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Bacterial, mycological and immunological status in a mixed sheep and goat herd in El Fayoum Governorate

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ABSTRACT

The different symptoms of the herd were the motive to study the immunological state of the herd. Fifty sheep and eight goats of different ages from Herd with a history of recurrent abortions and suffering from abscesses, respiratory manifestation and diarrhea in El Fayoum Governorate, Egypt were investigated. 58 serum samples, 58 nasal swabs, 58 fecal swabs, 12 milk samples, 4 pus swabs, and 6 animal feeds samples were gathered to determine the bacteriological and fungal causative agents. The traditional culture methods for diagnosing *Brucella melitiensis* were confirmed by the polymerase chain reaction (PCR) test. Sensitivity test was applied to detect the effective antibiotic and antimycotic for each field strain isolate and to avoid microbial resistance.

Our research showed different prevalence of bacterial and fungal isolated from obtained samples from sheep and goat herd. Tested animals showed mixed infections with bacterial and mycotic pathogens represented 60.34%, bacteriological infection only was 5.17 % and mycotic infection only was 27.58% that reflected on immunity parameters and drugs sensitivity results.

INTRODUCTION

Small ruminants can develop diarrhea, abortions, abscesses, mastitis, and lung symptoms because of an abrupt shift in diet, poisonous plant consumption, or bacterial, fungal, or viral diseases. Acute and chronic diarrhea can be fatal and last for days or even weeks **(Gay et al. 2000; Thornton 2010)**. Respiratory disorders diminish animal productivity and have the potential to result in large losses in animal husbandry. Sheep abortions can occur occasionally or during enzootic outbreaks caused

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by bacteria, viruses, chlamydia, rickettsia, fungus, and protozoan diseases, requiring consideration of economics, animal health, and zoonotic risks (**Vidić et al. 2007**). Brucellosis is caused by Brucella melitensis in small ruminants, with global implications for human health and the economy **(Nagati and Safaa 2016)**. Evidence of a positive relationship between seroprevalence of abortion-causing viruses and numerous risk variables necessitates further research to be better understanding the etiology of infectious abortions in flocks and to develop an efficient prevention and control programs **(Guesmi et al. 2023)**. Molecular techniques like PCR and ELISA are more accurate and sensitive than traditional approaches **(Sunday et al. 2016)**. Staphylococcus spp. is opportunistic bacteria that cause mastitis and can be found in apparently healthy carriage. It can survive in the environment and spread by air, direct contact with another person, or secretion. In ruminants, the nasal carriage, along with the skin and mucous membranes, serve as main reservoirs **(Seyffert et al. 2012)**.

Salmonellosis is the most common foodborne and economically significant zoonotic infection, affecting sheep of all ages and sexes. While septicemia is more prevalent in young animals, adult sheep can develop acute enteric salmonellosis, which can result in fever, anorexia, depression, and diarrhea. There have also been instances of asymptomatic carriage, diarrhea, and abortion. Salmonella incidence was found to be 23.3% in sheep and 7% in goats **(Carvajal-Restrepo et al. 2017; Hawwas et al. 2022)**. *Pseudomonas aeruginosa* causes a variety of disorders, including caseous lymphadenitis and hepatic abscess disease. Sheep pneumonia is usually associated with *Klebsiella oxytoca* and *K. pneumoniae*, which are rod-shaped, capsulated Enterobacteriaceae that are non-motile, gram-negative, and cause diarrhea in farm animals **(van der Weide et al. 2019)**. Multi-antibacterial resistance is quickly spreading and posing significant health risks **(Alyassari et al. 2019)**. *E. Coli* was the most commonly isolated in pure or mixed cultures from lambs with diarrhea ranging from moderate to profuse. In severe circumstances, the animal becomes dehydrated **(Chatzopoulos et al. 2016)**. Fungi are the second leading cause of disease in sheep and goats. Opportunistic mycoses produce Candidiasis, Melanized fungal infections, Aspergillosis, Mucormycosis, and Cryptococcosis. These diseases ranged from localised infections to fatal widespread illnesses (**Seyedmousavi et al. 2018**).

Animal aspergillosis refers to a variety of diseases, including Aspergillus-related allergies and deadly infections that are either localized or rapidly spread (**Seyedmousavi et al. 2015**). *Aspergillus fumigatus* is the predominant cause of animal aspergillosis, with a few additional species occasionally contributing to the disease (**Heitman 2011**). *Aspergillus fumigatus* has been linked to ruminant pneumonia, mastitis, diarrhea, placentitis, and miscarriages around the world. Mucormycosis, a saprophytic opportunistic infection, is caused by the Mucorales order of the Zygomycetes class (**Hoffmann et al. 2013**). The most commonly recognized genera include Rhizopus, Mucor, Apophysomyces, Cunninghamella, Rhizomucor, Lichtheimia (previously Absidia), and Saksenaea. Mucorales are usually separated from food, dust, and air from both indoor and outdoor sources (**Hoffmann et al. 2013**). *Candida albicans*, an opportunistic fungus, is a main cause of mucosal infections in the gastrointestinal system, urogenital tract, and skin. A gastrointestinal tract fungus can spread and cause candidemia or localised infections of internal organs (**Miranda et al. 2009; Brown et al. 2012**).

