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Antibody dynamics in BRSV-infected Danish dairy herds as determined by isotype-specific immunoglobulins

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Abstract

Using specific ELISAs, antibody levels of four different isotypes to bovine respiratory syncytial virus (BRSV) were determined in calves, following experimental BRSV infection.

Most calves experienced an increase in the specific IgM and IgG1 titres about 6–10 days after infection with BRSV. The IgM titre was transient showing positive titres for only 5–10 days, while specific IgG1 was present for a longer time. IgA was detected concomitantly with IgM but at a lower level. Production of IgG2 anti-BRSV antibodies was detected from 3 weeks after infection.

In two closed herds, repeated blood samplings were performed on young stock to analyse maternal immunity. The passively transferred antibodies were mainly of the IgG1 isotype and the half-life of IgG1 to BRSV was estimated to be 26.6 days. One of the herds had an outbreak of enzootic pneumonia, diagnosed to be caused by BRSV. Furthermore, another herd with acute BRSV was followed by weekly blood samples in six calves; in both herds IgM and IgG1 was detected shortly after the appearance of clinical signs.

Serum samples from 50 Danish dairy herds (453 samples) were tested for immunoglobulins of the isotypes IgG1, IgG2 and IgM. The presence of antibodies to BRSV was widespread and more than 54% of the samples had BRSV antibodies of both the IgG1 and IgG2 isotypes indicating a high

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herd prevalence to BRSV. Test samples from two herds out of 50 were free from all isotypes to BRSV. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Bovine respiratory syncytial virus (BRSV) is a pneumovirus belonging to the family paramyxoviridae. It is widely distributed in most countries with an intensive and industrialised cattle production (van der Poel et al., 1993; Utenthal et al., 1996; Baker et al., 1986). In young stock, BRSV infection may result in severe pneumonia with up to 100% morbidity and 5–20% mortality (Baker et al., 1986; Blom, 1981). The virus is often diagnosed in combination with other viruses, bacteria or mycoplasma (Kimman et al., 1989; Tegtmeier et al., 1999; Brown et al., 1998). Respiratory disease caused by BRSV is mainly observed in winter, but reinfections probably occur in the summer season without causing severe problems in the herd (van der Poel et al., 1993).

Maternal antibodies transferred with colostrum do not protect against infection, however, high levels of maternal immunity may modulate the severity of disease (Kimman et al., 1988). Furthermore, maternal antibodies markedly suppress serum responses of all isotypes after experimental or natural infection in seropositive calves (Kimman et al., 1987a,b), thereby disturbing the diagnosis of BRSV based on serological methods.

In order to study the pathogenesis of BRSV and the efficacy of vaccines a challenge model was established in cattle (Tjørnehøj et al., in preparation; Larsen et al., 1998) in which severe respiratory symptoms are induced after a single inoculation.

The aim of the present study was to analyse the antibody isotypes induced in colostrum-fed calves infected naturally or experimentally with BRSV. Furthermore, the half-life of maternal antibodies in calves was analysed and the seroprevalence of BRSV in Danish dairy herds was determined based on randomly selected test samples.

2. Materials and methods

2.1. *Experimental infections*

Male Jersey calves were purchased from two closed herds 7–10 days after birth, and transferred to isolation units, where they were managed by personnel without contact to other cattle. All experimental calves were tested free from persistent infection with bovine viral diarrhoea virus (BVDV) before challenge (Bitsch and Ronsholt, 1995). The BRSV inoculation of the calves is detailed elsewhere (Tjørnehøj et al., in preparation; Larsen et al., 1998). Briefly, five calves (IV.8–IV.12), 8–12 weeks of age, were inoculated using unpassaged lung lavage fluid obtained from an experimentally infected calf suffering from BRSV (9402031) (Larsen et al., 1998). The BRSV inoculum contained

10^4 – 10^5 50% tissue culture infectious doses (TCID₅₀) of virus per millilitre. The inoculum was administered using a single combined aerosol (1 ml in 4 ml phosphate buffered saline (PBS)) and intratracheal inoculation (1 ml in 19 ml PBS). One calf (IV.7) was mock inoculated likewise using BRSV-free lung lavage fluid from a healthy calf. The inoculum was tested free from BVD virus.

