



PATHOLOGICAL, HEMATOLOGICAL AND BACTERIOLOGICAL STUDY OF OVINE KIDNEY DISEASES IN BASRAH PROVINCE

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Abstract: This study was conducted to verify the presence of macroscopic and microscopic lesions and the accompanying changes in the blood and bacterial isolation to some factors that causing infection in the kidneys, in sheep with acute and chronic kidney diseases in Basrah province, during the period from November 2021 to April 2022. The results of the blood test for young and adult sheep also showed a significant ($P \leq 0.05$) decrease for each of the (RBCs), (HB), (PCV), (MCV) and (MCHC), and revealed a significant ($P \leq 0.05$) increase in the (DLCs) and neutrophils and lymphocytes. In addition, the results of sheep serum tests as an indicator of kidney function also showed a significant ($P \leq 0.05$) increase in both the level of urea, creatinine and uric acid concentration and a significant ($P \leq 0.05$) decrease in calcium level in affected animals compared to healthy animals, the bacteriological study showed the presence of anaerobic bacteria that cause kidney disease in sheep, including: *Corynebacterium sp*, *Bacteroides sp*, *Prevotella sp*. and *Porphyromonas sp*, each of them has an effect on histopathological changes in the renal tissue, The result of the macroscopic examination of the kidney samples taken from the affected sheep, including hemorrhagic lesions and hypertrophy with focal yellowish-white spots spread in the cortex and medulla. The microscopic appearance of glomerulonephritis and a large infiltration of inflammatory cells in different places of the renal tissue, especially in the interstitial region as well as between the renal tubes, in addition, showed the enlargement of the renal glomeruli and congestion of the renal blood vessels and infiltration of large numbers of inflammatory cells, The histochemical section revealed renal fibrosis as a result of the deposition of fibers and thickening in the basement membrane of Bowman's capsule.

Keywords: Pathological; Hematological; Bacteriological ; Ovine Kidney Diseases ;Basrah .

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INTRODUCTION

Ovine kidney disease is an inflammatory conditions caused by the high reproduction of several pathogens in the urinary system, which lead to changes in the optimal function of the urinary tract and kidneys (Ansari-Lari, 2007), In addition, there are many pathological factors that cause renal diseases, including: bacterial, viral, parasitic diseases and some toxic compounds (Newman., 2012).

In some blood diseases, several changes occur that can affect the body's organs, including the kidneys, where the values of creatinine and BUN in the blood increases, It could result from pathological nephron diseases brought on by oxidative stress radicals that prevent the kidneys from adequately filtering protein waste products, which would then lead to an increase in renal biomarkers. (Ahmed, 2017).

One of the most common hematological disorders is normochromic normocytic anemia, which is associated with chronic renal failure and may also be correlated with severity of renal failure. (Naghmi *et al.*, 2015). Moreover, high levels of urea in the blood are dangerous and cause many acute and chronic kidney diseases (Younis *et al.*, 2021).

Bacterial isolation from the kidneys revealed that the isolated bacteria were Gram-negative and Gram-positive, which cause urinary tract infections because they contain cilia and flagella, in addition to some protein attachments that help them to adhere to the mucous membrane of the urinary tract, it also possesses a R factor, which allows it to with stand antibiotics and cause infections (Mohammed *et al.*, 2020).

There have been a few reports of anaerobic bacterial infection in sheep's urinary systems, as well as for many families of bacteria, including gram negative enterococcus, which can grow aerobically and anaerobically. (Kumar *et al.*, 2012).

The current study aimed to screen the ovine kidney diseases slaughtered in Basrah province, as well as study the hematological markers associated with these diseases, and assessment of histopathological alterations in kidneys affected by various renal disorders in diseased sheep using macroscopical and microscopical assays as well as accessible special stains, and bacteriological isolation with identification of the predominant causes of kidney disease in local sheep, as well as molecular identification of pathological isolates of specific nephrotic bacteria.

MATERIALS & METHODS

Samples collection:

After the animal was slaughtered in the slaughterhouse in Basrah province, the total number of the study samples is (50 samples) collected from several places, the blood samples were obtained via jugular vein from male and female sheep by using the new disposable syringe. Samples were kept in 2 different test tubes to study all blood counts, WBCs counts, and some kidney function tests (Radostitis *et al.*, 2007).

Culturing:

Swabs were taken by using cotton swab from every sample (cortex and medulla) then it culture was streaked on to plates of blood agar after that the dishes were incubated under anaerobic condition using anaerobic Jar at 37oC for 3days, after which the bacteria grown on the media were purified and isolated (Jaber 2019), Bacteria were cultured on nutrient broth in preparation for the PCR process. (Markey *et al.*, 2014).