Candida species were detected in small ruminants with mastitis (**Hassan et al. 2012**). The available published works addressed experimental Candidal mastitis in sheep and goats infected by *C. albicans*, as well as Candidal abortion in sheep (**Singh et al. 1997**; **Ophelders et al. 2016**). In contrast, C. zeylanoides was isolated from raw goat milk (**Fadda et al. 2010**). Animal candidiasis should be investigated as a treatment option, particularly if the hosts are resistant to medicines. Rhodotorula, a member of the Basidiomycota phylum, is widespread environmental yeast found in fruit juice, milk, soil, lakes, and the air. Rhodotorula spp. has been associated with skin infections in poultry, lung infections in sheep, and bronchotracheitis in dogs (**Biegańska et al. 2018**).

Aim of the work:

The purpose of this research is to determine the microbiological reasons of repeated abortions, abscesses, respiratory problems, and diarrhea in diseased sheep and goat herd in El-Fayoum Governorate. The different recorded symptoms of the herd were the motive to study the immunological state of the herd.

MATERIALS AND METHODS

Ethical Approval:

The Institutional Animal Health Research Ethics Committee approved this research, which was conducted in compliance with local laws and regulations.

Collection of Samples:

Fifty diseased sheep and 8 goats of various ages were collected from herd in Egypt's Fayoum governorate. They include 17 animals with diarrhea and abscesses, 31animals with abortions and 10 animals with respiratory symptoms. 196 samples were collected, including 58 serum samples, 58 nasal swabs, 58 fecal swabs, 12 milk samples, four pus swabs, and 6 feed samples. The collected samples were placed in sterile polyethylene bags and retained in an icebox before transporting to the laboratory under stringent aseptic conditions to be evaluated. Table 1 illustrates the distribution of samples from sheep and goats

Table 1. Sample distribution of the studied sheep and goats

Species	No of animal	Types of samples						
		Serum	Nasal	Fecal	Milk	Pus swab	Feed	Total
Sheep	50	50	50	50	10	4	3 dry	167
Goat	8	8	8	8	2	0	3 green	29
Total	58	58	58	58	12	4	6	196

Bacteriological Examination

Milk samples were aerobically incubated at 37ºC for 24 hours and centrifuged at 3000 rpm for 20 minutes. After discarding the supernatant, a sterile loopfull of sediment was collected. Nasal, fecal, and pus swab samples placed in broth containing tubes were incubated overnight at 37°C. All samples were streaked across the surfaces of blood agar and MacConkey agar plates. The inoculation plates were maintained at 37ºC nearly two days. To obtain pure cultures, suspicious colonies were marked on nutrient agar slants and cultured at 37ºC for a day. Bacterial isolates are presumptively diagnosed using colony morphology, Gramm stain reaction, hemolytic properties, and biochemical testing (**Quinn et al. 2011**)

Brucella Isolation

A loopful of previously prepared milk sediment was added to the surface of Brucella spp. agar plates, along with an antibiotic medicine supplement, and incubated at 37°C in a 10% CO_2 atmosphere. Brucella spp. growth was monitored on cultured plates beginning on day 4 and continuing daily for the next four weeks. The suspicious colonies were recognized and subcultured onto Brucella spp. agar slants. The identification of Brucella spp. is based on morphological appearances, microscopic appearance, and reactivity. Brucella isolates were classified based on their CO_2 requirement, H2S production, dye-induced proliferation, reactivity with monospecific sera (IgA and IgM), and bacteriophage type (Tiblisi phage; Central Veterinary Laboratory, Wybridge, UK), using the technique outlined by **Alton et al. (1988)**.

Serological diagnosis:

1-Indirect diagnosis (Rose Bengal Agglutination Test, RBT):

The Rose Bengal-stained antigen was attained from the Veterinary Serum and Vaccines Research Institute (VSVRI) in Abbasia, Cairo,

Egypt. The antigen was taken to room temperature before being tested. Apply 30 µl of serum to a dry, white enamel plate with a micropipette, and then add a drop of Bengal-stained antigen. A toothpick or glass rod was used to thoroughly combine the antigen and serum in a circular motion. The plate was shaken by hand for four minutes. Presence of agglutination was considered a positive result (**Alton et al.** 1988).

2-Direct Diagnosis

Conventional PCR assay:

DNA extraction from samples was carried out by means of the QIAamp DNA Mini kit (Qiagen, Germany, GmbH). The bacterial pellet was treated with 10 µl of proteinase K and 200 µl of lysis buffer at 56 °C for 10 min. Following incubation, 200 µL of 100% ethanol was added to the lysates. The sample was cleaned and centrifuged in accordance with instructions. Nucleic acid was eluted using 100 µl of the kit's elution buffer. Primers were supplied from biobasic (Canada). Primers sequences, target gene and cycling conditions for PCR assay according to **(Bricker and Halling (1994)** targeting 1S711 gene as listed in table (2).

