GLYCOPROTEIN VACCINE AGAINST IBR : EFFECT OF VACCINATION ON LATENCY (RECRUDESCENCE AFTER CHALLENGE EXPOSURE)

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I - INTRODUCTION

Live as well as inactivated and subunit vaccines are used in the prophylaxis of diseases caused by BHV1. Live vaccines may be used intranasally or parenterally (7,9,11). The fact that they may establish latency (7), the finding that they may provoke in vivo genomic recombination (12,13,14) can probably be considered as a disadvantage for their use in eradication programs. Indeed, by corticoïd treatment, reexcretion of vaccine virus has been reported. Inactivated vaccines do not pose these problems. Their potency is mostly to be enhanced by the use of oil adjuvants. Whole virus oil adjuvanted vaccines have proven their efficiency (8,9) but post vaccinal reactions were noticed (4,5). Both live and inactivated vaccines proved to be efficacious for reducing the excretion of virulent virus from experimentally challenge exposed animals (8,9,10,11).

Results obtained by Chappuis et al. (2) using cat herpesvirus as an experimental model, showed the importance of the viral capsidal proteins in eliciting post-vaccinal reactions. Therefore, in our laboratories, herpesvaccines solely composed of the viral envelope (subunit vaccines) were developed and tested in various species such as feline, swine, bovine and equine. This paper describes further experimental results concerning the **effect of vaccination with a sub-unit glycoprotein oil-adjuvanted BHV1 vaccine (*), with particular emphasis on the latency phenomenon.**

(*) IBEPUR (Trade name of RHONE-MERIEUX)

2 - MATERIALS AND METHODS

Experimental design : see table 1.

Animals : 10 cattle - 3 months old at day D0.

Vaccine : Ibepur has been administered to the animals according to the experimental design presented in table 1. The dose is 2 ml, injected subcutaneously.

Challenge exposure : 1 non-vaccinated control has been intranasally inoculated wit 5.10^5 TCID50, in 5 ml PBS, of a virulent BHV1 virus and used as excretor for the other animals by contact. The challenge strain represents the second passage on primary calf kidney (CK) cells.

Dexamethazone treatment : All the animals were injected with dexamethazone during 5 days, from day D455 (= D'107 after challenge exposure), the injection was I.V. for the first day of treatment, I.M. for the 4 other days, at the dosage of 0.1 mg/kg B.W.

Serology : Blood samples were collected on days **D 326**, **D 368**, **D 390** (=one injection of vaccine for animals of groups 1,3), **D 411** (=one injection of vaccine for animals of groups 1,3) and **D 455** (=beginning of dexamethazone treatment). Sera were titrated for BVH1 antibodies by serumneutralisation test (SNT) : 0.1 ml of threefold dilutions of inactivated sera (30 mn -56°C) were incubated at 37°C with 0.1 ml containing 30 TCID50 of IBR virus. After 24 hours 30,000 CK cells were added in each microwell. Final reading was done after 7 days.

Clinical symptoms : From day D 463 to D 478 the following clinical parameters were monitored daily : 1. hyperthermia, 2. nasal discharge, 3. red nose or epiphora, 4. membranous lesion. From these parameters a total clinical score was established by adding each daily record.

Virus isolation : nasal swabs were taken daily from D 463 to D 478. Swabs were stored at -20°C. After centrifugation, microtiter plates containing CK-cells suspension were inoculated with tenfold dilutions. Final reading was done after seven days. Titers were calculated according to Kärber.

