Cytological analysis of tracheal wash and bronchoalveolar lavage fluid in health and respiratory disease in dromedary camels

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Background. Tracheal wash (TW) and bronchoalveolar lavage (BAL) have proven to be useful tools for the identification of disease-associated changes in the respiratory tract in human and different animal species. In the dromedary camel, little is known about cytological analysis of TW and BAL in health and disease. The aim of the present study was to evaluate the cytological composition of TW and BAL in health and respiratory disease in dromedary camels. **Methods**. TW and BAL samples were collected from dromedary camels and cytological analysis was performed by microscopic examination of prepared smears. Camels with clinical respiratory disease (n = 18) were compared with apparently healthy (control) camels (n = 9). **Results.** In the apparently healthy camels, differential cytological analysis of TW samples identified macrophages and neutrophils as the main cell populations with lesser proportions of lymphocytes and epithelial cells and very rare abundance of eosinophils and mast cells. In the TW of camels with respiratory disease, neutrophils were the most abundant cells followed by macrophages and lymphocytes. In the BAL of healthy camels, macrophages represented the main cell type followed by lymphocytes and neutrophils. In respiratory-diseased camels, BAL samples contained higher percentages of neutrophils with reduced percentages of macrophages and lymphocytes in comparison to camels from the control group. Collectively, the results of the current study revealed higher abundance of neutrophils in the TW and BAL from dromedary camels than many other veterinary species. The cytological patterns of TW and BAL from camels with respiratory diseases were characterized by increased proportion of neutrophils and decreased proportion of macrophages in comparison to healthy camels. The proportion of lymphocytes was also decreased in TW samples from diseased camels.

1 **Cytological analysis of tracheal wash and bronchoalveolar lavage fluid in**
2 **health and respiratory disease in dromedary camels**

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20 **Abstract**

21 **Background.** Tracheal wash (TW) and bronchoalveolar lavage (BAL) have proven to be useful tools

22 for the identification of disease-associated changes in the respiratory tract in human and different

23 animal species. In the dromedary camel, little is known about cytological analysis of TW and BAL in

24 health and disease. The aim of the present study was to evaluate the cytological composition of TW

25 and BAL in health and respiratory disease in dromedary camels.

26 **Methods**. TW and BAL samples were collected from dromedary camels and cytological analysis was

27 performed by microscopic examination of prepared smears. Camels with clinical respiratory disease

28 (n = 18) were compared with apparently healthy (control) camels (n = 9).

29 **Results.** In the apparently healthy camels, differential cytological analysis of TW samples identified

30 macrophages and neutrophils as the main cell populations with lesser proportions of lymphocytes and

31 epithelial cells and very rare abundance of eosinophils and mast cells. In the TW of camels with

32 respiratory disease, neutrophils were the most abundant cells followed by macrophages and

33 lymphocytes. In the BAL of healthy camels, macrophages represented the main cell type followed by

34 lymphocytes and neutrophils. In respiratory-diseased camels, BAL samples contained higher

35 percentages of neutrophils with reduced percentages of macrophages and lymphocytes in comparison

36 to camels from the control group. Collectively, the results of the current study revealed higher

37 abundance of neutrophils in the TW and BAL from dromedary camels than many other veterinary

38 species. The cytological patterns of TW and BAL from camels with respiratory diseases were

39 characterized by increased proportion of neutrophils and decreased proportion of macrophages in

40 comparison to healthy camels. The proportion of lymphocytes was also decreased in TW samples

41 from diseased camels.

42

43   Keywords: bronchoalveolar lavage; camel; cytology; respiratory; tracheal wash

44

Introduction

The dromedary camel (Camelus dromedarius) belongs to the most important domestic animal
species adapted to dry and hot regions in Asia and Africa (Kebedi 2010). The camel world
population is around 30 million heads (Faye 2015). Respiratory diseases are among the main
factors responsible for high mortality rates in dromedary camels (Al-Ruwaili et al. 2012; Bakhesh
Alhendi 2000; Gebru et al. 2018; Li et al. 2017; Scaglione et al. 2017). Among respiratory
infectious diseases, viral and bacterial infections are usually correlated with most losses (Schwartz
HJ 1992). The main viral respiratory pathogens in camels include adenovirus, parainfluenza 3
virus (Kebedi 2010), morbilivirus (Al-Rawashdeh et al. 2000), and MERS Coronavirus, which is
among the emerging public health hazards (Memish et al. 2013). Bacterial infections of the
respiratory tract of camels have been shown to be mainly caused by *Manhaemiya hemolytica,*
Corynebacterium spp., *Pasteurella* spp., and *Arcanobacterium pyogenes (Wareth et al. 2014;*
Wernery 2002).

