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Review Paper

# Hemorrhagic Septicemia in Livestock: Molecular Insights, Epidemiological Dynamics, and Next-generation Control Strategies

**Muhammad Wasif Gulzar[1*], Sana Riaz[2,3], Muhammad Mubeen Ahmad[4], Sidra Zulfiqar[5]**

*1. Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.*
*2. Animal Science Division, Nuclear Institute for Agriculture and Biology (NIAB-C), Faisalabad, Pakistan.*
*3. Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad, Pakistan.*
*4. Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.*
*5. Animal Science Division, Nuclear Institute for Agriculture and Biology, Faisalabad(NIAB-C), Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad, Pakistan.*

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## ABSTRACT

Hemorrhagic septicemia (HS), caused by *Pasteurella multocida* serotypes B:2 and E:2, remains one of the most destructive bacterial diseases of cattle and buffalo in tropical and subtropical regions. Its hyperacute course, high mortality, and recurring outbreaks impose major economic losses, particularly on smallholder farming systems, where food security and livelihoods are already fragile. Despite centuries of recognition, HS persists as a neglected threat due to its complex molecular pathogenesis, dynamic epidemiology, and the rapid rise of antimicrobial resistance (AMR). At the molecular level, *P. multocida* deploys adhesins, toxins, capsules, iron acquisition systems, and biofilm formation to evade host immunity and trigger systemic septicemia. Comparative genomics underscores substantial strain diversity, plasmid-mediated resistance genes, and virulence islands, which complicate therapeutic and vaccine development. Epidemiologically, HS is driven by geography, seasonal monsoon patterns, host susceptibility, and environmental reservoirs that maintain persistent transmission cycles. Conventional bacterin vaccines and antimicrobials, though historically central to control, often fail under field conditions, with resistance to sulfonamides, tetracyclines, macrolides, and β-lactams increasingly reported. Emerging strategies, including recombinant and DNA vaccines, live-attenuated and aerosolized platforms, and immunomodulatory approaches, show promise but remain insufficiently validated in endemic contexts. Parallel advances in multi-omics, precision livestock farming (PLF), and molecular surveillance provide new opportunities, yet face barriers such as infrastructure, cost, and regulatory inertia. This

*** Corresponding Author:**
***Muhammad Wasif Gulzar, DVM.***
***Address:*** *Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.*
***E-mail:*** *2022ag5900@uaf.edu.pk*

review consolidates current insights into HS pathogenesis, epidemiology, AMR, and vaccine development, while identifying critical gaps and research priorities. Its scope is to bridge molecular discoveries with field-level applications, offering a framework for sustainable HS control and mitigation of its global burden.

## 1. Context

### 1.1. Introduction

Hemorrhagic septicemia (HS) is one of the most challenging problems in veterinary medicine, presenting as a hyperacute to acute septicemic illness that primarily affects cattle and water buffalo in tropical and subtropical regions of the world [1-3]. For populations that depend on livestock, the disease's high fatality rates and rapid onset have significant economic ramifications, especially in underdeveloped countries where animal husbandry is the mainstay of rural economies. The causative agent behind this destructive ailment is *Pasteurella multocida*; its serotypes B:2 and E:2 exhibit exceptional pathogenic potential. Figure 1 shows circular representation of the *P. multocida* genome showing annotated virulence, antimicrobial resistance (AMR), core, and ribosomal RNA (rRNA) genes. Virulence genes (red) include *ptx*, *ompH*, *pfhA*, *toxA*, and *tlyA*, which are implicated in adhesion, immune evasion, and toxin production [4, 5]. AMR determinants (orange) include *blaTEM* (β-lactam resistance), *sul2* (sulfonamide resistance), and *tetB* (tetracycline resistance), consistent with recent surveillance reports of multidrug-resistant *P. multocida* [6, 7]. Core housekeeping genes (green), such as *recA*, *rpoB*, and *gyrB* are essential for DNA repair, transcription, and replication. The rRNA operon (blue) contains the *16S-23S-5S rRNA* genes, which are widely used for phylogenetic identification and strain typing. The smaller circle represents a plasmid harboring *blaTEM* and *mobA*, indicating the potential for horizontal gene transfer of resistance traits. Gene annotations were assigned using the Virulence Factor Database (VFDB) and ResFinder, and plasmid elements were identified with PlasmidFinder.

Figure 2 shows circular genome map of the *P. multocida* B:2 strain.

On the chromosomal circle:

Virulence genes (red): *plpE*, *ptx*, *ompH*, *toxA*, *hlyA*, associated with adhesins, toxins, and hemolysins [4, 8].

AMR genes (orange): *blaTEM* (β-lactamase), *sul2*, and *tetB*, conferring resistance to β-lactams, sulfonamides, and tetracyclines [7, 9].

Capsule/iron acquisition genes (green): *bcbA*, *bcbB* (capsule biosynthesis), and *hgbA* (hemoglobin receptor) [10].

rRNA operon (blue): *16S–23S–5S* ribosomal RNA cluster [11].

The small circle depicts a self-replicating plasmid encoding *blaTEM* and *repA*, indicating the potential for dissemination of resistance traits.

Virulence genes (red) include *nanB* (neuraminidase), *plpE* (lipoprotein antigen), *ompH* (outer membrane porin), and *toxA* (dermonecrotic toxin [DNT]), linked to adhesion, immune evasion, and host tissue damage [4].

Capsule/iron genes (green) include *hgbB* (hemoglobin receptor) and *ecbA/B* (serogroup E capsule biosynthesis) [6].

AMR genes (orange) *blaTEM*, *sul2*, and *tetB* confer β-lactam, sulfonamide, and tetracycline resistance [7, 9].

The rRNA operon (blue) contains the complete *16S–23S–5S* cluster [10].

The plasmid (purple) carries *blaTEM* and *repA*, enabling the transfer of resistance traits. This genetic profile underpins the strain's high pathogenicity in HS.

Figure 3 shows genome map of *P. multocida* E:2 highlighting key virulence and resistance features.

*P. multocida* strains are classified into five capsular types (A, B, D, E, and F) based on the indirect haemagglutination test, 16 somatic serogroups based on the agar gel precipitation test, and eight LPS genotypes (L1–L8) [5]. Within hours of infection, they can overpower the host's immune system and progress to systemic septicemia, which can have disastrous outcomes.

