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Research Article

INDIGENOUS COST-EFFECTIVE METHOD FOR TRACHEAL LAVAGE IN CANINES

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ABSTRACT: Pulmonary airway sampling is always considered the chief diagnostic test for respiratory problems in dogs. The trans tracheal wash (TTW) technique is commonly used for collecting airway aspirates for cytological analysis and bacteriological examination. Tracheal lavage being the least invasive technique, requires local anesthesia or mild to moderate sedation and has been found beneficial in dogs with pulmonary diseases including pneumonia, chronic bronchitis, eosinophilic broncho-pneumopathy, neoplasia, etc. In the present study, TTW was performed on ten clinically healthy adult dogs weighing more than 10 kg, using a 14 gauge IV cannula and disposable dog catheter (4FG, OD 1.30 MM) replacing the costly designed catheter. The study was performed with mild sedation using diazepam (0.5mg/kg) and ketamine (5mg/kg) combination intravenously in six dogs and only local anesthesia in another four dogs. Transtracheal wash fluid revealed low cellularity with alveolar macrophages as the predominant cell type, followed by respiratory epithelial cells, lymphocytes, eosinophils, and other cells (mast cells, plasma cells, goblet cells, basophils). This article summarizes an indigenous cost-effective TTW procedure for obtaining a representative cytological sample from the pulmonary airways.

Key words: Airways sampling, Dog, Cytology, Tracheal lavage, Trans-tracheal wash (TTW).

INTRODUCTION

Respiratory problems are quite prevalent in dogs, especially those residing in shelters, pet shops, breeding and boarding kennels, research facilities, or veterinary clinics. Very young and older dogs have a higher risk of respiratory infections as compared to healthy adult dogs (Kuehn 2018). Respiratory diseases predominate in the cold season and the common clinical manifestations include coughing, nasal discharge, dyspnoea, fever, and exercise intolerance (Ayodhya *et al.* 2013). The diagnostic protocol followed usually includes prudent history, thorough physical examination, thoracic radiography, and complete blood count (CBC). But for effective therapy and outcome, additional diagnostic tests are always needed for obtaining a definitive diagnosis.

In companion animal respiratory medicine, pulmonary airway sampling is always considered the chief diagnostic test because of the various complications of the lung biopsy procedure (Corcoran 2010). Diagnostic procedures used for the collection of respiratory tract samples for cytological analyses include trans-tracheal wash (TTW), endotracheal lavage (ETL), bronchial brushings, bronchoalveolar lavage (BAL), and transthoracic fine needle pulmonary aspirates (McMillan and Taylor 2008). Radiographic manifestations of pulmonary or tracheobronchial disease causing chronic cough or respiratory distress, pinpoints to perform TTW and BAL. The cardiogenic origin of respiratory signs must be first eliminated through a complete cardiological examination. TTW and BAL are quite beneficial procedures for confirming inflammation, hypersensitivity reactions, infection (bacterial, viral, fungal, parasitic, or protozoal), and neoplasia (Barcante *et al.* 2008, Dunn 2010, Rozanski 2014).

Transtracheal wash (TTW) is the mainly preferred least invasive technique, because of being relatively inexpensive compared with bronchoscopy and requires only local anesthesia or mild to moderate sedation (Graham *et al.* 2021). It is especially beneficial in animals presented with vague clinical signs with pulmonary abnormalities seen on thoracic radiographs (McGuire 1991, McCullough and Brinson 1999).

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TTW normally yields representative samples of the trachea and primary or secondary bronchi, while some amount of materials can also be collected from the lower bronchioles and alveoli (Bexfield and Lee 2014, Graham *et al.* 2021). The prerequisite is that the cough reflex should remain intact whether the animal is sedated or not, which helps in the expectoration of samples from lower airways (Finke 2013). The main contraindication for this technique is coagulation defects or compromised respiratory function (Constable *et al.*2017). Therefore, the risk-benefit ratio in compromised patients should always be considered before performing this technique (Rozanski 2014).

This article summarizes an indigenous cost-effective TTW procedure for obtaining a representative cytological sample from the lower airways.

