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Isolation of *Pasteurella maltocida* and *Mannheimia hemolytica* from Pneumonic Calves and Their Antibiotic Susceptibility in Harar and Haramaya, Eastern Ethiopia

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Abstract: Cross sectional study was conducted from November 2016-March 2017 on calve pneumonic pasteuerellosis on dairy farms in Haramaya and Harar districts to determine the causative agents and their antimicrobial susceptibility pattern. Laboratory examination of 120 nasal swab samples from pneumonic calves was analysed to isolate Pasteurella. The results indicated that the overall prevalence of Pasteurella is 47(39.2%) including 28(23.3%) in Harar and 19 (15.8%) in Haramaya. 74.5% of the isolates were *M. haemolytica* and 25.5% were *P. multocida*. Pneumonic pasteurellosis was significantly associated with herd size and bedding. According to this study antimicrobial susceptibility test results, chloramphenicol (89.4%) and tetracycline (80.9%) were the most effective drugs, whereas ampicilin (53.2%) was the intermediate drug. Penicillin-G (10.6%) and streptomycin (14.9%) were inefficient drugs; while vancomycin was almost inactive against both isolates. In conclusion besides the use of best drug for the treatment of pasteuerellosis periodic bacterial isolation and antibiotic susceptibility test should be done before treating with antibiotics except for critical ones.

Key words: Antibiotic · Dairy Calf · Pneumonia · Pasteurellosis

INTRODUCTION

Animal production is now facing new challenges since demographic growth, urbanization and economic development are all contributing to the increasing in food requirement of which animal products are very important. In Ethiopia cattle are important sources of dairy product and the future of any dairy production depends on the successful raising of calves and heifers for replacement, Calves are the future herd and keeping them in a fit and healthy condition not only makes an effect on a livestock farms' efficiency but also contributes to the economy and production outputs in a country [1].

Despite the large livestock population of Ethiopia, the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal production systems, reproductive inefficiency, management constraints and general lack of veterinary care [2].

The most frequent calf disease syndrome is pneumonia [3, 4]. Calves develop a bacterial pneumonia most often caused by *Mannheimia Haemolytica*, *Pasteurella Multocida*, *Haemophilus Somnus* and *Mycoplasma* species. *M. haemolytica* and *P. multocida* are the most common bacterial isolates from respiratory disease cases than the others [5, 6, 7, 8].

Many factors have been associated with an increased risk of infectious disease during the first 90 days of life. For example, lack of adequate passive immunity have increased death rate and cause calves more susceptible to infectious diseases [9]. Persistence of the agents that caused calf disease in the environment was the major reason for out broken of calf problems on the dairy production [10].

Vaccination and other preventative measures have been widely adopted for the control of calf disease worldwide, the benefit of appropriate antimicrobial therapy decreases treatment costs and, increases health and wellbeing but the emergence of microbial tolerance of different antimicrobial agents has become a well-known phenomenon, which represented a major concern [11]. Because of this antimicrobial susceptibility testing was very crucial in the selection of an appropriate antimicrobial agent for the treatment of pneumonia. Moreover in Ethiopia, calf disease and death were ranked

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next to mastitis as the second biggest problem for dairy production [12]. But there was a gap on identifying the major bacteria, involved in calf disease particularly on pneumonia and antimicrobial susceptibility testing for the appropriate treatment of the case. Therefore, the objectives of this paper are: To identify and rank associated risk factors according to their contribution to pneumonic calves' morbidity and to indentify and determine antibiotic susceptibility testing of bacterial isolates.

MATERIALS AND METHODS

Study Animals: The sampling units for the study were calves of up to six month of age. All dairy calves, most bred (Local Zebu, Holstein-Friesian and cross) born in farm during the study period would be enrolled. The sample size was determined based on the availability of the suspected case with pneumonia having the sign of cough and nasal discharge. A total 120 calves were suspected and the samples were collected from nasal cavity for further laboratory examinations.

