

# **HEMATOLOGICAL, BIOCHEMICAL AND URINALYSIS FOR THE DIAGNOSIS OF URINARY TRACT INFECTION IN GERMAN SHEPHERD DOG.**

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## **ABSTRACT**

The current study was conducted to evaluate the hematological and biochemical alteration in German shepherd dogs suffering from bacterial urinary tract infection in Diwanya City Police Dogs division. The total number of examined cases was 7 cases and three bacterial isolates were detected from urine samples. Of the isolated strains 5 (71.42%) were identified as *E.coli*, 2 (28.57%) identified as mixed infection with *E.coli* and *Streptococcus* spp. Infected animals showed hematuria, fever, depression, abdominal pain, loss of appetite and restlessness. Physical examination of the infected urine showed turbidity and red coloration, furthermore chemical examination indicated the presence of RBCs (hematuria +4), bilirubin, Urobilinogen, leukocytes (+2), protein (+2), nitrate (+1) and the specific gravity is 1.030, glucose was negative in all cases. Crystals (amorphous), epithelial cells and casts of different types have been detected in the microscopic examination of the urine. Moreover results also indicated increase in creatinine and urea values, however there was a decrease in the concentration of  $K^+$  (potassium) in serum in case (No.5), mild increase in GGT values in the cases (No.5, 3 and 6) have been indicated. On the other hand triglyceride value showed increase in case (No.3) comparing with other cases. The results revealed that the Hb rates have been increased in case (No.5) compared to the other cases. The results revealed that the percentage of bacteriuria in the affected dogs was (57.14%).

## **INTRODUCTION**

The German Shepherd Dog originated in the Alsatian Region of Germany. Today it is used worldwide as a sentry, police dog, tracker, drug dog, search and rescue dog, and guide dog for the blind people. The official name for this breed is German Shepherd Dog, though it is often called the German shepherd or just Shepherd. The German Shepherd Dog is unique because it has a very short colon in comparison to other breeds of the same body weight. The Weight Standards: male - 80 to 85 lbs., female - 65 to 70 lbs., Height Standards: male - 24 to 26 inches, female - 22 to 24 inches, and the Coat: dense, straight, short, tricolored (1). Urinary tract infections (commonly called "UTIs") are very common in dogs, especially in females; although they certainly occur in males as well. A urinary tract infection (UTI) is bacterial infection that affects part of the urinary tract. When it

affects the lower urinary tract it is known as a simple cystitis (a bladder infection) and when it affects the upper urinary tract it is known as pyelonephritis (a kidney infection). Symptoms from a lower urinary tract include hematuria, dysuria, pollakiuria, urinary incontinence, painful urination and either, while those of pyelonephritis include fever and flank pain in addition to the symptoms of a lower UTI. The main causal agent of both types is *Escherichia coli*; however other bacteria, viruses or fungi may rarely be the cause (2, 3). *E. coli* is known as the most common pathogen responsible for great majority (75-90%) of urinary tract infections (4). Therefore rapid identification and reporting is useful in the direction of therapy, and also streamlines the overall turn-around-time and allows microbiologists to devote more time to problematic cultures (5, 6).

The aims of the current study were to clarify the hematological and biochemical alterations that occur due to UTI and pyelonephritis and identify the types of bacteria that cause these cases, as well as, to relying on these alterations in the diagnosis of UTI and pyelonephritis in German shepherd dogs.

## **MATERIAL AND METHODS**

### **Animals and study area**

Seven 2-3 years old *German shepherd* dogs, including five males and two females, from Diwanyaia City Police Department- dogs division were studied for investigation of urinary tract infection and pyelonephritis.

