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EVALUATION OF TRANSTRACHEAL WASH (TTW) AND TRACHEAL WASH (TW) IN DROMEDARY CAMELS WITH RESPIRATORY DISORDERS

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ABSTRACT

This study aims to analyse Transtracheal Wash (TTW) and Tracheal Wash (TW) samples cytologically from healthy camels and those affected by respiratory disorders. Endoscopy was used to examine the lower respiratory tract and to take TW samples, while TTW was done through using a special needle and catheter. Cytological analysis of TTW and TW fluid were analysed for fifteen camels, six healthy camels and nine camels affected by respiratory disorders. Oral cells were found in the TW sample due to contamination while inserting the endoscope. The TTW procedure is easier, quicker and without the use of an endoscope, compared to the TW procedure. The concentration of neutrophils in the TTW and TW samples of affected camels were higher, compared to the concentration in samples from healthy camels. A lower concentration of macrophages was present in the TTW and TW samples from affected animals, compared to the samples from healthy camels. The cytological analysis of the TTW and TW samples indicated that there was no significant difference between healthy and affected camels.

Key words: Camel, cytology, endoscope, respiratory, tracheal wash, transtracheal

Dromedary camel is predisposed to various respiratory pathogens, such as viral, bacterial and fungal pathogens (Al-Ruwaili *et al,* 2012; Gebru *et al,* 2018; Li *et al,* 2017; Scaglione *et al,* 2017). Respiratory disease in dromedary has received little consideration in the past, although it is an emerging problem, causing significant losses in production and high mortality rates (Alnaeem *et al,* 2020; Bekele, 1999; Fassi-Fehri, 1987). The involvements of camels in racing, during the past three decades, has caused an increase in the prevalence of respiratory problems, which results from contact between animals, transportation of animals and stress during racing events (Elgioushy *et al,* 2020). Tracheal wash (TW) samples are valuable mirrors of the tracheal lumen (Doyle *et al,* 2017; Malikides *et al,* 2003). Transtracheal wash (TTW) is a minimally invasive procedure used to sample the larger airways by enabling exploration of the lower respiratory tract (Doyle *et al,* 2017; Pravettoni *et al,* 2020). Cytological analysis of tracheal aspiration samples has been extensively used in veterinary medicine since the 1970s (Beech, 1975). Cytological and microbiological analysis of TTW and TW samples provide veterinarians essential information about the respiratory tract and associated pathology (Angen *et al,* 2009; Cooper and Brodersen, 2010; Fulton and Confer, 2012). TTW and TW samples are useful in providing an understanding of the stage and severity of the inflammatory reaction in the respiratory tract and detecting subclinical respiratory diseases (Beech, 1975; Caldow, 2001). TTW can deliver samples for a more comprehensive diagnostic approach, compared to that of nasopharyngeal swabs (Cooper and Brodersen, 2010; Doyle *et al,* 2017). Generally, there is limited practical information with regards to the tools which can be utilised for TTW procedure (Pravettoni *et al,* 2020). For the TTW procedure in large animals, trocars or angiocatheters can be used through which a small urinary catheter can be introduced, while for the TW procedure an endoscope can be used (Angen *et al,* 2009; Fulton and Confer, 2012; Shawaf, 2019). There is a lack of research conducted on the cytological analysis of TTW and TW in healthy and respiratory-diseased dromedaries. This study aims to analyse the TTW and TW cytology, comparatively in healthy and respiratory-diseased dromedaries.

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Materials and Methods

2.1 Animals

This study examined a total of 15 camels out of which 6 males and 9 females were aged 2-18 years (median age ± SEM; 9 ± 5.5). Six camels were healthy and 9 were affected by respiratory disease. The healthy camels were selected from a herd stationed at the Camel Research Centre, King Faisal University, Al-Al-Ahsa, Saudi Arabia. A physical and clinical examination was conducted on each camel in the healthy group to ensure that they were free from any apparent disorders. TW was collected from the healthy group and after four weeks, TTW was collected from the same group of animals. Camels with respiratory disorders were randomly selected from camels brought to Veterinary Teaching Hospital, College of Veterinary Medicine, King Faisal University. The main criteria used to distinguish between healthy and diseased camels was clinical history and clinical examination of the respiratory tract. The animals manifesting symptoms such as coughing, dyspnoea and abnormal respiratory sounds, were considered into the respiratory diseased group. TW was collected from 4 affected camels and TTW was collected from the other five affected camels. This study was conducted from August 2019 to September 2020.

