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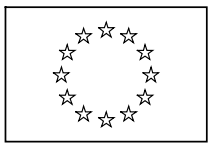

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C3 - Management of scientific committees II; scientific co-operation and networks

Report on Bovine Herpesvirus 1 (BHV1) marker vaccines and the accompanying diagnostic tests

Scientific Committee on Animal Health and Animal Welfare

Adopted 25 October 2000

Report on
Bovine Herpesvirus 1 (BHV1) marker vaccines and the accompanying diagnostic tests

Scientific Committee on Animal Health and Animal Welfare (SCAHAW)

Contents

1. Request for an opinion

The Scientific Veterinary Committee adopted the report VI/9098/95 on the use of marker vaccines and related tests in eradication programmes for Infectious Bovine Rhinotracheitis (IBR) in 1996. The report noted that much information was not available at that time and anticipated that more information would become available in the coming years. The committee is asked by the Commission to update this advice in the following areas:

1. Types of marker vaccines and their possible use in the European Union.

2. Efficacy and safety of Bovine Herpesvirus type 1 (BHV1) marker vaccines for use in eradication programmes.

3. Risks of latency and reactivation associated with the use of live marker vaccines.

4. Sensitivity and specificity of the available accompanying diagnostic tests.

5. Possibility of the existence of seronegative carriers and the risks posed by this.

2. Introduction and background

In the 1996 report it was concluded that, as for marker vaccines, the properties of gE-deleted vaccines and gD-subunit vaccines were best known and fitted rather well in a BHV1 eradication strategy in highly infected areas. It was expected that other strains with deletions in the genes coding for gC, gG, and gI would perhaps become available. Therefore no recommendation on preferred choice of deletion vaccines, whether live or inactivated was made in 1996. A recommendation on the choice of live versus inactivated vaccine, especially in case of gE-negative live marker vaccines could not be formulated. Indeed, the better efficacy of the gE-negative live marker vaccine compared to the gE-deleted inactivated and gD-subunit vaccine in vaccination challenge studies and in experimental transmission studies, and the evidence that vaccine virus reactivation is not a frequent phenomenon, makes that such vaccine could play a key role in an eradication programme. It was stated that in 1996 the importance of latency of gE-negative live marker vaccines was unknown and had to be further evaluated.

Meanwhile new data on BHV1 marker vaccines and on accompanying tests have become available. In this document the term “marker” vaccine is used, though the terms ‘deleted’ and ‘DIVA’(Differentiating of Infected from Vaccinated Animals) vaccines can also be used.

Denmark, Finland, Sweden, Austria and the province of Bolzano in Italy have been recognised as free from IBR under EU legislation (Commission Decision 93/42/EEC as amended).

3. Discussion on new data on marker vaccines and their implications for BHV1 eradication.

3.1. Types of marker vaccines and their possible use in the European Union

Since the former report no new BHV1 marker vaccines, have been developed. Therefore, reference can be made to the former report on the different vaccines with the following markers: gE-live, gE-killed, gG-killed, gC-live, gD-subunit, gB-subunit and gD-replication-incompetent.

To date, only gE-negative vaccines have been commercialised in the European Union. Much of the research relates to the commercial vaccines "Difivac", administered according to the directions of the manufacturer.

The inactivated gE-negative vaccine (active compound $10^{8.0}TCID_{50}$ gE-negative killed BHV1, strain Za (Difivac, inactivated) and adjuvant consisting of aluminium hydroxide and Quil A (Bayer, Germany)) is administered subcutaneously. The live gE-negative vaccine (active compound $10^{5.0}$-$10^{7.0}TCID_{50}$ gE-negative live BHV1, strain Za (Difivac, live), dissolved in distilled water (Bayer)) is given either intranasally or intramuscularly.
Unless otherwise noted, this strain has been used in the various experiments referred to in this report.

3.2. Efficacy of marker vaccines for use in eradication programmes

3.2.1. Experimental studies

Vaccination-challenge experiments

A combination vaccine containing gE-negative live vaccine, a BRSV and a BPI3 vaccine administered at two and six weeks of age has been shown to be efficacious in that it reduced severity of clinical signs and the amount of virus shedding after experimental challenge infection (Mars *et al,* 2000d).

A gE-deletion vaccine, wherein also the genes coding for gG and US2 had been deleted, has been found to be efficacious in preventing clinical signs and reducing challenge virus shedding in a vaccination-challenge experiment (Belknap *et al,*1999).

Kerkhofs *et al*., (2000) recently conducted a trial which aimed at the comparison, in the same bovine experiment, of the efficiency of four different immunisation protocols based on inactivated and live gE-negative vaccines with the first vaccination being administered to calves aged between four and six weeks. The first protocol consisted of two subcutaneous administrations of the inactivated vaccine while the second one involved two intramuscular injections of the live vaccine. The two other protocols both involved a first intranasal

administration of the live vaccine. In the third protocol, the first vaccination was followed by an intramuscular injection of the same live preparation whereas the inactivated vaccine was subcutaneously injected for the second vaccination of the cattle of the fourth group. The control group contained cattle that were not vaccinated. Both cellular and humoral responses were the greatest in the two groups where animals were vaccinated at least once with the inactivated vaccine. Following challenge-infection, a good clinical protection was observed in all vaccinated animals.
Although virological protection (reduction of virus titre in nasal swabs) was observed in all the vaccinated animals, the animals which received at least one administration of the inactivated vaccine showed a significant reduction of the challenge virus excretion compared to those that were only vaccinated with the live vaccine.

Prevention of reactivation/re-excretion of wild-type virus

Calves first vaccinated at the age of 7 weeks and challenge-infected and then treated with corticosteroids to reactivate putative latent virus shed less challenge-virus than non-vaccinated controls (Kaashoek *et al*, 1994).

Bosch *et al*., (1997b) carried out a comparative study to evaluate the ability of three BHV1 marker vaccines to reduce the re-excretion of virus after reactivation of latent BHV1. A live gE-negative vaccine, an inactivated gE-negative vaccine and an experimental gD-subunit vaccine were tested in three identical experiments in which yearling heifers, latently infected with BHV1, were vaccinated twice before they were treated with high doses of dexamethasone. Virus excretion after dexamethasone treatment was compared with that in BHV1-infected, unvaccinated cattle which served as controls. All cattle, controls and vaccinees, excreted virus. However, the inactivated vaccines reduced virus re-excretion more efficiently than did the live vaccine. This is somewhat unexpected in view of the earlier findings of efficacy of these vaccines (Bosch *et al*.,1996).

