erythrocyte dysmorphism.

Regarding the correlation between the occurrence of different isomorphic populations and urine density, a predominance of normal RBCs was observed in isosthenuric urine samples, whereas crenated RBCs or echinocytes were predominant in hypersthenuric urine samples. These results are in line with the findings of Gonçalves (1985), Bastos et al. (1998), Thrall et al. (2015), and Crivellenti & Giovaninni (2021), who reported that normal RBCs were present in urine samples that did not show changes in osmolarity and had normal densities. Echinocytes are seen in urine with increased density, causing RBCs to lose water and to become wrinkled. Annulocytes, which according to the same authors, are present in hyposthenuric urine, leading to swelling of the cell, were found in only three samples; however, as there were no urine samples with a density below normal, it was not possible to analyze the correlation between the occurrence of this population in urine samples with low density.

CONCLUSIONS

Similar to humans, it is possible to identify the presence of erythrocyte dysmorphism in dogs via urinalysis and to differentiate and classify the types of alterations present in red blood cells (RBCs). Regarding the microscopy techniques used, no difference was observed between bright-field and phase-contrast microscopy. Therefore, bright-field microscopy, which is used more frequently in the laboratory routine and is less expensive, can be used without significant detriment to the identification and differentiation of morphological changes in RBCs in urine.

In order to correlate the presence of erythrocyte dysmorphism with bleeding from glomerular origin in dogs and use this method as an early marker of glomerular injury, more studies are needed that use other diagnostic tests, such as biomarkers of glomerular injury, imaging examinations, and renal biopsy, with the latter being considered the gold standard in the diagnosis of glomerulopathies.

Therefore, this study provides a basis for further research aimed at using the method of identifying and correlating erythrocyte dysmorphism with the location of kidney lesions in pets, with a noninvasive and cost-effective test directing the investigation of diseases of the renal system to the nephrological or urological areas at an early stage. Although this is a simple test in terms of technique, it does not exclude the need for observer training to identify erythrocyte alterations better.

Conflict of interest statement.- The authors declare no conflicts of interest.

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