pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 34(3) : 161-164 (2017)
http://dx.doi.org/10.17555/jvc.2017.06.34.3.161

Journal of Veterinary Clinics
www.ksvc.or.kr

Bacterial Pathogens and Their Antimicrobial Susceptibility in Calves with Summer Pneumonia

Sungwhan Lee, Junhee Kim and Doo Kim[1]

College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Korea

(Received: February 19, 2017 / Accepted: May 28, 2017)

Abstract : Bovine respiratory disease (BRD) is one of the most important diseases in calves. It causes a huge economic loss in farms. BRD in calves is concentrated during winter because of the cold weather and lack of ventilation. However, BRD during summer in calves has continuously been a problem in farms. But there is no study about pathogens of summer pneumonia in calves and antimicrobial susceptibility in Korea. Therefore, aims of this study were to identify the pathogens and their antimicrobial susceptibility in calves with summer pneumonia. One hundred and one calves (2 weeks to 5 months after birth) with clinical sign of BRD from 5 farms were selected. After sampling by deep nasal swab, bacterial isolation and identification was conducted. Also, antimicrobial susceptibility test was performed. *Pasteurella* spp (49.4%), *Staphylococcus* spp (21.5%), *Actinomyces* spp (12.9%), *E coli* (10.7%), and *Mannheimia haemolytica* (5.3%) were isolated. The patterns of isolated pathogens from each farm were various. Also, the susceptibility of bacteria to antibiotics was showed a variety of patterns in each farm.

Key words **:** calves, summer pneumonia, bacterial pathogens, antimicrobial susceptibility.

Introduction

Bovine respiratory disease (BRD) is one of the most important diseases for cattle and it causes a huge economic loss for farms. BRD causes increased death losses as well as medication costs, labor, and lost production (5,7,10,16). The causes of BRD are multifactorial, but in cases of severe disease, viral infection and bacterial infection are almost always involved, usually in combination with stress. A wide variety of different stressors and agents may be involved in the disease process. In addition, environmental factors (eg, dust, temperature, humidity, and inadequate ventilation) enhance the transmission of infectious agents among animals (17). One or more bacterial or viral agents are commonly found as a commensal in upper respiratory tract in healthy cattle with no ill effects. The respiratory pathogens often enter the lower respiratory tract, but are usually expelled by muco-cilliary clearance or inactivated by immune system. However, under stress, the immune response against all the pathogens may be compromised, and the infection established, resulting in BRD (10,18). Mixing of the recently weaned calves from different sources, confinement in drafty or humid and poorly ventilated barns, and exposure to increment weather are associated with the increased risk of fatal BRD outbreaks in feedlots (10).

Numerous infectious agents have been isolated from cases of BRD. Among bacteria, *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Actinomyces pyogenes, and Mycoplasma bovis* are commonly isolated or generally accepted to be important contributors to BRD (1,3,19). *Pseudomonas aeruginosa, Escherichia coli, Streptococcus* spp*, Staphylococcus* spp*, Moraxella* spp*, and Salmonella* spp are the uncommonly isolated or uncertain importance in BRD (16). Virus involved in the development of BRD are mainly bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis virus (IBRV), bovine respiratory syncytial virus (BRSV), bovine coronavirus (BCV), and parainfluenzatype-3 virus (PI-3V) (3,10,16). Most of these pathogens are considered omnipresent in cattle populations. BRD is usually caused by two or more infectious agents acting together by these pathogens. However, some agents can also cause significant disease alone. Also, the relative importance of these organisms in BRD pathogenesis was different between farms, depending on which pathogens are most prevalent and how they interact together (10,16). Therefore, the clinical and epidemiologic characteristics, and diagnosis to pathogens will be first considered individually.

Most outbreaks of BRD occur within one month of housing in the autumn/early winter. Autumn-born calves are generally more severely affected than older spring-born calves (10,16). Also in cold weather, drawing veils in stable to prevent outside cold air lead a lots of ammonia gas, dust, moisture congestion and lack of ventilation; resulting aggravate the calves respiratory immune-system (17). However, BRD in calves during summer has continuously been a problem as well as during winter. Yet, there is no study on bacterial pathogens in calves with summer pneumonia and also neither their antimicrobial susceptibility in Korea. So, the purpose of this study is to identify the bacterial pathogens in calves with summer pneumonia and to conduct antimicrobial susceptibility test on isolated bacterial pathogens in Korea.

