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Pasteurella Multocida Strains collected from Lung Infections in Bovines: a comparative study

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Abstract

In the global intensive beef industry, bovine respiratory disease (BRD) is the main factor contributing to significant economic losses. In addition to several risk factors, the development of the disease may also be influenced by Pasteurella multocida, which is thought to be a secondary pathogen. An indication that some swine pneumonia strains may be more virulent than others is the discovery of a few clones in earlier studies on these strains connected to clinical illness. The BRD may have a relationship, but nothing is known about the makeup of the P. multocida populations in the herds. As a result, researchers decided on either phenotypic or genotypic Characterization of strains isolated from respiratory illness-infected calves in 31 different packs in Hungary. Two dominant strain types were found, according to the data. They could be recognized by their capacity to ferment trehalose and their activity as Molecular fingerprints of a-glucosidase obtained using ERIC and M13-PCR. They shared the same taxonomic origin (P. multocida subsp. multocida) and showed minimal phenotypic variation. Even after comparing them to strains recovered from healthy patients, the strain types' independent incidence and place of origin may be indicators of their significance in the illness.

Keywords: Pasteurella multocida, A-glucosidase activity, Trehalose fermentation capability, M13-PCR, ERIC-PCR, and Bovine Respiratory diseases.

Introduction

A Gram-negative bacterium called Pasteurella multocida frequently infects several animal species, including cattle. It is an important pathogen linked to respiratory infections in cattle, which causes the livestock industry to suffer considerable financial losses. An important public health issue is the complex bovine respiratory disease complex (BRDC), brought on by P. multocida lung infections in cattle. A multifactorial illness called BRDC involves interactions between bacterial and viral infections, the environment, and the host's immune system. One of the microorganisms is P. multocida implicated and is thought to be a major factor in both starting and escalating respiratory illnesses in cattle. P. multocida strains differ in their virulence, which causes a variety of infection outcomes and illness severity (1).The pathophysiology of respiratory disorders in cattle must be understood regarding the virulence and trait factors of P. multocida strains identified from lung infections in bovines. Understanding these strains' genetic diversity, gene expression patterns, and infection processes is possible by researching them (2).

Patterns of gene expression and transcriptional response in virulence-related genes in a P.multocida strain isolated from infected cattle's lungs. They compared P. multocida strains

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having varying levels of virulence, as well as genes associated with enhanced pathogenicity and possible virulence markers (3). The analysis used in vitro cultures and lung tissue samples taken from sick cows. The amount of lung injury, bacterial burdens, and mRNA abundance were all connected with the levels of several virulence-related genes' expression. The results contribute in order to comprehend the complex relationships between the bacterium and its host and offer useful insights into the virulence mechanisms of P. multocida strains in bovine lung infections (4). By revealing the factors that determine the pathogenicity of P. multocida strains, effective approaches can be developed for the prevention, diagnosis, and control of respiratory diseases in cattle. It also emphasizes the significance of taking strain-specific changes into account while researching the prevalence and treatment of bovine respiratory illnesses brought on by P. multocida (5).

The goal of the research (6) was to assess resistance phenotype of the P. multocida strain obtained from respiratory illnesses in animals, such as pets and animals used for food production. Time-series data were subjected to nonlinear analysis. data to analyze resistance patterns. It was noted that none of the species exhibited any detectable florfenicol resistance.
The study (7) was used for post-mortem lung swabs for collect 139 P. multocida isolates from cattle affected by BRD in feedlots. Phylogenetic relationships, multi-locus sequence types, lipopolysaccharide genotypes, and capsular serogroups were all identified using whole-genome sequencing (WGS). One of these has a global distribution and is spatially diverse and heterogeneous ST79.
The study (8) aimed to compare the pathogenicity of Pasteurella multocida serotype A strains CQ2 and CQ6, or PmCQ2 and PmCQ6, respectively. They performed in vivo and in vitro transcriptome sequencing analyses on the PmCQ2 and PmCQ6 strains. The results imply that the increased virulence of PmCQ2 over PmCQ6, as compared to PmCQ6, may be largely attributed to these virulence-associated DEGs, particularly those related to capsule formation.
The research (9) was to identify Pasteurella multocida from lung samples with respiratory illness symptoms such as nasal discharge, coughing, and fever, as well as to look into the phenotypic and genotypic characteristics of these bacteria. To identify the genes for capB and capE, a different mPCR procedure was created. In summary, unusual strains may frequently cause pneumonia in agricultural animals.