The calves were observed for clinical signs and rectal temperature was measured daily. The control calf (IV.7) and two BRSV infected calves (IV.8 and IV.9) were anaesthetised and killed by exsanguination at post infection day (PID) 15. The remaining three calves (IV.10, IV.11 and IV.12) were sacrificed PID 30. Plain blood samples were collected from the jugular vein 3–5 times per week during the observation period. Nasal swabs were obtained at PID 0, 1, 2, 3, 4, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26 and 30 and stored in 0.9% NaCl at -20°C for subsequent testing for presence of viral antigens.

2.2. *Decay of maternal immunity*

Calves from two dairy herds (herd A and B) with a known history of recurrent cases of enzootic pneumonia and confirmed presence of antibodies to BRSV in the adult dairy population, were blood sampled once a month during the winter season. Serum samples were kept at -20°C until analysed for IgG1, IgG2, IgM, IgA and neutralising antibodies to BRSV. Furthermore, the sera were tested for antibodies against bovine corona virus. Both herds were clinically supervised for 6 months.

In herd A, 13 calves were followed from September 1993 to February 1994. In addition, five out of these calves not in pasture was sampled again in July 1994 when the calves were 11 months old. At the first bleeding in September the age of the calves ranged from 3 to 31 days.

In herd B 11 calves, aged from 7 to 56 days old at the first blood sampling in October were sampled.

2.3. *Natural infection*

As a part of a concurrent project (Enevoldsen et al., in preparation) selected Danish dairy herds were monitored for presence of clinical signs of respiratory disease. To validate the isotype-specific assays under field conditions, one of the herds with confirmed BRSV infection was selected. Six calves aged 3 weeks to 4 months (herd C) were bled on days 0, 7, 14 and 28 after the onset of clinical symptoms and detection of BRSV antigen in lung lavage. Lung lavage was performed on severely affected, non-sedated calves as described earlier (Uttenthal et al., 1996).

2.4. *Seroprevalence study*

To investigate the prevalence of BRSV antibodies in Danish dairy herds, 453 blood samples from 50 randomly selected healthy herds, were analysed for BRSV-specific antibodies of the isotypes IgM, IgG1 and IgG2. The Cattle Health Laboratory under the Danish Dairy Board generously supplied the sera which were all collected for the Danish

national program for eradication of BVD virus (Houe, 1992). The identity of the farms from where the test samples originated was kept anonymous, but no herds with bovine viral diarrhoea persistently infected (PI) animals were analysed. The test panel consisted of serum samples from three young calves aged less than 15 months, three samples from heifers aged 16–26 months and three samples from adult cows.

2.5. *Isotype-specific BRSV ELISAs*

BRSV-specific IgM, IgA, IgG1 and IgG2 antibodies were detected and titrated using the ELISA systems adapted from Kimman et al. (1987b). The following murine monoclonal antibodies (MAbs) were purchased at ID-Lelystad, the Netherlands: 15–8 (anti-bovine IgG1), 12–5 (anti-bovine IgG2), 16–35 (anti-bovine IgA) and 17–3 (anti-bovine IgM).

Briefly, IgM and IgA anti-BRSV antibodies were analysed using immunocapture ELISAs. Microtitre plates (Maxisorp, Nunc) were coated with MAb against bovine IgM or IgA in 0.05 M sodiumbicarbonate buffer pH 9.6. The sera were diluted in duplicates in twofold titrations from 1:20 in dilution buffer (PBS pH [w/v] Tween 80) and incubated for 1 h at 37°C.