The early diagnosis of respiratory disorders is a key factor for choosing specific treatment to
prevent disease progression and chronic lung disease (Intisar et al. 2009; Intisar et al. 2010a; Intisar
et al. 2010b; Kebedi 2010). Tracheal wash (TW) and bronchoalveolar lavage (BAL) are valuable
tools widely used for the investigation of the respiratory tract in human (Rose & Knox 2007b),
horses (Orard et al. 2016), cattle (Pringle et al 1988; Abutarbush et al. 2019; Kokotovic et al.
2007), sheep (Katsoulos et al. 2009), pigs (Weissenbacher-Lang et al. 2016), alpaca (Pacheco
2012), dogs (Creevy 2009; Zhu et al. 2015), and cats (Lin et al. 2015). TW and BAL samples are
valuable mirrors of different areas of the airways and can be used in combination with other
methods for the diagnosis of lower respiratory tract inflammation (Malikides et al. 2003). Being

67   able to explore large areas of the lower respiratory tract (Hoffman 2008), BAL is widely used as a

68   source for specimens for cytological, microbiological and immunological investigation of the

69   respiratory tract (Couetil et al. 2007; Hoffman 2008; Hoffman et al. 1998; Rose & Knox 2007a).

70   BAL and TW samples are also useful in giving an insight on the severity and stage of inflammatory

71   reactions in the lung and in the detection of subclinical pulmonary diseases (Caldow 2001).

72   Species-specific differences have been reported regarding the selection of the appropriate

73   technique for sampling the respiratory secretions. In horses, TW is preferred for diagnosis of

74   respiratory infections, whereas BAL is the best choice for the diagnosis of non-infectious

75   pulmonary diseases (Davis & Sheats 2019). However, similar diagnostic values have been reported

76   for BAL and TW for the diagnosis of common bacterial and viral respiratory pathogens in cattle

77   (Doyle et al. 2017). Although respiratory cytology may rapidly provide a presumptive diagnosis

78   of infection, identification of the etiologic agent requires bacterial or viral culture or PCR (Jocelyn

79   et al. 2018).

80   Although some studies reported the cytological composition of TW samples in camels (Habasha

81   & Hussain 2014; Habasha & Hussain 2016) , there have been no studies conducted on the

82   comparative analysis of TW and BAL cytology in dromedary camels. The aim of the current study,

83   therefore, was to comparatively analyze the TW and BAL cytology in healthy and respiratory-

84   diseased dromedary camels.

85   **Materials & Methods**

86 **Ethical approval**

87 All experimental procedures used in this study were approved by the Ethics Committee at King

88 Faisal University, Saudi Arabia (Permission number KFU-REC / 2019 – 10 - 01). All applicable

89 international, national, and institutional (King Faisal University) guidelines for the care and use of

90 animals were followed.

91 **Animals and experimental design**

92 Twenty-seven camels (*Camelus dromedaries*) of different ages, sex, and breeds were involved in

93 this study. The control group included nine healthy camels (five males and four females with a

94 median age ± SEM of 10.3 ± 5.5 years and a median weight ± SEM of 390 ± 125 kg) selected from

95 animals maintained at the Camel Research Center at the King Faisal University, Al-Ahsa, Saudi

96 Arabia. The diseased animal group included eighteen camels (seven males and eleven females with

97 a median age ± SEM of 12 ± 6 years and a median weight ± SEM of 405 ± 185 kg) randomly

98 selected from respiratory-diseased camels brought to the Veterinary Teaching Hospital, College

99 of Veterinary Medicine, King Faisal University. Control camels were selected based on clinical

100 scoring, physical, and laboratory evaluation (no fever or signs of abnormal respiratory signs such

101 as cough, nasal discharge, dyspnea or abnormal lung sounds as well as normal white blood cell

102 counts and biochemistry panel).

103 **Clinical examination**

104 For all animals, detailed history and clinical examination signs (dyspnea, cough and nasal

105 discharge, lung sounds, and rate of breathing) were recorded. For the clinical evaluation of control

106 and respiratory diseased camels, a scoring system designed for healthy and respiratory diseased

107 horses (Ohnesorge 1998) was adapted with a modification by considering the animal rectal

108 temperature in the scoring procedure (Love et al. 2014) (Table 1). Clinical score points were

109 recorded by two persons. However, the data could not be taken blindly due to the difficulty of

110 taking the case history twice in the hospital. Camels with a clinical score less than three points

111 were considered healthy while camels with three or higher score points were considered diseased.

112 **Bronchoscopy and collection of TW and BAL samples**

113 For bronchoscopy, camels were positioned in sternal recumbency position. Due to the narrow nasal

114 passage in camels, especially in the Omani breed, and the lack of camel-specific endoscope and

115 BAL catheters, endoscope and BAL catheters were inserted via the oral cavity. After the

116 intravenous injection of xylazine 2% (0.1 mg/kg bodyweight; Rompun, Bayer Health Care)

117 (Shawaf 2017), a special mouth gag especially designed for camels in the Veterinary Teaching

118 Hospital (Fig. 1) was placed to keep the mouth open protecting and allowing easy passage of the

119 endoscope. The sedation was maintained throughout the whole endoscopic TW and BAL

120 collection procedures by intravenous injecting xylazine (0.05 mg/kg). A flexible 3.2 m long, 12

