The Journal of Animal & Plant Sciences, 27(6): 2017, Page: 2075-2080
ISSN: 1018-7081

Short Communication

PROPORTIONAL MORBIDITY RATE AND EFFECTIVENESS OF DIFFERENT DIAGNOSTIC TOOLS FOR PANDORA BOX SYNDROME AMONG CLIENT-OWNED DOMESTICATED CATS (*FELIS SILVESTRIS CATUS*) IN PAKISTAN

A. Naureen[1], M. S. Khan[1], M. A. Khan[1], A. Maqbool[2], M. Avais[1*] and J. A. Khan[1]

[1]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan
[2]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan
[*]Corresponding author's email: mavais@uvas.edu.pk

ABSTRACT

The present study has been designed to evaluate proportional morbidity rate**(**PMR) along with comparative evaluation study between different diagnostic tools (viz., urinalysis, radiography, hyperkalemia, clinical signs and ultrasonography for Pandora Box Syndrome (PBS).A total of PMR among PBS cases was estimated to be 8.7%. For diagnostic test evaluation, all respective tests were performed according to the defined procedures. To this end,total of 208 diseased and 98 apparently healthy felines were tested by taking urinalysis as gold standard. All tests but serum cortisol level (80.303; 70.70-89.89%) showed 100 percent specificity. Serum cortisol was found to be significantly more sensitive ($P<0.05$) than ultrasonography, clinical signs, hyperkalemia, and radiography with highest negative predictive value (NPV)as 58.2, respectively. On comparing apparently healthy felines (n = 98) with diseased (n = 208;affected with PBS in the past or at present), highestagreement 0.812 (0.70-0.92%) was found between clinical signs and ultrasonography, while lowest agreement was seen between ultrasonography and hyperkalemia (0.449; 0.35-0.54%).In conclusion,PMR values show more about specialty as well as interests of clinicians rather than the incidence of disease in that population.

Key words: Pandora box syndrome, proportional morbidity rate, comparison, diagnostic tests.

INTRODUCTION

Pandora box syndrome (PBS)is an important veterinary medical problem as well as a clinical disorder related to client owned domesticated cats (Buffington, 2011). Previously, it has been known as urolithiasis-cystitis-urethritis, feline urologic syndrome (FUS), urolithiasis, and feline lower urinary tract disease (Fisher, 1955; Holzworth, 1963;Osbaldiston and Taussig, 1970; Osborne *et al*., 1984).Currently it has been designated as Pandora Box Syndrome (PBS) due to complexity in its nature (Buffington, 2011) and is classified as obstructive and non-obstructive. It is characterized clinically by hematuria, dysuria (stranguria), pollakiuria, vocalization and periuria. Male cats frequently lick external genitalia, simulating constipation. Obstructive cases may lead to mucoid-crystal plug that lodges in the tapering distal urethra and if left untreated may lead to death (Norsworthy*et al*., 1998). Its main causes includecrystalluria, uroliths, urethral plugs, urinary tract infections, congenital (abnormalities by birth) and idiopathic/unknown in nature (Kalkstrein*et al*., 1999).Its predictable factors include biochemical and clinical abnormalities (like dehydration, electrolyte imbalance)leading to hypercalcemia, hyperphosphatemia, metabolic acidosis, accumulation of creatinine and urea (Osborne *et al*., 1984). The expected incidence in domesticated catsreported in United States and Great Britain wasapprox. 0.5 – 1% (Osborne *et al*., 1983; Osborne *et al*., 2000) and 9.1% in Japan by Buffington and Chew (1997). The data collected from different Private clinics as well as Veterinary Medical Teaching Hospitals (VMTH) at the University of Agriculture Faisalabad and Pet Center, University of Veterinary and Animal Sciences, Lahore shows the presence of this syndrome among domesticated cats in Pakistan especially its proportional morbidity rate (PMR)that shows poor cognizance of practicing veterinarians (Willeberg, 1984) together with different comparative diagnostic test evaluations (unreported thus far). Basic epidemiological data of PBS is based on description, analysis and diagnosis (Osborne *et al.,* 1984) however no reliable diagnostic indicators for subclinical PBS exist thus far.To this end, present study has therefore, been planned to calculate PMR values and comparative diagnostic evaluation againstPBS (viz., serum-cortisol level, urinalysis, radiography, hyperkalemia, clinical signs and ultrasonography).

MATERIALS AND METHODS

Proportional morbidity rate (PMR) is proportional number of cases of any disease calculated out of total number of cases attended (particular animal species) in practice or private clinic as perWilleberg(1984).