### **Blood samples:**

Blood samples were taken from the Femoral artery, Samples were divided in to two parts ,the first part (5 ml from each case) with EDTA were used for estimation of hemoglobin (mg/dl),and the second part (5 mL from each case) were centrifuged for separation of serum and estimation of Creatinine (mg/dl), Urea (mg/dl), Uric acid (mg/dl), K (mEq/L), GGT (U/l), Triglycerides (mg/dl), Glucose (mg/dl), ALT(mg/dl) and AST(mg/dl), all the parameters were estimated by using Reflotron apparatus (Reflotron and Precinorm by Roche Diagnostics GmbH D-68298, Mannheim, Germany). The Reflotron® is an *in vitro* diagnostic device designed for the quantitative determination of clinical chemistry parameters using Reflotron Test Tabs. Its dimensions approximately 300 x 350 x 210 mm, weight = approx. 5.3 kg, analysis time: 2 - 3 minutes/18-30 tests per hour. The optical system of the Reflotron® System should be checked using Reflotron® Clean Check. Reflotron® is required 32 microliter volume samples of blood or serum or plasma for completion of any of the above mentioned test, by using special micropipette achieved with the Reflotron® device. The sample is placed on the Reflotron test tabs, then these test tabs setting in the specific place in the device, then waiting for 180-210 minute (depending on the test type) for checking and estimation of the final result.

### **Urine samples:**

5 mL of Urine samples were taken by antepubic cystocentesis from dogs for chemical examination that include estimation of Blood in urine, Bilirubin, Urobilinogen, Keton bodies, Glucose, Protein, Nitrate, Leukocyte, PH, Specific gravity. Reagent strips (prepared by Nelisco, Inc, USA) were used for chemical examination of urine. Reagent strips are the most widely used technique for detecting constituents present in the urine and are available in a variety of types. A reagent strip is a firm plastic strip to which pads containing chemical reactants are attached. Reagent strips are designed to be used only once and discarded. After dipping of the strip in the urine sample, then leave it for 30-60 second (according to type of the test), the strip is pulled out and compare the color of the reagent strip with color on the reagent strip container. Physical examination included Turbidity and Color, odor and volume, Bilirubin and cholesterol granules. Moreover microscopic examination was conducted for detection of Crystals, casts, Epithelial cells and Parasites, using centrifuged urine sediments

#### **Bacterial culture and identification:**

Urine samples were streaked on MacConkey agar plates. Plates were incubated at 37C ° in aerobic conditions and examined daily for the presence of bacterial growth. The isolated bacteria were identified on the basis of cultural, morphological and biochemical characteristics (7), as well as, the effect of age and sex on the percentage rate of bacterial infection were studied by determination the age of affected dogs (< 2-3 years) and the gender.

## **RESULTS AND DISCUSSION**

Seven suspected cases have been examined and the results showed isolation of bacterial isolates from urine samples. Of the strains isolated 5 (71.42%) were identified as *E.coli*, 2 (28.57%) identified as mixed infection with *E.coli* and *Streptococcus* spp. (Table: 1). Moreover diseased animals showed hematuria, fever, depression, abdominal pain, loss of appetite and restlessness. The urinalysis revealed that the urine is turbid and reddish in color in case (No.5), while it was with less intensity in the other cases (2,3,4,6,and7), which indicate that the infection was very severe in one case (No. 5) than the others, there were blood in urine (+4), bilirubin, Urobilinogen, leukocytes (+2), protein (+2), nitrate (+1) and the specific gravity is 1.030, while the glucose was negative in all cases (table: 2). The microscopic examination of the urine revealed that the urine is containing crystals (amorphous), epithelial cells and casts of different types (hyaline, waxy, granular and fatty casts) (table:2). The results of blood samples revealed that there are increase in values of creatinine (No. 3, 4 and 5), urea in cases (No.,1, 5, 6 and 7), the uric acid values showed no difference between the affected animals, while the estimation

of K<sup>+</sup> (potassium) revealed decrease in the case (No.5), while it was still within the normal range in the other cases, also the GGT values mildly increases in the case(No.5, 3 and 6), while unchanged in other cases. The triglyceride values showed increase in case (No.3) as compared with other cases. The glucose levels in cases (No. 5 and 3) revealed slight decrease from other cases. The ALT and AST values showed no differences among all cases, while the Hb rate indicated increase in hemoconcentration of blood because the values is high in case (NO.5) as compared with other cases (table:3). The results revealed that the percentage of bacteriuria (bacteriuria denotes the presence of bacteria in urine not due to contamination from urine sample collection, but due to presence of bacterial infection) in affected dogs according to age and sex was (57.14%) (Table: 4).

