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Molecular characterization of some pathogenic bacterial strains and hematobiochemical profile in Barki sheep with diarrhea in Siwa Oasis

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ABSTRACT

Objective: To explore the incidence of several bacterial enteropathogens in Barki sheep with diarrhea and their antimicrobial resistance (AMR) trends and their hematobiochemical alteration in Siwa Oasis.
Design: Descriptive study.
Animals: A total of 500 adult Barki sheep were allocated into two equal-sized groups: 250 Barki ewes with diarrhea and 250 normal sheep taken as the control group. 250 faecal samples were taken from sheep with diarrhea. The bacterial enteropathogens were extracted, identified biochemically and determined using polymerase chain reaction (PCR). The Kirby-Bauer Disk-Diffusion Method was used to determine the sensitivity of positive samples from each organism to ten antimicrobials. Blood samples were collected from every animal for hematological and biochemical evaluation.
Results: Escherichia coli were the most prevalent agent (80%), followed by Campylobacter species, C. perfringens, Salmonella species, and Y. enterocolitica (68%, 40%, 30%, and 20%, respectively). The diarrheic sheep showed a significant (P < 0.05) increase of body temperature, pulse rate, respiratory rate, total leucocyte count, TEC (RBCs), Hb and PCV, neutrophil, lymphocyte and monocyte count as compared with control ones. In addition, the diarrheic sheep showed a significant (P < 0.05) reduction of serum values of glucose, total protein, Na, Cl, Ca and SOD with significant increase in the serum levels of K, creatinine, urea nitrogen, MDA and activities of AST and ALT as compared with control ewes.
Conclusion and clinical relevance: This study is helpful because it shows the most bacteria implicated in diarrheic enteropathogens that affect sheep with fast and accurate diagnosis. This information can be used to come up with ways to stop these infections.
Keywords: Barki sheep, diarrhea, PCR, Hematobiochemical changes, Siwa Oasis

1. Introduction

Barki sheep, which dominate the north western desert of Egypt with population of 470,000 heads (11% of the total Egyptian sheep population) are known to be well adapted to the desert harsh conditions and scarce vegetation including poor feeding, heat stress [1]. The basic information on body conformation and productivity of Barki ewes are available [2].

Diarrhea is one of the major problems facing sheep production especially those are bred under intensive or semi-intensive system of breeding, it also cause great economic losses due to deaths, poor growth rates, and veterinary costs [3-5]. Its etiology is multiple, including infectious agents, poor management, reproductive factors, nutritional factor and immune status of ewes and lambs [6]. The causative agents and the epidemiology of diarrhea have been widely studied worldwide; however, few studies have been carried out on farm animals in Siwa Oasis, Egypt. Enteropathogenic bacteria and viruses are important causes of diarrhea in livestock worldwide [7]. The most important enteropathogens accompanied by diarrhea in livestock include: Escherichia coli, Campylobacter, Salmonella species, and cryptosporidium, either singly or in combination [8]. Also, some pathogens are implicated in enteric diseases, including Clostridium perfringens, Klebsiella, Proteus, and Eimeria species [9].

The diarrheic animals loosed fluid; rapidly dehydrated and suffered from electrolyte loss, acidosis and infectious agents may cause initial damage to the intestine but death from scours usually result from dehydration, acidosis and loss of electrolytes [10]. Most of the diseased animals showed inappetance and depression. The feces of the animals varied from clay to yellowish gray or grayish to greenish in color, contained mucous and sometimes blood and many cases showed rise of body temperature.

The traditional methods of treatments of diarrhea is typically inexpensive and straightforward, but they require time since they rely on the microorganisms' ability to flourish in a variety of culture media, including pre-enrichment media, selective enrichment media, and selective plating media [11]. It takes 2-3 days to make a preliminary identification and over a week to confirm the pathogen species [12]. Additionally, false-negative results might occur as a result of viruses that are alive but unculturable [13]. Failure to detect pathogens increases the risk of pathogen transmission and treatment failure. Most importantly, delays in specific diagnosis, followed by incorrect antibiotic administration, may result in substandard health care and an increase in antimicrobial resistance (AMR). As a result, a variety of rapid diagnostic techniques with a high degree of sensitivity and specificity have been developed to aid in pathogen detection and identification and more efficient in terms of time, effort, and the capacity to reduce human mistake [14]. PCR is the most sensitive of the existing rapid methods to detect microbial pathogens in clinical specimens. In particular, when specific pathogens that are difficult to

culture in vitro or require a long cultivation period are expected to be present in specimens, the diagnostic value of PCR is known to be significant **[15].** Additionally, it can reduce the usage of antimicrobial drugs and expedite the transition to the appropriate treatment, thereby reducing both side effects and costs **[16].**

Hematological and serum biochemical analysis have been found to be a reliable indicator for assessing the animal health status and may give an assessment of the degree of damage of host tissues as well as severity of infection **[17, 18].** Diarrhea is usually associated with alterations in hematobiochemical constituents **[19].**

The aim of this work was the evaluation of the most effective method for the identification of the bacteriological causes of diarrhea and their antimicrobial resistance (AMR) patterns in Barki sheep in Siwa Oasis and their hematobiochemical alterations.