2.2 Bronchoscopy and tracheal wash (TW) sampling

Tracheal wash samples were collected through bronchoscopy from all healthy camels and four affected animals while being restrained in the sternal recumbency position. All animals received mild sedation through administering Xylazine 2% (Rompun; Bayer Health Care) @ 0.1 mg/kg body weight. Due to the narrowness of the nasal passage of the camel, the bronchoscope and TW samples were taken via the oral cavity using a mouth gauge specifically developed for camels (Fig 1A). A flexible endoscope (EVIS Olympus, OLYMPUS AUSTRIA Ges.m.b.H., Vienna) with a 12 mm diameter, 300 cm length and supported with an insufflation system, alongwith a light source and irrigation system was used for the bronchoscopy procedure during the TW collection. The endoscope was passed via an opened oral cavity, along the pharynx, through the rima glottidis into the tracheal lumen, up until the bifurcation was reached (Fig 1D). A catheter (EQUIVET; 2.3 mm x 350 cm) was advanced to the trachea through the biopsy channel of the endoscope, 10 mL of sterile saline, at room temperature, was injected through the catheter. The fluid was immediately aspirated back into the syringe from tracheal lumen (Fig 1E). TW samples were submitted to the laboratory for analysis and processed within 15 minutes of collection.

2.3 Transtracheal wash (TTW) sampling

TTW samples were collected from all camels in the healthy group and from 5 camels in the affected group. An EQUIVET IV catheter 14G x 10 cm (BBraun, Milan, Italy) and a 4FG 1.3 mm OD x 50 cm dog urinary catheter (SMI AG, Steinberg, BELGIUM) was used to collect samples from the tracheal lumen. The camels were sedated through intravenous injection of Xylazine @ 0.1 mg/kg. The camels were positioned in sternal recumbency and head was lifted while the neck was extended by an assistant (Fig 1B). A skin surface area of 10 x 10 cm was prepared aseptically on the ventral surface of trachea between the middle and distal third part of the neck. The prepared surface area was locally blocked by subcutaneous infiltration with 5 mL 2% Lidocaine. The trachea was held between the fingers of the operator while a hypodermic needle (14 G x 10 cm) was inserted into the trachea between cartilaginous rings (Fig 1B & C). Two different techniques were used during the insertion of the cannula. The first technique, which was performed on 3 healthy and 2 diseased camels, the cannula was inserted into the tracheal lumen and directed towards the thoracic inlet and was stopped at a point in the middle of the lumen (Fig 1B). During the second technique, which was performed on 3 healthy and 2 diseased animals, the same procedure was followed, but the cannula was kept directed towards the larynx (Fig 1C). During both techniques, the cannula was completely inserted up to the point where the needle grip made contact with the skin surface. A 50 cm catheter was inserted through the cannula into the tracheal lumen. A 50 mL sterile syringe was connected to the catheter and 30 mL of sterile saline solution, at room temperature was injected into the tracheal lumen and immediately aspirated out using the same syringe (Fig 1B & C). Atleast 10 mL of washing fluid was aspirated out. During the procedure, the camel's head was gradually returned to a horizontal position. At the completion of the procedure, the catheter and the cannula were removed. The time spent to perform the complete procedure ranged between 10 and 20 minutes. There were no reported cases of post-procedure complications. The samples were transferred into a sterile single-use tube and immediately transported to the laboratory.

2.4 Cytological analysis of TW and TTW samples

Slides from the TW and TTW samples were prepared for differential cell counts, through centrifugation of 10 minutes at 300 g of undiluted sample. Smears were made from the sample pellet after removal of the supernatant. The air-dried smears were stained with the Diff-Quick stain. The slides were examined under a microscope for mucus cells, bacteria, red blood cells and white blood cells. The differential cell count was performed under oil immersion (X1000) in order to accentuate the specific morphologic characteristics of each cell. The differential counts for 400 cells of macrophages (MAC), lymphocytes (LYM), neutrophils (NEU), mast cells (MAST), eosinophils (EOS) and epithelial cells (EPITH) were counted from each TW and TTW slide. The analysis results for each cell type was expressed as a percentage of total cells.

