MICROBIOLOGICAL EXAMINATION OF THE TRACHEAL FLUSHING SAMPLE AND ITS CLINICAL IMPORTANCE

Trakeal yıkama örneğinin mikrobiyolojik muayenesi ve klinik önemi

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Özet : Selçuk Üniversitesi Veteriner Fakültesi İç Hastalıklar Kliniğine getirilen 25 buzağıdan mikrobiyolojik muayene için burun sıvabı ve trakeal yıkama örnekleri alındı. Burundan alınan örneklerin 22'sinden çeşitli mikroorganizmalar izole edilirken, trakeal yıkama örneklerinden P. pneumonia (% 40), Staph. aureus (% 20), Klebsiell ssp. (% 13.3), Corynebacterium ssp. (% 6.6), Shigella ssp. (% 6.6), Ps. maltophila (% 6.6), Aspergillus ssp. (% 6.6) saf olarak izole edildi. Etken izolasyonu yapılamayan buzağıların 7'sine önceden değişik antibiyotikler uygulanmıştı. Onbir vakada Linko-spektin (% 47), 9 vakada Gentamisin (% 39) etkili bulundu. Aspergillus ssp. izole edilen bir buzağı Thiabendazole ile tedavi edildi. Bir buzağı tedavi edilemedi. Bu buzağının otopsisinde mikrobiyolojik ve patolojik olarak Tüberküloz olduğu teşhis edildi. Diğer 3 buzağı geniş spektrumlu antibiyotiklerle tedavi edildi. Çalışmanın sonucunda, trakeal yıkama metodunun enfeksiyöz buzağı pneumonilerinin teşhis ve tedavilerinde kolaylıkla ve güvenilir bir şekilde uygulanabileceği kanısına varıldı.

Summary : Nasal swab and tracheal flushing samples were taken from 25 calves with clinical symptoms of pneumonia which have been

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admitted to the Clinic of Internal Medicine, Faculty of Veterinary Medicine, University of Selçuk for microbiological examination. While microbiological isolation from nasal swab could be performed in 22 of 25 calves and revealed a large population of normal flora, microbiological isolation from tracheal flushing samples could be performed in 15 of 25 calves and obtained more pure culture; P. pneumonia (40 %), Staph. aureus (20 %), Klebsiella ssp. (13.3 %), Corynebacterium ssp. (6.6 %), Shigella ssp. (6.6 %), Ps. maltophila (6.6 %), Aspergillus ssp. (6.6 %) (1). In 7 of 10 calves which microbiological isolation cauld not be performed from tracheal flushing samples various antibiotics had been injected farmerly. Linco-spectin and Gentamicin were found to be effective in 11 (47 %) and 9 (39 %) cases of the bacterial pneumonia of calves, respectively. One calf from which Aspergillus ssp. was isolated was treated with Thiabendazole. One calf could not be treated. Tuberculosis was diagnosed in the pathologic and bacteriologic examination in its autopsy. The other 3 calves were treated with broad-spectrum antibiotics. In conclusion, it was found that tracheal flushing sampling method could be used easily and safely in the diagnosis and treatment of infectious calf pneumonia.

Introduction

Infectious calf pneumonia is a well known respiratory tract disease with high morbidity and is frequently in housed dairy animals in winter months especially. Viral, mycoplasmal and bacterial agents are involved in the complex etiology.

It is customary to collect samples from respiratory tract for sufficient antibacterial therapy in the infectious pneumonia. A direct swab from nasal passages is the simplest and common test (5). Sampling from the lower respiratory tract is the other method of choice for diagnostic evaluation of respiratory disease in man and animals. For this purpose, tracheal flushing sample is obtained by aspirating tracheal secretion thorough a catheter which passed into a cannula inserted trachea (9, 14). In addition to this, fibroptic endoscopes for sampling of the contents of small airways and alveoli of the lung by lavaging bronchi and alveoli in the live animals has been used recently (8, 13).

The objective of this study was to compare the bacterial flora in the nasal cavity with that of the lower respiratory tract in diseased animals and to show the clinical importance of lower respiratory tract sampling in the diagnosis and treatment of infectious calf pneumonia.