Genetic Identification:

Genomic DNA Extraction:

The DNA of the 7 isolated bacteria was extracted by a genomic DNA purification kit (Mini DNA Extraction Kit, Promega / USA), the result was detected by electrophoresis on 1% agarose

and showed under ultraviolet light where the DNA appears in form bands (Sambrook *et al.*, 1989) and (Jabber, B. 2021).

Polymerase Chain Reaction (PCR):

Using the technique of performing PCR, the DNA of the bacteria was amplified with master mix, Promega\USA. primers are paired together to identify significant bacterial species (Miyoshi *et al.*, 2005).

Table 1. PCR technique used for amplification of 16S RNA gene.

gene	Primer	bp
16SrRNA	F-AGAGTTTGATCCTGGCTCA	1500
	R-GGTTACCTTGTTACGACTT	

Purification and Sequencing:

Macrogen Laboratories in Korea purified and sequenced PCR products for comprehensive identification of bacterial isolates.

Histological Technique:

The histopathological specimens were sent to the laboratory and fixed with formalin 10 % for processing for diagnoses of the kidney lesions according to (Suvarna, *et al.*, 2018).

RESULTS

Hematological Results:

Complete blood count (CBC):

Table 2. Hematological changes in adult and young sheep with kidney diseases.

Parameters	Young sheep		Adult sheep		Remarks
	control	diseased	control	diseased	
RBCs (x10/M)	8.11±0.87	6.45± 1.56	8.33±1. 21	5.32±1.41	*
HB (gm/dl)	9.8± 1.77	6.23±1.76	10.23± 1.88	6.89± 1.35	*
PCV (%)	33.65±1.78	22.78± 5.89	35.36±2.77	24.65±4.78	*
MCV (fl)	41.48± 1.86	38.24±3.72	42.22±2.77	41.48± 1.86	*
MCHC (gm/dl)	29.12± 1.91	29.12± 1.91	28.93±2.76	30.24±3.88	*

*Significant at (P≤0.05).

There is a significant (P≤0.05) decrease in total RBCs count in young animals with kidney diseases group is compared with the healthy group, which showed (6.45± 1.56) and (8.11±0.87) of young sheep with kidney disease group and healthy group respectively, but in adult sheep infected (5.32±1.41) and (8.33±1. 21) of adult sheep with kidney disease group and healthy group respectively as in table (2).

In addition, the result of hemoglobin concentration (Hb) showed a significant (P≤0.05) decrease in the young sheep-infected group compared to the healthy group, which showed (6.23±1.76) and (9.8± 1.77) of the young infected group and healthy respectively. Also, the same thing is observed in infected adult animals, there is a significant (P≤0.05) decrease in hb which showed (6.89± 1.35) and (10.23± 1.88) in the adult infected group and healthy respectively as in table (2).

Moreover, the hematocrit values (PCV) of the young diseased group showed a significant (P≤0.05) decrease which showed

(22.78± 5.89), while the control group showed (33.65±1.78) and the adult diseased group showed a significant (P≤0.05) decrease which showed (24.65±4.78), while the control group showed (35.36±2.77) as in table (2).

In addition, the mean cell volume (MCV) of the young diseased group showed a significant (P≤0.05) decrease which showed (38.24±3.72), while the control group showed (41.48± 1.86), and the adult diseased group showed a significant (P≤0.05) decrease which showed (41.48± 1.86), while the control group showed (42.22±2.77) as in table (2).

Finally, The mean corpuscular hemoglobin concentration (MCHC) of the young diseased group showed a non-significant (P≤0.05) which showed (29.12± 1.91) in male and female, but in the adult diseased group showed a significant (P≤0.05) increase which showed (30.24±3.88), while the control group showed (28.93±2.76) as in table (2).

Differential WBCs count (D.L.C):

Table 3. Leucogram in adult animals infected with kidneys and healthy.

Parameters	Control	diseased	Remarks
DLC x10 ⁶	1.56± 10.9	3.85±14.66	*

Neutrophils' / absolute x10 ³	4.30±0.17	2.87±7.89	*
Lymphocytes / absolute x10 ³	0.44±5.32	1.65±5.37	*
Monocytes / absolute x10 ³	0.06±0.55	.233±0.61	-
Eosinophiles / absolute x10 ³	.022±0.51	0.12±0.52	-
Basophiles / absolute x10 ³	0.03±0.07	0.01±0.071	-

*Significant at (P≤0.05).

