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An Experimental Study of a Concurrent Primary Infection with Bovine Respiratory Syncytial Virus (BRSV) and Bovine Viral Diarrhoea Virus (BVDV) in Calves

By *M. Elvander*[1,2], *C. Baule*[2], *M. Persson*[1], *L. Egyed*[2], *A. Ballagi-Pordány*[2], *S. Belák*[2] and *S. Alenius*[1,2]

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Elvander M, Baule C, Persson M, Egyed L, Ballagi-Pordány A, Belák S, Alenius S: An experimental study of a concurrent primary infection with bovine respiratory syncytial virus (BRSV) and bovine viral diarrhoea virus (BVDV) in calves, Acta vet. scand. 1998, 39, 251-264. – Experimental infections with bovine respiratory syncytial virus (BRSV) and bovine viral diarrhoea virus (BVDV) were performed to study the effect of concurrent BRSV and BVDV infections. Twelve seronegative calves, in 3 groups, were inoculated on a single occasion with pure BRSV (group A), BRSV and noncytopathogenic BVDV (group B) or mock infected (group C).

Mild respiratory symptoms were recorded 4 to 5 days post inoculation (dpi) in group A and group B calves. One calf in group A was severely affected and required medical treatment. In group B, fever (40.7-41.4 °C) was prominent 7 to 8 dpi. Only calves in group B were BVDV positive in purified lymphocytes at 2 to 14 dpi and showed increased serum interferon levels, with a peak at 4 dpi, indicating BVDV to be responsible for inducing the rise.

BRSV was detected in lung lavage fluids up to 7 dpi for group A calves, compared to 11 dpi for group B and calves in this group also seroconverted later displaying lower BRSV titers. The time lag before an antibody response and the titers recorded in group B, indicated that the duration of BVDV infection in lymphocytes negatively influenced the capacity to mount a BRSV antibody response.

cattle; respiratory disease; interferon; PCR.

Introduction

Respiratory disease in commercial calf rearing units is an important cause of ill health and loss of production world-wide (*Andrews & Read* 1983, *Caldow et al.* 1988). Several microbiological agents have been incriminated as causal agents, amongst which bovine respiratory syncytial virus (BRSV) is undoubtedly an important cause of severe respiratory disease both in young cattle (*Baker et al.* 1986, *Martin & Bohac* 1986, *Caldow et al.* 1993) and in dairy cows (*Inaba et al.* 1972, *Elvander* 1996).

However, although several experimental infection models have been described for BRSV (*Bryson et al.* 1983, *Thomas et al.* 1984, *Castleman et al.* 1985, *Kimman et al.* 1986 &1987, *Potgieter et al.* 1988, *Le Blanc et al.* 1991, *Ciszewski et al.* 1991, *Gershwin & Giri* 1992), it has proven difficult to reproduce severe clini-

cal disease in calves with BRSV alone. In the studies cited, the virus was generally administered for 4 consecutive days and, although lung lesions developed, only mild to moderate clinical symptoms were recorded (*Bryson et al.* 1983, *Thomas et al.* 1984). Natural outbreaks of respiratory disease in young calves often occur following the mixing together of calves from different sources in specialized calf rearing units. Under these circumstances, several viral infections may occur, either concurrent or sequentially (*Caldow et al.* 1993).

Bovine viral diarrhoea virus (BVDV) is a common pathogen of calves which, because of its immuno-suppressive ability, may be a potent predisposing agent for other organisms (*Potgieter* 1995). Thus, the objective of the present study was to simulate a likely natural event, namely concurrent BRSV and BVDV infection, and compare this with a single BRSV infection.

Materials and methods

Animals

Twelve male calves[1], between 14 and 17 weeks old, were obtained from a closed dairy herd of approximately 180 dairy cows. Nine calves were of the Swedish Red and White breed (SRB) and 3 were Friesians. The dairy herd was affiliated to national health programs and free of bovine viral diarrhoea virus (BVDV), infectious bovine rhinotracheitis virus (IBR) and bovine leukemia virus (BLV). The calves were tested for antibodies to BRSV at 5 to 8 weeks of age, when still in the herd. At that time 8 were negative and 4 had low BRSV titers, which were colostrum-derived since all calves were antibody negative to BRSV and BVDV at time of inoculation.

On arrival at the Faculty of Veterinary Medicine (Swedish University of Agricultural Sciences), Uppsala, Sweden, the calves were housed in 3 different isolated experimental stables (A, B and C) in groups of 4. No other cattle were kept on the premises during the experiment. The stables were adjacent to each other, connected by an outer corridor. The ventilation system had a common influx of air, while the outlet of exhaust air was separate for each stable. Clean protective clothing and shoes were required before entering each room. The feeding (hay and concentrates) and sampling routines always followed the order stable C (control calves) to A to B. The calves were housed 2 weeks prior to experiment initiation to overcome the effects of transportation and change in environment and to establish base lines for clinical and blood parameters.

Experimental design

Four calves were infected with pure BRSV (strain SVA 187/92), (group A; calves Nos. 68, 69, 70 and 73) and 4 with mixed BRSV and BVDV (strain SVA 504/93), (group B; calves Nos. 64, 65, 66 and 72). Four calves in the control group (C; calves Nos. 67, 71, 74 and 75), were inoculated with uninfected tissue culture cell supernatant. The calves were inoculated when 16 to 19 weeks old, each animal receiving 10 ml of inoculum administered intratracheally and 10 ml intranasally (5 ml in each nostril) on one occasion only.