Study Design and Sampling Technique: Cross sectional type of study was conducted from November 2016 to April 2017. Total of 18 dairy farms, composed of 10 smallholder and 8 large dairy farms, were included. The farm was purposively selected based on the availability of calf, the willingness and support of the owners to participate on this research activity. All suspected pneumonic calves in selected farms were incorporated in the study. The health status of each calf was evaluated by clinical examination. Calves free from nasal discharges and coughing were classified as healthy whereas sick calves showed abnormal body condition or erected hair coat, nasal discharge, coughing and weakness. Laboratory examination of nasal swabs from sick calves for the diagnosis of disease causative agents was done and each bacterial isolate was tested for antimicrobial susceptibility. In addition questionnaire survey was also conducted to assess the farm management practices (Including, herd size, animal housing, cleaning and disinfection, feeding of the calves and colostrum feeding).

Sample Collection Procedure: Nasal specimens were collected from all suspected pneumonic calves aged from newborn to six month present on the farm at the time of the visit. Samples were collected directly from nasal cavity of suspected none treated pneumonic calves taking nasal swabs by using sterile test tubes with peptone water up to 5ml which were transported to Haramaya University, Veterinary Microbiology Laboratory. Collected samples were clearly labeled including the information on the name of farms, date of sampling and tag number of the calves and samples were processed as soon after collection. Major risk factors including lactation, sex, age, exotic genetic influence, colostrum feeding time and method of colostrum feeding, quantity of milk feeding, feeding and type of feeds, watering, health care and management practices were also recorded during sample collection.

Detection of Pasteurella Species: Sediments mixed with 5% peptone water was inoculated onto MacConkey agar plate, then, further sub culturing to 5% ovine blood agar plates (Oxoid) for examining of hemolysis after incubation at 37°C for 24 hours. A single colony was sub-cultured and tested by Gram staining and biochemical tests including catalase and oxidase test [13]. Isolate, characteristic of Gram negative, coccobacilli shape, catalase (3% H2O2) positive, cytochrome oxidase positive and having the typical colony morphology of Pasteurellaceae, was taken into consideration for identification procedure. Differentiation between *P. Multocida* and *M. Hemolytica* were determined according to hemolysis on blood agar (Blood agar base No: 2, Oxoid, containing 5% ovine blood), growth on Mac Conkey agar (Oxoid), catalase (3% H2O2) and oxidase (After incubation 37°C for 24 hours). Motility was examined in SIM medium (Oxoid); urease activity was tested in Urea Medium (Urea agar base, Oxoid); sugar fermentation, indole production was also examined by adding Kovac's reagent to a 48-hours of cultured samples [13, 14].

Antimicrobial Susceptibility of Pasteurella: Antibiotic susceptibility test was done by the disc diffusion technique [15, 16]. The pure culture colony suspension of the isolate was made using sterile physiological saline and adjusted to 0.5 McFarland standards then spread to Muller Hinton agar using sterile cotton swap and allowed to stand for 3-5 minutes to observe any excess moisture from the medium before the antimicrobial discs were applied. And the a ring of each disc (Oxoid, England) containing single concentration of each antimicrobial agent was then placed onto the inoculated surface using sterile forceps, gently pressed with the point of the forceps to ensure complete contact with the agar surface and left for 30 minutes for diffusion of the antibiotics in the disc. The plates were inverted upside down and

incubated at 37°C for 18 to 24 hours. The result is evaluated for clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a measuring caliper and interpreted as susceptible, intermediate and resistant according to Clinical and Laboratory Standards Institute break points [16]. Each isolate was tested against commonly used antimicrobials for treatment of pneumonia. These antimicrobials were chloramphenicol, penicillin-G, streptomycin, vancomycin, ampicillin and tetracycline

Data Management and Analysis: Data obtained from the research was exported to SPSS 20.0 for appropriate statistical analysis. The occurrence of Pasteurella from the total pneumonic calves was determined by using descriptive statistics. Chi square (χ^2) was used to measure the association between the different risk factors and occurrence of Pasteurella causing calf pneumonia. Effects were reported as statistically significant if p-value is less than 5 % ($P<0.05$).