Table (1): Types of identified bacteria in urine samples of dogs infected with UTI.

|   | PARAMETER | CASE NUMBER |        |        |         |                 |         |                  |
|---|-----------|-------------|--------|--------|---------|-----------------|---------|------------------|
|   |           | 1*          | 2**    | 3*     | 4*      | 5*              | 6**     | 7*               |
| 1 | Urine     | E.coli      | E.coli | E.coli | Strept. | E.coli+ strept. | Strept. | E.coli + strept. |

**Table (2):** represent the results of physical, chemical and microscopical exam of urine of infected dogs.

\*means male dog. \*\* means female dog.

|   | PARAMETERS   | CASE NUMBER |      |    |    |    |      |    |
|---|--------------|-------------|------|----|----|----|------|----|
|   |              | 1*          | 2 ** | 3* | 4* | 5* | 6 ** | 7* |
| 1 | Blood        | -           | -    | -  | -  | +4 | -    | -  |
| 2 | Bilirubin    | -           | -    | -  | -  | +  | -    | -  |
| 3 | Urobilinogen | +           | +    | +  | +  | ++ | +    | +  |
| 4 | Keton bodies | -           | -    | -  | -  | -  | -    | -  |
| 5 | Glucose      | -           | -    | -  | -  | -  | -    | -  |

|    |                        |        |        |        |        |         |        |        |
|----|------------------------|--------|--------|--------|--------|---------|--------|--------|
| 6  | Protein                | +      | +      | +      | +      | ++      | +      | +      |
| 7  | Nitrate                | +      | -      | +      | +      | +       | -      | +      |
| 8  | Leukocyte              | -      | -      | -      | -      | ++      | -      | -      |
| 9  | PH                     | 6      | 6      | 6      | 6      | 6       | 6      | 6      |
| 10 | Specific gravity       | 1.020  | 1.020  | 1.020  | 1.025  | 1.030   | 1.020  | 1.020  |
| 11 | Crystals/<br>amorphous | Little | Little | Little | Little | Many    | Little | Little |
| 12 | Hyaline casts          | Little | Little | Little | Little | Little  | Little | Little |
| 13 | Fatty casts            | Little | -      | -      | -      | Many    | -      | -      |
| 14 | Waxy casts             | ++     | -      | -      | -      | +3      | -      | -      |
| 15 | Granular casts         | Little | Little | Little | Little | Little  | Little | Little |
| 16 | Epithelial             | -      | -      | -      | -      | +2      | -      | -      |
| 17 | Turbidity              | Clear  | clear  | clear  | clear  | Turbid  | clear  | clear  |
| 18 | Color                  | Yellow | Yellow | Yellow | Yellow | Reddish | Yellow | Yellow |

\*means male dog. \*\* means female dog.

**Table (3):** Biochemical results of serum of diseased dogs infected with UTI.

|   | PARAMETER                | CASE NUMBER |      |      |      |       |       |       |
|---|--------------------------|-------------|------|------|------|-------|-------|-------|
|   |                          | 1*          | 2**  | 3*   | 4*   | 5*    | 6**   | 7*    |
| 1 | Creatinine (mg/dl)       | 1.13        | 1.17 | 1.51 | 1.53 | 1.84  | 0.8   | 0.7   |
| 2 | Urea (mg/dl)             | 52.5        | 35.2 | 40.2 | 37.5 | 50    | 49    | 50    |
| 3 | Uric acid (mg/dl)        | 2.00        | 2.00 | 2.00 | 2.07 | 2.0   | 2.0   | 2.0   |
| 4 | K (mav/l)                | 12.0        | 13.5 | 12.0 | 12.0 | 9.0   | 12.66 | 11.70 |
| 5 | GGT (U/l)                | 5.00        | 5.00 | 7.00 | 5.00 | 7.80  | 7.00  | 5.00  |
| 6 | Triglycerides<br>(mg/dl) | 70          | 78.8 | 112  | 74.7 | 70.00 | 70.00 | 73.20 |

|    |                 |       |       |       |       |       |       |       |
|----|-----------------|-------|-------|-------|-------|-------|-------|-------|
| 7  | Glucose (mg/dl) | 70.6  | 63.1  | 53.4  | 75.7  | 50.30 | 67.80 | 70.00 |
| 8  | ALT(mg/dl)      | 25.32 | 27.00 | 27.00 | 29.12 | 27    | 30.7  | 30.7  |
| 9  | AST(mg/dl)      | 27.10 | 28.34 | 30.00 | 29.18 | 27.8  | 30.7  | 30.7  |
| 10 | Hb(mg/dl)       | 0.9   | 0.8   | 0.9   | 0.7   | 1.84  | 0.8   | 0.7   |

\*means male dog. \*\* means female dog.