MATERIALS AND METHODS

The present study was undertaken at the Small Animal Clinics of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Ten (10) clinically healthy adult dogs (Non-descript-6, Labrador Retriever-4) without any evidence of respiratory disease on clinical examination were included in this group. The animals had no history of clinical illness for 6 months to 1 year. Physiological and hemato-biochemical parameters were recorded in all the dogs and were found within the normal range. Thoracic radiography (Lateral and ventrodorsal) was undertaken in all the dogs. The equipment used for performing TTW includes clippers, chlorhexidine gluconate 2% solution, a local anesthetic (lignocaine 2%), tranquilizing drugs (Diazepam (5mg/ml) and Ketamine (50mg/ml)), 2 ml syringe and 21 G hypodermic needle, surgical gloves, three to four 10 ml syringes, 100ml bottle of 0.9% sterile non-bacteriostatic saline, disposable dog catheter 4FG (OD 1.30 MM) (SURU International Pvt. Ltd.), 14G IV cannula, sterile plain, and EDTA collection tubes, dressing gauze and bandage and microscopic slides (Fig. 1.).

Ethical approval

The IAEC approved the protocol with proposal number GADVASU/2018/IAEC/47/03.

Statistical analysis

Means and standard errors for each evaluated variable of the healthy group were calculated using descriptive statistical procedures. Statistical analyses were performed using the SAS software.

The technique

Trans-tracheal wash (TTW) was performed by the standard method outlined by Taylor (2015) in all the dogs which were more than 10 kg body weight. In dogs weighing less than 10kg, an endotracheal wash is preferred due to the smaller diameter of the trachea, which prevents the placement of a large needle used in the TTW procedure. In the present study, trans-tracheal wash (TTW) was performed using a 14 gauge IV cannula and disposable dog catheter (4FG, OD 1.30 MM). Strict asepsis was maintained throughout the procedure. The patient was placed in a sitting position with the neck extended and the nose elevated toward the ceiling. Overextension of the neck was avoided as it leads to more resistance by the animal. Tranquilization using diazepam (0.5mg/kg) and Ketamine (5mg/kg) combination intravenously was done for restraining aggressive dogs (n=6). No premedication was given as most premedicants have the antisecretory effect (anticholinergics) and also depress the cough reflex (opioids) which will ultimately hinder the airway sampling. Diazepam can be used as a premedicant agent in respiratory patients wherever calming or anxiolytic activity is required.

The ventral neck region including the larynx and the cervical trachea was shaved and aseptically prepared. Out of four 10 ml- syringes, two were loaded with approximately 10 ml and the other two with 5-6 ml of sterile, 0.9 percent saline solution. The cricothyroid ligament was palpated as a triangular depression distal to the thyroid cartilage. About 1 ml of local anesthetic drug (2% lidocaine) was infused into the skin and the subcutaneous (SC) tissue. Beforehand the length of the dog tube (still in a sterile package) was measured from the cricothyroid ligament to the 5th intercostal space (ICS) (*i.e.* level of carina). The cannula was inserted through the skin and cricothyroid ligament into the tracheal lumen with the bevel facing ventrally after stabilizing the larynx with the non-dominant hand. For proper stabilization, the clinician must firmly grab at least 180 degrees of the girth of the airway between the thumb and the fingers.

A slight "pop" was felt as the cannula passed the ligament. Retching was seen in dogs while using the cricothyroid approach. Once the cannula is in the tracheal lumen, it was angled down approximately 45^{o} and the needle was removed from the cannula. The dog tube was inserted into the tracheal lumen through the cannula. Minimal resistance was noticed when the catheter was advanced properly. In some dogs, when resistance was encountered due to the lodging of the cannula against the distant tracheal wall, then the cannula was slightly retracted and the catheter was reinserted without any resistance. Sometimes cannula goes into the surrounding tissue then the continuous resistance was felt. In that case,

the needle cannula was removed and redirected. A coughing reflex was observed whenever the catheter was in the tracheal lumen. The catheter was passed down into the tracheal lumen up to the level of carina (approximately at the 5th or 6th ICS).