Clinical examinations were performed daily, from 4 days before until 18 days after inoculation. Rectal temperature, general condition, heart and respiratory rate, pulmonary auscultatory sounds, presence of coughing, conjunctival and nasal discharges and the appearance of the oral and nasal mucus membranes were recorded. Fever was defined as a rectal temperature >39.8 °C.

[1] The use of calves for experimental purposes was approved by the Local Ethical Committee on Animal Experiments, Uppsala, Sweden, protocol C 83/94.

Virus strains

Strain SVA 187/92 of BRSV was isolated from a dairy cow during an outbreak of acute respiratory disease (Herd B, *Elvander* 1996).

Strain SVA 504/93 of BRSV (also containing BVDV), was isolated from the lungs of a naturally infected calf that died of acute respiratory disease.

Both virus strains were isolated and multiplied in BVDV free monolayers of primary bovine turbinate cells as described previously (*Elvander* 1996). Fourth and fifth passages were used as inocula, following one cycle of freeze-thawing. Assay by immunoperoxidase (IPX) for BVDV demonstrated strain 504/93 to be BVDV positive, whereas strain 187/92 was BVDV negative.

The group A inoculum contained $10^{4,25}$ 50% tissue culture infectious doses ($TCID_{50}$) of BRSV per ml. The group B inoculum contained $10^{5,75}$ $TCID_{50}$ of BRSV per ml and $10^{5,50}$ $TCID_{50}$ of BVDV per ml. Supernatants from uninfected monolayers of the same cells were used as control inoculum.

Sampling procedures

Serum and EDTA blood samples were collected 4 days before inoculation, at infection and for 10 consecutive days thereafter, then 3 times a week for 2 weeks. Nasopharyngeal swabs, nasal tampons and lung lavage fluids were collected 3 times weekly for 3 weeks. Serum samples were stored at –20 °C until analysed for antibodies and interferon. Lung lavage was performed on unanaesthetised animals according to the protocol of *Kimman et al.* (1986). In brief, a sterile human gastric tube, 90 cm long with 8 mm diameter (Gastric Lavage Kit, Beiersdorf, Kungsbacka, Sweden), was passed through one nostril and into the trachea via the ventral nose duct until firm resistance was encountered. Approximately 60 ml of phosphate buffered saline (sterile, isotonic, pH 7.2) was slowly inoculated through the tube and immediately withdrawn using the same syringe. After collection, the 30-40 ml of recovered fluid was centrifuged for 10 min at 1.000 rpm (Sorvall Superspeed RC2-B9, Du Pont Co, Newton, Conn, USA). Both supernatant and pellet, resuspended in 2 ml fluid, were stored at –20 °C until assayed.

Nasal fluid samples were obtained by insertion of a tampon into one nostril for about 3 min. The absorbed fluid, (approximately 2 ml), was extracted by compressing the tampon with a syringe, then stored at –20 °C. Nasopharyngal swabs (Culturette cotton swabs, Marion Scientific, Kansas City, KA, USA) were stored at –70 °C until examined.

Detection of antibodies

Antibodies to BRSV and BVDV were detected using commercial indirect ELISA kits (Svanova Biotech, Uppsala, Sweden) as described previously (*Niskanen et al.* 1989, *Elvander et al.* 1995). For sera diluted 1:100 an OD_{450} value >0.1 was considered antibody positive. Titration of antibodies to BRSV was performed in two-fold dilutions, starting at 1:25. A positive titer was estimated as the highest dilution which gave an OD_{450} value >0,1.

Detection of viruses

Polymerase chain reaction (PCR). BRSV: A nested PCR assay for the detection of BRSV in clinical samples was used as described elsewhere (*Vilček et al.* 1994). In brief, the primers were selected from a highly conserved region of the gene encoding the F fusion protein. The first stage PCR yielded a 711 base-pair (bp) product while the second stage amplicon was 481 bp long. Amplified DNA was demonstrated by gel electrophoresis; ethidium bromide stained bands were visualized by UV light and recorded by a video camera (*Belák et al.* 1994).

BVDV: RNA was extracted from purified lymphocytes by proteinase K digestion followed by phenol/chloroform extraction, as described by *Vilček et al.* (1994). From the RNA extracts cDNA was synthesized using random hexamers (Pharmacia, Uppsala, Sweden). In brief, 5 μl of RNA was mixed with 2 μl of double-distilled H_2O (dd H_2O) and 1 μl (0,02U) of random hexamer; denaturated at 56 °C for 5 min and then cooled on ice for 5 min. The mixture was transferred to a corresponding tube containing a reaction mix of 5 μl of 5×first strand buffer (Gibco BRL, Gaithersburg, MD, USA), 2.5 μl of 2 mM each dNTP (Pharmacia), 1 μl of RNase inhibitor (RNAguard, Pharmacia) and 1 μl (200U) of Murine leukemia virus reverse transcriptase (M-MLV RT, Gibco, BRL). After incubation for 90 min at 37 °C and inactivation of the enzyme for 5 min at 98 °C, the cDNA was either used immediately on the PCR or kept at –70 °C until processed further.

