SAFETY OF A NEW ATTENUATED IBR LIVE VACCINE WITH DOUBLE GENETIC DELETION gE-tk- DISSEMINATION, LATENCY/RE-EXCRETION AND ABORTIGENICITY: 3 TRIALS ASSAYING VERY HIGH DOSES

Alberto Moreno, Eva Desmiquels, Carol Casado, Mercè Roca

HIPRA, Amer (Girona) - Spain

INTRODUCTION

Eradication programs in the EU regarding Infectious Bovine Rhinotracheitis (IBR) are based mainly on a differentiation of infected from vaccinated animals (DIVA) strategy by using marker vaccines. So far, all these IBR marker vaccines were based on Bovine HerpesVirus-1 strains lacking glycoprotein E (single deleted BoHV-1 gE- strains).

At HIPRA we have developed and registered in the EU a new attenuated IBR vaccine (Hiprabovis® IBR Marker Live) which contains a BoHV-1 strain with a double genetic deletion gE-tk-. This viral strain features, as well as the gE deletion, which confers attenuation on the parental strain and allows for the serological marking, an extra deletion affecting the gen of the enzyme thymidine kinase (tk), as this confers also attenuation on the parental strain (1), reduces the probabilities of latency, reactivation, re-excretion and re-isolation in the field (2) and makes highly improbable the recovery of virulence after an event of herpesvirus spontaneous recombination (3).

This new IBR vaccine has widely demonstrate its safety through different trials, according to the strict requirements of European Pharmacopeia: recommended dose, repeated dose, overdose, transmission to unvaccinated animals, reversion to virulence, safety in pregnant cows, dissemination.

We present here three studies focused on demonstrating the safety of the new vaccine Hiprabovis® IBR Marker Live due to the intrinsic characteristics of the double deleted strain (strain CEDDEL) that contains:

Trial 1: dissemination, shedding and latency
Trial 2: latency, reactivation and re-excretion
Trial 3: abortigenicity and passage through the placenta.

MATERIALS AND METHODS

Animals: all the animals included in these trials were crossed-bred Friesians, in good health status and free of BoHV-1 antigen and antibodies.

Vaccine: Hiprabovis® IBR Marker Live (strain CEDDEL, gE-tk-), HIPRA.

Virus detection: an extensive array of technics was applied to detect BoHV 1 in the samples:

- Isolation and titration by cell culture
- A new developed and validated specific differential PCR:
  - Specific for BoHV-1
  - Differentiation of gE+/gE- strains
  - Differentiation of tk+/tk- strains
- Immunohistochemistry.

Method:

Trial 1. Dissemination, shedding and latency after a very high vaccine overdose
6 seronegative calves (3 male and 3 female) were vaccinated with a very high vaccine overdose (>10exp8.3 $CCID_{50}$/calf). This is more than 10 times the maximum vaccine dose. Necropsies of one male calf and one female calf were performed at 2, 4 and 6 days post vaccination. The extensive sampling included swabs, body fluids, respiratory and reproductive tissues and trigeminal ganglia.

Trial 2. Latency, reactivation and re-excretion after vaccination and treatment with dexamethasone
In this study 10 animals were used. 4 seronegative calves were vaccinated and revaccinated with the maximum vaccine doses (10exp7.3 $CCID_{50}$/calf) 21 days apart. 4 positive control calves were in parallel inoculated with 10exp7 $CCID_{50}$/calf of a virulent BoHV-1 challenge strain. 2 calves were kept as control unvaccinated animals. Three months after vaccination, all calves were treated with dexamethasone and latency, reactivation and re-excretion of the vaccine virus were analysed through a 21 days follow up period and through necropsies of tonsils and trigeminal ganglia.

Trial 3. Abortigenicity and passage through the placenta
24 seronegative cows at different stages of pregnancy were vaccinated:

- 8 in the 4th month of pregnancy
- 8 in the 5th month of pregnancy
- 8 in the 6-7th month of pregnancy

Vaccination was performed with a 10 times vaccine overdose (10exp8.3 $CCID_{50}$/cow). All cows were observed daily until the end of pregnancy plus a 15 days post-partum period of observation of the cows and of the offspring.

RESULTS AND DISCUSSION

Trial 1. Dissemination, shedding and latency after a very high overdose
No dissemination of the vaccine virus to the different tissues and organs of the vaccinated animals was detected, despite the vaccination with a very high vaccine overdose (>10exp8.3 $CCID_{50}$/calf). This result was identical whether the tissues were respiratory (nasal mucosa, trachea, lung), reproductive (testis, seminal vesicle, prostate, ovary, uterine mucosa, vaginal mucosa) or nervous (trigeminal ganglia), both by virus isolation and by specific differential PCR.

No viral shedding was detected: All swab samples (including nasal swabs) and body fluids (whole blood, serum, urine; and faeces) were negative for presence of BoHV-1, both by virus isolation and by specific differential PCR.

Remarkably, **no genetic material of BoHV-1 was detected in the trigeminal ganglia**, thus indicating **no latency** of the vaccine virus. A summary of the results is presented in Table I below.

Table I. Dissemination, shedding and latency after very high overdose (trial 1)

♂	swabs				blood		faeces	urine	nasal mucosa	conjun tiva	traq.	lung	trigem. ganglia	testes	semin. vesic.	prostate
	nasal	oral	ocul.	balan.	with hepa.	serum										
$CCID_{50}$	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PCR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

♀	Swabs				Blood		faeces	urine	nasal mucosa	conjun tiva	traq.	lung	trigem. ganglia	ovary	uterine mucosa	vaginal mucosa
	nasal	oral	ocul.	vagin.	with hepa.	serum										
$CCID_{50}$	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PCR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Trial 2. Latency, reactivation and re-excretion after vaccination and treatment with dexamethasone
The vaccine virus was not shed (was not re-excreted) after the dexamethasone treatment. Nasal swabs were negative for presence of BoHV-1, both by virus isolation and by specific differential PCR. In contrast, the positive control calves did shed high amounts of the virulent BoHV-1 strain after the dexamethasone treatment.
Furthermore, neither infectious virus nor genetic material of BoHV-1 was detected in tonsils and in trigeminal ganglia of the vaccinated animals, further indicating a lack of latency of the vaccine virus.

Trial 3. Abortigenicity and passage through the placenta
22 cows remain well until the end of pregnancy giving birth to 23 healthy calves (there was a case of twins). The 2 cases of abortion were thoroughly investigated. There were no evidences of IBR infection in these abortions: no virus was detected in placenta or foetus by a number of technics, including immunohistochemistry. In contrast, a bacterial infection was determined in one of the abortions. All calves born to term were in good health and were completely seronegative to IBR antibodies before sucking the colostrum.

CONCLUSIONS

The safety of a new double deleted (gE-tk-) attenuated live IBR vaccine (Hiprabovis® IBR Marker Live, HIPRA) was demonstrated in relation to the intrinsic characteristics of the double deleted strain CEDDEL:

- There was no dissemination or shedding of the virus after vaccination with a very high overdose
- No latency (trigeminal ganglia) was detected in any trial
- After dexamethasone treatment there were no evidences of reactivation or viral re-excretion
- The vaccine virus is not abortigenic: it did not cross the placenta

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Laboratorios Hipra, S.A.
Avda. la Selva, 135
17170 Amer (Girona)
Spain

Tel (34) 972 43 06 60
Fax (34) 972 43 06 61
hipra@hipra.com
www.hipra.com