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Isolation and Identification of Bacterial Organisms from Cattle with Acute Respiratory Infections in Punjab, India

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Abstract

A study was undertaken on bovines affected with acute respiratory infections following lumpy skin disease (LSD) epidemics in 2022 to identify the causative organisms. Nasal swabs and blood samples (n=40) of cattle with acute respiratory disease from veterinary hospitals and dairy farms in and around the Ludhiana region were collected. The clinical samples were initially processed for bacterial isolation using blood agar and brain heart infusion agar. For confirmation, the organisms were subjected to Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) analysis. The study revealed the presence of several bacterial organisms viz., Mannheimia varigena, Escherichia coli, Corynebacterium (C. xerosis, C. stationis and C. camporealensis), Acinetobacter (A. towneri, A. boumanii, A. indicus), Pseudomonas (P. aeruginosa, P.otitidis), Staphylococcus (S. haemolyticus, S. sciuri, S. cohnii, S.xylosus, S. hominis, S. aureus), Rothia nasimurium, Streptococcus (S. pluranimalium, S. dysgalactiae) Achromobacter xylosoxidans, Comamonas kerstersii and Aerococcus viridians in nasal swabs of cattle with acute respiratory distress and Stapylococcus haemolyticus and Corynebacterium stationis in the blood samples of cattle. The results of the present study indicated that LSD could be one of the risk factors predisposing the cattle population to bacterial pathogens, which had led to respiratory diseases with high mortality among the cattle population of this region.

Keywords: Bovine Respiratory Disease, Cattle, Bacterial Pathogens, MALDI-TOF, Punjab.

Introduction

Bovine respiratory disease (BRD) is a complex, multifactorial disease of cattle that primarily affects the respiratory system (Werid *et al*., 2024). The syndrome is caused by different genera of bacteria and viruses and is facilitated by environmental and host factors (Werid *et al*., 2024). Several viruses, such as bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza-3 virus (PI-3), bovine coronavirus (BCoV) and bovine adenoviruses are key contributors to BRD (Kapil and Basaraba, 1997; Hodgins *et al*., 2002). These viruses often weaken the respiratory system and compromise the immune system, making cattle more susceptible to secondary bacterial infections, which cause the most severe symptoms. Among the bacteria, *Mannheimia haemolytica*, *Pasturella multocida*, *Mycoplasma spp*., and *Histophilus somni* are some of the major pathogens that cause BRD in bovine. These pathogens can also co-infect cells in the respiratory system thereby making specific treatment extremely difficult. Lumpy skin disease virus (LSDV), which is caused by a capripoxvirus can also be considered as a risk factor for BRD because of its potential to contribute to secondary infections. Although LSD is not directly associated with BRD, the systemic effects of LSDV can predispose cattle to respiratory complications. Infection with LSDV leads to lesions in the respiratory tract, including the trachea and lungs, which might facilitate secondary bacterial infections, a common pathway for BRD development (Liang *et al*., 2022). Besides, the immunosuppressive effects of LSDV can compromise the respiratory defense mechanisms, increasing the susceptibility to BRD pathogens (Ratyotha *et al*., 2022).

BRD is a major source of economic loss because it affects primarily the feedlot young cattle (Joshi *et al*., 2016). The percentage of morbidity and mortality depends on the management system in place, prevention programs and the extent of the pathogen involved (Maier *et al*., 2019). The economic impacts of BRD are substantial and multifaceted, affecting cattle producers, feedlots, and the beef and dairy industries globally (Madureira Ferreira *et al*., 2024). The direct economic losses associated with BRD include treatment costs, mortality, reduced growth performance, and lower carcass value (Blakebrough-Hall *et al*., 2020). Since BRD is a complex, multifactorial disease, early and accurate diagnosis plays a vital role in minimizing both animal health impacts and economic losses. Diagnosis of specific bacterial pathogens associated with BRD often relies on necropsy, bacterial culture, immunohistochemical testing, or PCR assay (Kamel *et al*., 2024).