A comparative evaluation was conducted between serum cortisol, urinalysis and other conventional diagnostic tests (i.e., hyperkalemia, clinical signs,

radiography and ultrasonography). All respective tests were performed according to the defined procedures previously done. To this end, 208 diseased (76 had present history, while rest of 132 had present as well as past-distant history) and 98 apparently healthy (had no history of PBS) were tested for the presence of disease status of felines according to the diagnostic test performed.

Collection of urine for urinalysis: For fresh sample collection feline patient was put in the dorsal recumbency. For Cystocentesis the bladder was griped and urine was collected under sterilized conditions by using 23 gauge needle at an angle of 45° as per Crow and Swlshaw (1987). The urine collected was evaluated for color,clarity (turbidity) and further evaluated by using urine test strips (Medi-Test Combi 10® VET; MACHEREY-NAGEL GmbH & Co. KG Nuemann-Neander-Str.6-8.52355 Duren, Germany) for bloodparameters like urobilinogen, bilirubin, protein, nitrite, ketone, glucose, pH-values, density and leukocytes. All samples collected were fresh (not older than 2 hour) and test strips were interpreted by comparing reaction color withstandard color scale (after 30-60 seconds).

Microscopic Examination of Urine: Freshly collected urine samples streaked onto the blood agar as well as MacConkey's agar to check presence of bacterial infections like*Escherichia coli*. All samples were centrifuged (3000 rpm; 5 minutes) and sediment was placed on a clean glass slide and examined under microscope. Gram stained smear by use of single drop of methylene blue used to detect Gram-negative rods (*E. coli*). Samples were further checked for presence of red blood cells, leukocytes and crystal type.

Clinical signs: Clinical signs related to PBS were recorded accordingly that mainly includeddysuria, gross hematuria, pollakiuria, vocalization, licking, and urethral obstruction. Further presumptive diagnosis was made based on complete history (as narrated by owner) and clinical signs as were on presentation.

Serum biochemical profiling for signs of hyperkalemia analysis: Serum sampleswere collected from each feline after drawing blood from jugular vein in separate vacutainers aseptically. These sera were used for biochemical profiling performed in a private human diagnostic laboratory for serum concentrations of creatinine and cortisol level, blood urea nitrogen, and serum electrolytes (bicarbonates, chloride, potassium, and sodium), etc.

Diagnostic Imaging: Diagnostic imaging was based on the survey radiograph of lateral as well as ventral abdomen that were taken at low KVp (using 100 MA fixed X-ray unit) in case of obstructive as well as non-obstructive cases of PBS of patients. Ultrasonography was conducted by using $5M_{Hz}$ sector probe. Each feline attended was put in the dorsal recumbency in order to visualize kidney and urinary bladder. The thickness of urinary bladder wall and pelvis of kidneys were observed quite attentively for a proper and accurate diagnosis of PBS.

Data analysis: The Proportional morbidity rate(PMR) is calculated as the ratio of cases divided by total number of cases attended in practice, private clinic, or clinic (Willeberg, 1984). While, the sensitivity and specificity of each test was calculated using criteria of true negative and true positive responders as per Naureen*et al.,*(2007). The sensitivity was estimated from the following equation:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + false negative}} \times 100$$

And specificity was calculated by using the following equation:

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + false negative}} \times 100$$

The accuracy and predictive values were calculated as per previously standardized formulas by Martin (1977). Agreement between different tests was calculated by using software Win Episcope (2.0, Wageningen University, Wageningen, The Netherlands) as per Naureen*et al*.,(2007).

RESULTS

A total of proportional morbidity rate (PMR) among PBS cases studied was estimated to be 8.7% that actually relates data more towards specialty and interests of clinicians rather than that of incidence of that particular disease amongfeline population (Willeberg, 1984). In the present study, a total of 306 cats were selected for comparative evaluation of different diagnostic tests. The selection criterion was based on data regarding 6 diagnostic tests under study (viz., urinalysis, hyperkalemia, clinical signs, radiography, serum crortisol levels and ultrasonography) along with complete back history (present and past-distant history) of each case attended. Out of 306 cats under study, 208 (76 with present history only and 132 with both present as well as past distant history) had history of PBS, while 98 were selected as healthy cats based on the previous history record of PBS. Among 132 of the diseased patients selected, 52 had stones (6 suffering with Ca-Oxalate crystals, 39 suffering from struvite and rest of the 7 with mixed crystalline matrix) as shown in Table (1).Agreement between all the 6 diagnostic tests (viz., urinalysis, hyperkalemia, clinical signs, radiography, serum cortisol levels and ultrasonography) was shown in

