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 the products.~~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 4ºC to 37ºC. It was contrasted with an HS alum precipitated vaccine under the same conditions. The present study i~~I~~n comparison of overall humoral immune response against recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 37ºC.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~~the products. Serum immunoglobulin~~'s~~, plasma fractions, clinical samples, including tissues and bodily fluids, live is the cause of the acute, lethal septicaemic disease known as hemorrhagic septicemia (HS), which affects cattle and buffaloes[3]. 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*[4]. 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[5]. 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[6]. 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~~zero-point vibration than typical

Comment [U1]: The method applied is not well explained

Sample size

Comment [U2]: Lack of precision about the result

Comment [U3]: Rephrase it for more clarity

Comment [U4]: What should one conclude from this study and the implication in vaccine development?

Comment [U5]: Insufficient detail on D2O

Comment [U6]: Improve the narrative flow
-General introduction to vaccine preservation challenges
-Discuss HS, its impact and the significance of vaccination
-Introduce D2O as a novel solution
-Introduce your objective

Formatted: Font: Italic

Comment [U7]: awkward

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[7]. Numerous polar hydrogen atoms interact more or less readily with deuterium, or water hydrogen, a solvent. It has been noted that D2,O increases cell thermostability and protects proteins from denaturing[8]. Owing to the significant parameters of Efficacy of deuterium (D2O) and Alum the study aims to determine the Comparative Efficacy of deuterium (D2O) and Alum as an adjuvant in shelf life of HS vaccine.

This study aims to compare the thermostability and immunogenicity of vaccines formulated with deuterium (D2O) and alum under varying storage conditions.

Comment [U8]: An example of your objective

Material and methods

Thermostability of vaccines

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

Comment [U9]: What about the mice? Same age or variation?

Comment [U10]: Where is the ethical approval as you use animals for the experiences

Comment [U11]: The titre of the vaccine used and the concentration of D2O incorporate

Comment [U12]: How the temperature has been monitored and maintained throughout the period

Group I was divided into two temperature range i.e. 4ºC and 37ºC. 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 [9-12].

Comment [U13]: Which kind of treatment

Table 01: Evaluation of humoral immune response against deuterium with recombinant OmpH at 4ºC and room temperature

Sub groups	No. ~~of animals~~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 4ºC (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

Comment [U14]: Still 4°?

Comment [U15]: Remove this table

The thermostable properties of alum precipitated vaccine was also evaluated ~~.~~

Group II of alum precipitated vaccine was divided into two temperature range i.e. 4ºC and 37ºC. 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 4ºC and at 37°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 37ºC (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~~using I-ELISA.

Statistical analysis

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

Results

The humoral antibody response of Group I deuterium with recombinant OmpH at 4ºC 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).

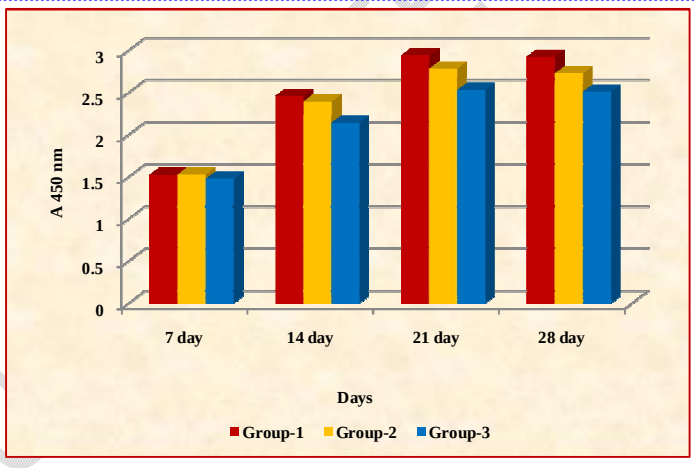
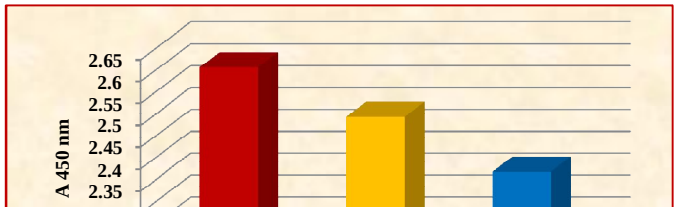


Figure 01: Humoral immune response of deuterium with recombinant OmpH at 4ºC

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



Comment [U16]: Not clear

Comment [U17]: Source

Also, describe the method

Formatted: Indent: First line: 0"

Formatted: Justified

Comment [U18]: Too weak,
The same result is repeated in all the results without a proper interpretation regarding vaccine storage, vaccine development, ... mention what is the key point in vaccine application

Comment [U19]: Legend:
Groups or sub groups

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).