2. Materials and methods

2.1. Animals and study design

A total of 250 Barki ewes with different levels of diarrhea and 250 normal Barki ewes taken as the control group with range of age 3 – 5 years (mean ± SD: 4 ± 0.6) and a range of body weight 28 - 40 kg (mean ± SD: 34 ± 4.9), were raised at Siwa Oasis, Egypt which lies between longitudes (Lat: 29 º 06" 29 º 24" N Long: 25º 16" 26 º 12" E), and located 330 Km southwest of the Mediterranean shoreline and at 65 Km east of the Libyan borders. All animals were housed in semi-open shaded pens and fed on 600 g concentrate feed mixture (CFM) plus 600 g alfalfa hay/head/day, while water was always available *ad libitum*. The composition of the basal diet is presented in Table 1. The natural pasture (green herbage, grass and remnant of plant, berseem and darawa) was fed when available. The investigated ewes were subjected to through clinical examination including recording of temperature, pulse and respiratory rates, mucous membranes **[10].**

2.2. Sampling

Fecal swabs were obtained aseptically from rectum of diarrheic Barki ewes kept in the refrigerator until they were examined within 24 hours of being taken in the laboratory of desert research center.

2.3. Isolation and identification of causative agents

Swabs kept in sterile nutrient broth tube and incubated at 37°C for 24 hours before being transferred aseptically on specific media (McConky's agar) and re-incubated at 37°C for 24 hours. Smears were prepared from suspected bacterial colonies, stained with Gram's stain and examined for the morphological appearance, arrangement and staining reaction of the isolates **[20].**

2.4. Antibiotic susceptibility test

Standard operating procedures were followed using Mueller-Hinton agar (Oxoid, Hampshire, England) and the Kirby Bauer disc diffusion method., Penicillin (P 10 unit/disc), ampicillin (AMP 10 meg/disc), streptomycin (S 10 meg/disc), ciprofloxacin (CIP 5 meg/disc), erythromycin (E15 meg/disc), cefotaxime (CTX, 5 g), amoxicillin (AMX The diameter of the zone of inhibition (in mm) around a disc was measured using a ruler, and the result was interpreted as, the diffusion zone breakpoints recommended by Clinical and Laboratory Standards Institute (CLSI) **[21].** Antibiotics in a panel used for a variety of bacterial species in conjunction with the dimensions of their zone of inhibition to be considered **[22].**

Table 1 Composition of the concentrate feed mixture (CFM).

Ingredients	Quantity
Corn	520 kg
Wheat bran	350 kg
Soya bean	120 kg
Sodium chloride	5 kg
Calcium carbonate	5 kg
Premix	1 kg
Netro-Nill	0.5 kg
Fylax	0.5 kg

Table 2. Primers sequences, target genes and amplicon sizes

Target gene	Primers sequences	Amplified segment (bp)	Reference
E. coli phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720	**[23]**
Salmonella invA	GTGAAATTATCGCCACGTTCGG GCAA TCATCGCACCGTCAAAGGAACC	284	**[24]**
Campylobacter 23S rRNA	TATACCGGTAAGGAGTGCTGGA G ATCAATTAACCTTCGAGCACCG	650	**[25]**
Y. enterocolitica 16S rRNA	AAT ACC GCA TAA CGT CTT CG CTT CTT CTG CGA GTA ACG TC	330	**[26]**
C. perfringens alpha toxin	GTTGATAGCGCAGGACATGTTA AG CATGTAGTCATCTGTTCCAGCAT C	402	**[27]**

2.5. DNA extraction.

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56OC for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

Oligonucleotide Primers: The primers used were supplied by Metabion (Germany) and are listed in table (2), Cycling conditions used during polymerase chain reaction for detection of bacterial genes listed in table (3).

2.6. PCR amplification

Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied Biosystems 2720 thermal cycler.

2.7. Analysis of the PCR Products

The products of PCR were separated by electrophoresis on a 1.5% agarose gel (AppliChem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products were loaded into each gel slot. The Gene ruler 100 bp ladder (Ferments, Germany) and the gel pilot 100 bp ladder (Qiagen, Gmbh, Germany) were used to determine the fragment sizes. A gel documentation system (Alpha InfoTech or Biometra) took pictures of the gel, and computer software was used to look at the data.

Table-3. Cycling conditions used during polymerase chain reaction for detection of bacterial genes.

Target gene	Primary denaturation	Amplification (35 cycles)			Final extension
		Secondary denaturation	Annealing	Extension	
E. coli phoA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.
Salmonella invA	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.
Campylobacter 23S rRNA	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.
Y. enterocolitica 16S rRNA	94˚C 5 min.	94˚C 30 sec.	62˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.
C. perfringens alpha toxin	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.