3.2.2 Field trials

A field trial with the live gE-marker vaccine.

In the Netherlands a randomised, double-blind, placebo-controlled field trial including 84 herds was conducted to assess the efficacy of an intramuscularly injected live gE-negative BHV1 vaccine administered to all cattle over 3 months, again after 4 weeks and again after 6 months (Mars *et al*., 2000a). The incidence of BHV1 infections during 17 months was monitored by detecting antibodies against BHV1 glycoprotein E. In the placebo-treated group 214 seroconversions in 3985 paired sera, and in the vaccinated group 67 seroconversions in 3601 paired sera were detected. The reproduction ratio[1] R_0 in placebo-treated herds was 2.5 (confidence interval 1.4-3.1) and in the vaccinated group 1.2 (confidence interval 0.5-1.5). The vaccinated and placebo-treated group differed significantly in transmission of BHV1, indicating that live gE-negative BHV1 vaccine reduced the incidence and transmission of BHV1 infections

[1] basic reproduction ratio of infection: average number of susceptible animals which are infected by one infected animal of a certain wild boar population

in the field , but not to an R_0 below 1, suggesting that additional measures may be necessary to achieve elimination in a herd

Field trials including both live and killed gE marker vaccines.

In 1997 a two year long BHV1 field trial was initiated in Belgium to compare the efficacy of 2 vaccination protocols based on repeated administration of the gE-deleted vaccines (Dispas *et al*., personal communication). Vaccination protocols were also compared with control groups which were given a farmer determined vaccination protocol. The two tested protocols only differ on primovaccination: all animals in the first group were treated with live attenuated vaccine (intranasal first administration at the age of 1 month, second by intramuscular route 3-5 weeks later, and all animals in the second group were treated with killed vaccine given by the subcutaneous route, the first vaccination given at 3 months, followed by a second vaccination 3-5 weeks later. All booster vaccinations were made with inactivated vaccines given subcutaneously every 6 months. Vaccine protection and virus circulation were estimated by individual serological testing using both gB- and gE-ELISA blocking tests. Two types of farms were investigated: pure dairy farms and dairy/beef combined farms, each accommodating about 100 animals. For each production type, 10 farms were followed up for each repeated vaccine administration schedule and 16 served as control group. Preliminary results suggest that

1. in dairy herds, vaccination reduced virus circulation with slightly better results for the killed vaccine protocol and

2. in dairy/beef combined herds, no reduction of virus circulation was observed compared to the control group. An additional study is underway to investigate the differences of apparent vaccine efficacy between the production types.

3.2.3. Discussion and conclusion

Experimental studies
The reported experimental trials with gE-deleted BHV1-marker vaccines demonstrated that these vaccines can reduce the severity of clinical signs, the shedding of challenge virus and reactivation after dexamethasone treatment, a common method for reactivating herpes viuses . Nevertheless, the efficacy varied with the vaccine type (live or killed), the vaccination scheme and the design of the experiment. Therefore, it remains difficult to recommend a particular vaccine type and vaccination scheme.
Other vaccine types like the gD-subunit vaccine have not been extensively tested. Only the gE-marker vaccines from one commercial manufacturer have been marketed to date.

Field Trials
Both live and killed gE-negative vaccines have shown efficacy under field conditions. However, significant differences between the vaccinated group and the control (placebo) groups were observed in different experiments, done by different groups with killed or live gE-deleted vaccines. The results varied with the vaccination scheme, the farm BHV1 status and the vaccine route and regime.
The available data seem not to be sufficient to recommend a common vaccination scheme, suitable for the different kinds of protection (clinical signs, virus shedding and transmission,

reactivation). Furthermore, some of the efficacious vaccination administrations/schemes are not recommended by the manufacturer and legislator in some countries.

3.3 Safety, risks of latency, reactivation, re-excretion, transmission and recombination associated with the use of live marker vaccines

3.3.1. Experimental studies

Reactivation of the vaccine virus

Live gE-deleted vaccine virus was isolated in nasal fluids of two cows eight months after intranasal vaccination which was followed 1 month later by intramuscular vaccination in a dairy farm experiencing an IBR outbreak. The genotypical characterisation of the reactivated strain was performed by gel electrophoresis after enzyme restriction analysis using Hind III and BSTII endonucleases, and by PCR using two different gE specific primers, confirming that the re-excreted virus was the Difivac strain as defined under 3.1 (Schynts *et al*., 1999). It suggests that commercially available live gE deleted marker vaccine can be re-excreted during spontaneous reactivation in field conditions (Dispas *et al*., personal communication).

In experimental conditions, 5 calves were inoculated intranasally with a genetically engineered gE deleted strain (Van Engelenburg *et al*., 1994). All the calves shed this virus strain after infection. The calves were treated with dexamethasone three months after infection. It was possible to re-isolate gE deleted strain in four out of the 5 treated calves indicating that this particular gE deleted strain which is not a validated vaccine strain and different from the Difivac strain could be reactivated and re-excreted in experimental conditions (Schynts and Thiry, personal communication), emphasising a possible strain effect.

After dexamethasone treatment of calves vaccinated intranasally with the gE-negative vaccine the vaccine virus was recovered from the nasal secretions in 2 calves and BHV1 DNA was detected by PCR in the trigeminal ganglia from 5 calves. Although maternally derived antibodies may have interfered with the vaccination in this experiment, the BHV1 gE-negative strain established latency and could be reactivated and re-excreted in the nasal secretions several months after only one intranasal inoculation. Moreover, BHV1 DNA sequences were found in the trigeminal ganglia of completely BHV1 seronegative animals which had been inoculated previously with the gE negative vaccine virus (Lemaire *et al*., 2000d).

