

## Comparative Efficacy of deuterium (D2O) and Alum as an adjuvant in shelf life of HS vaccine.

### Abstract

One of the difficult problems in preserving the quality of the products from manufacture till it reaches the end consumers is the preservation of vaccines, viruses, and other biologicals. Serum immunoglobulins, plasma fractions, clinical samples, including tissues and bodily fluids, live attenuated virus vaccines, and clinical samples are among the materials that typically need to be maintained in a cold chain to maintain their quality. In the present study the thermostable quality of the generated subunit vaccine was assessed by keeping the recombinant vaccine for 180 days at 4°C to 37°C. It was contrasted with an HS alum precipitated vaccine under the same conditions. In the present study In comparison of overall humoral immune response against recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 37°C. The antibody titres of deuterium with OmpH and HS alum were at par with each other.

**Keywords:** Efficacy , Deuterium (D2O) ,Alum , Shelf life

### Introduction

One of the difficult problems in preserving the quality of the products from manufacture till it reaches the end consumers is the preservation of vaccines, viruses, and other biological. Serum immunoglobulin's, plasma fractions, clinical samples, including tissues and bodily fluids, live s the cause of the acute, lethal septicaemic disease known as hemorrhagic septicemia (HS), which affects cattle and buffaloes<sup>3</sup>. It is a devastating epizootic that causes high rates of morbidity and death in many Asian and African nations. Hemorrhagic septicemia in cattle and buffaloes in Asia and Central Africa, respectively, is linked to serotypes B: 2 and E: 2 of *P. multocida*<sup>4</sup>. The pathogenesis of the disease is caused by interactions between host factors and specific bacterial virulence factors, such as lipopolysaccharides (LPS), capsules, outer membrane proteins (OMP), fimbrial proteins, etc. Among cattle and buffaloes in India, bacterially caused HS is the leading cause of death<sup>5</sup>. It was determined that *Pasteurella multocida* infections alone cause an estimated \$228 million in economic losses annually in India. An essential component of illness prevention is humoral immunity. Of all the measures, vaccination had the greatest impact on reducing mortality in HS. Broth bacterins, alum precipitated, aluminum hydroxide gel, and oil adjuvant vaccinations are among the immunizations used to prevent HS. The whole cell formalin-killed *P. multocida* P52 bacterin precipitated with alum or emulsified in aluminum hydroxide gel is the most often used vaccine in Asia<sup>6</sup>. Natural deuterium, often known as 2H or D, makes up around 0.0156% of all hydrogen. Deuterium nuclei exhibit a smaller amplitude of zero point vibration than typical hydrogen

nuclei due to their heavier (2 daltons) relative to the latter. This helps explain why deuterium bonds are stronger than hydrogen bonds. Interesting ramifications for biological macromolecules arise from the strengthened intra- and intermolecular hydrogen bonding. According to a recent analysis of protein X-ray structures, hydrogen bonds involve almost 95% of all atomic hydrogen<sup>7</sup>. Numerous polar hydrogen atoms interact more or less readily with deuterium, or water hydrogen, a solvent. It has been noted that D<sub>2</sub>O increases cell thermostability and protects proteins from denaturing<sup>8</sup>. Owing to the significant parameters of Efficacy of deuterium (D<sub>2</sub>O) and Alum the study aims to determine the Comparative Efficacy of deuterium (D<sub>2</sub>O) and Alum as an adjuvant in shelf life of HS vaccine .

## Material and methods

### Thermostability of vaccines

The produced subunit vaccine's thermostable quality was evaluated by storing the recombinant vaccine between 4°C and 37°C for 180 days. In the same settings, it was compared to an HS alum precipitated vaccine..