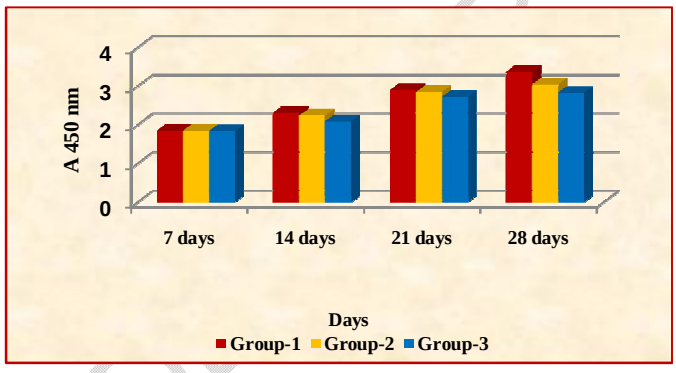


Figure 3 : Humoral immune response of deuterium vaccine at 37°C

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

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

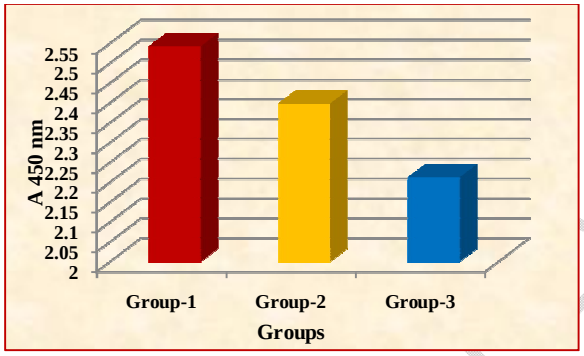


Figure 4: Overall humoral immune response of deuterium vaccine at 37°C
Means with different superscripts differed significantly ($p \leq 0.01$) at different time intervals

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

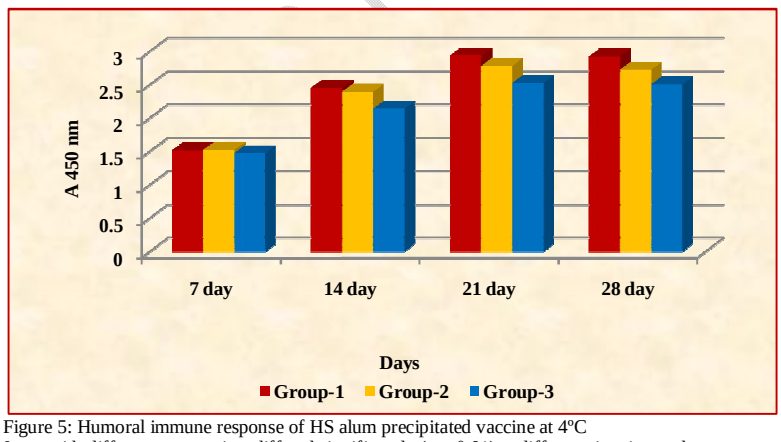


Figure 5: Humoral immune response of HS alum precipitated vaccine at 4°C
Means with different superscripts differed significantly ($p \leq 0.01$) at different time intervals

The humoral antibody response of Group II HS alum precipitated vaccine at 4ºC 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 ± 0.009), day 14 (2.457 ± 0.018), day 21 (2.940 ± 0.011) and day 28 (2.927 ± 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± 0.005), day 104 (2.395± 0.029), day 111 (2.783± 0.070) and day 118 (2.727± 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± 0.003), day 194 (2.143± 0.009), day 201 (2.530± 0.006) and day 208 (2.510± 0.011) post immunization.

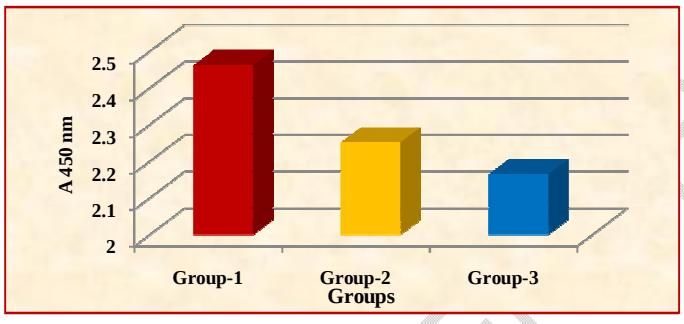


Figure 6: Overall humoral immune response of HS alum precipitated vaccine at 4ºC
Means with different superscripts differed significantly ($p \leq 0.01$) at different time intervals