Transmission of the vaccine virus

Mars *et al.,* (2000c) described four experiments to study possible reactivation and to quantify subsequent transmission of a live gE-negative bovine herpesvirus1 (BHV1) vaccine strain in cattle populations. Two groups of cattle aged 6-10 months at the start of the experiment were each tested twice for the possibility of reactivation. Inoculation with a gE-negative BHV1 vaccine was done either intramuscularly or intranasally and treatment with corticosteroids in an attempt to reactivate vaccine virus, was done after 6 or 11 weeks, and again after 6 months. To quantify transmission of vaccine virus following possible reactivation, each cattle was housed together with one susceptible contact-cattle. After corticosteroid treatments, re-excretion of virus was never detected in cattle that had been inoculated with gE-negative BHV1 vaccine strain. Contact cattle did not shed gE-negative BHV1, nor was an antibody response against BHV1 detected. In contrast, positive control cattle, inoculated intranasally with wild-type BHV1, re-excreted virus in high titres in nasal fluids and transmitted virus to contact cattle. Based on these

results the reproduction ratio R_0 of the vaccine strain was zero and it was concluded that it is unlikely that the live gE-negative BHV1 vaccine strain will be re-excreted after possible reactivation, and consequently, it is even less likely that reactivated vaccine virus will spread in the cattle population.

In another trial Van der Poel and Hage (1998) vaccinated cattle intramuscularly with live gE-negative BHV1 marker vaccine: of a herd of 114 BHV1-antibody negative cattle on one farm, 45 animals were vaccinated, and of a dairy herd of 55 cattle all 10 BHV1 positive animals were vaccinated. Vaccination was repeated after about a month. Antibodies against BHV1 were determined in all unvaccinated cattle. Only one unvaccinated cow (from the dairy herd) showed BHV1 seroconversion, detected by a gE-ELISA as well as by a gB-ELISA indicating that the animal had been infected by a field virus.
It was concluded that the intramuscularly administered live gE-negative BHV1 marker vaccine is unlikely to spread to unvaccinated cattle.

However, somewhat conflicting results were described by Wentzel(1996) and Wellemans and Vanopdenbosch (personal communication), who could isolate gE-negative intranasally administrated vaccine virus after corticosteroid treatment, although the duration and level of excretion was very limited, making it very unlikely that reactivated vaccine virus would have spread in the cattle population under natural conditions.

Three separate experiments showed shedding of gE-negative vaccine virus in high titres in nasal fluids after intranasal vaccination. Despite the high titres, only one out of fifteen contact calves became infected but did not shed the virus. The reproduction ratio was estimated to be 0.14, which is significantly below 1. After intramuscular vaccination no virus was detected in nasal fluids. It is therefore unlikely that the live gE-negative vaccine strain used will perpetuate in the cattle population (Mars *et al.,* 2000b)

Strube *et al*., (1996) reported that after intramuscular inoculation nasal virus shedding was not detectable in 3 month old calves but was observed at very low titres in a few calves that were vaccinated at 2 weeks of age.

Recombination

Homologous recombination is a well known feature in herpesvirus biology. It implies that an important aspect of the safety evaluation of gE negative live vaccines is the risk assessment of recombination between vaccinal and BHV1 field strains. With that goal, an experiment was set up to study genetic recombination between two BHV1 strains, including a gE deleted strain, following *in vitro* and *in vivo* co-infection (Schynts *et al*., 2000). Preliminary results indicate that recombination into the gE gene locus is a frequent event both *in vitro* and *in vivo.*

Contamination

Some batches of the gE-negative live vaccine were found to be contaminated with bovine virus diarrhoea virus in 1999 (Falcone *et al*., 1999).

3.3.2. Discussion and conclusion

The gE-negative vaccine virus can cause a latent infection and can be reactivated. The route of vaccine inoculation (intranasally or intramuscularly) influenced the probability of virus reactivation. The frequency of reactivation and subsequent re-excretion in the field is unknown. However, reactivation is followed by low excretion or no excretion, which means that the probability that vaccine virus transmission occurs is low.

While it could be desirable to carry out a risk assessment to quantify the risk of virus transmission as a result of reactivation, this is likely to be difficult due to lack of data.

3.4. Quality of the available accompanying diagnostic tests

There are various publications about gE-ELISAs (Van Oirschot *et al*., 1997, Wellenberg *et al*., 1998a,b, Ballagi *et al*., 1999, Böttcher *et al*., 1999, Conraths and Klähn, 1999, Mewes *et al*., 1999, Rauer and Crevat, 1999). The quality of antibody tests is primarily based on their sensitivity and specificity. Desirable properties of these tests have been listed (Van Oirschot *et al*., 1996).

3.4.1 Test Sensitivity and Specificity

After infection of seronegative cattle antibodies first appeared in serum between 11 and approximately 42 days after infection (van Oirschot *et al*., 1997, 1999, De Wit *et al*., 1998), which depended on the format of the tests used. De Wit *et al*., (1998) described that seroconversion after natural infection was detected in a gE-ELISA up to 2-3 weeks later than in a gB-ELISA. After challenge of cattle vaccinated twice with a gE-negative vaccine antibodies appeared between 7 and 14 days (Van Oirschot *et al*., 1997). On the other hand has it been described that the sensitivity of gE-ELISAs appeared limited in marker-vaccinated animals that were challenged with field virus (Conraths and Klähn, 1999). Antibodies to gE persisted at a stable level in experimentally infected cattle for at least 2-3 years (Kaashoek *et al*., 1996). Cattle have been detected or experimentally reproduced that were gE-seronegative and yet proved to be latently infected (Hage *et al*., 1998, Lemaire *et al*., 2000).

Individual milk samples have been compared with serum samples for the detection of antibodies to gE (Wellenberg *et al*., 1998a) in two different gE-ELISAs. It was found that the relative sensitivity of the tests differed considerably. One test had a relative sensitivity, compared with testing serum, of 0.96 and the other of 0.79. It therefore appears that in at least one ELISA milk can be used instead of serum for screening of cattle for gE-antibodies. A gE-ELISA was found suitable to estimate the prevalence of infected cattle in a herd by the use of bulk milk samples. The transition from a ‘ negative’ to a ‘positive’ result took place when approximately 10-15 percent of the animals within a herd become BHV1 gE-seropositive. (Wellenberg *et al*., 1998b). In contrast, some indirect BHV1-ELISA systems (whole virus) can detect BHV1-antibodies in a bulk milk sample of 50 animals containing milk from one weak positive animal.

