Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 23(1) January-April 2025]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

PANTNAGAR JOURNAL OF RESEARCH

Vol. 23(1) January-April 2025

CONTENTS

Bacterial isolates from tracheo-bronchial aspirates of healthy and pneumonic cattle

ASMITA NARANG*, CHARANJIT SINGH, MUDIT CHANDRA and DHIRAJ KUMAR GUPTA

Department of Veterinary Medicine, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab
**Corresponding author's email id: asmi.vet@gmail.com*

ABSTRACT: The study was conducted to isolate bacterial microflora from tracheo-bronchial aspirates (TBA) of healthy and pneumonic crossbred adult cattle. A total of fifty cross bred adult cattle diagnosed for pneumonia were selected. Ten apparently healthy cattle were included as healthy control animals. TBA samples were collected aseptically by percutaneous method. TBA sediment was inoculated on Blood agar and MacConkey's lactose agar. Bacterial isolates were identified and characterized on the basis of cultural examination and biochemical tests. TBA from six healthy animals and 44 pneumonic cattle were positive for bacterial growth. Out of 85 isolates obtained from pneumonic cows, *Staphylococcus spp.* other than *S. aureus* (25.9 %) were the most prevalent followed by *Pasturella multocida* (12.9 %), *Bacillus spp.* (11.8 %), *Pseudomonas spp.* (10.6 %), *Staphylococcus aureus* (10.6 %), *E. coli* (9.4 %), *Klebsiella spp.* (8.2 %), *Proteus spp.* (7.1 %) and *Streptococcus spp.* (3.5%)

Keywords: Bacteria, cattle, pneumonia, tracheo-bronchial aspirate

Respiratory infections are commonly presented in bovines. Lower respiratory tract diseases in cattle are primarily of infectious origin. The main incriminates are bacterial infection which may be primary or secondary. The stressors augment the rate of multiplication of resident bacteria and subsequent infection of the lungs. Generally, multiple bacterial infections have been reported from the respiratory system (Quinn *et al.,* 1994). The presence of only some of these pathogens can be associated with clinical respiratory disease in cattle. This often creates difficulty in interpreting microbiological findings during an outbreak of respiratory diseases. Many commensal bacteria are present in bovine respiratory tract which cause disease under stress conditions (Panciera and Confer, 2010). *Pasteurella multocida* was isolated from tracheo-bronchial aspirates in calves along with *Mannheimia haemolytica* and *Histophilus* (Angen *et al*., 2009). Other bacteria viz. *Staphylococcus aureus, Streptococcus* spp. and *Klebsiella pneumoniae* were isolated from bovine lung samples (Sayed and Zaitoun, 2009). Few hard data is available on prevalent respiratory bacterial pathogens in Indian context except for *Pasteurella multocida* (Vaid *et al.,* 2012).

So far in animals, nasal and nasopharyngeal swabs have been utilized for cultural studies. But these samples are not true representative samples from lungs. Tracheo-bronchial aspirate offers an advantage of avoiding nasopharyngeal contamination of secretions collected for microbiological culture. Studies investigating the bacterial flora of upper respiratory system in healthy and unhealthy animals are there (Holman *et al.,* 2015) but scarce literature is present aiming bacteria from lower respiratory tract in live animals. Although, there are studies on bacterial isolation from broncho-alveolar lavages in calves (Aslan *et al.,* 2002, Autio *et al.,* 2007, Angen *et al.,* 2009), but studies on percutaneous TBA samples in adult cattle could not be traced. To our knowledge, this is the first study on the use of TBA as a diagnostic sample for the isolation of bacteria in healthy and pneumonic adult cattle. Generation of such a data on respiratory disease profile would benefit in achieving quick and confirmatory diagnosis. Further, there are reports of antibiotic-resistant pneumonia in feedlot cattle (Azmat *et al.,* 2013). Currently the choice of antibiotics in respiratory infections is based on individual discretion as there is little published data on the antimicrobial susceptibility of respiratory bacterial pathogens in Indian context. So, there is need to study sensitivity

pattern of prevalent respiratory infections for rational treatment. In addition, duration of the treatment depends on the causative bacteria and severity of infection. The aim of this study was to examine the bacterial flora from lower respiratory tract in healthy and pneumonic crossbred cattle and determine the antimicrobial susceptibilities.