**Table (4): represent the percentage rate of bacteriuria in dogs affected with UTI according to age and sex.**

| Age (years) | Sex                 |                              |                     |                              | Total               |                              |
|-------------|---------------------|------------------------------|---------------------|------------------------------|---------------------|------------------------------|
|             | Female              |                              | Male                |                              | No. of dog examined | No. of dogs with bacteriuria |
|             | No. of dog examined | No. of dogs with bacteriuria | No. of dog examined | No. of dogs with bacteriuria |                     |                              |
| <2to3       | 2                   | 1                            | 5                   | 3                            | 7                   | 4 (57.14%)                   |

Urinary tract infections (UTIs) are the most common cause of lower urinary tract signs in dogs (8). Ascending UTI and pyelonephritis are usually caused by aerobic bacterial organisms, most commonly *Escherichia coli* and *Staphylococcus* sp., and less commonly with species of *Proteus*, *Streptococcus*, *Klebsiella*, *Enterobacter*, and *Pseudomonas aeruginosa* (9). Causative bacteria may possess specific virulence factors (VF's) that enhance their ability to adhere to and colonise the urothelium. *Escherichia coli*, the most common cause of cystitis and acute pyelonephritis in dogs and cats, produces VFs important to the development of pyelonephritis, and isolates associated with UTI possess more than those from healthy animals. Factors known to be more specific to the pathogenesis of pyelonephritis include haemolysin, various adhesins, pyelonephritis-associated pili, cytotoxic necrotising factor-1, aerobactin and secreted autotransported toxin (10). Symptoms of a bacterial UTI may or may not be present and are dependent on which part(s) of the urinary system is/are infected, obvious problems may not be present, but, When present, they may include the following signs fever, abdominal pain, inappetance, lethargy, blood in urine and vomiting (11). Results of the present study were in **accordance to some extent** with (12) that indicates the results of urinalyses performed at the same time urine samples were submitted for bacterial culture were available for all 7 animals. The median pH was 8.0, and median specific gravity was 1.026. Proteinuria was reported in all the affected animals, 1 cat had glycosuria, and 4 dogs and both cats had hemoproteinuria, as determined by means of dipstick analysis. Four dogs and 1 cat had pyuria, with urine WBC counts ranging from 5 to > 100 WBCs/hpf, and all 5 dogs and 1 cat had RBCs visible in the urine sediment. Three dogs and both cats had struvite crystalluria. Gram staining of the urine sediment revealed gram-positive rods in 3 dogs and both cats.

Once bacteria infect any site, all other areas are at risk. The diagnosis of lower UTIs (cystitis and urethritis) is usually made on the basis of signs and symptoms and then

confirmed by culture. Most episodes of short-term catheter-associated bacteriuria (greater than 10<sup>5</sup> organisms per mL of urine), however, are without symptoms. If present, symptoms usually consist of slight fever, burning, urgency and pain on urination. Similar symptoms or findings may occur in long-term catheterized patients, but these patients may also experience obstruction, urinary tract stones, renal failure and (rarely) bladder cancer (13). In upper UTIs (pyelonephritis), flank pain, fever, blood in the urine (hematuria) and other physical findings may be present. In frail, elderly patients, however, the typical signs and symptoms of a UTI may be absent. Moreover, bacteriuria, whether from an upper or lower UTI, is the most common cause of nosocomial gram-negative sepsis and has been linked to increased mortality (14). Our results resembled the result of the (15) that revealed the clinical signs on affected dogs were: depression, lethargy, anorexia, abdominal pain and Fever (39.5-40.5 C<sup>0</sup>). The dogs had leukocytosis (17,000-22,000) and left shift (80-90%). Serum creatinine and urea concentrations were 3.2-4.5 mg/dl and 82-156 mg/dl, respectively. The urine samples were concentrated and cloudy, while our result showed elevation in the urine specific gravity; their results revealed low urine specific gravity in most samples (1010- 1015). Urine pH was between 6 and 9. Nitrite and bilirubin were determined in some samples. During microscopic examination of the urine sediment, leukocytes, erythrocytes, epithelial cells, squamous epithelial cells, epithelial casts and leukocytic casts were observed. Bacterial virulence factors have been studied in many studies involving common bacteria of the canine (very little feline) urinary tract. Most of the studies are on factors effecting adhesion, penetration and virulence of *E. coli*, the most common canine urogenital pathogen (16). The mechanisms of renal damage caused by ascending UTIs have not been well studied in dogs or cats. In humans it has been shown that 55-75% of children that develop febrile UTIs have concurrent renal parenchymal damage, and 20-40% of these children will suffer permanent renal cell damage (17). The possible pathogenesis of renal damage from UTIs that has been studied in humans include: vesicoureteral reflux, direct effects of uropathogenic bacteria on renal cells causing: Local inflammatory response (cytokine and chemokine production), Infiltration of inflammatory cells, Liberation of proteolytic enzymes and free radicals and Eventually leading to cell death, and if sub-lethal then ultimately to fibrosis and renal scarring (18). It is unknown how similar the processes are in companion animal medicine.