In order to select primers, sequences for the 5′noncoding region (5'NCR) of 3 BVDV and 2 classical swine fever virus strains were aligned by applying the Pileup program of the GCG program-package. Primers were selected from the consensus parts applying the OLIGO 4.0 computer program (National Biosciences, Inc., Plymouth, MN, USA). A 296 bp PCR product was amplified with the primers OPES 13a (5' GCTAGCCATGCCCTTAGTAGGA 3') and OPES 14a (5'ATCAACTCCATGTGCCATTTACAGC 3'). The amplified products were subjected to a second PCR, using the internal primers OPES 11 (5'TGAGTACAGGGTAGTCGTCAGTGGTTCG 3') and OPES 12a (5′GGCCTTTGCAGCACCCTATCAG 3'). The size of the second PCR product was 169 bp. The primers were synthesised using a PCR-MATE DNA synthesiser (Applied Biosystem, Warrington, UK).

The PCR amplification was carried out in a 100 μl volume which comprised: 10 μl 10×PCR buffer (100 mM Tris-HCl, pH 9.0, 500 mM KCl and 1 mg/ml BSA), 10 μl $MgCl_2$ (25 mM), 1 μl of each dNTP (10 mM each, Pharmacia), 30 pmol of each outlet primer (OPES 13a, OPES 14a), 2 U Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT, USA) and 5μl cDNA; ddH_2O was added to adjust the final volume to 100 μl. This aqueous phase was overlaid with a drop of mineral oil (Sigma, St Louis, Mo, USA). Amplification was carried out in a DNA Thermal Cycler (Perkin-Elmer Cetus) using 5 cycles of 94 °C for 45 s, 50 °C for 30 s, 72 °C for 1 min and 30 cycles of 94 °C for 45 s, 45 °C for 1 min, 72 °C for 3 min and finally 72 °C for 7 min. The second PCR was performed in 50 μl PCR solution as above, containing inlet primers OPES 11 and OPES 14a. Approximately 1 ml of the first PCR product was transferred to the second PCR tube and the amplification was carried out using 5 cycles of 94 °C for 45 s, 55 °C for 30 s, 72 °C for 1 min and 30 cycles of 94 °C for 45 s, 50 °C for 1 min, 72 °C for 3 min and finally 72 °C for 7 min. The amplified product was demonstrated by ethidium bromide staining after gel electrophoresis. Water blanks were used throughout the procedure in order to control false positives caused by contamination. False negative reactions were revealed by an internal standard created for the OPES primers (*Ballagi-Pordány & Belák* 1996).

Virus isolation. Isolation of BRSV in primary bovine turbinate cell cultures was performed as described previously (*Elvander* 1996). Only samples positive by PCR were selected for cultivation.

Immunoperoxidase (IPX) assay. Serum samples were examined for the presence of BVDV by inoculation onto secondary cultures of embryonic bovine turbinate cells, incubated for 4 days then stained by an indirect im-

munoperoxidase (IPX) technique using a polyclonal antiserum (*Meyling* 1984).

Bacteriology

Nasopharyngeal swab material and lung lavage fluid (the resuspended pellet) were examined for bacteria by inoculation onto bovine blood agar (5%, incubated aerobically overnight at 37 °C) and hematin agar, which contained Fleischmanns yeast extract specially formulated for the culture of *Hemophilus somnus* (incubated at 37 °C in a humified 5% CO_2 chamber for 2 days). Lung lavage fluids were examined for *Mycoplasma spp* by cultivation in F-broth and HA-broth (*Bölske* 1988); F-broth with 0.05% urea, pH 6.0 (for *Ureaplasma diversum.*) and F-broth containing 9% fetal calf serum and 9% horse serum, and with bacitracin and methicillin substituted for ampicillin, (for *Mycoplasma dispar*). The solid medium used comprised F-broth with 0.6% agarose. Culture methods were performed according to *Bitsch et al.* (1976).

Interferon assay

Antiviral activity in serum, collected 4 days before, on the day of infection and 2, 4, 7, 9, 11, 14 and 18 days post infection (dpi), was measured as previously described for porcine sera (*Artursson et al.* 1989). In brief, the end-point dilution which protected a mean 50% of bovine kidney cells from destruction by vesicular stomatitis virus was determined by a conventional cytopathic effect inhibitory bioassay. As positive control, a laboratory standard of Sendai virus-induced porcine leukocyte interferon (corresponding to 23 Units of porcine interferon/ml) was titrated on each assay plate. Titres were expressed as the reciprocal of the end-point dilution.

Results

Clinical findings

Group A: All calves exhibited mild signs of respiratory disease at 4 to 5 days post inoculation (dpi). Moderate nasal and lacrimal discharges were noted and coughing was recorded for a mean 6 days. One calf (No. 70) showed a body temperature of 40.5 °C at 7 dpi, and continous fever (temperature > 40.2 °C) at 9, 10 and 11 dpi. On 11 dpi, this calf showed such severe signs of respiratory distress that penicillin treatment was initiated. The calf responsed well and no further signs of disease was recorded. Calf No. 68 had a body temperature of 40 °C at 8 dpi (Fig. 1).

Group B: Similar mild clinical signs as in group A were noted at 4 to 5 dpi. All 5 calves, however, became febrile with maximal temperatures of 40.7-41.4 °C at 7 and 8 dpi (Fig. 1). During this period, the calves were slightly depressed but their appetite was unaffected.

