Relative efficacy of inactivated bovine herpesvirus-1 (BHV-1) vaccines

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Abstract

The relative efficacy of four commercially available inactivated bovine herpesvirus-1 (BHV-1) vaccines was directly assessed in controlled studies in 5–6-month-old, BHV-1 naïve calves. The sero-response due to the basic course of two intramuscular vaccinations was closely similar for three of four vaccines whereas one vaccine did not induce seroconversion in five of six calves. At the level of challenge virus shedding in nasal mucus, all four vaccines were significantly protective but to a markedly variable degree. Clinically, however, the relative protection ranking of the vaccines was different to that observed for challenge virus shedding. There was no obvious correlation observed between pre-challenge circulating virus neutralising antibody titre and vaccine-induced protection against virus shedding or clinical signs following the intranasal BHV-1 challenge. Present comparative findings suggest that commercially available BHV-1 vaccines are likely to be of variable potency in the natural host.

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1. Introduction

Bovine herpesvirus-1 (BHV-1), commonly known as infectious bovine rhinotracheitis (IBR) virus, is an important cause of economic loss to the cattle industry worldwide [1]. The common illness due to IBR virus infection is febrile rhinotracheitis and the virus has, less frequently, been associated with abortions, still births, and diseases of the reproductive, central nervous and alimentary tract systems [2,1]. Moreover, the primary infection by the virus is strongly implicated in predisposing cattle to pneumonic pasteurellosis, also referred to as the shipping fever [3].

The first commercial vaccine against IBR virus was developed long ago [4] and the choice of BHV-1 vaccines, either alone or in combination, and as both live and inactivated formulations is now considerable [5]. However, none of the currently available BHV-1 vaccines are completely effective in preventing virus shedding and the febrile respiratory disease due to BHV-1 infection [6–8]. With respect to efficacy, live BHV-1 vaccines are generally considered superior to killed vaccines [9,10]. Nonetheless, many killed BHV-1 containing vaccines are marketed and afford a durable protection [11,6]. Still, new vaccines containing BHV-1 are being introduced indicating the market demand to control economic loss due to BHV-1 infections. There is, however, scanty published data directly comparing relative efficacy of commercially available BHV-1 vaccines [9,10]. Such data would be valuable since available vaccines contain different BHV-1 strains at different titres and their efficacy assessed using (i) different challenge inocula (virus strain and dose), (ii) challenge model and (iii) in vitro tests. In present paper, four commercially available killed BHV-1 containing vaccines were directly assessed for efficacy in seronegative bovine calves. Parameters assessed were the level of virus neutralising antibody response, reduction in challenge virus excretion in nasal mucus and reduction in the incidence and the severity of clinical signs due to intranasal BHV-1 challenge.

2. Methods

2.1. Tissue culture methods

Tests were conducted at 37 °C and comprised (i) titrations for BHV-1 infectivity in challenge inoculum (see below) and
nasal swabs, (ii) BHV-1 neutralising (VN) antibody in serum samples. Both of these tests were done using a line of lung cells explanted from a bovine embryo (BEL cells) and used between passages 20 and 60. BEL cells were grown at 37 °C in Dulbecco's minimal essential medium (MEM) supplemented with 10% donor horse serum (HS, Tissue Culture Services or Sigma Aldrich), 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 0.09% sodium bicarbonate (HSGM). This medium containing 2% HS (HSM) instead of 10% HS was used for (i) extracting nasal swabs (NS), (ii) diluting NS for infectivity titrations, (iii) diluting sera for VN antibody titrations and (iv) maintaining infected cultures.

Both infectivity and VN antibody titrations were performed in 96-well microtitration plates, the former using serial 10-fold dilutions and plating 200 μl/well and four wells/dilution and the latter using a constant virus (50 μl containing 100–200 tissue culture median infective dose [TCID₅₀] of BHV-1) and serum dilution (50 μl) procedure as described previously [12]. For both tests, HSM served as a negative control while a stock of BHV-1 was titrated in parallel as a positive control for infectivity and a bovine antiserum to BHV-1 as a positive control for VN titrations. For the VN titrations, inactivated (56 °C storage, arranged upright in a metal rack, until titrated for BHV-1 infectivity in BEL cells. Each swab was extracted in 2 ml HSMM contained in a sterile 5 ml screw cap (bijoux) bottle. For extraction, swab tips were snapped off and left submerged in the medium and bottles transferred for −70 °C storage, arranged upright in a metal rack, until titrated for BHV-1 infectivity in BEL cells.