Table (2). On comparing diseased (n = 208) and healthy (n = 98) cats, the highest agreement 0.812 (0.70-0.92%) was found between clinical signs and ultrasonography followed by 0.734 (0.62-0.84) between radiography and hyperkalemia, followed by 0.729 (0.62-0.83; between serum cortisol level and ultrasonography), then 0.622(0.51-0.72; between radiograph and ultrasound), 0.617 (0.51-0.72; between serum cortisol level and hyperkalemia), 0.613 (0.50-0.72; between clinical signs and serum cortisol level), 0.586 (0.47-0.69; between clinical signs and hyperkalemia), 0.567 (0.45-0.67; between urinalysis and serum cortisol level), 0.567 (0.46-0.66; between urinalysis and clinical signs), 0.543 (0.44-0.64; between urinalysis and ultrasound), 0.524 (0.42-0.62; between clinical signs and radiography), while lowest agreement was seen between ultrasonography and hyperkalemia (0.449; 0.35-0.54%).In order to evaluate sensitivity and specificity of diagnostic tests (viz., hyperkalemia, clinical signs, radiography, serum cortisol levels and ultrasonography) all the above mentioned tests were compared with that of the standard (Gold standard; urinalysis was taken as the gold standard in this study) as shown in Table (2). All tests but serum cortisol level (80.303; 70.70-89.89%) showed 100 percent specificity. The calculated sensitivities of serum cortisol, ultrasonography, clinical signs, hyperkalemia, and radiography against iLUTD were 84.167(79.54-88.78), 75.000(69.52-80.47), 73.333(67.73-78.92), 65.417 (59.39-71.43), 48.333 (42.01-54.65), respectively. Serum cortisol was found to be significantly more sensitive ($P<0.05$) than ultrasonography, clinical signs, hyperkalemia, and radiography. The negative predictive value of each test (serum cortisol, ultrasonography, clinical signs, hyperkalemia, and radiography) was as follows: 58.2, 52.3, 50.7, 44.2, and 34.7, respectively.

Table – 1. Different diagnostic tests (viz., Urinalysis, Hyperkalemia, Clinical Signs, Radiography, Serum cortisol levels, and Ultrasonography) performed for the diagnosis of Pandora box syndrome (PBS)

Groups of cats under study (I and II)	**Different tests performed for the diagnosis of PBS***						
	Total cats under study (n = 306)	**Urinalysis**	**Hk*** **	**Clinical Signs**	**Radiography**	**Serum cortisol levels**	**Ultrasonography**
Diseased cats with history (present + past distant) of PBS*	I (n = 208)	208	137	180	110	170	164
Apparently healthy cats**	II (n = 98)	32	20	0	6	45	12

*PBS – Pandora box syndrome

**without any clear clinical signs of Pandora box syndrome

***Hyperkalemia

Table – 2. Performance and Kappa statistics for different diagnostic tests (viz. Urinalysis, Hyperkalemia, Clinical Signs, Radiography, Serum cortisol levels, and Ultrasonography) performed for the diagnosis of Pandora box syndrome (PBS).

		Hyperkalemia		**Kappa values**	**Ultrasonography**		**Kappa Values**	**Serum cortisol levels**		**Kappa Values**	**Radiography**		**Kappa values**	**Clinical Signs**		**Kappa values**
		+	-		+	-		+	-		+	-		+	-	
Urinalysis	+	157	83	0.449	176	64	0.543	202	38	0.567	116	124	0.288	180	60	0.567
	-	0	66	(0.35-0.54)	0	66	(0.44-0.64)	13	53	(0.45-0.67)	0	66	(0.20-0.36)	0	66	(0.46-0.66)
Clinical Signs	+	137	43	0.586	164	16	**0.812**	170	10	0.613	110	70	0.524			
	-	20	106	(0.47-0.69)	12	114	**(0.70-0.92)**	45	81	(0.50-0.72)	6	120	(0.42-0.62)			
Radiography	+	116	0	**0.734(0.62-0.84)**	116	0	0.622(0.51-0.72)	116	0	0.411(0.32-0.50)						
	-	41	149		60	130		99	91							
Serum cortisol levels	+	157	58	0.617	176	39	**0.729**									
	-	0	91	(0.51-0.72)	0	91	**(0.62-0.83)**									