The preloaded syringe was attached to the catheter and the sterile saline approximately @ 0.8-1 ml/kg was infused into the tracheal lumen. In some cases, an assistant performed coupage during the instillation of the fluid to promote coughing. The infused saline was aspirated back into the syringe. Excess air if present, was expelled by detaching the syringe from the dog tube. Mostly 15-20 percent or less of the infused volume was recovered. The procedure was repeated mostly with the 3 preloaded syringes to recover a sufficient amount of aspirate. After an adequate sample was obtained, the syringe was removed along with the catheter from the trachea in a very smooth motion, and a soft, padded bandage laced with betadine was placed over the catheter site for approximately1-2 hours to minimize the formation of subcutaneous emphysema (Fig. 2.).

Specimen Handling

After a total of 2 to 5 ml of turbid fluid with flecks of mucus occasionally was obtained in the trans-tracheal wash (TTW), then the fluid was transferred from the syringe into EDTA vials and the sterile tube collector. It was properly labeled and then carried to the laboratory as soon as possible on ice packs. Separate samples were collected for culture and cytological examination. Samples were first measured for volume, although they were not filtered.

Cytology of trans-tracheal wash (TTW)

Two ml aliquots of TTW fluid obtained from the dog were taken in EDTA vials for cytological analysis. The samples were centrifuged (1000 rpm, 5 minutes) and smears were prepared from the sediment and stained with Leishman's stain. Two hundred cells from each sample were counted for differential cell counts (Graham *et al.* 2021). Rapid staining was done using Gram's stain and the smears were observed for the presence of bacteria. However, Lactophenol Cotton Blue Staining was also done on the same samples for the presence of fungal spores.

Bacteriological culture of TTW

At least 1 ml aliquot of TTW was transferred into sterile containers for bacteriology and was kept on ice packs till processed. All the samples were processed within 1-2 hours of collection. In a few cases, samples were centrifuged at 3000 rpm for 5 min and the supernatant was discarded but in others, the sample was directly used for culture. The sediment was cultured on 5 percent defibrinated sheep blood agar and Brain heart infusion agar and was incubated overnight at 37ºC in aerobic conditions for fungal isolation, the sediment was cultured on Sabouraud's dextrose agar (SDA) and was incubated for one week at 37ºC. A positive culture was absent in the healthy dogs except for some contaminations resulting in few colonies.

RESULTS AND DISCUSSION

Tracheal wash was performed without invading the oral cavity by passing a through-the-needle catheter through the cricothyroid ligament into the trachea (*i.e.,* trans-tracheal wash). The cricothyroid ligament approach was preferred over the between two tracheal rings approach, as there were comparatively rarer chances of tracheal perforation, and was easier to locate ligament in the case of obese dogs. Frequently, this procedure can be done with a local anesthetic. But in six dogs, mild to moderate sedation was also found beneficial. As propofol causes induction apnea further leading to hypoxemia (Gross *et al.* 2002), ketamine-diazepam has been used as a safer alternative in the present study. Ketamine has broncho-dilating properties and decreases airway resistance (Riviere and Papich 2009). This is due to direct stimulation of central adrenergic centers (*i.e.,* increased sympathetic tone) and also due to the inhibition of the reuptake of norepinephrine (Annetta *et al.* 2005); both these effects cause an increase in norepinephrine concentration at the synapse which dilates the bronchioles and decreases airway resistance. No side effects were observed in the present study as animals were only mildly sedated.

In this study, the procedure was modified using a 14 gauge IV cannula and disposable dog catheter (4FG, OD 1.30 MM) in place of the commercially sold designed catheter (Transtracheal wash kit, Dogs MILA International, Inc. USA). The commercially available catheter is quite costly and needs to be imported and is not easily available in India. The disposable dog catheter is very economical and easily available.

The use of a dog tube (made up of polypropylene (PP) was advantageous, due to sufficient length (500mm), making its passage down the trachea easier. McMillan and Taylor (2008) also used a sterile 28-inch (length), 3.5 French polypropylene catheter and a 14-gauge over-the-needle catheter (Medicut catheter; Sherwood Medical Industries, St. Louis, Missouri, USA) for collection of trans-tracheal aspiration in larger dogs weighing more than

Table 1. Comparison of Tracheal wash cells in the healthy group with the reference range.