2.3. Study animals

The experimental calves were mainly female, Friesian-Aberdeen Angus (Fr-AA) crosses ranging in age from 5 to 6 months at first IM vaccination and were all free of persistently infected by non-cytotoxic ruminant pestiviruses (NCRPVs) and also VN antibody to BHV-1. Male calves of other breeds (Friesian-Simmental [Fr-Sim], Friesian-Limosin [Fr-Lim] and Friesian-Hereford [Fr-Hr]) were, as far as possible, evenly distributed among experimental groups.

2.4. Experimental studies

The study specific details are given in the results section but both the short-term and the long-term efficacy studies in BHV-1 naïve calves directly compared responses in vaccinated and unvaccinated groups which were matched with respect to breed and sex. The vaccination regime involved two IM inoculations a month apart and the challenge times refer to the interval after the second vaccination. Routinely, the responses measured after vaccinations and challenge were (i) clinical reactions daily for 10–21 days; however, clinical findings for only up to 10 days are reported and only if the reaction did not pre-exist the treatment, (ii) serum VN antibody activity following vaccinations and/or challenge and (iii) BHV-1 shedding in nasal mucus daily for 10–12 days after intranasal (IN) challenge.

2.5. BHV-1 challenge inoculum

The challenge inoculum consisted of a pool of nasal swab isolate in BEL cells from a field calf (no. 532) with a severe febrile rhinotracheitis and lung lavage isolate from a calf (no. 31) that had been inoculated IN and intratra- cheally with the isolate from calf 532 as previously described [12]. For challenge each calf was administered 2 ml IN containing 8.0 log₁₀ TCID₅₀ in the short-term study and 7.0 log₁₀ TCID₅₀ in the long-term study. This stock was sterile and free of NCRPVs and mycoplasmas.

2.6. Animal procedures

Nasopharyngeal swabs were taken using a 12 cm long cotton-tipped plastic swab. Both nasal passages were swabbed in turn using a single swab by gently inserting the swab to its full length along the nasal passage wall while rotating it. Each swab was extracted in 2 ml HSMM contained in a sterile 5 ml screw cap (bijoux) bottle. For extraction, swab tip was repeatedly pressed against the bottle wall in between dunking it in HSMM in the bottle. After extraction swab tips were snapped off and left submerged in the medium and bottles transferred for −70 °C storage, arranged upright in a metal rack, until titrated for BHV-1 infectivity in BEL cells.

2.2. Vaccines

Inactivated BHV-1 vaccines tested were Bovilis IBR + Para influenza virus type 3 (PI3) virus (whole virus, Intervet; dose: 2 × 2 ml intramuscularly [IM] a month apart), IBEPUR (subunit, Merial; dose: 2 × 2 ml IM, a month apart), Iffavax (whole virus, Merial; dose: 2 × 5 ml IM, a month apart) and Bayovac IBR + infectious pustular vulvovaginitis virus (IPV), the genital form of BHV-1 (whole virus, Bayer; dose: 2 × 2 ml IM, a month apart).
There was no correction made to allow for increased mucus content in calves with nasal discharge.

Blood samples for serology were collected from the neck vein using a glass vacutainer and needle set. Bloods were allowed to clot at 37 °C for 2–3 h before processing for serum. Sera were stored at −20 °C until titrated for VN antibody activity.

2.7. Clinical monitoring

Rectal temperature, nasal discharge, ocular discharge, rate and nature of respiration, appetite, malaise/depression and general health were monitored. Calves with rectal temperature of 39.5 °C and above were considered as febrile. Scoring for nasal discharge was set as follows: 1 for marked serous, 2 for mild mucopurulent, 4 for marked mucopurulent. For ocular discharge, lacrimation was scored as 1, mild mucopurulent discharge as 2, marked mucopurulent discharge as 4, mild conjunctivitis as 2 and marked conjunctivitis as 4. Hyperpnoea was scored as 2, dyspnoea as 4 and respiration rates per min of 40–50 as 1, 51–60 as 2, 61–70 as 3 and above 70 as 4. Anorexia was scored as 4. A score of 1 was given for malaise/depression but eating and 2 for malaise/depression and reduced appetite. Findings for days 1–10 are reported and only if the reaction did not pre-exist the treatment.