Group I was divided into two temperature range i.e. 4°C and 37°C. It was further divided into three sub groups 1, 2 and 3 (six mice per group). The mice of subgroup 1, was immunized at 0 & 14 days (booster) with 0.2 ml of vaccine by S/C route. Serum was collected at 0, 7,14,21 and 28 days. Subgroup 2, was immunized at 90 and 104 days (booster) with 0.2 ml of vaccine by S/C route. Serum was collected at 90, 97,104,111 & 118 days and was treated as day 0, 7, 14, 21 and 28 for evaluation of antibody titre of subgroup 2, respectively. Likewise, Subgroup 3, was immunized at 180 & 194 days (booster) with 0.2 ml of vaccine by S/C route. Serum was collected at 180, 187, 194, 201 & 208 days and was treated as day 0, 7, 14, 21 and 28 for evaluation of antibody titre of subgroup 3, respectively<sup>9-12</sup>.

**Table 01: Evaluation of humoral immune response against deuterium with recombinant OmpH at 4°C**

Sub groups	No. of animals	Days of immunization	Serum collection days	Route	Dose
1	06	0 & 14	0, 7, 14, 21, 28	S/C	0.2 ml
2	06	90 & 104	90, 97,104,111,118	S/C	0.2 ml
3	06	180 & 194	180,187,194,201, 208	S/C	0.2 ml

**Table 02: Evaluation of humoral immune response against deuterium with recombinant OmpH at 4°C (room temperature)**

Sub groups	No. of animals	Days of immunization	Serum collection days	Route	Dose
1	06	0 & 14	0, 7, 14, 21, 28	S/C	0.2 ml
2	06	90 & 104	90, 97,104,111,118	S/C	0.2 ml
3	06	180 & 194	180,187,194,201, 208	S/C	0.2 ml

The thermostable properties of alum precipitated vaccine was also evaluated .

Group II of alum precipitated vaccine was divided into two temperature range i.e. 4°C and 37°C. It is further divided into three sub groups 1, 2 and 3 (six mice per group) and they were immunized in the same way as described earlier in the case of subunit vaccine. Serum samples were also collected following the same protocol described earlier.

**Table 03: Evaluation of humoral immune response against HS alum precipitated vaccine at 4°C**

Sub groups	No. of animals	Days of immunization	Serum collection days	Route	Dose
1	06	0 & 14	0, 7, 14, 21, 28	S/C	0.2 ml
2	06	90 & 104	90, 97,104,111,118	S/C	0.2 ml
3	06	180 & 194	180,187,194,201, 208	S/C	0.2 ml

**Table 04: Evaluation of humoral immune response against HS alum precipitated vaccine at 37°C (room temperature)**

Sub groups	No. of animals	Days of immunization	Serum collection days	Route	Dose
1	06	0 & 14	0, 7, 14, 21, 28	S/C	0.2 ml
2	06	90 & 104	90, 97,104,111,118	S/C	0.2 ml
3	06	180 & 194	180,187,194,201, 208	S/C	0.2 ml

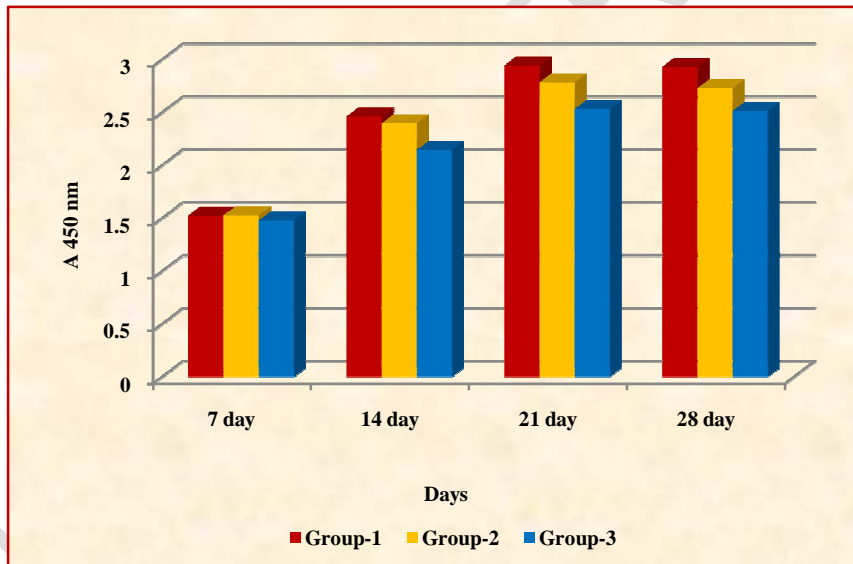
Blood samples were collected at weekly interval aseptically by intra orbital route @ 10% body weight of lab animals starting from day 0 to day 28. Serum was separated and pooled for evaluation of antibody response. The antibody titres were assessed with the help of I-ELISA.

