



## Cytological and Bacteriological Evaluation of Tracheal Aspirates for the Diagnosis of Lung Affections in Horses

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### ABSTRACT

Transtracheal wash technique is commonly employed for collecting aspirates and determining the bacterial agents involved in lower respiratory tract infections by culture examination. In addition, the cytologic analysis of transtracheal wash is also useful for diagnosis and differentiation of lower respiratory diseases of inflammatory and non-inflammatory origin. In present study four horses (two foals and two adult horses) with frank respiratory signs, inflammatory leukogram and radiologic findings suggestive of respiratory involvement were subjected to transtracheal wash collection. Cytology of the stained smears and culture examination revealed *Rhodococcus equi* in two horses whereas in other two horses, *Staphylococcus spp.* was isolated. Isolates of *Rhodococcus equi* were sensitive to erythromycin, amoxicillin, streptomycin, neomycin, norfloxacin and sulfadiazine, whereas *Staphylococcus* isolates were found sensitive to amoxicillin, ampicillin, doxycycline, gentamicin, neomycin, erythromycin, oxytetracycline, penicillin and streptomycin and resistant to ciprofloxacin and norfloxacin. Two horses (treated with amikacin and penicillin) and one foal (treated with erythromycin and rifampicin) responded to recommended doses of antibiotic therapy and recovered smoothly.

**Keywords:** Transtracheal wash, Pneumonia, *Rhodococcus equi*, Cytology

Disorders of respiratory system, particularly the lower airways, are the most frequently diagnosed conditions in sport horses evaluated for poor performance. These conditions have been identified as one of the main causes of training disruption and interruption of racing competitions in Thoroughbred horses (Wilsher *et al.*, 2006; Gerber *et al.*, 2014). The disorders of the respiratory system are responsible for 42% of decreasing performance of athlete horses, second to diseases of the musculoskeletal system (Allen *et al.*, 2006; Hewson and Arroyo, 2015).

Transtracheal wash (TTW) is a reliable technique for the diagnosis of lower respiratory tract affections in animals.

It is a commonly employed procedure for culture of pathogens of lower respiratory tract. Bacteriological evaluation of a transtracheal aspirate may provide useful information on etiology, antimicrobial sensitivity and aid in the selection of appropriate drugs (Ivester *et al.*, 2014). Similarly, cytology of TTW can be a useful diagnostic tool and help to differentiate inflammation, neoplasia and specific pathogens of lower respiratory tract (Hewson and Arroyo, 2015).

The present article reports the successful transtracheal wash aspiration and its utility in the diagnostic and therapeutic protocol in lower respiratory tract affections

in four horses. In spite of its use worldwide, the technique of TTW for diagnosis of lower respiratory tract affection in animals has not been employed in India and the study appears to be the first of its kind.

## MATERIALS AND METHODS

Four horses presented to Large Animal Clinics, GADVASU with frank respiratory signs and radiologic finding suggestive of respiratory involvement were subjected to thorough clinical examination.

### Samples collection

**Blood:** Blood samples (2 ml) were collected aseptically from jugular vein in EDTA coated vials. Immediately after collection whole blood was used for determination of haemoglobin (Hb), total erythrocytes count (TEC) and total leukocytes count (TLC) by ADVIA Haematology System. The blood smears were prepared and evaluated for differential leukocytes count (DLC) after staining by Leishman's stain.

**Transtracheal wash/aspirate:** TTW was collected percutaneously by following procedure. The horses were adequately restrained and sedated as required to immobilize the animal in a standing position. For transtracheal wash, about 10 cm<sup>2</sup> area was selected at the ventral aspect of the neck where the trachea could be grasped and the rings could be easily palpated. The skin over the selected site was clipped and surgically prepared and infiltrated with 2-5 ml of 2% lidocaine. A small stab incision was given just through the skin, using the scalpel blade.

Transtracheal wash was collected using Large Animal Trans-Tracheal Wash Kit (MILA International, Inc. USA) containing 10 gauge × 2.25 inches steel introduction catheter (like 10 gauge needle) with 12 gauge × 70 cm (28 inches) flexible plastic flushing catheter.

