Experiences With Two BHV1 Marker Vaccines

O.C. Straub
Federal Research Centre for Virus Diseases of Animals
P.O. Box 1149, D-72001 Tübingen

Abstract

Both marker vaccines tested were deleted vaccines, one had a gG, the other a gE deletion. The gG deleted vaccine was inactivated the gE deleted vaccine had a live and an inactivated form.

The object of the trial was to evaluate these vaccines in heifers and calves. Twenty young heifers were used for the inactivated deleted vaccines and 16 four week old female calves for the gE deleted live vaccine. Animals were three times vaccinated, first at 4 and 7 week intervals and again six or ten months later. Challenge was conducted by placing seronegative fields virus inoculated animals between the vaccines. Neither vaccine was able to prevent infection with filed virus, long virus shedding periods and the establishing of latency. Local reactions were insignificant concerning general health. The humoral response was better after the seven week interval but the protection form clinical disease was not. The live vaccine led to a better protection from clinical disease than the inactivated vaccines.

Introduction

BHV1 infections occur in most countries. Prevalences vary, also the type of clinical disease caused by BHV1. In some countries the most common infection is in the genital tract but in others it is the respiratory tract. Less frequent are the other manifestations: conjunctivitis, abortion, mastitis, encephalitis, enteritis, dermatitis and lesions in the interdigital space. Using biochemical methods it is possible to differentiate BHV1 strains, but the serological response is always the same, including conventional vaccines and BHV5 infections. This means that it is impossible to differentiate between antibodies derived from any of the field virus infections or vaccines.

For eradication programmes, however, the possibility to differentiate between antibodies acquired after field virus infections and antibodies following vaccinations, it is inevitable to use marker vaccines. A number of marker vaccines have been developed and two of them have been tested in the investigation to be reported, an inactivated one with a gG deletion and one with a gE deletion of which a live and an inactivated product was available.

Materials and Methods

Animals

For-eight calves and heifers were used in this investigation, 16 for the live vaccine and 20 for the inactivated vaccines. Twelve non-vaccinated animals were infected with field virus for the challenge experiment. Second vaccinations were carried out at four or seven weeks and the third vaccinations six or ten months later. The challenge was carried out 10 weeks after the third vaccinations by contact with the field virus infected animals. Immunosuppressions were carried out in the animals vaccinated with the live vaccine prior and after challenge and in the cattle vaccinated with the inactivated vaccines after challenge. Details are given in Tables 1 and 2.

Table 1. Vaccination scheme of the inactivated application.

Mode of vaccination	no. of animals	interval after 1st vaccin.	interval after 2nd vaccin.	interval to challenge	interval to immunosuppr.
sbc	10 1)	4 weeks	10 months	10 weeks	4 months
sbc	10 2)	7 weeks	10 months	10 weeks	4 months

1) and 2) 5 of each vaccine

Table 2. Vaccination scheme of the live vaccine application.

Mode of vaccinat.	no. of animals	interval after 1st vacc.	interval to immuno-suppress	interval after 2nd vacc.	interval to Challenge	interval to immuno-suppress
i.n.	4	4 weeks	2 months	6 months	2 months	3 months
i.n./i.m.	4	4 weeks	2 months	6 months	2 months	3 months
i.n.	4	7 weeks	2 months	6 months	2 months	3 months
i.n./i.m.	4	7 weeks	2 months	6 months	2 months	3 months

i.n./i.m. - the first two vaccinations were intranasal, the third intramuscular

Presented at the XIX World Buiatrics Congress, Edinburgh, Scotland, July 8-12, 1996.

Vaccines

The vaccines used were supplied by the companies. The live vaccine contained $10^{5.0}$TCID$_{50}$ per ml 2 ml were injected per animal, the inactivated vaccines $10^{8.0}$ TCID$_{50}$ before inactivation. The dose was also 2 ml.

Challenge virus

The BHVI strain used has the designation "Würzburg". It is the same strain used in other vaccination trials.