The sensitivity and detection limit, the latter being evaluated by the testing of serial dilutions, of some gE-ELISAs is in general lower than that of other ELISAs or of neutralisation tests (Perrin *et al*., 1996, van Oirschot *et al*., 1997, De Wit *et al*., 1998).

Two field strains were found that did not express an epitope of gE in cell culture with the use of a monoclonal antibody that was used in a gE-ELISA. Calves were inoculated with either of these strains and followed for their gE-response, as measured in that particular ELISA. It was found that all but one calf became seropositive for gE in the gE-ELISA. This finding demonstrated that BHV1 strains that do not express a particular gE-epitope in cell culture, still can be detected in a gE-ELISA (Van Oirschot *et al*., 1999).

De Wit *et al*., (1998) compared a gB-ELISA, a gE-ELISA and a Danish test system (consisting of a blocking and an indirect ELISA) for their specificity and sensitivity to detect antibodies against BHV1. The Danish test system showed the highest sensitivity and the gE-ELISA the lowest; the gB-Elisa showed an intermediate sensitivity. If the doubtful zone (25-50% blocking) of the gB-ELISA was considered as positive (gB-ELISA+), the sensitivity almost reached that of the Danish test system. The specificity, based on testing sera from 273 cattle of free herds, of all tests appeared to be very high, 99.7, 96.7, 100 and 99.7% for the gB-ELISA, gB-ELISA+ gE-ELISA and the Danish test system, respectively. Seroconversion was detected in the gE-ELISA up to 3 weeks later than in the gB-ELISA and the Danish test system. It was concluded that the combination of a gB-ELISA (for screening) and the Danish test system (for confirmation) provides for very high sensitivity (>99.0%)

Banks *et al*.(1998) compared 10 immunoassays against sequential bleedings taken from passively immune calves, one group of which were challenged with field virus. The relative sensitivities of a number of the assays varied with different sera. This variation was observed with samples taken from different animals. The Danish system was able to detect maternal antibodies more than 13 months post partum.

Sera from cattle that were seronegative in other ELISAs or neutralisation tests are virtually always score negative in gE-ELISA, resulting in a high relative specificity of gE-ELISA (Van Oirschot *et al*., 1997, De Wit *et al*., 1998). Sera from multivaccinated cattle have been described and reported to sometimes score positive results, which may be transient (Van Oirschot *et al*., 1997,). When testing these sera, the specificity of gE-ELISA thus appeared lower than when testing sera of uninfected or unvaccinated cattle. Sera have been found to give weak false positive reactions in that they were positive in the gE-ELISA but negative in the gB-ELISA. (Thiry, personal communication).

The specificity of gE-ELISAs for testing milk samples has been described to be high and scored 99 per cent as compared with a BHV1-gE ELISA using two monoclonal antibodies. (Wellenberg *et al*., 1998a).

3.4.2 Reproducibility

This feature has been described as high for a gE-ELISA, as evidenced by the blocking percentages of the dilutions of the internal positive serum and the negative serum found when these sera were tested in one microplate, in five microlates on one day or on 10 different days. (Van Oirschot *et al*., 1997). An indication for heterogeneity of test batches has been reported (Conraths and Klähn, 1999).

3.4.3 Discussion

It has been described that, in some cases, antibodies to gE can not be detected before 4 weeks after infection, and that they have a lower detection limit than other ELISAs. In addition, multivaccinated uninfected cattle can sometimes be scored (transiently) positive. These data indicate that there is room for improving the quality of the gE-ELISAs. In spite of this knowledge, some countries have decided to start an eradication programme, based on the use of gE-ELISA, thereby taking into account that a lower sensitivity can be compensated for by more frequent testing.

Three different gE-blocking ELISAs are commercialised in Europe. Until now, there are no reports available, which compare the sensitivity and specificity of the different gE-ELISA systems with each other.

Since exclusively gE-deleted marker vaccines are commercialised, only tests detecting gE-specific antibodies are relevant as accompanying assays. Some studies demonstrated lower sensitivity problems of the gE-ELISA tests compared to gB-blocking-ELISAs, indirect ELISAs or neutralisation assays. The useful and inexpensive testing of bulk milk samples for gE-antibodies seems to be only possible, if more than 10-15% of the cows are gE-seropositive thus allowing the discrimination between high and low seropositive herd levels. Therefore, bulk milk testing for gE-antibodies has to be repeated several times a year and the status of farms with a low seroprevalence could be false negative. The bulk milk test appears to be useful in discriminating between herds with high prevalence of BHV1 and those with low or no prevalence. Nevertheless, the use of ELISA in pooled samples needs further experimental evaluation.

In conclusion, the gE-blocking ELISAs have to be further standardised (EU-ringtest) and an improvement in sensitivity is desirable. Nevertheless, the lower immunogenicity of gE compared to other BHV1-glycoproteins (e.g. gB or gD) and unspecific reactions of multiple marker-vaccinated animals could make improvements difficult.

3.5 Possibility of the existence of seronegative carriers and the risks posed

3.5.1 Experimental data

BHV1 latently infected animals are usually identified by the detection of BHV1-specific antibodies in their serum. However, it has been postulated that some infected animals only possess residual antibodies, if any. While they are not detected, such seronegative latent carriers (SNLC) represent a threat for cattle husbandry. The presence of specific maternal antibodies can interfere with the development of an antibody response to vaccination. BHV1 infection of young calves under passive immunity could lead to viral latency. If this infection is not followed by an antibody response, it could generate SNLC after the disappearance of maternal antibodies (Lemaire *et al*., 1995).

Several studies were conducted to test the above hypothesis. Different strains of BHV1 were used: the highly virulent strain Iowa and the widely used conventional live-attenuated vaccine

strain RLB 106. In the context of BHV1 control associated with marker vaccines, it was also essential to investigate the effects of the vaccination with the live-attenuated gE-negative vaccine in neonatal calves. It was thereafter determined whether passively immunised gE-negative calves could produce an active antibody response to gE after infection with a field BHV1 strain (Ciney) (Lemaire *et al*., 1999). All the experiment were conducted in three phases: a first infection phase, a second monitoring phase (for 5 to 18 months) and a third phase where dexamethasone treatment was performed to reactivate putative latent virus. The antibody response was monitored by different serological tests. In addition, the cell-mediated immune response was assessed by an in vitro antigen-specific gamma-interferon (IFN-γ) assay (Godfroid *et al*., 1996).