There is a significant (P≤ 0.05) increase in differential blood cells of the diseases group compared with the healthy group, which showed (14.66±3.58) and (10.9±1.56) of the sheep kidney diseases group and healthy group respectively. In addition, the result of Neutrophil level count showed a significant (P≤0.05) increase in the diseases group compared to the healthy group, which showed (7.89±2.87) and (4.30±0.17) for the diseases group and healthy respectively as in table (3). Moreover, the lymphocyte level count of the infected group showed a significant (P≤0.05) increase which showed

(5.37±1.65), while the control group showed (5.32±0.44) as in table (3).

In addition, the result of the monocytes level count showed a significant (P≤0.05) decrease in the diseases group compared to the healthy group, which showed (0.61±2.33) and (0.55±0.06) for the diseases group and healthy respectively. Moreover, the eosinophil's and basophils level count of the infected group showed a significant (P≤0.05) decrease which showed (0.52±0.12) and (0.071±0.01), while the control group showed (0.51±0.22) and (0.07±0.33) as in table (3).

Biochemical analysis:

Table 4. Serum biochemical analysis of adult sheep with kidney diseases.

Parameters	Adult sheep		Remarks
	control	diseased	
Urea (gm/dl)	88.23±4.54	155.76±6.76	*
Creatinine (gm/dl)	1.45±0.23	2.1±0.76	*
Uric acid (gm/dl)	1.34±0.12	1.7±0.33	*
Calcium (gm/dl)	12.24± 1.45	9.96± 2.76	*

*Significant at (P≤0.05).

The results of the biochemical analysis showed a significant (p<0.05) increase in the concentration of (Urea, Creatinine, and uric acid), which showed (155.76±6.76), (2.1±0.76), and (1.7±0.33) in the renal disease group respectively when compared to the healthy group, although (calcium concentration) showed (9.96± 2.76) was a significantly (p<0.05) decreased in the renal diseases group when compared with the control group as in table (4).

Bacteriological results:

Isolation of bacteria that causing kidney infection in sheep:

Based on the culturing of kidney swaps on blood agars the results of bacterial isolation are distributed as following : out of 50 kidney samples that were collected from infected male sheep 23/35 (65.7%),while the cultured and isolated samples from slaughtered female sheep 12/18 (34.3%) as in table (5).

Table 5. Number and percentage of infected samples isolated from sheep kidney swaps according to gender

sample	No. of samples	No. of Infection	(%) of Infection
Male	32	23	65.7%
Female	18	12	34.3%
Total	50	35	100%

Molecular Identification by PCR Assay:

DNA Extraction and Detection:

Using a genomic DNA purification kit, DNA was extracted, electrophoresed with 1 percent agarose, and the result was

visible under UV light. Then, 16S rDNA was amplified using PCR as in (Figure 1), and the distinct band of the gene was observed at (1500 bp).

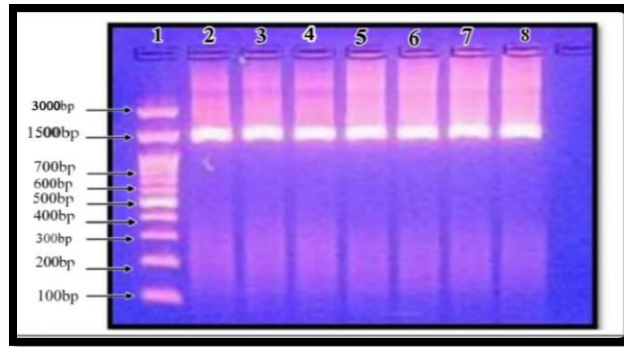


Figure 1. 16S rDNA Amplification in the area 1500bp

16S rDNA Sequencing and bacterial species identification:

In the genetic amplification of seven isolates of bacteria, four isolates were diagnosed, and three of them were not diagnosed with gene sequencing as in Table (6).

Table 6: Identified anaerobic bacterial strains by gene sequencing.