Antigen, consisting of poly ethylen glycol (PEG 6000) precipitated BRSV (Danish field strain 88Lu195) (Larsen et al., 1998) propagated in cell cultures of primary foetal calf lung cells, was added in parallel with control antigen produced from uninfected cell cultures. BRSV antigen was added to all wells except the upper row, which contained control antigen. Then biotin conjugated rabbit serum against human respiratory syncytial virus (DAKO, Denmark) diluted 1:1000 was added followed by horseradish peroxidase (HRP)-avidin 1:10000 (DAKO). Enzymatic reaction was detected using ortho phenylen diamine (OPD), (Kem-En-Tek, Denmark) according to the manufacturer's recommendations, and stopped using 1 M H₂SO₄.

For detection of BRSV specific IgG1 and IgG2 indirect ELISAs were used. Microtitre plates were coated with rabbit serum against human respiratory syncytial virus (DAKO). BRSV and control antigen was added as for the immunocapture ELISA. Serum samples were diluted in twofold starting from 1:40 for IgG2 and 1:20 for IgG1, for all serum samples the lowest dilution of serum was tested on the control antigen. Then MAbs were added and finally HRP conjugated rabbit anti-mouse (DAKO) applied. OPD was used as substrate for the enzyme.

All reagents were titrated to the optimal dilution, using checkerboard titrations. Each reagent was added at a volume of 100 µl per well. Between incubations, plates were washed six times in deionized water containing 0.05% Tween 80. All incubations were performed for 1 h at 37°C. Plates were read using a doublebeam ELISA reader at 490 and 650 nm. To eliminate non-specific reactions, the OD-value of the serum on the control antigen (OD_{neg}) was subtracted from the OD-value of the serum on the BRSV antigen (OD_{pos}). If the OD_{pos}–OD_{neg} was higher than 0.1 the well scored positive. The titre was defined as the reciprocal of the highest dilution of serum resulting in a positive score. An isotype-specific standard positive serum obtained from an experimentally infected calf was titrated in all sets of plates. Coronavirus antibodies were titrated by indirect ELISA as previously described (Uttenhal et al., 1996).

2.6. Virus neutralisation test

Serum samples were inactivated at 56°C for 30 min, centrifuged and diluted twofold from 1:2 to 1:256. The serum-dilutions were supplied with 100 TCID₅₀ of a Danish strain of BRSV (88Lu195) and incubated for 1 h at 37°C. This mixture was inoculated in duplicate to monolayers of semi-continuous calf foetal lung cells at 10–30th passage. The cells were seeded in 96-well micro plates (1.5×10^4 cells per well) the day before inoculation. The cultures were examined for cytopathogenic effect (CPE) after 6 to 7 days and the titre determined as the reciprocal of the highest dilutions completely inhibiting the growth of cytopathogenic BRSV.

2.7. Detection of viruses

Presence of BRSV, PI-3 virus and bovine coronavirus antigens in the lung lavage material or nasal swabs were analysed by antigen-ELISA as previously described (Uttenthal et al., 1996).

3. Results

3.1. Experimental infections

In general specific IgM was detected 6–10 days post infection, IgA was present in one calf only simultaneously with IgM. The IgG1 reaction was dependent on the preinoculation titre of maternally derived IgG1, in seronegative calves IgG1 was detected from PID 8 and lasted more than 30 days. An IgG2 response was present in only one calf (IV.10), being detected on PID 26. BRSV antigen was detected in all BRSV infected test calves for 2–6 days, two of three calves with high preinoculation titres had BRSV in nasal swabs for 6 days.

Consumption of colostrum resulted in various levels of antibodies to BRSV in the calves. At the time of infection, when the calves were 54–84 days old, only the control calf and two calves (IV.9 and IV.10) out of five infected calves had lost their maternally acquired antibodies to BRSV.