2.5 Statistical analysis

The obtained data were analysed by using Student's t-test in order to determine the significant difference. It was done through using Graph Pad Prism 7 software in order to determine the range, mean and standard error of the mean. In addition, values normal distribution was evaluated by D'Agostino & Pearson omnibus normality test.

Results

There was no adverse effect on the camels during or after the TTW and TW procedures. Two different techniques were used for the TTW procedure based on the direction of the catheter during the insertion phases (Fig 1B & C). When the catheter was inserted towards the direction of the thoracic inlet (Fig 1B), no red blood cells were detected in the samples but these were seen in the method during

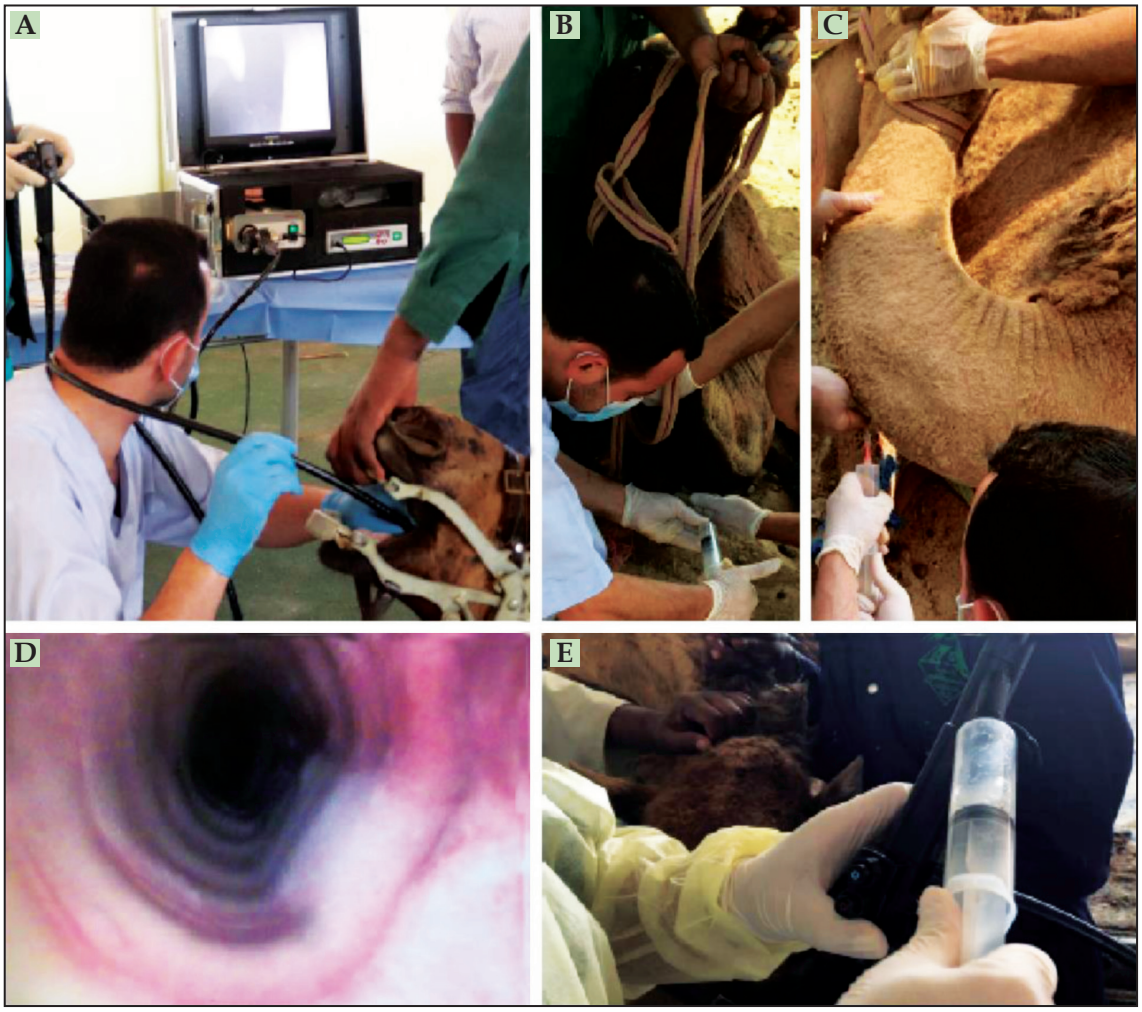

Fig 1. **A:** Bronchoscopic procedure of the lower respiratory tract via the oral cavity using a mouth gauge. **B:** Transtracheal wash (TTW) procedure using a 12G needle and a dog urinary catheter directed towards the lungs. **C:** Transtracheal wash (TTW) procedure in the larynx direction. **D:** Endoscopic image from a diseased camel showing moderate mucopurulent exudate in the thoracic trachea. **E:** Aspiration of a tracheal wash sample (TW) using a bronchoscope.