The objective of study (10) was to assess the incidence and features of multocida Pasteurella isolates from likely pigs in Vietnam. Multiplex polymerase chain reaction (PCR) was used to analyze isolates for virulence-associated gene identification, antibiotic resistance gene detection, and capsular typology. These isolates have high levels of antibiotic resistance, which should serve as a warning against the current overuse of antibiotics in the battle against pathogenic bacteria.
Research (11) revealed that the capsular serogroups, lip polysaccharide (LPS), the multi-locus sequencing kinds, and genotyping of 205 P. multocida isolates found in the lungs of respiratory illnesses in deceased rabbits allowed for their identification. PCR assays were

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used to examine the virulence factors and antibiotic susceptibility was determined. The creation of effective vaccines, methods for controlling pathogens, and epidemic strains
The objective of the research (12) was Pulse field gel electrophoresis (PFGE), and bacteriological methods were used to characterize an isolated P. multocida from sheep and cow lungs. Animals (sheep and cattle) that were both clinically well and sick were examined by PFGE to ascertain their connection. Limited to create a fingerprint was specified. The paper (13) set out to use air-liquid interface (ALI) cultures to look into how Pasteurella multocida (P. multocida) and Interactions between cells of the lungs of cattle. Examine how P. multocida and its host targets interact as they used cultures of properly developed the air-liquid interface (ALI) using cattle airway epithelial cells. They might talk about the germs' effects on the airway cells and how they respond.

Research (14) explores the Pasteurella multocida capsular type A strains with low and high levels of virulence (PMPAN001 and PMPAN007), respectively, of pathogen city were compared. The comparison between multocida songs was completed in vitro and on lung tissues. The results shed light on the mechanisms behind the pathogenesis of pulmonary infections produced by this bacterium in cattle. Study (15) looked at the prevalence of multidrug resistance in the Pasteurella multocida and Mannheimia haemolytica bacteria isolated from BRD cow mortality in North America. The antimicrobial resistance (AMR) study also looked at integrative and conjugative elements (ICEs). In-feed supplements containing metals, macrolides, tetracyclines, and tetracycline-based antibiotics may target these MDR isolates.

The study aimed to determine the prevalent strain types in P. multocida populations and explore their makeup. The study also considered the frequency, place of origin, and probable importance of these strain types in the emergence of bovine respiratory disease (BRD). As a result, to better understand the variety of the bacterial population, P. multocida strain identified in Hungarian cattle herds after being isolated from diseased animals underwent extensive phenotypic and genotypic analysis.

Materials and Methods

Strains of Bacteria

The 31 P. multocida strains under study were gathered between 2019 and 2023 from various Hungarian cattle herds. Due to the correlation between some breed types and years, the incidence of BRD increased from 2019 to 2023 (Figure 1). Sixty-six strains were discovered in the lung tissue of heifers, and five were found in the nasal swabs, lungs, and lungs of calves.

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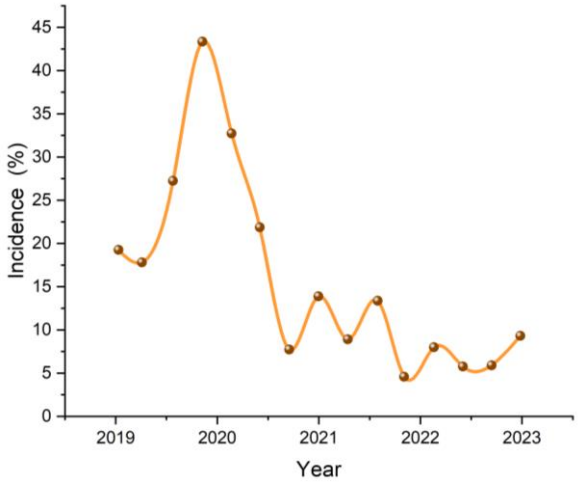

Figure (1) Incidence rate of BRD

Bacteriology was used to identify the strains, which were then maintained in solutions containing 20% skim milk powder at -70 degrees Celsius. They had streaks Colombia agar plates with 5% sheep blood defibrin for in-depth analyses. A biochemical study involved an infusion of isolated colonies of bacteria into the brain-heart infusion broth after the vessels had been cultivated for 24 hours at 37 degrees Celsius. Furthermore, they were streaked on dextrose-starch agar plates for serological examinations.

