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# Isotype-specific antibody responses to bovine herpesvirus 1 in sera and mucosal secretions of calves after experimental reinfection and after reactivation

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**Abstract**

Isotype-specific antibody responses to bovine herpesvirus 1 (BHV1) were measured in sera, nasal, ocular and genital secretions of calves that were reinfected with BHV1 and 6 weeks later treated with corticosteroids to reactivate putative latent virus. After reinfection and after reactivation, no BHV1-specific IgM antibody response was detected. The serum IgA response was only transiently detectable after reinfection and again appeared rapidly after reactivation in most calves. Most calves showed an increase in nasal and ocular IgA titres after reinfection and reactivation; some calves also had IgA antibodies in genital secretions. A salient finding was that after reinfection and reactivation more calves showed a serum IgA response than virus shedding or an increase in serum IgG1 or IgG2 titres. This suggests that the serum IgA response would be the most sensitive indicator to detect BHV1 reinfection and reactivation. No correlation was found between nasal IgA titre at the time of reinfection or corticosteroid treatment and the period of virus shedding, suggesting that nasal IgA does not play a major role in protection against reinfection with BHV1.

## 1. Introduction

Bovine herpesvirus 1 (BHV1) can cause the disease entities: infectious bovine rhinotracheitis, infectious pustular vulvovaginitis and infectious balanoposthitis. Because the immunity induced by a primary BHV1 infection is relatively shortlasting, cattle may be

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reinfected with BHV1. Cattle infected with BHV1 are lifelong carriers of the virus in a latent, non-infectious form, mainly in the trigeminal ganglia. As a result of stress, simulated by the administration of corticosteroids, BHV1 can be reactivated and again be shed into the environment. Because the isotype-specific antibody responses after reinfection with and reactivation of BHV1 have only been fragmentarily studied (Rodak et al., 1983; Guy and Potgieter, 1985a,b; Osorio et al., 1989; Edwards et al., 1991), we extensively investigated these responses. In this report we describe their kinetics in sera, nasal, ocular and genital secretions of experimentally reinfected and corticosteroid treated calves. The isotype-specific antibody responses after primary infection have been recently reported (Madic et al., 1995).

## 2. Materials and methods

### *2.1. Experimental animals and experimental design*

In a previous experiment (Madic et al., 1995), 32 calves were randomly divided into eight groups of four calves. Seven of these groups were intranasally infected with one of seven BHV1 strains and one group served as control (Table 1). Two calves inoculated with the Iowa strain died. In the present experiment the remaining 30 calves were all (re)inoculated by intranasal spray, 4 ml into each nostril, containing a total of $10^7$ $TCID_{50}$ of the virulent Iowa strain of BHV1 (kindly provided by Dr. J.M. Miller, National Disease Center, Ames, Iowa), 16 weeks after the primary infection. The titre of the inoculum was verified by virus titration on the day of inoculation. None of the calves developed clinical signs characteristic of BHV1, except the primary inoculated control calves.

Six weeks after reinfection all calves were treated intramuscularly with 0.1 mg of dexamethasone (Opticortenol 0.5%, Ciba) $kg^{-1}$ body weight for 5 consecutive days. After this treatment none of the calves developed moderate or severe clinical signs.

### *2.2. Sampling procedures*

Blood samples were collected on post-reinfection days (PRD) 0, 3, 8, 15 and 29 and on post-treatment days (PTD) 0, 2, 7, 11, 16 and 21, where PTD 0 marks the beginning of the corticosteroid treatment. Serum was obtained from whole blood by centrifugation and stored at −20°C until tested. Nasal, ocular and genital secretions were collected on PRD −8, 0, 3, 6, 8, 10, 13, 15, 17, 20, 29 and 35 and on PTD 0, 2, 4, 7, 9, 11, 14, 16, 18 and 21. The mucosal secretions were collected with cotton tips (Medical Wire & Equipment Co. (Bath) Ltd., Potley, Corsham, UK) and suspended in 3 ml of phosphate buffered saline solution, pH 7.2, clarified by centrifugation and stored at −70°C until tested. Between 100 and 200 μl of nasal or ocular secretions, and 50–200 μl of genital secretions were absorbed by a cotton tip. Thus, the dilution ($log_{10}$) factor for nasal or ocular secretions was between 1.2 and 1.5, and for genital secretions between 1.2 and 1.8. Blood contamination of secretions was checked with the Sangur-test (Boehringer GmbH, Mannheim, Germany).