DISCUSSION

The Pandora box syndrome (PBS) was clinically illustrated in 1925 by Kirk,since then number of studies have focused on its etiology, risk factors and diagnostic tests for trimming the occurrence of disease down to minimum (Buffington, 2011). Previously many studies reported presence of disease worldwide (Buffington, 1997a, b; Chew *et al*., 1986;Engle, 1977). An increase in incidence has been reported to be attributed to high frequency of indoor keeping of cats (Patronek *et al*., 1996)as compared to outdoor cats (Willeberg, 1984). The present study shows a total of 8.7% PMR among feline patients attended, actually indicates the interests of clinicians as compared to incidence of disease regarding population under study because denominator is highly selected and undetermined fraction of the population (Willeberg, 1984). This value of PMR among felines mostly misinterpreted with that of incidence of disease in the population at risk. Contrarily, true incidence is not too easy to estimate however, retrospective study is reported with preceding 12 months period (Willeberg, 1984).

In our study, a total of 306 cats were selected for evaluation of feline lower urinary tract disease taking urinalysis as gold standard, compatible with many other studies in which urinalysis was employed as an effective tool of diagnosis (Kraijer*et al*., 2003). According to present report 208 cats had history of PBS while 98 cats were healthy thus the ratio of diseasedversus healthy subjects came out to be 2.1:1 that was in contrast to another study conducted by Buffington, in which the ratio of diseased cats with that of healthy ones was of 1:1. The comparison between diagnostic tests including urinalysis, hyperkalemia, clinical signs, radiography, serum cortisol levels and ultrasonography were conducted on all cats. The present and past history of disease was also taken as confirmed to be an important indicative sign by literature (Buffington and Chew, 1993; Buffington and Chew, 1995).

Among 132 of the diseased patients selected in our study, 52 presented with stones varying in nature and quantity. Only 11.5% (6) felines were suffering from Ca-Oxalate crystals and 13.5% (7) with mixed crystalline matrix, while the majority of the cats (75%; 39) with stones were suffering with struvite. Studies by many other authors support these results stating struvite to be the commonest type of stones in felines suffering from lower urinary tract disease (Buffington *et al*., 1997b; Markwell*et al*., 1998). The dietary elements and environmental factors that directly influence the nutritional system and kidney function are supposed to be the prime causes for development of these stones in felines. The stones made of calcium have also been supported by Ching *et al*., (1989) and Defauw*et al*. (2011) in which calcium, ammonium and magnesium deposits have largely been found (Little, 2001).

A varying agreement was found in all the 6 diagnostic tests (urinalysis, hyperkalemia, clinical signs, radiography, serum cortisol levels and ultrasonography). When a comparison of diseased cats (n=208) with the healthy (n=98) ones was made, the highest agreement 0.812 (0.70-0.92%) came out to be between Clinical signs and ultrasonography proceeded by 0.734 (0.62-0.84) between radiography and hyperkalemia. On the third place, 0.729 (0.62-0.83; between serum cortisol level and ultrasonography) was found and then 0.622(0.51-0.72; between radiograph and ultrasound), 0.617 (0.51-0.72; between serum cortisol level and hyperkalemia), 0.613 (0.50-0.72; between clinical signs and serum cortisol level), whereas, 0.586 (0.47-0.69) was found between clinical signs and hyperkalemia and 0.567 (0.45-0.67) between urinalysis and serum cortisol level). Furthermore, 0.567 (0.46-0.66) was present between urinalysis and clinical signs) and 0.543 (0.44-0.64) between urinalysis and ultrasound). Following these, 0.524 (0.42-0.62) was seen between clinical signs and radiography while the lowest agreement was seen between ultrasonography and hyperkalemia (0.449; 0.35-0.54%).

Little (2001) in the review for non-obstructive Feline Lower Urinary Tract Disease has emphasized the conduction of these tests but at very later stage and if needed. Supporting our assumption of considering the urinalysis as gold standard, in this review too, the urinalysis is mentioned as a first place diagnostic test, after thorough physical and behavioral examination. Other diagnostic tests suggested in the review were blood analysis, pH testing, X-Ray,ultrasonography and other sugar related tests. All these are in favor of our diagnostic preferences used in the study (Jones *et al*., 1997; Little, 2001).All tests but serum cortisol level (80.303; 70.70-89.89%) showed 100 percent specificity. The calculated sensitivities of serum cortisol, ultrasonography, clinical signs, hyperkalemia, and radiography against PBS were 84.167(79.54-88.78), 75.000(69.52-80.47), 73.333(67.73-78.92), 65.417 (59.39-71.43), 48.333 (42.01-54.65), respectively. Serum cortisol was found to be significantly more sensitive ($P<0.05$) than ultrasonography, clinical signs, hyperkalemia, and radiography. The negative predictive value of each test (serum cortisol, ultrasonography, clinical signs, hyperkalemia and radiography) were 58.2, 52.3, 50.7, 44.2, and 34.7, respectively.