RESULTS

Most of the farms kept exotic Holstein Friesian (HF) (76.6%), cross breed (HF and local) (23.4%) and local breed (0%) calves. In most of dairy farms, male calves were vended soon after birth and females were kept for replacement stock (Table 1). Regarding different management system, some of the farm practiced navel treatment during birth of calves; bedding was provided for calves in all large dairy farms and in none of the smallholder dairy farms. Most of the farms kept their calves' in-group pens separating from the cows, while others kept calves together with cows due to lack of enough space. Almost 98% of the dairy farmers had knowledge of the advantage of colostrum feeding but only 23.4% were feed colostrum with in less than 6 hours of birth and just at 6 hours 40.4%, while 36.2%, were feed after 6 hours of birth. All study farms fed whole milk for calves two times daily by bucket feeding with the exception of few smallholder farms that allowed calves to suckle their dams. No special starter feed was used in any of the farms; rather the same feed given to cows was used for calves. These include straw, hay and concentrates. Weaning age varied from farm to farm; most (74.5%) of farms weaned calves at the age of 4 month whereas few smallholder farms (25.5%) weaned at above 4 months of age. In general, the weaning age was lower for male calves, mostly under three months (Table 2).

Laboratory examination of 120 nasal swab samples from pneumonic calves indicated 47 (39.2%) of overall Pasteurella, with 28 (23.3%) in Harar and 19 (15.8%) in Haramaya. From these total isolates of Pasteurella 74.5% of the isolates was *M. haemolytica* and 25.5% was *P. multocida*.

Antimicrobial susceptibility testing was performed for all bacteria isolated from pneumonic calves. As shown on table 3, high resistance by Pasteurella isolates was seen against vancomycin (93.6%), penicillin (87.2%) and streptomycin (72.3%). On the other hand, 89.4, 80.9 and 53.2% of isolates were susceptible to chloramphenicol, tetracycline and ampicillin, respectively.

DISCUSSION

In all study dairy farms, there were no professionals animal health technicians either as fully employed or part time employed to deal with health aspects of the farms. Rather the farms call private veterinary practitioners whenever their animals face health problems. From farm managers or owners that mentioned calf health problems as a problem in dairy production and the majority of them complained calf pneumonia as major cause of calf death in the younger age. In this study, Pasteurella species were isolated in 39.2% of the calves, which was taken from dairy farms. *M. haemolytica* was accounted for 74.5% and it was highly prevalent than *P. multocida*. Pneumonic pasteuerellosis was significantly associated with herd size (0.046) and bedding (p-0.009) and not significantly associated with the sex, age, breed, CFT and DFT of suspected calves (Table 2).

In this particular study the recruitment of animals was based on their clinical signs and symptoms to pneumonia and the identification of Pasteurella species among these animals could be reliable as causative agents. Comparing the two Pasteurella spp, *M. haemolytica* constituted 74.5% of the total indicated that, *M. haemolytica* was the major causative agent involved in calve pneumonic pasteurellosis. This is consistent with previous reports [17, 18]. *M. haemolytica*, which is a normal flora of the upper respiratory tract, but in animals with suppressed host defence mechanism, most commonly in young growing cattle under certain conditions associated with debilitation, nutrition and climatic factors may lead to bronchopneumonia [19, 20, 21]. Although the percentage isolation was relatively low (12.5%), the possible role of *P. multocida* in calve pneumonia should not be under estimated.

Table 1: Prevalence of calves'pneumonia according to breed, age and sex categories

Category		*P. Haemolytica*	*P. Multocida*	Negative	Chi-Square (P-value)
Breed	Exotic	25(69.4%)	11(30.6%)	58(79.5%)	4.062(0.398)
	Cross	10(90.9%)	1(9.1%)	13(17.8%)	
	Local	0	0	2(2.7%)	
	Total	35	12	73(100%)	
Age	<3month	21(72.4%)	8(27.6%)	34(46.6%)	2.783(0.249)
	>3month	14(77.8%)	4(22.2%)	39(53.4%)	
	Total	35	12	73(100%)	
Sex	Female	27(71.1%)	11(28.9%)	63(86.3%)	2.052(0.358)
	Male	8(88.9%)	1(11.1%)	10(13.7%)	
	Total	35	12	73(100%)	