BRD remains a significant health concern in the cattle and buffalo population of India, leading to considerable economic losses due to decreased productivity and increased mortality. Recent studies have provided insights into the prevalence and impact of BRD and its associated pathogens across various regions of the country (Katoch *et al*., 2017; Gangil *et al*., 2020; Kamdi *et al*., 2020; Jaibhaye *et al*., 2022). In the present report, we aimed to investigate the cases of severe BRD with high mortality among the cattle population in Punjab region of India.

Materials and Methods

Sampling and isolation

Several cases of acute respiratory infections with high mortality were observed following the outbreaks of LSD in the Ludhiana region of Punjab during 2022-2023. To investigate the cases of BRD, both nasal swabs and blood samples from 40 nos. cattle described with acute respiratory disease were collected from veterinary hospitals and dairy farms in Ludhiana region (Fig.1). The detailed history was obtained directly from the owner and clinical manifestations were recorded. All the samples were inoculated initially on Blood agar and incubated at 37°C for 18-24 h and examined for haemolysis patterns in order to differentiate between pathogenic and commensal species. Isolates that exhibited positive growth on blood agar were further sub-cultured onto brain heart infusion (BHI) agar to obtain individual colonies and subsequently confirmed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis.

Identification of the Organism Using MALDI-TOF Analysis

The confirmation of the organisms was done using MALDI-TOF analysis. All the isolates exhibiting growth on blood agar were subsequently sub-cultured onto BHI agar to obtain pure colonies, which were then identified using MALDI-TOF mass spectrometry. Briefly, a single colony from the BHI culture plate was directly smeared onto a spot on the MALDI-TOF target plate using a sterile toothpick and then air-dried. To the spot 1 microlitre (µl) of 70% formic acid was applied and air dried. The spot was then overlaid with 1µl of matrix solution. After air drying

the spots at room temperature, the samples were analyzed by using the Bruker biotyper system (Bruker Daltonics, Germany).

Fig.1: The figure represents a cattle died within 72 hours of showing acute respiratory distress. The affected animal had a history of lumpy skin disease virus infection before the clinical manifestation of acute respiratory distress.

Results and Discussion

Primary isolation on blood agar revealed the presence of bacterial colonies (Fig. 2) in all the nasal swabs from 40 nos. of cattle. However, among the blood samples only two samples showed positive growth on blood agar. The positive isolates were examined for haemolytic pattern in order to differentiate between pathogenic and commensal species. Based on haemolytic patterns observed on blood agar, we were able to identify and differentiate potential pathogenic bacteria such as *Streptococcus dysgalactiae*, *Staphylococcus aureus*, and *E. coli*, which were subsequently confirmed using MALDI-TOF (Table 1).

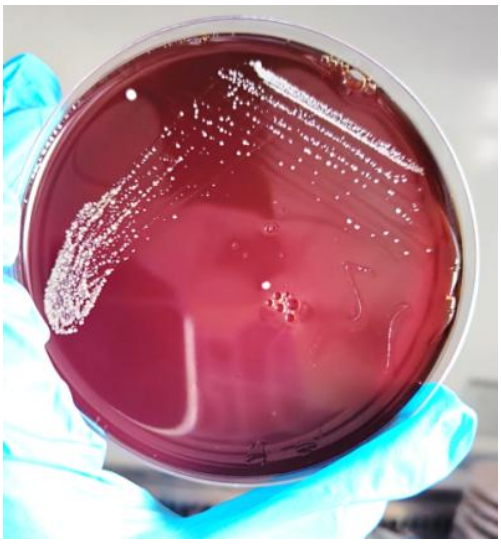

Fig. 2: Primary isolation of clinical samples in blood agar

On MALDI-TOF analysis, the presence of several bacterial organisms, viz., *Mannheimia varigena*, *Escherichia coli, Corynebacterium spp*. (*C. xerosis, C. stationis* and *C. camporealensis*), *Acinetobacter spp.* (*A. towneri, A. boumanii, A. indicus*), *Pseudomonas spp*. (*P. aeruginosa, P.otitidis*), *Staphylococcus spp. (S. haemolyticus, S. sciuri,*