The presence of maternally acquired antibodies in calves did not prevent either initial viral replication or latency of a virulent BHV1 strain (Iowa), as was earlier demonstrated by Lemaire *et al*., (1995). Furthermore, no increase in antibody response could be detected following infection and the results obtained suggested that BHV1 infection early in life could produce SNLC calves. A second experiment confirmed that the presence of passively acquired antibodies did not prevent virus excretion and establishment of a latent infection (Lemaire *et al*., 2000a). All calves and even those that did not show any increase in antibody after BHV1 infection developed a cell-mediated immune response as detected by the specific IFN-γ assay. One out of seven calves became seronegative by virus neutralisation test at 7 months of age like non infected control calves. This calf presented negative IFN-γ results at the same time and was seronegative by ELISA at around 10 months of age. In conclusion, this study demonstrated, for the first time, that BHV1 SNLC can be experimentally obtained. In addition, the IFN-γ assay was able to discriminate calves possessing only passively acquired antibodies from those latently infected by BHV1, but it could not detect SNLC.

It was then examined whether SNLC could be more easily obtained after infection with a less virulent strain *i.e.* the widely used live-attenuated temperature-sensitive (ts) BHV1 vaccine (strain RLB 106) (Lemaire *et al*., 2000b). The ts strain established acute and latent infections in all vaccinated calves either with or without passive immunity. In total, four out of 7 calves inoculated under passive immunity became clearly BHV1 seronegative, like the seven control calves, by several ELISAs and serological reference tests. In contrast to the antibody response, the presence of a passive immunity did not hinder the cell-mediated immune response. The results obtained in this study demonstrate that SNLC can be easily developed by vaccination with a live-attenuated BHV1 under passive immunity, whatever the serological test used and despite a high sensitivity.

Surprisingly, long periods of virus excretion were observed after infection in the presence of specific maternal antibodies. The consequences of the presence of a specific passive immunity on the virus shedding of the live-attenuated gE-negative BHV1 vaccine strain were therefore investigated. The replication of gE-negative strain was considerably and significantly reduced in the maternally immunised calves, in comparison with the non-immune calves. On the other hand, the excretion of a gE-positive conventional vaccine strain (RLB 106) was not reduced and even seemed to be prolonged in the presence of maternal antibodies (Lemaire *et al*., 2000c).

The effects of the vaccination with the gE-negative BHV1 vaccine in neonatal seronegative and passively immunised calves on immune response and virus latency were then examined (Lemaire *et al*., 2000d). Like uninoculated control calves, all passively immunised inoculated calves became seronegative to BHV1 and surprisingly remained so post-dexamethasone treatment (PDT). However, all calves which excreted the virus (seven of 10 passively immunised and all

six naïve calves) developed a cell-mediated immune response and a booster response was observed PDT.

3.5.2. Discussion and conclusion.

SNLC animals can be experimentally obtained by infection under passive immunity, even with a highly virulent strain. A strain effect was observed: one SNLC out of 7 calves was obtained with the virulent strain and four with a conventionally attenuated BHV1 vaccine. With the gE-negative vaccine, the seven calves which excreted the vaccine virus became seronegative to BHV1. The IFN-γ assay appears to be a good complementary test to the serological methods, at least in the acute phase of infection. However this test was not able to detect the SNLC. The failure to easily detect such animals could represent a threat for BHV1 free herds, selection stations, and artificial insemination centres. The vaccination with a live-attenuated BHV1 conventional vaccine could represent a good model to experimentally produce SNLC in aim to improve the serological diagnostic tools or to develop new approaches in the detection of latency. In addition, this study demonstrated that the BHV1 gE-negative strain can establish latency not only in seronegative but also in passively immunised calves after only one intranasal inoculation.

The importance of SNLC for BHV1-eradication remains unclear. In particular, more epidemiological data of SNLC reactivation rate in BHV1-free regions needs to be collected and studied.

4. General conclusion

The new data available since 1996 show that gE-negative marker vaccines are efficacious. They can reduce severity of disease virus shedding, and frequency of reactivation. However, field trials did not give consistent results.

The vaccines are safe, but live gE-marker vaccines can sometimes be reactivated after dexamethasone treatment. However, vaccine strain transmission in a population is regarded as unlikely.

Seronegative latent carriers (SNLC) have been experimentally demonstrated. Their importance with regard to control and eradication programmes is unclear

The accompanying ELISAs that are available may be improved with regard to sensitivity and specificity. Bulk milk testing can be used to classify herds as low-infected or high-infected but will not reliably identify non-infected herds.

gE deleted vaccines may have an important role to play in eradication programmes for IBR. However, this role must be assessed in the context of the region undergoing eradication as epidemiological factors such as type of cattle population, farming patterns, existing disease incidence, animal movement patterns, veterinary and laboratory resources, willingness to implement test and slaughter programmes are important influences on the prospects for success. In highly infected areas, the use of vaccine to lower the prevalence of infected cattle may be a first phase in an eradication programme. Additional measures, such as keeping closed herds, etc, certainly contribute to getting elimination of BHV1 from a region. In low-prevalent areas, vaccination may not be necessary for elimination of BHV1.

5. Summary responses to the five requests for an opinion:

5.1 Types of marker vaccines and their possible use in the European Union.

Only gE-negative marker vaccines have been marketed so far. Other types of marker vaccines are not expected to be commercialised in the near future. The gE-negative marker vaccines, live as well as killed are already used in control or eradication programmes in the EU.

Because of the risks of recombination between vaccine strains, it is not recommended to use live vaccines containing different deletions in the same animal population.