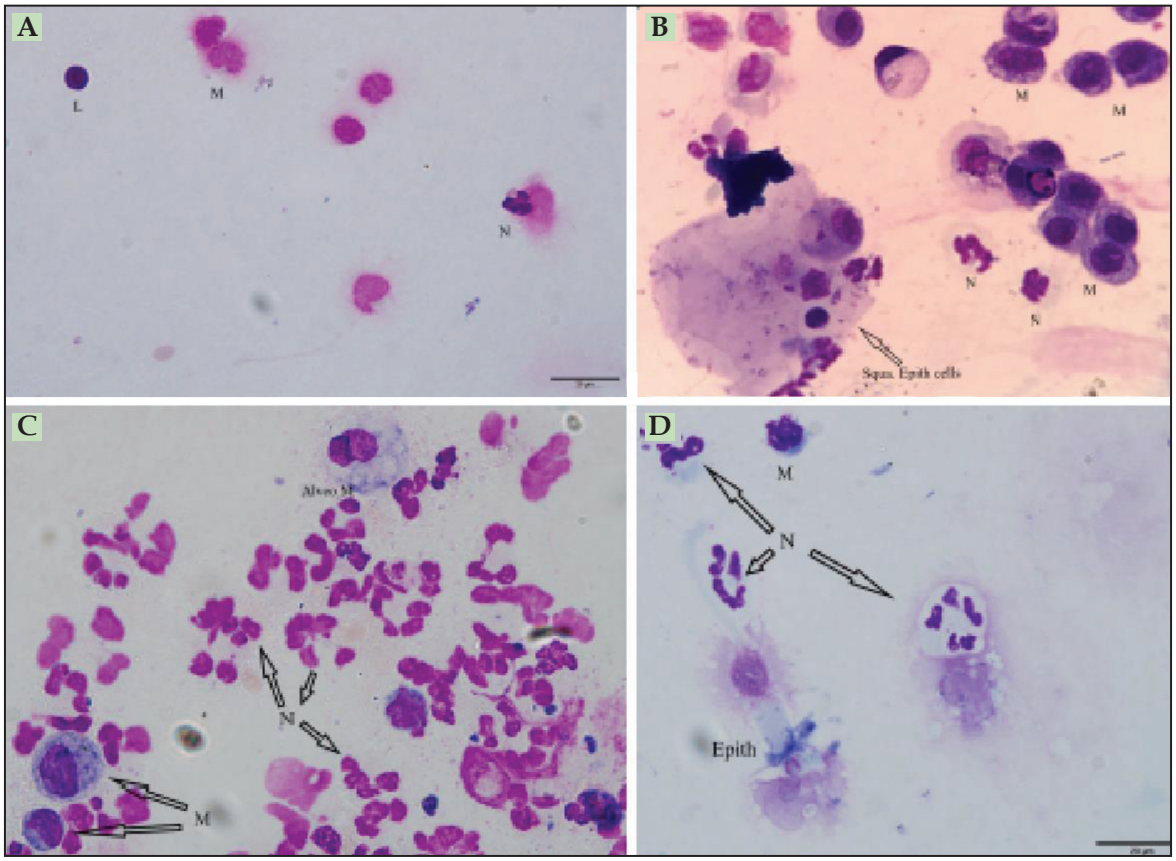

Fig 2. Cytological slides of tracheal and transtracheal wash from camels. **A:** Cell composition of a TTW sample from a healthy camel where lymphocyte (L); macrophage (M); neutrophil (N). **B:** Squamous epithelial cell (Squa. Epith. Cells), several macrophages and neutrophils in a TW sample, contaminated with oral/pharyngeal material from a healthy camel. **C:** Degenerated neutrophils, alveolar macrophage (Alveo. M) and macrophages (M) and pollen particle in a TTW sample from a camel with respiratory disorders. **D:** Neutrophils (N), macrophage (M) and ciliated epithelial cell (Epith) in a TW from a camel with respiratory disorders.

which when the catheter was inserted in the direction of the larynx in 3 samples (one healthy and two affected camels) (Fig 1C). The presence of mucous within the TTW samples were higher when catheter was directed towards the thoracic inlet as compared to the TTW samples collected when directed towards the larynx. Samples obtained from the TW procedure had a higher count of oral epithelial cells and bacteria, compared to the TTW samples (Fig 2D). The cytological analysis results from the TWW and TW samples, for both healthy and affected camels, are summarised in Table 1 and Fig 1, 2, 3 and 4. There was a significantly higher concentration of neutrophils in the TWW (63.6±3.7% P<0.0092) and TW samples (74.4±7.86% P<0.008) of affected camels, compared to the TTW and TW samples from healthy camels (31±4.79%, 26±6.92%).