### Statistical analysis

Antibody responses, as measured by I-ELISA, were analyzed by two way Anova to find the statistical significance of the differences between the groups of mice with Minitab version 17<sup>13</sup>.

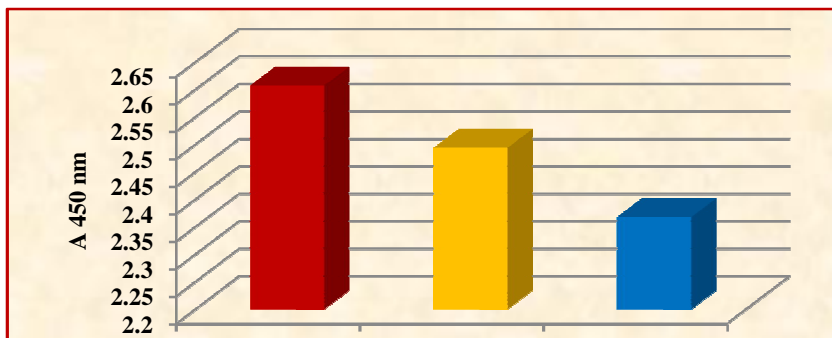
### Results

The humoral antibody response of Group I deuterium with recombinant OmpH at 4°C was evaluated for 180 days. In subgroup 1 (from day 07 to day 28), there was a significant increase in titres from day 07 (1.843 ± 0.012), day 14 (2.320 ± 0.011), day 21 (2.893 ± 0.009) and day 28 (3.370 ± 0.085) with mean values post immunization. In subgroup 2 (from day 90 to day 104), a significant increase in titres from day 97 (1.843 ± 0.012), day 104 (2.240 ± 0.021), day 111 (2.847 ± 0.023) and day 118 (3.047 ± 0.020) with mean values post immunization was observed. In subgroup 3 (from day 180 to day 194), a significant increase in mean of antibody titres from day 187 (1.837 ± 0.009), day 194 (2.087 ± 0.009), day 201 (2.723 ± 0.009) and day 208 (2.827 ± 0.009) post immunization (Fig: 1).



**Figure 01: Humoral immune response of deuterium with recombinant OmpHat 4°C**

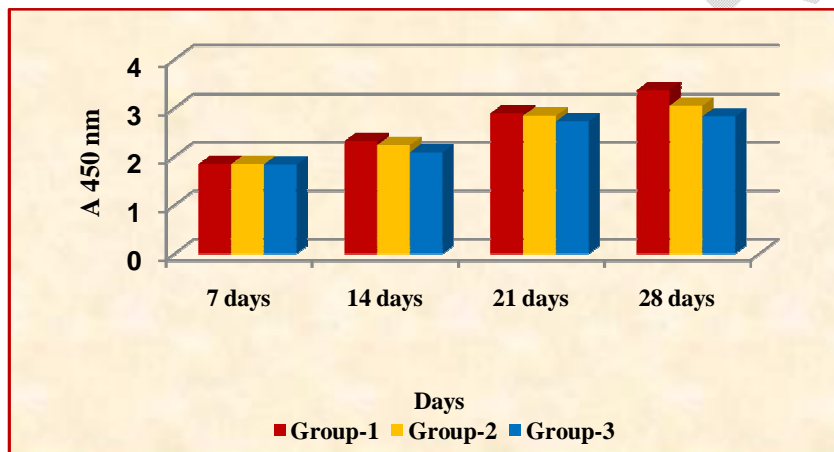
Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals