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

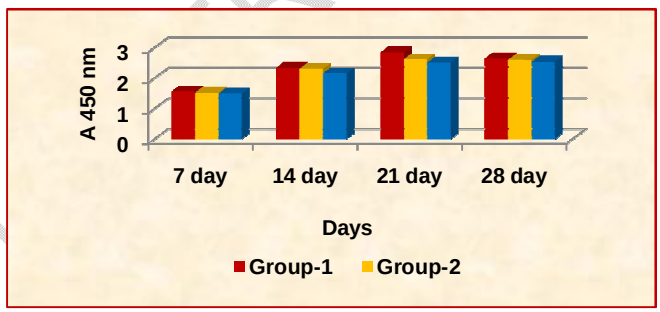


Figure 7: Humoral immune response of HS alum precipitated vaccine at 37ºC
Means with different superscripts differed significantly ($p \leq 0.01$) at different time intervals

The humoral antibody response of HS alum precipitated vaccine at 37ºC 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 ± 0.011), day 14 (2.340 ± 0.011), day 21 (2.843 ± 0.009) and day 28 (2.643 ± 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± 0.006), day 104 (2.303 ± 0.009), day 111 (2.617 ± 0.012) and day 118 (2.603 ± 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 ± 0.003), day 194 (2.153 ± 0.009), day 201 (2.510 ± 0.015) and day 208 (2.533 ± 0.003) at post immunization.

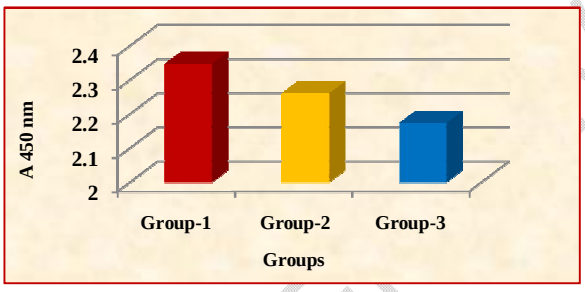


Figure 8: Overall immune response of HS alum precipitated vaccine at 37ºC
Means with different superscripts differed significantly ($p \leq 0.01$) at different time intervals

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

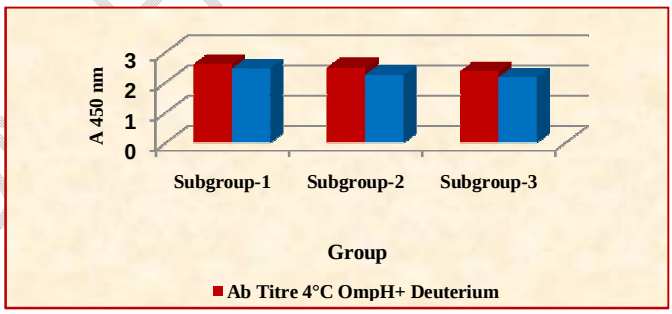


Figure 9: Comparison of overall humoral immune response against deuterium with recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 4ºC
Means with different superscripts differed significantly ($p \leq 0.01$) at different time intervals

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

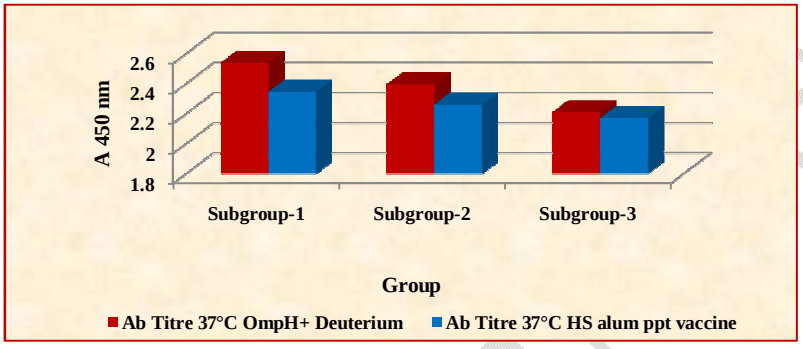


Figure 10:Comparison of overall humoral immune response against recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 37ºC
Means with different superscripts differed significantly ($p \leq 0.01$) at different time intervals
In comparison of overall humoral immune response against recombinant OmpH deuterium vaccine and HS alum precipitated vaccine at 37ºC.The antibody titres of deuterium with OmpH and HS alum were at par with each other (Figure 10).