MATERIALS AND METHODS

Animals

Tracheo-bronchial aspirates were collected from 10 apparently healthy cattle (control group) and 50 adult cross bred pneumonic cattle presented to large animal clinics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. The affected animals were diagnosed for aspiration pneumonia (n=8), suppurative pneumonia (n=10), fibrinopurulent pneumonia (n=8), chronic pneumonia (n=14), chronic active pneumonia (n=6) and bronchitis (n=4) on the basis of tracheal aspirate cytology supported by history and comprehensive clinical examination.

Bacteriological examination of TBA

Tracheobronchial aspirates were collected from standing cattle using (Large Animal Transtracheal Kit, MILA International, Inc. USA) as per the methodology used by Narang *et al.,* 2023. Ten ml aliquot of TBA was transferred into sterile containers for bacteriology and was kept on ice packs till processed. All the samples were processed within 1-2 hours. Samples were centrifuged at 3000 × g for 10 min. The sediment was cultured on 5 per cent defibrinated sheep blood agar and incubated for overnight at 37°C in aerobic conditions. Single colonies of bacterial isolates were picked up and re-streaked on fresh Sheep Blood agar (SBA) which was again incubated at 37/ °C for 24/ h to obtain pure colony of isolates. The cultures were observed for colonial morphology. Then, the smears were prepared from the purified colonies and subjected to Gram's staining followed by biochemical tests.

Identification of isolates

Gram-positive isolates were identified based on their colonial morphology, microscopic features and biochemical characteristics, including catalase, oxidase, and motility tests. *Staphylococcus* species were further subcultured on mannitol salt agar. Sugar fermentation tests were performed on the Gram-positive isolates to facilitate species-level identification. Gram positive cocci present in clusters which were catalase positive, oxidase and motility negative were identified as *Staphylococcus. Staphylococcus aureus* was confirmed on positive hemolysis on blood agar and fermentation of dextrose, mannitol and maltose. The cultures were streaked onto mannitol salt agar and incubated overnight for re-confirmation of *Staphylococcus aureus*. Gram positive cocci present in chains which were catalase, oxidase and motility negative were identified as *Streptococcus.* The *Bacillus spp.* produced thick, greyish white or cream coloured colony with uneven surface on Nutrient agar and abundant growth with creamy yellow coloured colony and produced haemolysis on Blood agar media. The organisms found as Gram positive rods and also in long chain indicating *Bacillus. Bacillus* revealed positive reaction in Catalase test and found negative for VP and indole test but positive for Methyl red test.

The colonies from Gram negative bacteria were streaked onto McConkey Lactose Bile Salt Agar (MLA) for identification and incubated for 16-24 hours to differentiate between lactose fermenters and non-lactose fermenters within the enterobacteria group. The lactose-fermenting colonies were then subcultured onto Eosin Methylene Blue (EMB) agar. The Gram negative rods were subjected to Indole, Methyl red, Voges Proskaeur and Citrate test (IMViC) tests. *E. coli, Klebsiella* and *Proteus* were differentiated on the basis of growth on Eosin methylene blue agar (EMB), MLA and IMViC test. Lactose fermenting mucoid or non mucoid colonies on MLA, haemolytic or non-haemolytic Gram negative rods on blood agar showing characteristic metallic sheen on EMB agar and IMViC test results as ++— were characterised as *E. coli.* Lactose fermenting mucoid colonies on MLA, non-haemolytic capsulated Gram negative rods on blood agar showing —++ IMViC reaction were characterised as *Klebsiella pneumoniae*. Typical swarming growth on nutrient agar was seen in all *Proteus* isolates and produced H_2S production in TSI

agar. Gram negative nonlactose fermenting rods producing large, pale colonies on MLA, greenish blue pigment showing positive reaction for gelatin liquefaction and oxidase test were identified as *P. aeruginosa*.