**Figure 02: Overall Humoral immune response of deuterium with recombinant OmpH at 4°C**

Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

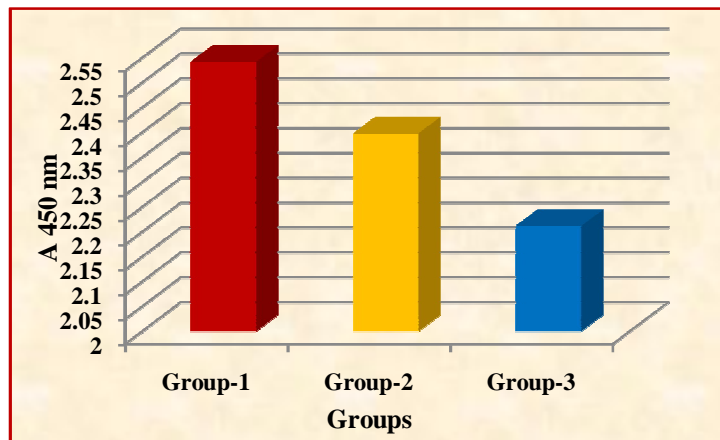
The analysis showed non significant difference in mean antibody titres between the groups (Fig: 2).



**Figure 3 : Humoral immune response of deuterium vaccine at 37°C**

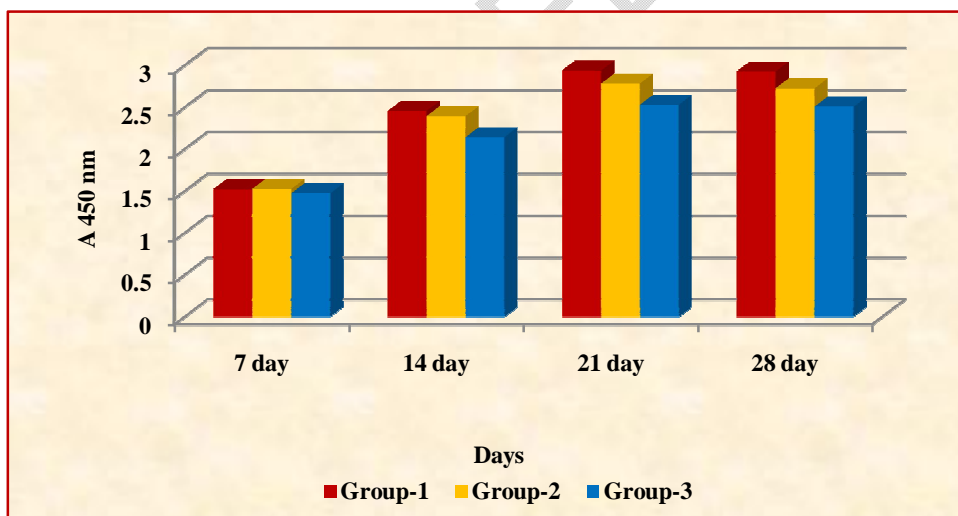
Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

The humoral antibody response of deuterium with recombinant OmpH at 37°C was evaluated for 180 days (Figure 03). In subgroup 1 (from day 07 to day 28), a significant increase in titres from day 07 ( $1.810 \pm 0.006$ ), day 14 ( $2.340 \pm 0.011$ ), day 21 ( $2.817 \pm 0.012$ ) and day 28 ( $3.200 \pm 0.058$ ) with mean values was observed post immunization. In subgroup 2 (from day 90 to day 104), there is significant increase in titres from day 97 ( $1.823 \pm 0.009$ ), day 104 ( $2.180 \pm 0.006$ ), day 111 ( $2.640 \pm 0.006$ ) and day 118 ( $2.950 \pm 0.015$ ) with mean values observed post immunization. In subgroup 3 (from day 180 to day 194), a significant increase was observed in mean of antibody titres from day 187 ( $1.737 \pm 0.009$ ), day 194 ( $1.983 \pm 0.014$ ), day 201 ( $2.440 \pm 0.015$ ) and day 208 ( $2.690 \pm 0.006$ ) post immunization.