Materials and Methods

Nasal swab and tracheal flushing samples were taken from 25 calves with clinical symptoms of pneumonia. Body temperature of diseased animals varied between normal temperature and 41.5 °C. Diseased animals had at least one of the following symptoms; elevated respiratory frequency or increased bronchial tones. Moreover, they often coughed and had nasal discharge.

The nasal samples were taken with cotton swabs and immediately brought to transport medium.

Sampling from the lower respiratory tract: The samples from the lower respiratory tract were taken in unanestethised animal. The lower third of the trachea was clipped and disinfected using iodine tineture and alchool. Local anesthesia was applied where the cannula was inserted. The lower third of the trachea was fixed with one hand and a cannula(*) was inserted in the midline downwards in 45° angle to the trachea. After passing the skin and tracheal wall, the cannula was inserted paralell to the trachea. The catheter (**) was pushed 20-25 cm into the cannula. Sterile saline solution (10 ml) was infused and immediately aspirated through the catheter. Aproximately 1 - 1.5 ml of fluid was aspirated. The sample was put into a test tube.

Microbiologic examination : Each sample taken from nose and lower respiratory tract was inoculated on two plates of 5 % sheep blood agar(***) one plate of Mac Conkey agar (****) and one plate of Saborraund Dextrose agar (***) and incubated at 37 °C for a maximum period of 5 day for microbiologic isolation, but mycologic plates were incubated at room temperature for seven days. One of the blood agar plates was incubated aerobically, the other blood agar plate was incubated anaerobically using a vacuum pump. The plates were examined every 24 hours and in the case of multiple isolates, population of the various colonies grown were estimated. Smears were made from tracheal flushing samples and various colonies grown on the plates and stained by Gram's method, Giemsa technique and Ziehl-Neelsen technique (1), and examined microscopically under an oil-immersion lens. Appearence of a few colonies of known non-pathogenic bacteria was considered insignificant and not recorded. The

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various microorganism isolated were tested biochemically according to their genera and species requirements (2, 4, 6, 10, 11).

Antibiotic susceptibility of isolates was carried out on Mueller Hinton Medium (*****) using antibiotic disks as described by Baure et al (3). When the bacterial isolation was perfomed in the tracheal flushing sample, antibiotic susceptibility test was done with the bacteria isolated from tracheal flushing sample. However, when any microorganisms could not be isolated from the tracheal flushing sample, antibiotic susceptibility test was carried out with the bacteria isolated from nose.

Treatment of the calves was immediately started with a broad spectrum antibiotic after sampling. Therapy was continued with antibiotic obtained by antibiotic sensitivity test.

Results

Microorganisms isolated from tracheal flushing sample and nasal swab, and the results of the antibiotic sensitivity test of salves suffering from pneumonia are shown in table 1.

Signs of discomfort was not seen during and after sampling. Only a few occasion, coughing was observed during the sampling.

Microbiologic isolation from tracheal flushing samples could be performed in 15 of 25 calves. The most common isolated microorganism from tracheal flushing samples was P. pneumonia (40 %). Microbiologic isolation from nasal swab revealed a large population of normal flora and could be performed in 22 of 25 calves.

Microbiologic isolation from tracheal flushing samples could not be performed in 10 of 25 calves. Seven of which had had antibiotic injection before sampling.

All calves were treated succesfully apart from one calf (calf number 18). The calf was euthanasied because of unsuccesful recovery. Tuberculosis was diagnosed in the autopsy and bacteriologic examination.

The calf (calf number 1) had been treated twice with antibiotic before tracheal flushing sampling and the therapy had been unsuccesful. Microbiologic examination of the tracheal flushing sample of the calf revealed Aspergillus ssp. The calf was treated with thiabendazole succesfully.

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-5-

Table 1 Microorganisms isolated from tracheal flushing and nasal swap samples and the antibiotic sensitivity in each calf suffering from pneumonia.