Group C: No signs of respiratory disease was observed during the first week of experiment. From day 9 to 16 dpi mild respiratory signs with coughing were noted in all 4 calves of this group. Only one of the calves (No. 67) had fever during the observation period, with a temperature of 39.9 °C at day 14 and 40.0 °C at day 15 (data not shown).

Virological findings

BRSV – PCR. Group A: nasal swab samples were BRSV-positive between 2 and 7 dpi. Lung lavage samples were positive between 4 and 7 dpi. All 4 calves were negative from 11 dpi onwards (Table 1a).

Group B: BRSV was detected in nasal swab material between 2 and 7 dpi. Lung lavage samples were positive between 4 and 11 dpi. All 4 calves were negative at 14 dpi (Table 1a).

In group C, BRSV was detected in nasal and/or lung lavage samples at 11 dpi. At 14 dpi all calves were BRSV positive in lung lavage samples. No samples were collected after 14 dpi. (Table 1a).

BRSV – Virus isolation. In groups A and

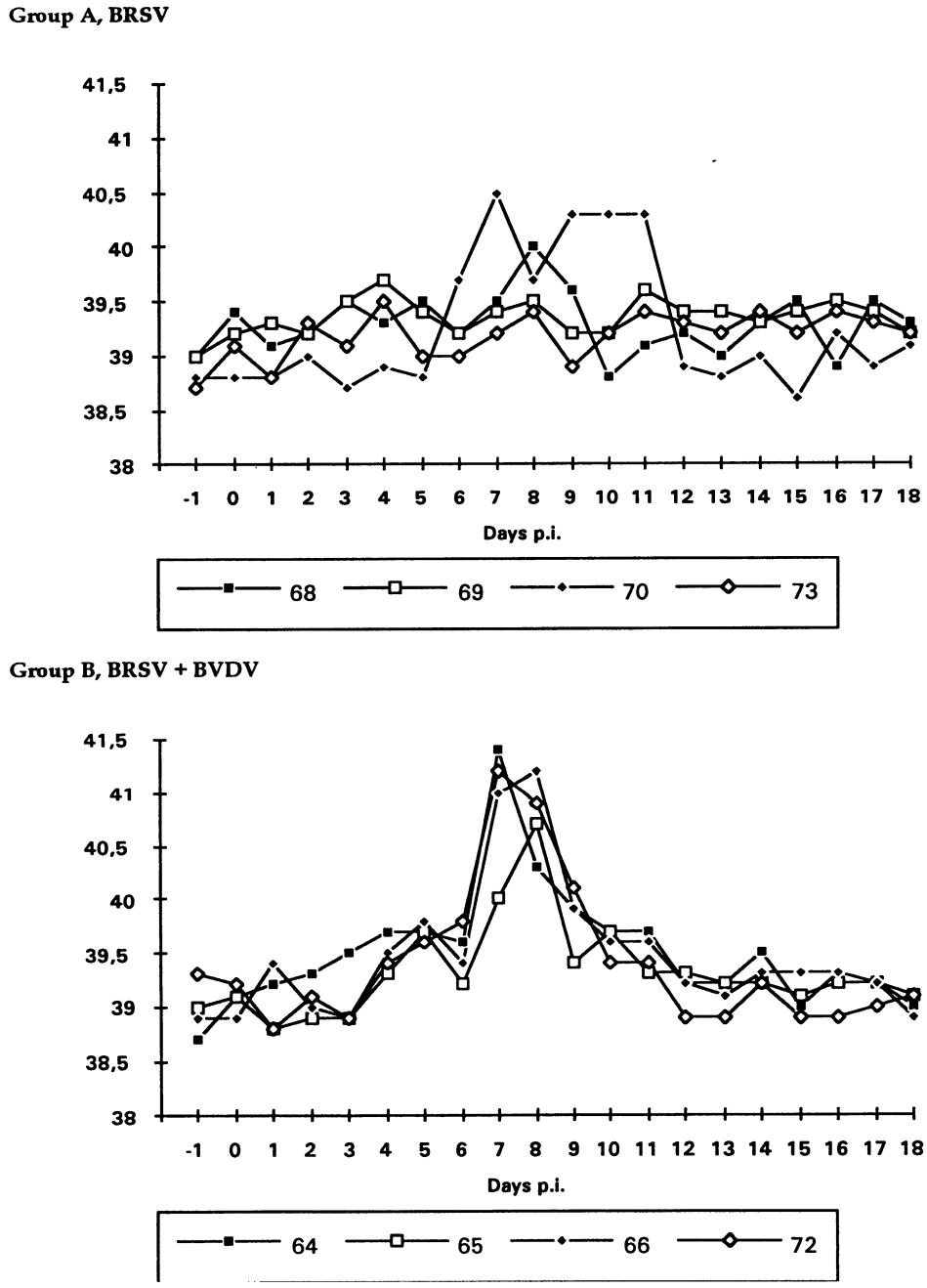

Figure 1. Individual body temperatures in group A and group B calves on day 1 prior to preinfection, on day of infection and on days 1-18 post infection. Temperatures >39.8 °C were regarded as fever.

Table 1a. Detection of bovine respiratory syncytial virus (BRSV) in nasal swab and lung lavage samples by PCR assays. The samples were collected on the day of infection and on 5 consecutive occasions.