2.8. Blood Sample

Ten milliliters of blood were collected from each animal via jugular venipuncture. The collected blood was added to plain tubes (i.e., without anticoagulants) and to others containing EDTA to yield serum or whole blood, respectively. All samples were cooled on crushed ice and were transported immediately to the laboratory for further processing. Tubes containing whole blood were used for CBC. Serum biochemical analyses using commercial test kits according to the standard protocols of the suppliers were carried out. The

following kits were used to quantify serum concentration of total protein, albumin, glucose and creatinine (Gamma Trade Company, Egypt); For BUN, (BioScien Egypt, Ref: BSU117100). calcium (BioMed, Egypt, REF: CAL103100); sodium and potassium (TECO Diagnostics Company, USA); chloride (Spinreact Company, Spain); and AST (aspartate aminotransferase) and ALT (alanine aminotransferase), (Spectrum Company, Egypt) on a selective chemistry analyzer (Apple 302, USA). Malondialdehyde (MDA) and super oxide dismutase (SOD) (Biodiagnostic Egypt, CAT No: MD2529 and CAT No: SD 25 21), respectively. Globulin was determined by the differences between total protein and albumin [28]. A/G ratio was calculated by dividing the albumin over globulin [29].

2.9. Statistical analysis

Statistical analyses were carried out using a statistical software program (SPSS, ver.20, Inc., Chicago, USA). The prevalence of different microorganisms was determined by dividing the number of positive samples by the total number of samples examined, and the results were expressed as a percentage. Similarly, according to the prevalence of AST (antimicrobial susceptibility testing) was performed and the susceptibility percentage was calculated as resistance, intermediate, and sensitive. The percentages of A chart displaying various antimicrobials was presented. Descriptive statistics were performed for all hematological and biochemical parameters. Student's t-test was used analyze the data. Results were considered statistically significant at P < 0.05.

3. Results

3.1. The clinical examination

Clinically, the common clinical signs that appeared on the control group were normal appetite, shiny coat, shiny eyes, their tail were fatty, normal defecation in form of small hard pellets and mucous membranes were light rosy, red in color. While ewes with diarrhea suffered from moderate to severe diarrhea, depression, dullness, loss of appetite and moderate degree of dehydration.

Table 4. Changes in temperature, pulse rate and respiratory rates (Mean±SE) in control (n= 250) and diarrheic (n= 250) Barki sheep.

parameters	Control sheep	Diarrheic sheep	P values
Temperature (°C)	38.9 ± 0.2	40.8 ± 0.1*	0.004
Pulse (Pulse/min)	83.3 ± 0.8	115 ± 2.8*	0.001
Respiration (Time/min)	25 ± 1.1	35.6 ± 0.8*	0.002

The feces were semi fluid to watery in consistency and grayish to yellowish green in color, contained mucous and sometimes blood, the perineum and tail were soiled with feces and mucous membranes were congested. Clinical examination results showed a significant (p < 0.05) increase of body temperature (38.9 ± 0.2 vs 40.8 ± 0.1 °C), pulse rate (83.3 ± 0.8vs 115 ± 2.8), and respiratory rate (25 ± 1.1vs 0.

35.6 ± 0.8) in healthy and diarrheic Barki ewes, respectively (Table 4).

3.2. Confirmation and prevalence of pathogens by PCR

A total of 250 samples of diarrheic Barki sheep were obtained. The pathogens were detected using PCR. E. coli, Salmonella, Campylobacter spp ,Y. enterocolitica, and Clostridium perfringens were identified by their band sizes of 720 bp, 284 bp, 650 bp, 330 bp, and 402 bp, respectively (Figure-1). There were 200 E. coli positives (80% prevalence), 170 Campylobacter positives (68%), 100 C. perfringens positives (40%), 75 Salmonella positives (30%), and 50 Y. enterocolitica positives (20%). (Figure-2)

Table 5. Changes in hematological value (Mean±SE) in control (n= 250) and diarrheic (n= 250) Barki sheep.

parameters	Control sheep	Diarrheic sheep	P values
WBC(×10^9/L)	9.4 ± 0.2	12.9 ± 0.2*	0.001
RBC (× 10^{12}/L)	9.9 ± 0.1	12.7 ± 0.1*	0.001
Hb (g/dl)	10.2 ± 0.1	14 ± 0.2*	0.001
PCV%	28.3 ± 0.4	34.5 ± 0.2*	0.001
MCV (fL)	38.7 ± 3.2	38.1 ± 3.7	0.82
MCH (pg)	11.7 ± 2.1	11.4 ± 1.4	0.88
MCHC (g/dl)	30.1 ± 3.4	31.4 ± 3.6	0.63
lymphocyte (× 10^9/L)	5.2 ± 0.1	6.9 ± 0. 1*	0.001
monocyte (× 10^9/L)	0.4 ± 0.04	0.6 ± 0.01*	0.006
neutrophil (× 10^9/L)	3.5 ± 0.05	5.3 ± 0.1*	0.001

Table 6. Some biochemical parameters (Mean±SE) of control (N=250) and diarrheic (N=250) Barki sheep.

