

SOME IMMUNOLOGICAL AND BACTERIOLOGICAL STUDIES ON CAMELS IN THE NORTH WESTERN COAST OF EGYPT

Amani A. Hafez^{1*} and Muhammed A. H. El-Rayes²

¹Infectious Disease Unit, Department of Animal and Poultry Health, Animal and poultry Production Division, Desert Research Center, Cairo, Egypt.

²Department of Animal and Poultry Physiology, Animal and Poultry Production Division, Desert Research Center, Cairo, Egypt.

*Corresponding author

Corresponding author: Amani A. Hafez. Email: amani.hafez@yahoo.com

ABSTRACT

This study aimed to shed light on the most important bacteria that cause respiratory manifestations and their effect on inflammatory cytokines in camels, and their relationship with other immunological parameters such as Acute Phase Proteins, complement, and immunoglobulins. Lung specimens (n = 572) were collected for this investigation, with normal lungs identified in 390 (68.18%) serving as a control group, and pneumonic camel lung samples (n = 182; 31.82%) classified as the diseased group, all of which were collected from slaughterhouses in Egypt's North Western Coast. The isolates comprised nine genera of pathogenic bacteria; *Staphylococcus* spp. 88 (19%), *Salmonella* spp. 40 (8.7%), *Klebsiella pneumoniae* 75 (16.4%), *Pastuerella multocida* 38 (8.3%), *Mycoplasma* spp. 58 (12.6%), *Bacillus* spp. 35 (7.6%), *Streptococcus pyogenes* 20 (4.3%), *Streptococcus pneumoniae* 68 (14.8%) and *Proteus* spp. 35 (7.6%). Using blood samples collected from both groups, the specified biochemical and immunological parameters were evaluated. The diseased camels exhibited substantial increases in pro-inflammatory cytokines (IL-2, IL-6, TNF-, and IFN-), acute phase proteins (fibrinogen, ceruloplasmin, haptoglobin, and serum amyloid), and acute phase cytokines (IL-2, IL-6, TNF and IFN). Immunoglobulins increase, but total protein (TP), albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT) were not significantly affected by infection. In contrast, the anti-inflammatory cytokine IL-10, complement 3, complement 4, albumin serum levels, and the A/G ratio all decreased substantially, and hematological analysis revealed a highly significant decrease ($P \leq 0.01$) in RBC count compared to the control group. Histopathological finding revealed a hyperplastic bronchiolar wall.

Key words: Camel, North Western Coast, Respiratory Bacteria, Cytokines and Hematology.

INTRODUCTION

It was previously believed that camels developed exceptional resistance to a wide range of diseases as a result of their prolonged exposure to the harsh desert ecosystem, and their ability to adapt to such arid climates gave them the ability to resist many infectious diseases (Hussen and Schubert, 2021). However, environmental stress and poor management conditions Overcrowding, poor sanitary conditions, poor feeding and feeding management, poor hygiene, extreme weather fluctuations, and the general health of the herd all play a role and are risk factors that expose animals to pneumonia (Muna et al., 2017; Ahmed and Musa, 2015). Camel respiratory disease is an emerging disease in the Egyptian desert and results in significant economic losses due to loss of productivity, treatment expenses, wastage of carcasses, and possible animal mortality (Ismail, 2017 and Gebru et al., 2018). The fact that lung diseases are very common in camels shows how important they are. (Al Shukairi et al., 2018). Camel respiratory disease is a difficult disease due to the fact that it has many causes,

such as bacterial, viral, mycoplasma, and fungal infections. The diagnosis is based on conventional detection methods that are time-consuming and insensitive, such as microscopy, bacterial culture, and biochemical tests. Therefore, we had to rely on a fast and accurate method, which is polymerase chain reaction (PCR), and it became one of the most promising methods for diagnosing respiratory infections (Liao et al., 2019 and Hu et al., 2020). It also turns out that the difference between the blood parameters and the reference number tells us important things about the animals' health. This knowledge can be used to find out what is wrong with the sentences and how to deal with them. (Faraz et al., 2021). The aim of this study was to examine some of the most prevalent bacteria associated with pneumonia in lung samples obtained during postmortem examinations on the North Western Coast of Egypt by using PCR and their effect on some immunological indicators, some cytokine measurements, some hematological parameters and their effect on histopathology of the lung.

MATERIALS AND METHODS

1. Collection of Specimens

Camel lung specimens (n = 572) were collected between 2019 and 2021, with normal lungs found in 390 (68.18%), and grossly pneumonic camel lung samples (n = 182 (31.82%)) collected from slaughterhouses on Egypt's North Western Coast and placed in sterile plastic bags, transported to laboratory of the Desert Research Center, Cairo on ice, and kept at 20 °C until used. Blood samples and sera were taken from camels in the same places that lung samples were taken aseptically based on presence of the pneumonic signs at the tissue level and some sera were also taken from Bedouin people on the North Western Coast. The sera were stored at 20 degrees Celsius until they were used.

2. Bacteriological Isolation

The bacteria were separated from the samples using different types of media. These were nutrient agar, blood agar, brain heart infusion, mannitol salt agar, MacConkey agar, and bright green agar. For decontamination, a red-hot scalpel blade was used to cut through the top of each lung sample. Then, a clean scalpel blade was used to make a deep cut in each lung's surface. A sterile swab was then dipped into the cut area and strewn on a sheep blood agar plate. From the cut area, a sample was taken and put in a sterile bottle with brain-heart infusion broth. At 37°C, kept for 24 hours. If a plate didn't show bacterial growth within 24 hours, it was kept in the incubator for a week and checked every day to make sure it was negative, followed by identification using morphological and biochemical characteristics (Catalase, Oxidase, and Oxidation Fermentation Tests) of the isolated strains, such as colony morphology, Gram staining, spore forming ability, and acid-fast staining were used to determine the species of the isolated bacteria (Mamo et al., 2011, Abo-Elnaga and Osman, 2012, Ievy, et al., 2013, Ahmed and Musa, 2015, Kabir and Bari, 2015, Nahed et al., 2016, Das, et al. 2018, Mohammed and Jassim, 2018; Zhao et al., 2021).

3. Polymerase Chain Reaction (PCR)

3.1. DNA extraction

The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used to get DNA from samples, but the instructions from the manufacturer were changed. In short, 10 minutes were spent at 56 °C mixing 200 µl of the sample suspension with 10 µl of proteinase K and 200 µl of lysis buffer. After the lysate had been incubated, 200 µl of 100 percent ethanol was added to it. The sample was then washed and put through a centrifuge, just as the manufacturer said to do. One hundred µl of elution buffer was used to get rid of the nucleic acid.

3.2. Oligonucleotide Primer

Primers used were supplied from Metabion (Germany). The sequences and conditions are listed in Table 1.

3.3. PCR amplification

In a 25-µl reaction containing 12.5µlof Emerald Amp Max PCR Master Mix (Takara, Japan), 1µlof each primer at a concentration of 20 pmol, 4.5 µl of water, and 6 µl of DNA

template, primers were employed. For the reaction, an Applied biosystem 2720 thermal cycler was used.

3.4. Analysis of the PCR products

The PCR products were separated by electrophoresis on 1.5 percent agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature with 5V/cm gradients. 20 µl of each product was loaded into a slot on the gel for analysis. The fragment sizes were determined using Gel pilot 100 bp (Qiagen, Germany, GmbH) and gene ruler 100 bp ladder (Fermentas, Germany). The gel was photographed by a gel documentation system (Alpha Innotech or Biometra), and computer software was used to analyze the data.