	Calves No.	0 dpi[1]		2 dpi		4 dpi		7 dpi		11 dpi		14 dpi	
		N	Lu	N	Lu	N	Lu	N	Lu	N	Lu	N	Lu
A	68	–	–	+	–	+	+	+	+	–	–	–	–
	69	–	–	+	–	+	+	+	+	–	–	–	–
	70	–	–	–	–	+	–	–	+	–	–	–	–
	73	–	–	+	–	+	–	+	+	–	–	–	–
B	64	–	–	+	–	+	+	+	+	–	–	–	–
	65	–	–	+	–	+	–	+	+	–	+	–	–
	66	–	–	–	–	+	+	+	+	–	+	–	–
	72	–	–	+	–	+	–	+	+	–	+	–	–
C	67	–	–	–	–	–	–	–	–	–	+	–	+
	71	–	–	–	–	–	–	–	–	+	+	–	+
	74	–	–	–	–	–	–	–	–	+	+	–	+
	75	–	–	–	–	–	–	–	–	+	–	–	+

N nasal swabs, Lu Lunglavage, [1] dpi days post inoculation, + detection of BRSV by PCR, – no detection of BRSV by PCR.

Table 1b. Detection of bovine viral diarrhoea virus (BVDV) in white blood cells by PCR. The samples were collected on the day of infection and on 7 consecutive occasions.

	Calves No.	0 dpi[1] WBC	2 dpi WBC	4 dpi WBC	7 dpi WBC	9 dpi WBC	11 dpi WBC	14 dpi WBC	18 dpi WBC
B	64	–	–	+	+	+	+	+	–
	65	–	–	–	–	+	+	+	–
	66	–	–	–	–	–	+	+	–
	72	–	+	+	+	+	+	+	–

WBC white blood cells. [1] dpi days post inoculation, + Detection of BVDV by PCR, – no detection of BVDV by PCR.

B, BRSV was cultured from PCR positive nasal samples collected at 4 and 7 dpi and in group C from samples collected 11 and 14 days after the initiation of the experiment.

BVDV – PCR. BVDV was detected in purified lymphocytes of group B calves at 2 to 14 dpi. One calf (No. 72) remained positive for the whole of this period, while the virus was detected from 4 to 14 dpi in No. 64, from 9 to 14 dpi in No. 65 and from 11 to 14 dpi in No. 66 (Table 1b). All calves were negative at 18 dpi.

BVDV – Immunoperoxidase (IPX) assay. BVDV was detected in serum only from 2 of group B calves (Nos. 64 and 72) at 4 dpi and 4 to 7 dpi, respectively.

Serological findings

Group A. Antibodies to BRSV were first detected in one calf (No. 68) at 9 dpi, while 3 of the calves (Nos. 69, 70 and 73) became positive at 11 dpi (Fig 2). At 14 dpi all 4 calves showed high antibody titers to BRSV that remained high throughout the observation period (Table

2). All calves were negative for antibodies to BVDV during the whole period, i.e. to 25 dpi.

Group B. In 3 calves (Nos. 64, 65 and 66) antibodies to BRSV were first detected at 14 dpi. One calf (No. 72) did not become antibody positive until 21 dpi (Fig. 2). At 25 dpi 3 of the calves had lower titers to BRSV than the calves in groups A and C (data not shown). It should be noted, that the only calf in group B exhibiting a high antibody titer to BRSV at 25 dpi was calf No. 66 in which BVDV was detected by PCR in the lymphocytes for the shortest time period (at 11 and 14 dpi), and that calf No. 72, which did not become antibody positive until 21 dpi, was BVDV positive in lymphocytes during the whole time period between 2 to 14 dpi. The calves in this group became antibody positive to BVDV at 14 dpi (Nos. 64 and 66) and at 16 dpi (Nos. 65 and 72) .

Group C. All calves were antibody negative to BRSV at 14 dpi, at which time all group A calves had high titers and 3 group B calves had low titers (Table 2). Antibodies to BRSV was first recorded in one calf (No. 71) at 16 dpi and in 3 calves (Nos. 67, 74 and 75) at 18 dpi (data not shown). At 25 dpi, the BRSV titers were comparable to that of group A calves (data not shown). All group C calves were antibody negative to BVDV up to 25 dpi.

Serum interferon

None of the calves in groups A and C showed any increase in serum interferon levels at any time. Calves in group B showed positive interferon levels between 2 and 6 dpi, with a peak on day 4 (110 to 360 U/ml) (Fig 3).

Interferon in nasal secretions

Interferon was infrequently detected in nasal secretions. Levels of 184 and 147 U/ml were seen in calves Nos. 68 and 73 (group A) at 7 dpi, of 65 U/ml in calf No. 70 (group A) at 9 dpi, of 60 U/ml in calf No. 64 (group B) at 4 dpi and of 184 U/ml in calf No. 74 (group C) at 11 dpi.

Bacteriology

Nasal swabs: Very small numbers of bacteria, including *P multocida* and P *hemolytica* in mixed culture, were found in all calves on most sampling occasions.

Lung lavage material: The 3 first lavages after inoculation were all bacteriologically sterile, after which small numbers of a mixture of species were recorded. In calf No. 70, a pure culture of *P multocida* was demonstrated at 7 dpi.

Neither H *somnus* nor *Mycoplasma spp* was detected at any sampling of any animal.