**Figure 4: Overall humoral immune response of deuterium vaccine at 37°C**  
Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

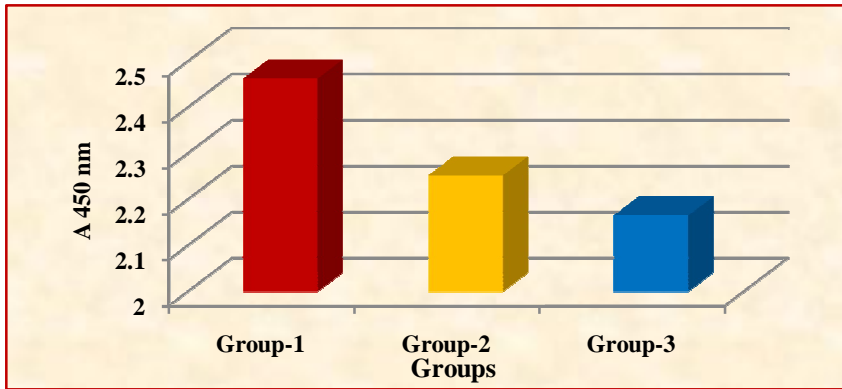
There was no significant difference ( $p \leq 0.01$ ) between the mean antibody titres of the groups (Figure 4).



**Figure 5: Humoral immune response of HS alum precipitated vaccine at 4°C**  
Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

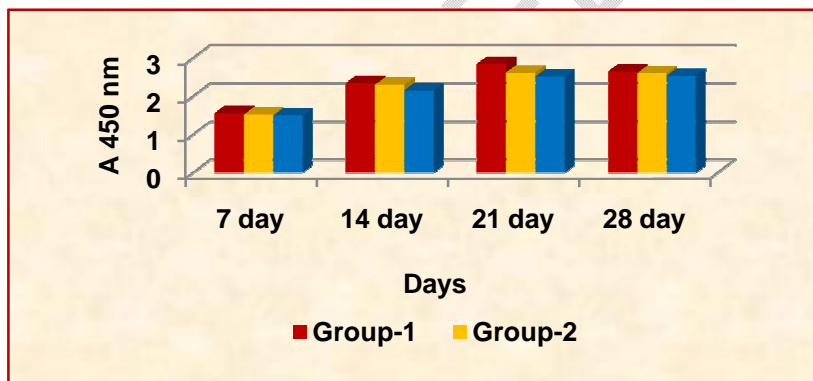
The humoral antibody response of Group II HS alum precipitated vaccine at 4°C was evaluated for 180 days (Figure 5). In subgroup 1 (from day 07 to day 28), there was a significant increase in titres from day 07 ( $1.523 \pm 0.009$ ), day 14 ( $2.457 \pm 0.018$ ), day 21 ( $2.940 \pm 0.011$ ) and day 28 ( $2.927 \pm 0.009$ ) with mean values post immunization. In subgroup 2 (from day 90 to day 104), a significant increase in titres

from day 97( $1.526 \pm 0.005$ ), day 104 ( $2.395 \pm 0.029$ ), day 111 ( $2.783 \pm 0.070$ ) and day 118 ( $2.727 \pm 0.101$ ) with mean values post immunization was observed. In subgroup 3 (from day 180 to day 194), a significant increase in mean of antibody titres from day 187 ( $1.477 \pm 0.003$ ), day 194 ( $2.143 \pm 0.009$ ), day 201 ( $2.530 \pm 0.006$ ) and day 208 ( $2.510 \pm 0.011$ ) post immunization.



**Figure 6: Overall humoral immune response of HS alum precipitated vaccine at 4°C**  
Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

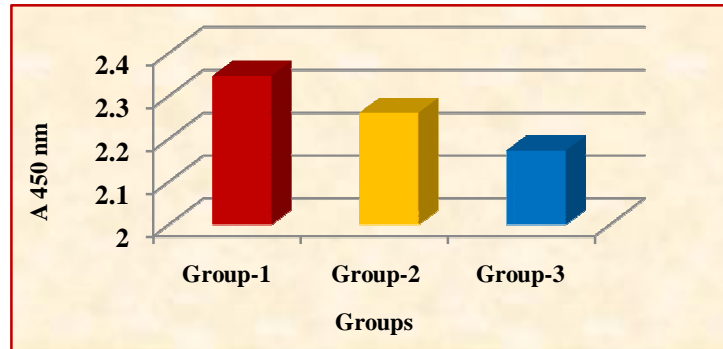
Overall humoral immune response of HS alum precipitated vaccine at 4°C was studied. There was no significant difference between the mean antibody titres of the groups (Figure 6).