parameters	Normal sheep	Diarrheic sheep	P values
Glucose (mg/dl)	100.3 ± 1.8	85.6 ± 1.7*	0.005
TP (g/dl)	4.6 ± 0.1	3.6 ± 0.2*	0.01
Albumen (g/dl)	2.6 ± 0.08	2.3 ± 0.2	0.2
Globulin (g/dl)	1.4 ± 0.02	1.2 ± 0.06	0.06
Creatinine (mg/dl)	1 ± 0.08	1.8 ± 0.2*	0.04
BUN (mg/dl)	24.6 ±2.1	40.6 ± 2.7*	0.01
AST (U/L)	38.3 ±1.4	53.6 ± 2.4*	0.005
ALT (U/L)	24.6 ± 1.4	36.6 ± 2.7*	0.01
Ca (mg/dl)	9.1 ± 0.1	7.4 ± 0.3*	0.01
Na (mmol/l)	182 ± 3.4	139 ± 3.9*	0.002
K (mmol/l)	3.3 ± 0.08	5.2 ± 0.06*	0.001
Cl (mmol/l)	120 ± 2.8	98 ± 5.5*	0.02
SOD (U/l)	67.6 ± 1.4	51.3 ± 4.3*	0.02
MDA (nmol/l)	2.4 ± 0.1	4.9 ± 0.2*	0.002

3.3. Ecoli AMR pattern

To determine the AMR pattern, a cultural sensitivity test against ten different antimicrobials was performed. *E.coli* had the highest resistance to penicillin (100%), followed by cefixime (88.8 %), tetracycline (69.2%), doxcycline and ampicillin (68%), amoxicillin (66%), trimethoprim-sulfaethoxazole (60%) and cefotaxime (52%). ciprofloxacillin

(64, 8%) and Gentamicin (56%) were relatively sensitive (Figure 3).

3.4. Salmonella spp. AMR pattern

Salmonella spp. was found to be highly resistant to ampicillin (64 %), amoxicillin (60 %), penicillin (56 %), tetracycline (52 %), and cefotaxime (12 %) in AMR and sensitivity testing. Ciprofloxacin (56%) was the most sensitive drug, while penicillin (8%) was the least sensitive. Doxycycline (60%) demonstrated moderate resistance to Salmonella spp., followed by Gentamicin (46%) and Penicillin (36%) (Figure 4).

3.5. Campylobacter spp. AMR pattern

Campylobacter spp. was found to be highly resistant to penicillin (88 percent), amoxicillin (84 %), erythromycin (84%), tetracycline (68%), and Ciprofloxacin (52 percent) in AMR and sensitivity testing. Gentamicin (92%), ampicillin, and Doxycycline (68 %) were the most sensitive drugs, while amoxicillin (4%) was the least sensitive (Figure 5).

3.6. Clostridium perfringens AMR pattern

Clostridium perfringens. was found to be highly resistant to gentamicin (76%), ampicillin (68%), amoxicillin and ciprofloxacin (60 percent). In AMR and sensitivity testing, penicillin (52 %), cefotaxime (64 %), tetracycline (40%), cefixime (44%), and gentamycin (zero %) was the least sensitive. Doxycycline and TP-SMX were found to have 72% moderate resistance to Clostridium perfringens (Figure 6).

3.7. Y.enterocolitica AMR pattern

Y.enterocolitica was found to be extremely resistant to penicillin (80%), ampicillin (76%), and amoxicillin (56%). In AMR and sensitivity testing, Ciprofloxacin (80%), tetracycline (76%), Gentamicin (34%), and TP-SMX (84%), were the most sensitive drugs, and streptomycin 50% demonstrated moderate resistance to Y. enterocolitica (Figure 7).

Hematological analysis of blood collected from diarrheic Barki sheep revealed that, there were a significant (P< 0.05) increase in total leucocyte count, TEC (RBCs), Hb and PCV, neutrophil, lymphocyte and monocyte count as compared with control ones; however no significant changes were observed in values of MCV, MCH, MCHC in diarrheic and control ewes. (Table 5)

Serum biochemical analysis of diarrheic sheep showed a significant reduction in the serum values of glucose, total protein, Na, Cl, Ca and SOD with significant increase in the serum levels of K, creatinine, blood urea nitrogen, MDA, AST and ALT as compared with control ewes. While there was non-significant (P≤ 0.05) decrease in levels of albumin and globulin (Table 6).