Characterization of phenotypes

Characteristics of Serology

PCR was used to determine the bacteria's capsular types. The agar gel precipitation test was used to investigate the somatic serogroups.

Characteristics of biochemical

The biochemical tests revealed the ability to ferment sugars or sugar-alcohols such as glucose, lactose, arabinose, sucrose, maltose, xylose, and trehalose in addition to indole production. As a result of the observations, the strains were divided into biovars.

Characterization at the molecular level

The molecular analysis method developed by Chelex was used to isolate the bacterial DNA. The important traits of animal, poison, and capsule were found using a particular multiplex PCR. To determine capsule types other than A, multiplex capsular PCR was utilized. The 16S rRNA gene's PCR-RFLP was used to classify the strain subgroups. The link between the strains was examined using the M13 and PCRs known as ERICs (enterobacterial repetitive intergenic consensus). The reaction mixes for the ERIC and M13 PCRs contained the following components: 25 pmol primers, 2.5 U Dream Taq (Fermentas), 1*PCR buffer, 3.5 mM MgCl2, 200 nM dNTP-mix, and 5 l template DNA. Following Thirty cycles of 93 degrees Celsius for Thirty seconds, 50 degrees Celsius for one minute, and 72 degrees Celsius for seventy seconds, pre-denaturation at 93 degrees Celsius was carried out for three minutes. The final five minutes were spent polymerizing at 72 degrees Celsius. The PCR

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fragments were found using gel electrophoresis on an agarose gel of 1.5% or 2%. Molecular markers called Hyper Ladders II (50–2000 bp, Bioline, Massachusetts, U.S.) were used to analyze the molecular patterns approach utilizing the TREECON software suite. This section examined the impact of various temperatures on the survival and reproduction of P. multocida (Figure 2)

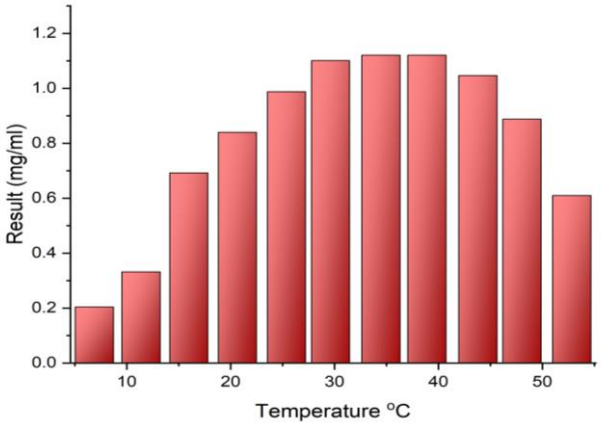

Figure (2) Result of P.multicodia

Results and discussion

Although P. multocida is known to cause respiratory illnesses in different host species (including pigs, rabbits, and poultry), nothing is known about its relationship to BRD. In this study, 31 cattle strains of P. multocida primarily isolated from the lungs of cattle with pneumonia in various Hungarian herds underwent thorough phenotypic and genotypic Characterization. Without regard to their origin, the findings revealed that the strains had similar biochemical, genetic characteristics, and serological. Figure 3 and Table 1 depicts the BRD rates for the bacterial pathogen P. multocida on an annual basis over the five years 2018-2023, there might be seen a considerable increase BDR in P. multocida from 3.70% in first year to 22.80% in final year.

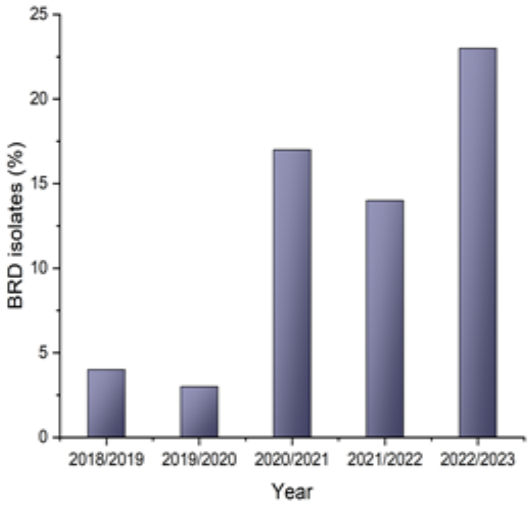

Figure (3) BRD Isolation

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Table (1) Outcome of BRD Isolation