**Figure 7: Humoral immune response of HS alum precipitated vaccine at 37°C**  
Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

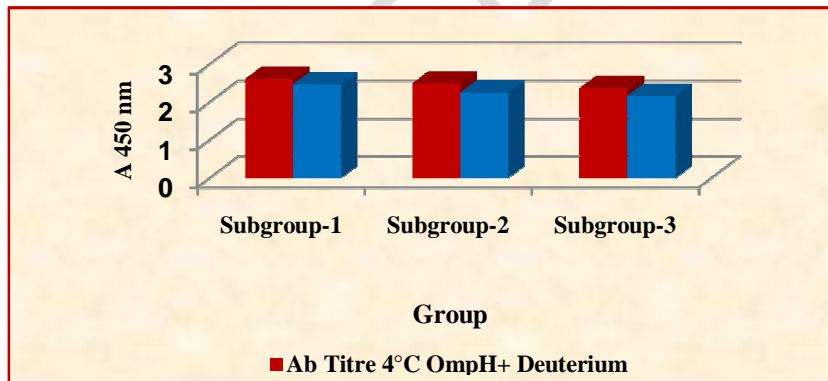
The humoral antibody response of HS alum precipitated vaccine at 37°C was evaluated for 180 days (Figure 7). In subgroup 1 (from day 07 to day 28), there was a significant increase in titres from day 07( $1.560 \pm 0.011$ ), day 14 ( $2.340 \pm 0.011$ ), day 21 ( $2.843 \pm 0.009$ ) and day 28 ( $2.643 \pm 0.018$ ) with mean values post immunization. In subgroup 2 (from day 90 to day 104), a significant increase titres from day 97( $1.520 \pm 0.006$ ), day 104 ( $2.303 \pm 0.009$ ), day 111 ( $2.617 \pm 0.012$ ) and day 118 ( $2.603 \pm 0.003$ ) with

mean values post immunization was observed. In subgroup 3 (from day 180 to day 194), a significant increase in mean of antibody titres from day 187 ( $1.493 \pm 0.003$ ), day 194 ( $2.153 \pm 0.009$ ), day 201 ( $2.510 \pm 0.015$ ) and day 208 ( $2.533 \pm 0.003$ ) at post immunization.



**Figure 8: Overall immune response of HS alum precipitated vaccine at 37°C**  
Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

Overall immune response of HS alum precipitated vaccine at 37°C was studied. There was no significant difference between the mean antibody titres of the groups (Figure 8).



**Figure 9: Comparison of overall humoral immune response against deuterium with recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 4°C**  
Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

In comparison of overall humoral immune response against deuterium with recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 4°C. There was no significant difference between the groups against deuterium (Figure 9).



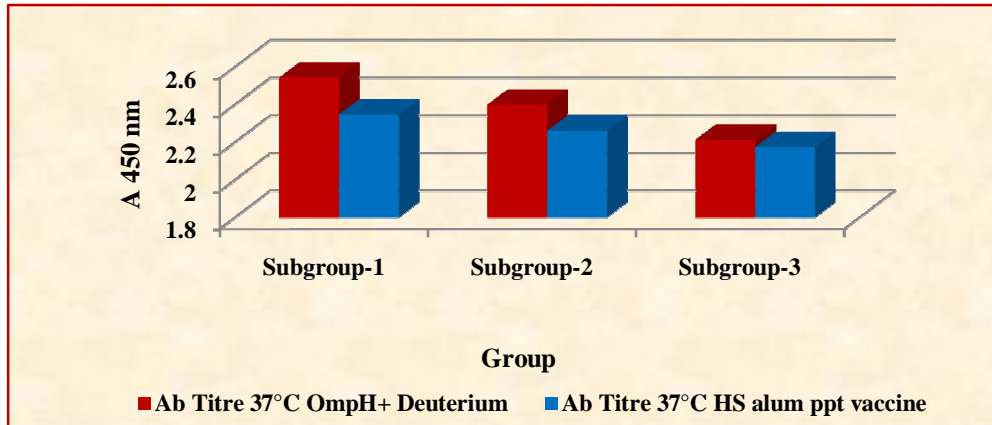


Figure 10: Comparison of overall humoral immune response against recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 37°C