Identical to strain	Accession	Length (bp)	Bacterial specie
SNJBp1	ON778719	1138	<i>Bacteroides sp.</i>
SNJBp2	ON778720	1122	<i>Prevotella sp.</i>
SNJBp3	ON778721	1197	<i>Porphyromonas sp</i>
SNJBp5	ON778723	1105	<i>Corynebacterium sp</i>

Phylogenetic Tree:

The phylogenetic tree of *Bacteroides sp.* bacteria illustrated in figure (2)

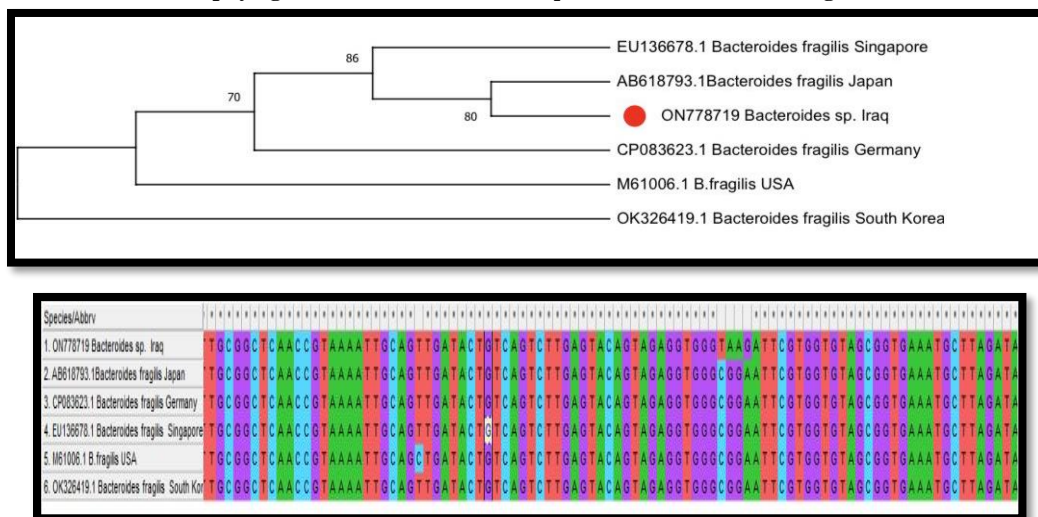


Figure 2. Maximum likelihood tree illustrating the phylogenetic relationship between the 16S tRNA sequence of one isolated *Bacteroides sp* bacterium (isolated from a local sheep kidney in Iraq) and other 16S rRNA sequences from closely related bacterial species. International nucleotide databases use their accession numbers to express them. Mega 6 sequencing, 6.5 software was used to create the phylogenetic tree.

The phylogenetic tree of *Prevotella sp.* bacteria illustrated in figure (3)

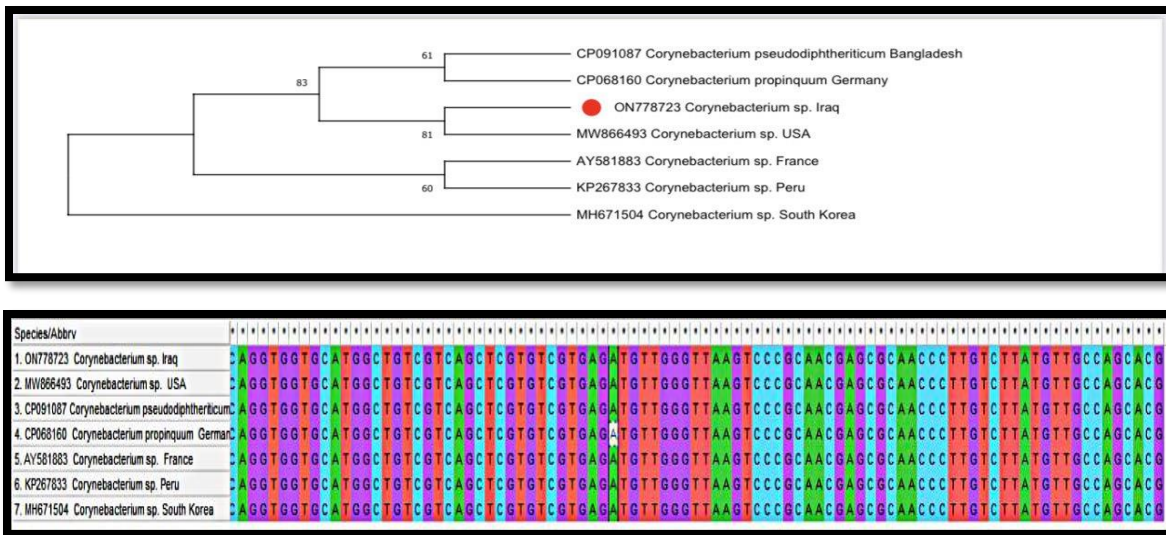


Figure 5. Maximum likelihood tree illustrating the phylogenetic relationship between the 16S tRNA sequence of one isolated Corynebacterium sp bacterium (isolated from a local sheep kidney in Iraq) and other 16S rRNA sequences from closely related bacterial species. International nucleotide databases use their accession numbers to express them. Mega 6 sequencing, 6.5 software was used to create the phylogenetic tree.