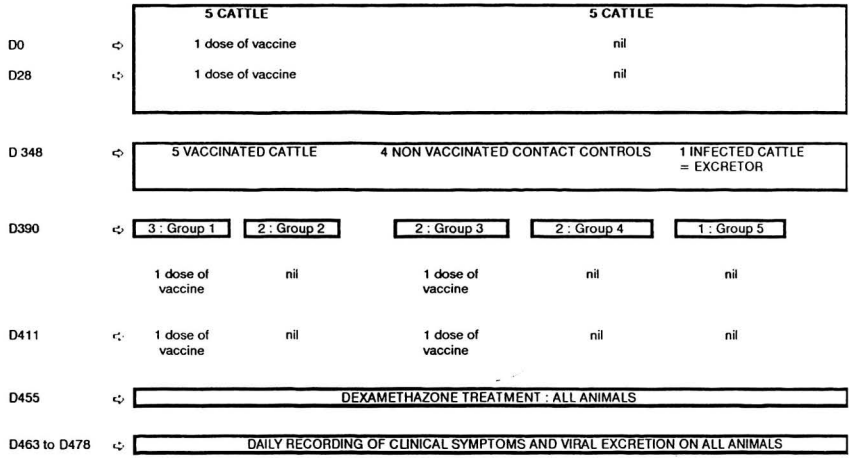

Table 1 : EXPERIMENTAL DESIGN

		D326	D368	D390	D411	D455
Group ①	Vaccinated before and after challenge	0.5	3.1	3.1 (v)	3.6 (v)	3.1
		0.7	4.1	3.6 (v)	3.6 (v)	3.6
		0.2	3.8	3.4 (v)	3.6 (v)	3.6
Group ②	Vaccinated before challenge, not after	1.7	4.1	3.6	4.1	3.6
		1	3.8	3.6	3.6	3.6
Group ③	Vaccinated after challenge, not before	0.2	2.6	2.4 (v)	3.6 (v)	3.6
		0.2	2.2	1.9 (v)	3.4 (v)	3.6
Group ④	Non vaccinated controls	0.2	2.6	2.6	2.9	2.6
		0.2	2.9	2.6	2.6	2.2
Group ⑤	Excretor non-vaccinated	0.2	2.6	3.6	2.6	2.6

Table 2 : SEROLOGICAL RESULTS (log 10 - SN)
(V) 1 injection of vaccine

		SN antibody titer			Total clinical score after Dexamethazone treatment (15 days)		Viral excretion after Dexamethazone treatment	
		D326	D368	D455	Individual	Mean (= m)		
Group ①	Vaccinated before and after challenge	0.5	3.1	3.1	1	m = 4.3	negative	m = 5 days
		0.7	4.1	3.6	6		positive 6 days	
		0.2	3.8	3.6	6		positive 9 days	
Group ②	Vaccinated before challenge, not after	1.7	4.1	3.6	7	m = 4	negative	
		1	3.8	3.6	1			
Group ③	Vaccinated after challenge, not before	0.2	2.6	3.6	1	m = 5.5	positive 4 days	m = 8 days
		0.2	2.2	3.6	10		positive 12 days	
Group ④	Non vaccinated controls	0.2	2.6	2.6	21	m = 17.6	positive 9 days	= 10.6 days
		0.2	2.9	2.2	15		positive 9 days	
Group ⑤	Excretor non-vaccinated	0.2	2.6	2.6	17		positive 14 days	

Table 3 : Serological ; clinical and viral excretion Results after Dexamethazone treatment.

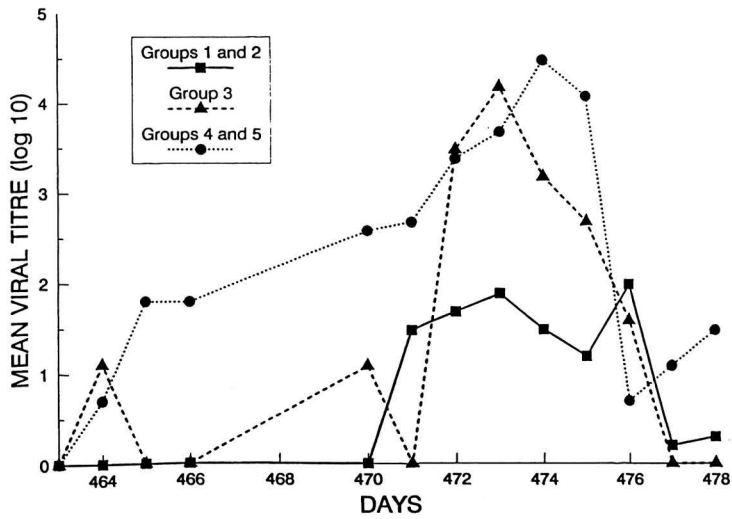

Fig. 1 : Viral excretion after Dexamethazone treatment - Mean titer in log 10

3 - RESULTS

Serological results : On previously vaccinated animals, the challenge exposure induced a notable booster effect (Group 1 and 2). Furthermore vaccination after challenge induced also a marked booster effect on those animals not vaccinated previously (Group 3). See table 2.

Clinical results after dexamethazone treatment : (see table 3)

One animal of groups 4 and 5, (which did not receive any injection of vaccine) presented the highest (=worst) clinical score (mean = 17.6). The situation was better for animals vaccinated after challenge exposure (Group 3) with a mean of 5.5. The animals vaccinated before challenge presented the most favourable results : 4.3 for group 1 ; 4 for group 2.

Viral excretion (see table 3 and figure 1) :

No virus could be isolated in the swabs of one animal of group 1 and the two animals of group 2 (vaccination before challenge, with or without vaccination after challenge). Two animals of group 1 excreted virus. The other animals, not vaccinated before challenge excreted BHV1 virus after dexamethazone treatment. The duration of viral excretion was shorter for groups 1 and 3 (vaccinated after challenge) than for groups 4 and 5 (non-vaccinated animals).

