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Transtracheal aspiration technique in diagnosis of lower respiratory tract disease in the cow

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Transtracheal aspiration technique에 의한 소의 하부호흡기계 질병진단

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초록 : 소의 하부호흡기계 질병을 진단하고자 기침과 비루를 주증상으로 하는 31두의 환우와 임상증상을 나타내지 않는 9두의 소에 5 fr. urinary catheter를 이용한 transtracheal aspiration technique를 적용하고 이 방법의 유용성 및 분리된 병원성 세균과 세포상을 임상증상과 관련하여 조사하여 다음과 같은 결과를 얻었다.

Transtracheal aspiration technique은 구강내 정상상재균에 오염되지 않은 가검물을 하부호흡기로부터 채취하는데 호흡장애와 합병증을 유발하지 않았다.

총 40두로부터 분리한 세균중 *Pasteurella multocida*가 48.7%로 가장 많이 분리되었으며 점액 화농성 염증이 40%로 가장 많이 나타났다.

심한 임상증상을 보인 소중 점액화농성 염증이 60%로 가장 많이 나타났으며 *Pasteurella multocida*가 63.2%로 높게 분리되었다. 경미한 임상증상을 보인 소에서는 염증세포에 따른 세포상이 고루 나타났으며 *Pasteurella multocida*가 40% 분리되었다.

세포학적 관찰에서는 정상인 경우는 섬모원주상피세포가 다수 관찰되었고 점액성 염증인 경우는 소수의 호중구 및 상피세포가 관찰되었으며 점액화농성 염증인 경우는 다수의 밀집된 호중구, 상피세포 및 점조한 삼출물이 동시에 관찰되었다. 복합세포성 염증에서는 대식구, 호중구, 임파구 및 상피세포가 혼재하였다.

이상의 결과로부터 transtracheal aspiration technique은 소의 하부호흡기계 질병을 진단하는데 실제 임상에서 응용할 수 있는 쉽고 안전하며 유익한 방법으로 사료된다.

Key words: transtracheal aspiration technique, respiratory disease, cattle.

Introduction

Diagnosis of lower respiratory tract disease of cow is not easy and has clinical problems especially in rearing calves at the change of seasons. Taking clinical history, physical examination and investigation of thoracic radiographs are routine diagnostic steps in the evaluation of animals with the clinical signs of respiratory diseases in the field situation,[1] but the limitations of these procedures in the definitive diagnosis of the pulmonary diseases make it difficult to differentiate bronchopulmonary diseases because of the inability of animals to expectorate sputa for the examination.[2,3]

In human medicine, several methods for the obtaining specimens from the lower respiratory tract can be employed, but the aspirates obtained by the bronchoscopy or oral catheterization are frequently

contaminated by the normally inhabitated organisms in mouth making these methods unable to reveal the actual pathogens in the lower respiratory tract.[4~6]

The transtracheal aspiration technique, however, has been considered to be a safe, simple and useful method clinically for obtaining the tracheobronchial materials for bacteriological diagnosis of the lower respiratory tract diseases.[7] Subsequent reports have confirmed safety and validity of this procedure for bacteriological and cytological diagnosis.[8~11]

In small animal medicine, Creighton and Wilkins[2,3] has reported that the transtracheal aspiration technique using the commercial 16-gauge intravenous catheter sets has been considered as a diagnostic aid in dogs in order to evaluate animals with the lower respiratory tract disease and investigate the cytological patterns and bacteriology.

The application of transtracheal aspiration technique was first introduced in equine medicine.[12] The technique has been recognized as a valuable method for retrieving bronchial cells and isolating pathogenic bacteria within the lower respiratory tract in horse.[13,14]

It was reported that the tracheobronchial fluid sampled by transtracheal aspiration using the orxcentracath(Vigon) was used to diagnose the respiratory diseases bacteriologically in bovine medicine[15] and it is helpful to determine which agents are present and responsible for the disease at lower respiratory tract by this technique only when a herd is exposed to an enzootic or epizootic respiratory disease. In a large herd, this technique is not usually applied to individual cases for diagnosis. It is important to diagnose accurately the lower respiratory tract disease by the transtracheal aspiration technique in cases of a high cost cow, infection of the whole herd by unknown causes and the client's commitment for treatment.