Starting from day 4 after BRSV infection, the test calves presented typical respiratory signs of BRSV, i.e. increased respiratory rate, anorexia, ocular discharges and coughing. In the calves kept after PID 15, the predominant signs of disease was coughing.

Four out of five calves had elevated rectal temperature ($>39.3^\circ\text{C}$) starting from day 4 with a duration of 3–8 days. BRSV antigen was detected in nasal swabs from all infected calves for at least two successive days between day 3 and 8 after infection. Results of three calves are shown in Fig. 1. PI-3 virus and bovine coronavirus were not detected in nasal swabs or lung lavage from any of the experimentally infected calves.

The control calf did neither show respiratory signs, nor did the body temperature rise. BRSV antigen was not detected in nasal swabs, and no BRSV antibody was detected in serum from this calf (data not shown).

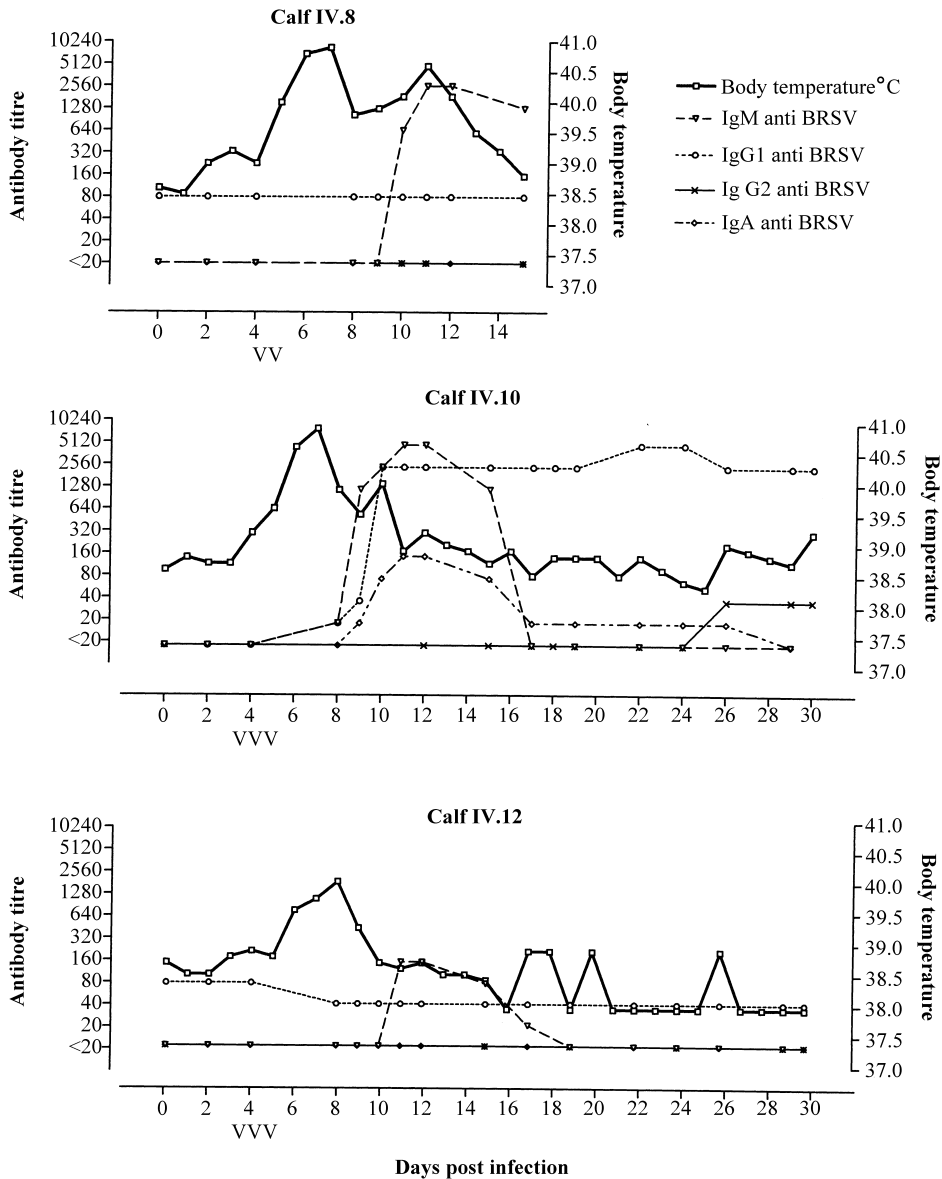


Fig. 1. Body temperature and kinetics of antibody activity to BRSV in three colostrumfed, two- to three-month old calves after experimental primary infection with BRSV. Nasal swabs were taken daily (except day 6 and 7); detection of BRSV-antigen is shown as "V".

An IgM response was present in calves IV.8 and IV.9 from day 8 to 10 after infection. Calf IV.8 had IgG1 titres of 80 before inoculation. This antibody level seemed to suppress the IgG1 response, but not the clinical course of infection as the calf had fever, increased respiratory frequency, and was depressed. Calf IV.9 (data not shown) had no specific

IgG1 titre before inoculation and seroconversion was detected at day 9. The calf had typical signs of BRSV including increased breathing frequency and fever.

Calf IV.10 was seronegative prior to infection. Six days after infection the body temperature was 40.9°C, followed by an increase in IgM and IgG1 PID 8, IgA increased PID 9 and IgG2 PID 27. The IgM and IgA responses were shortlasting compared to the IgGs and returned to negative at PID 18 and PID 26, respectively. IgG1 remained at high titres (2560) for the rest of the observation period.

Calf IV.11 had IgG1 titres of 160 at the time of infection and this titre decreased slightly after infection (data not shown). The calf had increased respiratory rates and was coughing but was not pyretic. BRSV antigen was detected in four consecutive samplings from PID 3 to 8. No IgM, IgA or IgG2 was detected in this calf.