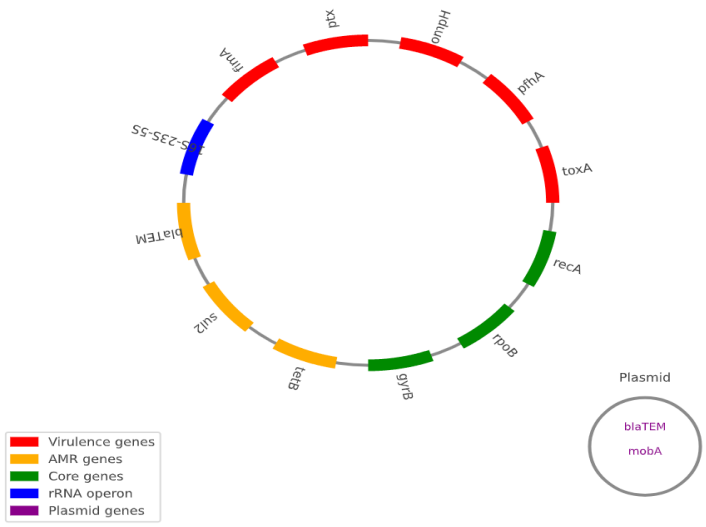


**Figure 1.** Circular representation of the *P. multocida* genome

It is impossible to overestimate the historical significance of HS, as outbreaks have been reported for centuries. Nevertheless, the disease is still evolving and adapting, posing new difficulties for researchers and veterinary professionals [12, 13]. A dynamic disease landscape needs ongoing monitoring and flexible management techniques due to the intricate interactions between environmental factors, host susceptibility, and pathogen virulence. Our capacity to examine the basic mechanisms of HS pathogenesis has been revolutionized by recent technological developments, which have revealed complex molecular systems that control host immune responses, bacterial virulence, and disease progression [1, 14].

Beyond the acute losses of cattle, HS has a substantial negative economic impact on food security in affected areas due to decreased productivity, disrupted breeding programs, and higher veterinary expenses. Smallholder farmers are disproportionately affected by HS-related losses, which conservative estimates suggest amount to hundreds of millions of dollars annually worldwide, because they have limited access to veterinary care and preventive measures. This economic reality emphasizes the need for effective, affordable, and sustainable management techniques that function across a range of agricultural systems and socioeconomic contexts. Current HS research has advanced as a result of the application of state-of-the-art molecular techniques, high-throughput sequencing technologies, and systems biology methodologies. The discovery of new virulence factors, the comprehensive examination of host-pathogen interactions at unprecedented resolution, and the in-depth characterization of pathogen genetics have all been made possible by these methodological advancements. At the same time, epidemiological studies using contemporary monitoring tools and analytical techniques have improved our knowledge of disease transmission dynamics, risk factor identification, and the capacity to anticipate outbreaks.

Microbiology, immunology, epidemiology, and veterinary medicine are among the scientific fields that must be integrated in order to establish effective prevention methods for HS [15, 16]. Innovative preventive strategies must be investigated because traditional techniques that mainly rely on antimicrobial therapy and conventional vaccination have demonstrated little success in various circumstances. Probiotics and prebiotics, immunomodulatory treatments, next-generation vaccination platforms, antimicrobial substitutes, and precision medicine techniques tailored to particular host populations and epidemiological settings are examples of emerging approaches.

This review aims to critically synthesize current knowledge on HS, with emphasis on the molecular determinants of *P. multocida* virulence, epidemiological drivers of disease spread, AMR trends, and the performance of existing and emerging vaccines. The scope of this review is to integrate molecular, epidemiological, and immunological perspectives, identify unresolved gaps in prevention and control, and highlight innovative approaches such as multi-omics tools, recombinant vaccine platforms, and advanced diagnostic strategies that could inform future research and practical applications.

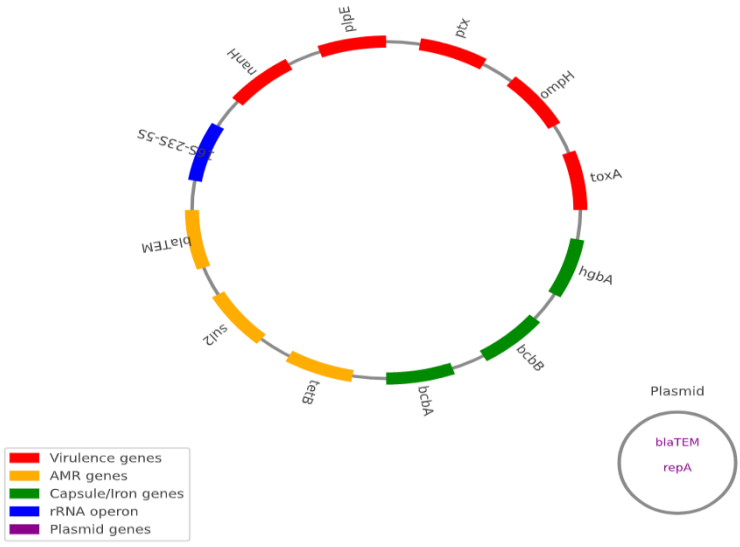


**Figure 2.** Circular genome map of the *P. multocida* B2 strain

## 1.2. Molecular pathogenesis

HS's molecular pathogenesis involves a complicated series of host immune reactions and bacterial virulence mechanisms that lead to systemic septicemia and often fatal outcomes. The advanced molecular machinery of *P. multocida* serotypes B:2 and E:2 allows for rapid colonization, immune evasion, and systemic spread in vulnerable hosts [17, 18]. Understanding these molecular processes is essential for creating targeted treatment plans and successful preventive measures.

Several adhesion factors, such as fimbriae, outer membrane proteins, and surface-associated polysaccharides, aid in the bacterial attachment and colonization of the upper respiratory tract during the early phases of HS pathogenesis. By mediating specialized interactions with host cell receptors and offering defense against early immune responses, the capsular polysaccharide, especially in serotype B:2 strains, plays a crucial role in initial colonization. According to recent molecular research, a number of adhesin proteins, such as PfhB1, PfhB2, and several autotransporter proteins, aid in bacterial attachment and the early onset of infection.