The concentration of macrophages was lower in the TWW (20.2±1.74% P<0.0097) and TW (22.5±7.98% P<0.032) samples from affected camels compared to healthy camels (51.25±4.87%, 55.4±7.25%) (Fig 3A & B). The lymphocyte cells in the TW samples from affected camels were lower (2.8±0.91% P<0.0038), compared to the samples from healthy camels. There was no significant difference in the mast, eosinophils and epithelial cells in the TTW or TW samples from healthy and diseased camels. There was no significant difference in the cell population for the TTW samples compared to the TW samples from either healthy or affected animals (Fig 3C & D), except for lymphocytes. The concentration of lymphocyte cells was higher in TTW samples as compared to TW samples from affected animals (7.6±1.43%, 2.8±0.91%).

Discussion

The results in the study were compared to the studies of other livestock species because limited information was available about TTW and TW in camels. The TW procedure in camels was found more complicated compared to the the same procedure in

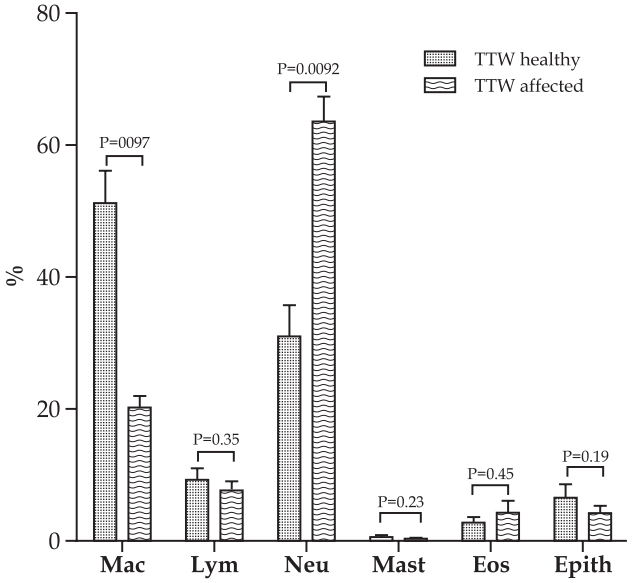

Fig 3a. The differential cell counts of transtracheal wash (TTW) in healthy and affected camels.