Antibiotic sensitivity test: The disc diffusion technique was used for the antibiotic sensitivity test and zones of sensitivity were observed as clear zones around the antibiotic discs. All bacterial isolates were tested for antimicrobial sensitivity against 15 antimicrobials (HiMedia, India) including Amikacin (30mcg), Amoxycillin (10mcg), Amoxyclav (10mcg), Ampicillin (25mcg), Cefoperazone (75mcg), Ceftriaxone (10mcg), Ceftriaxone/ tazobactum (10mcg), Cotrimoxazole (25mcg), Enrofloxacin (10mcg), Erythromycin (15mcg), Gentamicin (10mcg), oxytetracycline (30mcg), Penicillin (10unit), Piperacillin(10mcg) and Streptomycin (10mcg) by disc diffusion technique (Kirby and bauer, 1966). The interpretation of the results as sensitive or resistant was done as per the manufacturer's instructions. The isolates showing intermediate pattern to any antimicrobial were considered as resistant.

Data analysis

The relative abundance of each species and genus were expressed as a percentage of the total number of isolates.

RESULTS AND DISCUSSION

Tracheo-bronchial aspirates from six healthy control animals were positive for bacterial growth and nine bacterial isolates were obtained. *Pasteurella multocida* (33.3%) and *Staphylococcus spp.* (22.2%) were predominant followed by *Staphylococcus aureus, Bacillus* spp., *Pseudomonas spp*.and *E. coli* each with a proportion of 11.1%. Single bacterial growth was yielded in 66.7% of the positive samples and mixed bacterial growth in 33.33% samples (Table 1).

Out of 50 TBA samples from pneumonic cattle, 44 (88%) were found positive and yielded 85 bacterial isolates. In the present study, *Staphylococcus spp.* other than *S. aureus* (25.9%) was most common followed by *Pasturella multocida* (12.9%), *Bacillus spp*. (11.8%), *Pseudomonas spp.* (10.6%), *Staphylococcus aureus* (10.6%), *E. coli* (9.4%), *Klebsiella spp.* (8.2%), *Proteus spp.* (7.1%) and *Streptococcus spp.* (3.5%) (Table 1). The presence of both Gram-positive (51.8%) and Gram-negative bacteria (48.2%) was recorded in the study. Of these positive samples, 31.8% yielded single bacterial growth and 68.2% had mixed bacterial growth (Table 1).

Various isolates in different pneumonias

Majority of *P. multocida* organisms were isolated from purulent infections [suppurative pneumonia (4/ 11) and fibrinopurulent pneumonia (3/11)] (Table 2). *Staphylococcus* spp. was the dominant bacteria (5/12; 33.33%) in aspiration pneumonia. Maximum isolates of *S. aureus* were isolated from purulent infections [suppurative pneumonia (2/9) and fibrinopurulent pneumonia (3/9)] and from chronic active pneumonia group (3/9) that was characterized by foci of suppuration (Table 2). All the 3 isolates were obtained from purulent infections [suppurative pneumonia (2/3) and fibrinopurulent pneumonia (1/ 3)]. *Bacillus spp.*was predominantly found in mixed infections in pneumonic cattle.

E. coli isolates were obtained from aspiration pneumonia and chronic pneumonia (3/8 each). *Klebsiella spp.* was invariably isolated from all the clinical groups (Table 2). Maximum isolates of *Pseudomonas spp.* were obtained from chronic pneumonia (4/9) followed by chronic active pneumonia (2/9) and fibrinopurulent pneumonia (2/ 9) (Table 2).

Cultural Sensitivity tests

Antibiogram of 85 isolates from 50 cattle showed enrofloxacin (82.3 per cent) as the most effective antibiotic *in vitro* followed by gentamicin, ceftriaxone/tazobactum, oxytetracycline, amikacin, ceftriaxone, streptomycin, erythromycin, cefoperazone, amoxyclav, ampicillin, cotrimoxazole, penicillin and least effective was piperacillin (Table 3).