Calf IV.12 had IgG1 titre 80 on the day of the infection. IgM was detected from day 11 to 17; IgG1 declined slightly through the sampling period, whereas IgG2 remained below the detection limit.

3.2. Decay of maternal immunity

Calves born into herds A and B were followed for 6 months. Colostrum feeding resulted in a mean serum IgG1 anti-BRSV titre of 197 in herd A. This titre decreased during the first 4 months leaving the calves below the detection level (>20) in February at 5 months of age (Fig. 2). IgG2 or IgA antibodies were not detected during the first 6 months. The neutralising titres to BRSV decreased in parallel to the specific IgG1 but neutralising antibodies could be detected at least 2 months after the disappearance of IgG1.

Similar to the BRSV titres the specific anti-corona virus titre decreased during the first 3 months, but samples collected in January showed an increase in antibodies to corona virus. Whether the coronavirus infection was enteric or respiratoric was not known, however no respiratory symptoms or diarrhoea were reported in herd A in January. In contrast, the veterinarian reported an acute outbreak of respiratory disease in March. Presence of IgG1 as well as IgG2 in July indicated an active BRSV infection in the herd after the intensive observation period. No IgA or IgM was detected in July.

The initial mean IgG1 titre in the 11 calves in herd B was 150. One calf had IgM antibodies in October and November when the calf was only 1 and 2 months old. Unfortunately, there was not enough serum left to perform IgA isotype ELISA on these two IgM positive sera. Decreasing IgG1 titres were registered until December when an outbreak of respiratory disease was registered. The following two blood samples showed a specific increase in IgG1-anti BRSV titres. IgM anti-BRSV was detected in January in 8 out of 11 calves. IgA was detected in 1 out of 11 calves. Specific IgG2 was not detected until February.

In herd B, the antibodies to corona virus were also declining until the sampling in December. In January a titre of 6400 resembling the level in the primary sample in the herd was seen. Thus coronavirus could have been a cofactor in the respiratory disease reported in December.