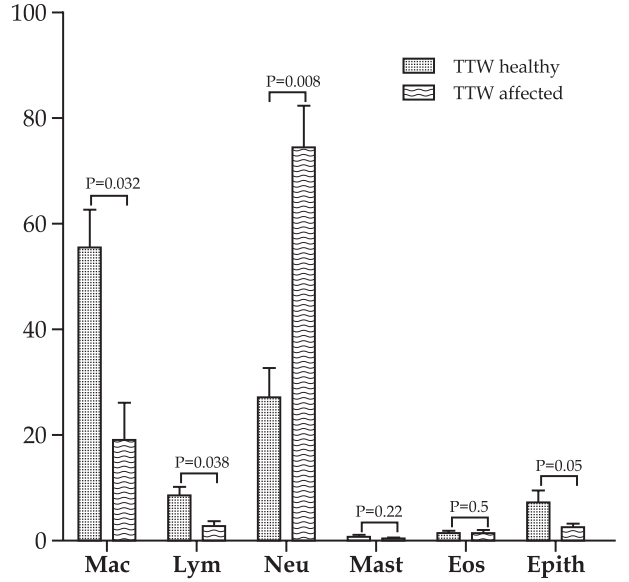

Fig 3b. The differential cell counts of tracheal wash (TW) in healthy and affected camels.

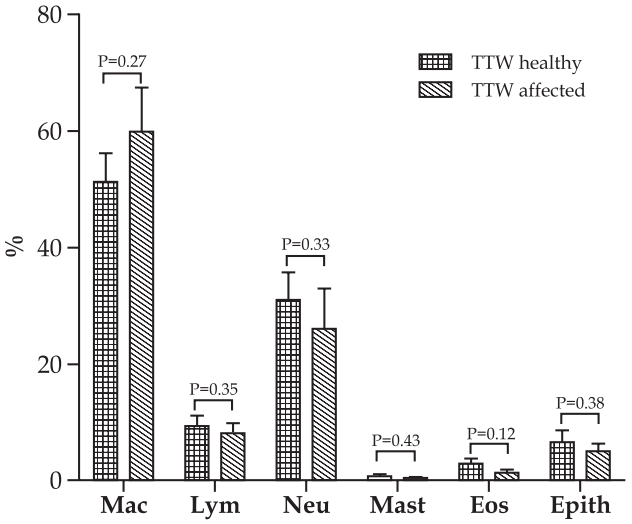

Fig 3c. The differential cells of transtracheal wash (TTW) and tracheal wash (TW) in affected camels.

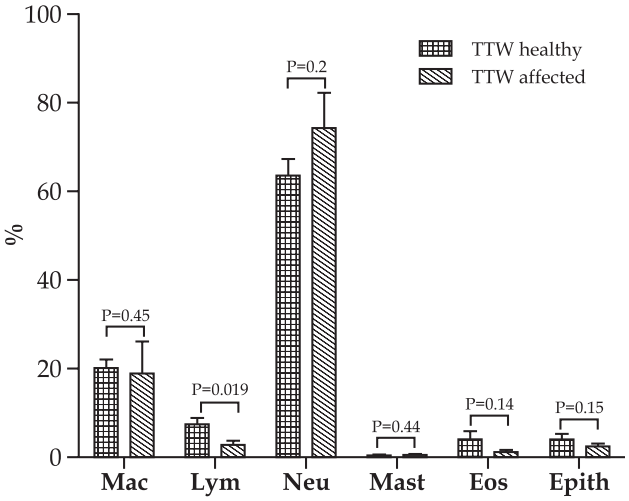

Fig 3d. The differential cells of transtracheal wash (TTW) and tracheal wash (TW) in healthy camels.

Table 1. Differential cell counts (Mean ± SEM and range) of transtracheal wash (TTW) and tracheal wash (TW) samples from healthy and affected camels.