For virus isolation, nasal swabs were collected on PRD 0–10, 13, 15 and 17 and nasal and ocular swabs on PTD 0–10, 13, 15 and 17. Virus was isolated from these swabs as described previously (Kaashoek et al., 1994).

Table 1
The isotype-specific antibody responses and virus excretion periods after reinfection and corticosteroid treatment in individual calves

| Virus strain | Animal no. | Reinfection | | | | | | | | Corticosteroid treatment | | | | | | | | Virus excretion | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | IgA[a] | | | | IgG1 | | | | IgG2 | | | | IgA | | | | IGg1 | | | | IgG2 | | | | Reinf. | Cort. | |
| | | S | N | O | G[b] | S | N | O | G | S | N | O | G | S | N | O | G | S | N | O | G | S | N | O | G | N | N | O |
| Babiuk | 421 | + | + | + | +[c] | – | – | – | – | – | – | – | – | + | + | + | – | – | + | – | – | – | + | – | – | 0[d] | 2 | 0 |
| | 43 | + | + | + | – | – | – | + | – | + | – | – | – | + | + | + | – | + | – | – | – | + | – | – | – | 1 | 5 | 0 |
| | 44 | + | + | + | – | – | + | + | – | + | – | – | – | + | + | + | – | + | + | + | – | – | + | – | – | 2 | 4 | 0 |
| | 45 | + | + | + | – | – | – | – | – | – | – | – | – | + | + | + | + | + | + | + | – | + | + | – | + | 3 | 5 | 0 |
| Cooper | 66 | + | + | + | – | – | – | – | – | – | – | – | – | + | + | + | – | + | + | + | – | + | + | – | + | 3 | 3 | 6 |
| | 68 | + | + | + | + | – | – | – | – | + | – | – | – | + | + | + | + | + | – | – | – | + | + | – | + | 2 | 4 | 4 |
| | 69 | + | + | + | + | – | – | – | – | + | + | – | – | + | + | + | + | + | + | + | + | + | + | – | + | 1 | 4 | 2 |
| | 70 | + | + | + | – | – | – | – | – | + | – | – | – | + | + | + | + | + | + | + | – | + | + | – | – | 1 | 5 | 4 |
| Edwards | 55 | + | + | + | – | – | – | + | – | – | – | – | – | + | + | – | – | + | + | + | – | + | – | – | – | 0 | 4 | 2 |
| | 57 | + | + | + | + | + | + | + | – | + | + | – | – | + | + | + | + | – | + | + | + | – | – | – | + | 1 | 1 | 0 |
| | 58 | + | + | + | + | + | – | – | – | + | – | – | – | + | + | + | + | – | – | – | – | – | – | – | – | 2 | 2 | 0 |
| | 61 | + | + | + | – | – | – | – | – | – | – | – | – | + | + | + | – | + | + | + | – | + | + | – | – | 0 | 3 | 1 |