121 mm tip diameter bronchoscope (EVIS Olympus, OLYMPUS AUSTRIA GmbH., Vienna),

122 supported with an insufflation system, light source, and irrigation system was introduced into the

123 oral cavity. When reached the pharynx, the endoscope was inserted via the rima glottidis into the

124 trachea. A tiny sterile plastic catheter (EQUIVET; 2,3 mm x 350 cm) was passed into the

125 endoscope's working channel and TW was done by the injection of 10-15 mL sterile normal saline

126 into the last part of the trachea followed by immediate aspirating to recover TW fluid. The retrieved

127 fluid was collected into plain tubes for cytological analysis (within 15 min of collection). BAL

128 was performed immediately after the endoscopy and TW procedure. To decrease coughing during

129 BAL, 20−40 mL of 1% lidocaine was infused as local anesthesia into the lower airway. A catheter

130 (EQUIVET B.A.L. catheter 240 cm, KRUUSE, Denmark) was passed through the speculum of

131 mouth gag into the oral cavity until the pharynx and then advanced into the larynx, trachea, and

132 bronchi until reaching a slight resistance (Fig. 2). As we faced difficulties to introduce the BAL

133 catheter blind into the larynx, we used an endoscope for guiding the BAL catheter into the larynx.

134 As soon as the tube was wedged into the bronchus, the cuff was then gently inflated using 10-20

135 ml of air to prevent the backflow of infused fluid. Five syringes (each of 50 ml) of sterile isotonic

136 saline were placed in a water-bath to warm up to approximately 37 °C and were then instilled via

137 BAL catheter. BAL was aspirated immediately after injection and the samples were immediately

138 positioned on ice and submitted to the lab within 30 min of collection. Samples were considered

139 acceptable when they contained a foamy surfactant layer. BAL samples were used for cytological

140 analysis within one hour after sample collection.

141 **Cytological analysis of TW and BAL samples**

142 The total cell count of BAL samples was estimated by taking a small volume from the well mixed

143 original fluid in a Bürker's counting hemocytomer (Laporoptic, Germany) after filtering the

144 sample through a gauze. For differential cell counting, the TW and BAL samples were centrifuged

145 at 1500 rpm for 15 minutes and the supernatant was discarded. Microscopic smears were prepared

146 from the cell pellet and air dried smears were stained using the Diff Quick staining kit (Hemal

147 Stain Co. Inc., Danbury, CT). The cytological analysis was performed by a specialist in clinical

148 pathology, who was blinded to the camel health status. Using a magnification of 1000× and a

149 standardized counting protocol (De Brauwer et al. 2000; De Brauwer et al. 2002), the percentages

150 of macrophages, lymphocytes, neutrophil, mast cells, eosinophils, and epithelial cells were

151 calculated after counting a minimum of 400 cells.

152 **Statistical analysis**

153   Using the statistical software Graph Pad Prism 5, differences between the means were analyzed

154   using the one-way analysis of variance (ANOVA) in combination with the Bonferroni post test for

155   multi-comparison analysis. Normal distribution was evaluated by D'Agostino & Pearson omnibus

156   normality test. The differences between the groups were considered significant if the P-value was

157   less than 0.05.

158   **Results**

159   For the cytological analysis of tracheal wash (TW) and bronchoalveolar lavage (BAL) in healthy

160   and respiratory diseased dromedary camels, a scoring system was used to group camels into a

161   healthy control group (n=9) and a respiratory diseased group (n=18). All examination parameters

162   and scoring points were within the normal ranges in the animals from the control group with no

163   abnormal respiratory sounds or any signs of respiratory disorders or infections (clinical score < 3

164   points with a mean ± SEM of 0.88 ± 0.75). The diseased group showed varying signs of respiratory

165   signs like cough, nasal discharges, and abnormal respiratory sounds (clinical score ≥ 3 points with

166   a mean ± SEM of 7.9 ± 3.4). Endoscopic visualization of the mucosa lining the trachea and bronchi

167   also revealed no abnormal mucus accumulation in healthy animals, while there was varying

168   degrees of mucus accumulation and narrowing in the airways of diseased camels. From the total

169   instilled 250 mL fluids, the retrieved fluids were 155 ± 35.2 mL, representing 62 ± 14.8 % of the

170   total instilled fluids. No differences in the volume of the retrieved fluid were observed between

171   healthy and diseased camels.