Serum cortisol level has also been a fundamental suggestive test, especially when blood tests give doubtful results. The presence of other renal diseases may also enforce to conduct this test in order to confirm PBS. Some other studies have also made their subjects undergone these tests and have conducted various other urine sample analysis (Jones *et al*., 1997; Little, 2001) for evaluation of PBS.However, one of the limitations in this study remains exclusion of certain other environmental

and dietary factors that may have given an in depth insight to other factors related to PBS. The breed, population, indoor or outdoor dwelling of felines and other demographic factors have a direct impact on development and severity of the disease in cats and are suggested to be explored further.

REFERENCES

Buffington, C. A. T. and D. J. Chew (1995). Does interstitial cystitis occur in cats? In: Current Veterinary Therapy XII. Kirk, R. W. and J. D. Bonagura (editors).WB Saunders Co. Philadelphia USA. pp:1009-1011.

Buffington, C. A. T. and D. J. Chew, (1993).Presence of mast cells in submucosa and detrusor of cats with idiopathic lower urinary tract disease. J. Vet. Intern. Med., 7:126.

Buffington, C. A. T., (2011). Pandora syndrome: Rethinking your approach to idiopathic cystitis in cats. Vet. Med., 106:515-522.

Buffington, C. A. T., D. J. Chew, and B. E. Woodworth, (1997b). Animal model of human disease-feline interstitial cystitis. Comp. Path. Bull., 29:3-6.

Buffington, C. A. T., D. J. Chew, M. S. Kendall, P. V. Scrivani, S. B. Thompson, J. L. Blaisdell, B. E. Woodworth, (1997a). Clinical evaluation of cats with nonobstructive urinary tract diseases. J. Am. Vet. Med. Assoc., 210:46-50.

Chew, D. J., S. P. DiBartola, and W. R. Fenner, (1986). Pharmacologic management of urination. In: Kirk's Current Veterinary Therapy IX. Small Animal Practice. Kirk, R. W. (editor). 9th Ed. WB Saunders Co. Philadelphia USA. Pp: 1207.

Ching, S. V., M. J. Fettman, and D. W. Hamar, (1989). The effects of chronic dietary acidification using ammonium chloride on acid-base and mineral metabolism in adult cats. J. Nutr., 119:902-915.

Defauw, P. A., V. Maele, L. Duchateau, I. E. Polis, J. H. Saunders, and S. Daminet, (2011). Risk factors and clinical presentation of cats with feline idiopathic cystitis.J. Feline Med. Surg., 13(12):967-975.

Engle, G. C., (1977). A clinical report of 250 cases of feline urological syndrome. Feline Pract., 7:24.

Jones, B., R. L. Sanson, and R. S. Morris, (1997). Elucidating the risk factors of feline urologic syndrome.New Zealand Vet. J., 45:100–8.

Kirk, H., (1925). Urinary deposits and retention. In: The diseases of the cat and it general management. London: Bailliere, Tindall and Cox pp. 261–7.

Kraijer, M., J. F. Gremmels, and R. F. Nickel, (2003). The short-term clinical efficacy of amitriptyline in the management of idiopathic feline lower urinary tract disease: A controlled clinical study. J. Feline Med. Surg., 3 (5):191-196.

Little, S., (2001). Non-obstructive Feline Lower Urinary Tract Disease; review in The Winn Feline Foundation, retrieved through The Indoor Cat Initiative (Idiopathic/Interstitial Cystitis in Cats). http://vet.osu.edu/indoorcat

Markwell, P. J., C. T. Buffington, and B. H. E. Smith, (1998). The Effect of Diet on Lower Urinary Tract Diseases in Cats, J. Nutr., 128:2753–2757.

Patronek, G. J., L. T. Glickman, and A. M. Beck, (1996). Risk factors for relinquishment of cats to an animal shelter. J. Am. Vet. Med. Assoc., 209:582–588.

Willeberg, P., (1984). Epidemiology of naturally occurring feline urologic syndrome.Vet. Clin. North Am. Small Anim. Pract.,14:455–469.