Table 2. Primers sequences, target gene and cycling conditions for PCR assay

Strain	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)			
			Primary Denaturation	Secondary Denaturation	Annealing	Extension
Brucella abortus	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT	498	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.
	B. abortus-specific Primer GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC					
Brucella melitensis	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT	731	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.
	B. melitensis-specific Primer AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA					

Mycological Examination:

The collected samples (nasal, fecal, and pus swabs, milk, and animal feeds) were tested for fungal isolation in line with **ISO 21527-2 (2008)** and **Refai et al. (2014)**. Moulds were identified based on colony morphology, growth rate, and microscopic morphology of isolates in both direct culture and micro-slide culture mount and procedure. Yeast isolates were also identified **(Koneman et al. 1992 and Refai et al. 2014)**.

Antimicrobial Sensitivity Tests:

Bacterial and fungal isolates were added to the nutritious broth. For bacterial examination, samples were cultivated at 37°C for 18 hours, and for fungi, at 25°C for 1-3 days. To assess antimicrobial sensitivity, samples were diluted with normal saline and adjusted to 1.5×10^8 CFU/ml using standard McFarland tube number 0.5, following **(Quinn et al. 2011)**. Each bacterial and fungal isolate's antimicrobial susceptibility to various widely used antibiotics was tested using the disc diffusion technique with commercially available discs, following guidelines **(Gupta and Kohli 2003; CLSI 2018**). Antimicrobial agents included amikacin (30 µg), ampicillin (10 µg), amoxicillin (10 µg), cefotaxime (30 µg), sulphamethoxazole + trimethoprime (25 µg), erythromycin (15 µg), amoxicillin + clavulinic acid (30 µg), enrofloxacin (5 µg), gentamycin (10 µg), and tetracycline (30 µg) were obtained from Oxoid. The antifungals utilised included Fluconazole (10 µg), Voriconazole (1 µg), Itraconazole (10 µg), Nystatin (100 µg), and Amphotericin B (100 µg) from Oxoid. The plates were incubated for 2-5 days at 28-37°C (fungi) and 24-48 hours at 37°C (bacteria). The experiment was conducted three times using pooled data.

Immunological test

1- Measurement of Lysozyme Activity:

Lysozyme activity was determined using the method described by **(Schultz 1987**).

2- Detection of serum oxidant and antioxidant:

Nitric oxide (NO), malondialdehyde (MDA), and total antioxidant capacity (TAC) concentrations were estimated according to **Rajaraman et al. (1998)**, **Ohkawa et al. (1979)**, and **Koracevic et al. (2001)**, respectively

Statistical analyses:

The obtained data was exposed to analysis of variance (one-way ANOVA test) by IBM SPSS 23 Statistics for Mac OS, Armonk, NY, USA.

RESULTS

Bacteriological examination of nasal swab revealed that *Staphylococcus aureus*, *Klebsiella pneumonaea*, *E. coli*, and *Pseudomonas Aureginosa* represented 8.62 %, 10.34%, 10.34% and 13.79%, respectively. The bacterial isolates from fecal swabs were *Salmonella typhimurium*, *Enterococcus Faecalis*, *Klebsiella pneumonaea* and *E. coli* as the value being 24.13%, 17.24%, 10.34 and 13.79%, respectively. Examination of 12 milk samples revealed isolates *Strept agalactiae*, *Staphylococcus aureus*, *E. coli* and *Brucella mellitensis represented 66.67%, 16.67%, 8.33% and 41.67%* , respectively while *Staphylococcus aureu*s was isolated from four sheep suffering from abscess as found in table 3.

Table 3. Prevalence of bacteria isolated from nasal, fecal, milk, and pus swabs samples from sheep and goat herd

Isolates Bacterial spp.	No. of isolates	Nasal swabs (N.58)		Fecal swabs (N.58)		Milk samples (N.12)		Pus swap (N.4)	
		No	%	No	%	No	%	No	%
Salmonella typhimurium	14	-	-	14	24.13	-	-	-	-
Strept agalactiae	8	-	-	0	0	8	66.67	-	-
Enterococcus Faecalis	10	-	-	10	17.24	-	-	-	-
Staphylococcus aureus	11	5	8.62	-	-	2	16.67	4	100
Klebsiella pneumonaea	12	6	10.34	6	10.34	-	-	-	-
E. coli	15	6	10.34	8	13.79	1	8.33	-	-
Pseudomonas Aureginosa	8	8	13.79	-	-	-	-	-	-
Brucella mellitensis	5	-	-	-	-	5	41.67	-	-

N. = number of examined samples
%: was calculated according to the number of examined samples

The detection of brucella spp in serum and milk samples was confirmed using Rose Bengal test. It is found 16 (27.6 %) samples were positive in serum samples and 5 represented 41.66 % in milk samples as reported in table 5 and figure 1.