5.2 Efficacy and safety of Bovine Herpesvirus 1 (BHV1) marker vaccines for use in eradication programmes.

The reported experimental trials with gE-deleted BHV1 marker vaccines demonstrated that these vaccines can reduce the severity of clinical signs, the shedding of challenge virus and reactivation after dexamethasone treatment. Nevertheless, the efficacy varied with the vaccine type (live or inactivated), the vaccination scheme and the design of the experiment. Therefore it remains difficult to recommend a particular vaccine type and vaccination scheme.
Both live and inactivated gE-negative vaccines have shown efficacy under field conditions. However, significant differences between the vaccinated group and the control groups were observed in different field experiments, done by different groups with killed or live gE-deleted marker vaccines. The results varied with the vaccination scheme, the farm status and the vaccine administration. The available data seem not to be sufficient to recommend a common vaccination scheme, suitable for the different kinds of protection (clinical signs, virus shedding and transmission, reactivation). Attention should be paid to the possible contamination of batches of gE-live vaccine with bovine viral diarrhoea virus.
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5.3 Risks of latency and reactivation associated with the use of live marker vaccines

The gE-negative vaccine virus can cause a latent infection and can be reactivated. The route of vaccine inoculation (intranasally or intramuscularly) influences the chance of virus reactivation. The frequency of reactivation and subsequent re-excretion in the field is unknown. However, reactivation is followed by low excretion or no excretion, which makes the chance that vaccine strain transmission occurs, low. While it could be desirable to carry out a risk assessment to quantify the risk of virus transmission as a result of reactivation, this is likely to be difficult due to lack of data.

5.4 Sensitivity and specificity of the available accompanying diagnostic tests

Three different gE-blocking ELISAs are commercialised in Europe. Until now there are no reports available, which compare the sensitivity and specificity of the different gE-ELISA systems with each other. Some studies demonstrated lower sensitivity problems of the gE-ELISA

tests compared to gB-blocking-ELISAs, indirect ELISAs or neutralization assays. The useful and inexpensive testing of bulk milk samples for gE-antibodies seems to be only possible, if more than 10-15% of the cows are gE-seropositive, this allowing the discrimination between high and low seropositive herd levels.
The gE-blocking ELISAs have to be further standardised (EU ringtest) and sensitivity needs to be improved. Nevertheless, a reduced immunogenicity of gE compared to other BHV1 glycoproteins (e.g. gB or gD) and unspecific reactions of multiple marker-vaccinated animals could make improvements difficult.

5.5 Possibility of the existence of seronegative carriers and the risks posed by this.

SNLC (seronegative latent carrier) animals can be experimentally obtained by infection under passive immunity, even with a high virulent strain but also readily with gE-negative vaccine, and also in seronegative calves. The failure to easily detect such animals could represent a threat for BHV1 free herds, selection stations and artificial insemination centres. The importance of SNLC for BHV1 eradication remains unclear.

6. Recommendations for Future Research

Further research is required to clarify existing gaps in our knowledge relating to BHV1 vaccination, testing and eradication.

There is insufficient information available to recommend a common EU vaccination scheme i.e. type of marker vaccine, administration route and timing

The failure to easily detect seronegative latent carrier (SNLC) could represent a threat for BHV1 free herds, selection stations and artificial insemination centres. The importance of this for BHV1 eradication is unclear and needs to be further evaluated.

Interlaboratory comparisons should be held to get more insights into the quality of gE-ELISA and the performance in different laboratories.

Sensitivity problems with the gE-antibody ELISAs have been reported. Further research to improve such tests is recommended.

Bulk milk testing is currently sufficient only to distinguish between high and low prevalence on farms. Consequently frequent testing is required to detect new infection and then often only when the prevalence is high. The improvement of sensitivity would lead to earlier detection in newly infected herds.

The development of new vaccines (eg DNA-vaccines[2], multiple deletion vaccines, subunit vaccines) and new accompanying test systems may offer advantages over the current gE deleted system.

A confirmatory test for the presence of antibodies to gE using different principles to the ELISA does not exist. The absence of such a test can cause problems in the clarification of the real status of animals that give borderline responses to the gE ELISA. The development of such a confirmatory test would be an important advance.

[2] No DNA vaccines have yet been licensed

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Annex – Report of Scientific Veterinary Committee 1996

COMMISSION OF THE EUROPEAN COMMUNITIES

VI/9098/95 - EN

Directorate-general for Agriculture

VI/B.II.2

REPORT OF THE SCIENTIFIC VETERINARY COMMITTEE ON IBR (BHV1)

REPORT OF THE SCIENTIFIC VETERINARY COMMITTEE ON IBR (BHV1)

Scientific Veterinary Committee - subgroup BHV1

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Meeting on the use of bovine herpesvirus 1 (BHV1) marker vaccines and the related serological tests in BHV1 eradication programmes.

(3 October 1995)

Members present:
Dr E. Vanopdenbosch (Chairman and rapporteur)
Prof J. van Oirschot
Prof E. Thiry
Prof W. Schuller
Dr G. Keil
Dr S. Edwards
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Apologies:
Prof O. Straub

I. Agenda:

1. Review of vaccine deletion types and comment on choice for future use in E.U.
2. Efficacy and safety of deleted IBR vaccines for use in a control programme.
3. Latency and reactivation of live deleted vaccines.
4. Exchange of views on current information on specificity and sensitivity of diagnostic tests for virus deletions.
5. Miscellaneous.

II. Introduction.

The meeting of 3 october 1995 was the consequence of a request of the Scientific Veterinary Committee (ScVC) at the meeting of 22 and 23 june 1995 for the convening of a subgroup to advise it on the use of deleted BHV1 vaccines and the related serological tests. Dr E. Vanopdenbosch, as member of the ScVC, was asked to chair this subgroup and to report the views of this group to the ScVC.

Since the winter of 1971-1972, IBR has spread over Europe, coming from north-America. After the acute outbreaks, the disease changed obviously and now IBR is present under a less acute form with, from time to time, severe cases occuring. BHV1 is an important cause of infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), and infectious pustular balanoposthitis (IPB), as well as encephalitis (this former BHV1 is now called BHV5), conjunctivitis, enteritis, and abortion in cattle. BHV1 also causes immunosuppression and infected animals become highly susceptible to secondary pneumonia and significant occasional mortality. Like other herpesviruses, BHV1 can establish latency in clinically normal animals, with subsequent intermittent episodes of re-excretion. The virus cannot be eliminated from the host following infection, and in a vaccination-challenge experiment, vaccination can only prevent the clinical

disease and decrease the re-excretion and spread of the virus after reactivation. At present, IBR is not generally considered a major cause of economic loss on comercial farms, but it becimes important mainly as a trade issue in the AI, ET and pedigree sectors.

