Haemorrhagic septicaemia (HS) is a major disease of cattle and buffaloes occurring as catastrophic epizootics in many Asian and African countries, resulting in high mortality and morbidity. The disease has been recorded in wild mammals in several Asian and European countries. In many Asian countries disease outbreaks mostly occur during the climatic conditions of high humidity and high temperatures. The disease is caused by Pasteurella multocida, a Gram-negative coccobacillus residing mostly as a commensal in the nasopharynx of animals. The Asian serotype B:2 and the African serotype E:2 (Carter and Heddleston system). The geographical distribution of HS includes some areas of Asia, Africa, the Middle East and southern Europe.

Clinically, HS caused by B:2 or E:2 strains is typified by fever, respiratory distress with nasal discharge, and frothing from the mouth, leading eventually to recumbancy and death. Infection with serotypes A:1 and A:3 predominantly involves pneumonia and death. Septicaemia is the main characteristic feature in all forms of the disease. The incubation period varies from 3 to 5 days. In peracute cases, sudden death without clinical signs may be observed. Water buffaloes are generally more susceptible to HS than cattle and show more severe forms of disease with profound clinical signs. Severe subcutaneous oedema of the mandible, neck and brisket is a distinctive feature of the disease. In endemic areas mortality is largely confined to older calves and young adults.
At *post mortem* most animals succumbing to HS typically show marked swelling of the neck caused by severe blood-tinged oedema. There are also abundant petechial haemorrhages in many tissues and organs, particularly in serosal membranes. The thoracic, pericardial and abdominal cavities may contain serosanguinolent fluid. The lungs are notably congested and oedematous, and foam is generally present in the nasal cavity, trachea and bronchi.

Massive epizootics may occur in endemic as well as non-endemic areas. HS has been identified as a secondary complication in cattle and water buffaloes following outbreaks of foot and mouth disease (FMD). Case fatality approaches 100% if treatment is not followed at the initial stage of infection.

**The diagnosis** of the disease is based on:
1. the clinical signs, gross lesions.
2. morbidity and mortality patterns.
3. Confirmation requires the isolation and characterization of the pathogen using conventional and molecular techniques. There are no confirmed reports of human infections with *P. multocida* B2 and E2; however, other serotypes do cause human infections and precautions should be taken to avoid exposure.

**The isolation** of the causative organism, *P. multocida*, generally from the, by cultural and biological methods, and the identification of the organism by biochemical, serological and molecular methods.
**Isolation and identification of the agent:** Pure cultures of *P. multocida* can be obtained by streaking materials (blood or bone marrow of a dead animal) on to artificial media and the subsequent identification on the basis of the morphological, cultural, and biochemical characteristics of *P. multocida*.

Conventionally, the identification of the specific serotype is carried out using one or more serological methods. These include:

**Serotyping methods:**

a) Rapid slide agglutination test (capsular typing)
b) Indirect haemagglutination test (capsular typing)
c) Agar gel immunodiffusion tests
d) Counter immunoelectrophoresis
e) Agglutination tests (somatic antigen)
f) Serotype designation
g) Antimicrobial susceptibility testing

**Serology:** Serological tests for detecting specific antibodies are not normally used for diagnostic purposes.

Confirmation of the isolates can be made using molecular techniques.
C. REQUIREMENTS FOR VACCINES

The three types of vaccines used against HS are bacterins, alum-precipitated vaccine (APV) and oil adjuvanted vaccine (OAV). To provide sufficient immunity with bacterins, repeated vaccination is required. Administration of dense bacterins can give rise to shock reactions, which are less frequent with the APV and almost nonexistent with the OAV.

* A live HS vaccine prepared using an avirulent P. multocida strain B:3,4 (Fallow deer strain) has been used for control of the disease in cattle and water buffaloes over 6 months of age in Myanmar since 1989. It is administered by intranasal aerosol application. However, there is no report of its use in other countries and killed vaccines are the only preparations in use by the countries affected with HS.

* A single dose of vaccine administered to young calves 4–6 months of age will protect susceptible animals for 3–4 months when APV is used, and for 6–9 months when OAV is used.

* The OAV vaccine should be administered by deep intramuscular injection, and the recommended age for primary vaccination is 4–6 months. For routine, prophylactic vaccination, a single dose of OAV at 4–6 months, a booster 3–6 months later, and annual revaccination thereafter, is recommended. Where husbandry practices are such that reaching individual animals at appropriate times is impracticable, annual vaccination of all animals over 4 months of age, preferably before the breeding season.
Leakage of OAV into subcutaneous tissue can occasionally give rise to fibrous lumps at sites of injection. Rarely, abscesses may develop if sterility conditions are not observed, though most animals are resistant to such infections. APV may occasionally cause shock reactions.

The OAV emulsion should be pure white, and should stick to glass like paint. *If the emulsion shows signs of cracking, it should be discarded. Separation of a thin layer of oil on the surface is permissible.*

It can be stored at 4–8°C for 6 months without any significant loss of potency. *It must not be frozen.* Increase in the content of lanolin improves stability, but also increases the viscosity – a distinct disadvantage. Use of other emulsifying agents such as ‘Arlacel’ helps to produce thinner, stable emulsions.