Blood cells (Per cent)	Transtracheal wash (TTW)				Tracheal wash (TW)			
	Healthy		Affected		Healthy		Affected	
	Mean±SEM	Range	Mean±SEM	Range	Mean±SEM	Range	Mean±SEM	Range
Macrophages	51.25±4.87	38-61	20.2±1.74	14-24	55.4±7.25	38-72	22.5±7.98	6-39
Lymphocytes	9.25±1.8	6-14	7.6±1.43	3-11	8±1.83	4-12	2.8±0.91	1-5
Neutrophils	31±4.79	23-42	63.6±3.7	52-72	26±6.92	12-45	74.4±7.86	52-92
Mast cells	0.5±0.29	0-1	0.2±0.2	0-1	0.5±0.29	0-1	0.2±0.2	0-1
Eosinophils	2.75±0.85	1-5	p4.2±1.88	0-10	1.25±0.48	0-2	1.2±0.58	0-3
Epithelia cells	6.5±2.1	1-11	4.2±1.16	1-8	5.25±1.13	3-8	2.4±0.81	0-5

other livestock species, due to difficulties in insert endoscope through the oral cavity possibly due to the anatomical and physiological differences in camels compared to other livestock species (Burger *et al,* 2019; Faye, 2016). Microscopic examination of the TW samples revealed that the samples were contaminated during the TW procedure with squamous epithelial cells and with oral/pharyngeal material from the oral cavity (Fig 2B), which complicates the bacterial analysis (Smith, 2019). On the other hand, the process of aspiring the TTW samples was easier, less complicated, require fewer instruments and was completed in less time compared to the TW procedure. TTW sample collected while the catheter was directed towards the larynx contained red blood cells due to haemorrhage during sampling. No blood was observed in the TTW samples when the catheter was directed towards the lungs. This study agreed with Doyle's statement that each method of sample collection from the lower respiratory tract had its own advantages and disadvantages (Doyle *et al,* 2017). However, Doyle *et al* (2017) reported that the TTW procedure was more effective than the TW procedure to detect pathogenesis in bovines. Prevalence of most abundant macrophage cells in the TTW and TW samples from healthy camel were in agreement with previously published data (Chemuturi *et al,* 2005; Couetil and Thompson, 2020; Rossi *et al,* 2018; Shawaf, 2019; Vaught *et al,* 2018). The decreased cell count of macrophages in the TTW and TW samples from affected camels in the study was in agreement with the results of previous studies (Doyle *et al,* 2017; Rossi *et al,* 2018; Shawaf, 2019; Vaught *et al,* 2018). The lower cell count of macrophages in samples from affected camels was associated with the elevation of other cells, such as neutrophils (Smith, 2019). The lymphocyte cell population of the TTW and TW samples from healthy and affected camel were lower than that reported for other livestock species (Angen *et al,* 2009; Chemuturi *et al,* 2005; Rossi *et al,* 2018; Shawaf, 2019). Contrary to previously published data on other livestock species (Brazzell *et al,* 2006; Rossi *et al,* 2018; Shawaf, 2019; Vaught *et al,* 2018), we found higher levels of neutrophils in the TTW and TW samples from healthy camels. The increased values of neutrophils in TTW and TW samples in healthy camels can be explained by the higher levels of neutrophils in the blood of camels as compared to other species (Hussen *et al,* 2017). The increase of neutrophils in both the TTW and TW samples from affected camels compared to the samples from healthy camels in present study was in agreements with previous studies (Brazzell *et al,* 2006; Couetil and Thompson, 2020; Rossi *et al,* 2018; Shawaf, 2019; Smith, 2019; Vaught *et al,* 2018). The lower count of mast cells and eosinophils in the TTW and TW samples from healthy and affected camel was in agreement with other researchers (Rossi *et al,* 2018; Shawaf, 2019), who stated that these cell populations played a more critical role in bronchoalveolar lavage (BAL) than in TW. However, Shawaf (2019) reported a higher eosinophils cell count in TW samples from healthy and affected donkeys, when compared to the results of present study. The TTW and TW epithelial cell count in this present study was lower for affected camel compared to the same cell count for healthy camels, which was in contradiction to previous studies (Riihimaki *et al,* 2008; Wysocka and Klucinski, 2015).

In conclusion, TTW and TW were helpful techniques in diagnosing lower respiratory tract diseases in camels. No significant difference was found between TTW and TW in camels. The TTW procedure was more practical and did not require an endoscope in comparison to the TW procedure.

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