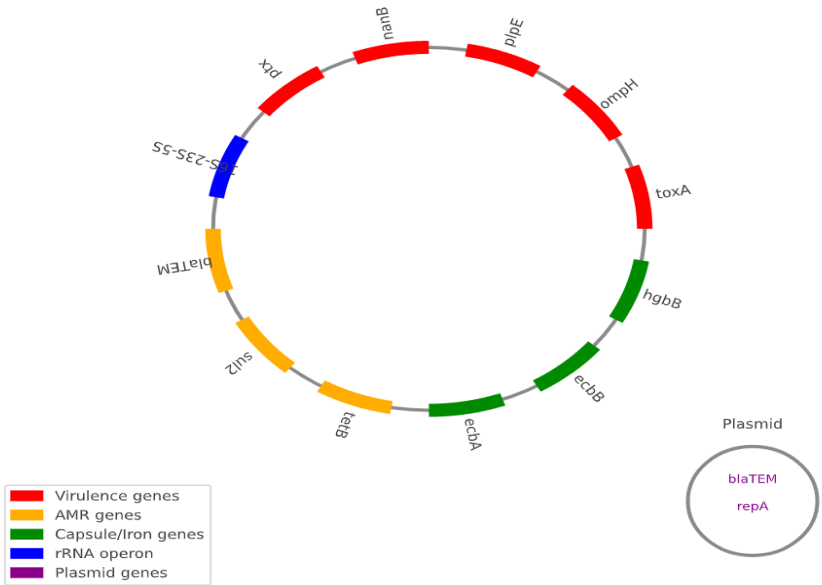


**Figure 3.** Circular genome map of the *P. multocida* E2 strain

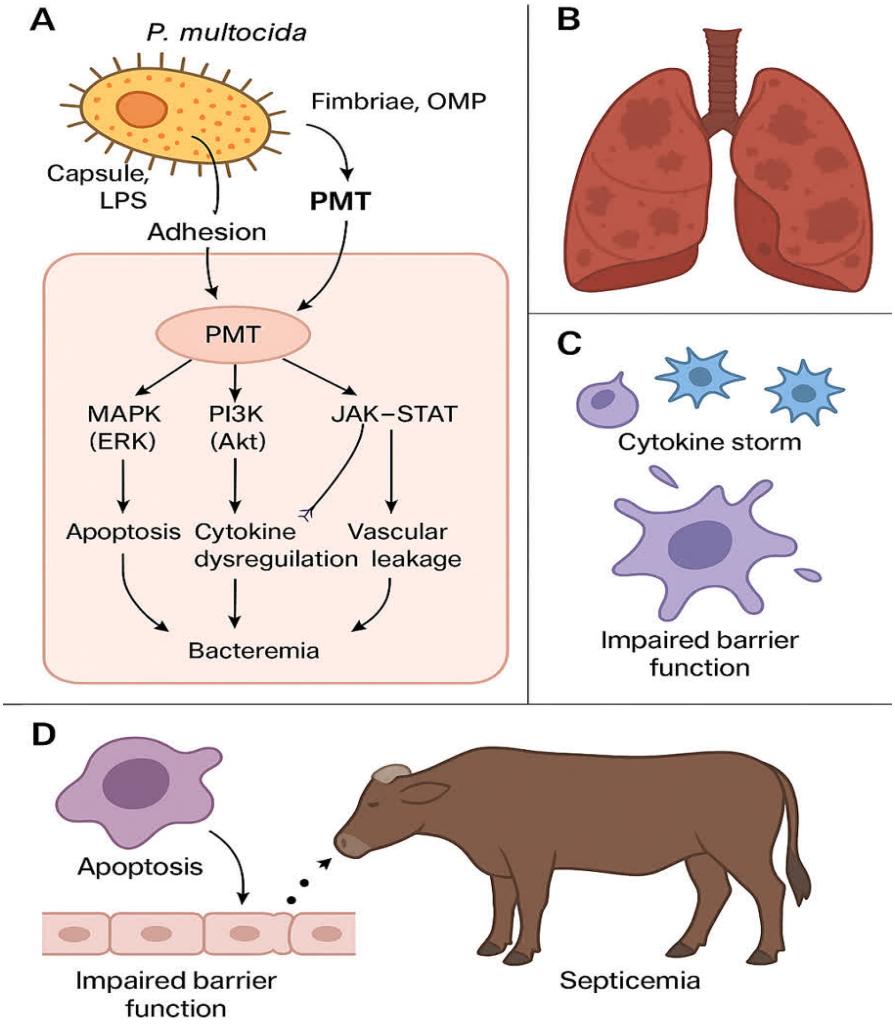


**Figure 4.** Molecular pathogenesis of hemorrhagic septicemia by *P. multocida*: Respiratory entry, immune evasion, endotoxin release, septicemia, vascular damage, and organ failure

*P. multocida* uses a variety of virulence strategies to evade the host's immune system and cause a systemic infection after colonization is achieved. A key virulence mechanism is the generation of potent exotoxins, with cytolethal distending toxin (CDT) and DNT having particularly significant roles in pathogenesis [19, 20]. The strong vasoconstrictive and inflammatory effects of DNT, which is encoded by the *toxA* gene, help explain the typical vascular damage and hemorrhagic lesions observed in HS patients (Figure 4). The toxin causes smooth muscle contraction, endothelial dysfunction, and increased vascular permeability by activating specific signaling pathways, such as the Rho/ROCK pathway.

*P. multocida*'s capsular polysaccharide is an essential component of pathogenesis and vaccine development because it has two roles: It functions a protective antigen and a virulence factor. Complex genetic mechanisms involving numerous gene clusters regulate the production of capsular polysaccharides; recent genomic studies have shown that the arrangement of capsular genes varies significantly amongst serotypes [21, 22]. The formulation of vaccines and the development of cross-protective immunity are significantly impacted by these molecular variations.

Another essential virulence mechanism used by *P. multocida* during systemic infection is its iron acquisition systems [23]. The pathogen has a variety of iron uptake mechanisms, such as direct iron transport, heme utilization pathways, and siderophore-mediated acquisition. Recent transcriptome investigations have shown complex regulatory networks controlling iron homeostasis during infection, demonstrating how host environmental factors and iron availability tightly regulate the expression of these systems. Bacterial survival and proliferation during systemic infection depend on their capacity

