Sayed *et al. Irish Veterinary Journal* (2025) 78:11
https://doi.org/10.1186/s13620-025-00296-1

Irish Veterinary Journal

RESEARCH Open Access

Single nucleotide polymorphisms, gene expression and evaluation of immunological, antioxidant, and pathological parameters associated with bacterial pneumonia in Barki sheep

Ahmed El Sayed[1*], Amani Hafez[1], Ahmed Ateya[2*], Asmaa Darwish[1] and Amin Tahoun[3,4]

Abstract

Background In sheep, pneumonia is a major concern because of its high morbidity, mortality, and economic impact. It results from various infectious agents, including bacteria, viruses, and environmental stressors, that weaken the immune system.

Objective The objective of this study was to monitor nucleotide sequence variations, gene expression, and serum biomarkers of inflammation and oxidative stress in sheep with pneumonia. Additionally, this study aimed to identify various bacterial strains and virulent gene combinations in pneumonic sheep, as confirmed by PCR.

Methodology The enrolled animals were categorized as follows: 50 apparently healthy ewes, considered the control group, and 150 infected ewes with pneumonia. The infected ewes included 100 sporadic cases from the Center for Sustainable Development of Matrouh Resources, Desert Research Center, Matrouh, Egypt, and 50 ewes from the slaughterhouse, all exhibiting respiratory symptoms such as coughing, serous to mucopurulent nasal discharge, fever, and abnormal lung sounds. Blood samples were collected to assess various biochemical parameters, detect SNPs, and analyse the expression of specific immunological and antioxidant-related genes. Nasopharyngeal and lung swabs were taken from the affected ewes for bacteriological analysis, and lung samples were collected for histological examination.

Results Phenotypic characterization and identification revealed the presence of *Klebsiella pneumoniae*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Pseudomonas* spp., *Mycoplasma*, *Streptococcus*, and *Escherichia coli*, with frequencies of 40%, 28.6%, 34%, 18%, 44%, 29.3%, and 20%, respectively. Additionally, virulence genes for *Klebsiella pneumoniae*, *iutA* and *fimH*, were detected at rates of 39% and 68%, respectively, whereas the *toxA* gene for *Pseudomonas* spp. was present in 59.2% of the cases. Nucleotide sequence variations in immunity- and antioxidant-

*Correspondence:
Ahmed El Sayed
decernes@yahoo.com
Ahmed Ateya
ahmed_ismail888@yahoo.com

Full list of author information is available at the end of the article

related genes were observed between healthy and pneumonic ewes. The genes encoding *IL-1α*, *IL1B*, *IL6*, *TNF-α*, *LFA-1*, *CR2*, *IL17*, *IL13*, *DEFB123*, *SCART1*, *ICAM1*, *NOS*, and *HMOX1* were significantly upregulated in pneumonia-affected ewes compared with resistant ewes. Conversely, the genes encoding *IL10*, *SOD1*, *CAT*, *GPX1*, and *NQO1* were downregulated. Further analysis of the serum profile revealed a significant ($P < 0.05$) increase in IL-1α, IL-1β, IL-6, TNF-α, NO and MDA along with a significant ($P < 0.05$) decrease in the serum levels of C3, C4, CAT, GPx, GR and IL-10 in diseased ewes compared with healthy ewes. Histopathological examination revealed that the infected sheep exhibited broncho-interstitial pneumonia and purulent to fibrino-purulent bronchopneumonia.

Conclusions This study revealed the significant presence of various pathogens and virulence factors in infected sheep, along with distinct immunological and antioxidant gene expression patterns. The altered serum profile and gene regulation in pneumonia-affected ewes underscore the complex immune response and potential biomarkers for disease susceptibility and resistance.

Keywords Antioxidant, Barki sheep, Gene expression, Immunity, Nucleotide sequence variants, Pneumonia

Introduction

The Barki sheep breed, named after the Libyan province of Barka, is native to Egypt's northwest coastal region and is essential to the local population's way of life [1]. With a population of 470,000, accounting for 11% of Egypt's total sheep. This breed is spread across a vast area, from eastern Libya to the western part of Alexandria, Egypt. Barki sheep exhibit remarkable tolerance to extreme temperatures, limited forage, and heat stress and play a prominent role in this region [2]. Basic information about the body conformation and productivity of Barki ewes is available [3].

The health of livestock populations worldwide is severely threatened by bacterial and viral respiratory diseases [4, 5]. In addition, predisposing management factors such as stressful environmental conditions such as overcrowding, transportation, abrupt climate changes, inadequate ventilation, and poor nutrition typically result in lung infections that cause significant losses. Respiratory disorders in sheep frequently develop as a result of immunosuppressive stress conditions, which may lead to secondary infections or primary infections caused by bacterial and/or viral agents [6–9].

Pneumonia typically arises from an inflammatory response in the bronchioles and alveoli of the lungs caused by substances that lead to the solidification of lung tissue. According to [10], lung defense mechanisms become weakened, and disease can develop when a threshold level of pathogens, host vulnerability, and nonspecific defense responses is reached. Pneumonia is responsible for 40–70% of sheep losses and affects 30–40% of flocks. The economic costs associated with sheep pneumonia include reduced growth rates, milk production, increased morbidity and mortality, carcass condemnation, and the expense of vaccination and chemotherapy programs [11, 12]. In Egypt, the highest incidence of sheep pneumonia typically occurs during autumn and early winter, as well as in spring and early summer. This is due to sudden temperature shifts from warm to cold. Additionally, dust particles that carry infections deep into the respiratory system are more prevalent in spring and autumn [13, 14]. To maintain production and meet the demand for meat and milk, eliminating or mitigating the impact of diseases that affect sheep is crucial [15].

Several bacteria have been isolated from the infected lungs of sheep, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus* species, *Streptococcus pyogenes*, *Corynebacterium* species, *Pasteurella* species, and *Arcanobacterium pyogenes* [16, 17].

A wide range of clinical issues in small ruminants, especially respiratory conditions, are linked to various *Mycoplasma species*. These problems result in significant economic losses, particularly in African countries [18]. These bacteria are highly specific and require particular substrates to grow in controlled environments [19, 20]. Species such as *Mycoplasma arginini*, *Mycoplasma ovipneumoniae*, and *Mycoplasma agalactiae* have been identified and isolated in Egypt [21]. *E. coli*, a common member of the Enterobacteriaceae family, is frequently observed in respiratory infections in sheep, where it can be isolated from both pneumonia-affected lungs and nasal swabs [22, 23].

Pasteurella multocida, an opportunistic bacterium, is one of the most serious pathogens affecting domestic ruminants, causing acute pneumonia outbreaks, particularly in animals under stress from factors such as weaning, transport, environmental changes, or primary respiratory infections [24, 25]. In normally benign nasopharyngeal commensals, these bacteria can become harmful when an animal's immune defenses are weakened [26]. *Pseudomonas aeruginosa* causes a variety of diseases in sheep and goats, but respiratory illnesses, especially pneumonia, are the main cause of concern because they lead to significant mortality and increased financial losses [27]. *Klebsiella pneumoniae* carries the fimH gene, a virulence factor believed to increase bacterial adherence to epithelial cells. Another important

virulence gene, iutA, increases the expression of other pathogenic genes, contributing to greater harm [28].

Polymerase chain reaction (PCR) is the most efficient and rapid method for identifying microbial pathogens in clinical samples. PCR is especially useful for detecting pathogens that are difficult to culture or require extended cultivation periods [29]. This method allows for faster diagnosis, enabling quicker treatment and reducing the use of antimicrobial drugs, which helps lower both costs and side effects [30].

Oxidative stress often occurs when there is an imbalance between antioxidants and oxidants, such as free radicals or reactive oxygen species. However, animal bodies can regulate excess free radicals through defense mechanisms [31, 32]. Catalases and other antioxidant enzymes are crucial to these processes [33]. Cytokines, a class of soluble proteins, regulate cellular and tissue activity. They have a short half-life, are produced locally in response to stimuli, and can function in an endocrine, exocrine, or autocrine manner [34]. Sheep that have acute pneumonia are at risk for organ failure and death due to a severe proinflammatory state that increases the release of inflammatory cytokines [35].

Marker-assisted selection (MAS) is a genomic technique that helps in the development of disease-resistant animals by selecting for alleles at the DNA level rather than relying on a phenotypically expressed disease state. This approach enhances selection accuracy without being influenced by environmental factors, making it a valuable tool for improving the disease resistance of livestock [36]. Molecular genetic techniques also hold promise for partially addressing the shortcomings of conventional methods used by animal breeders in their efforts to improve the health of their animals [37]. The exposure of animals to infections is one of the main obstacles to using selection for disease-resistant animals. Since there is no guarantee that animals bred for reproduction are treated humanely, it is impractical to reject this practice. Many beneficial single nucleotide polymorphisms (SNPs) have been described as a result of extensive efforts to identify DNA markers for sickness resistance [38]. This study aimed to investigate the bacterial species and virulence gene combinations isolated from sheep with respiratory symptoms and to verify them via PCR. This work also explored the histological and macroscopic abnormalities in the affected lungs. Additionally, this study assessed how proinflammatory cytokines, oxidative stress biomarkers, and genetic polymorphisms could be used to identify pneumonia in Barki sheep.

Materials and methods

Animals and study design

A total of 200 Barki ewes, aged 3 to 5 years (mean ± SD: 3.8 ± 0.6) and weighing between 28 and 43 kg (mean ± SD: 35 ± 5), were included in this study. On the basis of a thorough clinical examination following the methodology outlined by [39], the ewes were divided into two groups according to assessments of heart, lung, and rumen health, along with other vital signs. Group 1 ($n = 50$), comprising clinically healthy ewes, was assigned to the healthy control group (HCG), which presented a normal body temperature, pulse, respiration rate, clear eyes, absence of nasal or lacrimal discharge, normal posture, and appetite. The pneumonic group (PG) consisted of 150 ewes displaying clinical signs of respiratory disease, including fever, abdominal breathing, mucopurulent nasal discharge, weakness, appetite loss, and abnormal lung sounds (crackles and wheezes). This group included 50 ewes from a slaughterhouse and 100 sporadic cases from the Center for Sustainable Development of Matrouh Resources, Desert Research Center, Matrouh, Egypt.

The ewes were housed in semiopen shaded pens and provided a daily diet consisting of 750 g of concentrated feed mixture (CFM) and 750 g of alfalfa hay per head, which was offered twice daily with unrestricted water access. When available, they also had access to natural pasture, including grass, berseem, darawa, and green herbage. The CFM composition included wheat bran (240 kg), soybean meal (230 kg), corn (530 kg), sodium chloride (5 kg), calcium carbonate (10 kg), premix (1 kg), Netro-Nill (0.5 kg), and Fylax (0.5 kg).

Sampling

Blood sampling

Ten milliliters of blood were collected from each animal via jugular venipuncture. The samples were divided into plain tubes (without anticoagulant) for serum collection and EDTA tubes for whole blood collection. The samples were immediately chilled on crushed ice and transported to the laboratory. Whole blood was used for DNA and RNA extraction, while serum samples were obtained via centrifugation in plain blood tubes at 3000 rpm for 15 min, aliquoted, and stored at −20 °C for biochemical analysis of oxidative and energetic stress markers.

Nasopharyngeal swabs

A total of 100 nasopharyngeal swabs were collected. Each animal was identified, restrained, and secured by an assistant. After the nasal exterior was disinfected with 70% alcohol, a sterile cotton-tipped swab was inserted into the nostril and rotated against the nasal cavity wall. Swabs were placed in sterile test tubes containing 10 mL of tryptone soy broth, brain heart infusion broth, and PPLO broth and stored in an icebox for transport to the Microbiology Department, Desert Research Center, Matrouh. Sampling was conducted on the basis of evidence of recurrent pneumonia symptoms.

Emergencies slaughtered (lung swabs)

Fifty lungs were subjected to standard postmortem meat inspection. Suspicious lung surfaces were incised with a sterile scalpel, and inner surfaces were sampled via sterile swabs. The samples were transported to the Microbiology Department following the same protocol as the nasal swabs [40]. Affected lung tissues and associated lymph nodes were collected in 10% neutral-buffered formalin (NBF) for histopathology, while frozen tissues were stored at −20 °C for further analysis.

Pathological investigation

Tissue samples (~ 1 cm^3) were fixed in 10% NBF for histological analysis. The samples were dehydrated in graded ethanol, embedded in paraffin, sectioned at 5 µm thickness, stained with hematoxylin and eosin, and examined under a standard light microscope [41].

Bacterial isolation

Samples from affected lung lesions were aseptically collected and transported to the Bacteriology Department. The pneumonic lung surface was cauterized with a heated spatula before the inner tissue was cultured on blood agar with 5% sheep blood, Baird−Parker agar, mannitol salt agar, Pseudomonas cetrimide agar, oxacillin resistance screening agar basal medium (ORSAB) [42], and MacConkey agar. The plates were incubated aerobically at 37 °C for 24–48 h, while the PPLO agar plates were incubated at 37 °C for 5–7 days in 5% CO2. Bacterial identification followed standard protocols, including colony morphology assessment; hemolysis type; Gram staining; and biochemical tests, such as oxidase, catalase, indole, urease, triple sugar iron (TSI), oxidation/fermentation, and motility tests [43].

Molecular identification of bacterial isolates and detection of specific virulence and resistance genes confirmed the presence of *Klebsiella pneumoniae* (16–23 S ITS) [44], *Klebsiella iutA* [28], *Klebsiella fimH* [45], *P. multocidaKmt1* [46], *Mannheimia haemolytica ssa* [47], *Pseudomonas 16 S rDNA* [48], *Pseudomonas toxA* [49], *Mycoplasma 16 S Rrna* [50], *Streptococcus 16 S rRNA* [51], *and E. coli phoA* [52].

PCRbased bacterial detection

DNA extraction

DNA was extracted via the QIAamp DNA Mini Kit (Qiagen, Germany) with minor modifications according to the manufacturer's protocol. Briefly, 200 µL of sample suspension was incubated with 10 µL of proteinase K and 200 µL of lysis buffer at 56 °C for 10 min. After adding 200 µL of ethanol, the samples were washed and centrifuged, and the nucleic acids were eluted with 100 µL of elution buffer.

The oligonucleotide primers used were supplied by Metabion (Germany) and are listed in Table 1.

PCR amplification

Uniplex PCR

The 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 at a concentration of 20 pmol, 5.5 µL of water, and 5 µL of the DNA template. Amplifications were conducted in an Applied Biosystems 2720 thermal cycler.

Analysis of PCR products

The products of PCR were separated via electrophoresis on a 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature via gradients of 5 V/cm. For gel analysis, 20 µL of the uniplex PCR products and 40 µL of the duplex PCR products were loaded into each gel slot. A GelPilot 100 bp plus ladder (Qiagen, gmbh, Germany) and a Generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed with a gel documentation system (Alpha Innotech, Biometra), and the data were analysed with computer software.

Positive controls included *Klebsiella pneumoniae* ATCC® BAA-1705D-5™, *Pasteurella multocida subsp. multocida* (ATCC® 43137™), *Mannheimia haemolytica* (ATCC® 33396™), *Pseudomonas aeruginosa* (ATCC®27853™), *Mycoplasma ovipneumoniae* (ATCC® 29419™), *Streptococcus* sp. ATCC ® 15,913, and *E. coli* ATCC 25,922. For virulence genes, positive and/or negative controls were field isolates supplied by the Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute.

Nucleotide sequence variants between healthy and pneumonic ewes

DNA extraction and polymerase chain reaction (PCR)

A Gene JET whole blood genomic DNA extraction kit was used to extract genomic DNA from whole blood in accordance with the manufacturer's instructions (Thermo Scientific, Lithuania). A Nanodrop was used to test high-quality, pure, and concentrated DNA. The immune (IL-1α, IL1B, IL6, TNF-α, IL10, LFA-1, CR2, IL17, IL13, DEFB123, SCART1, and ICAM1) and antioxidant (SOD1, CAT, GPX1, NOS, HMOX1, and NQO1) fragments were amplified via PCR. The primer sequences were created on the basis of the *Ovis aries* sequence published in PubMed. Table 2 lists the primers that were utilized in the amplification.