Pathological results:

Macroscopical results:

The macroscopical lesions showed a wide superficial solitary ecchymotic hemorrhage in the middle part of the kidney also the entire kidney was hypertrophied and congested; as well as yellowish coloration and paleness in the lower convex of the kidney as in (figure 6), as well as showed hypertrophy and congestion of the entire both kidneys and present a solitary focal white spot located in the lower part of the left kidney, while the right kidney appeared congested, friable and consisted a grey-whitish focal cystic lesion in the middle part of the kidney as in (figure 7). Also, The right and left kidneys showed petechial hemorrhage in the subcortical region as in (figure 8), in the Other gross section showed a congested separated spot in the renal medulla as in (figure 9).

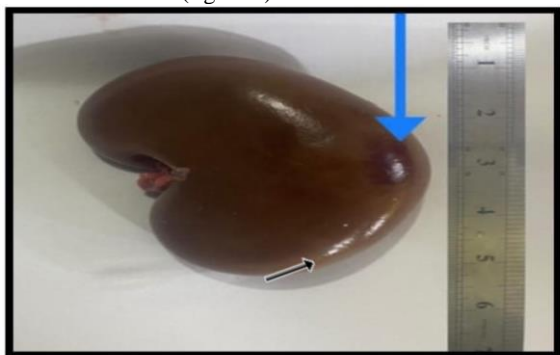


Figure 6. The right kidney showed a wide superficial solitary, ecchymotic hemorrhage in the middle part of the kidney(blue arrow): As well as hypertrophy, yellowish coloration and paleness in the lower part of kidney(black arrow).

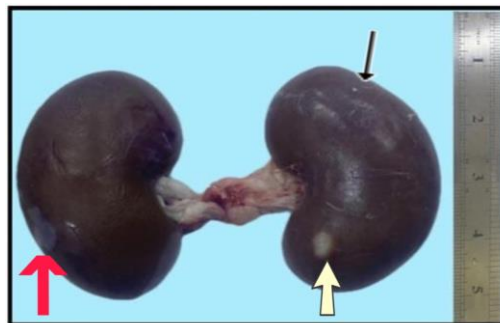


Figure 7. Gross section of the kidneys showed :The kidneys revealed hypertrophy with congestion of the entire both kidneys(black arrow);As well as a focal white spot present in the lower part of left kidney(white arrow),The right kidney was congested, friable and consisted a grey-whitish focal cystic lesion in kidney (red arrow).

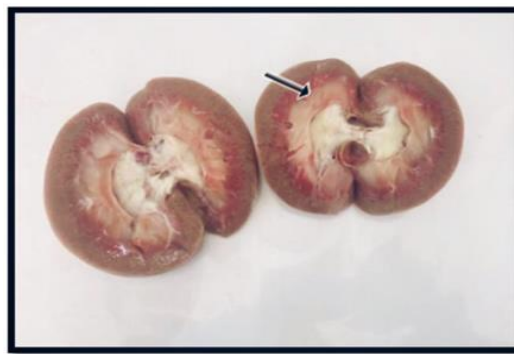


Figure 8. The right and left kidney showed petechial hemorrhage in the sub cortical region (black arrow).

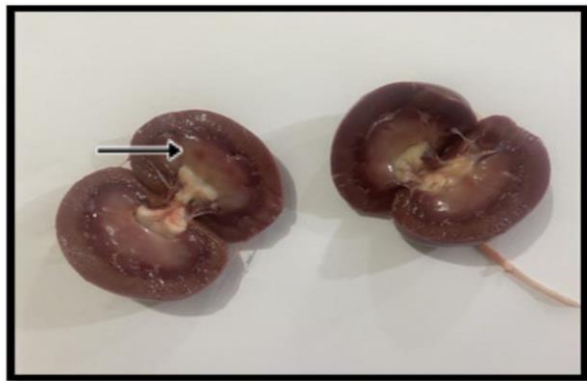


Figure 9. Gross section of the left kidney showed a congested separated spots in the renal medulla (black arrow).