Following stab incision on the skin the steel introduction catheter was inserted on the ventral midline, while stabilizing the trachea with the other hand, and passed into the tracheal lumen between two tracheal rings. Through this catheter, flexible flushing catheter was passed towards the lungs so as to reach thoracic inlet. For TTW the sterile saline (50 ml in adult horses and 20-30 ml in foals) was injected through the flushing catheter into the trachea. After injecting, it was aspirated immediately, in an

attempt to recover at least one third of the volume of fluid administered. If recovery was unsuccessful, an additional amount of saline equal to the first volume was injected to obtain fluid adequate for all diagnostic procedures. The same volume of fluid can be administered three times to receive adequate fluid for proper diagnosis and without any side effects to the animal. After the sample was obtained, the catheter was withdrawn and the site was dressed with pressure bandage for 24 hours.

The aspirated fluid or the transtracheal washings were transferred into EDTA vials and sterile containers for cytology and bacteriology, respectively. All the samples collected in EDTA vials were processed immediately for cytology, the leftover TTW samples were centrifuged (1000 rpm for 5 minutes). Smears were prepared from the sediments by discarding the supernatants and stained with Leishman's stain.

An aliquot (100 µl) of TW fluid was cultured on Blood Agar and MacConkey Lactose Agar. Plates were incubated in aerobic conditions at 37 °C for 18 h. The isolates were identified by Gram staining, growth on specific media and biochemical tests. Antibiotic sensitivity testing was done by disk diffusion method and zones of inhibition were measured. (Bauer *et al.*, 1966)

## RESULTS AND DISCUSSION

### Case 1

A 3-months old filly was presented with a history of fever (104-106 °F), bilateral purulent nasal discharge, ocular discharge, coughing and inappetence for past 15 days. The animal was treated with broad spectrum antibiotics for one week by referring veterinarian with mild remission of clinical signs. On clinical examination, the filly had normal rectal temperature (101.6 °F), tachycardia (124 beats/minute), tachypnoea (75 breaths/minute) and congested mucous membranes. Auscultation of the lungs revealed wheezes in caudal left lung lobe, and harsh lung sounds in both the lungs. Haemogram revealed neutrophilic leukocytosis with toxic changes in most of the neutrophils, few immature neutrophils and microcytic hypochromic anaemia (Table 1). Thoracic radiography showed fluid density in cranial chest and moderate interstitial pattern in caudal lung lobe. Thick purulent transtracheal wash

**Table 1:** Haematological alterations in affected horses

Parameters		CASE 1	CASE 2	CASE 3	CASE 4
Hb (g/dl)		9.8	9	17.4	12.8
TLC (per $\mu$ l)		27170	35010	12860	23990
TEC (per $\mu$ l)		$8.85 \times 10^6$	$5.32 \times 10^6$	$10.26 \times 10^6$	$8.06 \times 10^6$
PCV (%)		30.5	32	47.4	35.8
MCV (fl)		34.46	60.15	46.19	44.42
MCH (pg)		11.07	16.91	16.96	15.88
MCHC (g/dl)		32.13	28.12	36.71	35.75
<b>Platelets (per <math>\mu</math>l)</b>		<b><math>1.30 \times 10^5</math></b>	<b><math>5.82 \times 10^5</math></b>	<b><math>9.9 \times 10^5</math></b>	<b><math>1.03 \times 10^5</math></b>
DLC	N (%)	86	86	70	96
	E (%)	00	00	00	00
	L (%)	14	14	30	04
	B (%)	00	00	00	00
	M (%)	00	00	00	00