| Harberink | 59 | + | + | + | – | – | – | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – | – | – | – | 1 | 0 | 2 |
| | 60 | + | + | + | + | + | – | – | – | + | – | – | – | + | + | + | + | – | – | – | – | – | – | – | – | 1 | 0 | 0 |
| | 65 | + | + | + | + | – | – | – | – | – | – | – | – | + | + | + | – | – | – | + | – | – | – | – | – | 1 | 0 | 0 |
| | 67 | + | + | + | – | – | – | + | – | – | – | – | – | – | + | + | – | – | + | + | – | – | – | – | – | 0 | 2 | 2 |
| Iowa | 71 | – | + | + | – | – | + | – | – | – | – | – | – | + | + | + | – | + | + | + | – | + | – | – | – | 3 | 5 | 1 |
| | 93 | – | + | + | – | – | – | + | – | – | – | – | – | + | + | + | – | + | + | + | – | – | + | – | – | 0 | 5 | 3 |
| Lam | 48 | – | + | – | – | + | – | – | – | – | – | – | – | + | + | + | – | + | + | – | – | – | – | – | – | 2 | 4 | 0 |
| | 49 | + | + | + | – | – | – | + | – | – | – | – | – | + | + | + | – | + | – | + | – | + | – | – | – | 0 | 3 | 0 |
| | 50 | + | + | + | – | – | – | + | – | + | – | – | – | + | + | + | – | + | + | + | – | – | + | – | – | 3 | 3 | 0 |
| | 51 | + | + | + | + | – | + | + | – | + | – | – | – | + | – | + | – | – | + | + | – | – | + | – | – | 0 | 0 | 0 |
| Espuna | 52 | – | + | – | – | – | + | – | – | – | – | – | – | + | + | + | – | + | + | + | – | – | + | – | – | 0 | 4 | 3 |
| | 53 | + | + | + | – | – | – | + | – | – | – | – | – | + | + | + | – | + | + | – | – | – | – | – | – | 0 | 0 | 0 |
| | 54 | + | + | + | – | + | + | + | – | + | – | – | – | + | + | – | – | – | + | + | – | + | – | – | – | 0 | 0 | 0 |
| | 56 | – | – | – | – | – | – | – | – | + | + | – | – | + | + | + | + | + | + | – | – | + | + | – | – | 3 | 2 | 2 |
| Controls | 46 | + | + | + | – | + | + | – | – | + | – | – | – | + | + | + | – | – | + | – | – | – | – | – | – | 8 | 0 | 0 |
| | 47 | + | + | + | – | + | – | – | – | + | – | – | – | + | + | + | – | – | – | – | – | + | – | – | – | 8 | 3 | 0 |
| | 62 | + | + | + | – | + | – | – | – | + | – | – | – | + | + | + | – | – | + | + | – | + | – | – | – | 9 | 3 | 1 |
| | 92 | + | + | + | – | + | + | – | – | + | – | – | – | + | + | + | + | + | + | + | – | – | – | – | – | 8 | 7 | 0 |