[1]Corresponding author.
E-mail : kimdoo@kangwon.ac.kr

Table 1. Bacterial pathogens isolated from calves with summer pneumonia by farms

Group	No. of samples	*Pasteurella* spp	*Staphylococcus* spp	*Actinomyces* spp	*E coli*	*Mannheimia haemolytica*	**Total**
Farm 1	20	15	1	1	0	0	**17**
Farm 2	20	7	1	1	7	1	**17**
Farm 3	21	10	13	5	1	1	**30**
Farm 4	20	7	4	5	1	0	**17**
Farm 5	20	7	1	0	1	3	**12**
Total	**101**	**46**	**20**	**12**	**10**	**5**	**93**

Materials and Methods

Herd and sample selection

From June to August in 2016, one hundred and one calves (2 weeks to 5 months after birth) with clinical sign of BRD from 5 farms (Seosan, Farm 1; Paju, Farm 2; Pyeongchang, Farm 3 and farm 4; and Andong, Farm 5) were selected (Table 1). These calves had 4 and above of total respiratory score according to calf respiratory scoring criteria (CRSC) (15), and were sampled by deep nasal swab (4,20).

Bacterial isolation and identification

Samples were cultured on 5% sheep blood agar and chocolate agar within 6 hours after sampling. And plates were incubated at 37°C for 24~48 hours in aerobic condition. Following presumptive identification of bacteria by morphological features of colonies, Gram's stain, and other biochemical tests, identification of bacteria was conducted with appropriate API® kit and API-web software of bioMeriuex (St. Louis, MO, USA) (2).

Antimicrobial sensitivity test

Antimicrobial susceptibility was conducted by disc diffusion method according to the protocols of National Committee for Clinical Laboratory Standards (NCCLS) (8). The 8 paper antimicrobial susceptibility test discs including ampicillin (AM, 10 μg), cephalothin (CF, 30 μg), chloramphenicol (C, 30 μg), erythromycin (E, 15 μg), nofloxacin (NOR, 10 μg), penicillin (P, 10U), sulfamethoxazole (23.75 μg) / trimethoprim (1.25 μg, SXT), and tetracycline (TE, 30 μg) were purchased from Becton, Dickinson and Company (MD, USA).

Results

To identify the bacterial pathogens in calves with summer pneumonia, bacterial pathogens were isolated from 101 calves with 4 or above of total respiratory score and identified with API kit, and their antimicrobial susceptibility test were conducted by disc diffusion method.

Bacterial isolation and identification

Ninety three bacterial strains were isolated from 101 deep nasal swab samples from calves with summer pneumonia. The 46 strains of *Pasteurella* spp (49.4%), 20 strains of *Staphyloccocus* spp (21.5%), 12 strains of *Actinomyces* spp (12.9%), 10 strains of *E coli* (10.7%), and 5 strains of *Mannheimia haemolytica* (5.3%) were identified (Table 1). Comparing pathogens isolated by farms, *Pasteurella* spp were the most frequently isolated in 4 farms. In the Farm 2, *Pasteurella* spp and *E coli* was isolated in same frequency (7 strains). However, in Farm 3, *Staphylococcus* spp were the most prevalent isolates (Table 1).

Antimicrobial sensitivity of bacterial strains

All strains of *Staphyloccocus* spp were sensitive to CF, NOR and SXT, and showed a variety of susceptibility to other antibiotics. *Mannheimia haemolytica* strains were 100% sensitive to NOR and showed 80% sensitivity to other antibiotics except EM. *Pasteurella* spp strains showed more than 80% of sensitive to antibiotics except AM and E. The strains of *E coli* showed the lowest sensitivity to antibiotics except NOR and the strains of *Actinomyces* spp also showed relatively low sensitivity to all antibiotics (Fig 1).

Bacterial strains of each farm showed a variety of susceptibility to antibiotics. In Farm 1, bacterial strains showed more than 80% of sensitivity to 8 antibiotics, however, some strains showed resistant to antibiotics. In case of Farm 2, all pathogens showed very low sensitivity to antibiotics compared with other farms. In Farm 3, pathogens showed more than 80% of sensitivity to antibiotics except AM and P, however, some strains showed resistant to antibiotics. In Farm 4, pathogens showed 100% of sensitivity to C and CF, and TE showed the lowest sensitivity. In Farm 5, pathogens showed 100% of sensitivity to NOR and the other antibiotics showed around 80% of sensitivity (Fig 2).

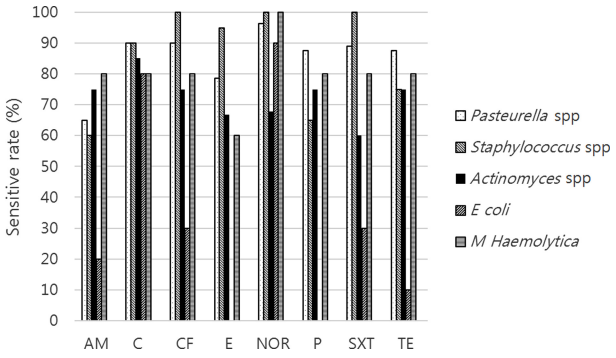

Fig 1. Antimicrobial susceptibility of 93 isolates from calves with summer pneumonia. AM, ampicillin; C, chloramphenicol; CF, cephalosporin; E, erythromycin; NOR, norfloxacin; P, penicillin; SXT, trimethoprim/sulfamethoxazole; and TE, tetracycline.