Table 2: Prevalence of calves' pneumonia based on management of risk factors

		P. haemolytica	*P. multocida*	Negative	Chi-Square (P-value)
Herd Size	Large	20(90.9%)	2(9.1%)	31(43.5%)	6.156(0.046)
	Small	15(60%)	10(40%)	42(57.5%)	
	Total	35	12	73(100%)	
Navel	Untreated	18(64.3%)	10(35.7%)	41(56.2%)	3.858(0.145)
	Treated	17(89.5%)	2(10.5%)	32(43.8%)	
	Total	35	12	73(100%)	
CFT	<6 hours	9(81.8%)	2(18.2%)	20(27.4%)	0.964(0.915)
	At 6 hours	14(73.7%)	5(26.3%)	31(42.5%)	
	>6 hours	12(70.6%)	5(29.4%)	22(30.1%)	
	Total	35	12	73(100%)	
DCF	24 hours	16(88.9%)	2(11.1%)	35(47.9%)	5.427(0.246)
	24hr-4days	13(72.2%)	5(27.8%)	24(32.9%)	
	>4days	6(54.5%)	5(45.5%)	14(19.2%)	
	Total	35	12	73(100%)	
Weaning	<4 month	27(77.1%)	8(22.9%)	51(68.9%)	0.782(0.677)
	>4 month	8(66.7%)	4(33.3%)	22(30.1%)	
	Total	35	12	73(100%)	
Housing	Separate	21(80.8%)	5(19.2%)	39(54.4%)	1.251(0.535)
	Non separate	14(66.7%)	7(33.3%)	34(46.6%)	
	Total	35	12	73(100%)	
Bedding	<once/week	2(33.3%)	4(66.7%)	8(11%)	13.569(0.009)
	>Once/week	28(90.3%)	3(9.7%)	53(72.6%)	
	once/week	4(44.4%)	5(66.6%)	12(16.4%)	
	Total	35	12	73(100%)	

TCF1st = Time of first colostrum feeding, DCF = Duration of colostrum feeding

In our study, according to the antimicrobial susceptibility test results, chloramphenicol (89.4%) and tetracycline (80.9%) were the most effective drugs; where as ampicilin (53.2%) was the intermediate drug while penicillin-G (10.6%) and streptomycin (14.9%) were inefficient drugs and vancomycin was totally inactive against both isolates (Table 3). Increase in resistance against antibiotics in both *P. multocida* and *M. haemolytica* isolates have been reported by the work of many scholars [6, 22, 23]. The result is in line with the literature which state as chloramphenicol is highly effective and well-tolerated broad spectrum antibiotic to many genera of Gram-positive and Gram-negative bacteria [19]. However, this result contradicts the findings of Aschalew [18] who reported tetracycline as ineffective drug of choice.

One of the interesting findings of the present study demonstrates the highest resistance of Pasteurella isolates against vancomycin (93.6%). In contrast Esra *et al.* [24] reported that vancomycin 95 and 90% as the most effective antibiotics against *M. haemolytica* isolates. This might be due to difference in the strain of the isolate or due to the existence of host factors that may affect the action of drug in bovines. Besides in this study *P. multocida* showed resistance to penicillin-G, in contrary to literature [25] which indicates that most strains

Table 3: Antibiotic susceptibility of Pasteurella

		P. *Haemolytica*	*P. multocida*	Total
Penicillin G	Resistant	31(66%)	10(21.3%)	41(87.2%)
	Intermediate	0(0%)	1(2.1%)	1(2.1%)
	Susceptible	4(8.5%)	1(2.1%)	5(10.6%)
	Total	35(74.5%)	12(25.5%)	47(100%)
Streptomycin	Resistant	24(51.1%)	10(21.3%)	34(72.3%)
	Intermediate	4(8.5%)	2(4.3%)	6(12.8%)
	Susceptible	7(14.9%)	0(0%)	7(14.9%)
	Total	35(74.5%)	12(25.5%)	47(100%)
Chloramphenicol	Resistant	2(4.3%)	0(0%)	2(4.3%)
	Intermediate	2(4.3%)	1(2.1%)	3(6.4%)
	Susceptible	31(66%)	11(23.4%)	42(89.4%)
	Total	35(74.5%)	12(25.5%)	47(100%)
Vancomycin	Resistant	32(68.1%)	12(25.5%)	44(93.6%)
	Intermediate	3(6.4%)	0(0%)	3(6.4%)
	Susceptible	0(0%)	0(0%)	0(0%)
	Total	35(74.5%)	12(25.5%)	47(100%)
Tetracycline	Resistant	1(2.1%)	3(6.4%)	4(8.5%)
	Intermediate	4(8.5%)	1(2.1%)	5(10.6%)
	Susceptible	30(63.8%)	8(17%)	38(80.9%)
	Total	35(74.5%)	12(25.5%)	47(100%)
Ampicillin	Resistant	9(19.1%)	3(6.4%)	12(25.5%)
	Intermediate	8(17%)	2(4.3%)	10(21.3%)
	Susceptible	18(38.3%)	7(15%)	25(53.2%)
	Total	35(74.5%)	12(25.5%)	47(100%)