Discussion

Experimental inoculation of BRSV in calves has invariably produced mild to moderate clinical symptoms of respiratory disease (*Thomas et al.* 1984, *Kimman et al.* 1987, *Ciszewski* 1991). This failure to provoke severe disease experimentally suggested that under natural conditions some undefined factor was predisposing or aggravating the course of disease. Our aim was to investigate whether concurrent BVDV infection could influence the outcome of an experimental BRSV infection administered by a single inoculation of virus via the respiratory tract. Infections with both viruses are commonly detected in calf rearing units. The BRSV isolates used in this experiment were obtained from natural cases of severe BRSV infections and inoculated at low passage levels.

All calves were negative for BRSV and BVDV antibodies at the beginning of the study. Four to 5 days post infection, all calves in both groups A and B showed symptoms of mild respiratory disease. In terms of clinical signs, no aggrava-

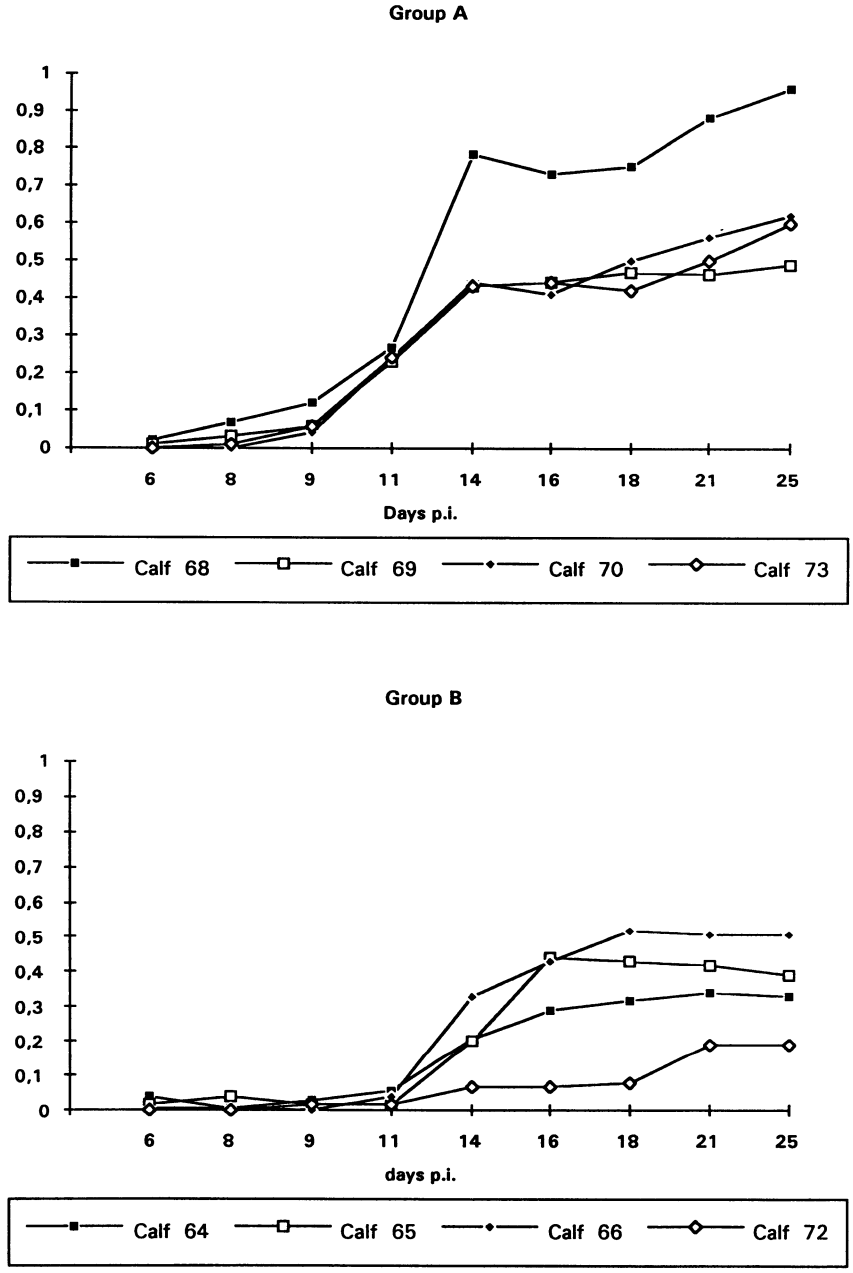

Figure 2. Detection of antibodies to bovine respiratory syncytial virus (BRSV) by ELISA in individual calves in experimental groups A and B. Antibody titers are expressed as optical density at 450 nm absorbtion (OD_{450}) in sera tested in 1:100 dilution.

Table 2. Serum samples collected at 14 days post infection were analyzed for the presence of antibodies to bovine respiratory syncytial virus (BRSV), Titers are expressed as the reciprocal value of the highest dilution giving an $OD_{450} > 0.1$.

Group	Calf No.	Titer
A: BRSV	68	1600
	69	1600
	70	1600
	73	1600
B: BRSV + BVDV	64	100
	65	100
	66	400
	72	<25
C: Control	67	<25
	71	<25
	74	<25
	75	<25

tion due to BVDV was noted for most variables monitored except rectal temperature. All group B calves exhibited high fever at 7 to 8 dpi, while only calves No. 70 and No. 68 in group A were febrile at 7 and 8 dpi respectively (Fig. 1). The only calf that showed severe signs of respiratory disease similar to that seen in natural cases was No. 70 in group A. This animal required treatment with penicillin at 11 dpi due to secondary bacterial infection involving *P multocida* and recovery followed rapidly after initiation of treatment.