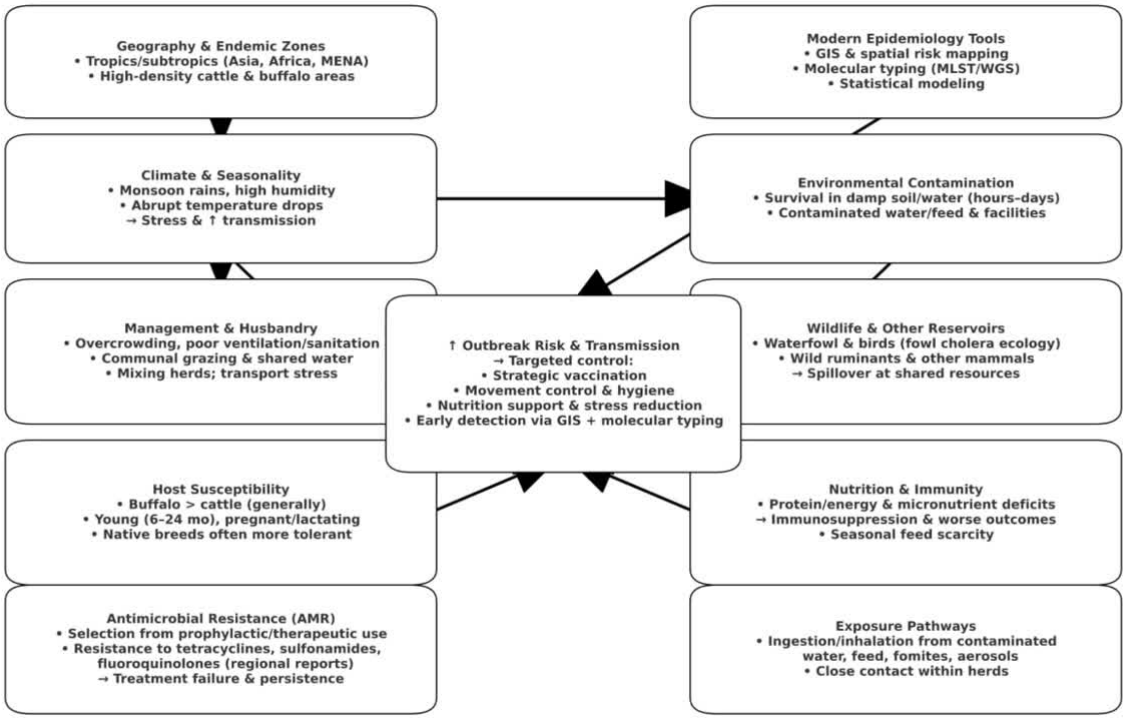


**Figure 5.** Flowchart illustrating the epidemiological determinants and risk factors driving HS outbreaks, as well as potential control strategies

to effectively compete for iron in the iron-limited host environment [24, 25].

Both innate and adaptive immunological mechanisms are involved in the host's immune response to *P. multocida* infection; nevertheless, the rapid development of HS frequently overwhelms these defenses [26, 27]. Pattern recognition receptors (PRRs) are responsible for innate immune recognition by identifying pathogen-associated molecular patterns (PAMPs) on bacterial surfaces. Toll-like receptors (TLRs), especially TLR4 and TLR2, are crucial for initial immune recognition that triggers the production of cytokines and inflammatory signaling pathways [28, 29]. Nevertheless, *P. multocida* has developed a number of mechanisms to evade these immunological reactions, such as altering the structure of lipopolysaccharides and producing immunosuppressive substances [30, 31].

Massive cytokine production, including pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and IL-6, is part of the inflammatory response triggered by *P. multocida* infection. Even though these responses are initially protective, the excessive inflammatory cascade may contribute to the pathological alterations that are typical of HS, such as aberrant coagulation, increased vascular permeability, and endothelial dysfunction. Certain inflammatory pathways have been identified by recent research as potential therapeutic targets for modulating overactive immune responses during HS.

Significant genetic variation has been found in the genomes of *P. multocida* strains linked to HS, and many putative virulence genes that contribute to pathogenesis have been found. Comparative genomic research has revealed the presence of horizontally acquired virulence factors, mobile genetic elements, and pathogenicity islands that enhance the pathogenic potential of the bacteria. Developing comprehensive preventive strategies that account for the genetic variability within the pathogen population and understanding strain-specific virulence features are greatly influenced by these findings.

Increasing attention has been paid to the role of biofilm formation in *P. multocida* pathogenicity since recent studies have demonstrated that HS-associated strains can form biofilms under specific environmental conditions. In addition to increasing their resistance to antibiotics and evading host immunological responses, the formation of biofilms may aid bacteria in surviving in environmental reservoirs. New therapeutic strategies that target these bacterial populations may be developed with an understanding of the molecular mechanisms underlying biofilm formation and dissemination.

The relationship between *P. multocida* and the host complement system is a key battleground in the pathophysiology of HS. The complement system is one significant innate immune defense mechanism that can both directly eliminate pathogens and aid in their removal by phagocytic cells. However, sophisticated complement evasion strategies, such as complement inhibitor production, surface antigen modification, and complement regulatory protein expression, have been established by successful *P. multocida* strains. Recent molecular research has revealed certain complement evasion pathways that may be targeted for therapeutic intervention.

### 1.3. Epidemiology and risk factors

The dynamics of disease occurrence and transmission are influenced by the epidemiological landscape of HS, which is defined by various geographic distributions, seasonal patterns, and host susceptibility characteristics. Developing efficient monitoring systems, risk assessment procedures, and targeted preventive initiatives requires an understanding of these epidemiological characteristics. Geographic information systems (GIS), advanced statistical modeling techniques, and improved molecular typing procedures have greatly aided modern epidemiological investigations by offering deeper insights into disease patterns and transmission pathways.

HS exhibits a clear regional distribution, with the highest incidence rates found in tropical and subtropical regions of Asia, Africa, and the Middle East. India, Pakistan, Bangladesh, Myanmar, Thailand, Vietnam, Egypt, Sudan, and numerous other countries with large populations of cattle and water buffalo are among those where the disease is endemic.

There is ample evidence of seasonal fluctuations in the incidence of HS, with the majority of outbreaks occurring during monsoon seasons and periods of high temperature and humidity. Due to a number of environmental factors, such as stress from weather changes, increased pathogen survival in humid conditions, and enhanced transmission through contaminated water sources and feed materials, the onset of monsoon rains usually corresponds with an increase in disease incidence. Changes in temperature, especially abrupt reductions in ambient air temperature, have been found to be important risk factors that increase susceptibility to HS by weakening animals' immune systems and making them more vulnerable to infection.

Significant differences are observed between various animal species, breeds, age groups, and physiological conditions, and host variables are important in determining susceptibility to HS. Although both species can experience severe sickness, water buffalo often exhibit higher susceptibility to HS than cattle. Young animals are more susceptible to HS, especially those between six months and two years of age. This is likely because their immune systems are still developing, and their exposure-induced immunity is limited. Due to immunosuppression and physiological stress related to reproductive processes, pregnant and lactating animals also exhibit increased susceptibility.

There is evidence of breed-specific susceptibility patterns, with native breeds frequently exhibiting higher levels of HS resistance than exotic or crossbred animals.

HS susceptibility is strongly influenced by nutritional status; animals that are malnourished exhibit higher susceptibility to infection and more severe disease outcomes. Protein, energy, vitamin, and trace mineral deficiencies in particular impair immune function and lessen the animal's capacity to mount an effective defense against *P. multocida* infection. By producing periods of heightened host susceptibility, seasonal fluctuations in feed quality and availability, which are common in many endemic regions, contribute to temporal patterns of HS incidence.

Management and husbandry techniques are important risk factors for the development and spread of HS. The risk of disease is raised by overcrowding, inadequate ventilation, poor sanitation, and the mixing of animals from various sources. Sharing water supplies and engaging in communal grazing facilitate the spread of pathogens among animals and herds. A major contributing factor to HS epidemics is transportation stress, particularly related to the long-distance movement of animals for commerce or seasonal migration.

Beyond climate, environmental factors such as vegetation patterns, soil properties, and water quality are significant in HS epidemiology. Under ideal circumstances, the pathogen can persist in the environment for long extended periods, and contaminated water sources, feed sources, and facilities are significant infection reservoirs. *P. multocida* has been found in a variety of environmental samples in recent environmental monitoring investigations, underscoring the significance of environmental contamination in disease transmission cycles.