of *P. multocida* are susceptible to penicillin-G. This difference strengthens the recommendation. Kaan [26] stated that "Antibiotic susceptibility profiles of *P. multocida* and *M. haemolytica* help veterinarians to choose appropriate antibiotic against bovine respiratory disease; however, antibiotic susceptibility studies should be renewed periodically".

CONCLUSIONS

Pneumonic pasteuerellosis is the major disease of pneumonic calves in the area and *M. haemolytica* is the most common cause. Large herd size and uncleaned bedding were among the risk factors for the disease. Chloramphenicol, tetracycline and ampicillin were effective drugs whereas penicillin G and vancomycin were mostly inactive against the isolates. Measures such as, improving management practices by providing optimal sanitation, providing good quality hay and water and supplement to reduce the disease risk. Besides the use of best drug for the treatment of pasteuerellosis periodic bacterial isolation and antibiotic susceptibility test should be conducted before treating with antibiotics except for critical ones. Moreover, further stereotyping and molecular techniques are needed to identify the isolate to the strain level.

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REFERENCES

1. Bhatti, S.A., M. Sarwar, M.S. Khan and S.M.I. Hussain, 2007. Reducing the age at first calving through nutritional manipulations in dairy buffaloes and cows: A review. Pakistan Veterinary Journal, 27(1): 42-47.
2. Sisay, A. and A. Ebro, 2008. Growth performance of Boran and Their Semmintal cross calves. In The Proceeding of 6th national conference of the Ethiopian society of animal production (ESAP). Addis Ababa, Ethiopia, pp: 157-162.
3. Ferede, Y., H. Mazengia, T. Bimrew, A. Bitew, M. Nega and A. Kebede, 2014. Pre-Weaning Morbidity and Mortality of Crossbred Calves in Bahir Dar Zuria and Gozamen Districts of Amhara Region, Northwest Ethiopia. Open Access Library Journal, 1: e600.