[a]IgM antibodies were not detected after reinfection and corticosteroid treatment.
[b]S, serum; N, nasal secretion; O, ocular secretion; G, genital secretion. Sera were collected on post-reinfection days 0, 3, 8, 15 and 29, and on post-treatment days 0, 2, 7, 11, 16 and 21. Nasal, ocular and genital secretions were collected on post-reinfection days −8, 0, 3, 6, 8, 10, 13, 15, 17, 20, 29 and 25, and on post-treatment days 0, 2, 4, 7, 9, 11, 14, 16, 18 and 21.
[c]+, appearance of antibodies or a four-fold or higher increase in titre when the pre-sample was positive.
[d]Number of days of virus excretion.

## 2.3. Isotype-specific BHV1 enzyme-linked immunosorbent assays (ELISAs)

These were performed exactly as previously reported (Madic et al., 1995).

## 3. Results

### 3.1. IgM antibody responses

An IgM antibody response was not detected in sera or secretions of any of the reinfected calves. The control calves, which were infected for the first time, developed an IgM antibody response in serum, nasal and ocular secretions that was first detected on PRD 8 and lasted until PRD 27.

After corticosteroid treatment none of the calves developed detectable IgM antibodies against BHV1 in sera or secretions.

### 3.2. IgA antibody responses

Twenty-one of the 26 calves reacted after reinfection with a detectable IgA response in serum (Table 1). The mean PRD of first detection was 7.1. The control calves all developed IgA serum antibodies with a mean PRD of first detection of 12. The IgA antibodies in reinfected calves peaked around PRD 10–12 (Fig. 1) and disappeared from the serum

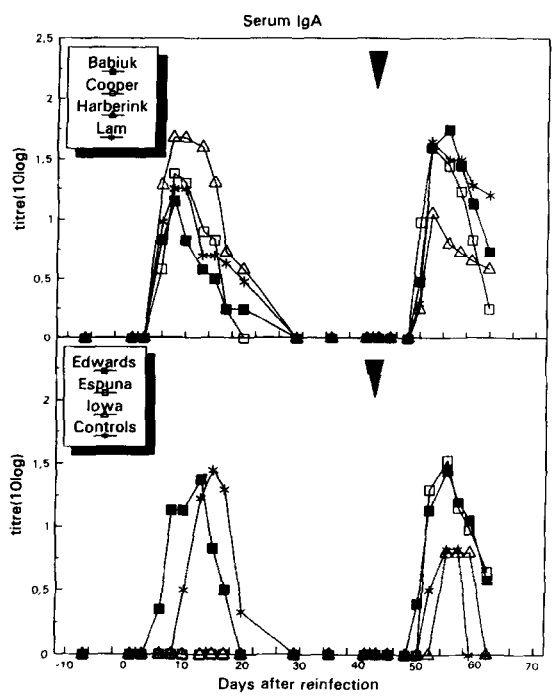


Fig. 1. The mean IgA antibody responses against BHV1 in sera of calves that were reinfected with the Iowa strain of BHV1 and given corticosteroid treatment. Arrows indicate time of corticosteroid treatment.

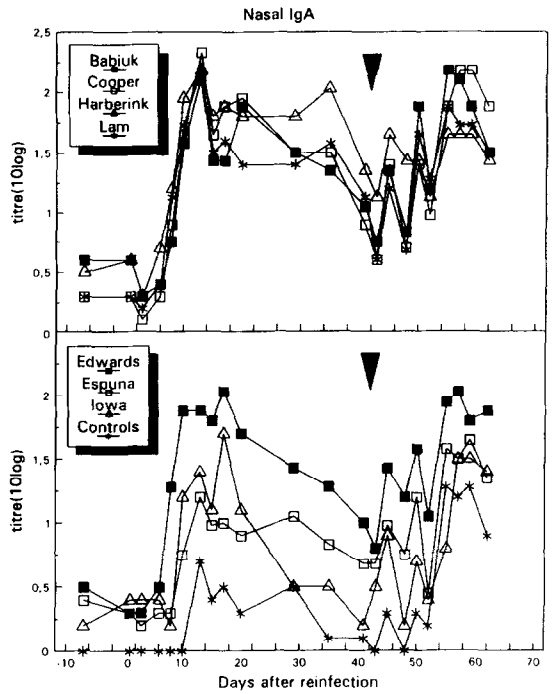


Fig. 2. The mean IgA antibody responses against BHV1 in nasal secretions of calves that were reinfected with the Iowa strain of BHV1 and given corticosteroid treatment. Arrows indicate time of corticosteroid treatment.

approximately 8 days after their first detection. The mean PRD of last detection was 15.4 for the reinfected calves and 17.8 for the control calves.

In nasal secretions a four-fold or higher increase of IgA antibody titres was found in all but one reinfected calves (Table 1). This increase occurred between PRD 4 and 11, with a mean PRD of first detection of 9.0. In the control calves IgA was first detected in nasal secretion on PRD 13. The IgA antibody peaked between PRD 10 and 15 and gradually decreased thereafter (Fig. 2). In all but three calves, the IgA antibodies persisted until the day of corticosteroid treatment (PTD 0). A fourfold or higher increase in ocular secretions was seen in all but 3 reinfected calves (Table 1) between PRD 6 and 13, with a mean PRD of first detection of 10.6. All control calves developed IgA antibodies in ocular secretions with a mean PRD of first detection of 14. The mean titres in ocular secretions were lower than in nasal secretions (Figs. 2 and 3). In 16 of 26 calves IgA persisted in ocular secretions until PTD 0. The individual IgA antibody titres in secretions varied considerably. In 8 of 26 reinfected calves IgA antibody was detectable at low levels in genital secretions for a few days (Table 1). IgA antibodies were not detected in genital secretions from control calves.