Table 4. Seroprevalence of Brucella infection in serum and milk by Rose Bengal test and culture

Type of animals	No. of serum samples	No. of +ve RBT	No. of milk sample	+ve Result by PCR
Sheep	50	14	10	4
Goat	8	2	2	1
Total	58	16	12	5

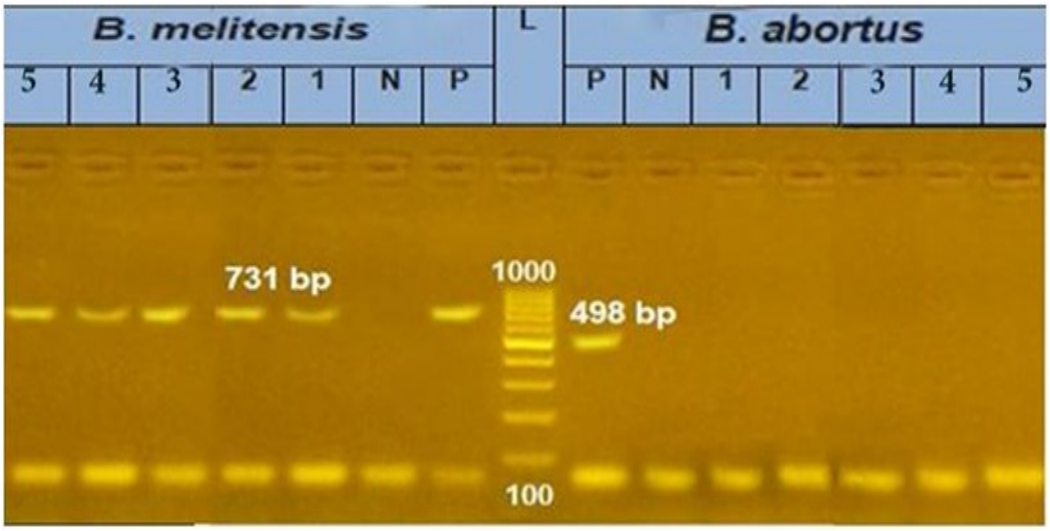

Figure (1): By using PCR amplification for detection of *B. melitensis* and *B.abortus* five DNA extracted from four sheep and one goat milk culture showed positive for *B.melitensis* at 731bp in lane 1, 2, 3, 4 sheep milk and 5 milk goats while negative for *B. abortus* at 498bp respectively. Lane P: Control Positive *B. Melitensis* and *B. abortus*. Lane N: control Negative *B. Melitensis* and *B. abortus* Lane L: Marker 100 bp.

The results of mycological examination of the collected nasal, fecal, pus swabs, milk and animal feeds samples from 50 sheep and 8 goat revealed that the total isolated yeast species were 36 Candida species, 4 *Geotrichum candidum*, and 18 *Rhodotrula mucilaginosa* represented 26.08%, 2.90%, 13.04%, respectively. On the other hand, the total mould isolates from different samples were 30 Mucor species (21.74%), 20 Rhizopus species (14.49%), 27*Aspergillus niger* (19.57%), 22Aspergillus flavus (15.9%), 8 *Aspergillus candidus* (5.80%), 7 *Aspergillus fumigatus* (5.07%), 7 *Aspergillus terrus* (5.07%), 12 Penicillium species (8.70%), and 3 Fusarium species (21.7%) as described in tables (5).

Table 5. Fungal isolates from nasal, fecal, pus swabs, milk and animal feeds samples from sheep and goat herd

Samples Fungal species	Nasal (58)		Fecal (58)		Pus (4)		Milk (12)		Animal feeds (6)		Total samples (138)	
	No	%	No	%	No	%	No	%	No	%	No.	%
Total *Yeast species*												
Candida species	16	27.59	14	24.14	2	50	3	25	1	16.67	36	26.08
Geotrichum candidum	1	1.72	1	1.72	1	25	-	-	1	16.67	4	2.90
Rhodotrula mucilaginosa	9	15.52	7	12.07	1	25	1	8.33	-	-	18	13.04
Total *Mould species*												
Mucor species	12	20.69	15	25.86	1	25	-	-	2	33.33	30	21.74
Rhizopus species	9	15.52	10	17.24	-	-	-	-	1	16.67	20	14.49
Aspergillus niger	14	24.14	11	18.97	-	-	1	8.33	1	16.67	27	19.57
Aspergillus flavus	10	17.24	8	13.79	1	25	1	8.33	2	33.33	22	15.94
Aspergillus candidus	4	6.90	2	3.44	1	25	-	-	1	16.67	8	5.80
Aspergillus fumigatus	6	10.34	1	1.72	-	-	-	-	-	-	7	5.07
Aspergillus terrus	3	5.17	4	6.90	-	-	-	-	-	-	7	5.07
Penicillium species	4	6.90	5	8.62	1	25	-	-	2	33.33	12	8.70
Fusarium species	1	1.72	1	1.72	-	-	-	-	1	16.67	3	2.17

N. = number of examined samples
%: was calculated according to the number of examined samples

Fungal isolated from all the diseased animals. Animals with diarrhea and abscesses had highest level of *Rhodotrula mucilaginosa*, *Aspergillus niger*, *Aspergillus flavus* and Mucor species while animals with abortions had highest level of *Rhodotrula mucilaginosa*, Candida species, Rhizopus species, *Aspergillus niger*. Animals with respiratory symptoms had highest level of Candida species, Mucor species, *Aspergillus niger*, and *Aspergillus flavus*. Moreover Mucor species, Candida species, *Aspergillus niger*, and *Aspergillus flavus* were the most prevalence in all diseased animals as shown in table (6)