172   Due to specific anatomical and physiological characteristics of the pharynx cavity and the long

173   soft palate (Dulla) in the camel, we needed to use visual endoscopy in eight cases to introduce the

174   BAL catheter into the larynx.

175   **Identification of different cell types in camel TW and BAL samples**

176   In both TW and BAL samples, camel alveolar macrophages were slightly variable in size with

177   abundant vacuolated cytoplasm and irregular cell margins (Fig.3A and 3E). Macrophages

178   cytoplasmic vacuoles occasionally contained cellular debris (Fig.3E). BAL neutrophils were

179   normally segmented and non-degenerative, which is similar to blood neutrophils, whereas TW

180   neutrophils were segmented and degenerated (Fig.3A). Lymphocytes were characterized by small

181   round central to eccentric nuclei with dense clumped chromatin and scant amounts of blue

182   cytoplasm with smooth margins (Fig. 3E). Eosinophils were identified based on their uniformly

183   sized small red orange cytoplasmic granules (Fig. 3C). Epithelial cells were found as ciliated

184   columnar cells with basally located nucleus. Some epithelial cells showed loss of cilia, which were

185   seen in the background of the slides (Fig.3A and F). In general, TW cells were frequently

186   degenerated and more difficult to be differentiated in comparison to BAL cells. In some TW slides,

187   contamination with bacteria, saliva, food material, red blood, or oral squamous epithelial cells was

188   observed (Fig.3D).

189   **Cellular composition of TW and BAL samples from healthy and diseased camels**

190   BAL samples from diseased camels contained significantly more cells (824 ± 401.1 cells/µL) than

191   BAL samples from the control group (200.4 ± 39.2 cells/µL) (Table.2). Due to high mucus

192   accumulation in the samples, we found difficulties in the estimation of total cell counts in the TW

193   samples.

194   The differential cell counts of the TW fluids in healthy camels consisted primarily of macrophages

195   ($51.6 \pm 10.2\%$) and neutrophils ($27.3 \pm 7.2\%$) with lesser frequency of lymphocytes ($8.2 \pm 1.3\%$),

196   epithelial cells ($8.8 \pm 5.1\%$), eosinophils ($1.7 \pm 0.4\%$), and mast cells ($1 \pm 0.4\%$). In the TW

197   samples from diseased camels, neutrophils were the most abundant cells (73.3 ± 7.4%) followed

198   by macrophages (22.4 ± 7.6%) with lower percentages of lymphocytes (3.2 ± 1.2%), mast cells

199   (1.3 ± 0.1%), epithelial cells (1.2 ± 0.3%), and eosinophils (0.5 ± 0.4%) (Fig.4A-F).

200   The differential cell counts of the BAL in healthy camels revealed the dominance of macrophages

201   (60.0 ± 3.1%) and lymphocytes (23.7 ± 1.9%) with low proportions of neutrophils (7.9 ± 1.8%)

202   and epithelial cells (5.4 ± 1.2%) and rarely seen mast cells (0.6 ± 0.2%) and eosinophils

203   (1.6 ± 0.5%). BAL samples from diseased camels showed reduced percentage of macrophages

204   (52.6 ± 4.2%) and lymphocytes (15.8 ± 6.1%) but increased percentage of neutrophils

205   (24.7 ± 4.7%) when compared to healthy animals. However, the percentage of epithelial cells

206   (5.2 ± 1.2%) was similar in healthy and diseased camels (Fig.4A-F). Based on their rectal

207   temperature, the studied animals were classified into animals with low temperature (<39) and

208   animals with high (>39.1) temperature. BAL samples from the animal group with high rectal

209   temperature contained significantly more cells (1653 ± 235.6 cells/µL) than BAL samples from

210   the normal group (587 ± 115.7 cells/µL) (Table.3). For both TW and BAL samples, the percentage

211   of neutrophils was higher in the camels with high rectal temperature . In addition, there was a

212   decrease in the percentage of macrophage in TW and BAL samples in animals with high rectal

213   temperature compared to animals with normal rectal temperature (Table.3).

214   **Discussion**

215   In the current study, the cytological composition of tracheal wash (TW) and bronchoalveolar lavage

216   (BAL) samples were comparatively analyzed in apparently healthy camels and camels with clinical

217   respiratory diseases. The cytological patterns of TW and BAL from camels with respiratory

218   diseases were characterized by increased proportion of neutrophils and decreased proportion of

219   macrophages in comparison to healthy camels. The proportion of lymphocytes was also decreased

220   in TW samples from diseased camels, when compared to healthy camels.

221   The detecting of oral epithelial cells with bacterial contamination in TW slides may affect the

222   cytological analysis and argues against using TW for bacterial examination (Smith 2019). In the

223   horse, other sampling techniques have been suggested for obtaining uncontaminated lower airway

224   secretions for bacterial culture. This includes the transtracheal aspiration after the insertion of a

225   sterile flushing tube through a tracheal cannula between tracheal rings below the larynx. In

226   addition, the protected aspiration catheter technique using a guarded sterile tube, due to its reduced

227   complication risk, has been suggested as an alternative to the transtracheal aspiration for the

228   isolation of equine pulmonary bacteria (Darien et al. 1990). However, comparative studies are

229   required to determine the most optimal sampling technique for the collection of camel lower

230   airway fluids for bacterial culture.

231   TW contamination with saliva might have resulted from oral saliva coming down from the oral

232   cavity into the airways during endoscopy. As shown in Fig. 3D, the presence of some red blood

233   cells in TW could be due to minimal bleeding during sample collection (Hughes et al. 2003). Our

234   results are also in agreement with Walker et al. (2006), who observed that BAL cells are better

235   preserved and usually easier to be identified than TW cells.