Discussionand conclusion

According to Clements *et al.*[14] 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 equipmen[15]. 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[16]. A study conducted by Adebayo *et al.*[17]evaluated various stabilizers for reconstitution of the freeze-dried vaccine. These stabilizing agents included 0.9% NaCl, double distilled water (ddH_2O) and various percentages (10–90%) of D_2O [18]. A dramatic loss in infectivity titer was recorded with 0.9% NaCl and 10% D_2O. Higher stability of the reconstituted vaccine was observed when ddH_2O was used for reconstitution instead of 0.9% NaCl solution. Of the three stabilizing agents used for reconstitution, 90% D_2O resulted in the best stabilization of the reconstituted vaccine under thermal treatment of 37°C up to 24 hrs[19, 20].

The ability of heavy water, heavy water–$MgCl_2$ and conventional saline diluents to confer thermostability on reconstituted live-attenuated PPR vaccine was tested. These were carried out at three temperatures: 25,

Comment [U20]: The conclusion is missing

Discussion: Pls, make a clear comparison of your results with others researchers

Comment [U21]: Not referenced

Comment [U22]: Incomplete

Formatted: Indent: First line: 0"

37 and 40°C[21]. It was seen that the reconstituting diluent heavy water–$MgCl_2$ maintained titers over 102.5 TCID 50/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[22]. A heavy water–$MgCl_2$ 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 [23].

Findings of instability of alum ppt HS vaccines corroborated with Van and Van [24], who observed that the toxoid components of DTP or DTO-Polio vaccine stored at 37ºC upto 22 weeks, caused a 50 % reduction in potency[25]. In some DTP vaccines the deterioration process was rapid at 45ºC. 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[26]. The findings stated that 70% D_2O incorporated vaccine lost potency at greater rate than H_2O vaccine [27,28] . 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 4ºC D_2O and H_2O vaccine maintained their potency upto 45 days. But at 40ºC D_2O and H_2O vaccine lost their potency by 0.8 and 0.5 protection units respectively in 45 days.

Refernces

Comment [U23]: Update the table

1. Jogi J, Nayak A, Rai A, Dubey A. Immunoprotection in mice immunized with native OmpH, recombinant OmpH and HS alum precipitated vaccine of Pasteurella multocida P52 against P. multocida challenge. *Indian Journal of Experimental Biology* ,61(2023) 436-441.
2. Yadav DK, Yadav N, Khurana SM. Vaccines: present status and applications. *Animal biotechnology* ,Jan 1 (2020) 523-542.
3. Cuevas I, Carbonero A, Cano D, Pacheco IL, Marín JC & Borge C, First outbreak of bovine haemorrhagicsepticaemia caused by Pasteurella multocida type B in Spain–Short communication. *Acta VeterinariaHungarica*, 68 (2020) 8.
4. Gallego C, Patiño P, Martínez N &IreguiC , The effect of carbohydrates on the adherence of Pasteurella multocida to the nasal respiratory epithelium. *Anais da Academia Brasileira de Ciencias* , 7 (2021) 93.
5. Verma H, Rawat M, Samal A, Upmanyu V, Verma R , Standardization and development of Pasteurella multocida inactivated adjuvanted vaccine against septic pasteurellosis in pigs.*Indian J Exp Biol* , 57 (2019) 315.
6. Cuevas I, Carbonero A, Cano D, García-Bocanegra I, Amaro MÁ & Borge C , Antimicrobial resistance of Pasteurella multocida type B isolates associated with acute septicemia in pigs and cattle in Spain. *BMC Veterinary Research* , 16 (2020) 1.