The per cent sensitivity was variable for 22 *Staphylococus* spp. isolates with maximum sensitivity towards gentamicin (90.91 per cent) and

least towards piperacillin (18.18 per cent). In case of *Staphylococcus aureus* isolates (9), maximum sensitivity was seen towards gentamicin and least towards piperacillin (11.1 per cent). Streptococci showed 100 per cent sensitivity towards enrofloxacin and ceftriaxone/tazobactum. Complete resistance was seen in case of cefoperazone, cotrimoxazole and piperacillin for Streptococci. *Bacillus* spp. (n=10) was most sensitive towards enrofloxacin and least towards erythromycin. *E. coli* showed 100 percent sensitivity towards enrofloxacin, gentamicin, oxytetracycline, streptomycin, amikacin and ceftriaxone/tazobactum in descending order (Table 3).

In case of *Pasteurella* spp. isolates (11) the maximum sensitivity of 90.9 per cent was seen towards enrofloxacin followed by gentamicin and cefoperazone (72.7 per cent), and least towards

Table 1: Bacterial isolates from TBA in healthy and pneumonic cattle

Name of the isolate	Healthy control (n=10)		Pneumonic cattle (n=50)	
	Number of isolates	Proportion (%)	Number of isolates	Proportion (%)
S. aureus	1	11.1 %	9	10.6 %
Staphylococcus spp. (other than *S. aureus)*	2	22.2 %	22	25.9 %
Streptococcus spp.	0	0	3	3.5 %
Bacillus spp.	1	11.1 %	10	11.8 %
E.coli	1	11.1 %	8	9.4 %
P. multocida	3	33.3 %	11	12.9 %
Klebsiella spp.	0	0	7	8.2 %
Pseudomonas spp.	1	11.1 %	9	10.6 %
Proteus	0	0	6	7.1 %
Total number of isolates	9		85	

Table 2: Bacterial isolates from TBA in different forms of pneumonia

Clinical Groups Isolates	Aspiration pneumonia (n=8)	Suppurative Pneumonia (n=10)	Fibrinopurulent Pneumonia (n=8)	Chronic Pneumonia (n=14)	Chronic Active Pneumonia (n=6)	Bronchitis (n=4)	Total
S. aureus	1	2	3	1	2	0	9
Staphylococcus spp.	5	3	2	7	3	2	22
Streptococcus spp.	0	2	1	0	0	0	3
Bacillus spp.	2	1	2	4	1	0	10
E. coli	3	0	1	3	0	1	8
P. multocida	0	4	3	1	3	0	11
*Klebsiella*spp.	0	2	1	2	1	1	7
Pseudomonas spp.	0	1	2	4	2	0	9
Proteus	1	0	0	3	0	2	6
Total isolates in each group	12	15	15	25	12	6	85
No growth	3	1	0	1	1	0	5

Table 3: Antibiotic sensitivity pattern of isolates from TBA from pneumonic cattle

Organism/isolates	A	AMX	AMC	AMP	CPZ	CTR	CIT	COT	EX	E	G	O	P	PI	S
S. aureus (9)	77.8	55.6	66.7	55.6	22.2	66.7	77.8	33.3	88.9	22.2	100	77.8	33.3	11.1	44.4
Staphylococcus spp. (22)	72.7	31.8	40.9	59.1	40.9	59.1	81.8	22.7	86.4	59.1	90.9	86.4	22.7	18.2	54.5
Streptococcus spp. (3)	66.7	33. 3	66.7	33.3	0	66.7	100	0	100	33.3	66.7	33.3	33.3	0	66.7
Bacillus spp. (10)	50	10	20	10	20	50	40	10	90	0	50	60	40	10	20
E. coli (8)	62.5	12.5	12.5	25	50	50	62.5	37.5	100	25	87.5	75	12.5	12.5	62.5
P. multocida (11)	27.3	27.3	36.4	36.4	72.7	45.4	63.6	45.4	90.9	36.4	72.7	54.5	18.2	27.3	45.4
Klebsiella spp. (7)	57.1	28.6	42.9	14.3	28.6	71.4	100	42.9	100	85.7	85.7	71.4	28.6	14.3	57.1
Pseudomonas spp. (9)	33.3	22.2	22.2	11.1	22.2	11.1	44.4	44.4	66.7	22.2	77.8	33.3	22.2	0	44.4
Proteus (6)	66.7	16.7	16.7	33.3	33.3	83.3	66.7	16.7	100	33.3	83.3	50	0	16.7	50
Total isolates (85)	57.6	27.1	35.3	35.3	36.5	52.9	67.1	29.1	82.4	37.6	81.2	65.9	23.5	14.1	48.2