There have been no published studies that analyze relationships between bacteriological findings, cytological examinations by the technique using 5 fr. urinary catheter with 13-gauge 2.5 inch hypodermic needle and clinical signs in cow. In this study, application of the transtracheal aspiration technique in bovine medicine is introduced and the relations between bacteriological findings, cytological examinations by the technique and clinical signs in cow are investigated.

Materials and Methods

Experimental animals: Transtracheal aspiration technique was performed on 40 cows, 31 of them showed clinical signs such as cough and nasal discharge and 9 cows showed no respiratory signs. In all cases, taking clinical history, physical examinations and observations of clinical signs were performed prior to transtracheal aspiration.

Transtracheal aspiration: The technique for the transtracheal aspiration utilizing a commercial polypropylene 5 fr. urinary catheter (Sherwood Medical, USA) with 13-gauge 2.5 inch hypodermic needle was performed in the field situation. Animals were restrained such as head and neck were secured in straight, elevated position, preferably standing to expose the cervical trachea. Anatomical location for the procedure was apporximately half-way between larnyx and the point where the tracheal rings can no longer be palpated on the ventral cervical area.

The area was clipped, prepped surgically with povidone iodine thoroughly and infiltrated with 2 to 5ml of 2% lidocaine hydrochloride subcutaneously. The trachea was grasped by one hand with a sterile gloves, and then a stab incision was made through the skin with the #15 BP scalpel blade to facilitate passing the needle. The 13-gauge sterile hypodermic needle was inserted through the incision into the tracheal lumen pushing firmly between two tracheal rings perpendicular to the long axis of the trachea and it was assured that the needle was within the tracheal lumen by hearing or feeling air-flow through the needle. The 5 fr. urinary catheter was then threaded through the needle until slight resistance was felt, and the needle was withdrawn, leaving the catheter.

Twenty to fifty mililiters of sterile nonbacteriostatic saline was injected with a sterile syringe through the catheter into the lower repiratory tract and aspiration was immediately applied withdrawing or threading the catheter with syringe to facilitate

recovering of the tracheal fluids.

The procedure described above was conducted aseptically and repeated until the material was successfully aspirated.

Bacteriological Examination: An aliquot of the tracheobronchial fluids was plated immediately on 5% calf blood agar after sampling in the field or transporting in an ice-box to the laboratory. The blood agar plates were incubated for bacterial culture for 48 hours at 37°C. Indentification of bacteria cultured primarily on the blood agar was performed by routine bacteriological identification method.

Cytological Examination: A part of aspirated tracheobronchial fluids was centrifuged at 1,500rpm for 5 minutes.[16] After centrifuging, all the supernatant was discarded and the sediment was resuspended in a minimal volume of remaining supernatant. A drop of sediment was smeared on the slide glass with a loop, and then air-dried rapidly. Staining was carried out with Wright's stain.[17] And the examination of the stained sediment was performed under light microscope with the low power objective first and the high power objective for a closer examination.

Results

It was taken 15 to 20 minutes to collect samples and the technique was well tolerated. The punctured wound was rapidly healed itself without any complications. The animal always coughed temporarily when the catheter tip reached the distal trachea or mainstem bronchus and sterile saline was injected. The catheter is rigid so its tip is not flipped up into the larynx as animal coughs and the attempt is not confounded. Infusion of up to 50ml of saline was required in cattle to obtain an adequate sample.

Amount of recovered sample was 5 to 10ml and it was adequate for all diagnostic procedures. The greatest amount of material was aspirated when the suction is applied withdrawing or threading the catheter as animal coughs and the catheter is withdrawn to the descending trachea curves dorsally just proximal to its bifurcation which is a frequent site of trachebronchial fluid accumulation. In case the first flush was unsuccessful, an additional volume of saline equal to the first was injected.