7. Pirali T, Serafini M, Cargnin S, Genazzani AA. Applications of deuterium in medicinal chemistry. *Journal of medicinal chemistry* , 62 (2019) 5276-97.
8. Chen CH. Deuterium Oxide Effects on Thermostability of Vaccines. *In Deuterium Oxide and Deuteration in Biosciences* , Aug 22 (2022)151-161.
9. Kumar R, Srivastava V, Baindara P, Ahmad A. Thermostable vaccines: An innovative concept in vaccine development. *Expert Review of Vaccines* , Jun 3;21(2022) 811-24.
10. Jogi J, Nayak A, Rai A, Shakya P, Gangil R, Sikrodia R, Chhabra D, Sharda R, Rawat K, Bordoloi S, Jogi J. The adjuvant potential of deuterium in subunit vaccine prepared from outer membrane protein of *pasteurellamultocida*. *Ann. For. Res* ,65(2022) 7889-97.
11. Fanelli A, Mantegazza L, Hendrickx S, Capua I. Thermostable Vaccines in Veterinary Medicine: State of the Art and Opportunities to Be Seized. *Vaccines* , Feb 5;10(2022) 245.
12. de la Torre Arrieta J, Briceño D, de Castro IG, Roser B. A thermostable tetanus/diphtheria (Td) vaccine in the StablevaX™ pre-filled delivery system. *Vaccine* , May 22;41(2023) 3413-21.
13. Chavda VP, Jogi G, Dave S, Patel BM, VineelaNalla L, Koradia K. mRNA-Based Vaccine for COVID-19: They Are New but Not Unknown!.*Vaccines* ,22;11(2023)507.
14. Gulia D & Aly SS , Modeling Vaccination Programs in Outbreaks of Hemorrhagic Septicemia in India. *Journal of Animal Research* , 10 (2020) 19.
15. Gulliver EL, Wright A, Lucas DD, Mégroz M, Kleifeld O, Schittenhelm RB, Powell DR, Seemann T, Bulitta JB, Harper M & Boyce JD , Determination of the small RNA GcvB regulon in the Gram-negative bacterial pathogen Pasteurella multocida and identification of the GcvB seed binding region. *RNA* , 24 (2020) 704.
16. Tanwar H, Yadav AP, Brijbhushan, Shweta Singh SB &Ganju L,Immunity against Pasteurella Multocida in Animals Vaccinated with Inactivated *Pasteurella multocida* and Herbal Adjuvant 'DIP-HIP'. *J Vaccines Immun*, 2 (2016) 10.
17. Shalaby HA, Hassan NM, Nasr SM, Korany T, El Ezz A, Talaat NM, Zeina A & Hala A , An Anthelmintic Assessment of Balanitesaegyptiaca Fruits on Some Multiple Drug Resistant Gastrointestinal Helminthes Affecting Sheep. *Egypt J Vet Sci*., 51 (2020) 93.
18. Pallela P.N &Ummey, S , Antibacterial activity assessment and characterization of green synthesized CuOnano rods using *Asparagus racemosus* roots extract. *SN Appl. Sci.*, 1(2019) 421.
19. Tahamtan A, Charostad J, HoseiniShokouh SJ & Barati M. An overview of history, evolution, and manufacturing of various generations of vaccines. *JAMM* , 5 (2017) 30.
20. Rita DV, Swee KC, Shamini C, Kang TL, Nurshamimi NR, Hussin AR, Nurul K & Salmah I , A Recombinant Subunit HSABA392 as a potential Vaccine for Haemorrhagicsepticaemia disease in livestock. *Trop Biomed* , 35 (2018) 1075.
21. Aiswarya V, Mathakiya RA, Bhanderi BB & Roy A , Characterization of Pasteurella multocida isolates of buffalo origin from Gujarat state of India by outer membrane protein profile analysis. *Buffalo Bull.*, 36 (2017) 313.
22. Yassein AA, Teleb AA, Hassan GM & El FikyZA , The immune response and protective efficacy of a potential DNA vaccine against virulent Pasteurella multocida. *J Genet Eng Biotechnol* ,19 (2021) 1.
23. Devi LB, Bora DP, Das SK, Sharma RK, Mukherjee S & Hazarika RA ,Virulence gene profiling of porcine Pasteurella multocida isolates of Assam. *Vet wrld* , 11 (2018) 348.
24. Mostaan S, Ghasemzadeh A, Sardari S, Shokrgozar MA, Brujeni GN, Abolhassani M, Ehsani P & Karam MR , Pasteurella multocida vaccine candidates: A systematic review. *Avicenna J. Med. Biotechnol* ,11 (2020) 140.

25. Ayalew S, Confer AW & Couger MB, Genome Sequence of a Bovine Isolate of Pasteurella multocida Strain 232. *Microbio res ann*, 16 (2019) 20.
26. Jogi J, Nayak A, Shukla PC, Dubey A, Singh R & Rai A, Shakya P & Bordoloi S, Molecular characterization of *Pasteurella multocida* serotype B:2 strain. *J EntomolZool Stud*, 8 (2020) 1307.
27. Ali AA & Ramadhan BB, Effect of ultrasound on protoscoleces of Echinococcus granulosus in vitro and in vivo. *Iraqi J. Vet. Sci.*, 2 (2021) 2.
28. Jogi J, Nayak A, Shukla PC, Dubey A, Singh R, Rai A & Shakya P, Isolation and Characterization of the Major Outer Membrane Protein (OMP) of *Pasteurella multocida* Serotype B:2. *Int J Curr Microbiol App Sci*, 8 (2019) 474.