After corticosteroid treatment all but one calf developed serum IgA antibodies (Table 1). On average, these appeared in the blood on PTD 9.2. The serum IgA antibodies remained

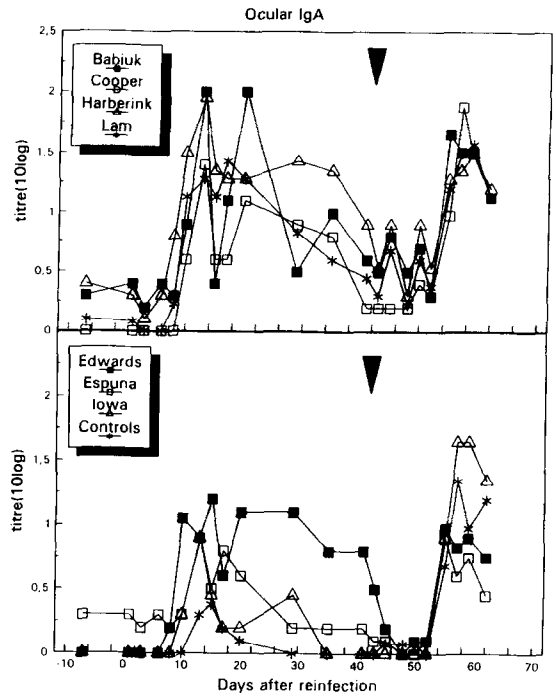


Fig. 3. The mean IgA antibody responses against BHV1 in ocular secretions of calves that were reinfected with the Iowa strain of BHV1 and given corticosteroid treatment. Arrows indicate time of corticosteroid treatment.

detectable for a period varying from 1 to 12 days. In 14 animals they were still present on PTD 21, which marked the end of the experiment. In all but two calves a fourfold or higher increase of IgA antibodies was noted in nasal secretions after corticosteroid treatment; in ocular secretions such an increase was found in all but three calves (Table 1). The mean PTD of this increase was 7.3 (range 3–15) for nasal secretions and 11.8 (range 3–17) for ocular secretions. The peak of the mucosal IgA titres was around PTD 14 and reached values comparable to those after reinfection (Figs. 2 and 3). The individual mucosal IgA antibody titres fluctuated considerably and persisted until the end of the experiment. In nine calves IgA antibodies were detected in genital secretions; five of them also had genital IgA antibodies after reinfection (Table 1).

### *3.3. IgG1 antibody responses*

In 21 of the 26 calves, reinfection did not lead to a significant increase in IgG1 antibody titre in serum; in the remaining five calves a fourfold or higher increase was detected between PRD 8 and 15. In all control calves IgG1 antibodies were first detected on PRD 13. The course of mean serum IgG1 titres per group is given in Fig. 4.

