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Diagnostic overview of Haemorrhagic Septicaemia (HS): A veterinary review

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Abstract

Haemorrhagic septicaemia (HS) is an acute, highly fatal septicemic disease of cattle and buffaloes caused by *Pasteurella multocida*. The disease is widely distributed in Asia and Africa and remains one of the most economically important bacterial diseases of bovines. Rapid diagnosis is essential because outbreaks are often associated with high morbidity and mortality, especially in buffaloes. Clinical diagnosis based on fever, respiratory distress, and characteristic edema is useful during outbreaks, but laboratory confirmation is necessary for definitive diagnosis. Conventional diagnostic methods include microscopy, bacterial isolation, and biochemical characterization, while modern approaches such as polymerase chain reaction (PCR), real-time PCR, multiplex PCR, and loop-mediated isothermal amplification (LAMP) provide rapid and highly sensitive detection. Serological techniques including ELISA and indirect hemagglutination tests are also employed for surveillance and epidemiological investigations. This review summarizes the etiology, epidemiology, clinical manifestations, sample collection procedures, conventional and molecular diagnostic methods, differential diagnosis, and future perspectives in the diagnosis of HS.

Keywords: Haemorrhagic septicaemia, *Pasteurella multocida*, PCR, ELISA, diagnosis, buffalo, cattle

1. Introduction

Haemorrhagic septicaemia is a contagious bacterial disease characterized by acute septicemia, high fever, edema, and respiratory distress in cattle and buffaloes. The disease is caused mainly by *Pasteurella multocida* and is responsible for substantial economic losses due to mortality, reduced productivity, and trade restrictions (Harper *et al.*, 2006) [3]. Buffaloes are generally more susceptible than cattle, and outbreaks frequently occur during periods of high humidity, rainfall, transportation stress, or overcrowding (OIE, 2019) [5].

Because HS progresses rapidly and animals may die within 24–48 hours after the onset of clinical signs, timely and accurate diagnosis is critical for effective control measures (Benkirane and De Alwis, 2002) [1].

2. Etiology and Epidemiology

2.1 Etiological Agent

The causative organism is *Pasteurella multocida*, a Gram-negative, non-motile, encapsulated coccobacillus belonging to the family Pasteurellaceae. HS is primarily associated with capsular serogroup B (B:2) in Asia and serogroup E (E:2) in Africa (Harper *et al.*, 2006) [3].

2.2 Epidemiology

HS is endemic in many tropical and subtropical countries.

The disease is most prevalent in South Asia, Southeast Asia, and parts of Africa. Stress factors such as transportation, poor nutrition, sudden climatic changes, and concurrent infections increase susceptibility (Benkirane and De Alwis, 2002) [1].

3. Clinical Features Relevant to Diagnosis

Clinical diagnosis is often the first step during outbreaks. Typical signs include:

- High fever (40–41.5 °C)
- Depression and anorexia
- Profuse salivation
- Dyspnea and open-mouth breathing
- Submandibular and brisket edema
- Nasal discharge
- Cyanosis
- Sudden death in peracute cases

Buffaloes commonly develop severe edema of the throat and brisket region, which is considered highly suggestive of HS during endemic outbreaks (De Alwis, 1999) [2].

4. Post-Mortem Findings

Necropsy findings that aid diagnosis include:

- Extensive edema of the throat and brisket

- Hemorrhages in lymph nodes and serosal surfaces
- Congested lungs
- Hydrothorax and hydropericardium
- Enlarged hemorrhagic lymph nodes
- Septicemic lesions in multiple organs

These lesions, although not pathognomonic, strongly support a presumptive diagnosis when correlated with clinical history (Radostits *et al.*, 2007) ^[7].

5. Sample Collection and Transportation

Field diagnosis

Proper sample collection is essential for laboratory confirmation.

Recommended samples

- Blood from live febrile animals
- Heart blood from freshly dead animals
- Lung tissue
- Spleen
- Lymph nodes
- Edematous fluid
- Nasal swabs (less reliable)

Transportation

- Samples should be collected aseptically.
- Transport at 4 °C.
- Use transport media when delay is expected.
- Avoid decomposition because *Pasteurella multocida* dies rapidly in putrefied tissues (OIE, 2019) ^[5].

6. Conventional Diagnostic Methods

6.1 Microscopy and Staining

Smears prepared from blood or tissues can be stained with:

- Gram stain
- Giemsa stain
- Leishman stain
- Methylene blue

The organism appears as a Gram-negative coccobacillus showing bipolar staining, often described as a “safety-pin” appearance (Quinn *et al.*, 2011) ^[6].