4. Analytical Methods

4.1. Pro-inflammatory cytokines

Serum levels of pro-inflammatory and anti-inflammatory cytokines, including interleukins (IL-2, IL-6, and IL-10), Tumor Necrosis Factor alpha (TNF-α) and Interferon (IFNγ) were evaluated from undiluted serum samples (Biosource, Diagnostic Corporation, USA). Plates were examined using a computerized automated microplate ELISA reader at 450 nm (BioTek, ELx808™, USA). RayBio, Catalog Number for ELISA Kit: CSB-E04628r, CSB-E04640r, CSB-E04595r, CSB-E07324r, and CSB-E04845r, respectively.

4.2. Complement and immunoglobulins

Serum levels of complement 3 (C3), complement 4 (C4), immunoglobulins G (IgG), and M (IgM) were measured using the ELISA with commercially available reagents and in accordance with the manufacturer's instructions.

4.3. Acute phase response

Plasma concentrations of Fibrinogen (Fb) and serum values of Ceruloplasmin (Cp), Haptoglobin (Hp), Serum Amyloid A (SAA), Total Protein (TP), Albumin (Alb), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Gamma Glutamyl transferase (GGT) were determined by spectrophotometric methods and commercial test kits (Sigma Chemical Co. Ltd., Poole, Dorset, UK) and following the manufacturer's instructions. After determining total globulin by subtracting albumin from total serum protein, the A/G ratio was calculated.

5. Preparation of Lung Tissues for Histopathology

Lung tissue samples were obtained and partially preserved with 10% formalin neutral buffer solution. The specimens that had been fixed were processed and encased in paraffin wax. For histological evaluation, a piece of 3-5 micron thickness was produced and stained with H & E according to Sheehan and Harpach (1980), Bancroft and Gamble (2002).

6. Statistical Analysis

All data are represented using the mean standard error of the mean (SEM). Student's t-test was used to examine the statistical significance of the means; $p \leq 0.05$ was considered significant. All tests were conducted utilizing the statistical analysis system's computer software (SAS, Inc., 2002).

RESULTS

1. Bacteriological Findings

In this study, a total of 572 lungs were taken, normal lungs were found in 390 (68.18%), while 182 (31.82%) had evidence of one or more lesions. The main lesions encountered include: 127 pneumonia (70%), 35 with hydatid cysts (19.2%), and 20 with emphysema (11%). A total of 457 bacterial isolates belong to 9 different microbes confirmed by using PCR, there are *Staphylococcus* spp. 88 (19%), *Salmonella* spp. 40 (8.7%), *Klebsiella pneumoniae* 75 (16.4%), *Pastuerella multocida* 38 (8.3%), *Mycoplasma* 58 (12.6%), *Bacillus* spp. 35 (7.6%), *Streptococcus pyogenes* 20 (4.3%), *Streptococcus pneumoniae* 68 (14.8%) and *Proteus* spp. 35 (7.6%) the percentage calculated according to the total number of isolated bacteria as shown from Fig. 1 to Fig. 9.

2. Histopathology and Gross Lesions

Gross findings: Infected lung obtained from slaughtered camels showed swollen lungs with different degrees of congestion indicated by dark red patches. The emphysematous areas were higher, lighter and paler than the surrounding tissue (Fig 10). Upon palpation, there were crepitating sounds with hydatid cysts (Fig. 11). Histopathological examination of the lungs (Fig. 12) revealed hyperplastic bronchiolar wall (severe change). A, Thickening in the bronchial wall, and alveolar wall with congestion in the perialveolar blood capillaries and hemorrhage in the bronchial wall (Fig. 13 and 14). In addition to diffuse hemorrhage was observed in the bronchiole and interstitial tissue (Fig. 15).

3. Pro-inflammatory Cytokines

In Table 2, the results showed that the serum concentrations of pro-inflammatory cytokines (IL-2, TNF- α , IFN γ and IL-6) were significantly ($P \leq 0.05$) higher in diseased than in control camels, while the serum concentrations of anti-inflammatory cytokines (IL-10) was decreased.

4. Complements and Immunoglobulins

As shown in Table 3, compared to the control group, there were significant decreases in complements C3 and C4 in the diseased animals. Infected camels showed a highly significant increase in both IgG and IgM (2.70 ± 0.21 and 1.41 ± 0.25) respectively compared to control groups (0.73 ± 0.24 and 0.47 ± 0.29) respectively.

5. Serum Biochemical Studies

The examined biochemical parameters showed that total protein, albumin and globulin contents were not affected significantly by the infection, measuring 6.02 ± 0.39 , 2.67 ± 0.21 and 2.61 ± 0.19 respectively, compared to control (5.27 ± 0.39 , 3.04 ± 0.24 and 2.98 ± 0.23 respectively). Accordingly, the albumin to globulin ratio (A/G) increased non-significantly (Table 4).

6. Hematology

The haematological analysis of selected parameters showed a highly significant decrease ($p \leq 0.01$) in RBCs count when compared to the control group. This decrease was also observed in Hb concentration, Hct %, and MCHC % as shown in Table 6. On the other hand, MCV and MCH values, which measure 76.31 ± 3.38 and 24.97 ± 1.24 respectively in control groups, expressed a non-significant difference among infected camels, being 77.94 ± 2.90 and 27.38 ± 1.07 respectively. The platelet count of the infected group (627 ± 52.33) showed a significant decrease ($P \leq 0.05$) compared to the control one (801 ± 61.11). Generally, erythrocyte indices decreased significantly when compared to control, with the exception of MCV and MCH, which exhibited a non-significant difference. When compared to the control ($9.090.56$), the infection significantly reduced WBC count ($4.230.48$).

DISCUSSION

Camel commerce is growing in North Africa and the Middle East, and camels are used for meat and milk production, as well as transportation and sport. A postmortem examination of numerous camels slaughtered in abattoirs revealed the presence of one or more pulmonary lesions despite their evident health. (Hamad et al., 2011, Samara et al., 2012, Pasha et al., 2013 ; Ali et al., 2019).

Despite this, a number of studies have shown that camels are more resistant to many of the diseases that afflict other than livestock species in the same ecosystem or region (Farooq et al., 2012; Padalino and Menchetti, 2021).

However, other studies have confirmed that camels, like other animals, can be infected by a variety of infectious diseases caused by various species of bacteria, viruses, parasites and fungi. In Egypt, respiratory disorders continue to be a significant issue for camels (Ibtihal et al., 2017).

In this study, 31.82% of camel carcasses examined had internal lesions in the lungs; this was similar to the findings of Wareth et al. (2014) from El-Warrak slaughterhouse, one of Cairo's primary abattoirs. Also, with Ahmed and Musa (2015) from Sudan, but lower than the reports of Tiwari et al. (2015) as they recorded lesions in lungs with 59.7% from India. On the same way, Awol et al. (2011) and Bekele (2008) who found lesions on 77.5% and 98.0% of the camel carcasses in Ethiopia respectively. Abubakar et al. (2010) reported lesions on 64.0% of the carcasses in Nigeria. On the other hand, Higher than El-Tigani et al. (2004) who found 25%; Mohamed et al. (2014) in Egypt, who found 14.5%; and Al-Tarazi (2006) in Jordan, who found 10.66%. These differences in the incidence of pulmonary lesions may be attributable to differences in sample size (number of examined carcasses) or to diseases that afflicted the animal in the past. Or because of the stress of the long-distance journey.

The fact that these bacteria are always found in the lungs of different kinds of animals could mean that they cause different kinds of respiratory infections, especially when the animal's immune system is weak. due to other external factors. The normal mix of bacteria in a healthy individual can be changed by a number of things, like changes in the animal's nutrition and immune system are affected by how clean it is, as well as its environment and climate. These things could make the lung tissue less resistant and, most likely, the existing organism would come out .which can cause a number of diseases to show up (Bosch et al. 2013).