236   Due to limited information about cytological values in camels, the results from the current work

237   were compared with data reported for other species. In our study, the total cell count of BAL in

238   healthy camels was similar to results reported for the bovine BAL cytology (Abutarbush et al.

239   2019). Similar to other species, including cattle, horses, and donkeys (Abutarbush et al. 2019;

240   Hoffman 2008; Rossi et al. 2018; Shawaf 2019), respiratory diseases in camels were associated

241   with increased total cell count in the BAL fluid. For cattle with respiratory diseases, however,

242   greater increase in the total BAL cell count was reported (Thirunavukkarasu et al. 2005).

243   Similar to their distribution in healthy horses (Malikides et al. 2003), neutrophils were found in

244   higher proportions in TW than in BAL fluids from healthy camels. The increased fraction of

245   neutrophils in TW and BAL fluids from camels with respiratory diseases is also in line with

246   findings in respiratory diseased horses (Rossi et al. 2018), donkeys (Shawaf 2019), cattle

247   (Kokotovic et al. 2007), and alpaca (Pacheco 2012).

248   In the present study, the decreased fraction of macrophages in TW and BAL from diseased camels

249   is in line with reports from other species, including human (Rose & Knox 2007b), horses (Rossi

250   et al. 2018), cattle (Kokotovic et al. 2007), and alpaca (Pacheco 2012). The lower frequency of

251   macrophages in diseased animals may be a result of the increased accumulation of neutrophils in

252   the respiratory secretions. Further studies are needed to see, whether this change in macrophages

253   count is also associated with modifications in their functional type.

254   Although the proportion of lymphocytes in the BAL fluids from healthy camels is comparable

255   with their percentage in equine BAL fluids, healthy camel TW contained only a minor population

256   of lymphocytes, which is in contrast to the equine system, where TW lymphocytes are also the

257   second dominant population after macrophages (Richard et al. 2010). In contrast to finding in the

258   horse, where changes in the percentage of BAL and TW lymphocytes were of lower relevance for

259   the diagnosis of equine respiratory disorders (Hoffman et al. 1998; Rossi et al. 2018; Shawaf 2019),

260   we found significantly less lymphocytes in TW of respiratory diseased camels. Whether these

261   differences in the cellular composition of respiratory secretions rely on species-specific defense

262   mechanisms in the respiratory tract, further comparative studies are required. Furthermore, as

263   lymphocytes are a heterogeneous cell population, it still to be investigated, whether selective

264   lymphocyte subsets like helper or cytotoxic T cells, B cells, or NK cells, were especially affected

265   by this decrease.

266   The low frequency of eosinophils (Abutarbush et al. 2019; Hughes et al. 2003) and mast cells

267   (Hughes et al. 2003; Leclere et al. 2006; Malikides et al. 2003) in BAL and TW from healthy

268   camels of the present study is in agreement with results from other species. Although the diagnostic

269   value of mast cells in BAL and TW are not fully studied (Rossi et al. 2018), we found significantly

270   more mast cells in the BAL from diseased camel, which is similar to results reported for diseased

271   horses (Leclere et al. 2006).

272   As normal cells lining the trachea, epithelial cells are present in high numbers in a normal tracheal

273   wash (Zhu et al. 2015) but only in low numbers in BAL samples (Hoffman 2008). The higher

274   frequency of epithelial cells in TW from healthy than respiratory diseased camels is in contrast to

275   reports from previous studies conducted in horses (Wysocka & Klucinski 2015). Interestingly, we

276   found in some TW samples from diseased camels separated epithelial cell cilia, which could have

277   originated from the inflamed airways (McCauley et al. 1998; Simet et al. 2010; Sisson et al. 1994).

278   In human, ciliocytophthoria of nasal epithelial cells has been reported after viral infections

279   (Gelardi & Ciprandi 2019). Investigating the clinical significance of the observed ciliated epithelial

280   cells and its association with viral pathogens, however, requires further studies.

281   Finally, one of the limitations of the present study is the cell identification method. Although it is

282   widely used for cytological analysis in several species (Jackson et al. 2013), staining with

283   Diffquick may fail to reliably identify all cell types (Leclere et al. 2006). Specifically, the estimated

284   frequency of mast cells in the BAL samples may have been affected by Diffquick staining.

285 However, mast cells represent only a minor fraction within BAL cells, which argues against a

286 significant impact of the staining method on the results of cytological analysis in the present study.

287 The identification of mast cells in other species relies on histochemical stains for their heparin,

288 glycosaminoglycans, or esterase. In addition, different antibodies have been used to identify mast

289 cells in human (Ribatti 2018). Therefore, the identification of cross-reactive antibodies to camel

290 mast cell markers would enable their confirmed immunophenotypic identification in camels. In

291 addition, the identification of functional cell subtypes, including pro-inflammatory (M1) and anti-

292 inflammatory (M2) macrophages, would help in better understanding and interpretation of

293 cytological findings. For this, a combination of cell labeling with monoclonal antibodies to

294 selected cell surface markers with flow cytometric analysis may be a good alternative to Diffquick

295 staining.