was obtained from the foal. Cytology of the stained smear of transtracheal wash revealed massive number of degenerated neutrophils suggestive of frank suppurative pneumonia (Fig. 2). The diagnosis was confirmed by microbial culture. Bacteriological evaluation of the transtracheal wash sample revealed *Rhodococcus equi* infection. Isolate was subjected to antibiotic sensitivity testing and was found sensitive to erythromycin, amoxicillin, neomycin, streptomycin and norfloxacin; and resistant to ampicillin, doxycycline, oxytetracycline and penicillin. The foal was treated with erythromycin @ 25 mg/kg body weight (bwt) and rifampicin @ 5 mg/kg bwt and responded initially. But, relapsed after 15 days and also developed diarrhoea. Animal was then put on gentamicin and ampicillin, selecting least toxic among the tested antibiotics irrespective of their sensitivity pattern. Animal made good recovery with resolution of pulmonary abscessations on repeated radiography and showed negative on culture examination of transtracheal wash fluid after 8 weeks. It however again developed fever and succumbed to infection following prolonged antibiotic therapy after 5 months.

### Case 2

A six and half month old colt had a history of fever (106-107 °F) from past one month and productive coughing since last 20 days. Moreover, there was no nasal discharge and appetite was normal. On clinical examination, the foal was bright, its rectal temperature was 102.6 °F,

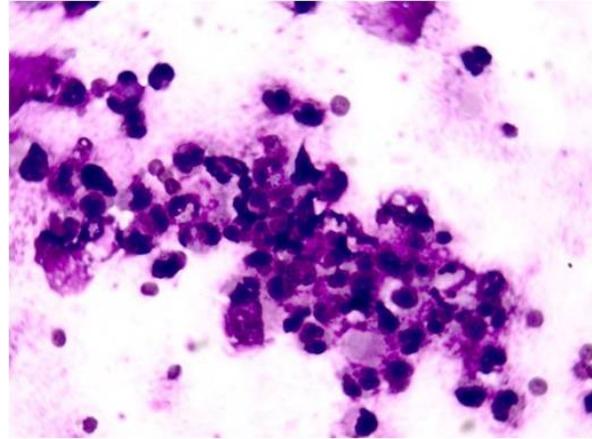
heart rate was 90 beats per minute, and respiratory rate was 40 breaths/minute. Soft moist cough was evident and lung auscultation revealed crackles in left lung; and crackles and wheezes on right side with severely affected cranio-ventral lung. Haemogram revealed macrocytic hypochromic anaemia and neutrophilic leukocytosis with toxic changes (Table 1). On thoracic radiography, there were severe nodular interstitial patterns in lungs with nodules upto 3 cm in diameter indicating lung abscessation. On radiographic impression it was suspected for *R. equi* infection. Thick purulent transtracheal wash was obtained from the foal, and cytological examination and cultural isolation was done. The transtracheal wash sediment smear showed relative neutrophilia besides some intracellular, pleomorphic rods, characteristic of *Rhodococcus equi* in neutrophils and presence of alveolar macrophages along with fibrin strands (Fig. 3a and Fig. 3b). Bacteriological isolation from the transtracheal wash sample yielded *Rhodococcus equi* infection. The isolate on antimicrobial sensitivity test were found sensitive to erythromycin, amoxicillin, neomycin, streptomycin and norfloxacin; and resistant to ampicillin, doxycycline, oxytetracycline and penicillin. The foal was treated with erythromycin @ 25 mg/kg bwt and rifampicin @ 5 mg/kg bwt and responded well to the antibiotic therapy and recovered uneventfully.

### Case 3

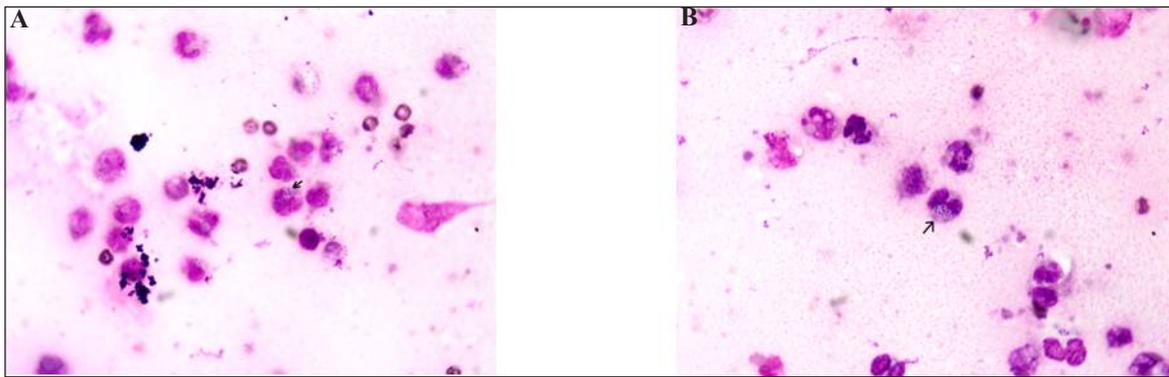
A 15 years old male horse was presented with a history