Table 1. Identification of microorganism isolates recovered from 36 of 40 cows by the transtracheal aspiration technique

Microorganisms isolated	No. of isolated bacteria	Percentage
Pasteurella multocida	19	48.7
Staphylococcus aureus	8	20.5
Streptococcus spp	6	15.4
E coli	2	5.1
Corynebacterium pyogenes	1	2.6
Bacillus spp	1	2.6
Non-identification	2	5.1
Total	39	100.0

Respiratory distress did not occur in animals dosed with a large amount of saline. The dilution of the aspirated material in saline did not interfere retrieval of the sample and subsequent cytological and bacteriological evaluations.

The identification of microorganisms isolated from 36 cows by the technique is set out in Table 1. Of these, 33 cows yield single bacterial species and three had two species. The bacteria were nor isolalated in pure culture in four animals. *Pasteurella multocida* accounted very highly for 48.7% of the microorganisms isolated. *Staphylococcus aureus* and *Streptococcus* spp were found in 20.5%, 15.4% of the cases, respectively, and *E coli.*, *Corynebacterium pyogenes* and *Bacillus* spp accounted for 5.1%, 2.6%, and 2.6%, respectively. Two microorganisms (5.1%) were not identified.

Forty animals gave successful aspirates for cytological evaluation and two animals gave poor cytological preparation. Of the forty, 36 had positive culture. In 27 cows of these, bacteria were obvious on cytological evaluation and no bacteria were present in four animals with negative cultures.

The cytological classification based on the inflammatory cells present is set out in Table 2. Six animals of the 40 cows were normal, 16 animals were classified as a mucopurulent inflammation, 8 as a mucoid inflammation, 6 as a mixed cell inflammation and 2 as a miscellaneous reaction.

Table 2. Cytological classification based on inflammatory cells recovered from the bovine tracheobronchial fluids collected by the transtracheal aspiration technique

	No. of cases	Percentage
Normal	6	15
Mucoid inflammation	8	20
Mucopurulent inflammation	16	40
Mixed cell inflammation*	6	15
Miscellaneous**	2	5
Non-preparation	2	5
Total	40	100

*: Neutrophils, lymphocytes, macrophages and epithelial cells were examined.
**: The pattern did not fit any of the criteria

The cells in the aspirates of normal cows were mainly columnar ciliated epithelial cells, which occasionally formed sheets (Fig 3). Neither eosinophils nor goblet cells were seen. Epithelial cells seen lengthwise had terminal plates or cilia and the nucleus enlarged slightly in the cytoplasm and the chromatin pattern was easily seen (Fig 4).

A clumps of neutrophils mixed with epithelial cells, a few lymphocytes and thick mucocid exudates were seen in the mucopurulent inflammation(Fig 5). Many of the neutrophils had a hypersegmented nuclei and the mucus was usually abundant(Fig 5). A few neutrophils and epithelial cells were seen in the mucoid inflammation(Fig 6). A various number of neutrophils, lymphocytes, macrophages and epithelial cells were found in the mixed cell inflammation(Fig 7). Macrophages with one or more smooth or indented nuclei contained various amount of phagocytosed materials(Fig 8). Red blood cells and epithelial cells were appeared miscellaneously.

The relation between cytologic examinations and clinical signs is set out in Table 3. Of 9 cows without respiratory signs, four were normal, one had a mucoid inflammation, and two had a miscellaneous reaction in cytological classification. Of 11 cows with mild clinical signs, two were normal, 4 cows showed mucopurulent inflammation, 3 cows had a mucoid inflammation, two had a mixed cell inflammation, and two had a miscellaneous reponses. Of 20 cows with severe clinical signs, 12 cows were involved highly in a mucopurulent inflammation, four had a mucoid inflammation and four were classified as a mixed cell inflammation.