In this study, The difference in detection rates, there are *Staphylococcus* spp. 88 (19%), *Salmonella* spp 40 (8.7 %), *Klebsiella pneumoniae* 75 (16.4%), *Pastuerella multocida* 38 (8.3%), *Mycoplasma* spp. 58 (12.6%), *Bacillus* spp. 35 (7.6%), *Streptococcus pyogenes* 20 (4.3%), *Streptococcus pneumoniae* 68 (14.8%) and *Proteus* spp. 35 (7.6 %). All of these bacteria were isolated from diseased camels. Similar of these results, more or less were confirmed in previous studies (Tigani et al., 2004, Abubakr et al., 2010, Awol et al., 2011, Mohamed et al., 2014, Wareth et al., 2014, Ahmed and Musa, 2015, Nahed et al., 2016, Gebu et al., 2018 and Mohamed et al., 2018). The differences between the records were mainly due to culture only, ,age of camels or the geographical distribution.

The use of PCR as a fast and accurate technique for diagnosing bacteria, especially for pneumonia (Abdelazeem et al., 2020) will give us a clear vision to write the appropriate treatment and follow the vaccination program.

In our study serum concentrations of IgG, and IgM were significantly elevated. An actual increase in the number of white blood cells was noticed (9.09 ± 0.56) and this can be attributed to the factors of stress prevailing as a result of hypoxia cases, which in turn lead to immune responses in an attempt to maintain balance. Serum globulin levels were significantly affected by the infection. Infected camels showed a highly significant increase in both IgG and IgM (2.70 ± 0.21 and 1.41 ± 0.25) respectively, compared to control groups (0.73 ± 0.24 and 0.47 ± 0.29) respectively). This came with Islam *et al.* (2019). This is supported by our findings for IL-2 and IL-6, which increased to 157.12 ± 9.29 and 142.19 ± 7.06 , respectively. Such inflammatory conditions induce the release of cytokines. This is what was confirmed by previous research by Radostits et al. (2007), El-Deeb (2015) and Allam, et al. (2017) who dealing with camel calves and she-camel.

Also, anti-inflammatory cytokines are produced to suppress the expression of inflammation and thus calm the inflammatory process. IL-10, which showed a significant decrease in this study's than control group, indicates the presence of an overwhelming inflammatory response and excessive lung damage. According to Jeong et al. (2009), IL-10 is a significant anti-inflammatory cytokine that inhibits the production of numerous pro-inflammatory cytokines. Lack of IL-10 results in a more pronounced pulmonary inflammatory response.

The decline in serum complement levels was closely related to the pathogenesis of bacterial infection, as certain bacterial strains attack the complement system with their toxins or surface proteins, consuming complement proteins, increasing bacterial load in the lung and serum, and triggering lung inflammation (Alcantara et al., 2001). This explanation was logically supported by the substantial negative correlation found between serum concentrations of C3 and C4 and pro-inflammatory cytokines in diseased camels in the present study. In addition, low levels of C3 and C4 in the serum of pneumonic camels were predictive of extensive lung lesions such as emphysema, prominent small airways dysfunction, gas entrapment, and a significant defect in lung elastic recoil (Allam et al., 2017).

As a crucial indicator of animal health, various haematological parameters can be relied upon for the diagnosis and treatment of camel diseases (Kamal, 2008). Previous studies (Salem et al., 2012, Almujaali and Al Ghamdi, 2012; Al Ghamdi, 2013) demonstrating the same effect in dromedary camels confirmed the significant decrease in Hb. Weiss and Goodnough (2005) concluded from these results that the infected camels were anemic.

In this study It has been shown that inflammatory cytokines like Tumor Necrosis Factor TNF- α and (IFN γ) which are made by infections, cause tissue damage. All of these cytokines are very important for protecting animals. This was similar to the findings of El-Deeb (2011) and El-Bahr and El-Deeb (2013), who found that the airways and lung lesions of infected calves had a lot more TNF and IL-6 than normal. This due to stress or damage could result in the release of signals that stimulate the activation of other cells, particularly those of the innate immune system.

Also, plasma proteins as affected by bacterial infection are presented in Table (5). The value of total protein (5.27 ± 0.39) was decreased non-significantly compared to control. In camels, serum total protein and serum albumin concentrations are used for the assessment of healthy status (Kaneko, 1989), and the reduced concentrations are a measure of protein deficiency. Albumin is considered as a negative Acute Phase Protein and its value markedly declines during different diseases, such as tumors, malnutrition, and liver diseases (Toussaint et al., 2005).

Globulin serves an essential part in the immune defense of the body (Shetaewi and Ross, 1991; Caldeira et al., 2007). The greater stability of serum globulins relative to albumin in animals is well-known and can be rationalized by the vital role these proteins play in the immune defence (Lynch and Jackson, 1983; Shetaewi and Ross, 1991).

The decrease in total proteins and albumin in dromedary camels is consistent with previously published papers (Kamal, 2008; Salem et al., 2012, Almujaali and Al Ghamdi, 2012; Al Ghamdi, 2013). The hypoproteinemia may be caused by a decline in albumin. The drop in albumin may be due to albumin leakage via injured tissues, and the increase in gamma globulins is a compensatory response to restore the decreased plasma osmotic pressure caused by the albumin loss. This can explain the drop in serum albumin levels found in sick camels. Table 5 represents the variations in liver enzymes, including Alanine Transferase (ALT), Aspartate Transferase (AST), and Gamma Glutamyl Transferase (GGT). The activity of these enzymes is relevant to the clinical diagnosis of specific muscle and liver conditions (Ford, 1974, Johnson, 1976 and Lessard et al., 1986). In general, the bacterial infection in camels greatly increases the levels of many enzymes.

The considerable rise in AST, ALT, and GGT activities in the serum of infected camels as compared to healthy camels showed liver damage and provided an explanation for lower total protein and albumin levels in infected animals. Similar outcomes were observed in dromedary camels with digestive inflammatory diseases. (Bengoumi et al., 1997 and Kamal, 2008) compared to their corresponding values in healthy camels. In addition, the levels of total proteins, albumin, and glucose in ill camels were much lower than their values in healthy camels. These findings are consistent with those of Craig et al. (1992) for cattle and West (1989) for horses. Also, in chronic respiratory disease (Huzzey et al., 2009 and Chan et al., 2010), in metritis cases (Tabrizi et al., 2008), in lameness (Smith et al., 2010) and traumatic reticuloperitonitis (Nazifi et al., 2009) were reported.

Gross lesions of tissues recovered from slaughtered camels revealed enlarged lungs with varying degrees of congestion indicated by dark red spots, as determined by histopathology. The areas of emphysema were elevated, lighter, and paler than the surrounding tissue. A histopathological study of the lungs revealed hyperplastic bronchiolar walls, bronchial wall thickening, and congested alveolar wall. These results are consistent with those of other studies calculated by Abubakar et al. (2011) and Nahed et al (2016).

CONCLUSION

The study demonstrated the most important of bacteria that cause pneumonia by using PCR and their effects on pro-inflammatory, cytokines, which signal the onset of respiratory disease in camels, and these cytokines can therefore function as prognostic and diagnostic markers for the disease. In addition, their role as the primary initiator of the inflammatory response may increase their value as therapeutic targets for treating the camel disease. The imbalance between pro-inflammatory and anti-inflammatory cytokines in the blood of diseased camels is likely the primary cause of enormous lung damage and a stifling immune response. The disease appeared to stimulate both innate and humoral immune responses, from an immunological standpoint. Overall, this study can increase our knowledge and understanding of the pathogenesis of respiratory disease in camels, which can contribute to better disease management and treatment.