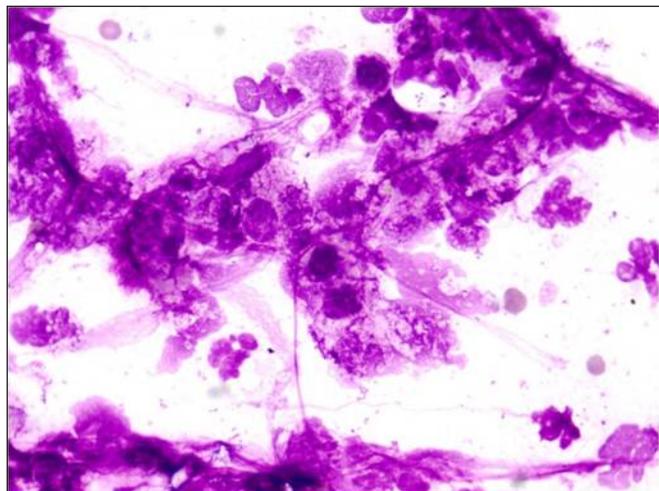
**Fig. 1:** Cotton wool abscesses (nodular interstitial pattern) in lungs in *Rhodococcus pneumonia* in second foal- case 2.



**Fig. 2:** Stained smears from transtracheal aspirate showing massive number of degenerated neutrophils suggesting frank purulent pneumonia



**Fig. 3a & 3b:** Stained smears from transtracheal aspirate showing *Rhodococcus equi* in neutrophils (Black arrow)



**Fig. 4:** Stained smears from transtracheal aspirate showing fibrinous purulent pneumonia

of dyspnoea, fever and coughing for past 4 months. There was a history of long journey following racing and jumping event leading to onset of dyspnoea. Feed and water intake, urination and defecation were normal. Physiological vitals included 100.3°F rectal temperature, 60 bpm heart rate, 62 breaths per min respiration rate and mucus membranes were slightly congested. Auscultation of chest revealed severe harsh lung sounds. Inspiratory and expiratory dyspnoea was evident. Thoracic radiography revealed moderate interstitial pattern in lungs. Grossly the transtracheal washing appeared cloudy with thick strands of mucus in it. Cytology of the transtracheal wash smears showed chronic active inflammation overlapped by marked suppurative inflammation. Bacteriological evaluation of a transtracheal aspirate yielded *Staphylococcus* spp. The isolates were found sensitive to amoxicillin, ampicillin, doxycycline, gentamycin, neomycin, erythromycin, oxytetracycline, penicillin, streptomycin, ciprofloxacin and norfloxacin. The animal was treated with amikacin @ 25 mg/kg bwt. and procaine penicillin @ 20,000 IU/kg bwt and showed marked improvement in about 7-10 days. Antibiotics were continued for two weeks and the animal made successful recovery.

