

Montanide[®] Adjuvanted Combined Hemorrhagic Septicemia-Mastitis Vaccine



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Field surveys of major dairy animal diseases in Pakistan have indicated that hemorrhagic septicemia (HS; colloquially known as "gulghotoo"; plate 1) and mastitis (colloquially known as "Sauroo"; plate 2) are economically the most important diseases of buffalo and cattle



Plate 1: An HS affected buffalo



Plate 2: Mastitis in a cow

- 50% reduction in HS incidence would be sufficient to bridge the gap between growing milk demand and supply.
- Vaccination using different types of vaccines (e.g. formalin-killed alum precipitated bacterin, oil adjuvanted vaccines) is the single most important control measure against HS in Pakistan and in some other countries.
- Alum precipitated bacterin is still the most commonly used HS vaccine in Pakistan but it gives immunity for a short duration *i.e.* 3 months
- Mastitis (inflammation or swelling of milk producing organ) is another common dairy animal disease which although not fatal, causes colossal economic losses to our resource-poor dairy farmers and milk processing industry
- Research conducted over the past four decades has shown that at the very least 20% of cows and buffaloes are afflicted with this disease.
- The huge mastitis affected population of buffaloes and cattle not only sustains nearly 25% reduction in their milk yield but the milk produced is also unwholesome for human consumption as it contains pathogenic bacteria, toxins and other harmful substances some of which are not destroyed by UHT treatment
- Whereas vaccination against HS is routinely practiced in Pakistan, control measures against mastitis including vaccination are not routinely adopted at most farms in Pakistan.

A breakthrough of University of Agriculture Faisalabad in the simultaneous control of HS and Mastitis

- A combined HS-mastitis vaccine incorporating local isolates of *Pasteurella multocida*, *Staphylococcus aureus* and *Streptococcus agalactiae* was prepared and tested under laboratory and field conditions at the Mastitis Research Lab. Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad (Plate 3).

- The study was compartmentalized into two phases. **In phase I (laboratory settings)**, isolates of *Pasteurella multocida*, *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from field cases of HS and mastitis were scrutinized for virulence/ pathogenicity and immunogenicity in laboratory animals.
- Bacterin- toxoids of *S. aureus* and *Str. agalactiae* were blended with prepared antigen of *P. multocida*, Montanide® ISA 201 VG, thimerosal and sodium azide to prepare combined HS- mastitis vaccine that was evaluated for sterility, safety and side effects under *in vitro* conditions and *in vivo* in cattle, buffaloes and mice.
- A challenge-protection assay conducted in immunized mice indicated 100% survival of challenged mice.
- The vaccine was physically stable in terms of pH, sedimentation, color, appearance, and syringibility for 6 months observation period at room temperature.
- **In Phase II (field evaluation)**, the combined vaccine was evaluated in adult cattle and buffaloes and calves of cattle and buffaloes. A total of 70 *S. aureus* and *Str. agalactiae* free lactating buffaloes (n=45) and cows (n=25), 50 lactating cows (n=25) and buffaloes (n=25) positive for *S. aureus/Str. agalactiae* and dairy calves (buffalo calves n=70; cow calves n=50) aged up to 1 year were treated with 2 doses of combined HS-mastitis vaccine at 21 day interval and evaluated (where relevant) for 6 months in terms of ELISA based antibody titers against *P. multocida*, *S. aureus* and *Str. agalactiae*, incidence of HS, local and systemic reactions, incidence and prevalence of *S. aureus* and *Str. agalactiae* mastitis, severity of mastitis, milk somatic cell count, milk yield, cost effectiveness and vaccine efficacy.
- ELISA based antibody titers against *P. multocida*, *S. aureus* and *Str. Agalactiae* were significantly ($P<0.05$) higher in vaccinated groups than in unvaccinated groups.
- Two cases of HS were recorded in vaccinated animals *vis-à-vis* 7 cases in un-vaccinated animals. Incidence of *S. aureus* and *Str. agalactiae* over 180 days in vaccinated and un-vaccinated cows and buffaloes initially cultural –ve for these pathogens was 3 and 10, respectively; the corresponding figures in groups initially culture +ve for these pathogens being 2 and 12, respectively.
- Cumulative mean somatic cell counts in vaccinated groups were significantly lower ($P<0.05$) than those in respective unvaccinated controls.
- Milk yield was significantly higher ($P<0.05$) in vaccinated cows and buffaloes than in un-vaccinated controls. Mastitis severity scores were significantly lower ($P<0.05$) in vaccinated groups than in unvaccinated controls.
- The vaccine tested had a vaccine efficacy 84.78 and 90.25% against HS and mastitis, respectively with



Plate 3: Montanide® Adjuvanted Combined Hemorrhagic Septicemia-Mastitis Vaccine

a financial benefit worth Rs 2,060,300.

- In sum, Montanide® adjuvanted combined HS-mastitis vaccine had preventative role against HS and both preventative and curative role against *S. aureus* and *Str. agalactiae* associated mastitis.

Desirable attributes of Montanide® Adjuvanted Combined Hemorrhagic Septicemia-Mastitis Vaccine

- Control of HS and mastitis in tandem with a single vaccine would be achieved,
- Less frequent handling of animals required for vaccination against HS and mastitis.
- Combined HS-mastitis vaccination may potentially be more protective and cost-effective by virtue of its Montanide® adjuvant.
- Prospects of initiation of control of mastitis against which a "**do nothing**" policy is in place virtually throughout the country