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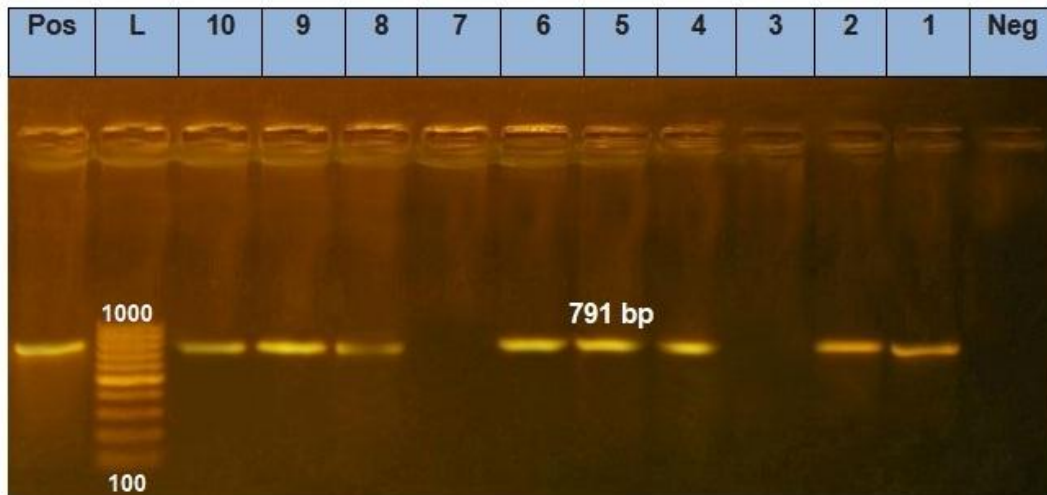


Figure 1.

PCR amplification of *staph. spp* DNA from different samples; M, Molecular weight marker showed 100bp-1000bp DNA ladder. The type code is shown above the lanes. Lanes 1, 2, 4, 5, 6, 8,9 and 10 represent the positive *staph. spp* product at 791bp, lane 3 and 7 represents the negative.

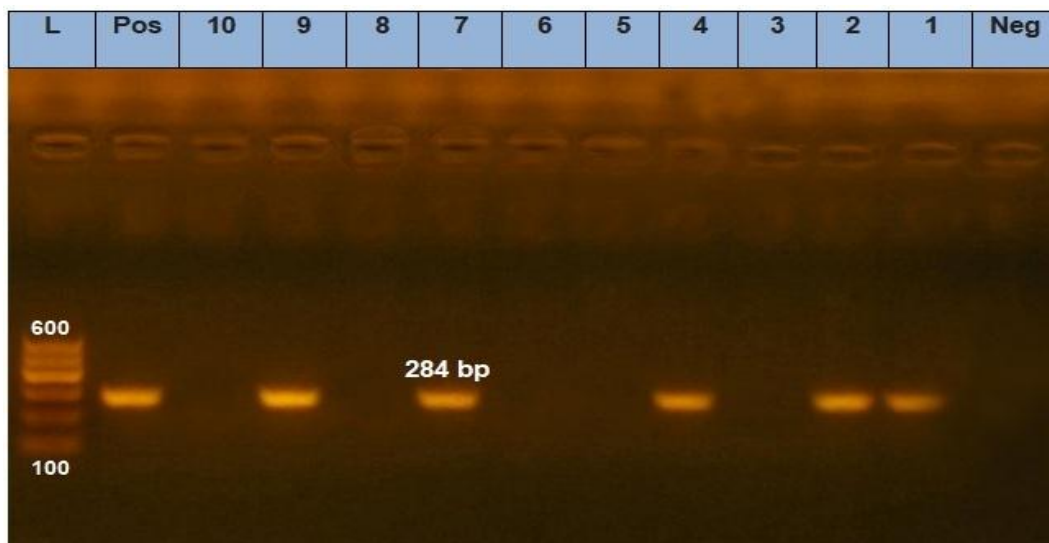


Figure 2.

PCR amplification of *Salmonella spp.* DNA from different samples; M, Molecular weight marker showed 100bp-600 bp DNA ladder the type code is shown above the lanes, lanes1, 2,4,7 and 9 represents the positive product of *salmonella* at 284 bp, lane 3, 5,6,8 and 10 represents the negative.

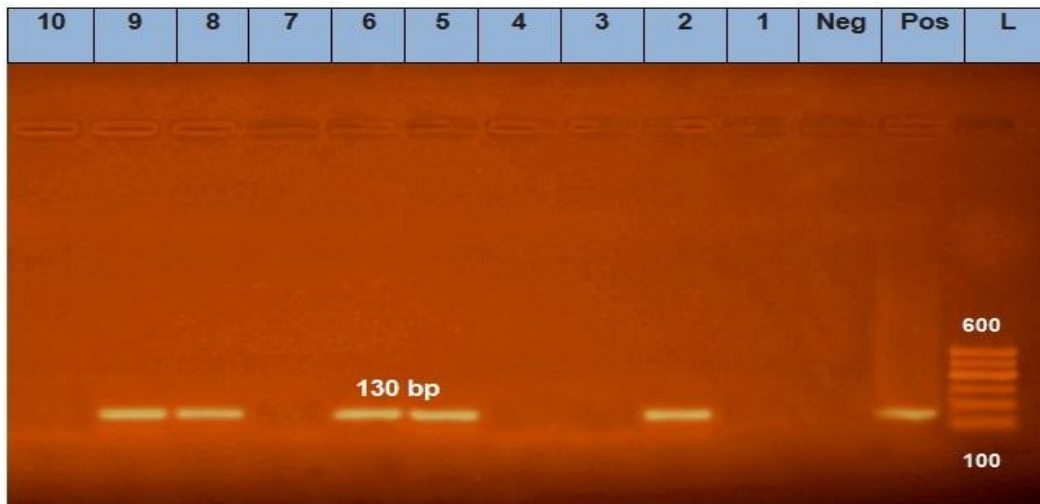


Figure 3.

PCR amplification of *Klebsiella pneumoniae* DNA from various samples; M, Molecular weight marker displayed 100bp-600 bp DNA ladder; the type code is indicated above the lanes; lanes 2, 5, 6, 8, and 9 represent the positive product of *Klebsiella pneumoniae* at 130bp; lanes 1, 3, 4, 7 and 10 represent the negative.

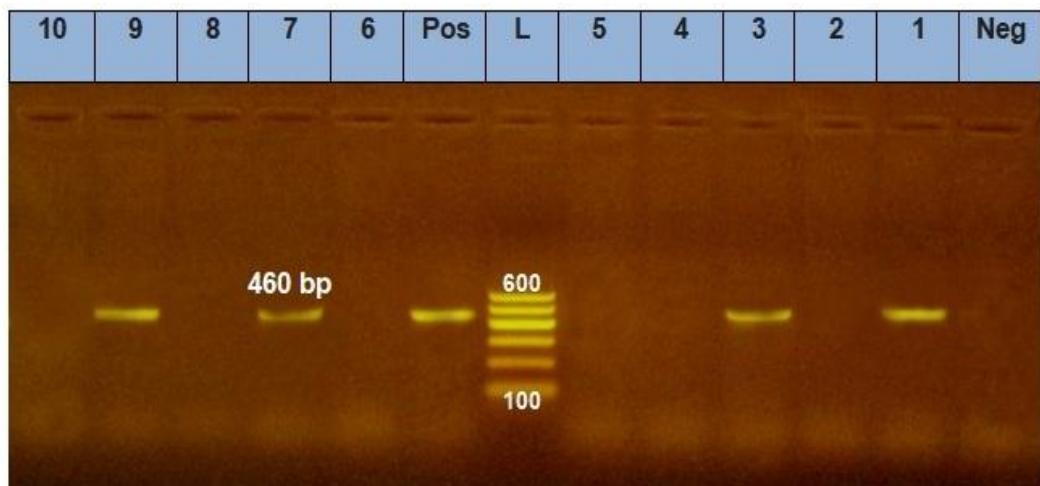


Figure 4.