Numerous species, including birds, other mammals, and wild ruminants, have been identified as *P. multocida* wildlife reservoirs [32-35]. In addition to potentially contributing to spillover infections in domestic

livestock populations, these animal populations can act as maintenance hosts for the pathogen. Comprehensive disease control techniques require an understanding of *P. multocida* ecology in wildlife populations, especially in regions where domestic animals and wildlife share resources or habitats. A schematic representation of the major epidemiological risk factors influencing HS occurrence is provided in Figure 5.

An increasingly significant epidemiological problem is the development of antibiotic resistance in *P. multocida* populations. Selection pressure favoring resistant strains has resulted from the widespread use of antimicrobial agents for both prevention and treatment. Resistance to several classes of antimicrobials frequently used in the treatment of HS, such as tetracyclines, sulfonamides, and fluoroquinolones, has been reported in recent surveillance investigations [36-39]. The emergence of this resistance has significant ramifications for the effectiveness of therapy and underscores the importance of prudent antibiotic use and employing alternative management methods.

## 2. Data Acquisition

The data for this review were collected from peer-reviewed scientific databases, including PubMed, Scopus, and Web of Science. Keywords such as "hemorrhagic septicemia," "*Pasteurella multocida*," "virulence factors," "epidemiology," "antimicrobial resistance," and "vaccine development" were used in various combinations. Articles published between 2000 and 2025 were prioritized, with emphasis on studies employing molecular, genomic, and epidemiological methodologies. Reference lists of key papers were screened to identify additional relevant sources. Reports from the World Organization for Animal Health (WOAH/OIE) and regional surveillance bulletins were also incorporated to ensure comprehensive coverage of both global and endemic perspectives.

## 3. Results

### 3.1. Current prevention and control strategies

Contemporary prevention and control of HS operates at the complex intersection of conventional bacterin regimens, evolving *P. multocida* biology, and emergent biotechnological approaches. Conventional strategies rely on heterologous and reactive vaccination, with whole-cell, oil-adjuvanted bacterins persists as the backbone of prophylaxis in endemic zones, despite demonstrable inconsistencies in efficacy (often declining below 60 percent field effectiveness) and frequently waning immunogenicity within 6–12 months post-administration. However, the molecular underpinnings of this variable efficacy-whether poor epitope conservation, formulation instability, or cold-chain breaches-remain poorly elucidated [16, 40].

Recent advances have introduced refined vaccine prototypes, notably recombinant PmSLP3-based formulations active against both B and E serogroups, which show extended immunogenicity and superior cross-protection in bovines. Yet, the absence of large-scale field validation, dose-sparing studies, and correlates of protection assays severely limits their translatability. Technological surveillance reveals a cautious increase in novel vaccine platforms globally, yet effective commercial deployment remains hindered by regulatory inertia and uncertainty regarding cost-effectiveness. In parallel, antimicrobial therapy continues to be employed both therapeutically and prophylactically. A recent Pakistan-based molecular epidemiology investigation exposed an alarming prevalence of resistance: Trimethoprim/sulfamethoxazole (≈70 %), erythromycin (≈68 %), and the emergence of β-lactamase genes including, *bla_TEM*, *bla ROB-1*, *bla OXA-2*, and *NDM* (New Delhi Metallo-β-lactamase) variants in *P. multocida* isolates [41]. Surveillance in Hungarian waterfowl, while somewhat reassuring in continued general antimicrobial susceptibility, noted resistance emerging to enrofloxacin, suggesting a gradual drift in resistance. Nevertheless, systematic AMR surveillance across endemic regions, and especially wildlife reservoirs, remains limited, creating a blind spot in stewardship and therapeutic guidelines.

Management interventions, structural biosecurity reforms, movement restrictions, nutritional optimization, housing ventilation, and all-in/all-out systems are undeniably fundamental, low-technology pillars of HS control. Yet, rigorous evaluation of their measurable impact on HS incidence, seasonality modulation, or economic cost-benefit remains rare. Moreover, the epidemiological role of wildlife (e.g. wild ungulates, antelopes, cervids) as cryptic reservoirs capable of sustaining and transmitting HS remains speculative; interspecies transmission pathways are poorly characterized except for anecdotal reports of serotype detection. Similarly, predictive molecular surveillance, real-time genotyping, serotype drift mapping, and virulence island monitoring are largely absent in many endemic regions, leaving emergent lineage shifts undetected until outbreak emergence. In summary, while the architecture of HS prevention integrates vaccines, antimicrobials, husbandry, biosecurity, and surveillance, each pillar is undermined by gaps in

validation, implementation fidelity, and ecological realism. This fragmentary mosaic renders HS an archetypal neglected disease, significant in scale and impact, yet insufficiently studied at the molecular epidemiological level to reliably prevent its re-emergence.

### 3.2. Emerging prevention strategies

The trajectory of HS prevention is shifting from conventional strategies to a combination of advanced biotechnologies, systems immunology approaches, and precision livestock medicine frameworks. These emerging modalities, while still in early stages, reflect a shift toward highly safe, targeted, and context-adaptable interventions. The molecular toolkit includes rational antigen selection, gene-delivery platforms, nanoscale engineering, and host-centric immunomodulation.

Recombinant vaccine platforms now transcend whole-cell bacterins, targeting specific *P. multocida* antigens such as conserved outer-membrane proteins (e.g. OmpH) and transferrin-binding components, offering antigenic specificity, reproducibility, and streamlined production. Experimental data on recombinant OmpH from *P. multocida* B:2 strains, tested in murine and rat challenge models, demonstrated robust IgG responses and partial to near-complete protection, substantially outpacing killed vaccine benchmarks [42].

DNA vaccines, engineered to encode OmpH or transferrin-binding protein A (TbpA), represent a promising frontier. Constructs delivered via mammalian expression vectors (e.g. pUCP24-OmpH or bicistronic TbpA IL2) induced potent humoral and cellular immunity in rodents, with enhanced antibody titers and measurable lymphocyte proliferation even without adjuvants, suggesting durable genetic immunogenicity [42, 43]. Notably, TbpA constructs fused to IL-2 augmented immunostimulation, indicating a potential route for molecular adjuvant integration.

Live attenuated and aerosol vaccine vectors, such as aroA-deficient *P. multocida* B:2 strains and non-pathogenic aerosolized formulations, are re-emerging as viable strategies. Intramuscular administration of an aroA mutant conferred robust protection in calves, whereas intranasal delivery proved inconsistent, underscoring route-dependent efficacy. A live aerosol vaccine trial in buffalo and cattle reported significantly elevated antibody titers and complete challenge protection, with the added benefit of reducing vaccination frequency in practical field settings [44].