Table 6. Prevalence of fungal isolated in different animal groups with different symptoms

Fungal species	Animals with diarrhea and abscesses (17)		Animals with abortions(31)		Animals with respiratory symptoms (10)		Total No. of Animals (58)	
	+ve	%	+ve	%	+ve	%	+ve	%
Yeast species								
Candida species	2	11.76	2	6.45	10	100	14	24.13
Geotrichum candidum	1	5.88	1	3.22	1	10	3	5.17
Rhodotrula mucilaginosa	4	23.53	2	6.54	3	30	9	15.51
Mould species								
Mucor species	4	23.53	2	6.54	9	90	15	25.86
Rhizopus species	3	17.64	3	9.68	5	50	11	18.96
Aspergillus niger	5	29.41	3	9.68	6	60	14	24.13
Aspergillus flavus	5	29.41	2	6.54	6	60	13	22.41
Aspergillus candidus	1	5.88	2	6.54	1	10	4	6.90

N. = number of examined samples
%: was calculated according to the number of examined samples

Only 6.89% from the herd was free from bacterial and mycotic infections while mixed infections with mycotic and bacterial pathogens were 60.34%, bacteriological infection only was 5.17 % and mycological infection only was 27.58%as shown in table (7)

Table 7. Distribution of different category of infection among herd

Total No. of animal	-Ve bacteriological & mycological isolation		+Ve bacteriological isolation only		+Ve mycological isolation only		+Ve bacteriological & mycological isolation	
	No	%	No	%	No	%	No	%
58	4	6.89	3	5.17	16	27.58	35	60.34

N. = number of examined samples
%: was calculated according to the number of examined samples

E coli was more sensitive to amikacin, gentamycin, cefepime and amoxicillin with clavulenic acid while *Salmonella typhimurium* was more sensitive to ciprofloxacin and enrofloxacin followed by amikacin, cefotaxim, cefepime and gentamycin. *Staph aureus* was highly sensitive to amikacin, gentamycin, and amoxicillin with clavulenic acid, followed by cefotaxim and cefepime. *Strept agalactiae* was highly sensitive to amikacin, cefepime, gentamycin, and amoxicillin with clavulenic acid followed by cefotaxim and ciprofloxacin. *Enterococcus fecalis* was highly sensitive to amikacin, ciprofloxacin, gentamycin, and amoxicillin with clavulenic acid followed by cefepime and cefotaxim.

Pseudomonus aureginosa was more sensitive to amikacin and amoxicillin with clavulenic acid. *Brucella mellitensis* was more sensitive to amikacin and ciprofloxacin. *Klebsiella pneumonia* was highly sensitive to amikacin, cefotaxim, cefepime, and gentamycin followed by ciprofloxacin, enrofloxacin and amoxicillin with clavulenic acid as illustrated in table 9.

Table 8. Antibiotics sensitivity tests for bacterial isolates

Bacterial isolates	E. coli (15)		Salmonella typhimurium (14)		Staph aureus (11)		Strept agalac-tiae (8)		Entero-coccus Fecalis (10)		Pseudo-monus aureginosa (8)		Brucella mellitensis (5)		Klebsiella pneumonia (12)	
Antibiotics	S	%	S	%	S	%	S	%	S	%	S	%	S	%	S	%
Tetracycline (30 µg)	9	60	4	28.57	6	54.55	2	25	6	60	2	25	3	60	5	41.67
Amikacin (30 µg)	14	93.33	13	92.86	11	100	8	100	10	100	6	75	4	80	12	100
Cefotaxime (30 µg)	11	73.33	13	92.86	9	81.81	7	87.5	9	90	5	62.5	3	60	12	100
Ciprofloxacin (5 µg)	9	60	14	100	7	63.64	7	87.5	10	100	5	62.5	4	80	10	83.33
Amoxicillin (10 µg)	2	13.33	1	7.14	4	36.36	4	50	3	30	1	12.5	0	0	2	16.67
Ampicillin (10 µg)	0	0	0	0	1	9.01	1	12.5	1	10	0	0	0	0	0	0
Cefepime (30 µg)	13	86.67	13	92.86	9	81.81	8	100	9	90	5	62.5	3	60	12	100
Gentamycin (10 µg)	14	93.33	13	92.86	11	100	8	100	10	100	6	75	3	60	12	100
Erythromycin (15µg)	2	13.33	1	7.14	4	36.36	6	75	5	50	3	37.5	0	0	3	25
Enrofloxacin (5 µg)	9	60	14	100	7	63.64	2	25	7	70	5	62.5	3	60	10	83.33
Sulphamethoxazole +Trimethoprime (25 µg)	4	26.67	1	7.14	5	45.45	4	50	5	50	3	37.5	1	20	5	41.67
Amoxicillin + Clavulenic acid (30 µg)	12	80	11	78.57	11	100	8	100	10	100	6	75	2	40	10	83.33