PCR amplification of *Pasturella multocida* . DNA from different samples; M, Molecular weight marker showed 100bp-600 bp DNA ladder the type code is shown above the lanes, lanes1, 3,7 and .9 represents the positive product of *Pasturella multocida* at 460 bp, lane 2,4,5,6,8 and10 represents the negative.

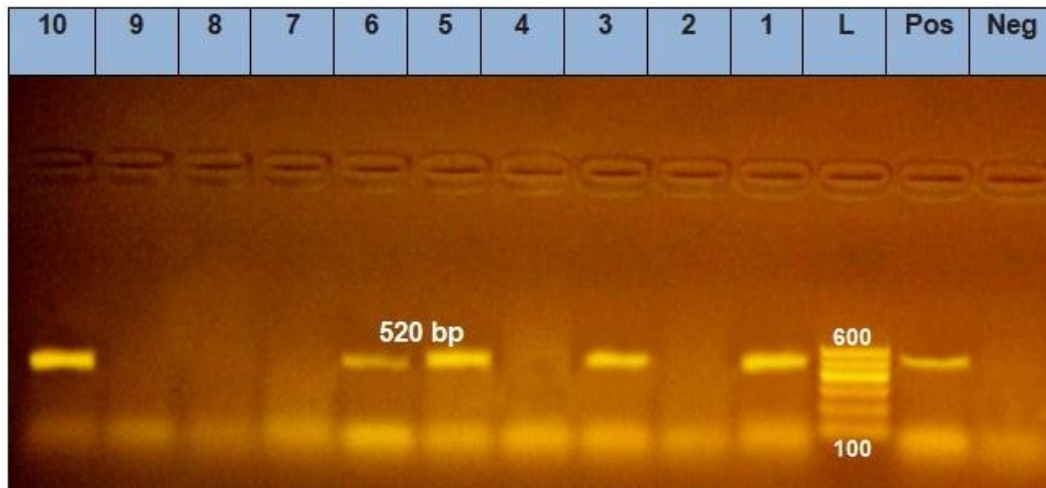


Figure 5.

PCR amplification of *Mycoplasma* spp. DNA from different samples; M, Molecular weight marker showed 100bp-600 bp DNA ladder the type code is shown above the lanes, lanes 1, 3, 5, 6 and 10 represents the positive product of *Mycoplasma* spp. at 520 bp, lane 2, 4, 7, 8 and 9 represents the negative.

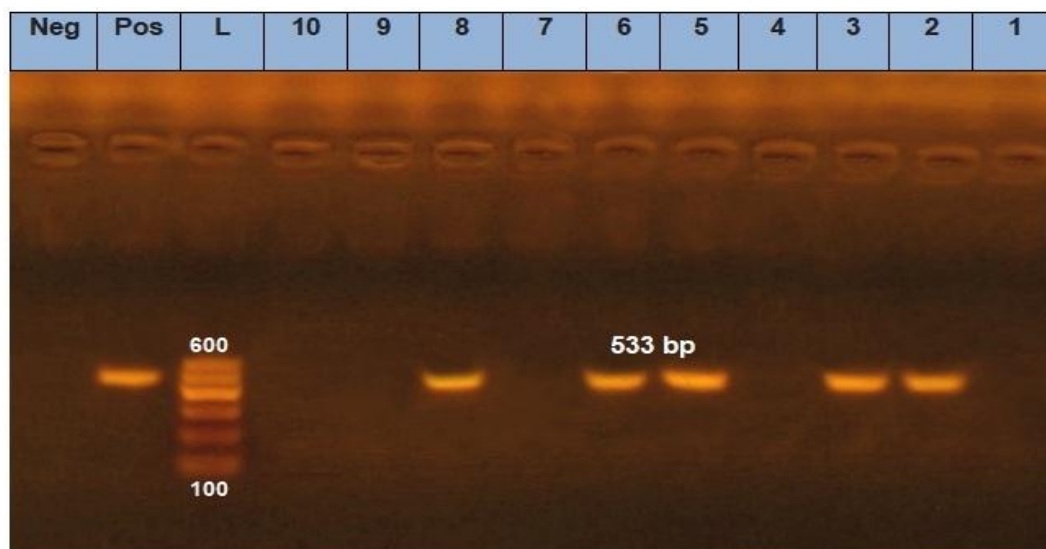


Figure 6.

PCR amplification of *Bacillus* spp. DNA from several samples; M, Molecular weight marker indicating 100bp-600 bp. The type code is displayed above the lanes on the DNA ladder, lanes 2, 3, 5, 6 and 8 indicate the 533 bp positive of *Bacillus* spp. while lanes 1, 4, 7, 9, 10 represent the negative.

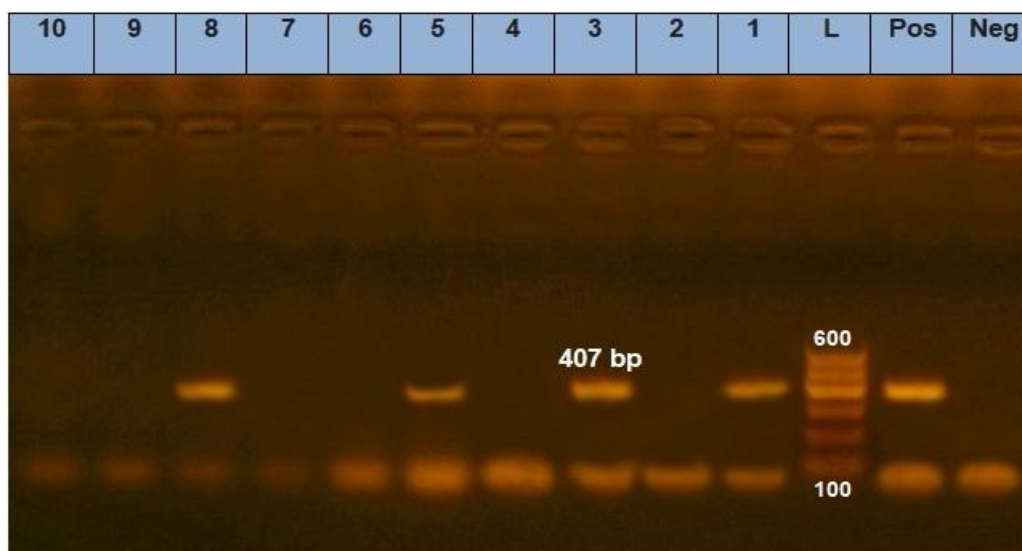


Figure 7.

PCR amplification of *Streptococcus pyogenes* DNA from several samples; M, Molecular weight marker indicating 100bp-600 bp. The type code is displayed above the lanes on the DNA ladder, lanes 1, 3, 5 and 8 indicate the 407 bp positive of *Streptococcus pyogenes*, while lanes 2, 4, 6, 7,9 and 10 represent the negative.