4. Discussion

Livestock is critical for food security and indeed a symbol of people's livelihoods in the deserts of the northwest coast

and Siwa oasis. However, animal infectious diseases are inflicting significant economic losses on the desert by interfering with production. Although vaccination and hygienic practices are two examples of the most effective preventative measures against these diseases, antibiotics are widely used either prophylactically or curatively in the livestock industry, as agents or therapeutics. This led to antibiotic resistance, which is a very critical problem. Because of this, rapid confirmatory diagnosis and the AMR pattern of pathogens have become very important for treating and preventing these infectious diseases and keeping them under control. The purpose of this study was to confirm the diagnosis of certain bacteria by using a rapid molecular diagnosis kit in sheep with diarrhea
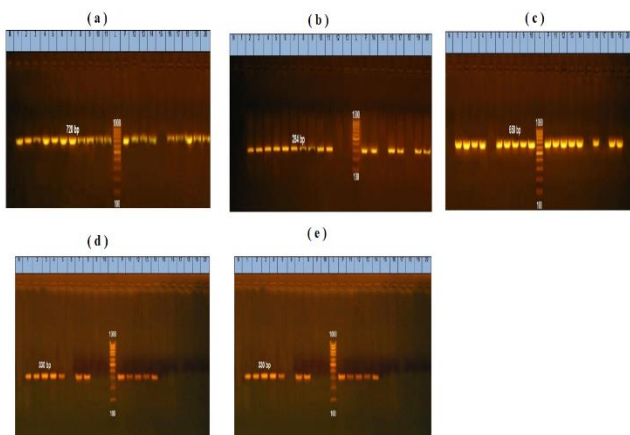
Figure-1. The result of the polymerase chain reaction assay in all samples.

Lane L denotes a 1 kb DNA marker, Lane N denotes a negative control, and Lane P denotes control DNA. (a) E. coli gene-sized (720 bp) amplicon, (b) Salmonella spp. invA gene identified from samples gene-sized (284 bp) amplicon, (c) Campylobacter 23S rRNA gene identified from samples (650 bp) and (d) Y. enterocolitica gene-sized (330 bp) amplicon, and (e) Clostridium perfringens alpha toxin gene was discovered in the samples' gene-sized (402 bp) amplicon
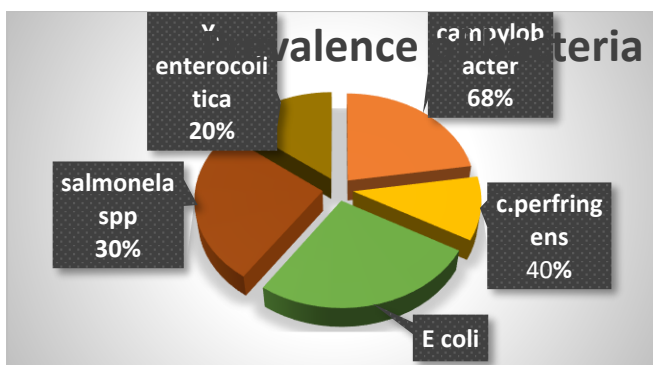

Figure-2 Prevalence of microorganisms confirmed by polymerase chain reaction

The clinical examinations of affected animals suffered from moderate to severe diarrhea were depression, dullness, loss of appetite and moderate degree of dehydration. The faeces was semi fluid to watery in consistency and grayish to

yellowish green in color, contained mucous and sometimes blood. The perineum and tail were soiled with feces. Mucous membranes were congested. This finding was in part similar to that given in sheep **[30-33]**. The recorded anorexia, depression and dullness may be attributed to muscular weakness due to escape of intracellular potassium, hyperkalemia and hypoglycemia **[10]**. There was a significant ($p < 0.05$) increase of body temperature, pulse rate and respiratory rate in diarrheic Barki ewes as compared with healthy ones which could be attributed to infection and inflammation **[34]**. These finding were in consistent with that shown in previous reports in ewes **[32]** and in buffalo calves **[30].**
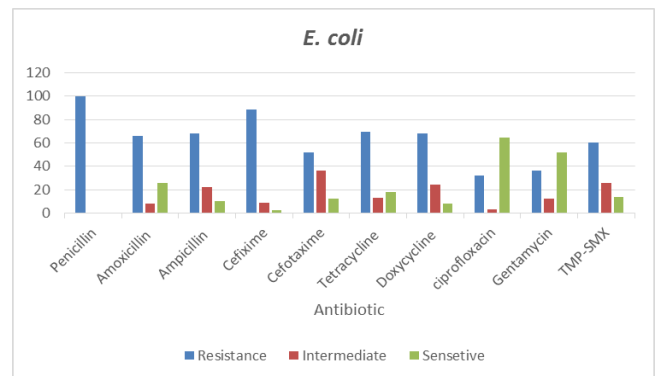

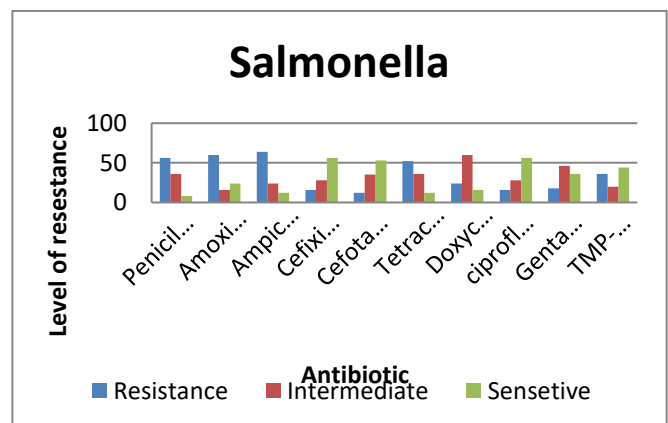**Figure-3:** Antimicrobial resistance pattern of *E.coli*

