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Estimation of Antibody Titres in Sheep Immunized with Capsular Serogroup B *Pasteurella multocida* Oil Adjuvant Vaccine

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Abstract

Background: Pneumonic pasteurellosis in sheep is an important economical disease in Bangladesh where *Pasteurella multocida* killed vaccine is prescribed as a preventive measure. **Objective:** The present research was conducted in 20 non-descript native sheep to estimate antibody titre in response to haemorrhagic septicaemia oil adjuvant vaccine by *Pasteurella multocida* Indirect Antibody Enzyme Linked Immunosorbent Assay. **Methodology:** This animal study was performed during January to March 2024 at Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh. Twenty non-descript native female lamb born to non-immunized mother aged 3 to 4 months was acclimatized for on station experiment. The blood was collected at up to 63 days with seven days' interval post vaccination. The vaccine seed molecularly identified for capsular sero-grouping by Polymerase Chain Reaction. **Results:** The seed bacterium was *Pasteurella multocida* capsular serotype B. The vaccine generated statistically significant ($p<0.05$) increased level of antibody titer compared to control group from day 14 that peaked at 42 days and starts to decline after 49 days and onwards. The mean ± standard deviation of antibody titre of treatment group was 1.79±0.33. In Levene teat, there was no variance equality in the samples was found. **Conclusion:** The oil adjuvant Hemorrhagic Septicemia vaccine produced by Livestock Research Institute; Bangladesh contained killed *Pasteurella multocida* capsular serogroup B. It was found to generate statistically significant antibody response in vaccinated sheep that started to rise from day 14 and peaked at 42 days. After 49 days, it started its declining trend up to the study period (69 days). Investigation of the field isolates and their subsequent immunoinformatic study could generate better knowledge in designing more effective vaccine candidate that would give durable protection. [*Bangladesh Journal of Infectious Diseases, June 2024;11(1):3-8*]

Keywords: Pasteurella infections; vaccine; sheep

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Introduction

Sheep is a providing and resilient livestock that are mainly reared for meat and wool, rarely milk in the Mediterranean, African, and Southeast Asian countries. The most notable feature of their physiology is the resistance towards common infectious diseases. However, they susceptible to respiratory diseases accounting for 5.6% of the total diseases affecting sheep and goats irrespective of etiological agents[1].

Respiratory illness can cause significant financial losses in sheep farms. From sporadic occurrence to seasonal outbreak, sheep flock are afflicted to this critical issue from time to time. It causes fast spreading with flock, higher morbidity, reduced growth rate, higher feed requirement for finishing of fattening lamb, progressive ill thrift, and increased treatment cost[2]. Among the infectious agents causing respiratory illnesses, *Pasteurella multocida* is the most abundant. *Pasteurella multocida* is a commensal in the respiratory tract of warm-blooded animals causing infections when the host immune system is weakened by viral infections or any physical stressors[3].

The bacteria *Pasteurella multocida* causes pneumonic pasteurellosis; an acute, deadly septicemic disease that affects cattle, buffaloes, sheep, and goats. It is found around the world with high rates of morbidity and mortality. Serotype A:3 of *Pasteurella multocida* is associated with pneumonic pasteurellosis in sheep[4]. The complex interaction between host factors and various virulent components like capsular outer membrane proteins, lipopolysaccharides, fimbrial proteins, iron regulated proteins and so one[5].

Protection against the disease is significantly aided by humoral immunity. More than any other intervention, vaccination reduces the mortality rate in pneumonic pasteurellosis[6]. The Livestock Research Institute produced alum precipitated vaccine and the oil adjuvant killed vaccine for hemorrhagic septicemia for large animals are the only vaccine available which is prescribed for use in small ruminants to prevent pneumonic pasteurellosis[7]. In cattle, this vaccine provides sufficient antibody response which was measured by Indirect Hemagglutination Test[8]. Though it is prescribed to use for small ruminants, the antibody titre in sheep has not been estimated yet. Besides, the capsular serotyping is required for vaccine seed to better correlate the serological findings.