In a thermal cycler, the polymerase chain reaction mixture was prepared at a final volume of 100 µL. Five microliters of DNA, 68 µL of d.d. water, 25 µL of PCR master mix (Jena Bioscience, Germany), and one µL of

Table 1 Primer sequences, target genes, amplicon sizes and cycling conditions for bacteria and some virulence genes

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Klebsiella. pneumoniae 16–23 S ITS	ATTTGAAGAGGTTGCAAACGAT TTCACTCTGAAGTTTTCTTGTGTTC	130	94˚C 5 min	94˚C 30 s.	55˚C 30 s	0.72˚C 30 s.	72˚C 7 min	Turton et al. 2010 [44]
Klebsiella iutA	GGCTGGACATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300	94˚C 5 min	94˚C 30 s.	63˚C 30 s.	72˚C 30 s.	72˚C 7 min	Yaguchi et al. 2007 [28]
Klebsiella *fimH*	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA GTAAATGCACTTGCTTCAGGAC	508	94˚C 5 min.	94˚C 30 s.	50˚C 40 s	72˚C 45 s	72˚C 10 min	**Ghanbarpour and Salehi, 2010** [45]
Pseudomonas 16 S rDNA	GACGGGTGAGTAATGCCTA CACTGGTGTTCCTTCCTATA CGCTGGCCCATTCGCTCCAGCGCT	618	94˚C 5 min.	94˚C 30 s.	50˚C 40 s.	72˚C 45 s	72˚C 10 min.	Spilker et al. 2004 [48]
E. coli phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC ccggccatattcacataa	720	94˚C 5 min	94˚C 30 s.	55˚C 40 s	72˚C 45 s	. 72˚C 10 min	Hu et al. 2011 [52]
P. multocida Kmt1	ATCCGCTATTTACCCAGTGG GCTGTAAACGAACTCGCCAC	460	94˚C 5 min.	94˚C 30 s.	55˚C 40 s	72˚C 45 s.	72˚C 10 min.	Duan et al. 2024 [46]
Mannheimia haemolytica ssa	TTCACATCTTCATCCTC TTTTCATCCTCTTCGTC	325	94˚C 5 min.	94˚C 30 s.	50˚C 40 s.	72˚C 40 s.	72˚C 10 min	Hawari et al. 2008 [47]
Mycoplasma *16 S rRNA*	GGGAGCAAACAGGATTAGATACCCT TGCACCATCTGTCACTCTGTTAACCTC	280	94˚C 5 min	94˚C 30 s.	55˚C 30 s.	72˚C 30 s.	72˚C 7 min.	Van Kuppeveld et al. 1994 [50]
Streptococcus *16 S rRNA*	CGGGGGATAACTATTGGAAACGATA ACCTGTCACCCGATGTACCGAAGTA	912		94˚C 30 s.	55˚C 40 s.	72˚C 1 min.	72˚C 10 min.	Osakabe et al. 2006 [51]

Table 2 Forward and reverse primer sequences, lengths of the PCR products and annealing temperatures of the investigated genes used for PCR-DNA sequencing

Gene	Forward	Reverse	Annealing temperature (°C)	Length of PCR product (bp)	Reference
IL-1α	5′-ATGGCCAAAGTCCCTGACCTC-3′	5′-ATGTAGCAGCCGTCATGTATGG-3′	60	472	Current study
IL-1β	5′-GAACCTTCATTGCCCAGGTTTC – 3′	5′-ACTGACTGCACGGCTGCATCA – 3′	58	388	Current study
IL-6	5′-GAGAGCTCCATCAGCCCTCCAG-3′	5′-GGAGTGGTTATTAGACCTGCGA-3′	62	526	Current study
TNF-α	5′-CAGACCAAGGTCAACATCCTCT-3′	5′-CAGGCCTCACTTCCCTACATC-3′	62	388	Current study
IL-10	5′-ACAACAGGGGCTTGCTCTTGC-3′	5′-CTCTCTTGGAGCATATTGAAGAC-3′	60	526	Current study
LFA-1	5′-TTCCTACACCATCGTGATGAG-3′	5′- ATACATTCCTGATAACCAGGGT-3′	60	438	Current study
CR2	5′-TAGCAGCTTAGGGACAGCTCT-3′	5′-CTATTGTAATATTCACAGATA-3′	58	381	Current study
IL-17	5′-ATGGCGTCTATGAGAACTGCCT-3′	5′-TTAAGCCACATGGCGGACAAT-3′	62	462	Current study
IL-13	5′-CTTAGGCCAGCCTATGCGTCTG-3′	5′- TGGTGTCTCGGACGTACTCACT-3′	58	376	Current study
DEFB123	5′- CAGAGCACTCCACCATTAGCT – 3′	5′- TTATTGATAGAGCTCAAGAATC – 3′	62	371	Current study
SCART1	5′-CAGAGCACTCCACCATTAGCT-3′	5′- GGCTCTGTCCCCACGCAGTGC-3′	62	220	Current study
ICAM1	5′-CGCCTCCGCCTGCGATGGCTC-3′	5′- CTTCACCCACAGGCTGCCAGAGT-3′	60	399	Current study
SOD1	5′-GCGTCGGCGTGTGTTCTGCGG-3′	5′-ATCTACGATATCCACAATGGCA-3′	62	387	Current study
CAT	5′-TCTGCAGCGCCGCTCAGACAC-3′	5′-TGGAGTATCTGGTAATGTCATG-3′	60	308	Current study
GPX1	5′-AGCTCACTGCTCTCAACTTGGA-3′	5′- ACGTGATGAACTTAGGGTCGGT – 3′	60	494	Current study
NOS	5′-GGCACGAGCGCCCGTCCGCCC-3′	5′-TCGCTCTCTCGGAGGTGGTAG-3′	58	540	Current study
HMOX1	5′-AGCACTGGTGACACCTTCTAG-3′	5′- TCGGCCACAATATCTGGGCTC-3′	62	387	Current study
NQO1	5′-CTACGCAGCCGCAGGATGGAGC-3′	5′- GCTCCATCCTGCGGCTGCGTAGAACAT-3′	64	377	Current study

--*IL-1α* Interleukin 1 alpha, *IL-1β* Interleukin 1 beta, *IL-6* Interleukin 6, *TNF- α* Tumor necrosis factor- alpha, *IL10* Interleukin 10, *LFA-1* Lymphocyte function-associated antigen 1, *CR2* Complement C3d receptor 2, *IL-17* Interleukin 17, *IL-13* Interleukin 13, *DEFB123* β-defensin, *SCART1* Scavenger Receptor Family Member Expressed On T Cells 1, *ICAM1* intercellular adhesion molecule 1, *SOD1* Superoxide dismutase 1, *CAT* Catalase, *GPX1* Glutathione peroxidase 1, *NOS* Nitric oxide synthetase, *HMOX1* Heme Oxygenase 1, *NQO1* NAD(P)H Quinone Dehydrogenase 1

each primer were included in each reaction volume. For eight minutes, the reaction mixture was exposed to an initial denaturation temperature of 94 °C. As indicated in Table 2, the cycling process involved 30 cycles at 94 °C for 1 min for denaturation, 45 s for annealing, 45 s for extension at 72 °C, and a final extension at 72 °C for 8 min. The samples were stored at 4 °C, and agarose gel electrophoresis was used for identification. The samples were stored at 4 °C, and agarose gel electrophoresis was used to identify representative PCR analysis results. A gel documentation system was then used to visualize the fragment patterns under ultraviolet light.

DNA sequencing and polymorphism detection

The removal of primer dimers, nonspecific bands, and other contaminants was performed prior to DNA sequencing. Using a PCR purification kit and the manufacturer's instructions, PCR products of the desired size (target bands) were purified as [53] described previously (Jena Bioscience # pp-201×s/Germany). The PCR product was quantified via a Nanodrop (UV−Vis spectrophotometer Q5000/USA) to guarantee sufficient concentrations and purity of the PCR products and to produce high yields [54]. PCR products with the target band were sent for forward and reverse DNA sequencing via an ABI 3730XL DNA sequencer (Applied Biosystem, USA), which relies on the enzymatic chain terminator technique developed by [55], to detect SNPs in genes examined in both healthy controls and pneumonic ewes.

The software programs Chromas 1.45 and blast 2.0 were used to analyse the DNA sequencing data [56]. Single-nucleotide polymorphisms (SNPs) are defined as differences between the PCR products of the genes under study and between the PCR products and reference sequences found in GenBank. On the basis of DNA sequencing data alignment, the MEGA4 software package was used to compare the amino acid sequences of the genes under investigation among the enrolled ewes [57].

Total RNA extraction, reverse transcription and quantitative real-time PCR

TRIzol reagent was used to extract total RNA from ewe blood in accordance with the manufacturer's instructions (RNeasy Mini Ki, Catalogue no. 74104). A NanoDrop® ND-1000 spectrophotometer was used to quantify and quantify the amount of isolated RNA. Each sample's cDNA was produced in accordance with the manufacturing technique (Thermo Fisher, Catalogue no. EP0441). Using quantitative RT−PCR with SYBR Green PCR Master Mix (2x SensiFastTM SYBR, Bioline, CAT No: Bio98002), the gene expression patterns of genes encoding immunity and antioxidants were evaluated. Using SYBR Green PCR Master Mix (Quantitect SYBR Green PCR Kit, Catalogue no. 204141), real-time PCR was used to measure the relative amount of mRNA. As indicated

in Table 3, primer sequences were created using the Ovis aries sequence published in PubMed.

A constitutive control for normalization was the housekeeping gene ß-actin. Three microliters of total RNA, four microliters of 5x Trans Amp buffer, 0.25 µL of reverse transcriptase, 0.5 µL of each primer, 12.5 µL of 2x Quantitect SYBR green PCR master mix, and 8.25 µL of RNase-free water made up the reaction mixture, which had a total volume of 25 µL. Reverse transcription was performed at 50 °C for 30 min, initial denaturation at 94 °C for 10 min, 40 cycles of 94 °C for 15 s, the annealing temperatures indicated in Table 3, and 72 °C for 30 s. A melting curve analysis was carried out at the conclusion of the amplification phase to verify the specificity of the PCR products. The $2^{-\Delta\Delta Ct}$ technique was used to calculate the relative expression of each gene per sample in comparison to that of the *ß.actin* gene [58].

Immunological and antioxidant parameters

In accordance with the author's guidelines, the obtained plasma and serum samples were used to evaluate the following parameters: ELISA kits from MyBiosecure Company® were used for the evaluation of serum pro-infammatory cytokines (IL-1α, IL-1β, IL-6, TNF-α) and anti-inflammatory cytokines (IL-10), serum concentrations of free radicals (nitric oxide (NO), malondialdehyde

Table 3 Oligonucleotide primer sequences, accession numbers, annealing temperatures and PCR product sizes of the investigated genes via real-time PCR

Gene	Primer	Product length (bp)	Annealing Temperature (°C)	Accession number	Source
IL-1α	F5′- CCATACATGACGGCTGCTACA – 3 R5′- TGTCTCAGGCATCTCCTTATGC – 3′	180	62	NM_001009808.1	Current study
IL-1β	F5′- ACAGATGAAGAGCTGCACCC – 3′ R5′- AGACATGTTCGTAGGCACGG – 3′	161	58	NM_001009465.2	Current study
IL-6	F5- TGCAGTCCTCAAACGAGTGG – 3′ R5′- CCGCAGCTACTTCATCCGAA – 3′	110	62	NM_001009392.1	Current study
TNF-α	F5′- CTTTGGGATCATCGCCCTGT – 3′ R5′- CAGCCCTGAGCCCCTAATTC – 3′	129	60	NM_001024860.1	Current study
IL-10	F5′- CTCGACTAGACTCCCCGACA – 3′ R5′- ACTCTAGGGGAGAGGCACAG – 3′	102	58	NM_001009327.1	Current study
LFA-1	F5′- TTGGGTACCGTGTTCTGCAA – 3′ R5′- TCACATGTTCGAGACAGCCC – 3′	223	58	NM_001035124.1	Current study
CR2	F5′- TACCCTAGAAGGCAGTCCCC-3′ R5′- AAGGTGACAACACCCAAGCA G – 3′	153	60	NM_001009724.1	Current study
IL-17	F5′- TTATCACAAGCGCTCCACCT – 3′ R5′- GTGATGGTCCACCTTCCCTT – 3′	133	58	XM_004018887.5	Current study
IL-13	F5′- TCCAGCACTAAAGCAGTGGG – 3′ R5′- TGCTGGCTGTCAGACAAGAG – 3′	141	62	NM_001082594.1	Current study
DEFB123	F5′- ACTGTGCTGTGTGAAGTCCA – 3′ R5′- GGGGTTAGACTCAGGGTAGC – 3′	112	58	XM_027976443.1	Current study
SCART1	F5′- CCACTGGGACTTGGCAGAC – 3′ R5′- GCTCTGTCCCCACGCA – 3′	140	58	EF215856.1	Current study
ICAM1	F5′- CACCATATACTGGTTCCCGGAG – 3 R5′- CATGGTCCTCTCTTCTCGGC – 3′	224	62	NM_001009731.1	Current study
SOD1	F5- TGATCATGGGTTCCACGTCC – 3′ R5′- CACATTGCCCAGGTCTCCAA – 3′	139	60	NM_001145185.2	Current study
CAT	F5- TTCGCTTCTCCACTGTTGCT – 3′ R5′- CCGGATCCTTCAGGTGTGTC – 3′	207	58	XM_004016396.5	Current study
GPX1	F5- AACGTAGCATCGCTCTGAGG – 3 R5- CAAACTGGTTGCACGGGAAG – 3	115	62	XM_004018462.5	Current study
NOS	F5′- CTCCCAAGGTGACTTCCCAGA – 3′ R5′- ACCAAAGGGCTGACTGTAGG – 3′	142	58	NM_001129901.1	Current study
HMOX1	F5′- CAAGCGCTATGTTCAGCGAC – 3′ R5′- GCTTGAACTTGGTGGCACTG – 3′	206	60	MK630326.1	Current study
NQO1	F5′- GGCAGTGGCTCCATGTACTC – 3′ R5′- TAGATGGCCACCTCACATGC – 3′	116	62	XM_012190046.4	Current study
ß. actin	F5′- GCAAAGACCTCTACGCCAAC-3′ R5′- TGATCTTCATCGTGCTGGGT – 3′	114	60	NM_001009784.3	Current study

-IL-1α Interleukin 1 alpha, *IL-1β* Interleukin 1 beta, *IL-6* Interleukin 6, *TNF- α* Tumor necrosis factor- alpha, *IL10* Interleukin 10, *LFA-1* Lymphocyte function-associated antigen 1, *CR2* Complement C3d receptor 2, *IL-17* Interleukin 17, *IL-13* Interleukin 13, *DEFB123* β-defensin, *SCART1* Scavenger Receptor Family Member Expressed On T Cells 1, *ICAM1* intercellular adhesion molecule 1, *SOD1* Superoxide dismutase 1, *CAT* Catalase, *GPX1* Glutathione peroxidase 1, *NOS* Nitric oxide synthetase, *HMOX1* Heme Oxygenase 1, *NQO1* NAD(P)H Quinone Dehydrogenase 1

(MDA)), antioxidants (catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR)), (spectrophotometrically using commercial kits from Biodiagnostic Company®), serum complement-3 (C3) and complement-4 (C4) (ELISA technique using commercial kits from New Test Company®), and immunoglobulin (Igs) serum concentrations (IgG, IgM, IgA) (Turbidmetrically using kits supplied by Biorex Diagnostics®, UK).

Statistical analysis

Independent-samples t tests were used to compare the means of the measured variables between the PG and HCG via SPSS version 23. The difference was considered significant at $P<0.05$. All variables of the PG and HCG are presented as the means ± SDs. Pearson's simple correlation test was used to determine correlations between the genetic, immunological and antioxidant parameters. The cut-off points, sensitivity, specificity and likelihood ratios (LRs) for the measured cytokines were estimated in the PG and HCG via the graphed prism version 8 program. The positive predictive value (PPV), negative predictive value (NPV), accuracy rate and percentages of increase were calculated via the following equations:

$$\mathrm{PPV} = \text{True positive} \div \text{Total positive} * 100$$

$$\mathrm{NPV} = \text{True negative} \div \text{Total negative} * 100.$$

$$\text{Accuracy rate} = (\text{True positive} + \text{True negative}) \div \text{Total population} * 100$$

$$\text{Percentage of increaese} = (\text{The mean value of the marker concentration in DG} - \text{The mean value of its concentration in CG}) \div \text{The mean value of its concentration in CG} \times 100$$

Results

Clinical findings

This study demonstrated that infected sheep typically display signs of respiratory distress, including rhinitis, clogged mucous membranes, and a wet, harsh cough. The most common symptoms were elevated rectal temperature (over 41 °C), serous or mucoid nasal discharge, and increased respiratory and pulse rates. Additionally, ataxia, depression, ruminal atony, and abnormal milk production were observed. Early-stage auscultation revealed pleuritic friction rubs, which later progressed to muffled sounds in the more severe stages.