Parameters	Healthy group	Reference range [Dunn 2010]	Reference range (English *et al.* 2008)
Macrophages (%)	49.57±5.28	70-80	71
Neutrophils (%)	11.43±1.00	5-8	5-10
Lymphocytes (%)	7.71±2.25	5-14	11-17
Eosinophils (%)	0.14±0.14	∠5	≤5
Respiratory epithelial cells (%)	29.14±7.13	0-15	11-18
Others (%)	2±0.79	∠2	2
Mean cell number (cells/HPF)	8.35±2.68	Low cellular count	Low cellular count

15 kg in their study. In another study, Tseng and Waddell (2000) used a 12-inch length, 18-gauge Intracath (Deseret Medical, Inc, Sandy, UT) catheter and Bexfieldand Lee (2014) through-the needle 19G, 12 or 24 inches long jugular catheters for collection of airway samples from larger dogs.

TTW fluid contains very fragile cells so the smears should be made as soon as possible. Due to the cellular release of enzymes, low protein medium and delayed cell preparation changes can develop in the cell quality of any fluid sample (TTW, bronchial brushing, BAL) (Latimer 1993). Non-perseverance of cell morphology increases the risk of cytological misinterpretation as macrophages and neutrophils may start phagocytizing extracellular bacteria, erythrocytes, and cell fragments (English *et al.* 2008).

Normal trans-tracheal wash (TTW) includes cells that are easily washed away from the proximal mucosal surface and comprise respiratory epithelial cells (ciliated and nonciliated columnar to cuboidal epithelial cells), alveolar macrophages, neutrophils, lymphocytes, eosinophils, goblet cells, and mucus (Burkhard *et al.* 2001, English *et al.* 2008). Cytologic interpretation should include estimated cellularity, differential cell counts, and morphologic description of the cells encountered (Creevy 2009). Presence of *Simonsiella* spp. bacteria (Fig. 3.) or superficial squamous epithelial cells in TTW samples indicates oropharyngeal contamination (Graham *et al.* 2021).

In the present study, a better yield of tracheal washings was obtained, *i.e.*, up to 15-20% of the infused volume. Normally the portion of infused saline remaining in the

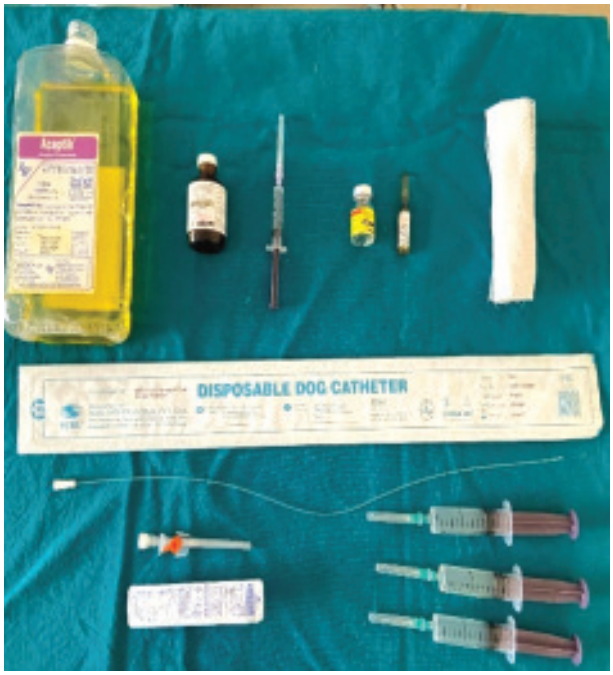

Fig. 1. Equipments used transtracheal wash in dogs.'

Fig. 2. Collection of transtracheal wash (aspirate) in dogs.