penicillin (18.2 per cent). Out of 7 isolates of *Klebsiella* spp.. 100 per cent were sensitive to enrofloxacin and ceftriaxone/tazobactum. *Pseudomonas* isolates exhibited comparatively lower sensitivity towards all the antibiotics (Table 3).

Overall sensitivity pattern for bacterial isolates from control groups was also determined. The bacterial isolates from non respiratory affected animals were most sensitive to gentamicin (100 per cent) followed by enrofloxacin (96.7 per cent), oxytetracycline (76.7 per cent) and ceftriaxone/tazobactum (73.3 per cent). In isolates obtained from healthy animals, sensitivity was highest for enrofloxacin (100 per cent) followed by gentamicin (88.9 per cent); oxytetracycline, ceftriaxone/tazobactum and cotrimoxazole (77.8 per cent), amikacin (66.7 per cent), amoxyclav (55.6 per cent), ampicillin (44.4 per cent), amoxicillin, erythromycin and streptomycin (33.3 per cent); ceftriaxone and cefoperazone (22.2 per cent), and penicillin (11.1). The aim of the study was to examine the bacterial flora of lungs from healthy and respiratory affected cattle. In the present study, tracheo-bronchial aspirates healthy control animals had bacterial growth. Likewise, studies have reported the isolation of potentially pathogenic bacteria from trans-tracheal aspirates in 27 to 68% of the healthy calves (Autio *et al.,* 2007; Angen *et al.,* 2009). Various researchers have reported *Pasteurella* and Staphylococci as predominant bacteria from healthy animals (Seker *et al.*, 2009; Mahmud *et al.*, 2016) which were predominant isolates in this study.

Bacterial growth from TBA in pneumonic calves has been reported as high as 90% which is in agreement with the present study (Szeredi *et al.,* 2010). On the contrary, some studies have reported it from only 21 to 59.26% (Härtel *et al.*, 2004; Aslan *et al.,* 2002). Common bacterial isolates *viz*. *Pasteurella*, *K. pneumonia*, streptococci, *Staphylococcus spp.*, *Proteus* and *E. coli* in varying proportions have also been isolated from nasal swabs (Seker *et al.,* 2009), lung swabs (Sayed and Zaitoun, 2009), tracheal swabs (Ali and Sultana, 2012), nasopharyngeal swabs (Holman *et al.,* 2015) from pneumonic cattle and tracheal aspirates from pneumonic calves (Aslan *et al.,* 2002). In the present study, streptococci, *Proteus* and *Klebsiella spp.* were only isolated from pneumonic cattle. The other isolates were common for healthy and pneumonic groups. Therefore, it is suggested that the isolation of bacteria should be associated with presence of clinical signs.

The results for Gram-positive and Gram-negative bacteria isolation were in corroboration with the study by Sayed and Zaitoun (2009). However, Seker *et al.* (2009) recorded predominance of Gram positive bacteria from apparently healthy animal and Gram negative bacteria from respiratory disease affected cattle. These findings have clinical and epidemiological significance.