3. Calculation of means

3.1. Virus shedding and its duration

Daily mean virus shedding (expressed as log10 TCID50 per ml ± standard deviation) in nasal mucus by a group was calculated as sum of titres including negative (titre < 1.1 log10 TCID50 per ml) calves for which a value of zero was used for the calculation divided by number of calves in the group.

Mean duration was calculated as sum of numbers of days of shedding divided by number of shedding calves and the variation is given in standard deviation.

3.2. Daily group mean pyrexia and duration

This was calculated as sum of individual significant (≥39.5 °C) rectal temperature divided by number of reactive calves. Mean duration was calculated as sum of number of days of reaction divided by number of febrile calves. Variation for both parameters is shown as standard deviation.

3.3. Daily group mean scores for one or more signs of rhinotracheitis and duration

This was calculated as sum of daily mean reaction (sum of daily score for respiratory signs divided by number of calves in the group) divided by the number of days of reaction. The number of days was chosen based on first observation of respiratory signs in one or more calves in a group until the cessation of reaction. Variation is shown as standard deviation.

3.4. Group mean VN antibody titres

This was calculated as sum of individual titres in log2 scale including the negative (titre shown as zero) animals for which a value of zero was used for the calculation divided by number calves in a group. Variation of activity is shown as standard deviation.

3.5. Statistical analyses

Mann–Whitney U-test in conjunction with ‘Minitab’ computer software package 14 for independent means was used to compare groups. Differences in the VN antibody concentration were considered significant at and above four-fold. Differences in P value of ≤0.05 were considered significant.

4. Results

4.1. Short-term immunity

The efficacy study in 5.5 months old, mostly Frisian-Aberdeen Angus female BHV-1 naïve calves directly compared the quality of protection afforded by Boviplus IBR + P13 and BIEPUR vaccines 18 days after the basic course of two IM vaccinations. The study involved seven calves given Boviplus IBR + P13 (group 1), seven calves BIEPUR (group 2) and a group of six matched unvaccinated calves (control, group 3). Neither vaccine induced a significant systemic or injection site reactions. Thus, at the injection site after each of the two IM injections, three to five calves in each group reacted with a diffuse thickening or a palpable swelling accompanied with slight oedema for 2–4 days (data not shown). In group 1, calves no other reactions due to the vaccine were observed while in group 2, the same calf became febrile a day after each IM inoculation but the calf was normal the following day.

Serologically, the response due to the vaccines differed significantly (Fig. 1a). Hence, 12 days after the primary vaccination, six of seven, group 1 calves were detectably positive for VN antibody to BHV-1 with group mean titre of 1.9 when all seven calves in group 2 were VN antibody negative. Twenty-eight days after the primary vaccination, all seven calves in group 1 compared to one of seven calves in group 2 were sero-positive and this was also the case after the booster vaccination and prior to challenge. The booster vaccination, however, did not result in a significant rise in titre in group 1 calves.

The IN BHV-1 challenge was 18 days after the second IM vaccination. About 3 h after the challenge with 8.0 log10 TCID50, only trace (1.5 log10 TCID50 virus per ml) amount of inocula could be recovered from one calf in both
Fig. 1. Circulating antibody response after vaccination (VAC) and challenge (CH): (a) short-term immunity and (b) long-term immunity. Numbers above columns represent percentage (%) of positive calves.
groups 1 and 3 while all seven group 2 calves failed to yield virus (Fig. 2a). The following day onwards considerable infectivity was recovered from nasal swabs from all calves for up to 6–7 days and at much reduced titres for a further 1–4 days depending on the treatment group (Fig. 2a). Between days 3 and 9 after challenge, calves in group 1 (Bovilis IBR + P13) on average shed 30–3000-fold less virus compared with unvaccinated calves in group 3. A consistent and significant (63–630-fold) decrease in virus shedding in group 2 calves (IBEPUR) occurred between days 5 and 9 after challenge. The respective duration of shedding by calves in groups 1, 2 and 3 was 7, 7.9 and 9.2 days.