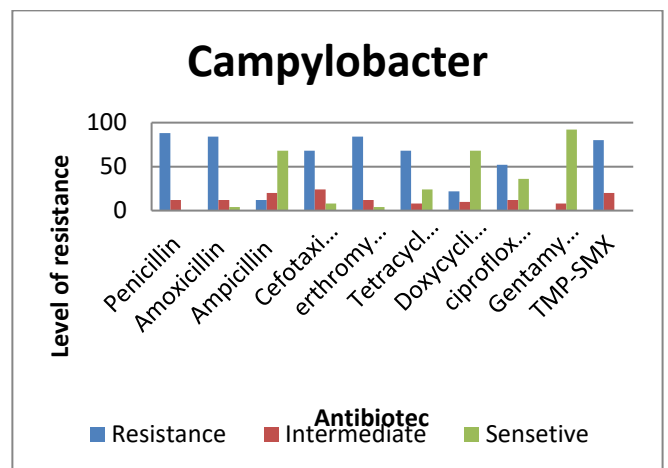**Figure4:** Antimicrobial resistance pattern of *salmonella spp*

Figure 5: Antimicrobial resistance pattern of *Campylobacter* spp
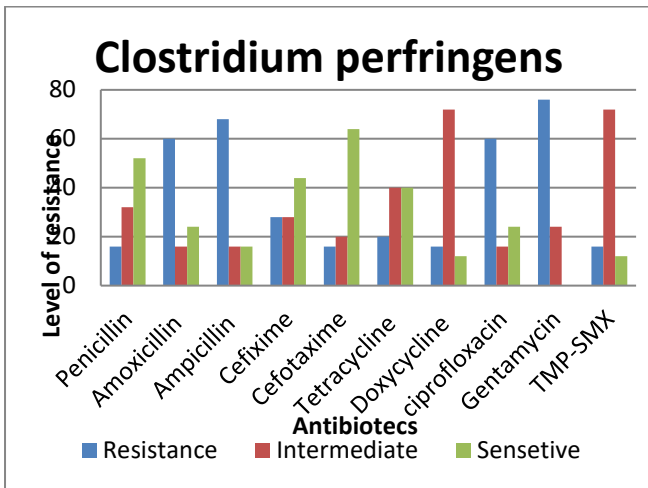
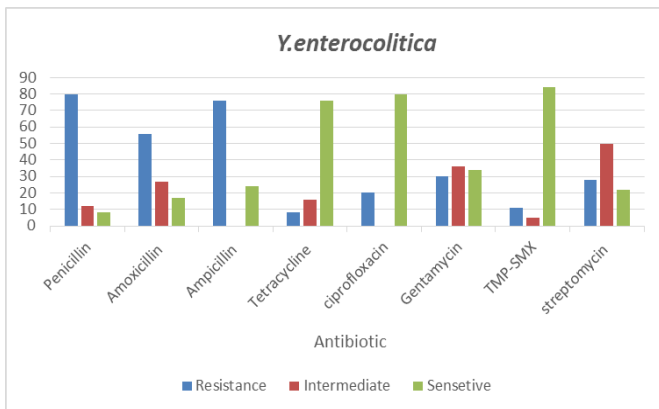**Figure 6:** Antimicrobial resistance pattern of *Clostridium perfringens*

Figure 7: Antimicrobial resistance pattern of *Y.enterocolitica*

Overall, 80% of the sheep in the Siwa oasis in Egypt were infected with E. coli. Nearly similar finding was observed by [35] in sheep (90%) in England, but away from that reported in sheep (69.7%) in Egypt **[36]**, in chickens (64.1%) in China **[37]**, in sheep (34.7%) and goats (30.7%) in Medina, Saudi Arabia **[38]** and in lambs (36.84%) in Nigeria **[39].** This difference could be caused by differences in geography and the environment. To determine the AMR pattern, a cultural sensitivity test against ten different antimicrobials was performed. E.coli had the highest resistance to penicillin (100%), followed by cefixime (88.8 %), tetracycline (69.2%), doxycycline and ampicillin (68%), amoxicillin (66%), trimethoprim-sulfaethoxazole (60%) and cefotaxime (52%) which was similar to the resistance pattern of E.coli isolated from sheep in northern Spain **[40, 41].** On the other side, the current study found that, ciprofloxacillin (64, 8%) and Gentamicin (56%) had relatively sensitive. This finding was in part similar to that given from drinking water sources in Ghana's Tamale Metropolis **[42].**