Isolation and identification

Bacterial isolates were detected in 80 out of 150 cases (53.3%). All bacterial isolates were subjected to PCR assays to identify specific genes via targeted primers, with positive control DNA samples incorporated into each reaction.

Prevalence of bacterial species isolated from infected animals

Table 4 summarizes the bacterial species identified from pneumonic cases in sheep via traditional bacteriological methods. The species isolated from sheep, nasal swabs and lung samples included *Klebsiella pneumoniae* (35% & 50%), *Pasteurella multocida* (28% & 30%), *Mannheimia haemolytica* (32% & 38%), *Pseudomonas* spp. (12% & 15%), *Mycoplasma* (30% & 72%), *Streptococcus* (25% & 38%), and *Escherichia coli* (12% & 36%).

Molecular identification and detection of several virulence genes

Conventional PCR tests were conducted on biochemically and serologically identified bacterial isolates to confirm their identity and detect specific virulence genes. The results revealed the following: *Klebsiella pneumoniae* 16–23 S ITS (40%, Fig. 1A), the *Klebsiella* virulence gene iutA (39%, Fig. 1B), the fimH gene (68%, Fig. 1C), *Pseudomonas* 16 S rRNA (Fig. 2A), toxA (59.2%, Fig. 2B), *E. coli* phoA (Fig. 3A), *P. multocida Kmt1* (Fig. 3B), *Mannheimia haemolytica ssa* (Fig. 3C), *Mycoplasma 16 S rRNA* (Fig. 3D), and *Streptococcus* (Fig. 3E).

PCR-DNA sequencing of immunity-, antioxidant- and GIT health-related genes

PCR-DNA sequencing of the following genes revealed variations in their nucleotide sequences: IL-1α (472 bp), IL1B (388 bp), IL6 (526 bp), TNF-α (388 bp), IL10 (526 bp), LFA-1 (438 bp), CR2 (381 bp), IL17 (462 bp), IL13 (376 bp), DEFB123 (371 bp), SCART1 (220 bp),

Table 4 Isolation of bacterial species from pneumonic sheep

Bacterial infection	No. of isolated bacteria		Percentage %		Total 150	Percentage %
	Nasal swab 100	Lung 50	Nasal swab	Lung		
Klebsiella pneumoniae	35	25	35	50	60	40
Pasteurella. multocida	28	15	28	30	43	28.6
Mannheimia haemolytica,	32	19	32	38	51	34
Pseudomonas aeruginosa	12	15	12	30	27	18
Mycoplasma	30	36	30	72	66	44
Streptococcus pneumoniae,	25	19	25	38	44	29,3
Escherichia coli	12	18	12	36	30	20

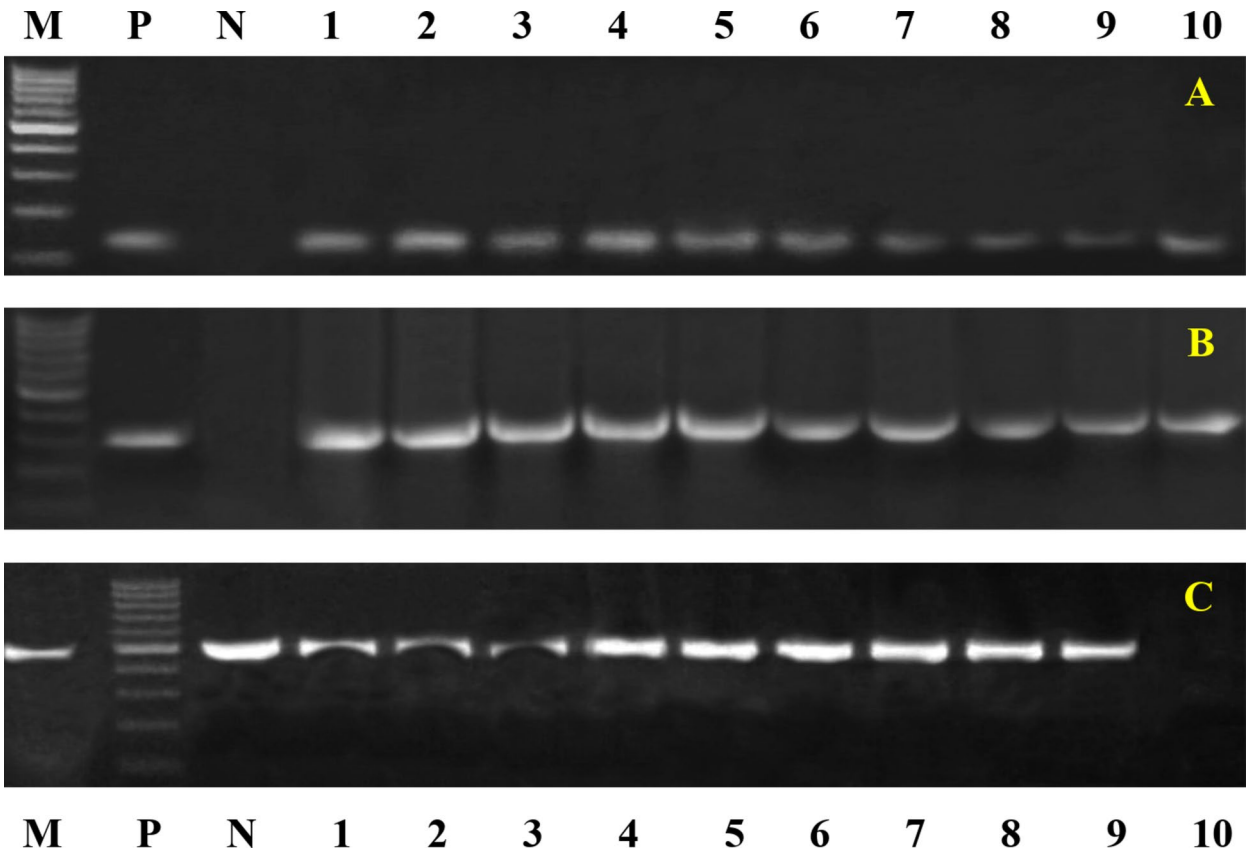

Fig. 1 Representative gel electrophoresis of *K. pneumoniae* strains isolated from sheep with pneumonia. **A** *16S–23S ITS* gene (genetic marker *of K. pneumoniae*), with an expected band size of 130 bp. **B** *iutA* gene with a band size of 300 bp. This gene was represented in 39% of the isolated *K. pneumoniae* strains. **C** *fimH* gene with the expected band size of 508 bp. This gene was present in 68% of the isolates

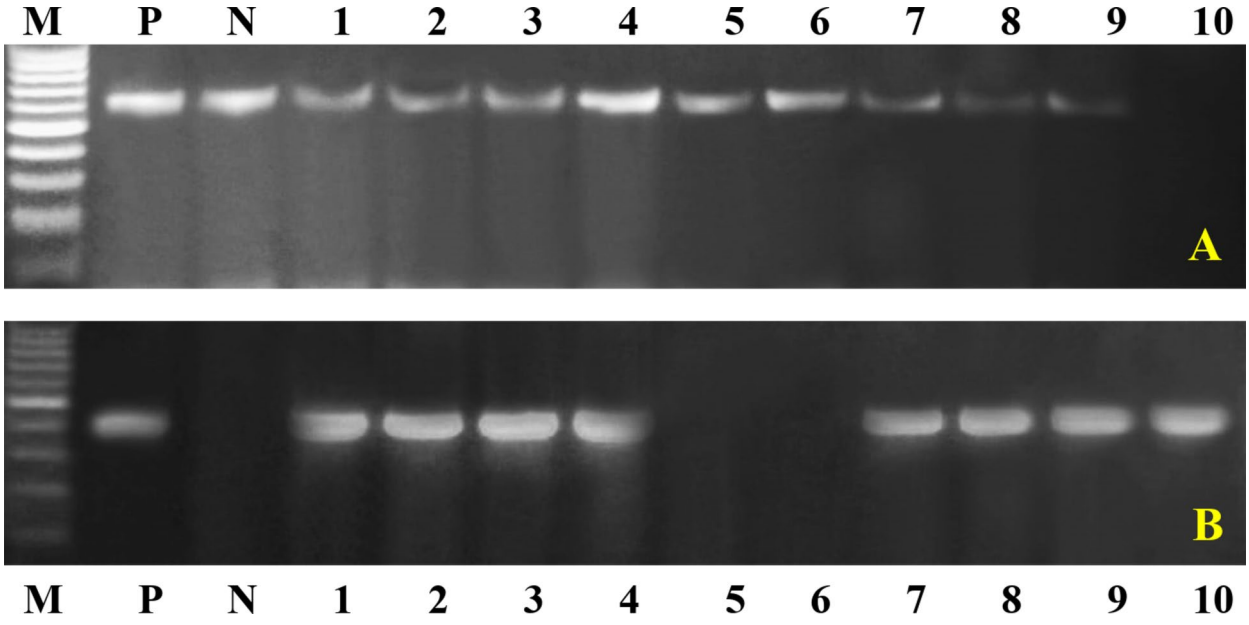

Fig. 2 Representative gel electrophoresis of several virulence genes from Pseudomonas strains isolated from sheep with pneumonia **A** *16 S rDNA gene* (genetic marker Pseudomonas), with an expected band size of 618 bp. **B** *ToxA gene* with a band size of 396 bp. This gene was represented in 59.2% of the isolated Pseudomonas strains

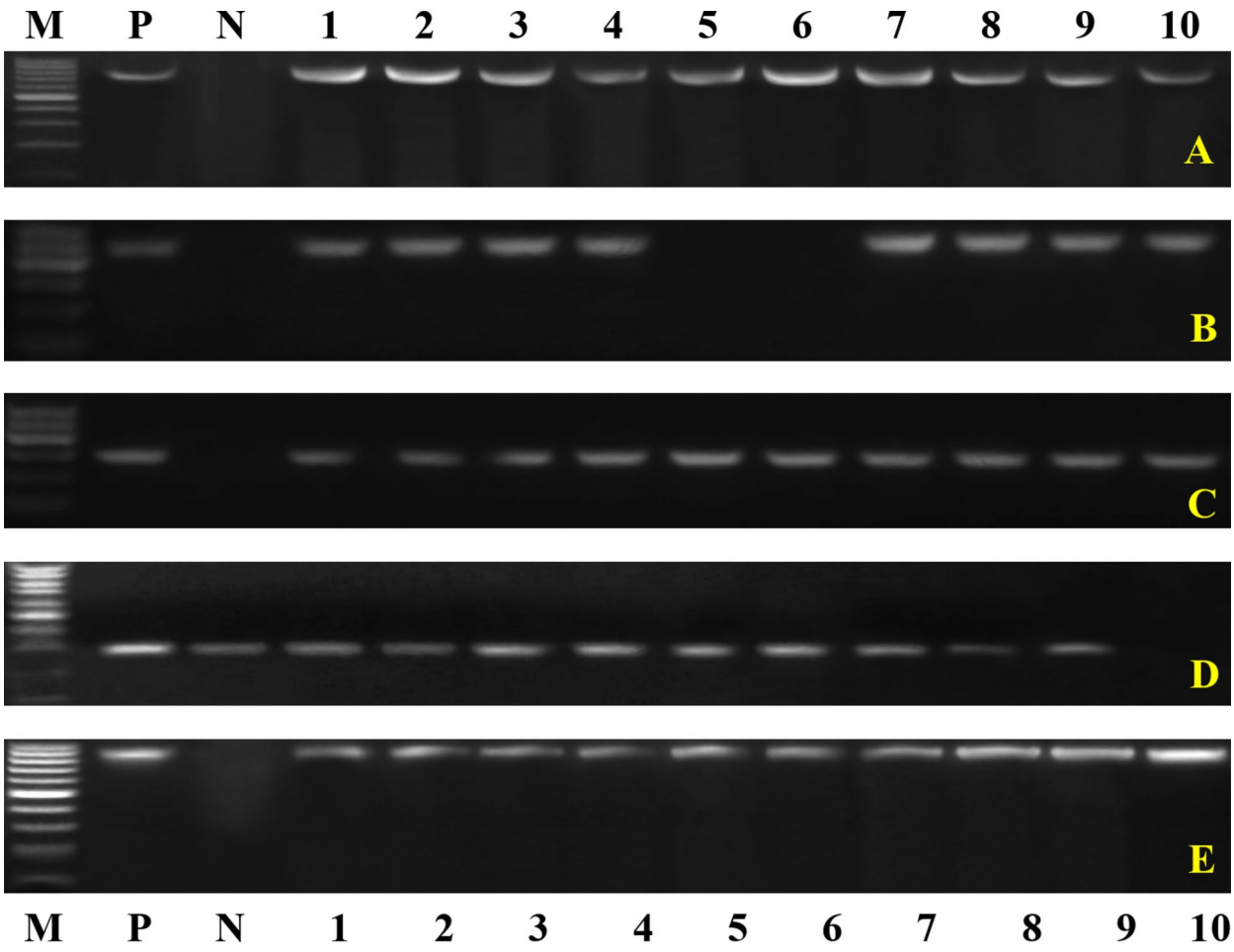

Fig. 3 Disagnostic PCR for some bacterial diseases causing pneumonia in sheep. Agarose gel electrophoresis of the group-specific primer set of the **A** *phoA gene* (genetic marker *of E. coli*), with the expected band size of 720 bp. **B** *P. multocida Kmt1* target gene at 460 bp. **C** Group-specific primer set of *Mannheimia haemolytica ssa* at the expected band size of 508 bp. **D** Group-specific primer set for *Mycoplasma16S rRNA, with* the target gene at 280 bp. **E** The group-specific primer set for Streptococcus16S rRNA, with positive samples except the 912 bp Lane (M): DNA ladder. Lane (N): negative control. Lane (P): control positive Lanes (1--10) samples

ICAM1 (399 bp), SOD1 (387 bp), CAT (308 bp), GPX1 (494 bp), NOS (540 bp), HMOX1 (387 bp), and NQO1 (377 bp) between healthy controls and pneumonic ewes. A comparison of pneumonic sheep with healthy controls revealed changes in exonic regions, resulting in altered DNA sequences. A total of 48 SNPs were identified, with 17 synonymous and 31 nonsynonymous mutations (Table 5).

Gene expression patterns of immune and antioxidant markers

Figures 4 and 5 show the gene expression profiles of immunological and antioxidant markers. In sheep with pneumonia, the expression levels of *IL-1α*, *IL-1B*, *IL6*, *TNF-α*, *LFA-1*, *CR2*, *IL17*, *IL13*, *DEFB123*, *SCART1*, *ICAM1*, *NOS*, and *HMOX1* were significantly greater than those in healthy control sheep. Conversely, the expression levels of IL10, SOD1, CAT, GPX1, and NQO1 were significantly lower in the pneumonia group than in the healthy control group. The gene expression of immune and antioxidant markers was significantly related to pneumonia resistance/susceptibility. In healthy Barki ewes, the most downregulated gene was LFA-1 (0.27 ± 0.07), whereas the most upregulated gene was SOD1 (2.15 ± 0.13). In sheep with pneumonia, CAT (0.35 ± 0.11) was the most downregulated, whereas IL-1α (2.66 ± 0.08) was the most upregulated.