S: mean sensitivity
%: was calculated according to the number of examined samples

Itraconazole was the drug of choice in descending manner for Candida species, *A. lavus*, *A. fumigatus*, Rhodotorula and *A. niger* while nystatin was most effective to *A. fumigatus* and Candida species, followed by *A. flavus* and *A. niger*. Amphotericin B was most effective to Rizopus and Mucor as illustrated in table 10

Table 9. Antifungal sensitivity tests for fungal isolates

Antifungal Agent / Fungal Isolates	Fluconazole (10 µg)		Voriconazole (1 µg)		Itraconazole (10 µg)		Nystatin (10.µg)		Amphotericin B (100 µg)	
	S	%	S	%	S	%	S	%	S	%
A. lavus (10)	1	10	3	30	9	90	9	90	6	60
A. niger (10)	2	20	4	4	8	80	8	80	5	50
A.fumigatus (10)	1	10	7	70	9	90	10	100	0	0
Candida spp. (10)	0	0	5	50	10	100	10	100	5	50
Rhodotorula (10)	0	0	0	0	9	90	0	0	4	40
Mucor(10)	0	0	0	0	7	70	0	0	9	90
Rizopus(10)	0	0	0	0	8	80	0	0	10	100

S: mean sensetivity
%: was calculated according to the number of examined samples

Animals of +Ve mycological isolation and animals of +Ve for both bacteriological and mycological isolation had significant highest level of lysozyme, nitric oxide, malondialdehyde levels and lowest level of total antioxidant comparing to animal with –Ve both bacterial and mycological isolation and animals of +Ve bacteriological isolation as recorded in table 10

Table 10. Serum lysozyme, Nitric oxide, Malondialdehyde and Total antioxidant levels in tested animals.

Groups / Immune parameters	-ve bacteriological &mycological isolation animals	+Ve bacteriological isolation animals	+Ve mycological isolation animals	+Ve bacteriological &mycological isolation animals
Lysozyme (µg/ml)	6.22 ± 0.61 ^{A}	13.7 ±3.4 ^{B}	59.5± 19.4 ^{ab}	53.7± 7.6 ^{a}
No (µmol/ml)	4.4 0± 0.92 ^{A}	7.23 ±0.62 ^{B}	11.11± 0.7 ^{ab}	11.12± 1.21 ^{ab}
MDA (nmol/ml)	0.5± 0.04 ^{A}	0.73 ±0.01 ^{B}	0.78 ±0.03 ^{C}	1.4 ±0.15 ^{abc}
TAC (nmol/ml)	2.01 ±0.07 ^{A}	1.68 ± .04 ^{aB}	0.8 ±0.01 ^{abC}	1.53 ±0.18 ^{ac}

Small litters indicate significantly different between groups against their capital litters in the same row.
The mean difference is significant at the level, $P<0.05$. Values represent means ± SD

DISCUSSION

Sheep and goats are the second most consumed farm animal meat after cattle However, they pose an economic and potentially public health risk because many diseases may be carried subclinically in these animal reservoirs (**Hawwas et al. 2022**). Most of sheep and goats in El Fayoum governorate in Egypt are living in herd which taken to the field early morning every day for feeding by green rations from the residues of crops and return home just before sunset (**Nagati and Safaa 2016**).

PCR is a valuable diagnostic means for virulence genes recognition since it is sensitive, specific and adopted as a complementary tool to conventional tests (**Awad and Awad 2021**). Our results were agreed with **Guesmi et al. (2023)** examined 793 blood samples from 26 sheep flocks and found the overall seroprevalence rate of brucellosis nearly 16.1%. **Mathur et al. (2022)** reported that serology could not distinguish between infected and vaccinated animals.

Several authors detected the prevalence of bacteria isolated from nasal, fecal, milk, and pus swabs samples from small ruminants. **Mahmoud et al. (2005)** isolated staphylococcus aureus, E. coli, and Pseudomonas aerogenosa from nasal swabs in 15 %, 11.7 %, and 5 %, respectively. **Sayed (1996)** discovered that E. coli was the utmost common agent in sheep and goats, at 34.7% and 30.7%, respectively, which was higher than our findings. Salmonella species were discovered in 3.6% of sheep and 2.6% of goats, whereas Klebsiella species were found in 1.6% of sheep but not in goats, which was lower than our findings. **Mahmoud et al. (2005)** isolated (15 %) *S. aureus*, (11.7 %) *E. coli* and (8.3 %) *K. pneumonia* isolated from nasopharyngeal swabs from sheep and goats with respiratory diseases in Abu-Ramad, Halaieb, and Shalateen Areas, Egypt. **Hassan et al. (2019)** isolated 46.6 % *S. aureus* and 43.3 % E. coli and detected *S. agalactiae* from bovine milk samples as a mixed infection samples.

The importance of antibiotic susceptibility tests is the selection of drugs with a low risk of resistance to suspected pathogenic organisms. Because of specific genes that may be carried on the bacterial chromosome, plasmids, transposons, or gene cassettes that are inserted into integrons code these features (**Williams 2000**).