4. Wudu, T., B. Kelay, H.M. Mekonnen and K. Tesfu, 2008. Calf morbidity and mortality in smallholder dairy farms in Ada'a Liben district of Oromia, Ethiopia. Tropical Animal Health and Production, 40: 369-376.
5. Shewen, P.E. and J.A.R. Conlon, 1993. Pasteurella. In: Pathogenesis of Bacterial Infections in Animals. 2 ed., C. L. Gyles & C. O. Thoen, Iowa State University Press, Ames, U.S.A.
6. Welsh, R.D., L.B. Dye, M.E. Payton and A.W. Confer, 2004. Isolation and Antimicrobial susceptibilities of bacterial pathogens from bovine pneumonia, 1994–2002. Journal of Veterinary Diagnostic Investigation, 16: 426-431.
7. K.B. Biyashev, Zh. S. Kirkimbayeva, A.Z. Maulanov, B.Biyashev, A.Zh. Makbuz and G.B. Kuzembekova 2014. Pasteurellosis as the Most Common Infection Affecting the Respiratory System of Calves in Southern Kazakhstan. Global Veterinaria, 12(6): 829-834.
8. Fentahun, M., F. Tsegaw, T. Zenebe, M. Tadesse and Robel Abebaw, 2017. Review on Ovine Respiratory Disease Complex in Ethiopia: Significance, Causes and Possible Management Methods. Academic Journal of Animal Diseases, 6(1): 13-22.
9. Svensson, C., K. Lundborg, U. Emanuelson and S. Olsson, 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Preventive Veterinary Medicine, 58: 179-197.
10. Heinrichs, A.J. and O.M. Radostits, 2001. Health and production management of dairy calves and replacement heifers. In: Herd Health, Food Animal Production Medicine, 3rd Ed., Radostits, O.M. (W.B. Saunders Company, Philadelphia), pp: 333-395.
11. Izzo, M., V. Mohler and J. House, 2011. Antimicrobial susceptibility of Salmonella isolates recovered from calves with diarrhoea in Australia. Australian Veterinary Journal, 89: 402-408.
12. International Livestock Center for Africa (ILCA), 1994. ILCA Annual Program Report 1993/1994. Addis Ababa, pp: 73-74.
13. Quinn, P.J., M.E. Carter, B.K. Markey and G.R. Carter, 2000. Pasteurella species. In: Clinical Veterinary Microbiology. 4th ed., Mosby- Yearbook Europe Limited, pp: 254-258.
14. MacFaddin, J.F., 1999. Biochemical Tests for Identification of Medical Bacteria. 2nd edn., The Williams and Wilkins Co.
15. Bauer, A.W., W.M. Kirby, J. C. Sherris and M. Turck, 1966. "Antibiotic susceptibility testing by a standardized single disk method," The American Journal of Clinical Pathology, 45(4): 493-496.
16. Clinical and Laboratory Standards Institute (CLSI), 2011. Performance Standards for Antibiotics Susceptibility Testing, Twenty- First Informational Supplement. CLSI, 31.
17. Tesfaye, S., 1997. Serological and bacteriological investigation of P. haemolytica serotypes in sheep in the high lands of wollo, North Eastern Ethiopia. DVM thesis, faculty of veterinary medicine Addis Ababa University, Ethiopia.
18. Aschalew, Z., 1998. A study on Pneumonic Pasteurellosis in North Shewa. DVM thesis, faculty of veterinary medicine Addis Ababa University, Ethiopia.
19. Aiello, S.E. and A. May, 1998. The Merck Veterinary Mannual. 8th edition. New Jersey. USA: Merck and Co, Inc, pp: 1053-1057.
20. Belege, T., K. Alamirew, A. Ketema, W. Kiflie and M. Endashaw, 2017. Ruminant Pneumonic Pasteurellosis: Review on Epidemiology, Pathogenesis and Virulence Mechanism. Academic Journal of Animal Diseases 6(2): 30-39.
21. Tadele, K., T. Fikadu, T. Zenebe and G. Kebede, 2015. Review on the Pneumonic Pasteurellosis of Cattle. Academic Journal of Animal Diseases, 4(3): 177-184.
22. Mohamed T. Ragab, Waleed H. Hassan and Wafaa A. Osman 2015. Isolation, Identification and Antibiogram Studies of Pasteurella multocida Isolated from Sheep and Goats in Siwa Oasis. Global Veterinaria, 14(4): 589-594.
23. Catry, B., A. Decostere, S. Schwarz, C. Kehrenberg, A. De Kruif and F. Haesebrouck, 2006. Detection of tetracycline resistant and susceptible pasteurellaceae in the nasopharynx of loose group housed calves. Veterinary Research Community, 30: 707-715.
24. Esra, S., K. Yahya and K. Selahattin, 2009. Bacterial examinations in the nasal cavity of apparently healthy and unhealthy Holstein cattle. Journal of Animal & Veterinary, 8: 2355-2359.
25. Quinn, P.J., B.K. Markey, M.E. Carter, W.J. Donnelly and F.C. Leonard, 2002. Veterinary Microbiology and Microbial Diseases. 1st edition. USA: Blackwell Science, pp: 49-52.
26. Kaan, O., K.K. Serpil and C. Tayfun, 2010. Frequency and antibiotic susceptibility of Pasteurella multocida and Mannheimia haemolytica isolates from nasal cavities of cattle. Turk Journal of Veterinary and Animal Science, 34: 91-94.