Immunological and antioxidant profiles

Table 6 highlights the significant innate immune response in ewes with pneumonia, as evidenced by elevated levels of proinflammatory cytokines (IL-1α, IL-1β, IL-6, and TNF-α) and free radicals (NO and MDA) in the pneumonia group (PG) compared with those in the healthy control group (HCG). In contrast, compared with the HCG, the PG presented significantly lower levels of C3 and C4, antioxidants (CAT, GPx, and GR), and anti-inflammatory cytokines (IL-10). Notably, the PG resulted in significant

Table 5 Distribution of SNPs and types of mutations in immune and antioxidant genes in healthy and pneumonia-affected sheep

Gene	SNPs	Healthy	Pneumonia	Total	Type of mutation	Amino acid number and type
IL-1α	T111C	21	-	21/100	Synonymous	37 D
	A122G	32	-	32/100	Non-synonymous	41 E to G
	T285C	-	26	26/100	Synonymous	95 D
IL-1β	C56T	-	17	17/100	Non-synonymous	19 S to L
IL-6	C66T	-	34	34/100	Synonymous	22 H
TNF-α	C174T	22	-	22/100	Synonymous	58 A
	C278T	-	28	28/100	Non-synonymous	93 S to L
	T330C	-	41	41/100	Synonymous	110 S
IL-10	G87C	38	-	38/100	Synonymous	29 P
	C137G	-	25	25/100	Non-synonymous	46 P to R
	G174A	-	36	36/100	Synonymous	58 Q
	T374C	33	-	33/100	Non-synonymous	125 M to T
	G404A	-	29	29/100	Non-synonymous	135 R to H
LFA-1	C85T	-	14	14/100	Non-synonymous	29 R to C
	A142G	43	-	43/100	Non-synonymous	48 S to G
	G154A	35	-	35/100	Non-synonymous	52 G to S
	T281C	-	24	24/100	Non-synonymous	94 L to P
CR2	C100G	37	-	37/100	Non-synonymous	34 L to V
	T111C	28	-	28/100	Synonymous	37 D
	T269A	34	-	34/100	Non-synonymous	90 L to M
IL-17	G72A	29	-	29/100	Synonymous	24 G
	T327C	-	31	31/100	Synonymous	109 N
	T360C	39	-	39/100	Synonymous	120 L
IL-13	C294A	-	36	36/100	Non-synonymous	98 H to Q
DEFB123	A56G	-	40	40/100	Non-synonymous	19 H to R
	T186G	-	21	21/100	Non-synonymous	62 H to Q
SCART1	C77G	42	-	42/100	Non-synonymous	26 P to R
ICAM1	C114T	-	31	31/100	Synonymous	38 L
	C232T	17	-	17/100	Non-synonymous	78 P to S
SOD1	T51C	36	-	36/100	Synonymous	17 S
	C196T	-	16	16/100	Synonymous	66 L
	G208T	24	-	24/100	Non-synonymous	70 D to Y
CAT	T64C	-	17	17/100	Synonymous	22 L
	C158T	-	44	44/100	Non-synonymous	53 A to V
GPX1	C62G	-	32	32/100	Non-synonymous	21 P to R
	C193A	13	-	13/100	Non-synonymous	65 H to N
	C256G	45	-	45/100	Non-synonymous	89 R to G
	C298T	33	-	33/100	Non-synonymous	100 R to C
	G391C	-	25	25/100	Non-synonymous	131 E to Q
NOS	G175A	19	-	19/100	Non-synonymous	59 A to T
	A244C	42	-	42/100	Non-synonymous	82 S to R
	C367T	30	-	30/100	Non-synonymous	123 P to S
	T457C	-	15	15/100	Non-synonymous	153 W to R
HMOX1	C33T	-	27	27/100	Synonymous	11 P
	C108T	44	-	44/100	Synonymous	36 A
	C352T	-	23	23/100	Non-synonymous	118 R to C
NQO1	G56T	25	-	25/100	Non-synonymous	19 R to L
	G244A	17	-	17/100	Non-synonymous	82 G to R

-A= Alanine; C= Cisteine; D= Aspartic acid; E= Glutamic acid; G= Glycine; H= Histidine; L= Leucine; M= Methionine; N= Asparagine; P= Proline; Q= Glutamine; R= Argnine; S= Serine; T= Threonine; V= Valine; W= Tryptophan; and Y= Tyrosine

-*IL-1α* Interleukin 1 alpha, *IL-1β* Interleukin 1 beta, *IL-6* Interleukin 6, *TNF- α* Tumor necrosis factor- alpha, *IL10* Interleukin 10, *LFA-1* Lymphocyte function-associated antigen 1, *CR2* Complement C3d receptor 2, *IL-17* Interleukin 17, *IL-13* Interleukin 13, *DEFB123* β-defensin, *SCART1* Scavenger Receptor Family Member Expressed On T Cells 1, *ICAM1* intercellular adhesion molecule 1, *SOD1* Superoxide dismutase 1, *CAT* Catalase, *GPX1* Glutathione peroxidase 1, *NOS* Nitric oxide synthetase, *HMOX1* Heme Oxygenase 1, *NQO1* NAD(P)H Quinone Dehydrogenase 1

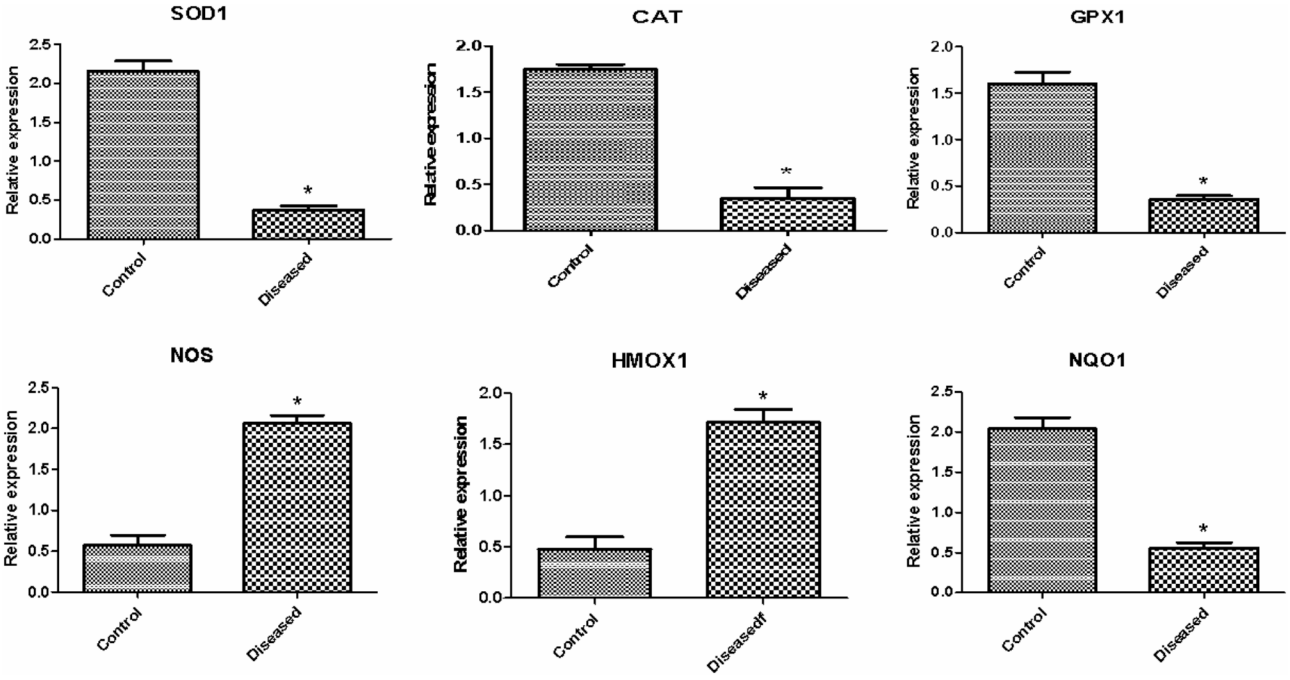

Fig. 4 Antioxidant gene transcript levels in healthy and pneumonic sheep. The symbol * denotes significance when $p < 0.05$

hyperimmunoglobulinemia, indicating a robust humoral immune response. The cytokines and oxidative stress markers demonstrated high sensitivity, specificity, PPV, NPV, and accuracy rates (AUC = 1), except for NO, which showed low specificity and LR. The levels of proinflammatory cytokines, particularly IL-1α, were greater than those of oxidative stress markers (Table 7).

Pathological findings

The affected lungs exhibited pronounced irregular consolidation with a lobular or lobar to diffuse pattern, primarily in the cranioventral to caudal lobes. The areas of consolidation varied in color from dark red to gray, pink, and marbled hues (Fig. 6). Histopathological examination revealed alveolar emphysema and giant alveoli in 47 out of 50 (94%) lung samples (Fig. 7a); catarrhal bronchiolitis, edema, and congestive atelectasis in 30 lung samples (60%) (Fig. 7b); catarrhal bronchitis, hyperplasia of the epithelial lining, and desquamated epithelial cells in 35 lung samples (70%) (Fig. 7c); active alveolar hyperemia and atelectasis in 35 lung samples (80%) (Fig. 7d); granuloma formation in 48 lung samples (96%) (Fig. 7e); and serous pneumonia with congestion in 38 lung samples (76%) (Fig. 7f).

Correlations between gene expression patterns and immune and antioxidant marker serum profiles

The mRNA levels of IL6 were negatively correlated with the serum levels of IL1α, IL1β, IL6, C4, CAT, NO, and MDA ($r = -1$, $p = 0.002$). Additionally, the mRNA levels of IL-1β were positively correlated with the serum IL-10 levels ($r = 0.997$, $p = 0.04$). The mRNA levels of IL17 were positively correlated with the serum levels of C3 ($r = 1$, $p = 0.005$) (Fig. 8).

Discussion

Pneumonia is considered a major source of economic loss because of its frequent occurrence across all age groups and types of sheep, its high morbidity and mortality rates, and the associated slow growth and veterinary costs [59]. Desert-dwelling animals face stressors such as extreme summer heat and winter cold, increasing their vulnerability to microbial infections, especially respiratory diseases [60]. Our objective was to investigate the bacterial species and associated virulence gene profiles isolated from sheep exhibiting respiratory symptoms, as confirmed by PCR. This study also aimed to characterize the macroscopic and histological abnormalities observed in the affected lungs. Additionally, we sought to evaluate the potential role of genetic polymorphisms, oxidative stress biomarkers, and proinflammatory cytokines as diagnostic tools for pneumonia in Barki sheep.

Compared with healthy control sheep, the pneumonic sheep in this study presented harsh lung sounds upon auscultation, coughing, cyanotic mucous membranes, dyspnea, bilateral nasal discharge, elevated body temperature, and increased heart and respiratory rates. Our clinical findings align with those reported in sheep [12, 35, 61], buffalo calves [62], and calves [63–65]. The observed anorexia, depression, and dullness may result

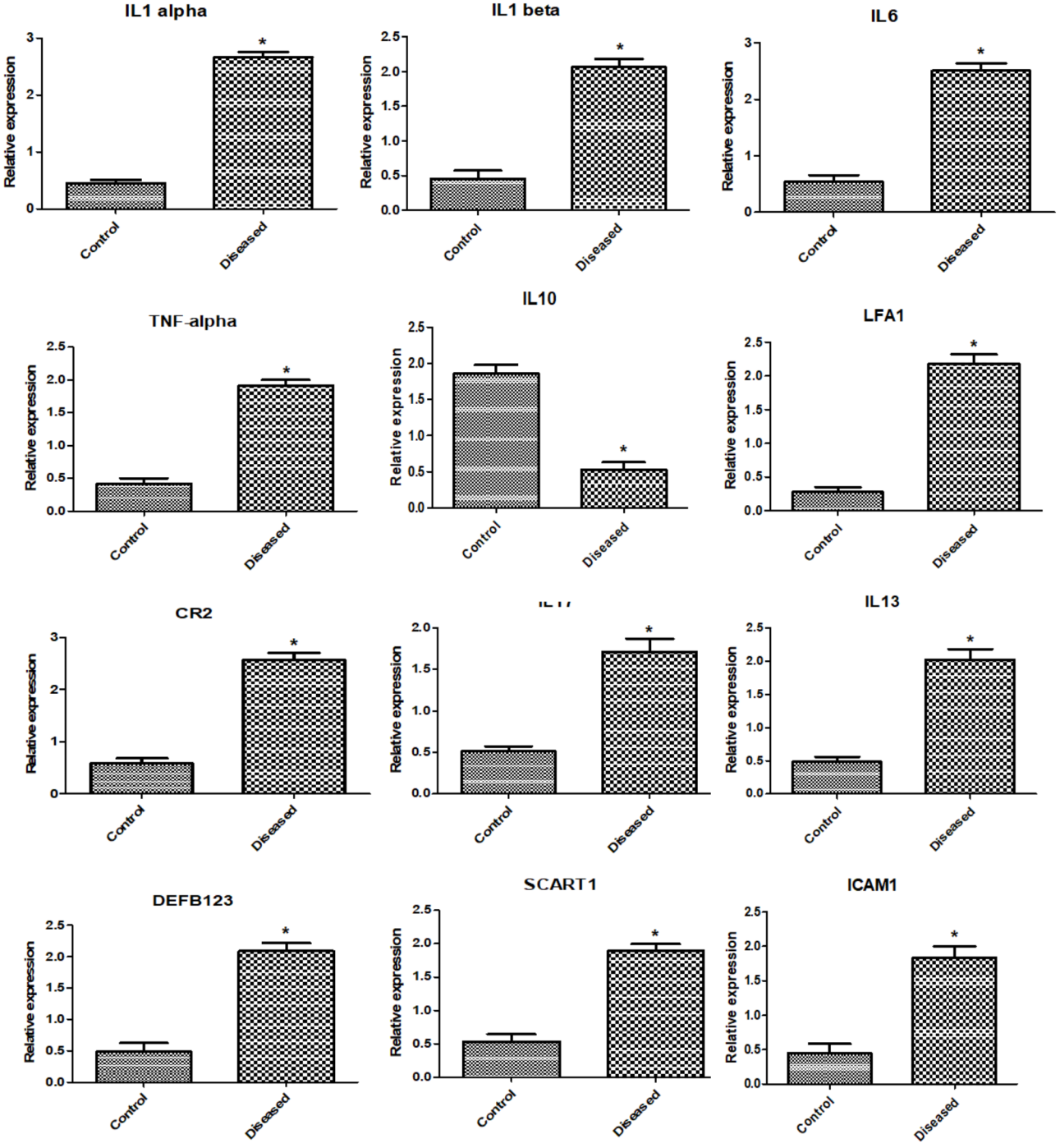

Fig. 5 Immune gene transcript levels in healthy and pneumonic sheep. The symbol * denotes significance when $p < 0.05$

from muscular weakness due to intracellular potassium leakage, hyperkalaemia, and hypoglycemia [66]. Additionally, hyperthermia and pain responses can be attributed to infection and inflammation, stimulation of the hypothalamic thermoregulatory center, and the release of pyrogenic substances such as prostaglandins [67].

Klebsiella pneumoniae was isolated from 40% (60/150) of the pneumonic sheep in this study, as confirmed by PCR. This prevalence is higher than the 24.2% reported by [68] in Taif, KSA, but it is consistent with [69], who isolated *Klebsiella pneumoniae* from pneumonic sheep in Egypt by 36% and 48%, respectively. According to [70], the rate was 27.15%. On the other hand [71] and [72], from Austria and Brazil reported rates of 3.1% and 6.2%, respectively, whereas [41] from Iran reported a rate of 15.09%. Multiple virulence factors that aid in colonization

Table 6 Comparison of the immunological and antioxidant profile parameters between the pneumonic group and the control group. The values are the means ± SDs

Parameters	CG	PG
IL-1α (Pg/ml)	24.05 ± 3.34	95.81 ± 1.68*
IL-1β (Pg/ml)	25.99 ± 2.96	99.11 ± 0.65*
IL-6 (Pg/ml)	24.63 ± 2.92	86.36 ± 0.09*
TNF-α (Pg/ml)	24.91 ± 2.98	87.44 ± 1.60*
IL-10 (Pg/ml)	103.70 ± 3.31	90.69 ± 2.24*
C3 (mg/dl)	151.17 ± 5.29	110.49 ± 1.83*
C4 (mg/dl)	12.07 ± 0.60	7.14 ± 0.83*
IgG (mg/dl)	229.02 ± 19.74	350.26 ± 36.07*
IgM (mg/dl)	13.79 ± 1.43	39.26 ± 3.04*
IgA (mg/dl)	4.49 ± 0.83	8.43 ± 0.63*
MDA (nmol/ml)	12.95 ± 1.14	23.70 ± 1.51*
NO (µmol/L)	26.80 ± 1.20	32.33 ± 1.66*
CAT (U/L)	412.25 ± 14.64	289.20 ± 6.16*
GPx (mU/L)	1015.45 ± 2.95	742.87 ± 16.76*
GR (ng/ml)	8.15 ± 0.62	5.16 ± 0.44*

IL-1α Interleukin 1 alpha, *IL-1β* Interleukin 1 beta, *IL-6* Interleukin 6, *TNF- α* Tumor necrosis factor- alpha, *IL10* Interleukin 10, *C3* Complement 3, *C4* Complement 4, *IgG* Immunoglobulin G, *IgM* Immunoglobulin M, *IgA* Immunoglobulin A, *MDA* Malondialdehyde, *NO* Nitric oxide, *CAT* Catalase, *GPx* Glutathione peroxidase, *GR* Glutathione reductase

Significant differences between the two groups, were indicated by (*) when $P < 0.05$

and pathogenesis are present in *K. pneumoniae*. Particularly in immunocompromised animals, virulence factors make the organism more resilient to effective host defenses and make infection easier [73]. Both the *iutA* and the *fimbrial adhesin fimH* virulence genes were present in 39% and 68% of the *Klebsiella pneumoniae* strains, respectively. These findings support those of earlier research in Iran [74], but they are less extensive than those of [75] in Egypt, who isolated them from all samples and demonstrated that these virulence factors are critical for the pathogenicity of *K. pneumoniae*.