#### Case 4

A 6 years old mare had cough since two weeks. There was normal feed and water intake. Urination and defecation was normal as well. Clinical examination revealed that temperature (100.5 °F), heart rate (48 bpm) and capillary refill time (<2 sec.) within the normal range. However, respiration rate was recorded as 62/min and mucus membranes were congested. On auscultation of lungs, there were mild harsh lung sounds and animal showed dyspnoea. Thoracic radiography depicted moderate interstitial pattern in lungs. Haematology revealed massive neutrophilic leucocytosis and the presence of mostly mature neutrophils (Table 1). Platelets count was on lower side of the normal range. Transtracheal wash sediment had massive number of moderate to marked degenerated neutrophils along with few macrophages, lots of fibrin strands and occasional respiratory epithelial cells indicating frank fibrinous purulent pneumonia with the possibility of abscessation (Fig. 4). The culture isolation yielded growth of *Staphylococcus* spp. The isolate was found susceptible to gentamicin, erythromycin, amoxicillin and neomycin; and resistant to ampicillin, doxycycline,

tetracycline, penicillin and streptomycin, ciprofloxacin and norfloxacin. The animal was treated with amikacin and procaine penicillin and showed marked improvement in about 5-7 days.

The present study confirmed the utility of transtracheal wash technique in horses that gave a comprehensive picture of pathological features of lower respiratory tract. This report described four clinical cases of horses diagnosed with pneumonia.

Two foals out of the four animals were diagnosed with *Rhodococcus equi* (*R. equi*) infection. *R. equi* is a facultative intracellular pathogen of macrophages (Pal and Rahman, 2015). It is a common cause of suppurative pneumonia in foals which is diagnosed routinely at post mortem (Muscatello, 2012). However, its diagnosis in living animal is rarely confirmed. In the present study, the transtracheal wash smears were used to demonstrate intracellular rods suggestive of *Rhodococcus equi* in neutrophils and alveolar macrophages which was confirmed by cultural isolation. The TTW yielded *R. equi* despite both foals being treated with antibiotics by the referring veterinarians. Thus, it can be inferred that *Rhodococcus equi* can be cultured from transtracheal washes in cases refractory to antibiotic treatment or to evaluate recovery with antibiotic treatment.

The two adult horses were diagnosed by pneumonia which was characterized by chronic active inflammation along with marked suppurative inflammation on cytology. The bacterial agent was identified by cultural methods. The common aerobic bacterial agents associated with lower respiratory tract affections in horses are *Streptococcus equi zooepidemicus*, *Escherichia coli*, *Pasteurella* spp. and *Klebsiella* spp. (Mathilde *et al.*, 2011 and Ivester *et al.*, 2014). The pure growth of *Staphylococcus* spp. obtained in these cases could be a contamination or normal resident flora. These two adult horses were the first one on which TTW technique was done and hence break in sterility during collection may be a possibility. Pneumonia or pleuropneumonia can be confirmed following evaluation of tracheal aspirates (Mathilde *et al.*, 2011). The cytological profile of pneumonia determined by transtracheal wash is characterized by an increase in the total nucleated cell count with mild neutrophilia (Malikides *et al.*, 2003 and Holcombe *et al.*, 2006). Thus, transtracheal wash was helpful in the diagnosis and confirmation of pneumonia by

cytology and bacterial isolation. The cytologic evaluation of transtracheal aspirate helps in the differentiation of lower respiratory tract diseases of inflammatory and non-inflammatory origin (Condasa *et al.*, 2015). Cultures from the aspirates may provide useful information about the offending organism and its antimicrobial sensitivity also aids in appropriate treatment (Mathilde *et al.*, 2011).

Cytological and microbiological evaluation of tracheal washes (TW) has proven to be important tool in the diagnosis of respiratory diseases in horses. In animals with lower airway diseases, the nasal flora may not reflect lung infection and cultures are best taken as transtracheal aspirates of the lower respiratory system. Tracheal wash samples collected using the percutaneous transtracheal technique are preferred for bacterial culture because these are not contaminated by oropharyngeal organisms. However, care should be taken while interpreting microbiological cultures taken from the lower respiratory tract as it may be due to the normal microflora. Furthermore, possible complications like subcutaneous emphysema or infection at puncture site may be encountered but are usually infrequent.

## CONCLUSION

Transtracheal wash is a reliable technique for diagnosis and differentiation of lower respiratory diseases in horses. It can facilitate in determining the therapeutic protocol in equine pneumonia. The present technique offers advantage of avoiding nasopharyngeal contamination of secretions collected for microbiological culture and examination. It can help in achieving accurate diagnosis particularly under field conditions.

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