Two weeks after challenge, all calves in both vaccinated groups responded anamnestically while all six control calves seroconverted with moderate circulating VN antibody activity (Fig. 1a).

The IN BHV-1 challenge resulted in high fever (≥40.0 °C) and nasal discharge between days 2 and 10 (Table 1). However, the incidence, the severity and the duration of fever was significantly reduced in group 1 calves compared with the reaction in unvaccinated calves in group 3. The respective duration of fever was 1.7 ± 1.2 and 5.2 ± 1.9 days for groups 1 and 3. Furthermore, the daily mean score for respiratory signs (predominantly nasal discharge) in group 1 calves between days 2 and 10 following the challenge was significantly lower at 3.7 than that in unvaccinated calves at 7.7. The standard course of two IM vaccination with IBEPUR was not significantly protective against the incidence, the severity and the duration of pyrexia compared to the reaction in unvaccinated calves.

### 4.2. Long-term immunity

Three inactivated BHV-1 vaccines were directly compared for the quality of protection they afford against BHV-1 challenge 6 months after a standard course of two IM vaccinations a month apart. The efficacy study was performed in 5–6 months old BHV-1 naïve Friesian cross-bred calves. Vaccines tested were Bovilis IBR + P13 (group 1, five calves), Bayovac IBR + IPV (group 2, four calves) and Iffavax (group 4, five calves) and responses due to the vaccines were directly compared with those in three unvaccinated calves (group 3).

None of the three vaccines caused noteworthy reactions at the injection site or systemically. Thus, there was no pyrexia or other clinical reactions observed after each of the two IM inoculations and at the injection site a diffuse thickening or slight swelling accompanied with slight oedema for a few days was recorded in a proportion of calves (data not shown).

The primary vaccination resulted in seroconversion in five of five, three of four and three of five calves, respectively, in groups 1, 2 and 4 (Fig. 1b). A month after the booster vaccination all calves in groups 1 and 2 responded with a significantly (≥4-fold) increased VN antibody titre whereas in group 4, there was no rise in titre in three previously highly (VN titre of 8–9) positive calves, and of two previously negative (titre < 1.0) calves, one seroconverted with titre of 7.0
Fig. 2. Challenge BHV-1 shedding in nasal mucus: (a) short-term immunity and (b) long-term immunity.

Key:
Fr-AA = Friesian-Aberdeen Angus cross; Fr-Sim = Friesian - Simmental cross; Fr-Lim = Friesian-Limousin cross; Fr-HR = Friesian-Herford cross.
while one did not respond (titre < 1.0). Over the following 5 months to challenge, group mean VN antibody titre in groups 1 and 2 decreased about eight-fold whereas the decrease in group 4, calves was only two-fold but all positive calves in all three vaccinated groups were still VN antibody positive at challenge. During this period, all three control calves remained VN antibody negative.

The IN BHV-1 challenge inoculum contained a tenth less (7.0 log10 TCID50 per calf) virus than that used in the previous study and no nasal swabs were taken 3 h afterwards. Furthermore, the amount of virus shed on days 1 and 2 after the challenge by the much older calves in experiment 2 was markedly less than that recovered at corresponding times in the previous study (Fig. 2). However, from day 4 onwards, the profile of shedding by unvaccinated calves between the studies was closely similar. Day 4 onwards calves in all three vaccinated groups overall shed significantly less virus compared to that shed by calves in unvaccinated group (Fig. 2b). The reduction usually ranged between nearly to over a hundred-fold to over a thousand-fold for groups 1 and 2 and between 30- and 300-fold for group 4. However, none of the three vaccines were significantly effective in reducing the duration of shedding.

Clinically, the IN challenge resulted in a high fever and a marked serous and/or mucopurulent nasal discharge in unvaccinated calves (group 3) between days 4 and 10 (Table 1). However, both Bayovac IBR + IPV and Iffavax were equally and significantly effective in shortening the overall duration of pyrexia but Bovilis IBR + PI3 was the most effective in this respect (Table 1).

In addition to the pyrexia between days 4 and 10 after challenge, an additional clinical reaction observed was nasal discharge; some control calves, however, also manifested anorexia, and/or malaise/depression and increased respiratory body activity was also recorded in all three unvaccinated calves (Fig. 1b).