BHV1 infected animals can be identified by the presence of BHV1 specific antibodies in their serum. Unfortunately, vaccination with whole virus interferes with the serological detection of infected cattle. If the antibody response induced by vaccination can be differentiated from the response induced by BHV1 infection, by using marker vaccines which lack one or more glycoproteins, infected animals in a vaccinated herd can be identified and eliminated. This strategy could be used for the establishment of IBR free herds, regions and countries.

To date, a number of mutant deletions of one of the non essential glycoproteins, as well as candidate subunit vaccines possessing only one immunogenic glycoprotein gB or gD, have been produced. As for the deleted vaccines, both live and inactivated vaccines are available for some deletions. So, not only the deletion type has to be evaluated but also the type of vaccine (live or inactivated); conventional modified live vaccines have advantages in that they induce good immunity after a single administration and they are easy to manufacture. They can be administered intramuscularly or intranasally, but the latter route induces better local immunity. Some conventional modified live vaccines have some residual virulence, some of them have been shown to persist in the host and cannot prevent infection and subsequent latency of wild-type virus in a laboratory vaccination-challenge experiment. Shedding of vaccine virus after intranasal vaccination and possible reactivation due to stress are additional disadvantages. Conventional inactivated vaccines are considered to be less immunogenic and protective than live vaccines: they neither can prevent infection nor subsequent latency of wild-type virus (in laboratory vaccination-challenge experiments), but obviously they do not establish latency of the vaccine virus.

Only Switzerland, Finland and Denmark have achieved the total eradication of IBR on a country basis. Other countries like Austria and Sweden are very near an IBR free status, and some other countries like France (Département du Morbihan) and Germany organize, on a regional basis, eradication programmes by elimination of seropositive animals. Finally, the Netherlands and Belgium, with a high percentage of about 80% of seropositive herds, envisage the start of an IBR eradication programme. Stimulated by all these activities, discussions are taking place in different Member States to determine the extent to which IBR control is appropriateto different circumstances prevailing in the EU. Due to the huge spread of IBR in some member states and the problems encountered in avoiding reinfection, only the eradication of IBR from artificial insemination and embryo transfer centers is envisaged by EC Directive 92/65/EEC, whereby AI and ET centers have to be free from 1 january 1999 onwards. To develop a future IBR policy, this subgroup was asked to give an overview of the present state of knowledge on the 4 points of the agenda.

III. Discussion

In the view of the subgroup the different points on the agenda are closely linked to each other. Therefore it is proposed to present the available data in table 1 (Efficacy) and table 2 (Safety).

III.1. BHV1 deletions.

A lot of information is now available on the role of a number of non essential glycoproteins as a result of experiments with the following deletion mutants and subunit candidate vaccines: gE, gC, gI, gG, gE/gI or gE/TK(thymidine kinase) and gC/TK double deletion mutants, and the gD and gB subunit vaccine. The presently known gE or gI, or gE/gI deleted strains are avirulent and gC and gG deleted viruses preserve a certain degree of virulence. gC plays a role in viral attachment and is highly immunogenic, gG, gI and gE have a function in cell to cell spread mechanisms. gC deleted vaccine seems therefore less indicated than gG, gE and gI deleted vaccine, although in the literature clinical protection and reduction of viral excretion was described after vaccination with a gC-/TK- live vaccine.

III.2. Efficacy of BHV1 marker vaccines.

TABLE 1.

Available data on
EFFICACY and SEROLOGICAL TESTS
of presently known BHV1 deleted vaccines.

	Virological efficacy					
Marker	Protection in seronegatives	Protection reactivation	Early immunity	Transmission experimental	Transmission field	Tests
gE-L*	+	? (96)	+	+	+(in progress)	+
gE-K**	+	? (96)	?	-	+	+
gG-K(USA)	+	?	?	?	?	?
gC-L	+	?	?	?	?	?
gD-sub***	+	? (96)	?	-	+	+
gB-sub	+	?	?	?	?	+
gD-L	?	?	?	?	?	-

* L = Live deleted vaccine
** K = Killed deleted vaccine
***sub = Subunit vaccine.

In a transmission experiment, an inactivated gE deleted vaccine, a live gE deleted vaccine and a gD subunit vaccine were compared and the reproduction ratio R, this being the average of secondary cases per infectious individual in a population, was calculated per vaccine group as a measure for virus transmission. For eradication R has to be <1.
In only the group given gE-deleted live vaccine was an R value < 1(0.92) was obtained, against R= 2.28 for the other groups (gE inactivated, gD subunit and placebo).

In a field study, involving 14,000 animals from 130 herds and comparing a gE inactivated and a gD subunit vaccine, the number of herds with virus circulation was reduced with about 50% and the number of new infections in herds with virus circulation was reduced significantly: R value respectively 2.4 and 1.7, against 3.7 for the placebo group. These results differ from the transmission experiment, in that they were better, but it also indicates that these vaccines do not reduce the number of new infections in a herd sufficiently to achieve eradication of BHV1 without accompanying sanitary measures. A similar field study with a live gE-negative vaccine has started recently and results will be available in 1996. Results of the protection against reactivation after vaccination with gE live, gE inactivated and gD subunit vaccine can also been expected in 1996.

III.3. Safety of BHV1 marker vaccines.

In the discussion on safety the following factors were included:

- Residual virulence (systemic signs, abortion);
- Recombination ;
- Shedding;
-Transmission;
- Local reaction;
- Reversion to virulence;
- Vaccination induced reactivation of wild type virus or so called “no stress” reactivation;
- Adverse effects (abortion, milk production, semen quality and fertility, growth).

Before allowing a marker vaccine, it has to be checked that no wild type deleted viruses are circulating. In a preliminary research, 2 out of 223 field strains appeared to lack one gE epitope and did therefore not react with the correspondent monoclonal used in the gE ELISA test. It is therefore recommended to use in the gE ELISA test monoclonals directed against different epitopes on gE. One Australian BHV5 strain lacked some gE epitopes.