4 - DISCUSSION

The results obtained in this experiment clearly demonstrate the interest of vaccination against BHV1 infection in cattle in relation with recrudescence of BHV1 infected cattle.

It has been previously demonstrated that inactivated vaccine were able to reduce viral recrudescence on previously infected animals (5).

In the present work, vaccination with a subunit glycoprotein vaccine was able to reduce strongly viral excretion after dexamethazone treatment on cattle previously challenge exposed, 348 days after a vaccination protocol consisting of two injections of vaccine, 28 days apart ; 3 of the 5 animals of groups 1 and 2 did not excret virus.

Furthermore, the recently published results about latency of live BHV1 strains and about the risk of genomic recombination within the BHV1 population (wild or not) (12,13,14) allow to make questionable the use of live stains in control programs against BHV1 infection in cattle.

On the contrary, sub-unit glycoprotein vaccines cannot induce latency, nor genomic recombination phenomenon.

The results of the present experiment may give new perspectives for the control of BHV1 infection in cattle.

Summary

Five cattle were twice vaccinated. The interval between the vaccinations was 28 days. Five cattle served as non-vaccinated controls.

348 days after the first vaccination, one non-vaccinated control animal was intranasally challenged with 5.10^5 TCID50 of virulent BHV1 virus. It served as contact excretor animal for all the other non vaccinated controls and the vaccinates.

After challenge, the following treatments were performed :

- three previously vaccinated animal, and two non-vaccinated controls received one dose of vaccine at day D 390 and day D 411 after challenge while the remaining five animals did not.
- all the animals were treated with dexamethazone during 5 days at day D 455 after challenge (I.V. 1st day, I.M. 4 days).

Vaccination significantly reduced virus excretion and clinical signs after challenge. Some of the vaccinated animals (vaccination before challenge) were protected against recrudescence after dexamethazone treatment.

Detailed results will be presented and discussed in the context of control of BHV1.

Résumé

Cinq bovins ont reçu deux injections de vaccin. L'intervalle entre les injections était de 28 jours. Cinq autres bovins servaient d'animaux témoins-non-vaccinés.
348 jours après la première injection, un animal témoin-non-vacciné a été éprouvé par la voie intranasale, par 5.10^5 TCID50 d'un virus BVH1 virulent. Il a servi d'animal contact excréteur pour tous les autres animaux, témoins-non-vaccinés et vaccinés.
Après épreuve, les traitements suivants ont été réalisés :
- trois des bovins précédemment vaccinés, et deux bovins témoins-non-vaccinés ont reçu une dose de vaccin aux jours D 390 et D 411 après épreuve, tandis que les autres bovins n'en recevaient pas.
- tous les animaux ont été traités par la déxamethazone pendant 5 jours, au jour D 455 après épreuve (I.V. le premier jour ; I.M. les quatre autres jours).

La vaccination a réduit significativement l'excrétion virale, et les signes cliniques après épreuve. Certains animaux vaccinés (vaccination avant épreuve) ont été protégés contre une recrudescence virale après le traitement à la dexaméthazone.
Les résultats détaillés seront présentés et discutés à la lumière du contexte lié au contrôle de l'infection par le virus herpès bovin 1.

Zusammenfassung

Fünf Rinder wurden 2 x geimpft. Der Abstand zwischen den beiden Impfungen war 28 Tage. Fünf Rinder dienten als ungeimpfte Kontroll-Tiere.
348 Tage nach der ersten Impfung wurde ein ungeimpftes Kontrolltier intranasal mit 5.10^5 KID 50 eines virulenten BHV1- Virus belastet. Dieses Tier diente zur Kontakt-Infizierung für die ungeimpften Kontrollen sowie die geimpften Tiere.
Nach der Belastungsinfektion wurde wie folgt verfahren :
- drei vorhergehend geimpfte Tiere und zwei ungeimpfte Kontrollen erhielten 390 und 411 Tage nach der Testinfektion 1 Impf-Dosis-die fünf anderen Tiere nicht.
- alle Tiere wurden 455 Tage nach der Belastungsinfektion 5 Tage lang mit 0,1 mg/kg Dexamethazone behandelt.

Die Impfung reduzierte deutlich die Virusausscheidung und die klinischen Anzeichen nach dem Test. Einige geimpften Tiere (Impfung vor dem Test) waren nach der Dexamethazon-Behandlung gegen ein Wiederaufleben der IBR geschützt.
Nähere Einzelheiten werden im Kontext mit der Bekämpfung von BHV1 dargelegt und diskutiert.

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