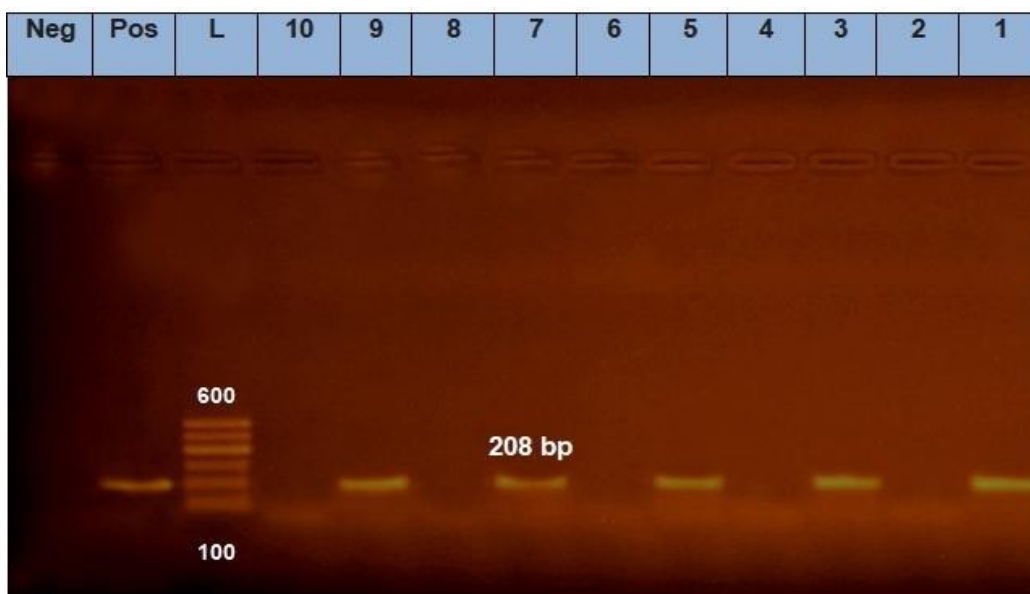


Figure 8.

PCR amplification of *Streptococcus pneumoniae* with pneumolysin DNA from several samples; M, Molecular weight marker indicating 100bp-600 bp. The type code is displayed above the lanes on the DNA ladder, lanes 1, 3, 5, 7 and 9 indicate the 208 bp positive of *Streptococcus pneumoniae* with pneumolysin, while lanes 2, 4, 6, 8 and 10 represent the negative.

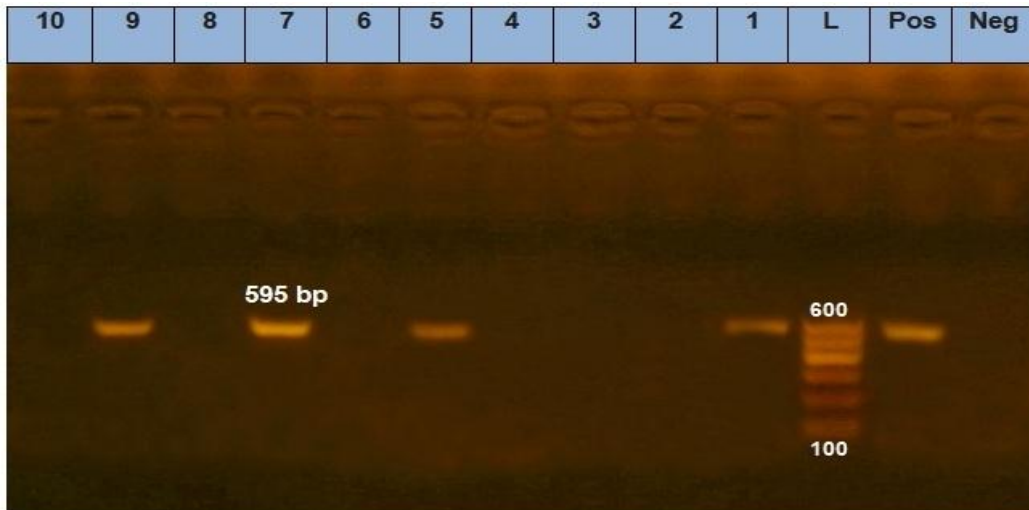


Figure 9.

PCR amplification DNA of *Proteus* spp. DNA from various samples; M, Molecular weight marker revealed 100bp-600bp Above each lane on the DNA ladder is a type code. Lane 1, 5, 7 and 9, for example, shows the positive product of *Proteus* spp. at 595 bp, while Lane 2,3, 4, 6, 8 and 10 shows the negative



Figure 10.

Camel lung with different degrees of congestion and emphysema.



Figure 11.

Camel lung with hydatid cysts.

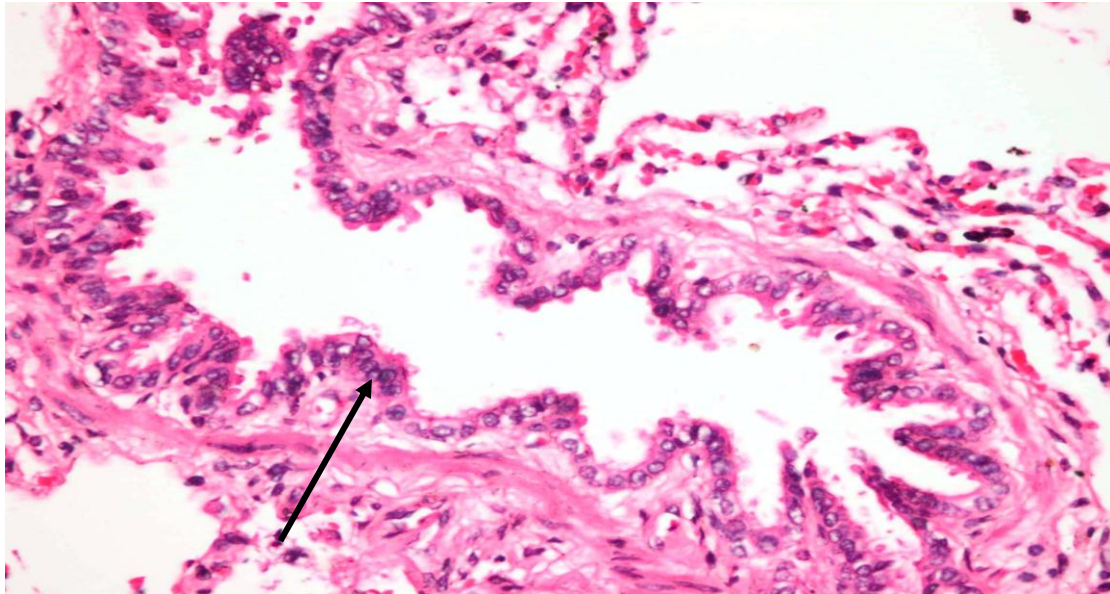


Figure 12. Histopathological analysis of camel lungs with Hematoxylin and Eosin stain (magnification x40) showing hyperplastic bronchiolar wall (sever change) and Thickening in the bronchial wall (arrow head).

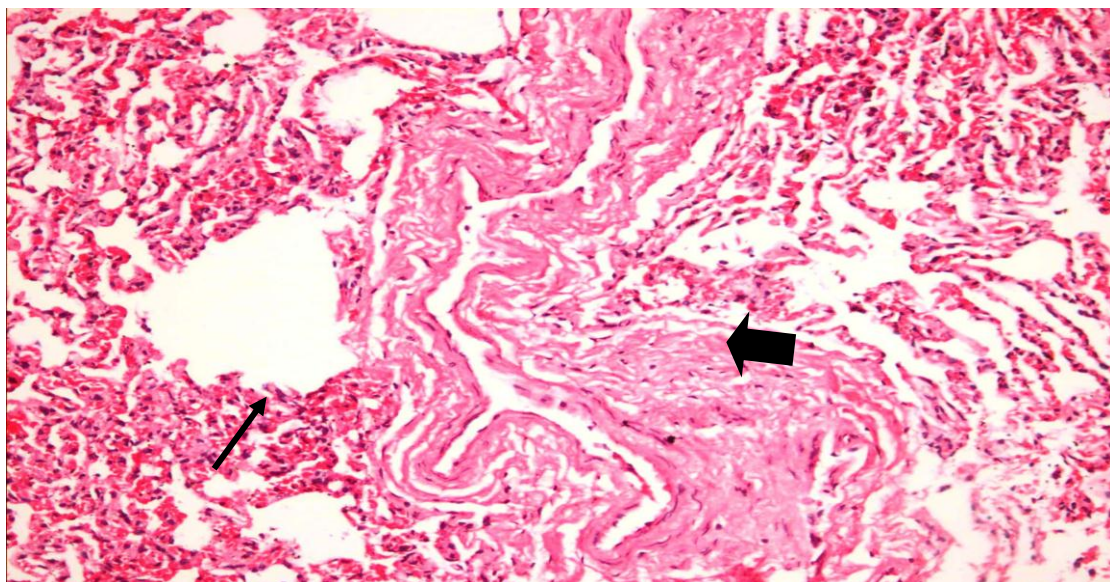


Figure 13. Histopathological analysis of camel lungs with Hematoxylin and Eosin stain (magnification x40) showing alveolar wall with congestion in the perialveolar blood capillaries. (sever change).