Based on sera from herd A and B the half-life of BRSV-specific IgG1 as well as neutralising antibodies was calculated to 26.6 days, in herd B the half-life could only be

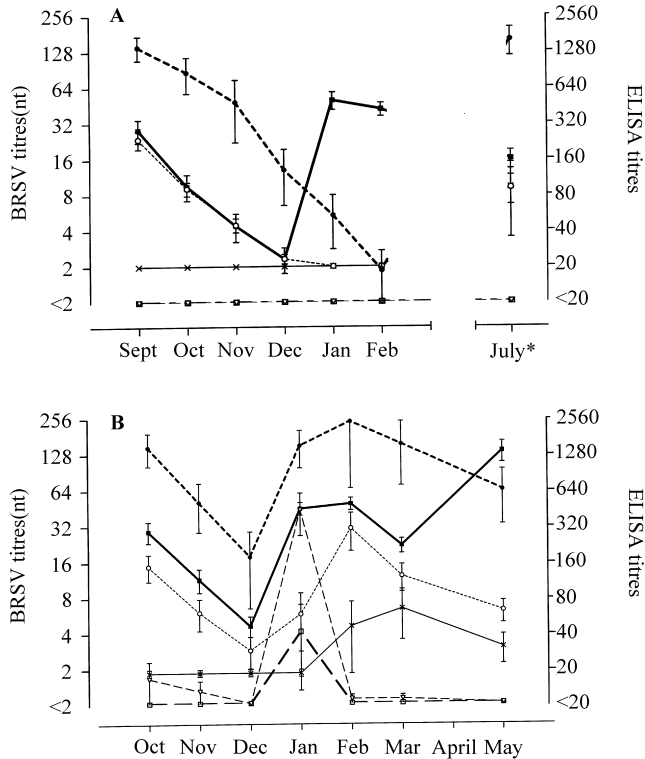


Fig. 2. Metabolism of maternal antibody activity in calves analysed during the first half year of life. Results are shown as the mean titre and S.E.M. The calves in herd A ($N = 13$), had no signs of pneumonia during the intensive observation period until February. In herd B ($N = 11$) an epizootic was present in the end of December. Antibodies to BRSV were analysed by isotype-specific ELISAs, antibodies to bovine corona virus were not subtyped. *Only sera from 5 calves were analysed. Symbols: (—●—) Neutralisation titers; (—▽—) IgM; (—○—) IgG1; (—×—) IgG2; (—□—) IgA; (—■—) corona. Antibody titres to corona virus are 10 times the value on the ELISA axis.

calculated based on sera sampled until December, when BRSV was introduced in the herd. The half-life of corona-antibodies, which only decayed for a few months, was similar. The specific time point of bovine coronavirus infection is unknown, but the half-life was not affected till after the sampling in December (Fig. 2).

3.3. Natural infection

Herd C experienced an outbreak of respiratory disease in March. Four of the six calves 3 weeks to 4 months old had IgG1 titre below 40 at the first blood sampling in relation to the outbreak. The remaining two calves were seronegative.

The clinical symptoms in the calves included pyrexia (rectal temperature above 40°C), depression, and serous nasal discharges accompanied by a markedly increased breathing

Table 1

Antibody responses (IgM, IgA, IgG1 and IgG2 isotypes) against BRSV after natural infection in herd C from the onset of respiratory disease (week 0). Six calves were followed by weekly blood samples for 4 weeks