Various isolates in different pneumonias

An attempt was made to draw a correlation between the bacterial isolate and type of pneumonia. *Pasteurella multocida* has been reported to be naturally present in small numbers in the nasal passages of healthy cattle. Under stress, proliferation occurs in the region and later gets extended to the lower respiratory tract and causes respiratory infection (Araghy, 2007). *Pasteurella multocida* was responsible for acute suppurative infections which is in corroboration with post mortem studies (Ali and Sultana, 2012). Ali and Sultana (2012) have also isolated S*taphylococcus spp.*at the frequency 16.2% from different types of lung lesions from slaughtered buffaloes. *Streptococcus* spp. was isolated from only pneumonic animals. *Bacillus spp.*was predominantly found in mixed infections in pneumonic cattle, however, in healthy animals, it was predominantly isolated as single bacteria (80% and 100%, respectively) (Table 2). *Bacillus* is generally a contaminant, however, Mahmud *et al.* (2016) suggested the pathogenicity of *Bacillus* spp. along with other bacteria from nasal and lung swab by mice inoculation.

Klebsiella spp. was isolated from only pneumonic cattle suggesting the role of the bacteria in pathogenecity. Although, *Pseudomonas aeruginosa* is usually considered as opportunistic pathogen, it can cause pneumonia in cattle (Quinn *et al.,* 1994). Amstel *et al.* (1987) found *Pseudomonas aeruginosa* as etiological agents of 25 per cent of the cases of pneumonia in calves. The present study may help to determine the type of pneumonia based upon the

bacteria isolated and vice versa. This would help in better therapeutic management even in cases where tracheo-bronchial aspirates are not attempted or the results are awaited to start the treatment.

There are fewer studies determining the susceptibililty of bacterial isolates from lower respiratory tract in cattle. Though, researchers have determined the antimicrobial susceptibilities of *Pasteurella multocida* strains isolated from nasal swab samples and lung samples (Seker *et al*., 2009, de Jong *et al.,* 2014, Kamran *et al*., 2014). Enrofloxacin had been recorded to be most susceptible for *Pasteurella* (Seker *et al*., 2009, Kamran *et al.,* 2014), which is in accordance with the present study. Mahmud *et al.* (2016) recorded high sensitivity of erythromycin to *Staphylococcus* spp. and *Bacillus* spp. and enrofloxacin to *E coli*, which corroborates with the present study.

CONCLUSION

Tracheal wash proved to be an excellent diagnostic sample for the isolation of bacterial lung pathogens. *Pasteurella* and *Staphylococcus spp* were predominant bacteria from control animals. In affected animals, *Staphylococcus* was the most prevalent bacteria followed by *Pasteurella, Bacillus, Pseudomonas, Klebsiella and E.coli*. *Staphylococcus spp* was the dominant bacteria in aspiration pneumonia. Maximum isolates of *S. aureus* were isolated from suppurative pneumonia and fibrinopurulent pneumonia. *Streptococcus* was isolated from only respiratory affected animals. *P. multocida* was isolated from purulent infections. The isolation of bacteria should be associated with presence of clinical signs.

REFERENCES

Ali, M.Z. and Sultana, S. (2012). Isolation and identification of bacteria from tracheas and lungs of buffaloes in Dinajpur. *Stamford J. Microbial.,* 2(1): 35-40.

Amstel, S.R., Benton, M., Witcomb, M.A., Fabian, B. and Vervoort, P. (1987). Antibiotic sensitivity of *Pasteurella haemolytica* isolated by means of a fiberoptic endoscope from cases of pneumonic pasteurellosis in cattle. Onderstepoort. *J. Vet. Res.,* 54: 551-552.

Angen, O., Thomsen, J., Larsen, L.E., Larsen, J., Peter, M., Heegaard, H. and Enemark, M.D. (2009). Respiratory disease in calves: Microbiological investigations on trans-tracheally aspirated bronchoalveolar fluid and acute phase protein response. *Vet. Microbiol.,* 137: 165-171.

Araghy, S.A. (2007). Etiological study of calf pneumonia by analysis of bronchoalveolar fluid. Ph.D. Thesis Veterinary Faculty, University of Tehran.

Aslan, V., Maden, M., Erganis, O., Birdane, F.M. and Corlu, M. (2002). Clinical efficacy of florfenicol in the treatment of calf respiratory tract infections. *Vet. Q.,* 24(1): 35-39.