S. cohnii, S.xylosus, S. hominis, S. aureus), Rothia nasimurium, Streptococcus spp. (S. pluranimalium, S. dysgalactiae), Achromobacter xylosoxidans, Comamonas kerstersii, and *Aerococcus viridians* were recorded in all the nasal swabs of cattle with acute respiratory distress (Table 1). The predominant organisms in the nasal swabs of cattle were recorded to be *Pseudomonas aeruginosa, Rothia nasimurium* and *Staphylococcus cohnii* (Fig. 3). On the other hand, only two organisms, *viz*., *Stapylococcus haemolyticus* and *Corynebacterium station*is were identified among the blood samples of cattle. The majority of the isolated organisms confirmed using MALDI-TOF were commensals (*Corynebacterium spp.*, *Pseudomonas spp*. and *Rothia spp*.) which are typically part of the normal flora and not associated with disease under healthy conditions.

Table 1: Bacterial organisms identified in nasal swabs of cattle with acute respiratory distress using MALDI-TOF.

Sl. No	Organisms identified in nasal swabs of cattle	Total
1	*Acinetobacter towneri*	3
2	*Mannheimia varigena*	1
3	*Pseudomonas aeruginosa*	4
4	*Staphylococcus haemolyticus*	1
5	*Acinetobacter baumanii*	1
6	*Staphylococcus cohnii*	1
7	*Streptococcus pluranimalium*	1
8	*Rothia nasimurium*	4
9	*Staphylococcus sciuri*	2
10	*Pseudomonas otitidis*	1
11	*Achromobacter xylosoxidans*	2
12	*Staphylococcus cohnii*	4
13	*Comamonas kerstersii*	1
14	*Corynebacterium xerosis*	1
15	*Corynebacterium stationis*	1
16	*Aerococcus viridans*	2
17	*Acinetobacter indicus*	2
18	*Staphylococcus xylosus*	2
19	*Streptococcus dysgalactiae*	1
20	*Corynebacterium camporealensis*	1
21	*Staphylococcus hominis*	1
22	*Staphylococcus aureus*	2
23	*Escherchia coli*	1
	Total	**40**

BRD is a significant health concern in India's cattle and buffalo populations, leading to substantial economic losses due to decreased productivity, treatment expenses and high morbidity and mortality. The contributing factors of BRD in India is multifactorial involving viral agents like IBRV, BRSV and BCoV, bacterial pathogens and environmental stressors such as transportation and overcrowding (Patil *et al*., 2022). LSD can be considered as a risk factor for BRD in several indirect but clinically important ways. While LSD and BRD are caused by different pathogens, their interaction can occur due to overlapping impacts on the immune system, stress levels and overall animal health. In severe cases of LSDV affected animals, ulcerative lesions could be observed on the mucosal surfaces of the eye and in the oral/nasal cavities, resulting in prolonged mastication, lacrimation and nasal discharges (Babiuk *et al*., 2008). Systemic immune suppression can be observed in severely affected animals with LSDV that increases the susceptibility to opportunistic infections, including those pathogens that are associated with BRD such as *Mannheimia haemolytica*, *Pasteurella multocida*, IBRV and BRSV (Haider *et al*., 2024).