The prevalence rate of Salmonella spp. in diarrheic Barki sheep in Siwa oasis area was 30 %, which was significant and

should not be overlooked because of its significance public health and the high possibility of disease dissemination in animals and birds. This finding was in comparable to other studies conducted by **[43, 44]** from lamb in district Lahore and Pakistan, respectively, but higher than reported by **[45]** in Ethiopia from camel (15.9 %), **[39]** in Nigeria from lamb (15.79%), **[46]** from Iraqi sheep (7.2 %) and lower than that reported by **[47]** in Pakistan from goats (36.7%). All Salmonella isolates were tested against ten different antibiotics. Salmonella spp. was found to be highly resistant to ampicillin (64 %), amoxicillin (60 %), penicillin (56 %), tetracycline (52 %), and cefotaxime (12 %) in AMR and sensitivity testing. Ciprofloxacin (56%) was the most sensitive drug, while penicillin (8%) was the least sensitive. Doxycycline (60%) demonstrated moderate resistance to Salmonella spp., followed by Gentamicin (46%) and Penicillin (36%). In this instance, **[48]** reported that, salmonella isolates from goats were highly sensitive (83.33%) to ciprofloxacin and moderately sensitive (16.67%) to gentamycin; highly sensitive (66.7%) and moderately sensitive (33.33%) to gentamycin; moderately sensitive (66.67%) and less sensitive (33.33%) to oxytetracycline; resistant to suiphamethoxazole and penicillin-G (66% and 83.33%, respectively).

Campylobacter spp. prevalence was found to be 78 % in sheep in the Siwa oasis area, which was significant given that it is a major cause of gastroenteritis worldwide. Our findings were in consistent with those reported from lamb in Iran **[49],** but was lower than that recorded in (32%) of diarrheal Patients in Bangladesh **[50].** Campylobacter was found to be more prevalent in chicken meat (90%), lamb meat (38%), pork meat (31%), and beef meat (14%), according to **[51].** Campylobacter spp. was shown to be extremely resistant to penicillin (88%), amoxicillin (84%), erythromycin (84%), tetracycline, cefotaxime (68%) and Ciprofloxacin (52%). This conclusion is verified by **[52]** in Kumasi and is consistent with a comparable study undertaken in diarrheal patients in Finland **[53].** According to **[54],** the diarrheal sample from the Arabian Gulf region had more resistance to ciprofloxacin (80%) and tetracycline (70%) than other samples.

Clostridium perfringens prevalence was found to be 40% of 250 diarrheic Barki sheep. According to our study, it was greater than (26.7%) that was isolated from Egyptian camels **[55],** (33.33%) that isolated from dromedary camel calves in the Al Ahsa region of eastern Saudi Arabia **[56]**, in 30.3% of layers and 38.7%, of broilers in Egypt **[57],** but lower than that isolated from of (46.1%) of sheep and goats in Pakistan **[58],** that isolated from (64.3%) of camel minced meat in Egypt **(Fayez et al., 2021)[59]** and that isolated from sheep and goats (72.36% and 60%, respectively) in India **[60].** In AMR and sensitivity testing, clostridium perfringens was found to be highly resistant to gentamicin (76%), ampicillin (68%), amoxicillin and ciprofloxacin (60 %). These results were in consistent with previous findings **[61, 62].** On the other hand, cefotaxime (64 %), penicillin (52 %) and cefixime (44%) were the highest sensitive drug while gentamycin (zero %) was the

least sensitive drug. to Clostridium perfringens. Our findings were similar to earlier findings **[63, 64].** Doxycycline and TMP-SMX were found to have 72% moderate resistance to Clostridium perfringens.

Y. enterocolitica prevalence was found to be 20 % of sheep in the Siwa oasis area, which was lower than that reported by **[65]** in sheep in Sweden, **[66]** in 35% of sheep in southern New South Wales, **[67]** in 37.7 % of sheep in France. Our results were significantly higher than those reported by **[68]** in (8%) sheep and (10.2%) pigs in the United Kingdom and **[69]** in (11%) sheep in Finland. This difference in prevalence percentages could be attributed to differences between breeds, ways of getting samples, or time. In AMR and sensitivity testing, Y.enterocolitica was found to be extremely resistant to penicillin (80%), ampicillin (76%), and amoxicillin (56%) which nearly similar to the resistance pattern of Y. enterocolitica isolated from sheep in Greece **[70].** TMP-SMX (84%), Ciprofloxacin (80%), tetracycline (76%) were found to be the most sensitive drugs while penicillin (8%) was the lowest sensitive drug and streptomycin 50% demonstrated moderate resistance to Y. enterocolitica.

Antibiotics' widespread use in animal husbandry for therapy, prophylaxis, and growth enhancement has frequently been linked to the spread of resistance. Another thing that helps resistance grow and spread is intensive animal husbandry, which makes animals more likely to get clinical infections and leads to a lot of preventive use of medicines that might not be necessary. Antibiotics used as growth promoters in animal feed also contribute significantly to the spread of resistance. Antibiotics are often given to cattle and chickens around the world to help them grow, use their feed better, and make more.