Serum antibody levels in immunized animals are measured using the Microtiter Agglutination Test (MAT), the Enzyme Linked Immunosorbent Assay (ELISA), and the Indirect Hemagglutination Assay (IHA). The current study evaluated sheep's antibody titers to the commonly used oil adjuvant HS vaccine for large animals using Indirect ELISA. The present research was conducted in 20 non-descript native sheep to estimate antibody titre in response to Haemorrhagic Septicaemia oil adjuvant vaccine by *Pasteurella multocida* Indirect Antibody Enzyme Linked Immunosorbent Assay.

Methodology

Study Settings and Population: This animal study was designed for the intended study which was conducted at the sheep-goat quarantine facility of the Sheep Production Research Division at Bangladesh Livestock Research Institute, Savar, Bangladesh during Jan-March 2024. The facility is located behind the Dairy Research & Training Centre and well-isolated from the pasturing animals and working environment. The research was approved by the Bangladesh Livestock Research Institute Animal Ethics Committee.

Study Procedure: A total of 20 (n=20) semi-intensively reared, apparently healthy, seronegative (to *P multocida* antibodies on ELISA) female non-descript native lambs less than three months old born to unvaccinated mother and themselves with no history of vaccination against pneumonic pasteurellosis were included in the study. Lambs were individually identified with ear tags, and they were dipped in 5% malathion (Square Pharmaceutical Crop Care Division, Bangladesh) before bringing to the designated facility and dewormed after seven days of arrival using Trilev-Vet Bolus (Triclabendazole INN 900 mg & Levamisole BP 600 mg equivalent to Levamisole HCl BP 708 mg; 19.5 mg/kg, Square Pharmaceuticals, Bangladesh). During the two weeks of adaptation period, feed and water was given ad libitum and animals were allowed to move out during the day but ensuring no interaction with other animals. Animals were kept in two separate well ventilated pans (10 animals per room). The animals were divided into two groups, control and treatment group.

Vaccine Seed Capsular Sero-Grouping: The Livestock Research Institute (LRI) produced formalin killed oil adjuvant Hemorrhagic Septicemia (HS) vaccine was used to test vaccine titre. At first, the bacteria were revived from vaccine seed to confirm bacterial species. Briefly,

the bacterial master stock was collected from HS vaccine production division, LRI, Mohakhali, Dhaka. A loopful of master stock was inoculated in Brain Heart Infusion broth and incubated aerobically overnight. The enriched BHI broth was ensured for growth by comparing turbidity with the control. Then it was streak plated in Blood Agar containing 5% sheep blood and incubated overnight. A well isolated colony was then streaked onto MacConkey agar to observe no growth after overnight incubation. The genomic DNA was extracted using Qiagen Genomic DNA Extraction Kit and purity was measured using Nanodrop embedded in Multiskan Skyhigh Microplate Spectrophotometer (Thermo Scientific™). The PCR was performed against four gene which are provided in Table 1[9]. All the bacteriological media were sourced from Oxoid, UK.

Table 1: ***Primers of Pasteurella multocida***

Sequence Name	Sequence (5' To 3')	Amplicon	Tm	Ref
hyaD-hyaC_F	TGCCAAAATCGCAGTGAG	1044	52.2	[1]
hyaD-hyaC_R	TTGCCATCATTGTCAGTG			
dcbF_F	TTACAAAAGAAAGACTAGGAGCCC	657	59	[1]
dcbF_R	CATCTACCCACTCAACCATATCAG			
bcbD_F	CATTTATCCAAGCTCCACC	760	52.9	[1]
bcbD_R	GCCCGAGAGTTTCAATCC			
KMT1T7	ATCCGCTATTTACCCAGTGG	460	56.1	[1]
KMT1SP6	GCTGTAAACGAACTCGCC			

Follow up and Outcome Measures: The sheep were classified into two groups of 10 animals randomly. The blood from all animals were collected prior to vaccination. The whole blood was collected by experienced vet technician in red topped blood collection tube (6 ml BD Vacutainer). The blood was allowed to clot at room temperature undisturbed for 15-30 mins.