Nanoparticle and vectored systems, mucosal administration, immunomodulators, and precision medicine remain largely theoretical. No robust data exist for nanoparticle-facilitated HS vaccines, viral-vectored platforms (e.g. adenovirus, NDV), or mucosal delivery systems specifically tailored for HS, despite their theoretical promise. Likewise, interventions such as cytokine adjuvants, probiotic-based modulation, phage therapy, or antimicrobial peptides are conspicuously absent in HS-specific literature, reflecting a substantial translational gap [13, 45].

Precision prevention frameworks, although strain-typing using MLST, PFGE, and whole-genome sequencing has enhanced our understanding of HS epidemiology (e.g. dominance of ST122 across Pakistan and Thailand; genomic divergence between circulating field strains and vaccine strain P52), no peer-reviewed study has leveraged these data to guide precision prevention strategies, such as host-genotype-informed breeding or pathogen strain-matched vaccination. Consequently, precision prevention frameworks remain purely theoretical [46].

### 3.3. Future directions and research priorities

The trajectory of HS research must increasingly align biotechnological sophistication with pragmatic, field-level realities, as conventional diagnostic and prophylactic strategies remain insufficient to curb recurrent outbreaks in endemic regions. Several interdependent domains illustrate both the opportunities and significant gaps in the current knowledge landscape:

#### 3.3.1. Multi-omics and systems biology integration

Despite rapid advances in host-pathogen interaction studies, true multi-omics integration (genomics, transcriptomics, proteomics, metabolomics) in HS remains in an early stage [47]. The absence of standardized pipelines, open-access omics repositories, and curated reference genomes of virulent *P. multocida* B:2 and E:2 strains hampers systems-level inference of virulence dynamics and host immune evasion. Cross-species comparative frameworks, widely used in human and zoonotic pathogen research, remain underdeveloped for HS.

#### 3.3.2. Artificial intelligence (AI) and predictive epidemiology

AI-enabled modeling offers theoretical promise for outbreak prediction and antimicrobial stewardship. However, algorithms remain limited by data sparsity,

geographical bias, and minimal real-world validation in low- and middle-income countries (LMICs) [48]. Integration of meteorological and livestock mobility datasets into machine learning pipelines is particularly underexplored.

### 3.3.3. Precision livestock farming (PLF) technologies

Wearable sensors, automated thermal imaging, and biosignal monitoring are reshaping livestock surveillance in high-income contexts, but their translational potential for buffalo and cattle in HS-endemic rural Asia and Africa is largely limited [49]. The prohibitive costs, infrastructural constraints, and absence of local technical support impede their practical deployment at scale.

### 3.3.4. Rational vaccine design and next-generation platforms

Recombinant outer-membrane lipoproteins (e.g. PmSLP-3) have demonstrated broad serogroup protection and extended immunogenicity in bovine models under experimental conditions [50]. Similarly, OmpH-DNA vaccine constructs outperform conventional bacterins in rodents, yet bovine challenge studies remain absent. Stability under field conditions, cold-chain independence, and cross-protection trials remain critical bottlenecks.

### 3.3.5. Aerosolized and route-dependent vaccination

Recent buffalo trials suggest attenuated *P. multocida* B:2 aerosol vaccines elicit superior mucosal immunity and long-lasting protection compared to oil-adjuvanted bacterins [51]. However, ecological safety, horizontal transmission risk, and thermostability in field deployment have not been systematically investigated.

### 3.3.6. AMR monitoring and alternatives

Global AMR surveillance increasingly flags *P. multocida*, the etiological agent of HS, as a rising livestock health threat across Asia and Africa. A recent study from Punjab, Pakistan, reported a 7.57% prevalence of *P. multocida* in HS-affected cattle and buffalo, with roughly 70 % of isolates resistant to trimethoprim/sulfamethoxazole, and 67.5% to erythromycin; about 31.5% harbored β-lactamase genes, including *blaTEM*, *blaROB-1*, *blaOXA-2*, and *blaNDM* [36]. These findings mirror regional and global patterns where overuse of antimicrobials in livestock fuels escalating resistance.

## 4. Conclusion

HS remains one of the most formidable bacterial diseases of livestock, disproportionately affecting regions where surveillance, vaccination, and therapeutic options are limited. Despite decades of research, *P. multocida* continues to challenge control strategies through its genetic diversity, complex virulence mechanisms, and emerging AMR. Current vaccines, although widely used, often provide incomplete protection, underscoring the urgent need for innovative immunization approaches tailored to field realities. Advances in molecular epidemiology, genomics, and recombinant vaccine platforms offer promising avenues for overcoming these barriers, yet their translation into cost-effective, field-ready solutions remains slow. To effectively curb HS, future efforts should prioritize integrated molecular and epidemiological studies, standardized AMR monitoring, and the development of next-generation vaccines that confer broad and durable immunity. By bridging laboratory insights with practical field realities, researchers and policymakers can close critical knowledge gaps and establish sustainable strategies to protect livestock health and productivity. Such progress is not only essential for improving animal welfare and farmer livelihoods but also for ensuring the resilience of livestock-dependent economies in endemic regions.

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### Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

### Data availability

This review is based on previously published studies, all of which are cited in the reference list. No new datasets were generated. Additional insights represent the authors' perspectives and interpretations of the existing literature. AI tools were used only to generate illustrative figures consistent with the manuscript's original concepts; no AI assistance was used in the writing, analysis, or interpretation of the text.

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## Authors' contributions

Conceptualization and study design: Sana Riaz; Methodology and data interpretation: Muhammad Mubeen Ahmad; Investigation: Sidra Zulfiqar; Writing the original draft: Sana Riaz, Muhammad Wasif Gulzar, and Sidra Zulfiqar; Supervision, review, editing, and final approval: Muhammad Wasif Gulzar.

## Conflict of interest

The authors declared no conflict of interest.

## References

[1] De Alwis MC. Haemorrhagic septicaemia--a general review. Br Vet J. 1992; 148(2):99-112. [DOI:10.1016/0007-1935(92)90101-6] [PMID]

[2] OIE. Haemorrhagic septicaemia. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris: OIE; 2018. [Link]

[3] Davies RL, MacCorquodale R, Caffrey B. Diversity of avian Pasteurella multocida strains based on capsular PCR typing and variation of the OmpA and OmpH outer membrane proteins. Vet Microbiol. 2003; 91(2-3):169-82. [DOI:10.1016/S0378-1135(02)00300-0] [PMID]

[4] Harper M, Boyce JD, Adler B. Pasteurella multocida pathogenesis: 125 years after Pasteur. FEMS Microbiol Lett. 2006; 265(1):1-10. [DOI:10.1111/j.1574-6968.2006.00442.x] [PMID]

[5] Peng Z, Wang X, Zhou R, Chen H, Wilson BA, Wu B. Pasteurella multocida: Genotypes and Genomics. Microbiol Mol Biol Rev. 2019; 83(4):e00014-19. [DOI:10.1128/MMBR.00014-19] [PMID]