Group B differed from group A in yielding BRSV. The virus was detected by PCR in lung lavage fluids up to 11 dpi compared with 7 dpi for group A (Table 1a).

Since PCR is a highly sensitive method for BRSV detection (*Vilček et al.* 1994), the animals were assumed to be non-infectious when viral RNA could no longer be detected in lung wash samples.

Antibodies to BRSV were detected at least 3 and up to 10 days later in group B calves compared with group A calves (Fig. 2). All group A calves had high antibody titres to BRSV at 14 dpi, whilst 3 group B calves exhibited only low to moderate titers at that time and one calf was still seronegative (Table 2). Antibodies to BVDV were detected at 14 to 16 dpi in group B, which is in accordance with the results of *Tråvén et al.* (1990). No BVDV could be detected by PCR in the lymphocytes 18 dpi when all 4 calves in this group were seropositive to this virus. The slower BRSV antibody response and the longer detection period of BRSV in lung samples by PCR from group B calves might be explained by the immunosuppressive effect of BVDV infection (*Potgieter* 1995). This suggestion is exemplified by calf No. 72, which was BVDV positive in lymphocytes by PCR during a longer time period (2 to 14 dpi) than the others of this group (Table 1b) and did not seroconvert to BRSV until 21 dpi (Fig. 2). In contrast to this calf No. 66, which showed the best BRSV antibody response, was only BVDV positive in lymphocytes at 11 and 14 dpi.

The calves of group C, which were sham-inoculated, developed mild clinical signs similar to those seen in groups A and B towards the end of the experiment. These signs were confirmed as due to accidental BRSV infection both by virus detection using PCR (Table 1a) and by seroconversion. Since the feeding and handling protocols, which stipulated group C always to be treated first, were strictly followed throughout, we assume that this adventitious BRSV infection was transmitted by aerosol. The mode of transmission could be due to a failure in the ventilation system, allowing the mixing of exhaust air with influx air. This possible route has been studied since and we believe this to be a likely explanation of the adventitious infection. However, there was no concomitant spread of BVDV. These observations suggest that BRSV, compared to BVDV, is more easily spread between herds by air or passively via humans, an

