International Journal of Microbiological Research 10 (1): 01-04, 2019
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2019.01.04

Isolation and Characterization of Pasteurella from Respiratory Tract of Cattle Slaughtered at Ambo Municipal Abattoir, West Shewa, Ethiopia

Masresha Yehualashet

National Institute for Control and Eradication of Tsetse Fly and Trypanosomiasis,
Kality Tsetse Fly Mass Rearing and Irradiation Center, Addis Ababa, Ethiopia

Abstract: The study was conducted in Ambo town from February to May 2013 on isolation and characterizationof *Pasteurella* from the respiratory tracts of cattle slaughtered at Ambo Municipal abattoir. The objectiveof the study was to isolate and characterize *Pasteurella* species causing pneumonic pasteurellosis in cattle in the study area.Atotal of 4 nasal swabs and 24 lung tissue samples were aseptically collected, labeled and transported in ice-box to the department of Veterinary Laboratory Technology Ambo University. The collected samples were microbiologically examined and biochemical tests were conducted following the standard procedures to determine prevalence of *Pasteurella* species accordingly, all collected 4 nasal swabs showed growth, whereas, 9 out of 24 examined lung tissue samples were positive for growth. The result of biochemical tests carried out reveled the recovery of 10 isolates of *M.haemolytica* with prevalence rate of 35.7%, 2 isolates of *Pasteurella multocida* with aprevalence rateof 7.14%and 1 isolate of *Pasteurella trehalosi* with prevalence rate of 3.6%. These showed that *M. haemolytica* was the most predominate species isolated followed by *P. multocida* and *P. trehalosi*. Therefore, further detailed study should be conducted in the study area specially to look at the association of risk factors that could pre dispose cattle to pneumonic pasteurellosis.

Key words: Ambo Municipal Abattoir · Cattle · Pasteurella · Pneumonic Pasteurellosis · Respiratory Tract

INTRODUCTION

Respiratory diseases in food animals are due to complex factors that often interact to produce diseases. Various conditions such us inclement weather, weaning, transportation, poorly ventilatedhousing and nutritional deficiencies are known to play apredisposing role as the animal's immunityweakens.In such condition, flare upof the normal flora of the upper respiratory tract and subsequent infection of the lungs is well documented [1].

Bovine pneumonicpasteuerellosis is one of the bacterial diseases of cattle, characterized by bronchopneumonia and pleurisy. It is commonly caused by *Mannheimia haemolytica*, *Pasteurella multocida* and *Pasteurella trehalosi* [2].

These bacteria are normal inhabitants of the upper respiratory tract of cattle in normal unstressed animal and do not usually cause adisease. However, animals which are exposed to various stress factors such as environmental temperature, shipping, crowding and exposure to different viral infectionsbecome more susceptible to growth of the organisms in the lower respiratory tract, resulting in sever pneumonia [3, 4].

Pasteurella multocida has wide host range area and *Mannheimia haemolytica* is largely restricted to ruminates causing bovine pneumonic pasteurellosis which can cause significant economic losses [5]. For instance, pasteurellosis due to bacteriausually results in mortality and contributes significantly to the economic losses estimated between$500 million and $1 billion annually in North America.

Respiratory tract infections are of common occurrence invarious species of domestic animals. However, pneumonic pasteurellosis, also known as respiratory mannheimiosis, is the most common example with a wide prevalence in ruminant animals. The disease

Corresponding Author: MasreshaYehualashet, National Institute for Control and Eradication of Tsetse fly and Trypanosomiasis, Kality Tsetse fly mass rearing and Irradiation Center, Addis Ababa, Ethiopia P.O. Box: 19917, Addis Ababa, Ethiopia. Tel: -(+251)0923244959.

in its typical clinical form, is highly infectious, often fatal and with very serious economic mortality in fed lot animals in which the disease accounts for approximately 30% of the total cattle deaths worldwide [6].

Therefore, eventhough, pneumonic pasteurellosis is economically very important disease and distributed worldwide, to the authors' knowledge, there are only limited works on identification and characterization of Pasteurella organism from respiratory tract of cattle in Ambo town. Hence the objective of the study was to isolate and characterize Pasteurella species causing pneumonic pasteurellosis in cattle.

MATERIALS AND METHODS

Study of Animals: The study was carried out on adult cattle brought to Ambo Municipal abattoir for slaughter. Theanimals included in the study were selected based on the clinical signs of pasteurellosis such as sudden onset of the disease, dyspnea, coughing, nasal discharge, increase body temperature that can be observed during anti-mortem inspection.