Table 3. Cytological classification based on inflammatory cells collected by transtracheal aspiration and clinical signs in 40 cows

Clinical signs	Cytologic classification				
	MPI	MI	MCI	Normal	Misc.
None	—	1	—	4	2
Mild	4	3	2	2	2
Severe	12	4	4	—	—

Normal : epithelial cells and rare neutrophils appears
MI : mucoid inflammtion
MPI : mucopurulent inflammation
MCI : mixed cell inflammation
Misc : the pattern did not fit any of the criteria

The relation between microorganisms isolated from the tracheobronchial fluids collected by the transtracheal aspiration technique and clinical signs is set out in Table 4. In cows without respiratory signs, *Pasteurella multocida*, *Staphylococcus aureus* and *Streptococcus* spp were one isolant, respectively. Of 15 cows with mild clinical signs, 6 isolants were *Pasteurella multocida*, three were *Staphylococcus aureus*, four were *Streptococcus* spp, one were *E coli* and *Bacillus* spp, respectively. Of cows with severe clinical signs, the major isolants were *Pas-*

Table 4. Microorganism isolates recovered from tracheobronchial fluids collected by transtracheal aspiration and clinical signs in 40 cows

Microorganisms isolated	Clinical signs		
	None	Mild	Severe
Pasteurella multocida	1	6	12
Staphylococcus aureus	1	3	4
Streptococcus spp	1	4	1
E coli	—	1	1
Corynebacterium pyogens	—	—	1
Bacillus spp	—	1	—

teurella multocida, and four were *Staphylococcus aureus*, and *Streptococcus* spp, *E coli* and *Corynebacterium pyogenes* were one isolants, respectively.

Discussion

This study indicates that the transtracheal aspiration can be a valuable diagnostic aid in clinical bovine medicine and its diagnostic value lies on the point that the uncontaminated tracheobronchial materials can easily be collected for cytological and bacteriological examinations. Materials can be successfully aspirated from most cows suspected of having a respiratory disease.

Many methods have been used for culturing the tracheobronchial fluid sample but the main problem was the nasohparyngeal floral contamination,[6,9,18] and transtracheal aspiration technique was introduced as a method to obtain materials uncontaminated by normal flora of nasopharynx in man and animals.[2,3,6,12,15] The technique applied in this study is similar to that of Zundel *et al*[15] using orx-centracath with trocar, but this study uses the 5 fr. urinary catheter with the 13-gauge 2.5 inch hypodermic needle. It is important to note that the tube should be stiff enough not to be retroflexed into larynx, and the same is true on immediate removal of the needle when the catheter was in place to prevent cutting. When materials is not aspirated, air alone is drawn into the syringe, thus it is necessary to expel the excess air to aspirate the tracheobronchial materials lodged in the catheter tip.

The bovine lung has a great capacity of absorption,[15] so that an infusion of upto 50ml of saline can be tolerated and a respiratory distress does not occur. Due to the rapid absorption of the saline injected, it is important to aspirate immediately after injection of saline or as animal coughs to obtain much materials.

Complications have been reported rarely in man, but included artrial fibrillation, acute bradycardia, cardiopulmonary arrest, aspiration pneumonia, transient hemoptysis, subcutaneous emphysema and infection of needle tract.[19~21] Subcutaneous emphysema and superficial skin infection was reported in the horse[12] and the slight hemorrhage of trachea was only seen in dogs.[2,3] In this study, the transtracheal aspiration technique did not produce these complications in cow.

The results of isolation of pathogens by transtracheal aspiration in cows developing a clinical signs in this study are consistent with the observations of Zundel et al,[15] who reported that the bacteria isolated by this technique in cows with the respiratory signs were *Pasteurella multocida*(62%), *Streptococcus* spp(21%), *E coli*(9%), *Staphylococcus aureus*(4%), *Corynebacterium pyogenes*(2%) and *Bacillus* spp(2%). The species of pathogenic bacteria in the present study are similar to the report of Scholz *et al*,[22] that the bacteria isolated from tracheobronchial secretions in cows with lower respiratory tract disease were *Pasteurella* spp, *Staphylococcus auerus*, *E coli*, and *Erwinia* spp. The availability of transtracheal aspiration technique proves valid for the isolation of pathogenic bacteria in lower respiratory tract disease in cow.

The most bacteria that have been isolated from pneumonic lungs were *Pasteurella* spp. and other bacteria less frequently isolated included *Hemophilus somnus*, *Salmonella* spp, *Streptococci*, *Staphylococcus aureus*, *E coli*, *Neisseria* spp and *Listeria monocytogenes*.[23] Similary, others,[15,22] and the present results agree that the most isolated species are *Pasteurella* spp and it is considered that the differences in isolation of other bacteria were due to the husbandry environments, resistance to the pathogens and geographical characteristics of individual cow.