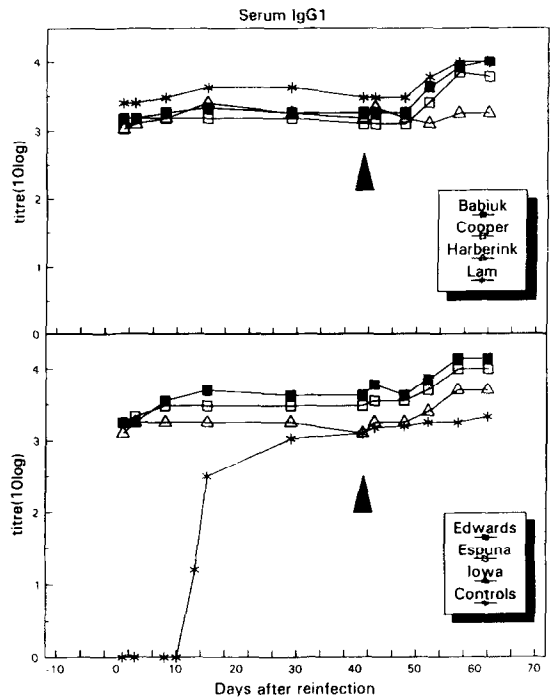


Fig. 4. The mean IgG1 antibody responses against BHV1 in sera of calves that were reinfected with the Iowa strain of BHV1 and given corticosteroid treatment. Arrows indicate time of corticosteroid treatment.

Nine calves, two of which were controls, had minimal IgG1 antibody titres (mean $\log_{10}$ titre of 0.15) in nasal secretions and 11 reinfected calves in ocular secretions (mean $\log_{10}$ titre of 0.18) for a short period. In none of these secretions were erythrocytes detected. The genital secretions were negative (Table 1).

After corticosteroid treatment 18 of 30 calves showed a significant increase in IgG1 antibody titre in serum, usually on PTD 15. The IgG1 antibody titres of the other 12 remained stable. The course of mean serum IgG1 titres per group is given in Fig. 4. In nasal secretions of 22 of 30 calves low levels of IgG1 antibodies (mean $\log_{10}$ titre of 0.40) were detected and in ocular secretions of 19 of 30 calves (mean $\log_{10}$ titre of 0.41), during a period varying from 1 to 14 days. In approximately one-third of the nasal secretions, 10–50 erythrocytes $\mu l^{-1}$ were demonstrated. Erythrocytes were not detected in ocular secretions. Two calves had IgG1 antibody in genital secretions, which were free of erythrocytes (Table 1).

### *3.4. IgG2 antibody responses*

The reinfection did induce a fourfold or higher rise in IgG2 serum antibody titre in 12 of the 26 calves between PRD 8 and 15. All control calves induced an IgG2 serum response

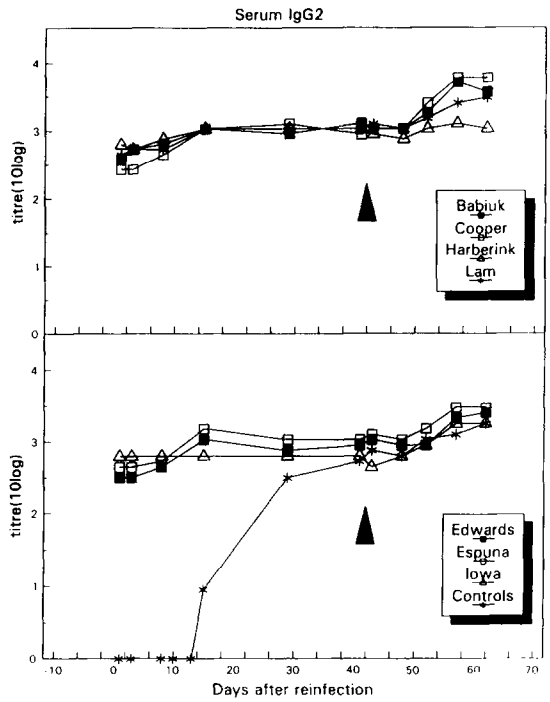


Fig. 5. The mean IgG2 antibody responses against BHV1 in sera of calves that were reinfected with the Iowa strain of BHV1 and given corticosteroid treatment. Arrows indicate time of corticosteroid treatment.

between PRD 15 and 29. The development of mean serum IgG2 titres is shown in Fig. 5.

Three calves showed low levels of IgG2 antibodies (mean $\log_{10}$ titre of 0.48) in nasal secretions after reinfection, and none had these antibodies in ocular or genital secretions after reinfection (Table 1).

After corticosteroid treatment the serum IgG2 antibody titre significantly increased in 14 of 30 calves, between PTD 10 and 15. The development of mean serum IgG2 titres is shown in Fig. 5.