Microscopical results:

On Transverse histopathological section of the kidney of diseased animals shows peri-glomerular mononuclear inflammatory cells infiltration and hyperplasia of mesangial cells, also there is a minimal degree of bowman capsule thickness as in the (Figure10), As well as showed subcortical inflammatory exudate consists of mononuclear inflammatory cells infiltration in the renal interstitium and between renal tubules as in (Figure 11), Another kidney section is characterized by a minimal degree of bowman capsule thickness, congested renal blood vessel, and subcortical inflammatory cells infiltration mainly mononuclear cells as in (Figure12). and showed diffuse interstitial mononuclear cells infiltration in the renal parenchyma and between renal tubules as in (Figure 13)

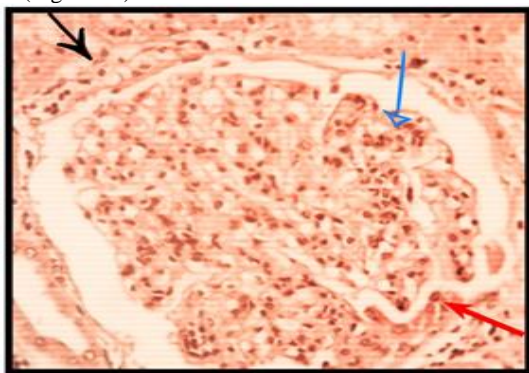


Figure 10. Transverse histopathological section of kidney of diseased animal shows peri-glomerular mononuclear inflammatory cells infiltration (red arrows), and hyperplasia of mesangial cells (blue arrows), also there is minimal degree of bowman capsule thickness (black arrows). H&E stain, 400X.

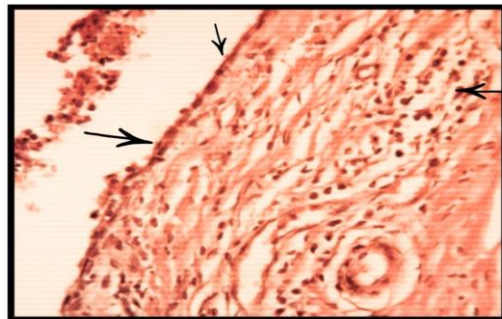


Figure 11. Transverse histopathological section of kidney of diseased animal shows subcortical inflammatory exudate consist from mononuclear inflammatory cells infiltration in the renal interstitium and between renal tubules (black arrows). H&E stain. 400X.

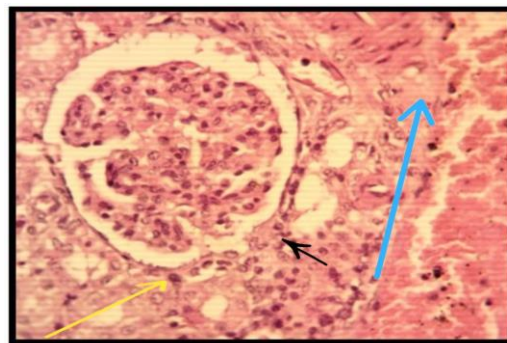


Figure 12. Transverse histopathological section of kidney of diseased animal shows minimal degree of bowman capsule thickness (black arrows), congested renal blood vessel (blue arrow), and inflammatory cells infiltration (yellow arrows). H&E stain. 400X.

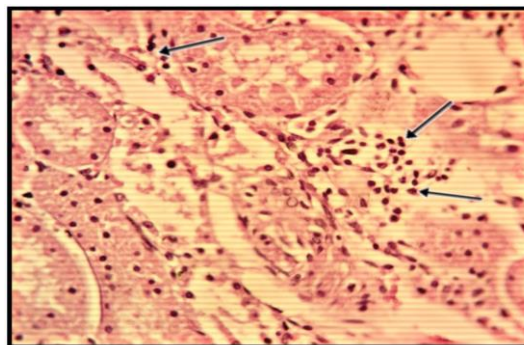


Figure 13. Transverse histopathological section of kidney of diseased animal shows diffuse interstitial mononuclear inflammatory cells infiltration in the renal parenchyma and between renal tubules (black arrows). H&E stain. 400X.

Histochemical Results:

The histochemical section of the kidney of diseased animal shows thickened bowman capsule basement membrane of the atrophied glomerulus in Masson trichrome stain as in (Figure

14), in addition shows renal vascular basement membrane thickness between renal tubules as in (Figure 15).

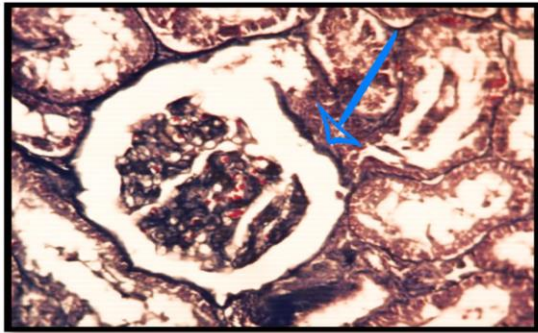


Figure 14. Transverse histochemical section of kidney of diseased animal shows thickened bowman capsule basement membrane of atrophied glomerulus (blue arrows). Masson trichrome stain, 400X.