296 **Conclusion**

297 The present study provides the first report on the comparative analysis of TW and BAL cytology

298 in dromedary camels. Dromedary camels show higher abundance of neutrophils in their TW and

299 BAL than many other veterinary species. The cytological patterns of TW and BAL from camels

300 with respiratory diseases are characterized by increased proportion of neutrophils and decreased

301 proportion of macrophages in comparison to healthy camels. The proportion of lymphocytes was

302 decreased only in TW samples from diseased camels. Collectively, BAL and TW represent

303 valuable techniques for detailed investigation of disease-associated cytological changes in the

304 respiratory tract of camels.

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480

Figure 1

Placement the endoscope to preform the endoscopic examination and TW sample collection from dromedary camel

Figure 2

Bronchoalveolar lavage collection using BAL catheter from dromedary camel

Figure 3

The identification of different cell types in TW and BAL samples.

Cytological slides were prepared from TW and BAL samples, stained with the Diff Quick stain, and examined microscopically: (A) A TW slide showing different cell types, including neutrophils (N) and epithelia cell (Ep) with a magnification of 1000×; (B) A TW slide from affected camel showing increased quantity of degenerated neutrophils with a magnification of 200×; (C) A TW slide from a camel with respiratory disease showing degenerated neutrophils. This field also shows an eosinophil cell with granules within the cytoplasm with a magnification of 1000×; (D) A TW slide from a camel with respiratory disease showing oral epithelial cells (O Ep), bacteria (B), red blood cells (RBC), and separated cilia (C). Stain Diff Quick stain; magnification, 1000×; (E) A BAL slide prepared from a healthy camel showing macrophages (M) and Lymphocytes (L); (F) A BAL slide from a camel with respiratory disease showing increased numbers of neutrophils. In addition, macrophages (M), alveolar macrophage (AM), lymphocytes (L), and Epithelia cell (Ep) can be identified in the field; magnification, 1000×.