The clot was removed by centrifugation by 2000 rpm for 10 mins in a refrigerated centrifuge. The supernatant was collected Pasteur pipette, aliquoted @0.5 ml to 1.5 ml Eppendorf tubes, stored in -20°C for further use. Each subject of the first group received 1 mL of the licensed LRI produced HS oil adjuvant vaccine subcutaneously and branded as treatment group and other 10 remained as control.

The subjects were closely monitored for any unusual incidents. The whole blood from each animal was collected at 7, 14, 21, 28, 35, 42, 49, 56, and 63 days, serum separated and stored at -20°C. The aliquoted serum lot was brought into room temperature and PTM Indirect Antibody ELISA was performed according to the manufacturer's instructions (Sunlong Biotech, China). The OD values were measured using Multiskan Skyhigh Microplate Spectrophotometer (Thermo Scientific™). Using a regression equation, the antibody titre was measured by choosing a single dilution of test sera.

Statistical Analysis: Data analysis was performed using Microsoft Excell LTSC 2021. A one tailed t-test was performed for hypothesis testing at 5% level of significance. In this case, the effect size of the test was measured by not assuming equal variance. The Levene test was conducted to explain the variance equality.

Ethical Clearance: The Bangladesh Livestock Research Institute 'Research Ethics Committee' approved this study under USDA pain and distress category 'C' (Memo No. 33.05.2672.304.05.001.21. 100).

Results

The vaccine seed was in good quality and able to grow spontaneously at 18 hrs. of incubation in both BHI broth and 5% sheep Blood Agar without any nutritional supplements. The pure culture revived from bacterial stock was found to be positive for *Pasterurella multocida* capsular serogroup B (Figure I).

It was found that the Antibody titre Control Group had lower values for the dependent variable (Mean±SD =1.4±0) than the Antibody titre Treatment Group ((Mean±SD = 1.79±0.33).

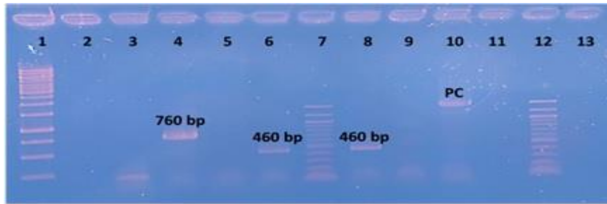

Figure I: Agarose Gel Electrophoresis (1%)- Lane 1: 1 kb ladder, Lane 2 & 11: Negative Control, Lane 3: dcbF (Neg), Lane 4: bcbD, Lane 5: Blank, Lane 6 & 8: KMT1 (460 bp), Lane 7 & 12: 100 bp ladder, Lane 9: hyaD-hyaC (Neg), Lane 10: 16S rRNA (PC)

The Levene test of equality of variance yielded a p-value of <0.001, which is below the 5.0% significance level. The Levene test was therefore significant and the null hypothesis that all variances of the groups are equal was rejected. Thus, there was no variance equality in the samples.

Since one tailed was selected, the t-test is used to determine if group Antibody titre Treatment Group has a statistically significant ($p<0.05$) greater mean value as the group Antibody titre Control Group. The effect size d was found to be 1.68 (equal variances not assumed). With $d = 1.68$ there was a large effect.

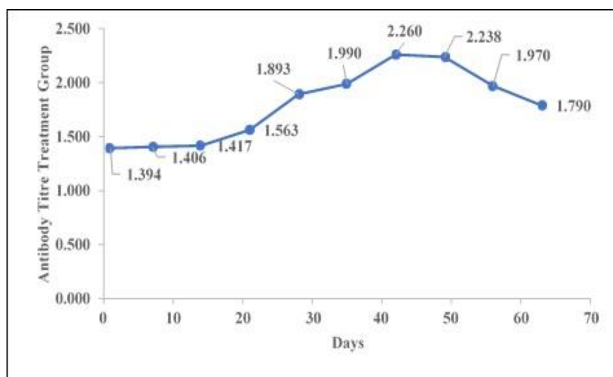

Figure II: Trend of Antibody Titre in Treatment Group

From the figure II, it can be observed that antibody titre of the treatment group started to rise from day 14 sharply and then maintained its rising course up to 42 days where it peaked and remained almost steady up to 49 days. After this tipping point, it started to decline in the next two weeks.