Our results showed that Amikacin was highly effective (75 -100 %) due to rarely used in veterinary field and Gentamycin, cefotaxime and Enrofloxacin sensitivity were (50–100%) with all isolates. While ampicillin, amoxicillin resistance were (50 –100%), Erythromycin and tetracycline were (37 -75%) as illustrated in table 8. The drug sensitivity test results for the investigated bacteria were consistent with **Tello et al. (2020)** findings. The study found resistance to extended-spectrum cephalosporins and other antimicrobials in 110/135 isolates, including tetracycline, sulfamethoxazole, ciprofloxacin, and trimethoprim. *Escherichia coli* produce extended spectrum lactamases (ESBLs) as a resistance mechanism to lactam antibiotics, followed by cephalosporinases and carbapenemase enzymes. ESBLs can break down penicillins, third-generation cephalosporins, and monobactams. They are not as effective against cephamycins as cefoxitin or carbapenems, but they are vulnerable to lactamase inhibitors such as clavulanic acid. Unlike ESBLs, ampC-type lactamases are active against cephamycins and resist clavulanate inhibition. **Mahmoud et al. (2005)** conflicted with our results as stated that S. aureus isolates were sensitive to Ampicilline, Enrofloxacin, erythromycin, and tetracycline, we agree with them in the case of *E. coli* isolates sensitivity for Enrofloxacin. This high resistance against bacterial isolates, due to random uncontrolled use from paramedical and owners, possibly to decrease stress and infection and probability of recirculation of resistant isolates.

In this study, the mycological examination revealed the isolation of 194 fungal isolates 58 (42%) yeasts and 136 molds (98.6 %) as represented in tables 5, 6&7. Moulds were the predominant fungal infections (98.6%) among all examined samples (Nasal swabs, fecal swabs, pus swabs, animal feeds and milk samples) than yeast infections (13.5%). The high reappearance of moulds isolates coincides with **Sarfati *et al.* (1996)** and **Abd El Tawab et al. (2021)** who stated that Aspergillosis, candi-

dosis, and zygomycoses were the most common mycotic infections in ruminants. According to **Jensen et al. (1992)**, the respiratory and gastrointestinal systems are entry points for mycotic infections. **Hassan et al. (2012)** discovered that yeasts isolated from sheep and goats suffered from diarrhea were more prevalent than those extracted from healthy animals. Yeasts can infect digestive tracts of animals when they are placed on contaminated soils with yeast pathogens as plant materials in animal feed can be inoculated by contaminated dust generated when soil is disturbed by strong wind or rain. Other healthy animals might lick the perianal region of animals affected with anal candidiasis resulting in oral candidiasis and, eventually, gastrointestinal tract candidiasis (**Donskey 2004**). Fungi, opportunistic pathogens with a diverse set of virulence traits, allow them to multiply and infect the host. Pathogenic funguses produce proteases and phospholipases, which breakdown protein and lipid-containing tissues and may explain the diarrhea of pathogenic fungal-infected animals (**Hube 1998**). Fecal samples had more yeast than milk and rectal swabs of small ruminants, indicating that the gastrointestinal tract contributes as a significant reservoir for Candida species (**Abou-Elmagd et al. 2011**).

Asperigillus spp. causes gastroenteritis in ruminants. *C. albicans* was found in diseased sheep and goats by examination of the vaginal swabs of (40% and 20%, respectively), and only 8% in apparently healthy goats. The mastitic milk caused a nearly identical widespread C. albicans infection, which isolated from 24% and 32% of mastitis cases in goats and sheep, respectively **(Atwa and Rady 2007; Maneenil et al. 2015; Ophelders et al. 2016)**. Also, *Aspergillus fumigatus* was the main fungi reported in mycotic abortion as recorded by **Dehkordi et al. (2012),** and **(Faris et al. 2013)**.

Hassan et al. (2012) detected *C. albicans* in the nasal swabs from sheep and goats with respiratory disorders (20% and 16%), respectively. Pneumonia caused by Aspergillus spp. was seldom in sheep and goats, notably in young animals in feedlots. Opportunistic pathogens of humans and/or animals include *A. fumigatus*, *A. niger*, *A. flavus*, *A. terreus*, and *A. nidulans* **(Carmo et al. 2020)**. Aspergillosis is a disorder observed in goats, can manifest as a nasal form, leading to a decline in physical well-being due to necrosis of turbinate bones and mucosa of nose. This pathological process can ultimately result in significant respiratory distress (**Elad and Segal 2018**).

Consumption of feed contaminated with mould act as contributing factors in pulmonary aspergillosis in ruminant animals mainly from airborne infection. Systemic aspergillosis in dairy sheep characterized by necrosis and a pyogranulomatous inflammation in lungs along with demonstration of fungal hyphae in the tissue sections **(Mahmoud et al. 2005)**. Nasopharyngeal swabs and pneumonic lung autopsies from diseased or murdered sheep and goats with respiratory symptoms had Aspergillus and *Candida albicans* that were the most prevalent fungus. The clinical signs included severe respiratory distress. Microbiological investigations led to the isolation of *Aspergillus niger* (**Do Carmo et al. 2014**). In addition, pathologic lesions such as multifocal necrosis in lungs with intralesional fungal hyphae have been reported in an Alpaca (**Hughes and Mueller 2008**). Zygomycosis is a granulomatous fungal infection that affects both people and animals. Two orders of pathogenic zygomycetes are of therapeutic and veterinary significance that were the Mucorales, which frequently cause systemic zygomycosis including Mucor, Rhyzopus, Absidia, and Entomophthorales, which mostly cause subcutaneous zygomycosis (Conidiobolus and Basidiobolus) (**Pawaiya et al. 2015**).