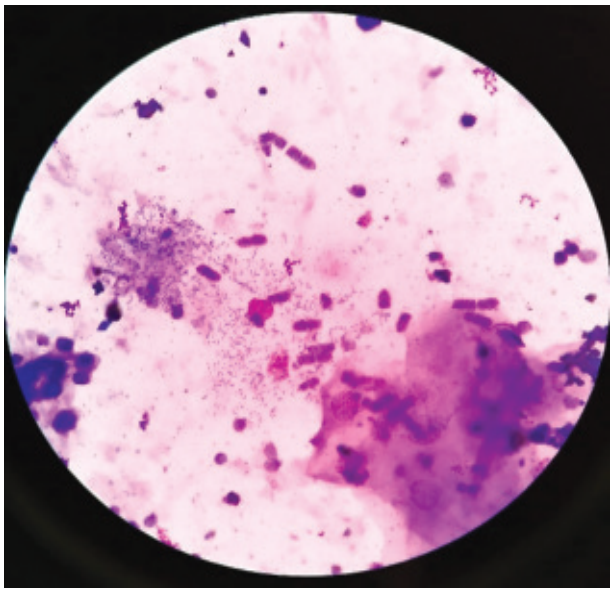

Fig. 3. Presence of *Simonsiella* spp. bacteria in TTW samples indicative of the oropharyngeal contamination.

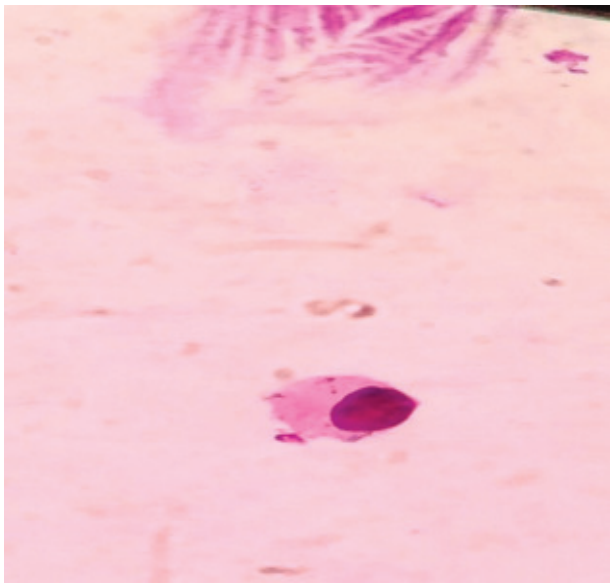

Fig. 4. Normal macrophage and slight mucus in TTW fluid of a healthy dog -100x (Leishman staining).

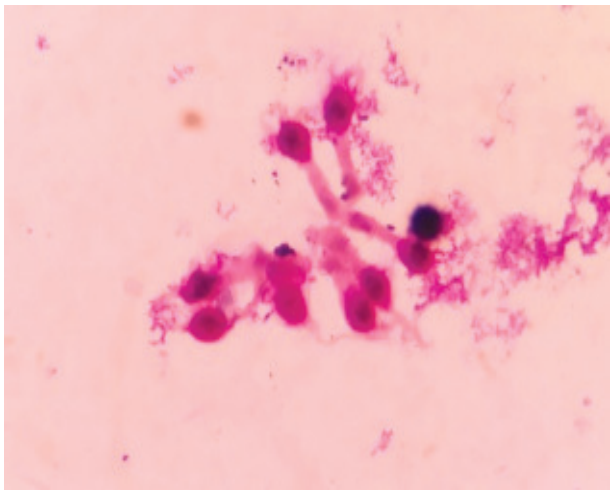

Fig. 5. Ciliated columnar epithelial cells from transtracheal wash in a healthy dog -100x (Leishman staining).

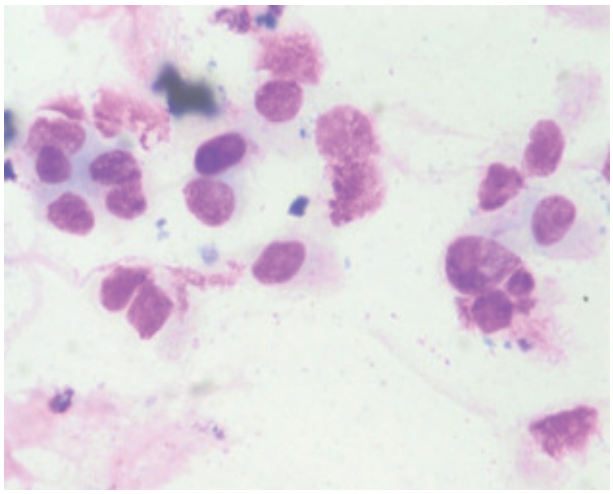

Fig. 6. Sloughed cuboidal epithelial cells in transtracheal wash of a healthy dog-100x. (Leishman staining.

airways gets absorbed there. But the better yield of washings indicates an adequate sample for cytological examination and bacterial cultures and also it prevents any concern regarding alveolar flooding.