Serology

For the virus neutralization test the usual strain BFA4SCH (9th passage) was employed.

Immunosuppression

Based on earlier trails prednisolon was used: 25 mg per 50 kg of bodyweight injected for 6 consecutive days.

Clinical observation, collection of samples, isolation of virus, tissue cultures and serological tests. All of them were carried out as described.

Results

Clinical symptoms after vaccinations

After the vaccination with the live vaccines (Table 2) no adverse effects were observed. The same is true for the first two vaccinations with the two inactivated vaccines for most of the animals. The third vaccination, however, led in 17 heifers to local reactions. They were less severe in cattle vaccinated with the gE deleted vaccine.

Virus shedding

After the first vaccination the live vaccine was recovered from the nasal swabs for up to 16 days with a maximal titre of $10^{7.75}$ TCID$_{50}$/g mucus. Following the second vaccinations virus shedding in low quantities could only be proven in one animal after the four and in three animals after the seven seeks interval ($10^{1.0}$ and $10^{2.0}$ TCID$_{50}$ per g mucus.).

Clinical symptoms and virus recovery before and after challenge and immunosuppressions

All animals inoculated with field virus developed the typical symptoms of IBR within the first three days. They shed virus for one to twelve days with highest titres ranging from $10^{8.0}$ to $10^{9.0}$ TCID$_{50}$ per g of nasal discharge.

The majority of the animals vaccinated with the live gE-deleted vaccine (Table 2) showed no or only mild clinical symptoms such as a slightly elevated body temperature, increased nasal discharge and salivation. None of the vaccinated animals were inappetent. Following the first immunosuppression (Table 2) vaccine virus could be isolated from nine of the 16 animals for one to 13 days between days nine and 31 after the application of prednisolon.

The majority of the animals vaccinated with the inactivated marker vaccines, however, developed clinical symptoms of IBR with varying severity. Most severe were the symptoms in animals vaccinated at the seven week interval with the gE-deleted vaccine. From the five animals all but one had highly elevated body temperatures (up to 40,9°C for several days and decreased appetite, one became totally inappetent. Other symptoms were conjunctivitis (four animals), seromucoid (all animals), mucopurulent (two animals) nasal discharge, dysponea (two animals). They shed virus from two to 13 days with titres ranging from $10^{3.0}$ to $10^{7.75}$ TCID$_{50}$ per g of nasal discharge.

All animals vaccinated with the gG-deleted vaccine also at the seven week interval - showed increased - mostly seromucoid - nasal discharge and conjunctivitis for up to 11 days. Feed consumption remained normal. Virus shedding was observed from days one to 17 with titres ranging from $10^{4.75}$ to $10^{7.5}$ TCID$_{50}$ per g of nasal discharge.

From the five animals vaccinated firstly at the four week intervals with the inactivated gE-deleted vaccine three showed increased body temperatures for two to five days (max. 40.1°C), seromucoid nasal discharge - all animals - for up to eight days and conjunctivitis for one to five days. Feed intake was not disturbed. Virus was recovered from nasal mucus fro three to nine days with titres ranging from $10^{2.5}$ to $10^{7.5}$ TCID$_{50}$ per g. The corresponding group, similarly vaccinated with the gG-deleted vaccine, showed the least symptoms: slightly elevated body temperatures (up to 39.5°C), increased serous and seromucoid nasal discharge for eight to nine days and conjunctivitis in four animals for three to five days. Feed intake was normal. Virus could be isolated for seven to eleven days with titres ranging from $10^{6.5}$ to $10^{8.5}$ TCID$_{50}$ per g of mucus.

Clinical symptoms and virus recovery following immunosuppression after challenge

The application of prednisolone did not lead to any clinical symptoms independent of whether virus was shed.

All but one animal of the field virus infected controls shed virus for seven to nine days beginning at day six after the administration of prednisolon with titres ranging from $10^{1.25}$ to $10^{6.5}$ TCID$_{50}$/ g nasal mucus.