Discussion

This study finds that that HS vaccine is produced from *P multocida* serogroup B:2 whereas serotypes in sheep in Bangladesh yet to be explored. Based on cellular capsule, there are five serotypes have been recorded to cause various forms of disease in humans and animals which are serotype A, B, D, E, and F[10]. It is well known that *P multocida* serogroup B:2/E:2 causes hemorrhagic septicemia in both cattle and buffalo while the capsular serogroup A causes respiratory tract infection principally in small ruminants[11]. In healthy animals, this bacterium can live in the respiratory tract without causing any disease[12]. When the animals fall under any stressful condition like transport, inclement weather, viral infections, shortage of water, malnutrition, chronic parasitic infestation, their immunity is reduced causing the increased growth of the bacteria and associated disease-causing symptoms[13].

This study chose ELISA over other serological techniques like Indirect Hemagglutination Assay or other agglutination assays because it has long been known that the antibody titer measured by ELISA is better representative due to the measurement of only IgG after immune response is triggered[14]. There was a significant different found between mean antibody titre of control and treatment group. The antibody titre of the treatment group started to rise up from day 14 which is a usual case of killed vaccine. This rising trend remained up to 42 days where it got peaked and then became steady for the next seven days. After 49 days, a declining trend was seen up to the end of the study period (63 days). A similar vaccine study with alum precipitated HS vaccine in India showed that the titer peaked at 42 days but declined at 83 days and onwards[14]. So, the vaccine was able to generate significantly higher antibody response up to 9 weeks post vaccination and peaked at 7 weeks.

Another study estimated level of IgG in the sera of vaccinated sheep which was significantly higher than control group and the mean antibody titre (1.794) was found to be present at 28th day post vaccination. This study compared humoral and cellular immune response with antigens inactivated with two different adjuvants[15].

In a protective antibody level study in sheep, it was found that vaccine produced from serogroup A provided good protection in field condition though this serotype was least prevalent. This study also recommended that it might not confer adequate immunity towards *M hemolytica* so multivalent

vaccine from field strain should be taken into consideration[16].

While studying the cross protection between *P multocida* serogroups, H Du found cross protectant in A & B serogroups' outer membrane proteins[17]. It can be inferred that due to the structural difference in capsular serogroups, there is a possibility to reduced antibody titer at the midway of vaccine coverage as the vaccine was intended to protect for 25 months[18]. Besides selecting ideal candidate for vaccine production is crucial part as instead of bacterin, different specific proteins associated with virulence of *Pasteurella multocida* like outer membrane protein, iron regulated proteins, capsules etc. can serve as a better candidate conferring adequate protection[19]. Moreover, the choice of adjuvant and inactivating chemicals are also an important factor that must be considered before formulating an ideal vaccine. So, an immunoproteomic approach with sufficient in vitro and in vivo trial could be better to screen best vaccine candidate in this case. Besides, anamnestic study regarding whether booster dosing of the vaccine generates good immunity should be investigated[20].

Conclusion

The LRI produced oil adjuvant HS vaccine contained killed *Pasteurella multocida* capsular serogroup B which was found to generate significant antibody response in sheep which peaked at up to 42 days. A booster dosing and measuring the protective antibody titre would serve better purpose in studying vaccine effectiveness. Moreover, investigating the field isolates are better approach to produce effective vaccine.

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Conflict of Interest
The authors have no relevant conflicts of interest to declare.

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Contribution to authors:
Zihadi MAH designed the project, conducted the research, drafted the manuscript, analyzed the data, Udoy SMAK conducted the research, and drafted the manuscript, Akther S conceptualized the project and analyzed the data, and Rahman MZ conceptualized and supervised the project. All the authors reviewed, edited, and finalized the manuscript.

