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Novel Work on Collection of Tracheo-bronchial Aspirates in Pneumonic Cattle

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ABSTRACT

Routine diagnosis of pulmonary disorders in bovines by physical examination, hematology and radiography is not sufficient to establish confirmatory diagnosis. Tracheo-bronchial aspirates (TBA) can be used as a reliable sample for the diagnosis of lower respiratory tract affections in cattle. Percutaneous tracheo-bronchial aspirate sampling was performed in 173 standing conscious cattle using two types of catheters. Fifty four cows were sampled with Large Animal transtracheal wash Kit (MILA International, Inc. USA) and 119 with infant feeding tube. Cytologic analysis of TBA smears was performed to assess the diagnostic utility of samples. Diagnostic samples were obtained in 87.8 per cent cows (152/173). Sampling using commercial catheter and infant feeding tube retrieved diagnostic samples in 90.7 per cent and 86.6 per cent of cows, whereas repeated infusions were required in 61.2 per cent and 78.7 per cent of cases, respectively. None of the cows exhibited complications post sampling. Infant feeding tube (7 Fr) with 10 G steel introduction catheter was effectively used in the collection of TBA and was comparable with commercial catheter. Present communication reports first systematic study on collection of TBA by percutaneous method in adult pneumonic cattle.

Key words: Cattle, Pneumonia, Tracheal wash catheter, Tracheo-bronchial aspirate.

Bovine respiratory affections cause enormous loss to dairy cattle industries. In India, routine diagnosis of pulmonary disorders in bovines is based on physical examination, hematology and radiography. So far in animals, nasopharyngeal swabs have been utilized for cytologic and cultural studies. However, nasopharyngeal swabs are not true representative samples from lungs and thus, are of limited value for diagnostics. With these modalities, the etiological diagnosis remains unestablished and leads to treatment failures. It could be better achieved if lower respiratory samples are available for laboratory examination. Tracheo-bronchial aspirates can prove to be promising diagnostic sample from lungs for microbiological culture as percutaneous sampling avoids nasopharyngeal contamination of secretions during collection. TBA collects secretions, cells and debris that accumulate in the distal trachea and bronchi, which may also be derived from more distal airways and alveoli. Several researchers have used tracheo-bronchial aspirates (TBA) as preferred sample for identifying ongoing pathology in lower respiratory system in horses (Robinson, 2003, Cian *et al*., 2015). The mucociliary apparatus moves secretions from all parts of the lung to the pharynx *via* trachea, therefore cytologic examination of tracheal aspirates are more likely to reveal inflammation in different lung regions (Derksen, 2001).

TBA collection has been established in equines with encouraging results diagnosing the conditions like Inflammatory Airway Disease (Sweeney *et al*., 1992). However, there are few reports on the use of percutaneous TBA in bovine calves giving an edge to disease diagnosis (Aslan *et al*., 2002, Brazell *et al*., 2006, Angen *et al*., 2009, Doyle *et al*., 2017). Abutarbush *et al*. (2019) demonstrated endoscopy guided tracheal wash sampling in healthy adult cattle for establishing normal cytological and microbiological values. Nonetheless, literature documenting percutaneous TBA sampling in adult cattle was hard to find. TBA is more reliable sample for investigation of lower respiratory diseases that can be subjected to clinico-pathological, microbiological, serological and molecular tests to achieve etiological diagnosis. The technique is simple and less invasive which is performed without any costly equipment. Therefore, it is rational to assess the diagnostic value of TBA in bovine respiratory diseases for better diagnosis. Keeping in view the above facts, present study was planned to standardize collection of TBA in cattle using commercial catheter and infant feeding tube.

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Tracheo-bronchial aspirate

Tracheo-bronchial aspirates were collected in 173 adult crossbred cattle, aged more than 2 years presented to Large Animal Clinics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab for respiratory signs *viz*. coughing, dyspnea, fever *etc*. These animals were

diagnosed for pneumonia based on physical examination, chest radiography and/or hematology. In 54 cows, samples were collected using commercial catheter (Large Animal Transtracheal Kit, MILA International, Inc. USA) with 10 gauge × 2.25 inches steel introduction catheter and 12 gauge × 70 cm (28 inches) flexible plastic flushing catheter. Infant feeding tube was used in place of plastic flushing catheter with 10G steel introduction catheters in 119 animals.

TBA was collected percutaneously in standing conscious animals (Fig 1). For this, about 10 cm^2 area at ventral midline of neck was aseptically prepared. Two to five ml of 2% lidocaine was administered into the prepared site. The steel introduction catheter was inserted through a small incision into the tracheal lumen between two tracheal rings (Fig 2). Then, the flushing catheter or infant feeding tube was threaded through the steel introduction catheter. Normal saline was infused and was aspirated back with immediate suction using 50 ml syringe. If recovery was unsuccessful, an additional amount of saline equal to the first volume was infused to obtain sample. After the procedure, gauze was placed over the incision with self-adhesive elastic bandage for at least 30 minutes to 1 hour. The owners were advised to monitor the incision site for any evidence of inflammation or infection over the next few days. No untoward reaction was observed in any of the cow.