Year	BRD isolates (%)
2018/2019	4
2019/2020	3
2020/2021	17
2021/2022	14
2022/2023	23

Most of them belonged to the serological serogroups A3 (14/31) and A3 (14/31), A3 (4/7/31) and A4 (4/31) are regarded to be typical pneumonia-causing strains in both pigs and cattle. But there were also a few isolates from serogroups D and A1. These serogroups have, respectively, been correlated with illnesses in pigs or birds (fowl cholera). The fascinating thing about the strains was their fermentation characteristics were consistent. In comparison, strains from other hosts (such as pigs, poultry, and rabbits) are more varied. All strains, with 80%, relate to the biovars 2 and 3, which are two biochemical variations. The only way these biovars varied from one another was in their capacity to ferment trehalose. Additionally, strains from different host's animals exhibit the same dominance of these two kinds. Five other biovars (numbered 1, 12, 4, 7, and 9) were found, however in small numbers, and they only differed from the two main kinds in a few biochemical characteristics (Table 2).

Table (2) P. multocida strains' patterns of fermentation

Biov-ars	No.of strains	ODC	Fermen-tation	D(-) Arabin-ose	Lact-ose	Malt-ose	Trehal-ose	D(+)Xyl ose	Dulc-itol	D(-) Sorbi-tol
3	(12)	+		-	-#	-	-	+∫	-	+
2	(13)	+‡		-	-	-	+	+	-	+

It's noteworthy to note that, except for P1185 and P1006, the existence of a-glucosidase activity corresponded with the strains' ability to ferment trehalose. This relationship has yet to be explored in this context. The two dulcitol-negative subspecies of P. multocida subsp. multocida and septic were previously thought to be distinguishable by this biochemical characteristic. However, molecular science's findings do not necessarily corroborate this coherence.

The strains showed comparable patterns in a 16S ribosomal RNA gene PCR-RFLP assay designed to distinguish subspecies consistent with P. multocida subsp. multocida. Various molecular fingerprinting techniques are used to identify the genetic relationship between these nearly identical strains. The M13 minisatellite marker test and the ERIC (enterobacterial repetitive intergenic consensus) -PCR were chosen for this Investigation

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based on personal experience and Taylor's comparative examination of molecular methodological procedures, respectively.

The outcomes of genotypic research were connected with those of phenotypic Characterization and with each other. The strains were divided into two main sub-populations using both methods. Each group shares some characteristics across the board. Biovar 3 bacteria could not ferment trehalose because they lacked a-glucosidase activity and had the M13 pattern. In contrast, the biovar two bacteria were members of the same ERIC-PCR group, fermented trehalose, and showed a-glucosidase activity. Observe that the first group solely contained lung-specific strains. Still, the second group also included commensals isolated from other non-respiratory channels, including the milk, fetus, vagina (unreported statistics), and the nasal route.

A few subgroups with different molecular characteristics were found in addition to the Using PCR and M13 minisatellite markers; there are two primary types. Strain a toxin-producing strain using Biovar 9 with B2, capsule type D or F (not defined), a pressure using A1 and A2, and a tune with B1 are examples of biochemical traits or biovars that may be connected to certain molecular types.

Conclusion

The study found two dominant strain types of Pasteurella multocida in 31 herds in Hungary that recovered from calves with respiratory illnesses. These strains showed some phenotypic variation despite having the same taxonomic background (P. multocida subsp. multocida). Their ability to ferment trehalose, the activity of the enzyme -glucosidase, and the patterns of their molecular fingerprints discovered by ERIC-PCR and M13-PCR may all be used to distinguish them. These strain types may be significant in the emergence of bovine respiratory disease (BRD) based on their distinct geographic origin and prevalence. There need to be more comparisons with strains derived from healthy individuals for the study's limitations. The relationship between particular songs and respiratory illness could be better understood by contrasting the discovered strain types with those obtained from animals in good health. Future research could build on these findings and concentrate on better characterizing the strain types in terms of their virulence traits, antibiotic resistance profiles, and capacity to elicit immunological responses. This would help us comprehend their function in BRD more thoroughly.

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