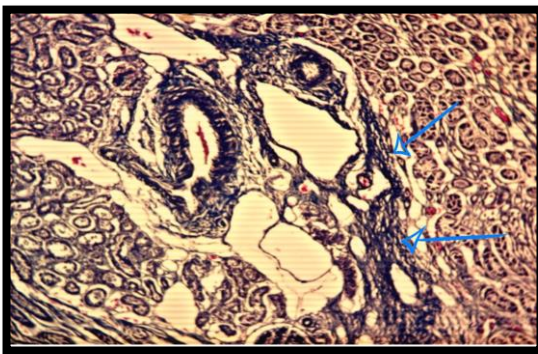


Figure 15. Transverse histochemical section of kidney of diseased animal shows renal vascular basement membrane thickness between renal tubules (blue arrows). Masson trichrome stain. 400X.

DISCUSSION

In the current study of animals with kidneys included the total number of red blood cells, Hb, PCV and MCV values have all decreased a significantly ($P \leq 0.05$) in this study at different ages of the affected sheep, this results observed in hemolytic type of anemia (normocytic normochromic anemia), These blood changes are agreement with the previous study of Heba and Amer (2009) which indicates the presence of hematological changes from the normal rates as a result observed in the hemolytic type of anemia, and these results are attributed to the toxin produced by bacteria that cause the dissolution of phospholipids in the membrane of red blood cells and cause hemolysis by damaging circulating red blood cells (Hafsan et al.,2022; Zadeh et al.,2022; Huldani et al.,2022; Bokov et al.,2022).

The current study's findings revealed a considerable increase in total leukocyte count, which might be explained by increase absolute neutrophil, eosinophilia, and leukocytes. Acute inflammatory disorders, particularly those resulting from bacterial infections, have been linked to increase in the total white blood cell count. This is due to the fact that infectious agents and tissue injury products induce of growth factors,

cytokines, and other inflammatory mediators that are released from a variety of cells that act as immediate stimuli, all of which together cause an increase in the total white blood cell count as well as further produced, multiplied, matured, and release of neutrophils in El-Deeb and Elmoslemany (2016).

Affected sheep had considerably higher mean serum creatinine, blood urea, and uric acid concentrations, the increase in this concentration could be due to rapid catabolism of body proteins as a response to illness, whereas the increase in serum creatinine could be due to reduced renal function following infection. Nearly same results were obtained by Radostitis *et al.*, (2007) who reported the biochemical changes confirmed by microscopic examination which revealed kidney damage.

It has been found that the blood calcium level is significantly decreased in diseased animal, that may due to acute phase hyperacidity that associated with the kidney diseases may contribute to decrease the vit D releases from the kidney which that lead to decrease calcium absorption from the intestine, and this leads to decreases serum calcium, these idea may agree with Naghmi, *et al.*,(2015), it has been shown that decrease in serum calcium during kidney disease are associated with acute phase hyperacidity of the disease and kidney infection, both of which contribute to decreased kidney function. In our current study, the important aim was to know the bacterial causes that affect the kidneys and the resulting macroscopic and microscopic changes, This study included phenotypic methods for the growth of microorganisms on blood agar, and described the morphologically grown microorganisms on it, the results of bacterial isolation on blood medium using anaerobic incubation method using anaerobic Jar and anaerobic pags showed that 7 isolates of gram-positive bacteria that appeared in the rod-shaped were isolated, able to live in anaerobic state, and other species in the form of gram-negative bacilli, confirmed with PCR, This result agreement with study Mohammed *et al.*, (2020), that documented the isolation and presence of these types of gram-positive and gram-negative anaerobic bacteria in urine and kidney samples, The results of 16S rRNA gene showed that all studied isolates showed successful amplification of the full length (1500 bp) of the 16S rRNA gene after DNA extraction from the isolates were completed, and electrophoresis analysis was used to verify them. The DNA domains that arose as a result of specific primers and isolates that bind successfully to the extracted DNA were identified by this research. When exposed to UV light, these production relationships appeared as single bands. Using a special DNA dye Ethidium bromide, Our aim was to use polymerase chain reaction (PCR) assays to discover genes that cause kidney disease. The results showed that the SNJB1,2,3,5 genes is involved. It was detected in strain isolated from *Bacteroides sp*, *Prevotella sp*, *Porphyromonas sp*, *Corynebacterium sp* In the previous study of Imirzalioglu *et al.*(2008), 1449 urine samples were examined by polymerase chain reaction. The majority of UTIs that were examined were caused by *Bacteroides*, *Prevotella sp*, *Porphyromonas sp*, *Corynebacterium sp* and a large part of the anaerobic bacteria that cause these diseases, In macroscopical study showed a wide superficial solitary, ecchymotic hemorrhage and hypertrophy, As well as consisted a grey-whitish focal cystic lesion that caused by the accumulation of edema, blood, fat, urine and fluid in the tubule or pelvis, as well as the enlargement of the kidneys with a dull gray color, which could be caused by