The possibility of recombination when using live deleted vaccines was discussed and regarded as of minor epidemiological importance : for the gE deletion, which is a large deletion of 2.7kb, reversion to virulence is virtually almost impossible because of the size of the deletion. And even if recombination should occur, which has to be considered as a normal but very rare phenomenon, this has no major epidemiological consequences, because that means that the wild type virus was already in the herd. Another problem, linked to recombination, could arise if the recombined vaccine virus should be more virulent than the parent strain, but no evidence is available on this possibility up to now. However, the possibility of reinsertion could be enhanced by using two different types of live deleted vaccines, and it is therefore advised to use only one type of live deleted vaccine.

Transmission of vaccine virus has not yet been demonstrated, but may be expected after IN vaccination and no transmission has been reported after IM vaccination.

TABLE 2.

Available data on SAFETY aspects of the different presently known IBR deleted vaccines

SAFETY		LIVE			INACTIVATED			
		gE-	gC-	gD-	gE-	gG-	gDsub	gBsub
Residual virulence -systemic signs		-	?	?	NA	NA	NA	NA
-abortion		-	?	?	NA	NA	NA	NA
Recombination		?	?	?	NA	NA	NA	NA
Shedding	IN	+	?	-	NA	NA	NA	NA
	IM (2w)	+	?	-	NA	NA	NA	NA
	(older)	-	?	-	NA	NA	NA	NA
Transmission	IN	-	?	?	NA	NA	NA	NA
	IM	-	?	?	NA	NA	NA	NA
Latency	IN	+	?	?	NA	NA	NA	NA
	IM	-	?	?	NA	NA	NA	NA
Reactivation/reexcretion	IN	-	-	-	NA	NA	NA	NA
	IM	?	-	-	NA	NA	NA	NA
Local reaction	IN	-	?	?	-	?	?	?
	IM	-	?	?	-	?	?	?
Reversion to virulence		-	?	?	NA	NA	NA	NA
Vaccination induced reactivation of wild-type virus		?	?	?	?	?	?	?
Adverse effects								
- Abortion		-	?	?	-	?	?	?
- Milk production		-	?	?	+*	?	?	?
- Semen		-	?	?	-	?	?	?
- Growth		-	?	?	?	?	?	?

NA = Not applicable
? = not tested or information not available
* : only 1 liter in total after 2 vaccinations

After intranasal vaccination with gE deleted vaccine, calves shed significantly less vaccine virus than calves vaccinated with a conventional live vaccine. After intramuscular vaccination with live gE deleted vaccine, only very low titers of vaccine virus are found in nasal secretions of calves under two weeks of age, but not in older calves.

As for latency, some work on a restricted number of animals has already been done in ID-DLO Lelystad with live gE deleted vaccine, and viral DNA has been detected by PCR in trigeminal ganglia after intranasal vaccination, but not after intramuscular vaccination. However, more work is needed to evaluate latency for other types of live deleted vaccines, administered IN or IM., but it is accepted that for all types of live vaccines, latency can not be excluded.

As for reactivation, until now all trials with gE deleted vaccines, using dexamethasone treatment remained negative, but the subgroup agrees that this does not guarantee that in the field reactivation never could occur. Results of vaccination induced reactivation and other reactivation experiments after dexamethasone treatment and transport stress will become available in 1996.

Most of the information in this report about the efficiency and safety of marker vaccines concerns gE deletion. The group felt it desirable to take all possible measures to find out similar information about alternative deletion systems. This work has probably been done but not published.

III.4. Specificity and sensitivity of diagnostic tests for virus deletions.

Very little information is available about gG, gC and gI tests. For gE, information is available on a commercial gE blocking ELISA test, using one monoclonal antibody and a ID-DLO Lelystad home made gE blocking ELISA test, using two monoclonal antibodies against different gE epitopes. It has been reported that the gE ELISA is somewhat less sensitive than the gB-blocking ELISA and equally sensitive to most conventional ELISAs. The gE-ELISA sometimes gave a positive reaction in some animals that were vaccinated seven times at one-month intervals with a gE-deleted live or inactivated vaccine. The overall specificity of the gE-ELISA with " hypervaccinated " sera was 95%.

ID-DLO compared the home made gE ELISA with a home made gB and seroneutralisation test, and scored 99% for specificity and 98% for sensitivity for the gE ELISA on a large number of sera, including the EU reference sera for IBR.

The subgroup felt that it was to early to organize a European comparative trial for gE antibody detection. However they suggest that all gE antibody tests should be as sensitive and as specific as the conventional tests when testing the EU reference sera.

To avoid the missing of some cattle infected with wild-type virus that is lacking one or more gE-epitopes, a combination of monoclonals against different gE epitopes should be used. Other approaches, not dependent on monoclonals at all and thus avoiding the vulnerability of monoclonal assays to minor alterationsin antigen epitope expression, may be preferred. For example, use of recombinant gE or other deleted glycoprotein as antigen in an indirect ELISA, or monospecific polyclonal antiserum raised against recombinant antigen in a blocking test.

IV. Conclusion.

The subgroup states that, as for marker vaccins, the properties of gE deleted vaccines and gD subunit are best known and fit rather well in an IBR eradication strategy in highly infected areas, although other deletion types such as gC, gG and gI cannot be excluded by lack of available scientific data on efficacy and safety. Therefore, no recommendation on preferred choice of deletion vaccines, whether live or inactivated, can be made at this time. A recommendation on the choice of live versus inactivated vaccine, especially in case of gE live marker vaccines cannot be formulated. Indeed, the good efficacy, especially the better reproduction ratio, under 1, of the gE live marker vacine compared to the gE deleted inactivated and gD subunit vaccine in experimental transmission studies, and the lack of evidence that vaccine virus reactivation is a frequent phenomenon, makes that such vaccine can play a key role in an eradication programme. The importance of latency of gE- live vaccines has to be evaluated further.

The efficacy of live gE-deleted vaccine in a large-scale field trial will start in November 1995 in The Netherlands and results will not be available until February 1997. However, some new data may become available concerning safety in reactivation trials. Therefore it is proposed to convene a meeting of this subgroup in spring 1996.

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