Cytological analysis of tracheo-bronchial aspirate

To evaluate the diagnostic efficiency of TBA, cytological analysis was performed. Two ml aliquot of TBA obtained from the animal was transferred from the syringe into EDTA vials for cytologic analysis. All the samples were processed immediately. The TBA samples were centrifuged (1000 rpm, 5 minutes) and smears were prepared from the sediment by discarding the supernatant and stained with Leishman's stain for cytologic analysis.

Chi-square test was applied to compare the proportion of successful samplings by commercial catheter and infant feeding tube. Also proportion of animals with single and multiple infusions with two catheters were compared.

Out of total 173 cows, TBA as diagnostic samples was recovered in 152 cattle (87.8 per cent). Sample could not be obtained in 9 animals despite repeated infusions for maximum of four attempts. Sample was non diagnostic in rest of 12 animals.

Diagnostic samples were recovered by commercial catheter in 90.7 per cent (29/54) of the animals. In three cows, no sample was recovered and sample was not diagnostic cytologically in two cows. A total of 119 cows sampled by infant feeding tube in place of commercial catheter, diagnostic samples were recovered in 86.6 per cent cows. Sample could not be recovered in 11 cows and sample was not of diagnostic value in five cows. Proportion of successful samplings was higher with commercial catheter though it was non-significant ($p>0.05$) (Table 1).

Repeated infusions were required in 61.2 per cent (30/49) and 75.7 per cent (78/103) of the cases by commercial catheter and infant feeding tube, respectively. Sample could be recovered with single infusion (60 ml aliquot) in significantly higher ($p<0.05$) proportion of animals with commercial catheter (38.8 per cent Vs 24.3 per cent) than infant feeding tube (Table 1).

Mean volume of normal saline infused with commercial catheter was 111.25±6.84 ml and recovery was 31.2±2.37 ml. Mean volume of saline infused using infant feeding tube was 120.6±4.46 ml and sample volume recovered was 29.5±1.47 ml. The recovery rate of the aspirate by commercial catheter was comparable with infant feeding tube based on the mean volume infused and recovered (Table 1).

A TBA sample of 5 ml or more with slight to good turbidity was found to be a sample from lower trachea with sufficient cell population. Samples with abundance of macrophages accompanied by columnar ciliated epithelial and fewer other cells such as lymphocytes, neutrophils and devoid of RBCs were considered diagnostic.

Fig 1: Collection of tracheo-bronchial aspirate in a standing conscious cow.

Fig 2: Tracheo-bronchial aspirate site at the ventral aspect of neck with placed steel introduction catheter (solid arrow).

Table 1: Commercial catheter vis-à-vis infant feeding tube for TBA collection in pneumonic cattle.

		Commercial catheter	Infant feeding tube	Pearson chi square value
Total number of animals sampled		54	119	-
Number of successful samplings		49	103	-
Proportion of successful samplings		90.7 %	86.6 %	0.435
Mean volume infused (ml)		111.2±6.84	120.6±4.46	-
Mean volume retrieved (ml)		31.2±2.37	29.5±1.47	-
Number of animals successfully sampled by	Single infusion	19	25	0.047
	Two infusions	20	45	0.922
	Three infusions	9	30	0.213
	Four infusions	1	3	0.786

This is the first systematic study reporting percutaneous tracheo-bronchial wash in adult pneumonic cattle using commercial catheter. The commercial catheter appeared advantageous over infant feeding tube as more proportion (40.8 per cent) of samples was recovered with single infusion by commercial catheter. This may be attributed to more length of the catheter and the rigidity provided by the stylet to tracheo-bronchial aspirate catheter. Few limitations of infant feeding tube were observed in long necked cattle, where the site of insertion had to be selected according to the length of the infant feeding tube. This factor may have attributed to failure of sample recovery with infant feeding tube in some cases. Dislodging of infant feeding tube while coughing could have been another factor that failed to recover the aspirate.

Angel *et al*. (2009) performed percutaneous tracheal wash in sedated calves using male dog urinary catheter and reported recovery of 5-7 ml fluid following administration of 20 and 40 ml sterile isotonic saline. No such studies were available for adult cattle. Abutarbush *et al*. (2019) reported the infusion of 20 ml isotonic saline for endoscopy guided tracheal wash sampling in healthy adult cattle. The present study recommends safe infusion of 180 ml sterile isotonic saline for percutaneous TBA sampling in adult cattle. Also 240 ml saline was infused in 2.63 per cent of the animals with no complications post sampling.

Samples were considered diagnostic based on cytologic analysis. Whitwell and Greet (1984) also reported pulmonary alveolar macrophages as the most abundant cells present on tracheal aspirates examination from normal horses which corroborates with our study. Histologically, bronchiolar ciliated epithelial cells have slightly shorter cilia compared with tracheal epithelium. These cells originate from the terminal area of the respiratory tract and therefore indicate a distal trachea or tracheo-bronchial sampling (Beech, 1991).

CONCLUSION

Tracheo-bronchial wash technique can advance the diagnosis of pulmonary diseases in bovines to a great extent. In older calves and adults, this procedure can be way helpful for the diagnosis of lung affections, their prognosis and even treatment success. Despite the limitations, infant feeding tube was as good as commercial catheter based on proportion of successful samplings and its accessibility. Infant feeding tube is cost effective and can be successfully used in clinical cases. Regardless of the ease of TBA technique, many practitioners are hesitant to perform these procedures. It is hoped that the practitioners will become acquainted with this underutilized skill in cattle.

Conflict of interest: None.

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