5. Discussion

BHV-1 infections account for significant economic loss in cattle industry worldwide [1,2]. Vaccination against the infection is widely and regularly practiced involving a variety of mono and polyvalent, live and inactivated BHV-1 containing, conventionally produced (tissue culture grown, harvested at maximum CPE, inactivated with ethylenimine or beta propiolactone or formaline and containing aluminium salt or mineral oil or saponin as adjuvant) whole virus vaccines (Bovilis IBR + PI3, Rospoval 4, Triangle 4 Hipzabovis 3 and 4, Bovax IFR, Bovax 3, Bayovac Tubor 4, Iffavax, Bayovac IBR + IPV). A growing trend in Europe is to use glycoprotein E deficient (gE−) whole virus live and inactivated gE− marker vaccines so that vaccinated and naturally infected animals could be differentiated based on their antibody responses [13]. Although some of the presently tested inactivated vaccines (IBRPUR, Iffavax, Bayovac IBR + IPV) are being replaced with conventionally produced gE− whole virus inactivated marker BHV-1 vaccines (Bayovac IBR marker inactivatum, Ibraxion) the main contribution of the present comparative data, in lack of other similar published data, is that they define what quality of protection should be expected for conventionally produced (see above) whole virus non-marker and marker vaccines containing classical adjuvants such as aluminium salts (Bovilis), mineral oil (Iffavax, IBRPUR, Ibraxion) saponin (Rospoval IBR marker) or aluminium hydroxide plus saponin (Bayovac IBR + IPV). Clearly many different adjuvants have been used in killed BHV-1 vaccines but in addition to the type of adjuvant, other key determinants affecting efficacy of killed BHV-1 vaccines include the nature of virus inactivating agent and the virus antigenic mass per vaccine dose [8,14].

A conclusion from the present directly comparative data show that conventionally produced inactivated BHV-1 vaccines do vary in the quality of protection they afford, both with respect to reduction in virus shedding and clinical signs (Fig. 2, Table 1). Experimentally, however, currently available live and killed BHV-1 vaccines are not fully protective [6]. In the present study, vaccine-specific daily reduction in virus shedding 1–3 days following challenge was of the order of 10–3000-fold for Bovilis IBR + PI3, 10–630-fold for IBEPUR, 16–50 000-fold for Bayovac IBR + IPV and 25–500-fold for Iffavax (Fig. 2). Thus, at the level of virus shedding, all the tested vaccines were significantly protective albeit to a variable degree and the performance ranking was in order Bayovac IBR + IPV, Bovilis IBR + PI3, Iffavax and IBEPUR (Fig. 2). Clinically, however, the best performing vaccine was Bovilis IBR + PI3, followed by Iffavax, then Bayovac IBR + IPV and lastly IBEPUR. Virological and clinical protection was, therefore, not linked. In this regard it should be mentioned that high amount (6–8 log10 TCID50/swab) of virus growth does not necessarily result in a significant febrile respiratory disease [7]. This may explain the fact that there was no direct correlation in present study between protection against virus shedding and that against clinical signs. Thus, Bayovac IBR + IPV vaccine was significantly more protective, and in fact, more so than other two vaccines against challenge virus shedding but much poorly compared with other two vaccines against pyrexia due to the challenge (Fig. 2b and Table 1). Results, however, do not allow a clear conclusion about a correlation between serum VN antibody activity and either type of protection. Thus, the circulating group mean VN antibody titre at challenge for the long-term efficacy study of
The drawback is their higher production cost as at least 100 effective in calves with maternally derived antibodies. A further drawback is their higher production cost as at least 100 times as much virus is required per dose than that used in live vaccines in addition to the cost of adjuvant. Nonetheless, they do have a useful role in controlling BHV-1 infections in the field.

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References


Compared to live BHV-1 vaccines inactivated BHV-1 vaccines have the advantage that they will not cause latency or be shed in nasal mucus or have the risk of reversion to virulence including the potential to cause abortions. However, unlike the live BHV-1 vaccines, they are not ideal for use in face of a field BHV-1 infection since at least two doses, usually a month apart, are necessary to confer protection. Also, since they are administered parenterally, they are unlikely to be effective in calves with maternally derived antibodies. A further drawback is their higher production cost as at least 100
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