Thirteen of 30 calves had detectable levels of IgG2 antibodies (mean $\log_{10}$ titre of 0.48) in nasal secretions, and 5 of 30 calves in genital secretions; none had IgG2 antibodies in ocular secretions (Table 1).

### *3.5. Antibody responses and virus excretion periods*

The number of days of virus excretion per individual calf after reinfection and after corticosteroid treatment is given in Table 1. After reinfection BHV1 was shed between PRD 1 and 5, with a mean PRD of first detection of 2.1. After corticosteroid treatment virus excretion was usually detected between PTD 4 and 8, with a mean PTD of first detection of 4.6. There was no correlation between nasal or ocular IgA antibody titre at the time of

reinfection or corticosteroid treatment, and the respective virus excretion periods in the individual calf, e.g. some calves that had no nasal IgA at reinfection shed BHV1 for a shorter time than some calves that had a high nasal IgA titre at reinfection (data not shown). Calves in which no virus excretion after reinfection and/or corticosteroid treatment was detected yet developed an antibody response against BHV1, mainly of the IgA isotype.

## 4. Discussion

The primary infections had induced a solid immunity, because intranasal reinoculation with a high dose of a virulent BHV1 strain prevented virus shedding in 10 of 26 calves. In the remaining 16 calves, reinfection led to 1–3 days of minimal virus excretion, whereas the primary infected controls had the typical virus excretion patterns (Kaashoek et al., 1994). In spite of this minimal virus replication, all calves did respond with antibody formation to the reinfection. After corticosteroid treatment virus replicated longer and to higher titres in nasal and ocular mucosae, which resulted in a more pronounced antibody response.

A BHV1-specific IgM antibody response was not detected in any of the reinfected or reactivated cattle in either sera or mucosal secretions. After intranasal re-exposure with BHV1, Guy and Potgieter (1985a) observed a slight increase in IgM antibody titre, whereas after intra-amniotic inoculation a rapid increase was detected after abortion. In addition, they detected a secondary IgM antibody response after reactivation (Guy and Potgieter, 1985b). These discrepancies may be explained by the difference in IgM detection method or by differences in experimental design, including breed of cattle, strain and dose of virus, route and duration of corticosteroid treatment. Osorio et al. (1989) could not detect an IgM antibody response after BHV1 reactivation either. After reactivation of herpes simplex virus, an IgM antibody response is seldom observed and then associated with severe encephalitis (Kalimo et al., 1977; Van Loon et al., 1981); however, reactivation of herpes zoster virus often leads to an IgM response (Tedder et al., 1981). After reinfection of calves with bovine respiratory syncytial virus (BRSV) IgM was not detected in sera, but was detected in nasal and ocular secretions (Kimman et al., 1987). However, the pathogenesis of a BRSV infection is different from that of a BHV1 infection: the latter giving rise to clinical signs of the upper respiratory tract, whereas a BRSV infection mainly affects the lower respiratory tract.

One of the most salient findings of this study is that in most calves a temporary IgA serum response was demonstrated both after reinfection and after reactivation. The serum IgA response in reinfected or reactivated calves was much earlier detectable than that in primary infected calves, indicating a memory response. Rodak et al. (1983) and Edwards et al. (1991) could not detect any serum IgA response in BHV1-reinfected calves. Eleven calves in which no virus excretion could be detected after reinfection and/or reactivation even showed a clear systemic IgA antibody response. An explanation for this unexpected finding may be that in latently infected calves that are given corticosteroids non-infectious physical rather than infectious BHV1 particles may be produced (Pastoret et al., 1979). These physical particles stimulate an antibody response. However, the discrepancy between virus excretion and antibody response may be due to our failure to detect infectious virus particles,

because these were not excreted via nasal or ocular secretions or because only one nasal or ocular swab was taken per 24 h. However, it may be the serum IgA response seems to be a much more sensitive indicator for reinfection with or reactivation of BHV1 than detection of nasal and ocular virus shedding. In the case of BRSV infection the secondary serum IgA response was not transient, but persisted for more than 3.5 months (Kimman et al., 1987).