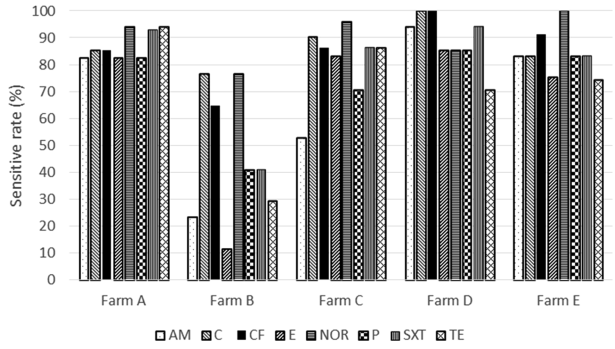

Fig 2. Antimicrobial susceptibility of the isolates from calves with summer pneumonia by farms. AM, ampicillin; C, chloramphenicol; CF, cephalosporin; E, erythromycin; NOR, norfloxacin; P, penicillin; SXT, trimethoprim/sulfamethoxazole; and TE, tetracycline.

Discussion

This study focuses on examination of the prevalence of bacterial pathogens in cases of summer pneumonia in calves. Bacterial pathogens isolated were *Pasteurella* spp (49.4%), *Staphylococcus* spp (21.5%), *Actinomyces* spp (12.9%), *E coli* (10.7%), and *Mannhaemia haemolytica* (5.3%). Like other studies in calves, *Pasteurella* spp was the most frequently isolated bacterial pathogen in this study (1,3,19). *P multocida* which commonly isolated from calves result both enzootic calf pneumonia to young dairy calves and shipping fever complex (9). *P multocida* is a pathogenic gram-negative bacterium rod or cocco-bacilli, however, sometimes this bacteria was not associated with BRD or economic losses in calves (1). The infection with several different viruses and mycoplasma may predispose to *P multocida*-induced pneumonia. The most important animal and environmental risk factors for summer pneumonia are supposed to weaning, overcrowding, hot weather, and poor ventilation (1).

Staphylococcus spp was the second most frequently isolated bacteria in this study. *S aureus* causes one of the most common types of chronic, subclinical, acute, per-acute mastitis. Although, *Staphylococcus* spp was uncommonly isolated or uncertain importance in BRD, these pathogens omnipresent in epithelial cells of calves skin and can be infected to calves which has a low immune system, resulting BRD (16).

Mannheimia haemolytica is the most common and important cause of respiratory disease in feedlot cattle and is a significant component of severe and fatal bronchopneumonia in all neonatal calves (11,14). A commensal of the nasopharynx, *M haemolytica* is an opportunist, gaining access to the lungs when host defenses are compromised by active viral and mycoplasmal infection and stress factors (14,16). The isolation rate of *M haemolytica* was only 5.3% in this study. However, previous study has been documented that *M haemolytica* play a prominent role in BRD and isolated the second frequently (1).

Actinomyces spp and *E coli* also contribute to respiratory disease complex. In this study, *Actinomyces* spp and *E coli* were isolated 12.9% and 10.7%, respectively. *Actinomyces pyogenes* is opportunistic bacterium that related to miscellaneous pyogenic infections in animals. Mastitis (45.1%), abscesses (18.0%), pneumonia (11.1%), and lymphadenitis (9.0%) were the most common clinical manifestations (13). In this study, *Actinomyces* spp was isolated from calves that showed a severe BRD sign. *E coli* strains were isolated most frequently in Farm 2. In this dairy farm, calves with clinical BRD sign were under 2 weeks after birth. These young calves tend to suckle farm environment and their nose, this behavior is thought to inoculate *E coli* in feces to respiratory tract of calves.

In this study, bacterial pathogens isolated from each farm showed a various patterns in susceptibility to antibiotics. Susceptibility of *Pasteurella* spp to antibiotics was relatively high. The isolates of *Pasteurella* spp showed more than 80% of sensitivity to CF, C, TE, P, SXT, and NOR. And *Staphylococcus* spp isolates also had high susceptibility to antibiotics. However, the strains of *E coli* showed the lowest sensitivity to antibiotics except NOR. These patterns of susceptibility to antibiotics of isolates are believed to be related to the long-term use of antibiotics in each farm (6,12). In Farm 2, antimicrobial susceptibility of bacterial isolates was relatively low compared with other farms. According to the local veterinarian, the prevalence of BRD of this farm has been high and the owner of the farm has used antibiotics over the long period without prescription. Therefore, for effective treatment of calves with BRD, antimicrobial sensitivity test and prudent choice of antibiotics are necessary for each farm.