From the inactivated gE-group four of the five animals vaccinated at the four week interval four of the five animals vaccinated at the four week interval four shed virus for one to 13 days beginning at day 6 with titres ranging form $10^{0.75}$ to $10^{3.0}$ TCID$_{50}$ per g nasal mucus.

From the group vaccinated at the seven week interval virus was only recovered from two animals. One shed virus for only for one day with a titre of $10^{4.0}$ $TCID_{50}$ and the other for seven days (from day 5 to 11) with a maximum titre of $10^{5.5}$ $TCID_{50}$ per g of nasal mucus. From the inactivated gG-group the result was the following: in the four week interval group three of the five animals shed virus from days 8 to 22 for dive (one heifer) or 15 days with titres from $10^{2.75}$ to $10^{4.75}$ $TCID_{50}$ per g of nasal mucus and from the seven week interval group virus could be isolated from four heifers for 5 to 13 days with titres from $10^{0.75}$ to $10^{6.5}$ $TCID_{50}$ per g of nasal mucus.

The results of the live gE-group were essentially the same as after the first immunosuppression: 10 of the 16 animals shed virus. The identification revealed that both viruses could be recovered, the vaccine and the challenge virus.

Results of the serological studies

The application of the inactivated vaccines led after the first vaccination to the induction of fairly low virus neutralizing antibodies (max. 1.054) First proof was 10 days p. vacc. and the highest titers reached in only three of the 20 animals at day 21. In the majority the highest level was observed between days 36 and 45 p vacc. A comparison of the titres revealed that the GG-deleted vaccine led in general to higher antibody titres than the gE-deleted one. This is also true for the titres after the second vaccination. The highest titres were reached 20 to 30 days after the second vaccination. The overall highest titres developed in those animals that were vaccinated at the seven week interval with significantly higher titres in all animals. The animals originally vaccinated at the seven week interval reached the highest titres of all groups. The maxima were reached in all animals after three(!) to twelve days p.vacc. There was no significant difference in titres of animals in the groups vaccinated with the gE-deleted vaccine.

Neutralizing antibodies were, however, already detected at day five after vaccination with the live gE-deleted vaccine. At the time of the second vaccination at day 28 all the animals had not reached the maximum titres because the seven week group showed increases until day 42 p. vacc. The booster led to a rapid increase in titres until day 12. The same was true following the third vaccination, where all but one animal showed another booster reaction.

Discussion

What was expected from the results of earlier studies and the properties of herpes viruses was confirmed. No vaccination is able to prevent infection and subsequently the establishment of a latent stage that under stress leads to virus shedding. It would have been astonishing if the gE-deleted vaccine would not have become latent as had been stated earlier. It is obviously a matter of the corticosteriod used.

The results also demonstrate that there is no correlation between antibody level, reaction to challenge and immunosuppression. The more severe local reaction but without any other symptom following the third vaccination with the inactivated gG-deleted vaccine is probably due to an improved cell-mediated immunity induced by the viral components since these animals had the highest humoral antibody titre. It might also be due to a response caused by the adjuvant, since the vaccination with the inactivated gE-deleted vaccine did not cause these local reactions.

The results obtained lead to the conclusion that the live gE-deleted vaccine first applied locally offers an earlier and better protection from clinical disease than both inactivated vaccines. The best interval between the first two vaccinations most likely lies between four and seven weeks because the humoral antibody production reaches the maximum in most animals after four weeks, but in individuals already after three weeks. The difference might be due to the estrogen or progesteron levels of the animal at the time of vaccination.

Most important is, however, the advantage of the marker vaccines to introduce eradication programmes since it is possible to differentiate between field and vaccine derived antibodies. It is desirable to improve the potencey of the inactivated vaccines to protect cattle from clinical symptoms caused by BHV1.

Keywords: BHV1 vaccines - deletion - protection · latency