Not only was there a vigorous IgA response in serum, but also a secondary type IgA antibody response was observed in nasal and ocular secretions in most reinfected and reactivated calves. The mucosal IgA response after reinfection was more rapid and stronger than in the primary infected calves (Figs. 2 and 3), indicating the existence of mucosal memory and thus confirms earlier reports on BRSV and bovine corona virus infections (Kimman et al., 1987; Heckert et al., 1991). After the primary infection (Madic et al., 1995), IgA antibodies were not detected in genital secretions, whereas, probably as a result of priming, after reinfection and reactivation genital IgA antibodies were found in 12 of 30 calves.

The increase of nasal IgA antibodies followed the onset of the virus shedding period with several days, suggesting that existing nasal IgA antibodies, which can neutralise BHV1 (Madic et al., 1995), did not play a major role in curtailing the amount of virus shedding after reinfection or reactivation. This is further substantiated by the finding that no correlation existed between nasal IgA titres at reinfection and onset of corticosteroid treatment on the one hand, and virus shedding periods on the other.

Whereas 16 of 26 calves had virus in nasal secretions, only five had an increase in serum IgG1 titre and 12 in serum IgG2 titre after reinfection. Guy and Potgieter (1985a) also found that the antibody increase after reinfection was more frequent in the IgG2 than in the IgG1 isotype. After corticosteroid treatment, virus-positive nasal secretions were detected in 20 of 26 reinfected calves, and a significant increase in serum IgG1 and IgG2 antibody titre in 17 and 12 calves, respectively. There was no correspondence between virus shedding and antibody titre increases (Table 1). The inability of some calves to elicit a booster IgG1 or IgG2 serum antibody response may partially be due to the high antibody titres that already existed after reinfection and corticosteroid treatment. In any case, more calves reacted with a systemic IgA response after reinfection and corticosteroid treatment than with a systemic IgG1 or IgG2 response. This further supports the systemic IgA response to be a sensitive indicator of BHV1 reinfection and reactivation.

Particularly after reactivation, but also after reinfection, IgG1 antibodies were seen in nasal and ocular secretions. IgG2 antibodies were only detected in nasal secretions in fewer calves than IgG1 antibodies. Most of the IgG containing secretions were free of erythrocytes, excluding leakage of blood as a cause of the IgG1 and IgG2 presence. Whether transudation or selective transport from serum or mucosal production is responsible for the mucosal IgG cannot be conclusively determined, but the IgG1/IgG2 antibody ratio in serum usually did not correspond with that in nasal secretion (data not shown). This indicates selective transport of IgG1 and IgG2 rather than transudation from serum. The IgG2 isotype was never detected in ocular secretions after primary infection (Madic et al., 1995), after reinfection and after reactivation, suggesting that this isotype may not or rarely pass the conjunctival mucosae. This finding is also in favour of selective transport of IgG1 through ocular mucous membranes. IgG1 and IgG2 antibodies against BHV1 were detected in genital secretions in a few calves, only after corticosteroid treatment.

There were clear differences in periods of virus shedding after corticosteroid treatment between the groups of calves primary infected with different BHV1 strains, e.g. calves given the Cooper strain had by far the longest virus shedding periods from nose and eye. These differences in virus shedding did, however, not result in marked differences in isotype-specific antibody responses between the groups of calves.

To our knowledge, this is the most extensive study performed on isotype-specific antibody responses to BHV1 after reinfection and reactivation. We may conclude that the IgM isotype is absent in secondary and tertiary antibody responses to BHV1 and that the serum IgA response is a more sensitive indicator of BHV1 reinfection and reactivation than virus shedding or the increases in IgG1 or IgG2 serum titres. In addition, nasal IgA antibody does not to appear to be involved in virological protection against reinfection or reactivation.

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