According to [76], *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause a variety of infections in the gastrointestinal tract, respiratory system, ocular region, and other anatomical sites. The isolation rate of *Pseudomonas aeruginosa* in the current study was 18% (27/150). While [77] reported 20% in Egypt and [78] reported 21.8% in the USA, the average percentage results are in line with those previously reported by [27] at 15% in Egypt; however, the values are higher than those reported by [78] at 11.4% [79], at 10%, and [70] in Egypt at 9.1%. The *P. aeruginosa* isolation rate was 5.8%, according to [27] and [22] (16/27). The enterotoxin gene *(toxA)* is present in 59.2% of *Pseudomonas aeruginosa* isolates. This result was lower than that of [80] (69.4%) and [81] (76.6%) in Iran, but it was consistent with that previously reported by [27] (61%) in Egypt.

According to our data, *E. coli* was isolated from 30 out of 150 cases (20%). Ali & Abu-Zaid [69] reported that the incidence of *E. coli* in Egypt was 18%, whereas [82] reported a 20.78% isolation rate from respiratory-infected sheep in Diwaniya Governorate, Iraq. Pneumonia was also found in 19% of cases in Bikaner, Rajasthan, India [83], and in Bangladesh (20%), as a component of a mixed infection [84]. On the other hand [85], reported a 15.38% organism isolation rate from the lungs in Egypt, compared with 8% in [79] and 10.6% in [86]. In another study, *E. coli* strains of different serotypes were isolated at a rate of 21.8% [70], which was slightly higher than the rates reported by [87] and [88] in Egypt (72.7% and 69.7%, respectively).

Some strains of *E. coli* carry virulence genes that can cause intestinal or extraintestinal diseases, whereas others possess genes encoding toxins. The genetic diversity of *E. coli* strains offers valuable insights into their evolutionary history, relationships, and potential transmission risks to humans and animals [89]. The identification of pathogenic *E. coli* strains is facilitated by the presence of virulence factors. This information can be used to stop the spread of *E. coli* infections and to create new treatments [90].

P. multocida was found in 28.6% (43/150) of the isolates. These findings are somewhat at odds with those of

Table 7 Cut-off points, sensitivity, specificity, LR, PPV, NPV, accuracy rate and percentages of increase or decrease in the estimated cytokines and oxidative stress markers in the PG compared with those in the CG

Parameters	Cut-off	Sensitivity	Specificity	PPV	NPV	LR	AR	% of increase or decrease
IL-1α (Pg/ml)	29.50	100%	90%	93.75%	100%	10	96%	298.38%
IL-1β (Pg/ml)	28.50	100%	85%	90.91%	100%	6.6	94%	281.34%
IL-6 (Pg/ml)	28.50	100%	90%	93.75%	100%	10	96%	250.63%
TNF-α (Pg/ml)	28.50	100%	90%	93.75%	100%	10	96%	251.02%
IL-10 (Pg/ml)	100.80	100%	80%	88.24%	100%	5	92%	−12.57%
MDA (nmol/ml)	14.10	100%	80%	88.24%	100%	5	92%	83.01%
NO (µmol/L)	27.50	100%	60%	78.95%	100%	2.5	84%	20.63%
CAT (U/L)	392.50	100%	90%	93.75%	100%	10	96%	−29.85%
GPx (mU/L)	1012.00	100%	90%	93.75%	100%	10	96%	−26.81%
GR (ng/ml)	7.59	100%	80%	88.24%	100%	5	92%	−36.69%

LR = 0.5–5: low; LR = 5–10: moderate; LR > 10: high

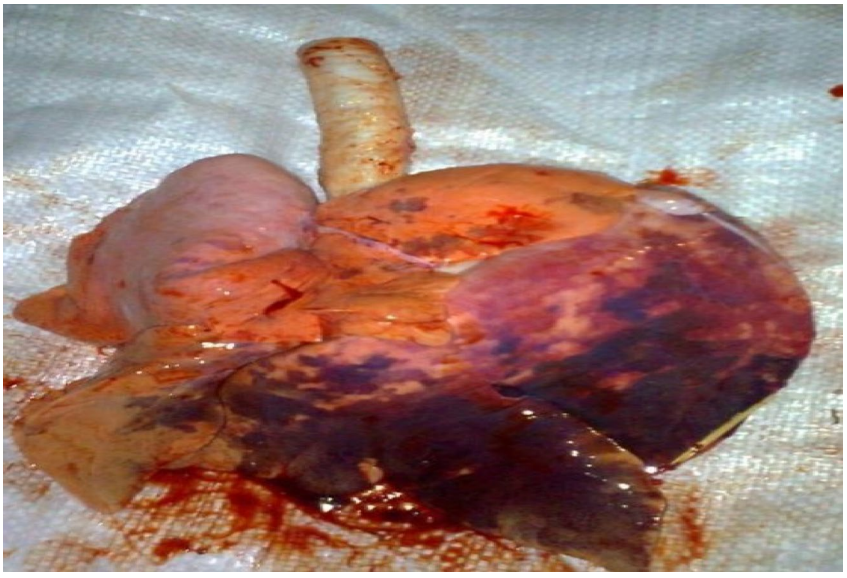

Fig. 6 Languages infected with congestion, hepatization, and marbling appearance

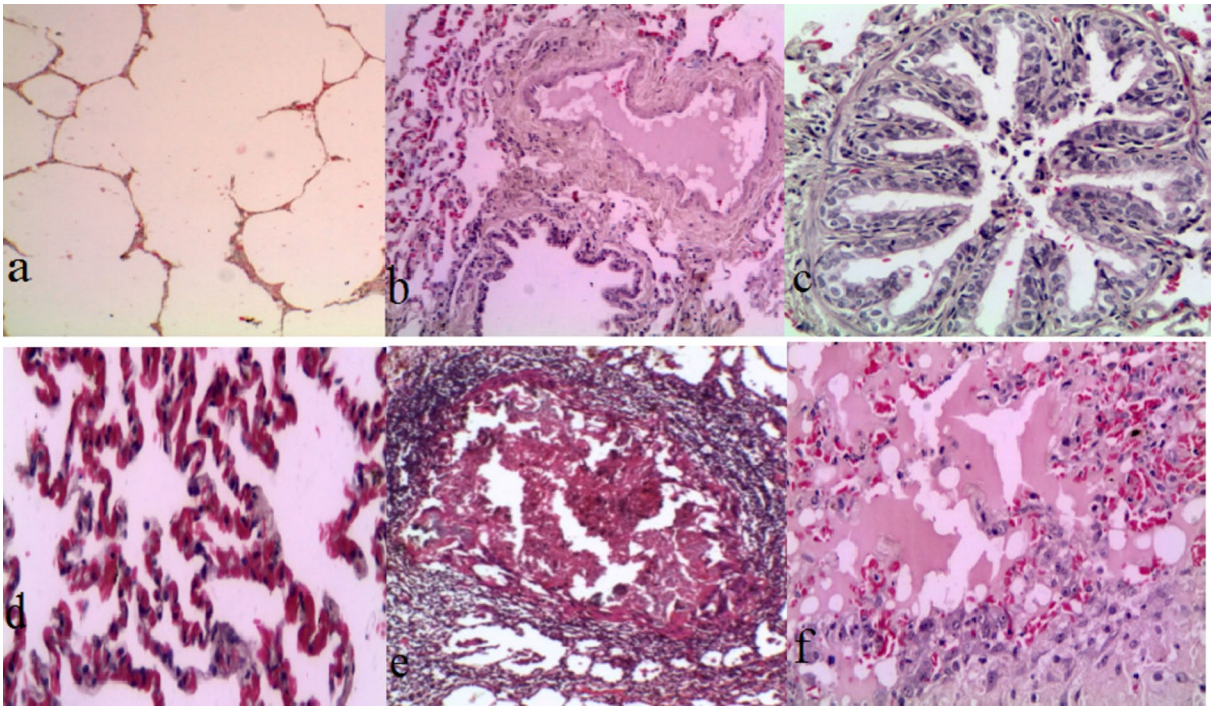

Fig. 7 Lung sheep. -positive samples in the pneumonia stage: **a** Histopathological examination of the lung showing alveolar emphysema and giant alveoli and **b** histopathological examination of the lung showing cattarhal bronchiolitis, edema and congestive atelectasis. **c** Histopathological examination of the lung showing cataract hyperplasia resulting from epithelial linning and the elimination of epithelial cells inside the lumen. **d** Histopathological examination of the lung showing active alveolar hyperemia and atelectasis. **e** Histopathological examination of a lung showing a granuloma. **f** Histopathological examination of a lung showing serous pneumonia and congestion (H&E× 4)

[91], who reported that *P. multocida* was isolated at rates of 28.9% in Egypt. These rates, however, are greater than those reported by [79], who reported a 4% isolation rate; [92], who reported a 5% isolation rate in Egypt; and [93], who isolated *P. multocida* at rates of 11.4% (25/219) from healthy Iranian animals and 4.4% (5/114) from animals with pulmonary symptoms. In addition [94], reported isolation rates of 22% from Iran, 15.25% from pneumonic calves in Ethiopia [95], and 18.2% from Egypt [96], which were lower than those reported by [97] in Egypt (40%). Moreover [98], reported a prevalence of 65.62% from Iran, whereas [99] reported 62.50% from Egypt.

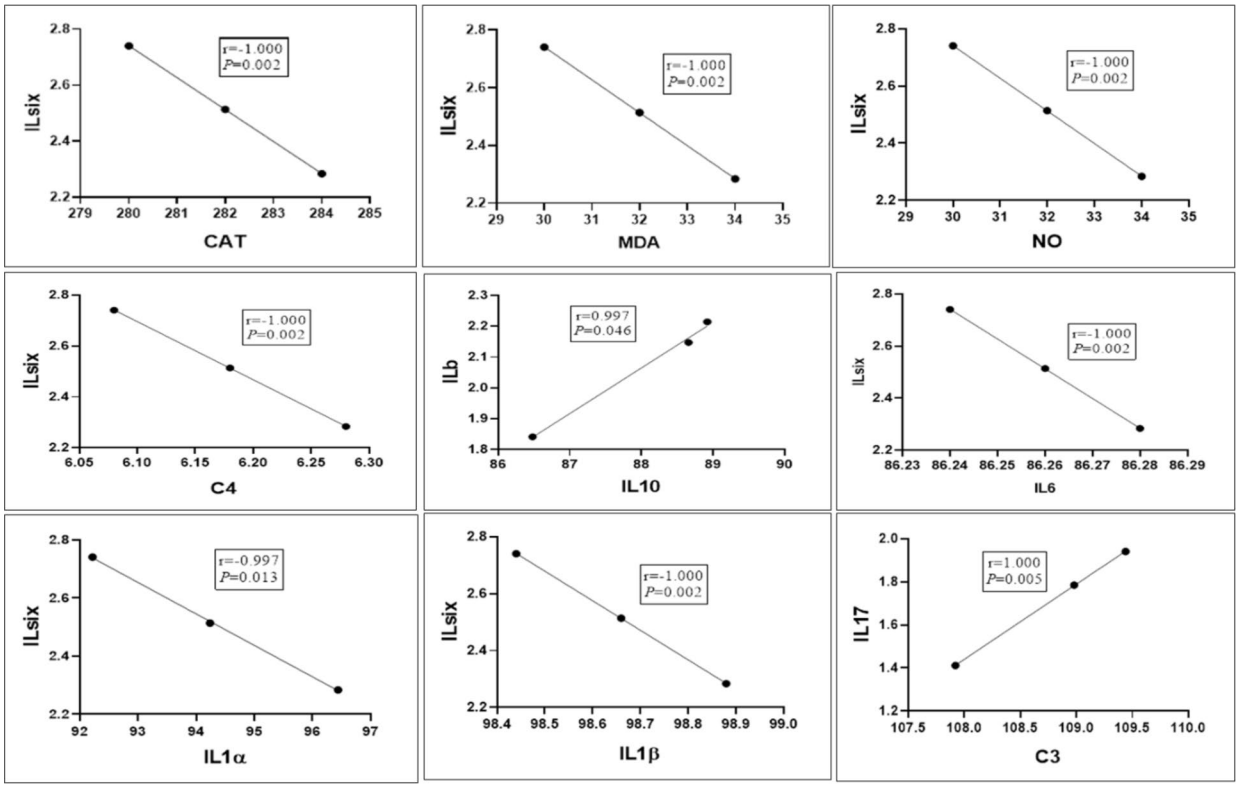

Fig. 8 Correlations between gene expression patterns and serum profiles of immune and antioxidant markers

Following an initial trigger, such as long-distance transportation, poor ventilation, overcrowding, severe weather, or a weakened immune response, *Mannheimia haemolytica*, a commensal of the upper respiratory tract, can become pathogenic, leading to bronchopneumonia [100]. Our findings indicated that *M. haemolytica* was isolated from 31% (47/150) of the samples in this study, which is the overall isolation rate of this species. The findings of [101] in Ethiopia, which revealed a 34.21% isolation rate, closely match this outcome. In Ethiopia, *M. haemolytica* was isolated from sheep with a 28% isolation rate [102], which is somewhat consistent with the current investigation. The findings of [103] in India and [104] in Chhattisgarh, who reported isolation rates of 66.6% and 62%, respectively, in pneumonic lungs, are substantially higher than this rate. In contrast [105], reported a lower isolation rate of 21.87% in Ethiopia.

According to [106], *Mycoplasmas* species are frequently linked to respiratory illnesses in sheep and cause large financial losses. The current study revealed that *Mycoplasma spp*. were present in 44% (66/150) of the samples. In addition [72], reported *Mycoplasma spp*. in 38% of pneumonic sheep in Brazil; [107] reported a decrease of 7.4% in the lower respiratory tract of calves in Brazil. These results are consistent with those of [108] in Jordan, who found *Mycoplasma spp*. via PCR in 45.2% of examined sheep tracheal samples. However [108], reported that the prevalence in Swedish flocks was much higher, at 76%.

Streptococcus was isolated from the sick sheep analysed in 44 out of 150 cases (29.4%), which was consistent with the findings of another earlier study by [85], which reported a prevalence of 26.92%. Our data, on the other hand, were significantly higher than those of other studies; it was lower than the 55% reported by [109] in New Zealand and higher than the 3.9% reported by [82] in Iraq and [70] in Egypt, as well as the 15.5% mentioned by [72] in Brazil and the 14.8% reported by [110].

A positive correlation exists between larger areas and a higher prevalence of illness in warm climates, indicating that climate may influence respiratory health in these regions. Factors such as stress, humidity, dust, and overcrowding are likely to exacerbate these health issues [111]. The differences between the results of the present study and those of previous studies may stem from several factors, including variations in management practices, stressors, hygiene protocols, and the immune status of the infected animals [112].

Our results revealed that PCR-DNA sequencing of fragments of the *IL-1α* (472 bp), *IL-1B* (388 bp), *IL6* (526 bp), *TNF-α* (388 bp), *IL10* (526 bp), *LFA-1* (438 bp), *CR2* (381 bp), *IL17* (462 bp), *IL13* (376 bp), *DEFB123* (371 bp), *SCART1* (220 bp), *ICAM1* (399 bp), *SOD1* (387 bp), *CAT* (308 bp), *GPX1* (494 bp), *NOS* (540 bp),

HMOX1 (387 bp), and *NQO1* (377 bp) genes revealed nucleotide sequence variations in the form of SNPs between pneumonia-affected and healthy controls Barki ewes.

In our opinion, this is the first study to identify SNPs in antioxidant (SOD1, CAT, GPX1, NOS, HMOX1, and NQO1) and immunological (IL-1α, IL1B, IL6, TNF-α, IL10, LFA-1, CR2, IL17, IL13, DEFB123, SCART1, and ICAM1) genes as potential suspects for pneumonia resistance/susceptibility in Barki ewes. Interestingly, compared with the corresponding GenBank reference sequence, our results showed that the polymorphisms found in the investigated genes were reported here for the first time. Nonetheless, the candidate gene method was applied to evaluate ruminant susceptibility to pneumonia. The MHC-DRB1 exon 2 polymorphism, for example, was linked to *Mycoplasma ovipneumonia* resistance or susceptibility genotypes in sheep, according to [113]. The impact of TMEM154 gene polymorphisms on sheep vulnerability to ovine progressive pneumonia virus after natural exposure was investigated by [114]. In Baladi goats with pneumonia and healthy control group, PCR-DNA sequencing revealed SNPs linked to pneumonia susceptibility and resistance in immunological genes (SLC11A1, CD-14, CCL2, TLR1, TLR7, TLR8, TLR9, defensin, SP110, SPP1, BP1, A2M, ADORA3, CARD15, IRF3, and SCART1) [115].