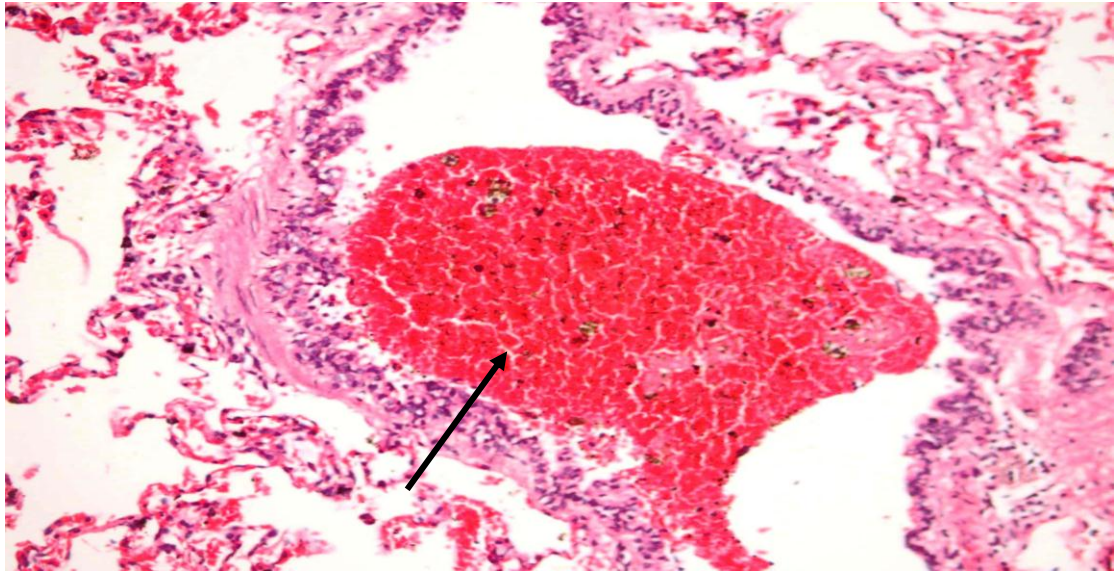


Figure 14.

Histopathological analysis of camel lungs with Hematoxylin and Eosin stain (magnification x40) showing diffuse hemorrhage.

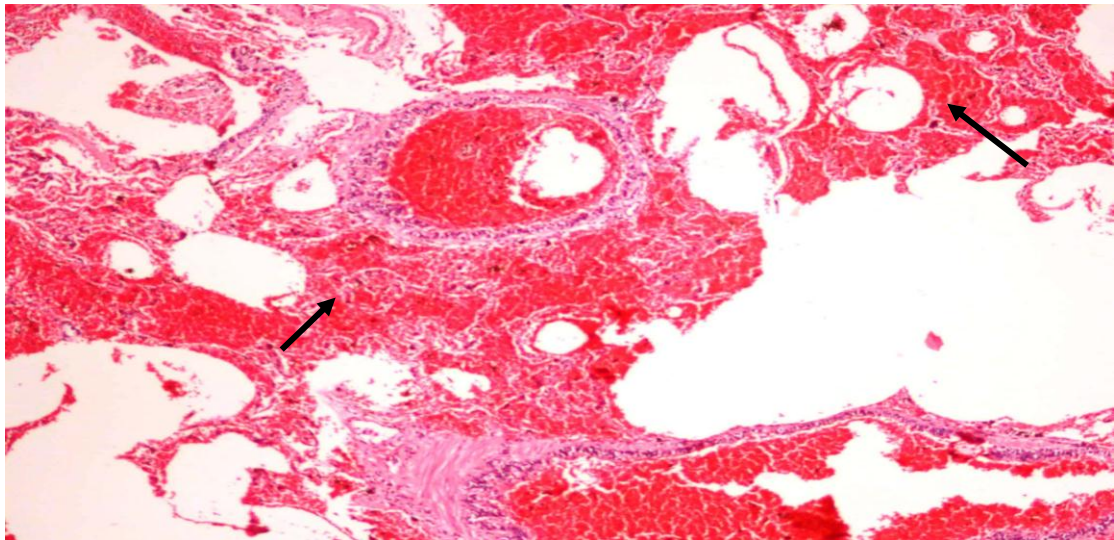


Figure 15.

Histopathological analysis of camel lungs with Hematoxylin and Eosin stain (magnification x40) showing diffuse hemorrhage in the bronchiole and interstitial tissue (moderate change).

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions according to reference.

Target gene	Primers sequences	Amplified segment (bp)	Reference
<i>Staph 16S rRNA</i>	CCTATAAGACTGGGATAACTTCGGG CTTTGAGTTTCAACCTTGCGGTCG	791	Mason et al., 2001
<i>Salmonella invA</i>	GTGAAATTATCGCCACGTTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	Oliveira et al., 2003
<i>Klebsiella pneumoniae</i> 16S-23S ITS	ATTGAAGAGGTTGCAAACGAT TTC ACTCTGAAGTTTTCTTGTGTTC	130	Turton et al., 2010
<i>Pastuerella multocida Kmt1</i>	ATC-CGC-TAT-TTA-CCC-AGT-GG GCT-GTA-AAC-GAA-CTC-GCC-AC	460	OIE, 2012
<i>Mycoplasma 16S rRNA</i>	CGCCTGAGTAGTTTCGC GCGGTGTGTACAAGACCCGA	520	Dvorakova et al., 2005
<i>Bacillus groEL</i>	TGCAACTGTATTAGCACAAGC T TACCACGAAGTTTGTTC ACTACT	533	Das et al., 2013
<i>Streptococcus pyogenes spy1258</i>	AAAGACCGCCTTAACCACCT TGGCAAGGTAAACTTCTAAAGCA	407	Schabauer et al., 2014
<i>Streptococcus pneumoniae pneumolysin</i>	CCC ACTCTTCTTGC GGTTGA TGAGCCGTTATTTTTTC ACTACTG	208	Murdoch et al., 2003
<i>Proteus atpD</i>	GTATCATGAACGTTCTGGGTAC TGAAGTGATACGCTCTTG CAG	595	Bi et al., 2013

Table (2). Cytokine profile contents (Pg/ml) in the diseased camels compared to apparently healthy camels.

Variations	Control camels	Diseased camels	Percentage Changes	Significances
Pro-inflammatory cytokines				
IL-2 pg/ml	77.74 ^B ± 10.84	157.12 ^A ± 9.29	(+) 102.11	**
TNF-α pg/ml	96.17 ^B ± 10.89	141.27 ^A ± 9.33	(+) 046.90	**
(IFNγ)	76.06 ^B ± 11.64	156.79 ^A ± 9.97	(+) 106.14	**
IL-6 pg/ml	75.55 ^B ± 08.24	142.19 ^A ± 7.06	(+) 088.21	**
Anti-inflammatory cytokines				
IL-10 pg/ml	105.29 ± 10.02	89.30 ± 11.70	(+) 017.91	-

IL: Interleukin, TNFα: Tumor necrosis factor α: Interferon gamma (IFNγ)

In each row different letters were significantly different.