The mean cell number (cells per HPF) in the tracheal wash smears of the healthy dogs was 8.35±2.68 cells (Table 1.). Burkhard *et al.* (2001) and Thomas (2004) reported that the tracheal wash from healthy dogs usually had low cellularity. Tracheal wash smears in our study comprised 49.57±5.28 percent pulmonary alveolar macrophages (Fig. 4.) and 11.43±1.00 percent neutrophils (Table 1.). Dunn (2010) and Rozanski (2014) in their study also recorded alveolar macrophages as the predominant cell in the tracheal wash of clinically normal animals whereas neutrophils constituted only 5-8 percent of the total nucleated cell count. Overall 29.14±7.13 percent respiratory epithelial cells were observed in the tracheal wash fluid in our study (Table 1.). Trachea and bronchi are lined by the ciliated columnar epithelial cells (Fig. 5.) and bronchioles by ciliated and non-ciliated cuboidal epithelial cells (Fig. 6.) (Thomas 2004). Respiratory epithelial cells are mainly present in the normal tracheal wash fluid (Nelson and Couto 2019) and can be present as single cells or clusters in the tracheal wash (Dunn 2010, English *et al.* 2008). Nearly 7.71±2.25 percent lymphocytes, 0.14±0.14 percent eosinophils, and 2±0.79 percent other cells (mast cells, plasma cells, goblet cells, basophils, etc.) were observed in the tracheal wash in

the present study. Thomas (2004) and Dunn (2010) proposed that the tracheal aspirates should have 5–14 percent lymphocytes, <5 percent eosinophils, and rarely mast cells (<2%).

The differential diagnoses can be narrowed based on the type of inflammatory cells accessible in trans-tracheal wash fluid (Ettinger *et al.* 2017, Nelson and Couto 2019). Inflammation of the tracheobronchial tract and lungs can be characterized as acute neutrophilic, chronic, chronic active (mixed), eosinophilic, neoplastic, or hemorrhagic inflammation (Raskin and Meyer 2015). Whatever technique is used for airway sampling, this categorization helps the clinician in establishing a diagnosis.

Probable complications after TTW are rare but consist of larynx or airway spasm, tracheal laceration, subcutaneous emphysema, pneumomediastinum, pneumothorax, hemorrhage, infection at the needle site, catheter breakage and aspiration of the catheter into the airway and worsening of respiratory status due to stress of the procedure (Dunn 2010, Bexfield and Lee 2014). But in our study, no such complications were recorded.

Overall, in respiratory patients, advanced airway cytological sampling techniques have established a more precise diagnosis and superior therapeutic options. Clinical presentation of the patient along with minimum history helps in deciding the appropriate advanced diagnostic test necessary for obtaining accurate and complete diagnosis. So, trans-tracheal wash being the least invasive technique, requiring local anesthesia or mild to moderate sedation was found beneficial in animals with vague presenting clinical signs.

CONCLUSION

Transtracheal wash (TTW) is the mainly preferred technique for obtaining pulmonary airway samples for cytological and microbial examination in other countries. The presently practiced costly technique can be modified by replacing costly commercially available catheters with readily available and economical disposable dog catheters. The authors hope that now veterinary clinicians can utilize this cost-effective procedure as a routine practice for dogs with pulmonary or respiratory signs. TTW is a valuable test for improving the quality of life of respiratory patients in which radiographs are inconclusive and also establishes the aetiologies for cough, wheezes, hemoptysis, hypoxia, respiratory distress, and other respiratory tract complications (infection, inflammation, and neoplasia).

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