Number of calves	Tracheal flushing sample	Antibiotic sensitivity (+++)	Nasal swab	Antibiotic sensitivity (+++)	Previous treatment
1	Aspergillus ssp.	—	—	—	Antibiotic has been injected
2	P. pneumonia	Ls,Ge,Cp,Ch	Staph. aureus K. pneumonia Streptococcus ssp.	—	—
3	Staph. aureus	Ls,Ge,Cp	K. pneumonia Shigella ssp. Acinetobacter calcoaceticus	—	—
4	K. pneumonia	Cp,Ox,E,Ne	K. pneumonia	—	—
5	Staph. aureus	Ox,E,Ls,Am, Ne	Staph. aureus Pasteurella ssp. Streptococcus ssp.	—	—
6	P. pneumonia	Ge,Ls,Ne	P. pneumonia Streptococcus ssp.	—	—
7	—	—	—	—	Antibiotic has been injected
8	—	—	Staph. aureus E. coli	Ls,TS,Ne	—
9	P. pneumonia	Cp,E,Am,TS	P. pneumonia	—	—
10	Shigella ssp.	—	E. coli Shigella ssp.	—	—
11	—	—	Staph. aureus K. pneumonia	Ge,E,Ls,Am, TS,Ne	Antibiotic has been injected
12	K. pneumonia	Cp,Ls	Acinetobacter calcoaceticus E. coli	—	—
13	Staph. aureus	Ox,Ls	Staph. aureus Corynebacterium ssp.	—	—
14	Ps. maltophila	Ge,Ls	—	—	—
15	—	—	P. pneumonia E. coli Corynebacterium ssp.	Ge,E,Ne	Antibiotic has been injected
16	—	—	Streptococcus ssp. Klebsiella ssp.	Ge	Antibiotic has been injected
17	—	—	Staph. aureus Corynebacterium ssp.	E,Am,Ne	—
18	—	—	Staph. aureus	Cp,Ne	Antibiotic has been injected
19	Corynebacterium ssp.	Ge	Aeromonas hydrophila Corynebacterium ssp.	—	Antibiotic has been injected
20	—	—	P.haemolytica	Cp,Ne	Antibiotic has been injected
21	P. pneumonia	Am,Ch,TS	Staph. aureus Streptococcus ssp.	—	—
22	—	—	Shigella ssp. E. coli	Ge,E,Am	—
23	—	—	not identified (gram + bacil)	—	Antibiotic has been injected
24	P. pneumonia	E,Am,Ls	Staph. aureus Corynebacterium ssp.	—	—
25	P. pneumonia	Cp,E,Ls,Am	Corynebacterium ssp. E. coli	—	—

Ge: Gentamicin Ox: Oxytetracycline E: Erytromycine Ls: Linco-Spectine Am: Ampicillin
Ne: Neomicin Cp: Cephaperazone Ch: Chloramphenicol TS: Trimethoprim-Sulphaxzole

Linco-spectin and Gentamicin were found to be effective in 11 (47 %) and 9 (39 %) cases of bacterial pneumonia of calves respectively.

Discussion

Identification of the microorganisms causing the respiratory syndrome and detection of the sensitive antibiotics are the first main objective of the treatment (7). The results of this study showed that tracheal flushing sampling method was more adequate than nasal swab sampling in individual animals for this purpose. More pure culture were obtained after cultivation by sampling from the tracheal flushing sampling. However, the resulting microbiological examination of nasal swabs revealed a large population of normal flora. This result is agreement with İmren (9), Lay et al (12), Pringle and Viel (13) and Viring et al (14).

Microbiological examination of tracheal flushing samples revealed negative results in 7 of 10 calves in which antibiotic had been injected. However, microbiologic examination of the tracheal flushing sample of the calf (calf number 1) revealed Aspergillus ssp. despite antibiotic injection. So, it can ve mentioned that microbiologic examination of the tracheal flushing samples of animals in which even if antibiotic has been injected is necessary for detection of mycotic pneumonia.

Viring et al (14) has taken the tracheal flushing samples in anestethised calves, but in the contrary to this, it was found the use of only local anesthetic where the cannula was inserted was sufficient.

The high frequency of Pasteurella ssp. isolates and the lower frequency of the other bacterial isolates in tracheal flushing and nasal swab samples indicated the importance of Pasteurella ssp. as causative agent of respiratory disease in calves.

Linco-spectin and Gentamicin were commonly found to be affective in the cases of bacterial pneumonia. This result showed that these new generation of antibiotic could be used in the cases of infectious pneumonia in which tracheal flushing sample could not be tested.

In conclusion, it was found that tracheal flushing sampling method could be used easily and safely in the diagnosis and treatment of infectious calf pneumonia, and more reliable than nasal swab sampling.

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