inflammation of the nephrons, glomeruli and renal tubules, these results are consistent with the results of the previous study carried out by Jarad, *et al.*, (2020) where his results showed that the kidneys were infected with renal hypertrophy and hemorrhage. In other cross section in both right and left kidneys showed petechial hemorrhage in the subcortical and showed a congested separated spot in the renal medulla. This hemorrhage occurs due to acute nephritis, septicemia, or bacterial sepsis, each of these infections leads to an acute response to inflammatory agents or a direct response of exposed tissue to pathogens or due to the effect of the toxin produced by different types of bacteria which causes an inflammatory response in the renal tissues and leads to an increase in the amount of blood supply of the kidneys, these results are in agreement with Greco *et al.*, (2005) who showed that a lot of kidney bleeding is a result of many bacterial infections and their toxins. A histopathological examination revealed that shows peri-glomerular mononuclear inflammatory cells infiltration and hyperplasia of mesangial cells, also there is minimal degree of Bowman capsule thickness and showed diffuse interstitial mononuclear inflammatory cells infiltration in the renal parenchyma and between renal tubules. This result may occur due to infection with bacteria which cause glomerulonephritis marked by a growth in the number and size of endothelial and mesangial cells in the glomerular tuft. Antigen-antibody responses to foreign proteins, as well as bacterial infections, are the most common causes of glomerulonephritis. This is attributed to the high number of inflammatory cells in the blood tests. These changes are consistent with the findings of Mahouz, *et al.* (2015), who showed that a lot of glomerulonephritis in his study due to many bacterial infection by (6%), as well as agreement with Hatipoglu *et al.*, (2001) results, they showed that sheep and goats had a higher percentage of glomerular lesions. They noticed that spontaneous proliferative glomerulonephritis, while the histochemical section of the kidney of diseased animal shows thickened Bowman capsule basement membrane of the atrophied glomerulus, this is attributed to the blood tests that gave an indication of anemia in the affected sheep, which leads to ischemia and necrosis of the nephrons, which causes atrophy of the renal glomeruli. In addition to the persistence of injury, immune response, and infiltration of defense cells, it causes thickening of the basement membrane of Bowman's capsule and renal vascular basement membrane thickness between renal tubules, the results of his study agreed with the previous study of Dutta *et al.*, (2016) discovered peri-glomerular fibrosis.

CONCLUSION

The current study concluded that kidney disease is accompanied by a significant decrease in total erythrocytes and hemoglobin (Hb) concentration and a significant decrease in (PCV), while the total differential white blood cell count showed a significant increase as the number of neutrophils and lymphocytes. Also, the biochemical analysis showed a significant increase in the concentration of urea, creatinine and uric acid, while the calcium concentration decreased significantly. The macroscopic lesions of the kidney were identified as extensive superficial ecchymotic hemorrhages, single white spots that were friable and had a whitish focal cystic lesion, and histopathological and histochemical abnormalities, low degrees of Bowman's capsule thickness,

infiltration of inflammatory mononuclear cells in the renal interstitium and between renal tubules, and thickening of the basement membrane of the Bowman's capsule. In the bacterial side of the study, anaerobic bacteria that cause diseases were isolated and identified using PCR technology and their genetic sequencing.

COMPLIANCE WITH ETHICAL STANDARDS STATEMENTS

I. Ethical approval:

The manuscript is written in original and all the data, results pertaining to this manuscript are original according to the research performed. The authors followed academic integrity and have not copied any content/results from another source.

II. Funding details (In case of Funding):

The authors of this manuscript did not receive any funding to perform the present research

III. Conflict of interest

The authors of the study do not have any conflict of interest

IV. Informed Consent:

The authors of the manuscript agree to publish this research in the journal if it's considerable by the editors of the journal. The authors provide full consent for reviewing and publishing this manuscript.

V. All the authors of this study contributed equally in terms of performing the research as well as in preparing the manuscript. All the authors of the study followed the guidelines of the corresponding author. Any query/suggestion related to the manuscript can be reached to the corresponding author

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