Autio, T., Pohjanvirta, T., Holopainen, R., Rikula,U., Pentikainen, J., Huovilainen, A., Rusanen, H., Soveri, T., Sihvonen, L. and Pelkonen, S. (2007). Etiology of respiratory disease in non-vaccinated, non-medicated calves in rearing herds. *Vet. Microbiol.,* 119: 256–265.

Azmat, J., Khattak, M., Munir, S., Jamal, Q. and Hussain, M. (2013). Antibiotic Susceptibility and Molecular analysis of bacterial pathogen *Pasteurella multocida* isolated from Cattle. *Journal of Applied Pharmaceutical Science*, 3(4): 106-110.

de Jong, A., Thomas, V., Simjee, S., Moyaert, H., El Garch, F., Maher, K., Morrissey, I., Butty, P., Klein, U., Marion, H., Rigaut, D. and Vallé, M. (2014). Antimicrobial susceptibility monitoring of respiratory tract pathogens isolated from diseased cattle and pigs across Europe: the VetPath study. *Veterinary Microbiology*, 172: 202-215.

Härtel, H.S., Nikunen, E., Neuvonen, R., Tanskanen, S., Kivelä, L., Sover, P.T. and Saloniemi, H. (2004). Viral and Bacterial Pathogens in Bovine Respiratory Disease in Finland. *Acta Vet. Scand.,* 45: 3-4.

Holman, D.B., Timsit, E. and Alexander, T.W. (2015). The nasopharyngeal microbiota of feedlot cattle. *Sci. Rep.,* 5:155-157.

Kamran, M., Ahmad, M.U.D., Anjum, A.A., Maqbool, A., Muhammad, K., Nawaz, M., Hudda, N.U., Sana, S. and Ali, M.A. (2014). Studies on the antibiotic sensitivity pattern of isolates of *P. multocida* from buffaloes. *The Journal of Animal and Plant Sciences,* 24(5): 1565-1568.

Kirby, W. M. M. and Bauer, A. W. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493-496.

Mahmud, M.A.A., Islam, M.N., Khan, M.F.R., Hussain, M.M. and Rahman M.M. (2016). Prevalence and antibiotic sensitivity of bacteria isolated from respiratory system of cattle. *Int. J. Nat. Soc. Sci.*, 3(2): 21-29.

Narang, A., Singh, C., Kumar, A., Gupta D.K. (2023). Novel Work on Collection of Tracheo-bronchial Aspirates in Pneumonic Cattle. *Indian J. Anim. Res.,* B-5142: 1-4.

Panciera, R.J. and Confer, A.W. (2010). Pathogenesis and pathology of bovine pneumonia. *Veterinary Clinics of North America: Food and Animal Practice,* 26: 191-214.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. (1994). Clinical Veterinary Microbiology. Wolfe Publishing, London. ISBN 0-7234-1711-3.

Sayed, S.M. and Zaitoun, A.M.A. (2009). Aerobic bacterial pathogens of pneumonic feedlot buffalo-calves, in Assiut Governorate, Egypt. *Ass. Univ. Bull. Environ. Res.*, 12 (1): 55-61.

Seker, E., Kuyucuoglu, Y. and Konak, S. (2009). Bacterial examinations in the nasal cavity of apparently healthy and unhealthy Holstein cattle. *J. Anim Vet Adv.,* 8(11): 2355-2359.

Szeredi, L., Jánosi, S. and Pálfi, V. (2010). Microbiological and pathological examination of fatal calf pneumonia cases induced by bacterial and viral respiratory pathogens. *Acta Vet Hung.*, 58: 341–356.

Vaid, R.K., Shanamugasundaram, K., Boora, A., Riyesh, T., Bera, B.C, Shukla, B.N., Anand, T., Virmani, N., Barua, S., Rana, N., Singh, K.P., Malik, P. and Singh, R.K. (2012). Sporadic outbreak of Haemorrhagic Septicaemia in Buffalo calves in an organized farm. *Veterinary Practitioner,* 13(2): 326-329.

Received: December17, 2024
Accepted: April 14, 2025