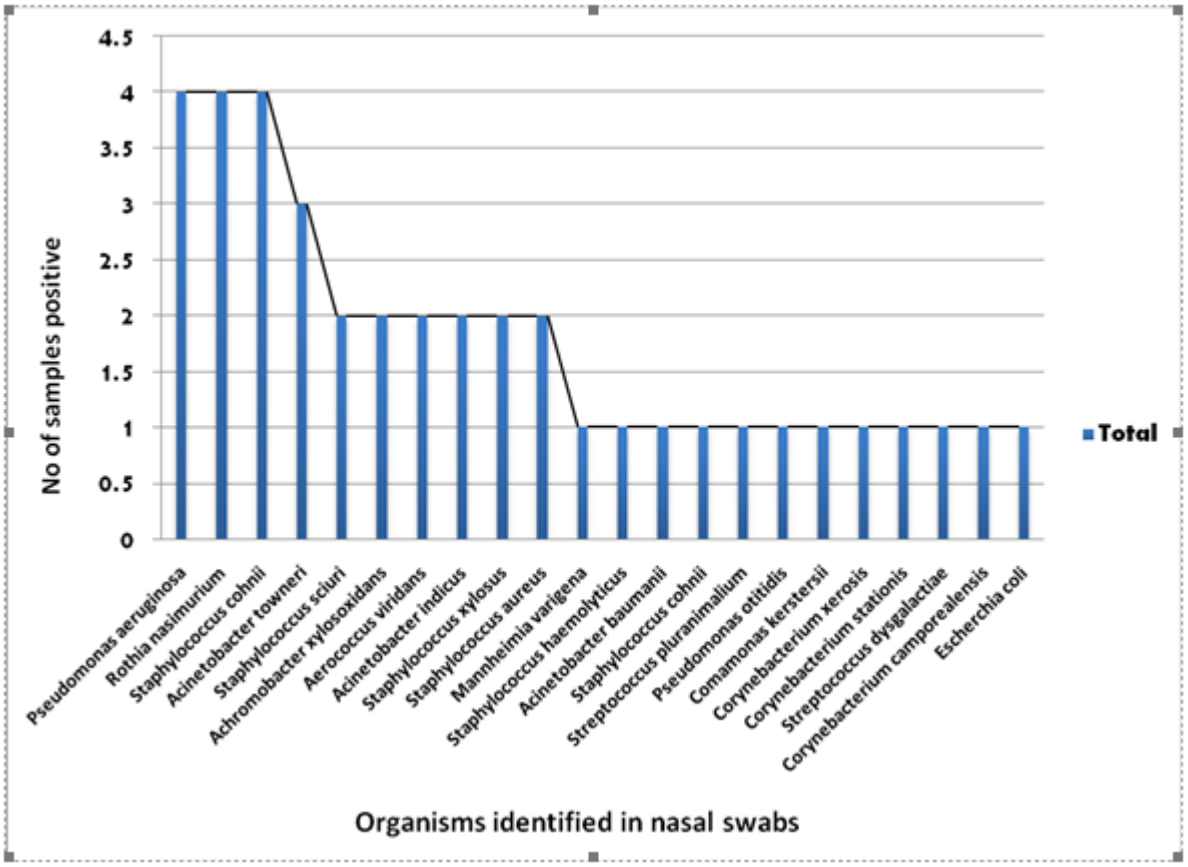

Fig. 3: Graph representing the predominant bacterial organisms (*P. aeruginosa, R. nasimurium* and *S. cohnii*) identified in nasal swabs of cattle

Previous reports on BRD from India revealed the association of different viruses and their pathological role in BRD complex. Investigation of 406 weaner calves up to ≤1 year with respiratory distress and pulmonary lesions exhibited 0.98% BCoV occurrence (Kamdi *et al*., 2024). Studies on sero-prevalence of BRD complex revealed a seroprevalence of 24% of IBRV and 50% of BRSV in Himachal region of India (Katoch *et al*., 2017). Another investigation of nationwide sero-surveillance of IBR in cattle and buffaloes showed 33.91% and 24.39% seropositivity, respectively, in India (Patil *et al*., 2022). In the present study, *P. aeruginosa, R. nasimurium* and *S. cohnii* were found to be predominant bacterial organisms isolated from the nasal swabs of cattle with BRD along with the detection of other bacterial organisms. The incidences of BRD associated with these organisms were observed following the epidemics of LSD in the region in the year 2022.

Conclusion

The present study revealed the presence of various bacterial organisms in the nasal swabs and blood of cattle associated with BRD with a history of infection with LSD. The results of this study suggest that a previous LSDV infection may act as a predisposing factor for the development of BRD in affected animals. Therefore, proactive measures such as stress reduction, nutritional support and monitoring for respiratory signs during and after LSD outbreaks are essential to mitigate the risk of BRD.

Contribution by Authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all authors and consented to publication.

Conflict of Interests

There is no conflict of interest.

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