### دراسة التغير الدمىة والكىماحوىة وتحلىل البول لتشخىص التهاب المسالك

### البولىة والتهاب الحوىضة والكلىة فى الكلاب البولىسىة نوع (*German shepherd*)

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### الخلاصة

أجريت الدراسة الحالية لمعرفة التغيرات الدمىة والكىماحوىة فى مجموعة من الكلاب البولىسىة التى تعانى من التهاب المسالك البولىة الجرثومى فى مدىنة الدىوانىة قسم الكلاب البولىسىة. كان مجموع الحالات التى تم فحصها هى (7) اذ تم عزل ثلاث سلالات جرثومىة من عىنات البول. من مجموع الحالات تم تحدىد خمس عزلات من الاشرىكيا

القولونية ( 71.42%) ، وعزلتين عبارة عن عدوى مشتركة بين الاشريكية القولونية و المكورات العنقودية (28.57%). العلامات السريرية التي لوحظت على الكلاب المصابة هي بيلة دموية والحمى والاكنتاب وآلام في البطن وفقدان الشهية وعدم الراحة . وكشف تحليل البول الفيزيائي ان البول عكر و لونه ضارب الى الحمرة فضلا عن ذلك فأن التحليل الكيميائي للبول كشف عن تواجد البيليروبين، واليوروبيلينوجين، الكريات البيض (+2) والبروتين (+2)، والنترات (+1)، وكانت الكثافة النوعية للبول ( 1،030)، في حين لم يتأثر مستوى الجلوكوز في جميع الحالات . وكشف الفحص المجهرى للبول احتواء البول على بلورات (متعددة الاشكال)، و على الخلايا الظهارية . وأظهرت الفحوصات الدموية زيادة في قيم الكرياتينين، واليوريا، بينما اظهرت عدم وجود فروقات في قيم حامض البوليك بين الحيوانات المصابة، في حين كان هناك انخفاض في تركيز عنصر البوتاسيوم في مصل الدم في الحالة رقم ( 5 )، في حين لا يزال ضمن المعدل الطبيعي في حالات أخرى، كذلك اوضحت النتائج وجود زيادة في قيم خميرة GGT في الحالات رقم ( 3 و 5 و 6)، في حين لم تتغير في بقية الحالات الأخرى . وأظهرت القيم زيادة الدهون الثلاثية في حالة رقم ( 3 ) بالمقارنة مع الحالات الأخرى . كشفت مستويات السكر في حالات (رقم 5 و 3) انخفاضه بشكل طفيف مقارنة مع بقية الحالات وأظهرت القيم ALT و AST عدم وجود فروق بين جميع الحالات، وكما أظهرت النتائج وجود زيادة في معدلات خضاب الدم في حالة رقم (5) بالمقارنة مع الحالات أخرى . أظهرت النتائج أن نسبة جراثيم البول (والتي تعني وجود البكتريا في البول نتيجة الاصابة البكتيرية للمسالك البولية وليس نتيجة تلوثها اثناء الجمع) في الكلاب المصابة 57.14%.

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