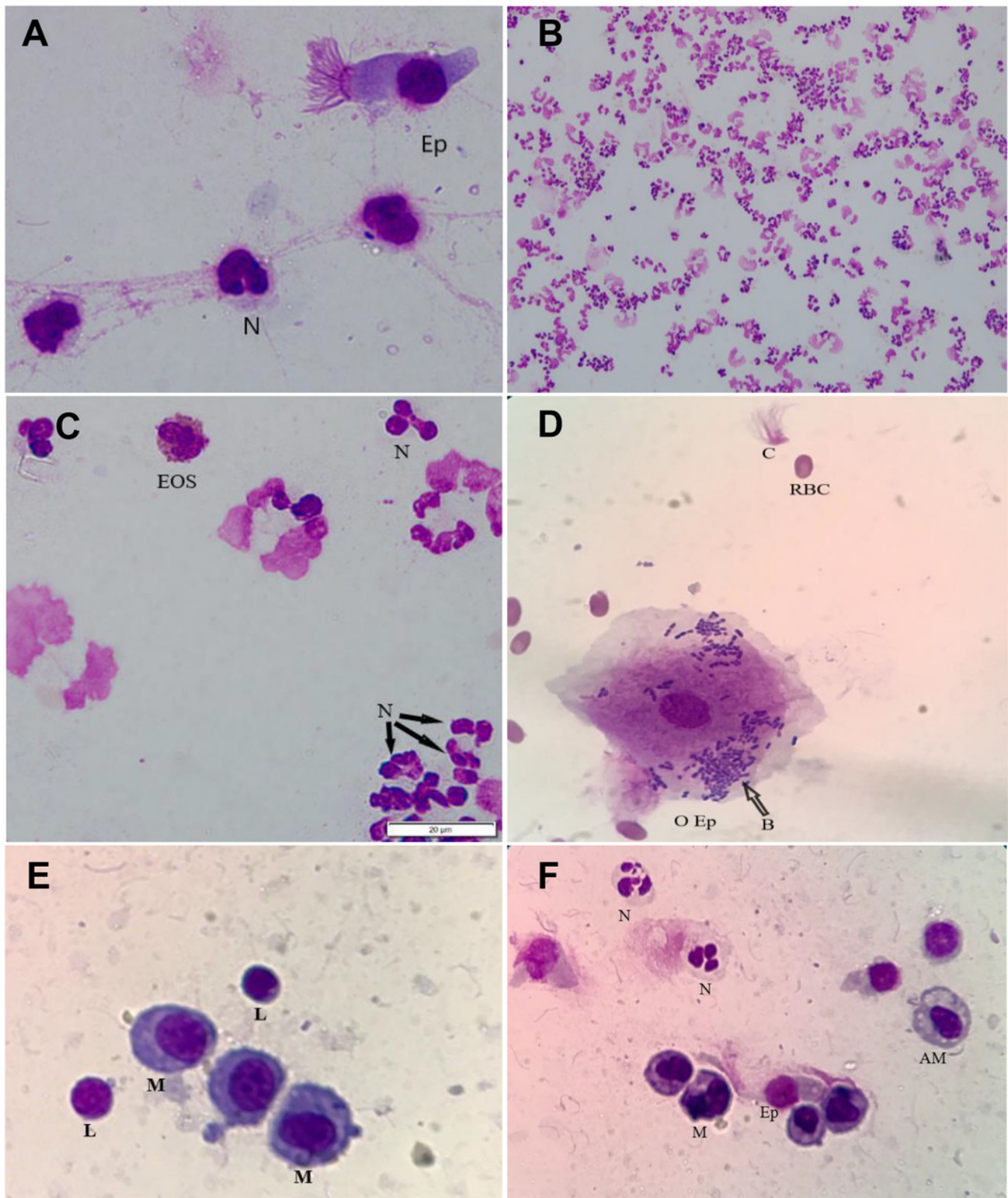
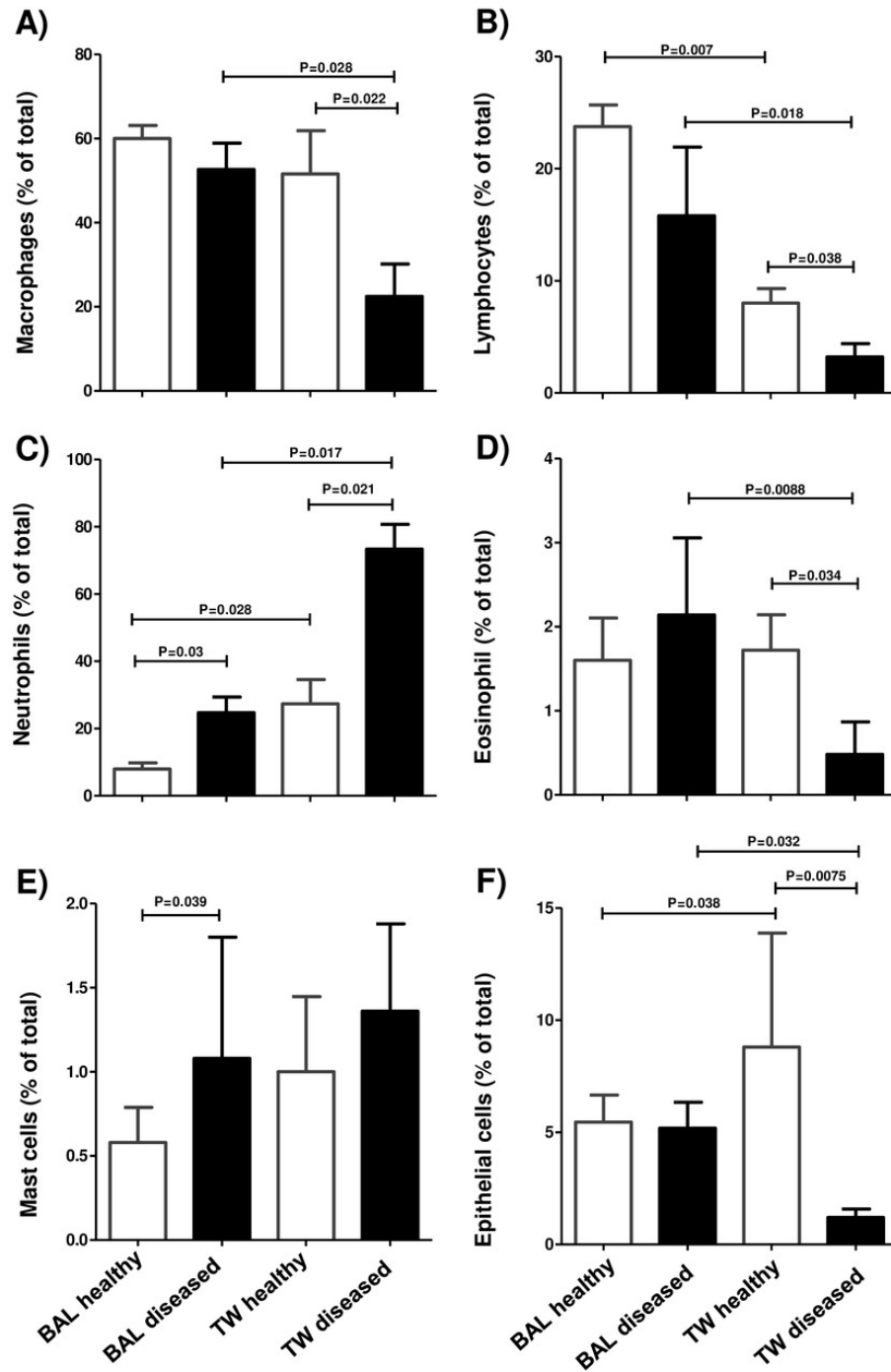



Figure 4

The differential composition of TW and BAL samples

The differential composition of TW and BAL samples. Cytological slides were prepared from TW and BAL samples, stained with the Diff Quick stain, and examined microscopically. The percentage of macrophage cells (A), lymphocytes (B), neutrophils (C), eosinophils (D), mast cells (E), and epithelial cells (F) were estimated in the total cellular content of BAL and TW samples collected from healthy camels and camels with respiratory diseases. Date is presented as mean ± standard error of the mean (SEM). There were considered to be differences in mean values when there was a P-value of less than 0.05.