Sample Collection: A total of 4 nasal swab samples were collected during ante- mortem inspection by inserting sterile cotton-taped application sticks in to nasal passageways after proper cleaning and disinfection of the external nares of clinically pneumonic cattle present for slaughterer. They were put in sterile test tubes containing transport medium Amies Transport Medium , labeled and kept in cool box or ice box and transported to Ambo University, Department of Veterinary Laboratory Technology for further processing and culture. In addition to this a total of 24 pneumonic lung tissues were collected aseptically from freshly slaughtered cattle after disinfecting the external surface with 70% alcohol to minimized surface contamination using sterile scissors and tissue forceps, pieces of lung tissue were collected separately in to sterile screw capped universal bottle and similarly transported in ice box for further processing and bacteriological study. Samples that were not immediately processed were stored in refrigerator to maintain the viability of the microorganisms in the sample.

Bacteriological Examination: The swabs were removed from bottle and streaked over the platecontaining blood agar base supplemented with 5-7% of sheep blood. Moreover, samples that were stored in refrigerator were removed from the refrigerator and thawed at room temperature for 30 minutes before streaking onto the blood agar. Whereas, the lung samples were incised, cut in to pieces in sterile Petridis and streaked onto a blood agar using sterile wire loop. The plates were then labeled andincubated aerobically at 37°C for 24-48 hours following the standard protocol [7, 8].

After taking note of cultural growth characteristics: colony size (moderate), shape(round)and color (grayish) were recorded and Pasteurella suspected colonies were sub cultured on nutrient agar media by selecting from mixed colonies grown on primary blood agar media. Subsequently, the colonies were stained using Gram-stain technique and examined for microscopicmorphology. Gram negative bipolar rod typical cellular characteristics of Pasteurella were observed under oil immersion objectives of light microscope, selected and subjected to further tests.

Identification was made on the basis of colony morphology, haemolysis, Gram's staining reaction and biochemical tests. Biochemical characteristics of the isolates were determined by using catalase, triple sugar iron(TSI) testand growth on MacConkey agar following the standard procedures [9, 10].

Data Analysis: Data generate or collected were organized and simple descriptive statistical tools such as frequency and percentage were applied to summarize and determine the prevalence of species of Pasteurella isolates.

RESULTS

Bacteriological Examination Results: The microbiological examination of the 24 cultured lung tissue samples on blood agar indicated that 9(37.50%) gave growth. Whereas, the remaining 15 (62.50%) of the lung tissue showedno growth. All 4 (100%) nasal swabs bacteriologically examined revealed growth on primary culture media used (Table 1).

Biochemical Test Results: Biochemical tests conducted to identify the species of Pasteurellaaredisplayed in (Table 2). Accordingly, 2(7.14%) of the isolates were Pasteurella multocida, 10(35.7%) were Mannheimia haemolytica and the remaining 1(3.6%) was found tobe Pasteurella trehalosi. This result revealed that *Mannheimia haemolytica* is the most prevailed Pasteurella species isolated followed by *Pasteurella multocida*and *Pasteurella trehalosi*wasthe least prevalent species isolated. Three of the *M. haemolytica* and one *P. trehalosi* were isolated from nasal swabs. All *P. multocida* and the remaining seven *M. haemolytica* isolates were recovered from lung specimens (Table 2).

Table 1: Number of samples that showed growth of bacteria

		Growth		No growth	
Typeof sample	Total sample	Frequency	Percentage	Frequency	Percentage
Nasal swab	4	4	100.00	-	-
Lung tissue	24	9	37.50	15	62.50

Table 2: Summary of biochemical tests results

	Pasteurella species identified		
Tests conducted	*Pasteurella multocida*	*M. haemolytica*	*Pasteurella trehalosi*
Growth on MacConkey	-	+	+
Colony morphology	Round, Greyish, Mucoid	Round, Greyish, Mucoid	Round, Greyish, non mucoid
Gram stain reaction	Gram negative rods	Gram negative rods	Gram negative rods
Catalase test	+	+	-
TSI test	Yellow/yellow	Yellow/yellow	Yellow/yellow
Motility test	-	-	-
Haemolysis	Non haemolytic	Haemolytic	Haemolytic
Total isolates	2 (7.14%)	10 (35.7%)	1 (3.6%)

Key: + = positive, - = negative, yellow/yellow = yellow slant and yellow butt

DISCUSSION

Examination of 4 nasal swabs and 24 lung tissue samples collected from the respiratory tract of cattle revealed the recovery of 10 isolates of *M. haemolytica* with prevalence rate of 35.7%, 2 isolates of *Pasteurella multocida* with a prevalence rate of 7.14% and 1 isolate of *Pasteurella trehalosi* with prevalence rate of %. Three of *M. haemolytica* isolates and one*P. trehalosi* were isolated from nasal swabs. Whereas, all*Pasteurella multocida* isolates were from the lung tissue.

This result is in agreement with the study of Roth and Perino [11] which showed the prevalence rate of *M. haemolytica* is the highest followed by *P. multocida* and *P. trehalosi*. The same research demonstrated that *P. trehalosi* is the mostly isolated from nasal swabs of cattle as compared to lung tissue. These results may reinforce the idea that *P. trehalosi* are rare in cattle and play no part in epidemic disease.