Calf No.	Virus in lung lavage	Calf age	Isotype	BRSV antibodies in serum after			
				0 weeks	1 week	2 weeks	4 weeks
2146	BRS, corona	3 weeks	IgM	0	0	20	Dead
			IgA	0	160	640	Dead
			IgG1	40	0	0	Dead
			IgG2	0	0	0	Dead
2152	BRS, corona	3 weeks	IgM	0	640	320	0
			IgA	0	0	0	0
			IgG1	20	0	0	40
			IgG2	0	0	0	40
2137	n.d.	10 weeks	IgM	0	320	160	160
			IgA	0	0	0	0
			IgG1	40	40	20	20
			IgG2	0	0	0	160
2136	n.d.	11 weeks	IgM	0	1280	640	320
			IgA	0	0	0	0
			IgG1	0	20	40	80
			IgG2	0	0	0	0
2133	n.d.	3 months	IgM	0	1280	320	0
			IgA	0	0	0	0
			IgG1	20	20	80	640
			IgG2	0	0	0	80
2127	n.d.	4 months	IgM	0	640	20	0
			IgA	0	0	0	20
			IgG1	0	0	0	640
			IgG2	0	0	0	80

frequency. One of the diseased calves (# 2146) died 3 weeks after the onset of pneumonia. BRSV and coronavirus was detected in lung lavage fluids from two of the diseased calves (Table 1). These calves had BRSV specific IgM in serum 1 to 2 weeks later. The remaining four clinically affected calves were older and they were all BRSV IgM positive for 2 to 4 weeks starting week 1.

BRSV-specific IgG1 present at week 0 in the two 3-week-old calves disappeared before the week 2 sampling. IgG1 and IgG2 were, however, present in the one surviving calf by week 4. In the 10-week-old calf, the IgG1 titre was halved during the four-week observation period. In the three older calves (>10 weeks of age) an active IgG1 response was detected between week 1 and 4. High titres of IgA were found in one severely affected calf, which died during week 3. In addition low titres of IgA was detected during week 4 in the 4-month-old calf. At week 4 IgG2 was detected in low titres in four out of five calves.

3.4. Seroprevalence samples

The randomly selected sera represented cattle from all parts of Denmark. A total of 453 blood samples representing 50 herds (7–10 samples per herd) were analysed for presence of isotypes IgM, IgG1 and IgG2 specific antibodies to BRSV. IgM anti-BRSV was not detected in any of the samples.

Only in two out of the 50 herds were all the tested samples free from IgG1 as well as IgG2 anti-BRSV antibodies; the remaining 48 herds had at least one seropositive animal. Among the 453 individual samples, BRSV specific IgG1 as well as IgG2 was present in 244 samples (54%) of the sera and 104 samples (23%) did not have any IgG antibodies to BRSV. In 30 samples (6.6%) IgG2 but no IgG1 was detected and 75 samples (16.6%) had IgG1 anti-BRSV antibodies, but no specific IgG2.

4. Discussion

The isotype-specific ELISAs were initially applied on sera from experimentally infected cattle and the lack of cross-reactivity between the isotypes was demonstrated. The kinetics of the different antibody isotypes in response to BRSV infection in our experimental design, capable of inducing severe clinical disease, were quite similar to those previously found in less affected BRSV inoculated calves (Kimman et al., 1987a).

All five experimentally inoculated calves became infected as BRSV antigens could be detected in nasal swabs for several days. Furthermore, the calves without IgG1 at the time of infection all mounted IgM response and seroconverted to IgG1, whereas the presence of IgG1 prior to inoculation decreased and/or abolished the IgM and the IgG1 responses. Interestingly, excretion of BRSV antigen and development of clinical symptoms were not affected by the presence of pre-existing antibodies. It has previously been shown that IgG1 transferred via colostrum may suppress the development of antibodies to BRSV and that the presence of very high levels of passive immunity has also been found to prevent virus shedding, clinical symptoms and lung changes (Kimman et al., 1987a; Belknap et al., 1991). Generally the immune responses to BRSV seemed to be independent of the age of the calf in contrast to earlier studies suggesting that the age of the calves being critical for the duration of the IgM response (Kimman et al., 1987b). Thus, in the present study the suppressive effect seemed to depend on the presence of pre-existing BRSV-specific antibodies rather than age.