[6] Khamesipour F, Momtaz H, Azhdary Mamoreh M. Occurrence of virulence factors and antimicrobial resistance in Pasteurella multocida strains isolated from slaughter cattle in Iran. Front Microbiol. 2014; 5:536. [DOI:10.3389/fmicb.2014.00536] [PMID]

[7] Kehrenberg C, Tham NT, Schwarz S. New plasmid-borne antibiotic resistance gene cluster in Pasteurella multocida. Antimicrob Agents Chemother. 2003; 47(9):2978-80. [DOI:10.1128/AAC.47.9.2978-2980.2003] [PMID]

[8] Vu-Khac H, Trinh TTH, Nguyen TTG, Nguyen XT, Nguyen TT. Prevalence of virulence factor, antibiotic resistance, and serotype genes of Pasteurella multocida strains isolated from pigs in Vietnam. Vet World. 2020; 13(5):896-904. [DOI:10.14202/vetworld.2020.896-904] [PMID]

[9] Ferreira TS, Felizardo MR, de Gobbi DD, Moreno M, Moreno AM. Antimicrobial resistance and virulence gene profiles in P. multocida strains isolated from cats. Braz J Microbiol. 2015; 46(1):271-7. [DOI:10.1590/S1517-838246120140084] [PMID]

[10] May BJ, Zhang Q, Li LL, Paustian ML, Whittam TS, Kapur V. Complete genomic sequence of Pasteurella multocida, Pm70. Proc Natl Acad Sci U S A. 2001; 98(6):3460-5. [DOI:10.1073/pnas.051634598] [PMID]

[11] Davies RL, MacCorquodale R, Reilly S. Characterisation of bovine strains of Pasteurella multocida and comparison with isolates of avian, ovine and porcine origin. Vet Microbiol. 2004; 99(2):145-58. [DOI:10.1016/j.vetmic.2003.11.013] [PMID]

[12] Verma R, Jaiswal TN. Haemorrhagic septicaemia vaccines. Vaccine. 1998; 16(11-12):1184-92. [DOI:10.1016/S0264-410X(98)80118-7] [PMID]

[13] Lestari TD, Khairullah AR, Damayanti R, Mulyati S, Rimayanti R, Hernawati T, et al. Hemorrhagic septicemia: A major threat to livestock health. Open Vet J. 2025; 15(2):519-32. [DOI:10.5455/OVJ.2025.v15.i2.3] [PMID]

[14] Wilkie IW, Harper M, Boyce JD, Adler B. Pasteurella multocida: Diseases and pathogenesis. Curr Top Microbiol Immunol. 2012; 361:1-22. [DOI:10.1007/82_2012_216] [PMID]

[15] Shivachandra SB, Viswas KN, Kumar AA. A review of hemorrhagic septicemia in cattle and buffalo. Anim Health Res Rev. 2011; 12(1):67-82. [DOI:10.1017/S146625231100003X] [PMID]

[16] Almoheer R, Abd Wahid ME, Zakaria HA, Jonet MAB, Alshaibani MM, Al-Gheethi A, et al. Spatial, temporal, and demographic patterns in the prevalence of hemorrhagic septicemia in 41 countries in 2005-2019: A systematic analysis with special focus on the potential development of a new-generation vaccine. Vaccines (Basel). 2022; 10(2):315. [DOI:10.3390/vaccines10020315] [PMID]

[17] Michael FS, Cairns CM, Fleming P, Vinogradov EV, Boyce JD, Harper M, et al. The capsular polysaccharides of Pasteurella multocida serotypes B and E: Structural, genetic and serological comparisons. Glycobiology. 2021; 31(3):307-14. [DOI:10.1093/glycob/cwaa069] [PMID]

[18] Boyce JD, Adler B. The capsule is a virulence determinant in the pathogenesis of Pasteurella multocida M1404 (B:2). Infect Immun. 2000; 68(6):3463-8. [DOI:10.1128/IAI.68.6.3463-3468.2000] [PMID]

[19] Townsend KM, Hanh TX, O'Boyle D, Wilkie I, Phan TT, Wijewardana TG, et al. PCR detection and analysis of Pasteurella multocida from the tonsils of slaughtered pigs in Vietnam. Vet Microbiol. 2000; 72(1-2):69-78. [DOI:10.1016/S0378-1135(99)00188-1] [PMID]

[20] Magyar T, Rimler RB. Detection and enumeration of toxin-producing Pasteurella multocida with a colony-blot assay. J Clin Microbiol. 1991; 29(7):1328-32. [DOI:10.1128/jcm.29.7.1328-1332.1991] [PMID]

[21] Peng Z, Liang W, Liu W, Wu B, Tang B, Tan C, et al. Genomic characterization of Pasteurella multocida HB01, a serotype A bovine isolate from China. Gene. 2016; 581(1):85-93. [DOI:10.1016/j.gene.2016.01.041] [PMID]

[22] Peng Z, Liang W, Wang F, Xu Z, Xie Z, Lian Z, et al. Genetic and phylogenetic characteristics of Pasteurella multocida isolates from different host species. Front Microbiol. 2018; 9:1408. [DOI:10.3389/fmicb.2018.01408] [PMID]

[23] Shen X, Guan L, Zhang J, Xue Y, Si L, Zhao Z. Study in the iron uptake mechanism of Pasteurella multocida. Vet Res. 2025; 56(1):41. [DOI:10.1186/s13567-025-01469-0] [PMID]

[24] Ibraim IC, Parise MTD, Parise D, Sfeir MZT, de Paula Castro TL, Wattam AR, et al. Transcriptome profile of Corynebacterium pseudotuberculosis in response to iron limitation. BMC Genomics. 2019; 20(1):663. [DOI:10.1186/s12864-019-6018-1] [PMID]

[25] Teng T, Xi B, Chen K, Pan L, Xie J, Xu P. Comparative transcriptomic and proteomic analyses reveal upregulated expression of virulence and iron transport factors of Aeromonas hydrophila under iron limitation. BMC Microbiol. 2018; 18(1):52. [DOI:10.1186/s12866-018-1178-8] [PMID]

[26] Nguyen QH, Lai CHR, Norris MJ, Ng D, Shah M, Lai CC, et al. A surface lipoprotein on Pasteurella multocida binds complement factor I to promote immune evasion. PLoS Pathog. 2025; 21(5):e1012686. [DOI:10.1371/journal.ppat.1012686] [PMID]

[27] Wang Z, Liu S, Xie M, Lang Z, Zhang X, Luo L, et al. Deleting fis downregulates virulence and effectively protects Pasteurella multocida infection in mice. BMC Vet Res. 2025; 21(1):323. [DOI:10.1186/s12917-025-04769-x]

[28] Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010; 140(6):805-20. [DOI:10.1016/j.cell.2010.01.022] [PMID]

[29] Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev. 2009; 22(2):240-73. [DOI:10.1128/CMR.00046-08] [PMID]