Results of antifungal sensitivity of different fungal isolates are summarized in table (10). These results coincide with previously investigated records by **Fahmy et al. (2020)**. **El-Hamaky et al. (2023)** used several antifungals to detect the resistance against A. flavus isolates and, the results were Voriconazole 1µg (90%), Nystatin 100µg (90%), Fluconazole 10µg (70%), and Itraconazole 10µg (30%), Amphotericin B 100µg (60%), respectively. **Tokarzewski et al. (2012)** recorded that A. niger was sensitive to Voriconazole and Itraconazo treatment and low susceptibility to Clotrimazole, Miconazole and Nystatin and

resistant to Fluconazole. Also, **Walsh et al. (2008)** have proved that Voriconazole is more successful than amphotericin B at treating invasive aspergillosis in haematology unit patients and is now mentioned as the reference treatment.

Miconazole, Clotrimazole, Ketoconazole, Fluconazole, and Itraconazole are all effective for treating oral candidiasis. Candida spp. and Rhodotorula were sensitive to Itraconazole. Candida spp. onlywas sensitive to Nystatin (**Fahmy et al. 2020**; **Abd El Tawab et al. 2021**).

Boucher et al. (2004), Hassan et al. (2021, 2023) revealed that *C. albicans* is highly susceptible to Nystatin, followed by Amphotericin B and Fluconazole, while it shows resistance to Clotrimazole. Currently, Amphotericin B (including lipid formulations) and the triazole Posaconazole are the only two systemic antifungals effective against Mucorales.

Mucorales were found to be resistant to a variety of antifungals in vitro, including flucytosine, ketoconazole, fluconazole, voriconazole, and echinocandins. Furthermore, they have varied susceptibility to itraconazole. Most fungal infections are amenable to Itraconazole and Nystatin, however repeated and longstanding azole treatments have resulted drug resistance in fungal pathogens treatment (**Almyroudis et al. 2007; Cowen 2008**).

The higher levels of lysozyme, nitric oxide, malondialdehyde levels and lowest level of total antioxidant in the infected animal as found in table 5 indicating the immunity profile. Lysozyme has numerous roles in the body's defensive mechanisms affecting bacteria (cell wall) and chitin-covered pathogens. Lysozyme action occurs spontaneously in Gram-positive bacteria on the peptidoglycan layer of the cell wall (**Dziarski and Gupta 2005**). Most gram-negative bacteria are resistant to lysozyme's action because their outer membrane (lipopolysaccharides) stops enzyme from accessing the peptidoglycan layer. However, this barrier could overcome the animal innate immune systems via antibacterial proteins synthesis that permeabilize the outer membrane, as lactoferrin (**Callewaert et al. 2008**). Lysozyme serum concentration is an indirect marker of inflammation, providing information on granulocyte activity and the monocyte-macrophage system and a possible indicator of the amount of pathogens in the environment (**Sotirov et al. 2005**).

Free radicals (reactive oxygen, ROS, and nitrogen species, RNS) can oxidise macromolecules (lipids, carbohydrates, proteins, and nucleic acids). Oxidative stress occurs when the ratio of free radicals to antioxidants shifts in favour of oxidants (**Ercan and Fidanci 2012**). In our result, an increase in MDA value in all infected animals are the most prone component to damage by free radicals but this increase was significant in case of mixed bacterial and mycological infection may be referred to that fungal metabolites impair phagocytic functions. Toxins prevent fungal adherence and phagocytosis while also influencing phagocytosis, intracellular death, and spontaneous superoxide production (**Tomee and Kauffman, 2000; Aktas et al. 2017**).

Antioxidants decrease free radicals, preventing cell damage in healthy mammals; the preventive antioxidant system maintains equilibrium with free oxygen radicals. Reduced total antioxidant capacity (TAC) in infected sheep in this research was agreed with **Abdel-Saeed and Salem (2019)** who detected a decrease in TAC in Ovine Verminous Pneumonia. we noticed significant decrease in G3 and this is may be due to Mycological species adopt a range of strategies to evade the immune system, including concealing critical pathogen-associated molecular patterns, blocking phagosome-lysosome fusion, and limiting antioxidant synthesis as catalase and SOD.

CONCLUSION

Small ruminants, particularly those rose in herd, are more susceptible to bacterial and fungal disease. These infections resulted in respiratory illnesses, diarrhea, and abortions. Fungal infections decrease immunity and predispose to bacterial infections. The drug sensitivity test has variable effect in vitro and vivo and the most essential factor in selecting antifungal and antibacterial drugs.

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