Compared to the experimental infections, the naturally infected animals produced IgM for a prolonged period in accordance with the findings by Kimman et al. (1987b). In contrast, we found higher IgG1 levels in experimentally infected calves than in naturally infected cattle. Other serological studies on BRSV revealed that experimentally infected animals had lower antibody titres (Schrijver et al., 1996); this discrepancy may be explained by differences in the experimental design, i.e. using the latter design induced only sparse clinical symptoms, if any.

The appearance of IgA antibodies in serum was rare. Thus IgA was only detected in one of five experimentally infected calves, concurrently with IgM, however, the IgA

response lasted longer. Prolonged IgA response, especially following reinfection, has previously been described (Kimman et al., 1987a).

Calves are born nearly agammaglobulinemic and receive passive immunity (mainly IgG1) through colostrum and milk during the first 48 h after birth (McGuire et al., 1976; Belknap et al., 1991). Accordingly the level of antibodies to BRSV was high in young calves born into herds with recurrent cases of pneumonia (herd A and B). The maternal immunity was confirmed to be solely of the IgG1 isotype, since no passively transferred IgM or IgG2 antibodies were detected. The IgG1 and the neutralising antibodies decreased over the initial 4 months with identical slopes if a new infection was not introduced into the herd. Coronavirus antibodies decreased for a few months then a natural infection interfered. The half-lives of the maternally derived antibodies against BRSV and coronavirus were, however, identical with a half-life of approximately 26 days. This figure is slightly higher than the 23 days previously found for passively transferred BRSV antibodies (Kimman et al., 1987b). Serological evidence for a coronavirus infection was present both in herd A and B. Furthermore, in herd C bovine coronavirus was detected in lung lavage fluid. Whether these infections were respiratoric or enteric are unknown, but no incidences of diarrhoea was reported from these farms. Respiratory enzootics involving several (viral) agents are often reported (Uttenthal et al., 1996; Martin et al., 1998; Larsen et al., 1999).

The blood samples taken for assessment of the seroprevalence of BRSV in Denmark were obtained in February, when the highest incidence of infections was expected; however, IgM was not detected in any of the samples. Similarly, van der Poel et al. (1993) found only eight positive IgM samples in 387 sera collected year-round from 190 two-to-four-month old calves. These findings are not surprising, since IgM is only present for a few weeks following infection. Furthermore, the sera included in the test sample were partly from adult cattle. The samples were not analysed for IgA, which may have revealed more positive animals due to the longer duration of this isotype and because IgA, in contrast to IgM, is induced in response to reinfection (Kimman et al., 1987b). The restricted detection of BRSV-specific IgA and IgM was in contrast to what was found following bovine PI-3 virus infection (Graham et al., 1999). Thus, in this study paired field sera from herds with known PI-3 infection revealed IgM-anti PI-3 virus in 44% and IgA in 26% of all sera analysed.

The test sample from only two herds out of 50 were completely free from BRSV-specific IgG antibodies, indicating that BRSV is very common in the Danish dairy herds. In a slightly different study all bulk milk samples obtained during June to December from 341 herds in England and Wales contained antibodies to BRSV (Paton et al., 1998), suggesting a similar high herd prevalence in UK as in Denmark. Accordingly, 54% of the samples had BRSV specific antibodies of both IgG1 and IgG2 isotypes. The presence of cattle with IgG1 but no IgG2 probably represent newly infected animal or animals with residues of maternally derived antibodies, whereas the very limited number (6.6%) of IgG1 negative and IgG2 positive cattle may be convalescent animals having lost their IgG1. Unfortunately the age of the specific animal was not provided; thus a more detailed comparison of age and isotypes was not possible.

In conclusion, the present report confirmed that the antibody dynamics following severe experimental BRSV infection is comparable to figures previously found in less

effected calves. Furthermore, the field study revealed a very high prevalence of BRSV in the Danish dairy herds.

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