In relation between the pathogenic bacteria and clinical signs, *Pasteurella multocida* was commonly isolated in cow with severe and mild clinical signs. This is consistent with the observations of Scholz *et al*,[22] who reported that *Pasteurella multocida* was usually isolated in severe respiratory signs but is not consistent with that *Pasteurella hemolytica* was usually isolated in mild clinical signs, and these agree with the report by Collier[24] who asserted that *Pasteurella multocida* was much more widespread than *Pasteurella hemolytica*.

Pasteurella multocida is more likely to cause a bronchopneumonia with moderate amount of fibrin and *Pasteurella hemolytica* fulfill the criteria of a

fibrinous pleuropneumonia.[25] In bacteriological findings from the present study, *Pasteurella multocida* was isolated in 18 cows with clinical signs and it is suspected of bronchopneumonia based on the report of Schiefer,[25] but it is likely to conjunct with the histopathologic findings for more definitive diagnosis.

The cytological examinations of tracheobronchial fluids were variable and each of the five types of cellular responses is present in the cows of this study. The report of Creighton and Wilkins[2,3] showed that cytologic classification of the tracheobronchial fluids in dogs were mucopurulent inflammation, mucoid inflammation, eosinophilic inflammation, mixed cell infllammation, neoplastic, hemorrhagic and miscellaneous reaction in dog. These were simliar to the present cytological findings, but it was difficult to examine the eosinophilic inflammation and neoplastic cells. That a mucopurulent inflammation were appeared more than others in this study agree with those of Creighton and Wilkins,[2,3] but *E coli* was most isolated in dog whereas *Pasteurella multocida* was most isolated in cows. Therefore, the major bacteria causing lower respiratory tract diseases of dog are of different species from those of cow; *Pasteurella multocida* is the causative microorganism in cow and *E coli* in dog.

Most lower respiratory tract inflammations are characterized by accumulation of neutrophils at the sites of the tissue injury and the alveolar macrophage is the resident mononuclear phagocyte of the lung and the primary celluar defender aganist inhaled phagocytable matter.[26]

In cow, pasteurellosis is a common type and exudative inflammatory react ion characterized by the formation of fibrinosuppurative exudates in alveoli and infiltration of neutrophils.[27] These agree with the present findings that the most bacteria isolated were *Pasteurella multocida*, and that many neutrophils and lymphocytes were seen in a mucopurulent inflammation, and macrophages, neutrophils and lymphocytes were seen in a mixed cell inflammation, and further a few neutrophils and epithelial cells were seen in a mucoid inflammation indicating that the cytological examinations of tracheobronchial fluids by this technique showed inflammatory states of lung and this technique is valuable in diagnosing pulmonary diseases in cow.

In the relation between the cytological examination and clinical signs, the most cows developing severe clinical signs had mucopurulent inflammation but the cows with mild clinical signs were included in each of the five cytologic examinations. These indicated that in early stages of pulmonary disease the mucus was secreted and a few neutrophils appeared, and as time went the amount of mucus and number of neutrophils increased.

There was a report[13] that tracheobronchial aspirates obtained from 27 normal horses and 57 horses with respiratory diseases by the transtracheal aspiration technique were examined cytologically. In the report, aspirate of normal horses contained mainly ciliated columnar epithelial cells, mononuclear cell, a few neutrophils and mucus, and the aspirates from horses with the respiratory signs had predominantly neutrophils, macrophages and eosinophils. These are similar to the present results, especially those of cow with respiratory signs. Goblet cells freqently seen in human patients with chronic inflammation especially with bronchiectasia[29] have not seen in cells examined in this study.

As results of this study, it is considered that the transtracheal aspiration technique proves to be a relatively non-invasive, inexpensive and rapid technique enhancing diagnostic accuracy and in some cases making a definitive diagnosis by isolation of pathogenic bacteria and cytological examinations in field conditions, and a therapeutic aid to control the lower respiratory disease of cow. Further, it may be necessary that bacteriological findings and cytological examinations should be correlated to clinical signs, physical examinations, and history taking.