The current study used real-time PCR to measure the levels of antioxidant and immunity-related mRNAs in both healthy controls and pneumonic Barki ewes. Our results revealed greater expression patterns of the IL-1α, IL-1B, IL6, TNF-α, LFA-1, CR2, IL17, IL13, DEFB123, SCART1, ICAM1, NOS, and HMOX1 genes in ewes with pneumonia than in healthy controls ewes. However, for the genes IL10, SOD1, CAT, GPX1, and NQO1, the opposite trend was observed. Our work is the first to use real-time PCR to determine immunological and mRNA levels in healthy controls and pneumonic ewes. Using genetic markers such as RFLP and SNPs, previous studies have investigated gene polymorphisms in domestic animals [113, 114]. Our study, however, was intended to address the drawbacks of earlier research by examining gene polymorphisms via gene expression and SNP genetic markers. Thus, the processes of gene regulation that have been studied in both healthy and pneumonia-affected lambs are well understood. The levels of immunological genes (SLC11A1, CD-14, CCL2, TLR1, TLR7, TLR8, TLR9, defensin, SP110, SPP1, BP1, A2M, ADORA3, CARD15, IRF3, and SCART1) vary in goats with and without pneumonia [115]. Complement genes and Toll-like receptors (TLRs) are linked to infectious pneumonia in sheep according to gene expression profiling [116].

In instances of inflammation, cytokines, including IL1α, IL-6, TNF-α, IL1B, IL17, and IL13, function as indirect indicators [117]. Among the primary proinflammatory cytokines that are involved in the immune response is TNF-α. TNF-α promotes the growth, development, and activity of numerous immune system cells, including B, T, NK (natural killer), and LAK (lymphokine-activated killer) cells, among others [118]. Additionally, a variety of additional cytokines are released in response to TNF-α [119]. TNF-α is encoded by a gene on chromosome BTA23q22 that has three introns and four exons [120]. Numerous mammalian cell types express TNF-α, with the highest levels observed in monocytes and macrophages. In these phagocytic cells, lipopolysaccharide (LPS) from bacterial cell walls triggers the synthesis of TNF-α. In LPS-stimulated macrophages, TNF-α gene expression increases threefold, mRNA levels increase approximately 100-fold, and protein secretion may increase by up to 10,000-fold [121]. According to [122], lipopolysaccharide-induced TNF-α factor (LITAF) is a new protein that binds to a crucial area of the TNF-α promoter and is thought to be involved in activating TNF-α expression after LPS induction. According to [123], β-defensins have been detected in vertebrates from a variety of tissues or cells, including blood plasma, urine, epithelial cells, neutrophils, and other leucocytes. During inflammation, defensins are released by neutrophils and monocytes (phagocytes) as microbicidal agents [124].

Together, these anti-inflammatory cytokines regulate the host inflammatory response to microbial antigens; interleukin-10 functions mainly as a feedback inhibitor of T-cell responses [125]. Leukocyte-specific integrins, known as beta 2 integrins, are expressed on the cell surface as heterodimers composed of the b subunit CD18 and the subunit CD11. Four distinct b2 integrins are produced by these subunit associations: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), CD11c/CD18 (CR4), and CD11d/CD18 [126]. Leukotoxin-beta 2-integrin interactions mediate the lethal effect of leukotoxin on ruminant leukocytes, according to previous research [127, 128]. Given its role in immune response regulation, CR2 is a viable candidate susceptibility gene for infection [129].

According to [130], the protein known as CD54 (cluster of differentiation 54), or intercellular adhesion molecule 1 (ICAM-1), is produced by the ICAM1 gene. This gene typically produces a cell surface glycoprotein on endothelial cells and immune system cells. T-cell-mediated host defense mechanisms and inflammatory processes are both mediated by ICAM-1 [130]. It is a costimulatory protein that activates MHC class II-restricted T cells on antigen-presenting cells and works in tandem with MHC class I to activate cytotoxic T cells on other cell types [130]. A protein that is present in only a specific subset of delta gamma T cells and is produced by the scavenger

receptor family member expressed on T cells (SCART1) gene serves to recognize important pathogens [131]. However [132], via genome-wide association analysis, reported that the SCART1 gene was linked to foot health features in Holstein dairy cattle.

Antioxidants can scavenge or detoxify ROS, prevent their generation, or sequester transition metals that are the source of free radicals [133]. These systems constitute the body's innate antioxidant defenses, such as glutathione peroxidase (GPx), catalase, and SOD, which are both enzymatic and nonenzymatic [134]. According to [135], type 2 nitric oxide synthase (NOS2) produces nitric oxide (NO), which is one of the primary ways that the host defends against infections. Through the 5-electron oxidation of the terminal guanidine-N2 of the amino acid L-arginine, constitutive endothelial or neural NO synthases or, at higher doses, inducible NO synthase (NOS2) produce nitric oxide.

LPS and other bacterial products, as well as cytokines, can trigger NOS2 expression [136]. Heme is broken down into equimolar amounts of carbon monoxide (CO), free iron, and biliverdin by the enzyme heme oxygenase (HO), which is the rate-limiting enzyme in the heme catabolic pathway [137]. It has been proposed to have a wide range of protective effects against many stressors, including anti-inflammatory, anti-apoptotic, anti-coagulation, anti-proliferative, vasodilative, and antioxidant effects. It is also referred to as a stress-responsive protein [138]. An antioxidant called NQO1 protects against the harmful oxidative and arylating effects of quinones [139]. NQO1's detoxification mechanism for quinones is based on the direct two-electron reduction of quinones to hydroquinones, which eliminates electrophilic quinones and prevents the production of semiquinone radicals and reactive oxygen species through redox cycling processes [140].

The fact that injured tissues experience more free radical responses than healthy tissues may be the reason for the noticeable change in the expression patterns of immunological and antioxidant markers in lambs with pneumonia [141]. While the resistant genotypes dramatically increase the expression of IL-2 and IL-10, the susceptible genotypes stimulate inflammatory responses by increasing the expression of TNFa, IFNc, IL-4, IL-6, and IL-1b. Pneumonia also creates a variety of cellular immunological components that mediate and regulate the inflammatory response and immune function by binding to cell surface receptors [142]. Several investigations have shown that the relationships between these immune factors are complex. For example, a complex network structure is created by the interaction of these proteins, which affects the production of different immune globulins, complement proteins, and acute-phase reactive proteins [143]. Consequently, we hypothesize that the majority of the ewes in this study may have had infectious pneumonia. Additionally, there is solid evidence from our real-time PCR data that sheep with pneumonia experienced a significant inflammatory response.

According to the results of the present study, compared with the healthy group, the PG group presented a significant increase in the levels of proinflammatory cytokines. The immune system's inflammatory response to an infection is triggered and intensified by these cytokines. By increasing the synthesis of humoral immune molecules, such as immunoglobulins (Igs), and innate immune molecules, such as acute-phase proteins, free radicals, complement factors, and matrix metalloproteinases can be produced. They are vital to the host's defense. Our findings were similar to those reported by [14, 35] in sheep and [144, 145] cattle calves and [146] in cattle. On the other hand, anti-inflammatory cytokines are responsible for inhibiting the proinflammatory response to prevent excessive inflammation. Fernández et al. [147] reported that the decreased levels of anti-inflammatory cytokines in the PG likely worsened the condition by enabling the innate immune response to become more aggressive. This mismatch between pro- and anti-inflammatory signals exacerbates the situation and increases the severity of the inflammatory response during infection. Research by [35] revealed that acute pneumonic sheep had reduced IL-10 levels.

One characteristic of oxidative stress is the imbalance between antioxidants and free radicals, which was noted in this investigation. According to [148], free radicals are unstable molecules produced in trace amounts during cellular processes under normal physiological conditions. They try to stabilize by stealing electrons from molecules that are close by. By neutralizing these free radicals, antioxidants protect host cells and tissues from their damaging effects [149]. On the other hand, immune cells generate many free radicals when proinflammatory cytokines are activated during infection. These reactive molecules first attack and harm pathogen lipids, DNA, and carbohydrates, thereby assisting in their death [150]. Free radical buildup can eventually overwhelm the body's antioxidant defenses. Free radicals then start attacking the host's own cells, causing oxidative stress, a condition that aids in the pathogenesis of disease [149]. There have been prior reports of oxidative stress in sheep [59, 148] and calves [64, 65]. In addition to worsening tissue damage, oxidative stress is a major factor in disease progression.

The complex network of interacting β-globulins that attach to cell membranes or circulate freely in bodily fluids comprises the complement system, which is essential to innate lung immunity. As a first line of defense against infections, these complement proteins are generated by macrophages in response to proinflammatory cytokines

[151]. Consistent with earlier findings, PG in this study significantly decreased the serum levels of the complement components C3 and C4 [152]. This decrease is most likely the result of hyperactivation of the complement cascade, which can be caused by bacterial lipopolysaccharides (LPS) via the alternative pathway or antigen–antibody complexes (IgG and IgM) via the classical pathway [151].

Many immune effectors, such as membrane attack complexes (C5b--C9), opsonins (iC3b, C3b, and C4b), and chemoattractants (C3a, C4a, and C5a), are produced by the cleavage of C3 and C4 upon activation. According to [152], these molecules aid in the elimination of pathogens, stimulate neutrophil chemotaxis, encourage adhesion to endothelial cells, improve the respiratory burst in phagocytes, and modulate the acute inflammatory response by causing mast cell degranulation (releasing histamine). According to [151], the decreased levels of C3 and C4 found in this study are suggestive of severe lung damage, such as emphysema, airway dysfunction, gas trapping, and impaired lung elasticity. This depletion of complement proteins may be integral to the pathogenesis of bacterial pneumonia. Certain bacterial strains can undermine the complement system by producing toxins or surface proteins that interfere with its protective role in the lungs. As a result, complement proteins are consumed, increasing the bacterial load and amplifying the production of proinflammatory cytokines such as IL-1β and TNF-α, which further exacerbates lung inflammation [152].

The activation of humoral immunity in the PG is also highlighted by the observed increase in immunoglobulins (γ-globulins) in the PG. This increase aligns with earlier research [35]. Igs that B cells generate in response to cytokines promote inflammation. By encouraging microbial antigen phagocytosis, neutralizing bacterial toxins, competing with pathogens for host cell attachment, precipitating microorganisms, and initiating the complement cascade, they strengthen the immune response. Together, these processes strengthen the body's defenses against infection and highlight the role that humoral immunity plays in preventing bacterial pneumonia [152].

With respect to the diagnostic and prognostic value of cytokines and oxidative stress markers, they had high values of sensitivity, specificity, PPV, NPV, and accuracy rate at AUC = 1 and moderate LRs. This result partially agreed with previous data suggesting that proinflammatory cytokines and oxidative stress indicators are sensitive markers for pneumonia in sheep [14, 35, 153], goats [154] and cattle calves [148], but this result disagrees with these studies in terms of marker preference, as it prioritizes proinflammatory cytokines among oxidative stress markers (due to their percentage increase, especially IL-1α) and excludes NO (due to its low specificity and LR). Several factors may result in this disagreement, such as animal age, nutrition and breed, disease stage, etiology, and method of marker estimation.

In the present study, the anatomic lung lesions in the pneumonic sheep matched the macroscopic pathological abnormalities in the lungs, which presented much more severe inflammatory lesions characterized by extensive red and gray hepatization and congestion and a marbling appearance [155]. Lung histopathological analysis revealed giant alveoli, alveolar emphysema, granulomas, serous pneumonia, and congestion, which supported earlier findings [61, 156].

Conclusion

This study revealed a diverse range of bacterial pathogens associated with respiratory illnesses in sheep flocks in Egypt, underscoring the importance of their identification and isolation. Our findings highlight the importance of SNPs in immune and antioxidant genes as genetic markers influencing pneumonia in healthy and pneumonic sheep. The genetic variability in these genes could serve as proxy biomarkers for pneumonia in Barki sheep. Additionally, the distinct expression patterns of immune and antioxidant genes in healthy and pneumonic Barki sheep may provide valuable insights for monitoring their health status. These findings offer a promising opportunity to mitigate pneumonia through selective breeding based on genetic markers linked to natural resistance. Finally, sheep pneumonia is characterized by alterations in the biochemical profile of immunological and antioxidant markers, triggering both innate and humoral immune responses.

Abbreviations

IL-1α	Interleukin 1 alpha
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
TNF-α	Tumor necrosis factor-alpha
IL10	Interleukin 10
LFA-1	Lymphocyte function-associated antigen 1
CR2	Complement C3d receptor 2
IL-17	Interleukin 17
IL-13	Interleukin 13
DEFB123	β-defensin
SCART1	Scavenger Receptor Family Member Expressed on T Cells 1
ICAM1	Intercellular adhesion molecule 1
SOD1	Superoxide dismutase 1
CAT	Catalase
GPX1	Glutathione peroxidase 1
NOS	Nitric oxide synthetase
HMOX1	Heme oxygenase 1
NQO1	NAD(P)H quinone dehydrogenase 1
C3	Complement 3
C4	Complement 4
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgA	Immunoglobulin A
MDA	Malondialdehyde
NO	Nitric oxide
CAT	Catalase
GPx	Glutathione peroxidase
GR	Glutathione reductase

Acknowledgements
The authors acknowledge the staff members of the Animal Health and Poultry Department, Desert Research Center, Egypt.

Authors' contributions
Asmaa Darwish and Ahmed El Sayed conceived, designed the experiment, collected blood samples, performed biochemical analysis, analyzed the data and wrote the manuscript. Ahmed Ateya, Amin Tahoun and Amani Hafez performed real-time PCR and contributed to writing the manuscript.

Funding
Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).
The author(s) received no financial support for the research, authorship, and/or publication of this article. Open access funding was provided by the Science, Technology & Innovation Funding Authority (STDF) in cooperation with the Egyptian Knowledge Bank (EKB).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All animal procedures included in the current study were approved by the Animal Health and Poultry Ethics Committee at the Desert Research Center (DRC) in Egypt with the approval reference number DRC045–6–11/2023. All methods were performed in accordance with the relevant guidelines and regulations and in compliance with the ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Animal Health and Poultry, Animal and Poultry Production Division, Desert Research Center (DRC), Cairo, Egypt
[2]Department of Development of Animal, of Veterinary Medicine, Mansoura University, Mansoura, Egypt
[3]Department of Animal Medicine, Faculty of Veterinary Medicine, Kafrelshkh University, Kafr El Sheikh 33516, Egypt
[4]Departments of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan

Received: 14 January 2025 / Accepted: 18 March 2025
Published online: 12 April 2025