[30] Harper M, Boyce JD. The myriad properties of Pasteurella multocida lipopolysaccharide. Toxins. 2017; 9(8):254. [DOI:10.3390/toxins9080254] [PMID]

[31] Harper M, Boyce JD, Adler B. The key surface components of Pasteurella multocida: capsule and lipopolysaccharide. Curr Top Microbiol Immunol. 2012; 361:39-51. [DOI:10.1007/82_2012_202] [PMID]

[32] Orynbayev M, Sultankulova K, Sansyzbay A, Rystayeva R, Shorayeva K, Namet A, et al. Biological characterization of Pasteurella multocida present in the Saiga population. BMC Microbiol. 2019; 19(1):37. [DOI:10.1186/s12866-019-1407-9] [PMID]

[33] Köndgen S, Leider M, Lankester F, Bethe A, Lübke-Becker A, Leendertz FH, et al. Pasteurella multocida involved in respiratory disease of wild chimpanzees. Plos One. 2011; 6(9):e24236. [DOI:10.1371/journal.pone.0024236] [PMID]

[34] Snipes KP, Carpenter TE, Corn JL, Kasten RW, Hirsh DC, Hird DW, et al. Pasteurella multocida in wild mammals and birds in California: prevalence and virulence for turkeys. Avian Dis. 1988; 32(1):9-15. [PMID]

[35] Smith E, Miller E, Aguayo JM, Figueroa CF, Nezworski J, Studniski M, et al. Genomic diversity and molecular epidemiology of Pasteurella multocida. Plos One. 2021; 16(4):e0249138. [DOI:10.1371/journal.pone.0249138] [PMID]

[36] Ali S, Tariq MHA, Yaqoob M, Haq MU, Zahra R. Molecular epidemiology and characterization of antibiotic resistance of Pasteurella multocida isolated from livestock population of Punjab, Pakistan. Int J Vet Sci Med. 2025; 13(1):1-12. [DOI:10.1080/23144599.2024.2437223] [PMID]

[37] Cuevas I, Carbonero A, Cano D, García-Bocanegra I, Amaro M, Borge C. Antimicrobial resistance of Pasteurella multocida type B isolates associated with acute septicemia in pigs and cattle in Spain. BMC Vet Res. 2020; 16(1):222. [DOI:10.1186/s12917-020-02442-z] [PMID]

[38] Dayao DA, Gibson JS, Blackall PJ, Turni C. Antimicrobial resistance in bacteria associated with porcine respiratory disease in Australia. Vet Microbiol. 2014; 171(1-2):232-5. [DOI:10.1016/j.vetmic.2014.03.014] [PMID]

[39] Bitew Z, Abayneh Tefera T, Deneke Y, T/Mariam T, Yihunie FB. Molecular serotyping and antimicrobial susceptibility profiles of Pasteurella multocida isolated from cases of hemorrhagic septicemia in cattle from selected districts of Keffa and Bench Sheko zones, South West Ethiopia. BMC Microbiol. 2025; 25(1):224. [DOI:10.1186/s12866-025-03947-z] [PMID]

[40] Domínguez-Odio A, Delgado DLC. Global commercialization and research of veterinary vaccines against Pasteurella multocida: 2015-2022 technological surveillance. Vet World. 2023; 16(5):946-56. [DOI:10.14202/vetworld.2023.946-956] [PMID]

[41] Kerek Á, Szabó Á, Jerzsele Á. Antimicrobial susceptibility profiles of Pasteurella multocida isolates from clinical cases of waterfowl in Hungary between 2022 and 2023. Vet Sci. 2024; 11(5):194. [DOI:10.3390/vetsci11050194] [PMID]

[42] Yassein AAM, Teleb AA, Hassan GM, El Fiky ZA.The immune response and protective efficacy of a potential DNA vaccine against virulent Pasteurella multocida. J Genet Eng Biotechnol. 2021; 19(1):81. [DOI:10.1186/s43141-021-00180-9] [PMID]

[43] Singh S, Singh VP, Cheema PS, Sandey M, Ranjan R, Gupta SK, et al. Immune response to DNA vaccine expressing transferrin binding protein a gene of Pasteurella multocida. Braz J Microbiol. 2011; 42(2):750-60. [DOI:10.1590/S1517-83822011000200043] [PMID]

[44] Sajid SM, Yousaf A, Irshad H, Zafar MAJPVJ. Preparation, safety and efficacy of live aerosol hemorrhagic septicemia vaccine in buffaloes and cattle. Pakistan Vet J. 2023; 43(3):449-55. [Link]

[45] Mostaan S, Ghasemzadeh A, Sardari S, Shokrgozar MA, Nikbakht Brujeni G, Abolhassani M, et al. Pasteurella multocida Vaccine Candidates: A Systematic Review. Avicenna J Med Biotechnol. 2020; 12(3):140-7. [PMID]

[46] Moustafa AM, Bennett MD, Edwards J, Azim K, Mesaik MA, Choudhary MI, et al. Molecular typing of haemorrhagic septicaemia-associated Pasteurella multocida isolates from Pakistan and Thailand using multilocus sequence typing and pulsed-field gel electrophoresis. Res Vet Sci. 2013; 95(3):986-90. [DOI:10.1016/j.rvsc.2013.07.003] [PMID]

[47] Baysoy A, Bai Z, Satija R, Fan R. The technological landscape and applications of single-cell multi-omics. Nat Rev Mol Cell Biol. 2023; 24(10):695-713. [DOI:10.1038/s41580-023-00615-w] [PMID]

[48] Akinsulie OC, Idris I, Aliyu VA, Shahzad S, Banwo OG, Ogunleye SC, et al. The potential application of artificial intelligence in veterinary clinical practice and biomedical research. Front Vet Sci. 2024; 11:1347550. [DOI:10.3389/fvets.2024.1347550] [PMID]

[49] Ermetin O. Evaluation of the application opportunities of precision livestock farming (PLF) for water buffalo (Bubalus bubalis) breeding: SWOT analysis. Arch Anim Breed. 2023; 66(1):41-50. [DOI:10.5194/aab-66-41-2023] [PMID]

[50] Fegan JE, Waeckerlin RC, Tesfaw L, Islam EA, Deresse G, Dufera D, et al. Developing a PmSLP3-based vaccine formulation that provides robust long-lasting protection against hemorrhagic septicemia-causing serogroup B and E strains of Pasteurella multocida in cattle. Front Immunol. 2024; 15:1392681. [DOI:10.3389/fimmu.2024.1392681] [PMID]

[51] Myint A, Jones TO, Nyunt HH. Safety, efficacy and cross-protectivity of a live intranasal aerosol haemorrhagic septicaemia vaccine. Vet Rec. 2005; 156(2):41-5. [DOI:10.1136/vr.156.2.41] [PMID]

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