Overall, main bacterial pathogen in calves with summer pneumonia was *Pasteurella* spp. Antimicrobial susceptibility of bacteria of each farm to antibiotics was showed various patterns. For effective management of calves with summer pneumonia, isolation and identification of pathogens and their antibiotic susceptibility test are necessary for each farm.

Acknowledgements

This study was supported by 2016 Research Grant from Kangwon National University (No. D1000656-01-01).

Reference

1. Dabo SM, Taylor JD, Confer AW. Pasteurella multocida and bovine respiratory disease. Anim Health Res Rev 2007; 8: 129-150.
2. Dance DA, Wuthiekanun V, Naigowit P, White NJ. Identification of Pseudomonas pseudomallei in clinical practice: use of simple screening tests and API 20NE. J Clin Pathol 1989; 42: 645-648.
3. Francoz D, Buczinski S, Bélanger AM, Forté G, Labrecque O, Tremblay D, Wellemans V, Dubuc J. Pathogens in Québec dairy calves and their relationship with clinical status, lung consolidation, and average daily gain. J Vet Intern Med 2015; 29: 381-387.
4. Godinho KS, Salasola P, Renoult E. Use of deep nasopharyngeal swabs as a predictive diagnostic method for natural respiratory infections in calves. Vet Rec 2007; 160: 22-25.
5. Griffin D. Economic impact associated with respiratory disease in beef cattle. Vet Clin North Am Food Anim Pract 1997; 13: 367-377.
6. Lamm CG, Love BC, Krehbiel CR, Johnson NJ, Step DL.

Comparison of antemortem antimicrobial treatment regimens to antimicrobial susceptibility patterns of postmortem lung isolates from feedlot cattle with bronchopneumonia. J Vet Diag Invest 2012; 24: 277-282.
7. Schneider MJ, Tait JRG, Busby WD, Reecy JM. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores. J Anim Sci 2009; 87: 1821-1827.
8. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing, 15th Informational Supplement, NCCLS Antimicrobial Susceptibility Testing Standards M2-A8 and M7-A6, 2005.
9. Quinn PJ, Carter ME, Markey B, Carter GR. Pasteurella species. In: Clinical Veterinary Microbiology. London: Mosby. 1994: 254-258.
10. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Pneumonic pasteurellosis of cattle. In: Veterinary Medicine, 10th ed. London: Saunders Elsevier. 2007: 934-956.
11. Rainbolt S, Pillai DK, Lubbers BV, Moore M, Davis R, Amrine D, Mosier D. Comparison of Mannhaemia haemolytica isolates from an outbreak of bovine respiratory disease. Vet Microbiol 2016; 182: 82-86.
12. Rerat M, Albini S, Jaquier V, Hussy D. Bovine respiratory disease: Efficacy of different prophylactic treatments in veal calves and antimicrobial resistance of isolated Pasteurellaceae. Prev Vet Med 2012; 103: 265-273.
13. Ribeiro MG, Risseti RM, Bolaños CA, Caffaro KA, de Morais AC, Lara GH, Zamprogna TO, Paes AC, Listoni FJ, Franco MM. Trueperella pyogenes multispecies infections in domestic animals: a retrospective study of 144 cases (2002 to 2012).Vet Q 2015; 35: 82-87.
14. Rice JA, Carrasco-Medina L, Hodgins DC, Shewen PE. Mannheimia haemolytica and bovine respiratory disease. Anim Health Res Rev 2007; 8: 117-128.
15. McGuirk S. Disease management of dairy calves and heifers. Vet Clin North Am Food Anim Pract 2008; 24: 139-153.
16. Smith BP. Lower respiratory tract diseases. In: Large Animal Internal Medicine, 4th ed. St. Louis, MO: Mosby Elsevier. 2009: 601-642.
17. Snowder GD, Van Vleck LD, Cundiff LV, Bennett GL. Bovine respiratory diseases in feedlot cattle: Environmental, genetic and economic factors. J Anim Sci 2006; 84: 1999-2008.
18. Srikumaran S, Kelling CL, Ambagala A. Immune evasion by pathogens of bovine respiratory disease complex. Anim Health Res Rev 2007; 8: 215-229.
19. Van Donkersgoed J, Potter AA, Mollison B, Harland RJ. The effect of a combined Pasteurella haemolytica and Haemophilus somnus vaccine and a modified-live bovine respiratory syncytial virus vaccine against enzootic pneumonia in young beef calves. Can Vet J 1994; 35: 239-241.
20. Vangeel I, Antonis AFG, Fluess M, Reigler L, Peters AR, Harmeyer SS. Efficacy of a modified live intranasal bovine respiratory syncytial virus vaccine in 3-week-old calves experimentally challenged with BRSV. Vet J 2007; 174: 627-635.