Farmers use antibiotics to enhance growth and manage disease on their farms. Tetracyclines are the most frequently used antibiotics (oxytetracycline, doxycycline, remacycline, and chlortetracycline). This can result in sulphadimidine, dihydrostreptomycin, piperazine, albendazole, tylosin, ivermectin, and benzylpenicillin resistance, with the possibility of resistance cross-and co-resistance **[71].**

Haematological examination of diarrheic sheep demonstrated significant increase in PCV%, RBCs, Hb. count, WBCs, granulocyte, lymphocyte and monocyte count in diarrheic sheep compared with control ones. This increase in hematological parameters may be attributed to haemoconcentration, excessive loss of body fluid and dehydration associated with hypovolemia. Our finding was in harmony with that given by **[10, 31, 32, 72]**, but away from those obtained by **[30, 33, 73]** in buffalo calves, goat and sheep, respectively. In the later study, the authors have found that there was significant decrease in RBCs and PCV with non-significant increase in neutrophil, lymphocytes, and monocytes count in diarrheic animals. The degree of leucocytosis within different individuals was influenced by the severity of the infectious agent and the susceptibility of the animal to the infection **[20]**.

Hypoglycemia and hypoproteinemia were evident in diarrheic sheep, while there was non-significant decrease in levels of albumin and globulin in compared to control ones. These findings are in agreement with those reported by **[30, 32].** The occurrence of hypoglycemia in diarrheic sheep due to bacterial infection may be attributed to lack of glucose absorption from damaged intestine, while reduction in the levels of serum total protein and albumin in diarrheic sheep could be attributed to the destructive effect of bacteria or bacterial toxin on the liver cells resulting in impaired synthesis of albumin or malabsorption from the intestinal tract as recorded by **[74]**.

The present study revealed a significant low value of serum Na, Cl and Ca with significant increase in serum K in diarrheic sheep compared with control ones. This finding was in part similar to that given by **[10, 32, 75, 76].** The significant decrease in the serum calcium may be attributed to malabsorption, dehydration and its loss in feces **[75].** Hyponatremia, hypochloriemia in diarrheic sheep were attributed to direct loss of sodium and chloride ions via feces as well as failure of intestinal absorption **[30].** Hyperkalemia in diarrheic sheep could be attributed to increase renal tubular reabsorption of potassium in response to acidosis. Also it could be attributed to oligouria or anuria in which kidney failed to eliminate excess potassium **[77].**

The increased level of AST and ALT in diarrheic sheep can be because of inflammation of gastrointestinal tract of diarrheic sheep and cellular destruction of the liver and intestinal mucosa **[30, 75].**

The increased level of creatinine and blood urea nitrogen in diarrheic sheep could be attributed to decrease renal function and reduction in glomerular filtration rate and decrease urine production resulting from hypovolemia, systemic arterial hypotension and vasopressin release **[78]**. It could be also due to excessive production of urea by catabolism of body proteins in toxic conditions **[20]**. These findings were in agreement with those given by **[30].**

The present study revealed a significant low value of serum SOD with significant increase in serum MDA in diarrheic sheep compared with control ones which could be attributed to stress condition related to diarrhea **[28, 79].** These finding was in consistent with that shown by **[30, 32].** The decreased SOD in diarrheic sheep suggests the role of oxidative stress in the pathogenesis of enteritis, its low level leads to accumulation of oxidant substances and free radical that caused cellular damage to the intestinal lining mucosa, while higher MDA concentration in serum of diarrheic sheep suggests increased production of lipid peroxidation in the liver, and indirectly pointed to enhanced free radical generation, lipid peroxidation and oxidative stress **[80].** This result signifies the importance role of antioxidants as a therapeutic agent during prescription drugs for diarrhea in sheep.

Conclusion

Infectious illnesses are a significant impediment to the development of sheep husbandry in Egypt's Siwa oasis. In this investigation, the prevalence of Ecoli, Campylobacter spp., Clostridium perfringens, Salmonella spp., and Y. enterocolitica was determined to be 80%, 68.5%, 40%, 30%, and 20.5 %, respectively, in sheep. Rapid diagnostic approaches such as polymerase chain reaction (PCR) were found to be successful in confirming the presence of certain infections in sheep exhibiting non-specific clinical symptoms. Additionally, our current study is capable of determining which antimicrobials are effective against certain species in order to reduce antimicrobial resistance. There were a significant ($P < 0.05$) increase in total leucocyte count, TEC (RBCs), Hb and PCV, neutrophil, lymphocyte and monocyte count in diarrheic Barki sheep as compared with control ones. There was a significant reduction in the serum values of glucose, total protein, Na, Cl, Ca and SOD with significant increase in the serum levels of K, creatinine, urea nitrogen, MDA and activities of AST and ALT in diarrheic Barki sheep as compared with control ewes

Acknowledgments

Conflict of Interest statement

The authors declare that they have no conflict of interest.

Ethical approval

All procedures were performed in accordance with the guidelines of animal and poultry health department, Desert Research Center, ministry of agriculture and land reclamation, Egypt and approved by its Ethical Committees.

Authors' Contributions

Each author contributed equally to the design, execution, statistical analysis, and manuscript writing.

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