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**IMMUNOLOGICAL STUDY ON DIFFERENT FORMULAE OF CONCENTRATED PNEUMO-
4 VACCINE**

DISSERTATION PRESENTED

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6. SUMMARY

Bovine respiratory diseases have a great economic importance in feedlot cattle due to high morbidity rate and losses of calves caused by them. Bovine respiratory viruses like BVD, BoHV-1, PI-3 & BRS viruses consider the most incriminated in Pneumo enteritis syndrome so production of a vaccine containing these viruses is a must to control this problem.

Our present study aimed for preparation of potent PEG concentrated combined inactivated viruses vaccine containing BVD, BoHV-1, PI-3, & BRS viruses(pneumo-4) by using two types of adjuvants (Montanide oil ISA 206 & Aluminum hydroxide gel) followed by evaluation of humeral immune response in calves compared with the commercial un concentrated vaccine and estimating the proper protective dose for each vaccine.

The applied experiment revealed that:

All viruses were successfully propagated on MDBK cell culture inducing cytopathic effects (72 h for BVD & BRS, and 24-36 h for BoHV-1 & 48 h for PI-3) post inoculation.

The highest viruses' titer obtained was $6 \log_{10}$ TCID₅₀/ml for BVD and BRS viruses, and $8 \log_{10}$ TCID₅₀/ml for BoHV-1 and PI-3 viruses.

All viruses were concentrated separately by using PEG 6000 then inactivated by BEI then used in preparation of the two vaccines, one by using Montanide oil ISA 206, and the other by using Aluminum hydroxide gel as adjuvants.

The prepared concentrated inactivated Pneumo-4 vaccines were found to be free from foreign contaminants; did not induce any clinical abnormalities or deaths among inoculated mice. As well as neither elevation of body temperature nor appearance of any clinical abnormalities reported in calves during 21 days of observation.

Evaluation of the prepared vaccines by vaccination of calves with different doses from each vaccine (2 ml, 2.5 ml & 3 ml from concentrated inactivated pneumo-4 gel vaccine) & (2.5 ml, 3 ml, & 3.5 ml from concentrated inactivated pneumo-4 oil vaccine) in three groups of calves (6 months age) per each vaccine. Comparing with another group injected with 5ml of the commercial unconcentrated vaccine. That occurred by giving 2 injections of each vaccine with 2 weeks interval by intramuscular route. Another group of calves was left as control till the end of the experiment.

Monitoring of the induced immunity in vaccinated calves with the prepared concentrated inactivated Pneumo-4 vaccines; it was found that SNT and ELISA tests revealed that the vaccine was able to induce detectable levels of specific BVD, BoHV-1, PI-3 & BRS viruses' antibodies by the 2nd wpv. These antibodies were increased to reach a maximum titer on 2 mpv for the concentrated inactivated pneumo-4 gel adjuvanted vaccine then remain protective till 7 mpv for all viruses contained in the vaccine and reach the maximum level on 3 mpv for concentrated inactivated pneumo-4 oil adjuvanted vaccine and remained protective more than 9 mpv.

Also It was noticed that the groups of calves vaccinated with 2.5ml or 3ml of gel vaccine mostly gave the same immune response but higher than that given 2ml of the gel vaccine, while the calves vaccinated with 3 ml & 3.5 ml of oil vaccine gave the same immune response but also higher than 2.5 ml in the titer and duration comparing with the group vaccinated with commercial vaccine and unvaccinated control group.

The prepared vaccines were applied in field in 2 different farms, by using the dose 2.5ml & 3ml from the concentrated inactivated gel & oil adjuvanted pneumo-4 vaccines respectively as they gave almost the same effect of the immune response and its duration of the largest dose (3ml & 3.5ml). The result proved the efficacy of the prepared vaccines to induce high immune response especially in oil adjuvanted vaccine which persist high till the end of experiment.