6.2 Bacterial Isolation

Isolation remains a classical confirmatory method.

Culture characteristics:

- Grows on blood agar
- Smooth, grayish, non-hemolytic colonies
- Poor growth on MacConkey agar
- Optimal growth at 37 °C

6.3 Biochemical Identification

Common biochemical reactions include:

Test	Reaction
Oxidase	Positive
Catalase	Positive
Indole	Positive
Urease	Negative
Glucose fermentation	Positive
Lactose fermentation	Variable

These tests help differentiate *Pasteurella multocida* from other *Pasteurella* species (Quinn *et al.*, 2011) ^[6].

7. Serological Diagnosis

7.1 Indirect Hemagglutination Test (IHA)

IHA has been used for detecting antibodies in herd surveillance. However, cross-reactions may occur, limiting specificity (De Alwis, 1999) ^[2].

7.2 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is widely used because it is:

- More sensitive than IHA
- Suitable for large-scale screening
- Useful for epidemiological studies
- Helpful in assessing vaccination status

7.3 Other Serological Tests

- Agar gel precipitation test (AGPT)
- Microagglutination test
- Complement fixation test

Serology is more useful for surveillance than for diagnosis of acute fatal cases, because animals may die before developing detectable antibody titers (OIE, 2019) ^[5].

8. Molecular Diagnostic Techniques

8.1 Conventional PCR

PCR targeting species-specific genes such as *kmT₁* has become a standard diagnostic tool.

Advantages

- Rapid detection
- High sensitivity
- Can detect organisms in partially treated animals
- Useful when culture fails

8.2 Multiplex PCR

Multiplex PCR simultaneously identifies:

- *Pasteurella multocida*
- Capsular type
- HS-associated serotypes

This method improves diagnostic efficiency during outbreaks (Townsend *et al.*, 2001) ^[8].

8.3 Real-Time PCR (qPCR)

Real-time PCR provides:

- Quantitative detection
- Greater sensitivity
- Reduced contamination risk
- Faster turnaround time

It is increasingly used in reference laboratories.

8.4 Loop-Mediated Isothermal Amplification (LAMP)

LAMP is particularly promising for field conditions because it:

- Requires minimal equipment
- Produces rapid results
- Has high sensitivity and specificity
- Can be used in resource-limited settings (Kawamoto *et*

al., 2010) ^[4]

9. Advanced and Emerging Diagnostic Approaches

Recent research focuses on:

- Whole genome sequencing
- MALDI-TOF mass spectrometry
- Nanoparticle-based biosensors
- Point-of-care molecular assays
- CRISPR-based diagnostics

These technologies may improve outbreak tracing and rapid field diagnosis in the future.

10. Differential Diagnosis

HS should be differentiated from:

Disease	Distinguishing Features
Anthrax	Dark unclotted blood, absence of rigor mortis
Black Quarter	Crepitating muscle swellings
Foot-and-Mouth Disease	Vesicles in mouth and feet
Pneumonic Pasteurellosis	Predominantly respiratory lesions
Septicemic Salmonellosis	Enteric signs often present

11. Diagnostic Challenges

Several factors complicate HS diagnosis:

- Peracute deaths with limited clinical observation
- Rapid decomposition of carcasses
- Prior antibiotic treatment
- Carrier animals
- Limited laboratory facilities in rural areas
- Cross-reactions in serological tests

12. Future Perspectives

Future diagnostic strategies should focus on:

- Portable molecular devices
- Field-deployable LAMP kits
- Digital surveillance systems
- Genomic epidemiology
- Integration of artificial intelligence with outbreak prediction

Such approaches could significantly improve early detection and control of HS in endemic regions.

13. Conclusion

Haemorrhagic septicaemia remains a major threat to cattle and buffalo production in many developing countries. Early and accurate diagnosis is essential because the disease progresses rapidly and may cause heavy mortality within a short period. Clinical examination and post-mortem findings provide valuable preliminary information, but laboratory confirmation is necessary for definitive diagnosis. Conventional methods such as microscopy, culture, and biochemical testing continue to be useful, while molecular techniques including PCR, real-time PCR, multiplex PCR, and LAMP have greatly enhanced diagnostic sensitivity and specificity. The integration of rapid molecular diagnostics with field surveillance programs offers the most promising strategy for effective control of HS in endemic areas.

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