References

1. Sallam AM. Risk factors and genetic analysis of pre-weaning mortality in Barki lambs. Livest Sci. 2019;230:103818.
2. Abousoliman I, Reyer H, Oster M, Murani E, Mohamed I, Wimmers K. Genome-wide analysis for early growth-related traits of the locally adapted Egyptian Barki sheep. Genes. 2021;12(8):1243.
3. El-Sayed A, Ateya A, Hamed M, Shoieb S, Ibrahim H, El-ashker M, et al. Gene expression pattern of acetyl-coA carboxylase alpha, fatty acid synthase, and stearoyl-CoA desaturase in pregnant Barki sheep under complete feed deprivation. Mansoura Vet Med J. 2019;20(3):8–13.
4. Varshney R, Varshney R, Chaturvedi VK, Rawat M, Saminathan M, Singh V, et al. Development of novel iron-regulated *Pasteurella multocida* B: 2 bacterin and refinement of vaccine quality in terms of minimum variation in particle size and distribution vis-a-vis critical level of iron in media. Microb Pathog. 2020;147:104375.
5. Rath PK, Panda SK, Mishra BP, Karna DK, Sahoo GR, Mishra U, Patra RC. Clinico-pathological evaluation of PPR in a flock of Ganjam sheep and goat in Odisha. J Anim Res. 2020;10(4):585–91.
6. Mousa WS, Zaghawa AA, Elsify AM, Nayel MA, Ibrahim ZH, Al-Kheraije KA, et al. Clinical, histopathological, and molecular characterization of Mycoplasma species in sheep and goats in Egypt. Vet World. 2021;14(9):2561.
7. Ben Chehida F, Gharsa H, Tombari W, Selmi R, Khaldi S, Daaloul M, et al. First report of antimicrobial susceptibility and virulence gene characterization associated with *Staphylococcus aureus* carriage in healthy camels from Tunisia. Animals. 2021;11(9):2754.
8. Wang J, Liu H, Raheem A, Ma Q, Liang X, Guo Y, Lu D. Exploring Mycoplasma ovipneumoniae NXNK2203 infection in sheep: insights from histopathology and whole-genome sequencing. BMC Vet Res. 2024;20(1):20.
9. Hassan H, Kamr A, Mousa W, Toribio R, El-Gendy AN, Khaled H et al. Silymarin antibacterial efficacy against some isolated bacterial strains from pneumonic sheep-vitro study. Pakistan Vet J. 2024;44(2):280–85.
10. Donia GR, El Ebissy IA, Wassif IM. Biochemical and immunological studies on the respiratory diseases in sheep in North Western Coast. Eur J Biomed. 2018;5(12):34–41.
11. Hu G, Do DN, Gray J, Miar Y. Selection for favourable health traits: a potential approach to Cope with diseases in farm animals. Animals. 2020;10(9):1717.
12. Madhi KS, Jasim AS, Nasear HA, Ibraheim HK, Gharban HA. Phylogenetic analysis of *Klebsiella pneumoniae* isolates of respiratory tract infections in humans and sheep. Open Vet J. 2024;14(9):2325.
13. Ramadan M, Ghanem M, El Attar HE, Abdel-Raoof Y. Evaluation of clinical and hematobiochemical alterations in naturally occurring bovine respiratory disease in feedlot cattle calves in Egypt. Benha Vet Med J. 2019;36(2):305–13.
14. Rizk MA, El-Sayed SA, E–S, Salman D, Marghani BH, Gadalla HE, Sayed-Ahmed MZ. Immunomodulatory effect of vitamin C on Proinflammatory cytokines production in ossimi lambs (Ovis aries) with Pneumonic pasteurellosis. Animals. 2021;11(12):3374.
15. Hernández-Castellano LE, Nally JE, Lindahl J, Wanapat M, Alhidary IA, Fangueiro D, et al. Dairy science and health in the tropics: challenges and opportunities for the next decades. Trop Anim Health Prod. 2019;51:1009–17.
16. Abd-Elfatah EB, Fawzi EM, Elsheikh HA, Shehata AA. Prevalence, virulence genes and antibiogram susceptibility pattern of *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from mastitic ewes. 2023.
17. Yassin S, Mahmoud D, Hafez A, Salama M, Barghash S. Flea-borne Mycobacterium and Mycoplasma in small ruminants on the Northern West Coast of Egypt. 2023.
18. Silló P, Pintér D, Ostorházi E, Mazán M, Wikonkál N, Pónyai K, et al. Eosinophilic fasciitis associated with Mycoplasma arginini infection. J Clin Microbiol. 2012;50(3):1113–7.
19. Halium MMA, Salib FA, Marouf S, Massieh ESA. Isolation and molecular characterization of Mycoplasma spp. In sheep and goats in Egypt. Vet World. 2019;12(5):664.
20. Akwuobu CA, Rabo JS, Chah KF, Oboegbule SI. Pathogenicity of local isolates of Mycoplasma ovipneumoniae and Mycoplasma arginini in experimental West African Dwarf goats. J Adv Vet Anim Res. 2016;3(3):242–51.
21. Ammar A, Eissa S, El-Hamid M, Ahmed H, El-Aziz A. Advanced study on mycoplasmas isolated from sheep. Zag Vet J. 2008;36:128–37.
22. Zaghawa A, Hassan H, El-Sify A. Clinical and etiological study on respiratory affections of sheep. Minufiya Vet J. 2010;7(1):93–103.
23. Singh F, Sonawane GG, Kumar J, Dixit SK, Meena RK, Tripathi BN. Antimicrobial resistance and phenotypic and molecular detection of extended-spectrum ß-lactamases among extraintestinal *Escherichia coli* isolated from Pneumonic and septicemic sheep and goats in Rajasthan, India. Turkish J Vet Anim Sci. 2019;43(6):754–60.
24. Akçakavak G, Karataş Ö, Tuzcu N, Tuzcu M. Determination of apoptosis, necroptosis and autophagy markers by real-time PCR in naturally, infected pneumonic pasteurellosis caused by *Pasteurella multocida* and Mannheimia haemolytica in Cattle. 2024.
25. Van Nguyen P, Le CT, Ho XTT, Truong PH, Van Loi B. Nguyen K C T. First report of antimicrobial resistance of Mannheimia haemolytica from Phan Rang sheep in Vietnam. Pakistan Vet J. 2023;43(1):41–48.
26. Kishimoto M, Tsuchiaka S, Rahpaya SS, Hasebe A, Otsu K, Sugimura S, et al. Development of a one-run real-time PCR detection system for pathogens associated with bovine respiratory disease complex. J Vet Med Sci. 2017;79(3):517–23.
27. Dapgh A, Hakim A, Abouelhag H, Abdou A, Elgabry E. Detection of virulence and multidrug resistance operons in *Pseudomonas aeruginosa* isolated from Egyptian Baladi sheep and goat. Vet World. 2019;12(10):1524.
28. Yaguchi K, Ogitani T, Osawa R, Kawano M, Kokumai N, Kaneshige T, et al. Virulence factors of avian pathogenic *Escherichia coli* strains isolated from chickens with colisepticemia in Japan. Avian Dis. 2007;51(3):656–62.

29. Yamamoto Y. PCR in diagnosis of infection: detection of bacteria in cerebrospinal fluids. Clin Vaccine Immunol. 2002;9(3):508–14.
30. Kerremans J, Verboom P, Stijnen T, Hakkaart-van Roijen L, Goessens W, Verbrugh H, Vos M. Rapid identification and antimicrobial susceptibility testing reduce antibiotic use and accelerate pathogen-directed antibiotic use. J Antimicrob Chemother. 2008;61(2):428–35.
31. Sharma N, Singh N, Singh O, Pandey V, Verma P. Oxidative stress and antioxidant status during transition period in dairy cows. Asian-Australasian J Anim Sci. 2011;24(4):479–84.
32. Surai PF, Earle-Payne K. Antioxidant defences and redox homeostasis in animals. Antioxidants. 2022;11(5):1012. https://doi.org/10.3390/antiox11051012.
33. Mohamed RH, Khalphallah A, Nakada K, Elmeligy E, Hassan D, Ebissy EA, et al. Clinical and correlated responses among steroid hormones and oxidant/antioxidant biomarkers in pregnant, nonpregnant and lactating CIDR-presynchronized dromedaries (Camelus dromedarius). Vet Sci. 2021;8(11):247.
34. Khalid A, Na Y, Jinyou Z, Khudhair N, Guixue Z. Responses of chicken Sertoli cells and fibroblasts after transfection with plasmids pEGFP-N3-HNP-1. Pakistan Vet J. 2015;35(4):504
35. Arbaga A, Hassan H, Anis A, Othman N, Kamr A. Clinicopathological and electrophoretic pattern of serum protein alterations in acute Pneumonic sheep. Pakistan Vet J. 2023;43(2):303–308.
36. Soller M, Andersson L. Genomic approaches to the improvement of disease resistance in farm animals. Rev Sci Tech. 1998;17(1):329–45.
37. Berry DP, Bermingham ML, Good M, More SJ. Genetics of animal health and disease in cattle. Ir Vet J. 2011;64:1–10.
38. Nicholas FW. Animal breeding and disease. Philosophical Trans Royal Soc B Biol Sci. 2005;360(1459):1529–36.
39. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences; Amsterdam. 2016.
40. Hamad M, Al-Jumaa Z. Isolation and identification of Mycoplasma from cases of pneumonia in feedlot lambs. Assiut Vet Med J. 2014;60(142):7–13.
41. Azizi S, Korani FS, Oryan A. Pneumonia in slaughtered sheep in south-western Iran: pathological characteristics and aerobic bacterial aetiology. 2013.
42. Kali A, Stephen S, Umadevi S. Laboratory evaluation of phenotypic detection methods of methicillin-resistant *Staphylococcus aureus*. Biomedical J. 2014;37(6):411–14.
43. Quinn PJ, Carter ME, Markey BK, Carter GR, editors. Clinical veterinary microbiology. 2nd ed. London: Mosby; 1998. p. 137–43.
44. Turton JF, Perry C, Elgohari S, Hampton CV. PCR characterization and typing of *Klebsiella pneumoniae* using capsular type specific, variable number tandem repeat and virulence gene targets. J Med Microbiol. 2010;59(5):541–7.
45. Ghanbarpour R, Salehi M. Determination of adhesin encoding genes in *Escherichia coli* isolates from omphalitis of chicks. Am J Anim Vet Sci. 2010;(2):91–96.
46. Duan R, Lyu D, Qin S, Liang J, Gu W, Duan Q, et al. *Pasteurella multocida* strains of a novel capsular serotype and lethal to marmota Himalayana on Qinghai–Tibet plateau in China. Int J Med Microbiol. 2024;314:151597.
47. Hawari A, Hassawi D, Sweiss M. Isolation and identification of Mannheimia haemolytica and *Pasteurella multocida* in sheep and goats using biochemical tests and random amplified polymorphic DNA (RAPD) analysis. 2008.
48. Spilker T, Coenye T, Vandamme P, LiPuma JJ. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other Pseudomonas species recovered from cystic fibrosis patients. J Clin Microbiol. 2004;42(5):2074–9.
49. Matar GM, Ramlawi F, Hijazi N, Khneisser I, Abdelnoor AM. Transcription levels of *Pseudomonas aeruginosa* exotoxin A gene and severity of symptoms in patients with otitis externa. Curr Microbiol. 2002;45:350–4.
50. Van Kuppeveld F, Johansson K, Galama J, Kissing J, Bölske G, Van der Logt J, Melchers W. Detection of Mycoplasma contamination in cell cultures by a Mycoplasma group-specific PCR. Appl Environ Microbiol. 1994;60(1):149–52.
51. Osakabe Y, Yaguchi C, Miyai T, Miyata K, Mineo S, Nakamura M, Amano S. Detection of Streptococcus species by polymerase chain reaction in infectious crystalline keratopathy. Cornea. 2006;25(10):1227–30.
52. Hu Q, Tu J, Han X, Zhu Y, Ding C, Yu S. Development of multiplex PCR assay for rapid detection of Riemerella anatipestifer, *Escherichia coli*, and *Salmonella enterica* simultaneously from ducks. J Microbiol Methods. 2011;87(1):64–9.
53. Boom R, Sol C, Salimans M, Jansen C, Wertheim-van Dillen P, Van der Noordaa J. Rapid and simple method for purification of nucleic acids. J Clin Microbiol. 1990;28(3):495–503.
54. Boesenberg-Smith KA, Pessarakli MM, Wolk DM. Assessment of DNA yield and purity: an overlooked detail of PCR troubleshooting. Clin Microbiol Newsl. 2012;34(1):1–6.
55. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci. 1977;74(12):5463–7.
56. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–10.
57. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 2007;24(8):1596–9.
58. Pfaffl MW. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res. 2001;29(9):e45–45.
59. Abdalla O, Haidy G, Yousseff F, Rehab A. Clinicopathological alterations in blood and Sera of sheep due to respiratory affections. J Clin Pathol Forecast. 2019;2:1006.
60. Mandal R, Gupta V, Joshi V, Kumar S, Mondal D. Study of clinico-hematobiochemical changes and therapeutic management of naturally infected cases of respiratory disease in nondescript goats of Bareilly region. Int J Livest Res. 2017;7(6):211–8.
61. AL-Dujaily A H, Abeed S A, Sahib A M. Hematological, biochemical, pathological, serology and molecular detection of mycoplasma ovipneumoniae from Awassi Sheep in Al-najaf Province, Iraq. Ann For Res. 2023;66(1):1410–1422.
62. Abdulla O, Kilany O, Dessouki A, Mohmoud N. Clinico-Pathological studies in some respiratory diseases in newly born Buffalo calves. Suez Canal Vet Med J. 2015;20(1):15–26.
63. Bozukluhan K, Merhan O, Kiziltepe S, Egritag HE, Akyuz E, Gokce HI. Determination of haptoglobin, some biochemical and oxidative stress parameters in calves with pneumonia. Fresenius Environ Bull. 2021;30:9485–9.
64. Su M, She Y, Deng M, Guo Y, Li Y, Liu G, et al. Effect of capsaicin addition on antioxidant capacity, immune performance and upper respiratory microbiota in nursing calves. Microorganisms. 2023;11(8):1903.
65. Saher AS, Raza A, Qiu F, Mehmood K, Hussain R, Qayyum A, et al. Detection of haptoglobin and serum amyloid A as biomarkers in naturally infected Mycoplasma Bovis calves. Acta Trop. 2024;254:107215.
66. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary medicine E-Book: veterinary medicine E-Book. Elsevier Health Sciences; Amsterdam. 2006.
67. Risalde M, Molina V, Sánchez-Cordón P, Pedrera M, Panadero R, Romero-Palomo F, Gómez-Villamandos J. Response of Proinflammatory and anti-inflammatory cytokines in calves with subclinical bovine viral diarrhea challenged with bovine herpesvirus-1. Vet Immunol Immunopathol. 2011;144(1–2):135–43.
68. Mansour AM, Zaki HM, Hassan NA, Al-Humiany AA. Molecular characterization and immunoprotective activity of capsular polysaccharide of Klebsiella pneumoniae isolated from farm animals at Taif Governorate. Am J Infect Dis. 2014;10(1):1.
69. Ali A, Abu-Zaid K. Study on *Klebsiella pneumoniae* causing respiratory infection in small ruminants. Anim Health Res J. 2019;7:57–67.
70. Fouad EA, Khalaf DD, Farahat E, Hakim AS. Identification of predominant pathogenic bacteria isolated from respiratory manifested small ruminants in Western North Egypt with regard to their susceptibility to antibiotics. Int J Health Sci. 2022;6:10818–28.
71. Loncaric I, Beiglböck C, Feßler AT, Posautz A, Rosengarten R, Walzer C, et al. Characterization of ESBL-and AmpC-producing and fluoroquinolone-resistant Enterobacteriaceae isolated from mouflons (Ovis orientalis musimon) in Austria and Germany. PLoS ONE. 2016;11(5):e0155786.
72. Franco MF, Gaeta NC, Alemán MA, Mellville PA, Timenetsky J, Balaro MF, Gregory L. Bacteria isolated from the lower respiratory tract of sheep and their relationship to clinical signs of sheep respiratory disease. Pesquisa Vet Brasil. 2019;39:796–801.
73. El Fertas-Aissani R, Messai Y, Alouache S, Bakour R. Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. Pathol Biol (Paris). 2013;61(5):209–16.
74. Soltani E, Hasani A, Rezaee MA, Pirzadeh T, Oskouee MA, Hasani A, et al. Virulence characterization of *Klebsiella pneumoniae* and its relation with ESBL and AmpC beta-lactamase associated resistance. Iran J Microbiol. 2020;12(2):98.
75. Sakr NH, Salama AA, Elsify AM, Hafez AA, Nayel MA, Mousa WS. Prevalence and molecular screening of some virulence genes among Klebsiella species isolated from camels. Alexandria J Vet Sci. 2024;81(1):e0155786.
76. Mittal R, Khandwaha RK, Gupta V, Mittal P, Harjai K. Phenotypic characters of urinary isolates of *Pseudomonas aeruginosa* & their association with mouse renal colonization. Indian J Med Res. 2006;123(1):67.
77. Basha OA. Some studies on the occurrence of Pseudomonas aeruginosa isolated from farm animals. Kafrelsheikh Vet Med J. 2011;9(2):1–18.
78. Wood SJ, Kuzel TM, Shafikhani SH. *Pseudomonas aeruginosa*: infections, animal modelling, and therapeutics. Cells. 2023;12(1):199.