Various studies [12, 13] have also reported *P. multocida* from the respiratory tract of sheep and cattle, isolation rates were much lower Comparing the three Pasteurella species. *M. haemolytica* constituted the highest share in this study, indicating that *M. haemolytica* is the major causative agent involved in bovine pasteurellosis. This is consistent with previous reports [14-17] which reported that *M. haemolytica* is the main causative agent of pasteurellosis in cattle.

CONCLUSIONS

The present study was conducted from February to may, 2013 in Ambo town, on isolation and characterization of *Pasteurella* species from respiratory tract of cattle slaughtered at Ambo Municipal abattoir, which revealed that 13 Pasteurella species were isolated and characterized. *M. haemolytica* was the most predominant species isolated followed by *P. multocida* and *P. trehalosi* with the prevalence rate of 35.7, 7.14 and 3.6% respectively. The present study has indicated that the prevalence of pasteurellosis is very high. However, due to shortage of time it was impossible to see the associational factors that can predispose animals for pneumonic pasteurellosis in the study area.

Therefore, based on these concluding remarks the following recommendations are forwarded

- Animals presented for slaughter should be provided with adequate rest before being slaughtered and thoroughly examined for any disease.
- Detailed study should be conducted to determine status of pasteurellosis in the study area.

REFERENCES

1. Radostits, O.M., C.C. Gray, D.C. Blood and K.W. Hinchcliff, 2000. Veterinary Medicine: A text book of disease of cattle, sheep, pig, goats and horses. 9th ed. Saunders, W.B.., London, England, pp: 337-470.
2. Yates, W.D., 1982. Review on infection bovine rhinotracheitis, shipping fever pneumonia and viral, bacterials synergism in respiratory disease of cattle. Can. J. Comp. Med., 46: 225-265.
3. Fillion, L.G., P.J. Willson, H. Bielfeedt, L.A. Babiuk and R.G. Thomson, 1984. The possible role of stress in the induction of pneumonic pasterellosis can. Inf. Comp. Med., 48: 268-274.

4. Babiuk, L.A., M. Morsy, M. Compos and R. Harland, 1995. Viral-Bacterial synergistic, infection pathogensis in cattle. Trop. Inf. Dis., 30: 250-285.
5. Quinn, P.J., B.K. Marky, M.E. Carter, W.J. Donnely and F.C. Leonard, 2002. Veterinary Microbiology and Microbial disease. Black well Science Ltd, UK and pp: 137-143.
6. Boudreaux, C.M., 2004. A novel strategy of controlling bovine pneumonic pasteurellosis: Transfecting the upper respiratory tract of cattle with a gene coding for the antimicrobial peptide cecropin. B.M.Sc. Thesis, Louisiana State University, USA.
7. Allan, S.W., S.W. Maheswaran and W.L. Kader, 1985. The role of Virulence factor produced pasteurella haemolytica in the pathogenesis of bovine pneumonic pasteurellosis: review and hypothesis. British Veterinary Journal, 149: 183-193.
8. Carter, G.R., 1984. Isolation and identification of bacteria from clinical specimens. 4th ed. Eds., Charles, C. and Thomas, USA, pp: 19-30.
9. Quinn, P.J., M.E. Carter, B.K. Markey and G.C. Carter, 1994. Clinical Veterinary Microbiology. Mosby, New York, Philadelphia, pp: 254-258.
10. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger and W.C. Winn, 1997. Miscellaneous Fastidious Gramm-Negative Bacilli. Color Atlas and Textbook of Diagnostic Microbiology. 5th ed. Lippincott- Raven, Philadelphia, pp: 1307-1371.
11. Roth, J.A. and L.J. Perino, 1998. Imminology and Prevetion of infection in feedlot cattle. Vet. Clin. Nort. Am., 14: 233-256.
12. Highlander, S.K., 2000. Inactivation of pasterella (Mannheimia) haemolytica Leukotoxin causes partial attenuation of virulence in a calf challenge model. Infect. Imm., 68: 3916.
13. Welsh, R.D., L.B. Dye, M.E. Payton and A.W. Confer, 2004. Isolation and antimicrobial susceptibilities of bacterial pathogens from bovine pneumonia. J.Vet. diag. Invest., 16: 426.
14. Radostits, O.M., D.C. Blood and C.C. Gay, 1994. Veterinary Medicine : A text book of disease of cattle, sheep, pig, goats and horses. 8th ed. Beilere, Tindal, London, England, pp: 501-599.
15. Schreuer, D., C. Schuhmacher, S. Touffet, A. Defert, C. Adlam and A. Banting, 2000. Evaluation of the efficacy of a new combined (Pasteurella) Mannheimia haemolytica serotype A1 and A6 vaccine in pre-ruminant calves by virulent challenge. Cattle Practice, 8(1): 9-12.
16. Dabo, S., J. Taylor and A. Confer, 2007. Pasteurella multocida and bovine respiratory disease An. Heal. Rev., 8: 129-150.
17. Donachie, E., 2000. Bacteriology of bovine respiratory disease. Cattle Practice, 8: 5-7.