Conclusion

In present study the transtracheal aspiration technique using a commercial polypropylene 5 fr. urinary catheter with 13-gauge 2.5 inch hypodermic needle was applied to bovine medicine in order to determine the availability of this procedure for diagnosis of

lower respiratory tract disease. The subjects were forty adult cattle which consisted of thirty-one cows with respiratory signs such as cough and nasal discharge and nine cows without these signs.

The results obtained were as follows: This procedure appeared suitable for collecting specimens of the lower respiratory tract secretions without uncontamination by the flora of the mouth, respiratory distress and any complications.

The most of microorganisms isolated was *Pasteurella multocida*(48.7%) and a mucopurulent inflammatory reponse was the most appeared accounting for 40% of cytological basis.

Most cows with severe clinical signs showed a mucopurulent inflammation(60%) and *Pasteurella multocida* were isolated dominantly in severe cases (63.2%). Cows with mild clinical signs were distributed in each of the five cytological classification and *Pasteurella multocida* was the most isolated accounting for 40% of mild cases.

In the cytologic examination, a few neutrophils and epithelial cells were seen in the fluid of mucoid inflammation, and many neutrophils with epithelial cells and thick mucoid exudate were seen in the fluid of mucopurulent inflammation. Various number of neutrephils, lymphocytes, macrophages and epithelial cells were examined in the fluid of mixed cell inflammation. The columnar ciliated epithelial cells were examined mainly in normal aspirates.

From these results the transtracheal aspiration technique as a diagnostic aid for lower respiratory tract disease of cow is considered as a simple, safe and useful method and can be peformed in the field situation.

Legend for figures

Fig 1. Equipment for transtracheal aspiration. a. Five french urinary catheter and 13-gauge hypodermic needle were sterilized with E.O. gas. b. 5 fr. urinary catheter frequently used for dogs and cats.

Fig 2. Five french urinary catheter in bovine lung through the 13-gauge hypodermic needle.

Fig 3. Normal ciliated columnar epithelial cells formed sheets and derived from tracheobronchial lining of cow. Wright's stain. ×400.

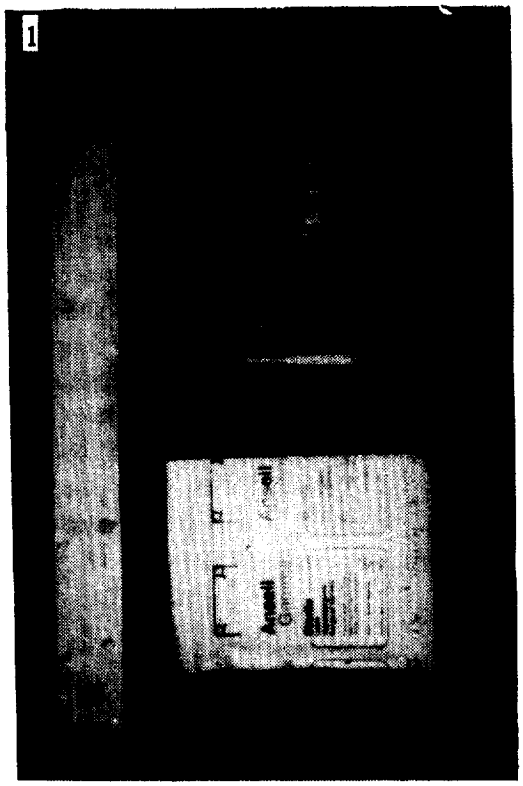
Fig 4. Normal ciliated columnar epithelial cells had terminal plates and cilia. The nucleus was enlarged slightly in the cytoplasm. Wright's stain. ×1000.

Fig 5. Mucopurulent inflammation with large number of neutrophils and lymphocytes. Wright's stain. ×400.

Fig 6. Mucoid inflammation with a few neutrophils and epithelial cells. Wright's stain. ×400.

Fig 7. Mixed cell inflammatory response. Cells include neutrophils, macrophages, lymphocytes and epithelial cells. Wright's stain. ×400.

Fig 8. Higher magnifications showing macrophages contained phagocytosed materials and neutrophils. Wright's stain. ×1000.

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