Data Availability
Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
The Institutional Review Board granted the study ethical approval. The BLRI Research Ethics Committee granted the study approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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References

1. Kumar A, Tikoo SK, Malik P, Kumar AT. Respiratory Diseases of Small Ruminants. Vet. Med. Int. 2014; Article id: 373642; 1-2
2. Bell S. Respiratory disease in sheep: 2. Treatment and control. In practice. 2008;30(5):278-83
3. Kumar AA, Shivachandra SB, Biswas A, Singh VP, Singh VP, Srivastava SK. Prevalent serotypes of *Pasteurella multocida* isolated from different animal and avian species in India. Veterinary research communications. 2004;28:657-67
4. Hasani SJ, Enferadi A, Sarani S, Nofouzi K. A review of pasteurellosis in humans and animals. Journal of Zoonotic Diseases. 2024
5. Harper M, Boyce JD, Adler B. *Pasteurella multocida* pathogenesis: 125 years after Pasteur. FEMS microbiology letters. 2006;265(1):1-10
6. Odugbo MO, Odama LE, Umoh JU, Lamorde AG. *Pasteurella multocida* pneumonic infection in sheep: Prevalence, clinical and pathological studies. Small Ruminant Research. 2006;66(1-3):273-277
7. Livestock Research Institute, Mohakhali, Bangladesh. Livestock and Poultry Vaccine: Production, Preservation, and Usage Guideline. Accessed at https://lri.gov.bd/

8. Rahman MH, Shahiduzzaman AN, Haque ME, Nazir KHMNH R. Development of experimental oil based inactivated HS vaccine from field Isolates of *Pasteurella multocida* from Cattle in Bangladesh. Int. J. Vaccines Vaccin. 2016;2(2)
9. Townsend KM, Frost AJ, Lee CW, Papadimitriou JM, Dawkins HJ. Development of PCR assays for species-and type-specific identification of *Pasteurella multocida* isolates. Journal of clinical microbiology. 1998;36(4):1096-100
10. Singh R, Remington B, Blackall P, Turni C. Epidemiology of fowl cholera in free range broilers. Avian diseases. 2014;58(1):124-128
11. Shivachandra SB, Viswas KN, Kumar AA. A review of hemorrhagic septicemia in cattle and buffalo. Animal Health Research Reviews. 2011;12(1):67-82
12. Chakraborty S, Kumar A, Tiwari R, Rahal A, Malik Y, Dhama K, Pal A, Prasad M. Advances in diagnosis of respiratory diseases of small ruminants. Veterinary medicine international. 2014(1); Article id:508304
13. Legesse A, Abayneh T, Mamo G, Gelaye E, Tesfaw L, Yami M, Belay A. Molecular characterization of *Mannheimia haemolytica* isolates associated with pneumonic cases of sheep in selected areas of Central Ethiopia. BMC microbiology. 2018;18:1-10
14. Qureshi S, Saxena HM. Estimation of titers of antibody against *Pasteurella multocida* in cattle vaccinated with haemorrhagic septicemia alum precipitated vaccine. Veterinary World. 2014;7(4)
15. Abbasi K, Tahamtan Y, Moazamian E, Hosseini MH. Formalin and ferric chloride inactivated *Pasteurella multocida* type a adjuvanted with bacterial DNA and alum as a new vaccine candidate in sheep pasteurellosis. Microbial Pathogenesis. 2023;183:106282
16. Ferede Y, Mekuriaw S, Mazengia H, Amane A. Serotyping and evaluation of the level of protective antibody titer in Northwest Ethiopian sheep before and after ovine pasteurollosis vaccination. Int J Pharm Med Bio Sc. 2013;2(4)
17. Sun T. Evaluation of a vaccine against *Mannheimia haemolytica* and *Pasteurella multocida* in sheep. 2009
18. Du H, Wu C, Li C, Fang R, Ma J, Ji J, Li Z, Li N, Peng Y, Zhou Z. Two novel cross-protective antigens for bovine *Pasteurella multocida*. Molecular Medicine Reports 2017;16(4):4627-4633
19. Sun J, Huang Y, Li X, Xu X, Cui X, Hao F, Ji Q, Chen C, Bao G, Liu Y. Characterization and immunological effect of outer membrane vesicles from *Pasteurella multocida* on macrophages. Applied Microbiology and Biotechnology. 2024;108(1):238
20. Lacasta D, Ferrer LM, Ramos JJ, González JM, Ortín A, Fthenakis GC. Vaccination schedules in small ruminant farms. Veterinary Microbiology. 2015;181(1-2):34-46