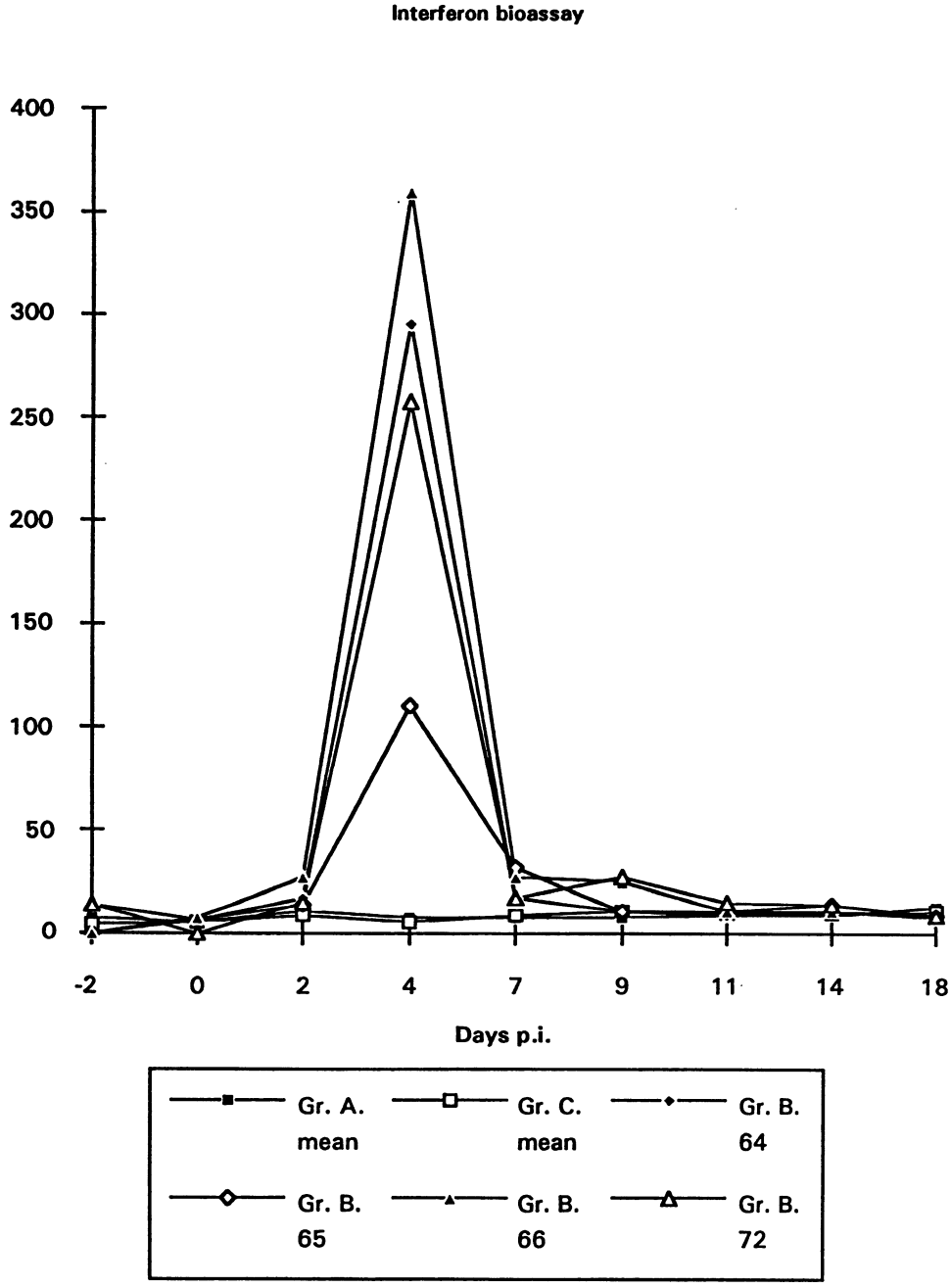

Figure 3. Interferon levels in serum samples expressed as the reciprocal of the mean end point dilution value (groups A and C) or as individual titers (group B).

observation that has also been made during natural outbreaks of infection (*Elvander & Alenius,* unpublished observations).

Serum interferon levels were increased in the mixed BRSV and BVDV infection group but not in the group receiving BRSV alone, suggesting that the BVDV infection was responsible for inducing this rise. The finding of peak interferon titers at 4 dpi is in accordance with similar results obtained in experimental BVDV infection by *Tråvén et al.* (1990).

The clinical signs recorded in the experimentally infected calves correlates with the observations made by *Thomas et al.* (1984). In spite of concurrent infections involving 2 viruses, only mild respiratory signs were seen in the majority of the calves. This suggests that other parameters such as stress and other environmental influences probably play an important role in the clinical outcome of a respiratory infection. Secondary bacterial infection is important, as demonstrated by calf No. 70, the only animal to show severe respiratory symptoms in this trial. In Sweden, it is generally animals older than 6 months that exhibit severe respiratory disease due to primary BRSV infections (*Elvander* 1996). Further studies involving the experimental infection of adult cattle are indicated to investigate this apparent difference between cows and calves in susceptibility to BRSV. Concurrent infection with BRSV and BVDV did not result in more severe respiratory disease than BRSV infection alone, but an increased duration of BRSV excretion and a depressed antibody response to BRSV were noted. The difference in temperature responses between groups A and B was probably caused by the BVDV infection since the temperature rise seen in group B corresponds with the fever period seen in an experimental BVDV infection (*Tråvén et al.* 1990).

Two possible reasons why the calves inoculated with mixed cultures of BRSV and BVDV seroconverted later to BRSV than those receiving BRSV alone are that the induction of serum interferon response by BVDV depresses BRSV replication, or that the BVDV infection caused a delayed immunresponse to BRSV due to its immunosuppressive ability. The time lag before an antibody response was induced and the recorded titers in the dual infected group, indicated that the duration of BVDV infection in lymphocytes negatively influenced the capacity to mount a BRSV antibody response.

In conclusion, BRSV did not elicit a serum interferon response although BVDV did; BRSV appeared to spread by air (adventitiously) more easily than BVDV under identical conditions, and concurrent BRSV and BVDV infection seemed to delay the BRSV antibody response, increase the time of BRSV excretion, and thus increase duration of BRSV dissemination. The observation that the experimental double infection with BRSV and BVDV did not result in a more severe disease does not exclude the possibility that aggravated signs might occur under natural conditions.

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Sammanfattning

Experimentell studie av en samtida primärinfektion med bovint respiratoriskt syncytialt virus (BRSV) och bovint virus diarré virus (BVDV) hos kalv.

En experimentell infektionsstudie genomfördes i syfte att klarlägga effekten av en samtida infektion med BRSV och BVDV. Tolv kalvar, i 3 grupper om 4 djur, inokulerades vid ett och samma tillfälle med ett av följande: enbart BRSV (Grupp A), BRSV tillsammans med BVDV (Grupp B) eller sterilt medium (Grupp C).

Samtliga kalvar i Grupp A och B visade milda respiratoriska symtom 4 till 5 dagar efter infektionstillfället (dpi). En kalv i Grupp A insjuknade i en sekundär Pasturellainfektion, varvid penicillinbehandling sattes in. De kalvar som fick både BRSV och BVDV (Grupp B) hade en mer uttalad feber (40,7-41,4 °C) 7 till 8 dpi än kalvar som fått BRSV enbart (Grupp A). Endast hos kalvar i Grupp B kunde BVDV påvisats i lymfocyter 2 till 14 dpi, och endast denna grupp visade positiva seruminterferon nivåer 4 dpi.

BRSV påvisades i lungsköljprov från Grupp A kalvar upp till 7 dpi, vilket kan jämföras med 11 dpi för Grupp B kalvar. Dessa serokonverterade även senare och visade inte lika höga antikroppsnivåer som Grupp A. Det fördröjda antikroppssvaret och den lägre antikroppsnivån hos kalvar som fått både BRSV och BVDV indikerar att individens förmåga att bilda antikroppar samt eliminera virus påverkas negativt av en pågående BVDV infektion.

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