** Highly significant

- Non significant

Significant differences in the values between the diseased and control groups were indicated by (*) at P < 0.05.

Values are mean ±SD.

Table (3). Complements and immunoglobulins (mg/dl) in the diseased camels compared to apparently healthy camels.

Variations	Control camels	Diseased camels	Percentage Changes	Significances
C3 mg/ml	00.26 ^B ± 00.11	00.77 ^A ± 0.09	(+) 196.15	**
C4 mg/ml	00.32 ^B ± 00.22	01.32 ^A ± 0.19	(+) 312.50	**
IgG mg/ml	00.73 ^B ± 00.24	02.70 ^A ± 0.21	(+) 269.86	**
IgM mg/ml	00.47 ^B ± 00.29	01.41 ^A ± 0.25	(+) 200.00	*

C: Complement IgG: Immunoglobulin G IgM: Immunoglobulin M

In each row different letters were significantly different, ** Highly significant * Significant

Significant differences in the values between the diseased and control groups were indicated by (*) at P < 0.05. Values are mean ± SD

Table (4). Least square means ± SE of some biochemical blood parameters in the diseased camels compared to apparently healthy camels.

Variations	Control camels	Infected camels	Percentage Changes	Significances
Total Protein g/dl	06.02 ± 00.39	05.27 ± 00.39	(-) 0 12.46	-
Albumin g/dl	03.04 ± 00.24	02.67 ± 00.21	(-) 012.17	-
Globulin g/dl	02.98 ± 00.23	02.61 ± 00.19	(-) 012.42	-
(A/g Ratio)	01.04 ± 00.12	01.11 ± 00.11	(+) 006.73	-
ALT U/ml	10.64 ^B ± 03.89	23.07 ^A ± 03.33	(+) 116.82	*
AST U/ml	48.66 ^B ± 13.31	95.73 ^A ± 11.40	(+) 096.73	**
GGT IU/ml	27.82 ^B ± 11.13	78.80 ^A ± 09.53	(+) 183.25	**

(A/g ratio): Albumin/Globulin ratio (%), ALT: Plasma alanine transferase (U/ml), AST: Plasma aspartate transferase (U/ml), GGT (IU/ml): Gama glutamyl transferase. In each row different letters were significantly different. ** Highly significant * Significant - Non significant

Table (5). Least square means SE of haematological indices in sick camels compared to camels that seem to be healthy.

Variations	Control camels	Infected camels	Percentage Changes	Significances
Erythrocyte Indices				
RBC's $10^6/\mu\text{L}$	$5.39^A \pm 0.19$	$4.02^B \pm 0.17$	(-) 25.42	**
Hb g/dl	$13.18^A \pm 0.40$	$10.15^B \pm 0.34$	(-) 22.99	**
Hct %	$40.50^A \pm 1.21$	$30.45^B \pm 1.03$	(-) 24.81	**
MCV fl	76.31 ± 3.38	77.94 ± 2.90	(+) 02.14	-
MCH pg	24.97 ± 1.24	27.38 ± 1.07	(+) 09.65	-
MCHC %	$32.85^B \pm 0.66$	$35.93^A \pm 0.57$	(+) 09.38	**
Platelet				
Platelet $10^9/\text{L}$	$801^A \pm 61.11$	$627^B \pm 52.33$	(-) 21.28	*
Leukocyte Indices				
WBC's $10^3/\mu\text{L}$	$09.09^A \pm 00.56$	$04.23^B \pm 0.48$	(-) 053.47	**
Lymphocytes %	29.63 ± 03.49	27.73 ± 2.59	(-) 006.43	-
Monocytes %	09.21 ± 02.65	10.85 ± 2.01	(+) 017.82	-
Granulocytes %	61.16 ± 06.30	64.07 ± 4.71	(+) 004.76	-

In each row different litters were significantly different.

** Highly significant

* Significant

- Non significant

بعض الدراسات المناعية والبكتيرية على الإبل في الساحل الشمالي الغربي لمصر

اماني حافظ¹ و محمد الرئيس²

¹ وحدة الامراض المعدية – قسم صحة الحيوان والدواجن – شعبة الانتاج الحيواني والدواجن- مركز بحوث الصحراء - القاهرة - مصر

² قسم فسيولوجيا الحيوان والدواجن – شعبة الانتاج الحيواني والدواجن- مركز بحوث الصحراء- القاهرة - مصر

الملخص العربي

هدفت هذه الدراسة إلى إلقاء الضوء على أهم أنواع البكتيريا المسببة لأعراض الجهاز التنفسي وتأثيرها على السيتوكينات الالتهابية في الإبل ، بالإضافة إلى علاقتها بالمعايير المناعية الأخرى مثل بروتينات الطور الحاد (APPs) والمكملات والجلوبولين المناعي. تم جمع عينات رئة الإبل (572) لهذا التحقيق ، مع تحديد الرئة الطبيعية في 390 (68.18%) كمجموعة ضابطة ، وعينات رئة الإبل الرئوية المصابة (182 ؛ 31.82%) ومصنفة على أنها مجموعة مريضة ، تم جمعها كلها من المسالخ في الساحل الشمالي الغربي لمصر. تتكون العزلات من تسعة أجناس من البكتيريا المسببة للأمراض. وهم كما يلي: بكتريا المكورات العنقودية حيث تم عزل عدد 88 عزلة بنسبة 19%، وبكتريا السالمونيلا بعدد 40 عزلة بنسبة 8.7%، ثم بكتريا الكليبيسيلا الرئوية *Klebsiella pneumoniae* بعدد 75 عزلة وبنسبة 16.4% ، والباستريلا ملتوسيدا *Pastuerella multocida* بعدد 38 عزلة وبنسبة عزل 8.3% ، والميكوبلازما 58 عزلة (12.6%) ، وبكتريا الباسلس *Bacillus spp.* بعدد 35 عزلة وبنسبة عزل 7.6% ، و *Streptococcus pyogenes* بعدد 20 ونسبة عزل 4.3% ، و *Streptococcus pneumoniae* بعدد 68 عزلة وبنسبة 14.8% ، واخيرا *Proteus spp.* 35 عزلة اي 7.6%. وباستخدام عينات الدم التي تم جمعها من كلا المجموعتين ، تم تقييم المعلمات البيوكيميائية والمناعية المحددة. أظهرت الإبل المريضة زيادات كبيرة في السيتوكينات المسببة للالتهابات (IL-2 و IL-6 و TNF و IFN) ، وبروتينات الطور الحاد (الفيبرينوجين ، والسيرولوبلازمين ، والهيبتوجلوبيين ، والأميلويد المصلي) ، والسيتوكينات الحادة (IL-2 ، IL-6 ، TNF و IFN). وقد زاد الجلوبيولين المناعي ، لكن البروتين الكلي (TP) ، الألبومين (Aib) ، الالانين امينو ترانسفيرز alanine aminotransferase (ALT) ، الأسبارتات امينوترانسفيرز (AST) aspartate aminotransferase ، و الجاما جلوتاميل ترانسفيرز (GGT) gamma glutamyl transferase لم تتأثر بشكل كبير بالعدوى. في المقابل ، انخفض السيتوكين المضاد للالتهابات IL-10 ، المكمل 3 ، المكمل 4 ، مستويات مصل الألبومين ، ونسبة A / G بشكل كبير ، وكشف تحليل الدم عن انخفاض كبير (p0.01) في عدد كرات الدم الحمراء مقارنة بـ المجموعة الضابطة. يكشف الأنسجة المرضية بالفحص الهستوباثولوجي عن احتقانات شديدة وانزفة دموية داخل نسيج الرئة.

الكلمات الدالة: الجمل ، الساحل الشمالي الغربي ، بكتيريا الجهاز التنفسي ، السيتوكينات وأمراض الدم.