In the face of an outbreak in vaccinated animals, one dose of APV, followed by one dose of OAV, is recommended.
Vaccines:

Three types of vaccines were prepared according to mixed methods of the OIE manual & alkindi production procedures. By preparing culture broth of Pasteurella multocida carter B strain used in vaccine production (a highly pathogenic strain) after counting of living bacteria & measuring the dry weight of the culture precipitate, it is divided into three parts to make three types of vaccines, alum precipitated HS vaccine (APV), alum precipitated HS + BQ vaccine, & OAV – HS.
Animals:

24 buffalo calves (4 – 8) months of age were prepared to be used for this work. After checking for antibody titers and stayed for about three months waiting to drop the titer to near zero titer (IHAT & TAT were used for the test), they were divided into four groups:

Group I: vaccinated with APV–HS and repeated after 3-4 weeks

Group II: vaccinated with APV–HS + BQ and repeated after 3-4 weeks


Group IV: not vaccinated, as control.

Challenge test:

A 24 hrs. broth culture of highly pathogenic strain of *P. multocida* (containing 7x 10⁹ CFU/ml) used as a challenge dose given subcutaneously for each one of three animals from each group.

These are isolated in another separated area.
**Indirect Hemagglutination test:**
This test is used according to the (OIE manual / 2013) for the detection of antibody titer to determine the immune response against each type of vaccine in buffalo calves to know which vaccine of HS is better for water buffaloes. Then serum sample was taken each month from each vaccinated calf that not used for challenge test in addition to the three remaining control ones.
This was used to know the period of immune response against each type of vaccine used.

**Results:**
1- Challenge test:
Blood smear from rabbit injected from same challenge culture
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<tr>
<td>1</td>
<td>1/16</td>
<td>HS-Al</td>
<td>HS-Al</td>
<td>1ml/ 7X10^8</td>
<td>R</td>
<td>R</td>
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<td>=</td>
<td>R</td>
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<td>30</td>
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<td>=</td>
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<td>D</td>
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<td>10</td>
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<td>HS+BQ-Al</td>
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<td>1ml/ 7X10^8</td>
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<tr>
<td>12</td>
<td>1/32</td>
<td>HS+BQ-Al</td>
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<td>=</td>
<td>R</td>
<td>R</td>
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<tr>
<td>24</td>
<td>1/32</td>
<td>HS+BQ-Al</td>
<td>HS+BQ-Al</td>
<td>=</td>
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<td>R</td>
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<tr>
<td>5</td>
<td>1/64</td>
<td>HS-Oil</td>
<td>-</td>
<td>1ml/ 7X10^8</td>
<td>-R</td>
<td>-R</td>
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<td>=</td>
<td>-R</td>
<td>-R</td>
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<td>22</td>
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<td>HS-Oil</td>
<td>-</td>
<td>=</td>
<td>-R</td>
<td>-R</td>
</tr>
<tr>
<td>14</td>
<td>1/64</td>
<td>--C</td>
<td>--C</td>
<td>1ml/ 7X10^8</td>
<td>D/29 hr.</td>
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<tr>
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<td>1/128</td>
<td>--C</td>
<td>--C</td>
<td>=</td>
<td>D/20 hr.</td>
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<tr>
<td>28</td>
<td>1/128</td>
<td>--C</td>
<td>--C</td>
<td>=</td>
<td>D/18 hr.</td>
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Note: Results labeled R and D are indicative of resistance and non-resistance, respectively.
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<tr>
<th>Animal No.</th>
<th>Vaccine Type</th>
<th>Reaction day 1</th>
<th>R 2</th>
<th>R 3</th>
<th>R 4</th>
<th>R 5</th>
<th>R 6</th>
<th>R 7</th>
<th>R 8</th>
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<tr>
<td>1</td>
<td>HS-APV</td>
<td>salivation, swelling &amp; oedema of neck</td>
<td></td>
<td></td>
<td>Decrease size of swelling 10x10 cm</td>
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<td></td>
<td></td>
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<td>HS-APV</td>
<td>10x10 &amp; enlarged L.N.</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>15 x 10</td>
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<tr>
<td>30</td>
<td>HS-APV</td>
<td>12x30</td>
<td></td>
<td></td>
<td>Increase swelling</td>
<td>Die after severe symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>HS+BQ-APV</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>12</td>
<td>HS+BQ-APV</td>
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<td></td>
<td></td>
<td>Increase swelling</td>
<td>Better swelling</td>
<td>15 x 15</td>
<td></td>
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<tr>
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</table>

14 Die in 29 hr.
16 Die in 20 hr.
28 Die in 18 hr.
Reaction to challenge dose after 4, 5 & 6 days
Control group IV; died on 5 am, 7 am & 4 pm respectively.
Group I; vaccinated two times with HS-Al vaccine
Group II; vaccinated two times with HS+BQ – APV
Group III; Vaccinated with OAV single injection
Subcutaneous fluid

Blood smear

Thoracic fluid