Detailed scoring system points for respiratory-affected camels

1   **Table 1:** Detailed scoring system points for respiratory-affected camels

Parameter	Clinical findings
Cough	
0	None
1	Rare
2	Daily repeated
3	Spontaneous during clinical examination
Dyspnoea	
0	No difficulty in respiration
1	Mild visible increase of the abdominal movement at the end of expiratory phase.
2	A clear abdominal movement during expiration.
3	A difficult abdominal breathing with wide nostrils.
4	High degree of respiratory distress
Lung auscultation	
0	Physiological (vesicular inspiration)
1	Mild degree of sharpness of inspiratory sounds.
2	Exaggerated inspiratory sounds and quite expiratory sounds.
3	Roughness in both expiratory and tracheal rattle sounds.
4	"Wheezes, Crackles" and or rattle sounds over the lung area.
Rectal temperature	
0	<36.5
1	36.7-39
2	39.1-40
3	>40.1

Total and differential cell counts (Mean ± SEM and range) in TW and BAL fluids from 27 camels (9 healthy and 18 affected)

1   Table. **2**: Total and differential cell counts (Mean ± SEM and range) in TW and BAL fluids from 27 camels

2   (9 healthy and 18 affected).

	Tracheal Wash (TW)				Bronchoalveolar Lavage Fluid (BAL)			
	Healthy		Affected		Healthy		Affected	
	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
Total cell count	-	-	-	-	200.4 ± 39.22	60-440	824 ± 401.1**	260-2400
Macrophages %	51.60 ± 10.25	22-72	22.46 ± 7.69	6-45	60.01 ± 3.11	46-75	52.66 ± 4.24	37-66
Lymphocytes %	8.20 ± 1.3	4-11	3.2 ± 1.2	1-7	23.74 ± 1.93	14-35	15.80 ± 6.11	11-33
Neutrophils %	27.34 ± 7.21	12-54	73.30 ± 7.42	51-88	7.95 ± 1.80	2-19	24.68 ± 4.67	16-39
Mast cells %	1 ± 0.44	0-2	1.36 ± 0.13	0-3	0.58 ± 0.21	0-2	1.08 ± 0.72	0-3
Eosonophils%	1.72 ± 0.42	1-3	0.48 ± 0.38	0-2	1.6 ± 0.5	0-4	2.14 ± 0.92	0-6
Epithelia cells %	8.8 ± 5.08	2-29	1.2 ± 0.37	0-2	5.45 ± 1.2	1-11	5.18 ± 1.16	1-8

**$p < 0.01$

Total and differential cell counts (Mean ± SEM and range) in TW and BAL fluids from 27 camels regarding rectal temperature (6 with normal (<39) rectal temperature and 22 with high (>39.1) rectal temperature) healthy and 18 affected).

1 Table. **3**: Total and differential cell counts (Mean ± SEM and range) in TW and BAL fluids from 27 camels

2 regarding rectal temperature (6 with normal (<39) rectal temperature and 22 with high (>39.1) rectal

3 temperature) healthy and 18 affected).

	Tracheal Wash (TW)				Bronchoalveolar Lavage Fluid (BAL)			
	Normal rectal temperature (<39)		High rectal temperature (>39,1)		Normal rectal temperature (<39)		High rectal temperature (>39,1)	
	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
Total cell count	-	-	-	-	587 ± 115.7	60-2250	1653± 235.6*	815-2400
Macrophages %	36.24 ± 4.55	6-72	17.43 ± 3.99*	6-31	56.71 ± 2.06	37-75	48.58 ± 4.34	37-65
Lymphocytes %	5.1 ± 0.82	1-11	4.17 ± 0.94	1-7	19.93 ± 2.02	1-35	11.67 ± 5.19*	1-35
Neutrophils %	51.22 ± 5.9	12-88	78.83 ± 3.89*	62-88	14.76 ± 1.99	2-36	31.42 ± 4.33*	17-49
Mast cells %	1.37 ± 0.21	0-3	1.1 ± 0.48	0-3	1.17 ± 0.24	0-3	1.33 ± 0.61	0-3
Eosonophils%	1.1 ± 0.22	0-3	0.47 ± 0.32*	0-2	1.62 ± 0.32	0-5	2.34 ± 1.05*	0-6
Epithelia cells %	4.48 ± 1.44	0-29	1.33 ± 0.33*	0-2	5.48 ± 0.61	1-11	4.67 ± 1.28	1-8

*P<0.05