79. Saleh NS, Allam TS. Pneumonia in sheep: bacteriological and clinicopathological studies. Am J Res Com. 2014;2(11):73–88.
80. Badamchi A, Masoumi H, Javadinia S, Asgarian R, Tabatabaee A. Molecular detection of six virulence genes in *Pseudomonas aeruginosa* isolates detected in children with urinary tract infection. Microb Pathog. 2017;107:44–7.
81. Khosravi AD, Shafie F, Montazeri EA, Rostami S. The frequency of genes encoding exotoxin A and exoenzyme S in *Pseudomonas aeruginosa* strains isolated from burn patients. Burns. 2016;42(5):1116–20.
82. Husseein M, Alrodhan M. Isolation and identification of some aerobic bacteria associated with respiratory infections of sheep in Al-Diwaniya Governorate. Al-Qadisiyah J Vet Med Sci. 2011;10(2):44–47.
83. Sonawane G, Singh F, Tripathi B, Dixit S, Kumar J, Khan A. Investigation of an outbreak in lambs associated with *Escherichia coli* O95 septicaemia. 2012.
84. Rashid M, Ferdoush M, Dipti M, Roy P, Rahman M, Hossain M, Hossain M. Bacteriological and pathological investigation of goat lungs in Mymensingh and determination of antibiotic sensitivity. Bangladesh J Vet Med. 2013;11(2):159–166.
85. El-Mashad A-B, I, Moustafa SA, Amin A, Samy EM. Pathological studies on lung affections in sheep and goat at Kalubia Governorate. Benha Vet Med J. 2020;38(1):17–23.
86. Hafez AA. Using multiplex PCR for detection of some microbial pathogen causing pneumonia in camel. Eur J Biomed Pharm Sci. 2018;5:135–45.
87. Hafez AA. Virulence and antimicrobial resistance genes of *E. coli* isolated from diarrheic sheep in the North–West Coast of Egypt. Sys Rev Pharm. 2020;11(11):609–17.
88. Mohammed R, Nader SM, Hamza DA, Sabry MA. Public health concern of antimicrobial resistance and virulence determinants in *E. coli* isolates from oysters in Egypt. Sci Rep. 2024;14(1):26977.
89. Muloi DM, Wee BA, McClean DM, Ward MJ, Pankhurst L, Phan H, et al. Population genomics of *Escherichia coli* in livestock-keeping households across a rapidly developing urban landscape. Nat Microbiol. 2022;7(4):581–9.
90. Madec J-Y, Haenni M, Métayer V, Saras E, Nicolas-Chanoine M-H. High prevalence of the animal-associated Bla CTX-M-1 IncI1/ST3 plasmid in human *Escherichia coli* isolates. Antimicrob Agents Chemother. 2015;59(9):5860–1.
91. Dalia T. Mohamed, Abeer I. Abou El-Gheit, Salwa A. M. Eid, Sally H. Abou-Khadra, Gehan M. Elsadik, Abeer A. E. Mohamed and Thoria A. Hamed. Prevalence of *Staphylococcus aureus* and *Pasteurella multocida* incriminated in calf pneumonia and their treatment. Egypt J Anim Health. 2023;13(1):21–39.
92. Hashem YM, Mousa WS, Abdeen EE, Abdelkhalek HM, Nooruzzaman M, El-Askary A, et al. Prevalence and molecular characterization of Mycoplasma species, *Pasteurella multocida*, and *Staphylococcus aureus* isolated from calves with respiratory manifestations. Animals. 2022;12(3):312.
93. Khamesipour F, Momtaz H, Azhdary Mamoreh M. Occurrence of virulence factors and antimicrobial resistance in *Pasteurella multocida* strains isolated from slaughter cattle in Iran. Front Microbiol. 2014;5:536.
94. Tabatabaei M, Abdolahi F. Molecular evaluation of sheep and goats isolates of Pasteurella multocida and their antibiotic resistance. In: Veterinary Research Forum. Urmia: Faculty of Veterinary Medicine, Urmia University; 2023.
95. Yilma MA, Vemulapati MB, Tefera TA, Yami M, Negi TD, Belay A et al. Phenotypic and molecular characterization of the capsular serotypes of *Pasteurella multocida* isolates from Pneumonic cases of cattle in Ethiopia. 2021.
96. FR E–S, AH A, HM H, AM N. Antimicrobial and immunological studies on *Pasteurella multocida* and Mannheimia haemolytica recovered from calves affected with respiratory manifestations. J Vet Med Res. 2019;26(1):55–63.
97. Radwan A, Zakria I, AboSakya R, Arnaout F, Selim A. Molecular characterization of Pasterulla multocida in fattening bovine. Egypt J Vet Sci. 2025;56(2):397–404.
98. Mirhaghgoye Jalali S, Jabbari A, Esmael Zad M. LPS-PCR typing of ovine *Pasteurella multocida* isolates from Iran based on (L1 to L8) outer core biosynthesis loci. Arch Razi Inst. 2017;72(3):165–71.
99. Bahr AD, Massieh ESA. Susceptibility of *Pasteurella multocida* isolated from cattle in Egypt to antibiotics, silver, Chitosan and Curcumin nanoparticles. Vet Res Forum. 2024;15(9):445.
100. Aiello SE, Mays A. The Merck veterinary manual. Whitehouse Station: Merck & Co. Inc; 1998. p. 2032–3.
101. Legesse A, Abayneh T, Mamo G, Gelaye E, Tesfaw L, Yami M, Belay A. Molecular characterization of Mannheimia haemolytica isolates associated with Pneumonic cases of sheep in selected areas of central Ethiopia. BMC Microbiol. 2018;18:1–10.
102. Demissie T, Dawo F, Sisay T. Biochemical and antigenic characterization of Mannheimia, pasteurella and Mycoplasma species from naturally infected Pneumonic sheep and goats, Bishoftu, Ethiopia. Afr J Basic Appl Sci. 2014;6(6):198–204.
103. Sahay S, Natesan K, Prajapati A, Kalleshmurthy T, Shome BR, Rahman H, Shome R. Prevalence and antibiotic susceptibility of Mannheimia haemolytica and *Pasteurella multocida* isolated from ovine respiratory infection: a study from Karnataka, Southern India. Vet World. 2020; 13(9):1947.
104. Rawat N, Gilhare VR, Kushwaha KK, Hattimare DD, Khan FF, Shende RK, Jolhe DK. Isolation and molecular characterization of Mannheimia haemolytica and *Pasteurella multocida* associated with pneumonia of goats in Chhattisgarh. Vet World. 2019;12(2):331.
105. Marru HD, Anijajo TT, Hassen AA. A study on ovine Pneumonic pasteurellosis: isolation and identification of pasteurellae and their antibiogram susceptibility pattern in Haramaya district, Eastern Hararghe, Ethiopia. BMC Vet Res. 2013;9:1–8.
106. Kumar V, Rana R, Mehra S, Rout PK. Isolation and characterization of Mycoplasma mycoides subspecies Capri from milk of natural goat mastitis cases. Int Sch Res Notices. 2013;2013(1):593029.
107. Gaeta NC, Ribeiro BL, Alemán MA, Yoshihara E, Nassar AF, Marques LM, et al. Bacterial pathogens of the lower respiratory tract of calves from Brazilian rural settlement herds and their association with clinical signs of bovine respiratory disease. Pesquisa Vet Brasil. 2018;38:374–81.
108. Al-Momani W, Halablab MA, Abo-Shehada MN, Miles K, McAuliffe L, Nicholas RA. Isolation and molecular identification of small ruminant Mycoplasmas in Jordan. Small Ruminant Res. 2006;65(1–2):106–12.
109. Murdoch DR, Anderson TP, Beynon KA, Chua A, Fleming AM, Laing RT, et al. Evaluation of a PCR assay for detection of *Streptococcus pneumoniae* in respiratory and nonrespiratory samples from adults with community-acquired pneumonia. J Clin Microbiol. 2003;41(1):63–6.
110. Hafez AA, El-Rayes MA. Some immunological and bacteriological studies on camels in the North Western Coast of Egypt. Egypt J Camel Sci. 2023;1(1):29–50.
111. Weiser GC, DeLong WJ, Paz JL, Shafii B, Price WJ, Ward AC. Characterization of *Pasteurella multocida* associated with pneumonia in Bighorn sheep. J Wildl Dis. 2003;39(3):536–44.
112. Hulbert LE, Moisá SJ. Stress, immunity, and the management of calves. J Dairy Sci. 2016;99(4):3199–216.
113. Wang K, Liu X, Li Q, Wan K, Gao R, Han G, et al. MHC-DRB1 exon 2 polymorphism and its association with Mycoplasma ovipneumonia resistance or susceptibility genotypes in sheep. J Genet. 2020;99:1–12.
114. Leymaster K, Chitko-McKown C, Clawson M, Harhay G, Heaton M. Effects of TMEM154 haplotypes 1 and 3 on susceptibility to ovine progressive pneumonia virus following natural exposure in sheep. J Anim Sci. 2013;91(11):5114–21.
115. Ateya A, Al-Sharif M, Abdo M, Fericean L, Essa B. Individual genomic loci and mRNA levels of immune biomarkers associated with pneumonia susceptibility in Baladi goats. Vet Sci. 2023;10(3):185.
116. Abendaño N, Esparza-Baquer A, Bernales I, Reina R, de Andrés D, Jugo BM. Gene expression profiling reveals new pathways and genes associated with Visna/Maedi viral disease. Animals. 2021;11(6):1785.
117. Salim T, Sershen CL, May EE. Investigating the role of TNF-α and IFN-γ activation on the dynamics of iNOS gene expression in LPS stimulated macrophages. PLoS ONE. 2016;11(6):e0153289.
118. Benedict CA, Banks TA, Ware CF. Death and survival: viral regulation of TNF signalling pathways. Curr Opin Immunol. 2003;15(1):59–65.
119. Bradley J. TNF-mediated inflammatory disease. J Pathol. 2008;214(2):149–60.
120. Lester DH, Russell G, Barendse W, Williams J. The use of denaturing gradient gel electrophoresis in mapping the bovine tumor necrosis factor α gene locus. 1996.
121. Bannerman D. Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. J Anim Sci. 2009;87(suppl13):10–25.
122. Myokai F, Takashiba S, Lebo R, Amar S. A novel lipopolysaccharide-induced transcription factor regulating tumor necrosis factor α gene expression: molecular cloning, sequencing, characterization, and chromosomal assignment. Proc Natl Acad Sci. 1999;96(8):4518–4523.
123. Li X, Duan D, Yang J, Wang P, Han B, Zhao L, et al. The expression of human β-defensins (hBD-1, hBD-2, hBD-3, hBD-4) in gingival epithelia. Arch Oral Biol. 2016;66:15–21.
124. Prame Kumar K, Nicholls AJ, Wong CH. Partners in crime: neutrophils and monocytes/macrophages in inflammation and disease. Cell Tissue Res. 2018;371:551–65.

125. Li MO, Flavell RA. Contextual regulation of inflammation: a duet by transforming growth factor-β and interleukin-10. Immunity. 2008;28(4):468–76.
126. Gahmberg CG, Fagerholm SC, Nurmi SM, Chavakis T, Marchesan S, Grönholm M. Regulation of integrin activity and signalling. Biochim Biophys Acta. 2009;1790(6):431–44.
127. Jeyaseelan S, Hsuan S, Kannan MS, Walcheck B, Wang J, Kehrli M, et al. Lymphocyte function-associated antigen 1 is a receptor for pasteurella haemolytica leukotoxin in bovine leukocytes. Infect Immun. 2000;68(1):72–9.
128. Deshpande M, Ambagala T, Ambagala A, Kehrli M Jr, Srikumaran S. Bovine CD18 is necessary and sufficient to mediate Mannheimia (Pasteurella) haemolytica leukotoxin-induced cytolysis. Infect Immun. 2002;70(9):5058–64.
129. Wu H, Boackle S A, Hanvivadhanakul P, Ulgiati D, Grossman J M, Lee Y, et al. Association of a common complement receptor 2 haplotype with increased risk of systemic lupus erythematosus. Proc Natl Acad Sci. 2007;104(10):3961–3966.
130. Van de Stolpe A, Van der Saag P. Intercellular adhesion molecule-1. J Mol Med. 1996;74:13–33.
131. Baldwin CL, Hsu H, Chen C, Palmer M, McGill J, Waters WR, Telfer JC. The role of bovine Γδ T cells and their WC1 coreceptor in response to bacterial pathogens and promoting vaccine efficacy: a model for cattle and humans. Vet Immunol Immunopathol. 2014;159(3–4):144–55.
132. Butty AM, Chud TC, Cardoso DF, Lopes LS, Miglior F, Schenkel FS, et al. Genome-wide association study between copy number variants and hoof health traits in Holstein dairy cattle. J Dairy Sci. 2021;104(7):8050–61.
133. Masella R, Di Benedetto R, Varì R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. J Nutr Biochem. 2005;16(10):577–86.
134. Glasauer A, Chandel NS. Targeting antioxidants for cancer therapy. Biochem Pharmacol. 2014;92(1):90–101.
135. Hobbs MR, Udhayakumar V, Levesque MC, Booth J, Roberts JM, Tkachuk AN, et al. A new NOS2 promoter polymorphism associated with increased nitric oxide production and protection from severe malaria in Tanzanian and Kenyan children. Lancet. 2002;360(9344):1468–75.
136. Orozco G, Sánchez E, López-Nevot MA, Caballero A, Bravo MJ, Morata P, et al. Inducible nitric oxide synthase promoter polymorphism in human brucellosis. Microbes Infect. 2003;5(13):1165–9.
137. Naito Y, Takagi T, Uchiyama K, Yoshikawa T. Heme oxygenase-1: a novel therapeutic target for Gastrointestinal diseases. J Clin Biochem Nutr. 2011;48(2):126–33.
138. Nakasone R, Ashina M, Abe S, Tanimura K, Van Rostenberghe H, Fujioka K. The role of Heme oxygenase-1 promoter polymorphisms in perinatal disease. Int J Environ Res Public Health. 2021;18(7):3520.
139. Ross D, Siegel D. The diverse functionality of NQO1 and its roles in redox control. Redox Biol. 2021;41:101950.
140. Foster CE, Bianchet MA, Talalay P, Faig M, Amzel LM. Structures of mammalian cytosolic Quinone reductases. Free Radic Biol Med. 2000;29(3–4):241–5.
141. Aslan O, Goksu AG, Apaydin N. The evaluation of oxidative stress in lambs with pestivirus infection. J Hellenic Vet Med Soc. 2017;68(3):299–306.
142. Blattman A, Kinghorn B, Gray G, Hulme D, JBeh K, Woolaston R. A search for associations between major histocompatibility complex restriction fragment length polymorphism bands and resistance to Haemonchus contortus infection in sheep. Anim Genet. 1993;24(4):277–82.
143. Zhang W, Li J, McManus DP. Concepts in immunology and diagnosis of hydatid disease. Clin Microbiol Rev. 2003;16(1):18–36.
144. El-Seedy F, Hassan H, Nabih A, Salem S, Khalifa E, Menshawy A, Abed A. Respiratory affections in calves in upper and middle Egypt: bacteriologic, Immunologic and epidemiologic studies. Adv Anim Vet Sci. 2020;8(5):558–69.
145. Akter A, Caldwell JM, Pighetti GM, Shepherd EA, Okafor CC, Eckelkamp EA, et al. Hematological and immunological responses to naturally occurring bovine respiratory disease in newly received beef calves in a commercial stocker farm. J Anim Sci. 2022;100(2):skab363.
146. Allam T, Said L, Elsayed M, Saleh N. Clinical investigation of the pathogenicity of *Pasteurella multocida* isolated from cattle in Egypt regarding its effect on hematological, biochemical, and oxidant-antioxidant biomarkers as well as Proinflammatory cytokines and acute phase proteins. Adv Anim Vet Sci. 2021;9(6):792–801.
147. Fernández S, Galapero J, Rey J, Pérez C, Ramos A. Cytokines study in lungs infected with Pasteurellaceae and Mycoplasma spp. From fattening lambs. J Med Microbiol Immunol Res. 2018;2(1):1–7.
148. Özbek M, Özkan C. Oxidative stress in calves with enzootic pneumonia. Turkish J Vet Anim Sci. 2020;44(6):1299–305.
149. Darwish A. Monitoring of antioxidant vitamins concentrations in some ovine diseases. Vet Sci. 2020;6(2):58–63.
150. Jarikre T, Ohore G, Oyagbemi A, Emikpe B. Evaluation of oxidative stress in caprine Bronchoalveolar lavage fluid of Pneumonic and normal lungs. Int J Vet Sci Med. 2017;5(2):143–7.
151. Gil E, Noursadeghi M, Brown JS. *Streptococcus pneumoniae* interactions with the complement system. Front Cell Infect Microbiol. 2022;12:929483.
152. Allam TS, Saleh NS, Abo-Elnaga TR, Darwish AA. Cytokine response and immunological studies in camels (Camelus Dromedarius) with respiratory diseases at Matrouh Province. Alexandria J Vet Sci. 2017;53(1):116–124.
153. El-Deeb W, Fayez M, Elsohaby I, Salem M, Alhaider A, Kandeel M. Investigation of acute-phase proteins and cytokines response in goats with contagious caprine pleuropneumonia with special reference to their diagnostic accuracy. PeerJ. 2020;8:e10394.
154. Mousa S, Soliman S. Oxidant and antioxidant status in Pneumonic goats with special reference to bacterial etiology. Int J Livest Res. 2016;6(5):15–23.
155. Li Z, Du Z, Li J, Sun Y. Comparative analysis on lung transcriptome of Mycoplasma ovipneumoniae (Mo)-infected Bashbay sheep and argali hybrid sheep. BMC Vet Res. 2021;17:1–11.
156. Mohammed ZM, Ibrahim WM, Abdalla IO. Pneumonia in slaughtered sheep in Libya: gross and histopathological findings. Eur J Vet Med. 2022;2(1):4–9.

Publisher's Note