Means with different superscripts differed significantly ( $p \leq 0.01$ ) at different time intervals

In comparison of overall humoral immune response against recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 37°C. The antibody titres of deuterium with OmpH and HS alum were at par with each other (Figure 10).

#### Discussion and conclusion

According to Clements *et al.*<sup>14</sup> the cold-chain forms an important part in maintaining the integrity of any vaccine. The creation and maintenance of the cold chain is a significant cost to immunization programs and maintenance of the cold chain is thought to be a 'Herculean task' in developing countries due to erratic power supplies and difficulties associated with the repair and maintenance of equipment<sup>15</sup>. This highlights the urgent need to develop vaccine formulations that are stable both at room temperature. To overcome the problems associated with the cold chain, different means of enhancing the thermal stability of vaccines have been explored<sup>16</sup>. A study conducted by Adebayo *et al.*<sup>17</sup> evaluated various stabilizers for reconstitution of the freeze-dried vaccine. These stabilizing agents included 0.9% NaCl, double distilled water (ddH<sub>2</sub>O) and various percentages (10–90%) of D<sub>2</sub>O<sup>18</sup>. A dramatic loss in infectivity titer was recorded with 0.9% NaCl and 10% D<sub>2</sub>O. Higher stability of the reconstituted vaccine was observed when ddH<sub>2</sub>O was used for reconstitution instead of 0.9% NaCl solution. Of the three stabilizing agents used for reconstitution, 90% D<sub>2</sub>O resulted in the best stabilization of the reconstituted vaccine under thermal treatment of 37°C up to 24 hrs<sup>19,20</sup>.

The ability of heavy water, heavy water–MgCl<sub>2</sub> and conventional saline diluents to confer thermostability on reconstituted live-attenuated PPR vaccine was tested. These were carried out at three temperatures: 25, 37 and 40°C<sup>21</sup>. It was seen that the reconstituting diluent heavy water–MgCl<sub>2</sub> maintained titers over 102.5 TCID<sub>50</sub>/ml until 28 days for exposure at 37°C and 40°C when deuterated virus was used, compared with 14 days for exposure at 37°C and 40°C when conventional virus was used<sup>22</sup>. A heavy water–MgCl<sub>2</sub> combination was a better reconstituting diluent than heavy water alone for both the deuterated and conventional PPR vaccines. This could be due to the combined solvent and

isotope effects of deuterium in deuterated virus reconstituted in heavy water as compared with only the solvent effect of deuterium in conventional virus reconstituted in heavy water diluents<sup>23</sup>.

Findings of instability of alum ppt HS vaccines corroborated with Van and Van<sup>24</sup>, who observed that the toxoid components of DTP or DTO-Polio vaccine stored at 37°C upto 22 weeks, caused a 50 % reduction in potency<sup>25</sup>. In some DTP vaccines the deterioration process was rapid at 45°C. The loss in potency of Tetanus component was 5% per day in the first 2 weeks of storage and 1% per day during the next month<sup>26</sup>. The findings stated that 70% D<sub>2</sub>O incorporated vaccine lost potency at greater rate than H<sub>2</sub>O vaccine<sup>27,28</sup>. In another study, alum precipitated HS water vaccine and deuterated HS (70%) vaccine were used for immunization in mice injected with 0.2ml I/M, which was repeated after 14 days. At 4°C D<sub>2</sub>O and H<sub>2</sub>O vaccine maintained their potency upto 45 days. But at 40°C D<sub>2</sub>O and H<sub>2</sub>O vaccine lost their potency by 0.8 and 0.5 protection units respectively in 45 days.

## Refernces

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