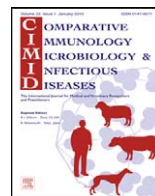




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Acute phase protein changes in calves during an outbreak of respiratory disease caused by bovine respiratory syncytial virus

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ABSTRACT

Bovine acute phase proteins (APPs), lipopolysaccharide binding protein (LBP), serum amyloid A (SAA), haptoglobin (Hp) and alpha₁-acid glycoprotein (AGP) were evaluated as inflammatory markers during an outbreak of bovine respiratory disease (BRD) caused by bovine respiratory syncytial virus (BRSV). Calves ($n = 10$) presented mild to moderate signs of respiratory disease. Secondary bacterial infections, *Pasteurella multocida* and *Mycoplasma dispar* as major species, were detected in tracheobronchial lavage samples. Concentrations of SAA and LBP increased at week 1 had the highest values at week 3 and decreased at week 4 of outbreak. Some calves had high Hp concentrations at week 3, but AGP concentrations did not rise during respiratory disease. Higher SAA, LBP and Hp concentrations at a later stage of BRD (week 3) were associated with the low BRSV-specific IgG₁ production, suggesting that these calves had enhanced inflammatory response to the secondary bacterial infection. In conclusion, APPs (especially SAA and LBP) are sensitive markers of respiratory infection, and they may be useful to explore host response to the respiratory infections in clinical research.

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1. Introduction

The early protection mechanism of the host against infection, trauma or other tissue damage comprises a set of reactions known as the acute phase response (APR). One of its main features is hepatic production of acute phase proteins (APPs) [1]. Determination of APPs provides a tool for evaluating inflammatory response of the host during infections. Bovine respiratory disease (BRD) is one of the most important causes of morbidity and mortality in beef

and dairy calves. Respiratory disease is a multifactorial disease, caused by a variety of aetiological agents. Environmental and husbandry factors and impaired resistance of predisposed calves to the infections are involved. APPs can potentially be used to investigate the complex pathogenesis of BRD and to evaluate the role of aetiological factors. However, more information about the synthesis pattern of APPs during natural BRD infections of calves is needed.

Studies on experimental infections have shown that some APPs have good properties as markers of respiratory infections in calves after viral [2–4], bacterial [3,5,6] or combined challenge [3,7]. However, only limited data are available on APPs as disease markers of spontaneous BRD. These studies have mainly been conducted to evaluate the potential of APPs as individual or herd health diagnostic

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tools in veterinary practice. Wittum et al. [8] and Young et al. [9] found that bovine APP haptoglobin (Hp) had limited capacity as a diagnostic clinical tool for BRD in feedlot cattle. Later, Hp was considered useful for identifying beef calves with BRD needing treatment and for monitoring treatment efficacy [10–12], whereas serum amyloid A (SAA) and alpha₁-acid glycoprotein (AGP) was not found to be a useful marker of BRD in feedlot calves [10,11].

Bovine respiratory syncytial virus (BRSV) has been shown to be a primary infectious agent causing mild to severe clinical respiratory disease [13]. BRSV infection is commonly accompanied by bacterial infection [14]. Thus, an outbreak of pneumonia caused by BRSV gives a good opportunity to characterize time related patterns of different APPs and to evaluate them as inflammatory markers during a naturally occurring respiratory infection.

The objective of this study was to investigate changes of four bovine APPs, SAA, lipopolysaccharide binding protein (LBP), Hp and AGP during an outbreak of BRD in young dairy calves. In addition, calves were retrospectively divided into two groups based on BRSV-specific IgG₁ production (high and low), and different patterns of APP concentration changes were compared to explore host response differences between these groups.

2. Materials and methods

2.1. Animals, sampling procedure and clinical examination

A group of 10 Holstein Friesian calves (7 males, 3 females) from the Helsinki University Suintia Research Farm was followed weekly over a 6-week period starting 1 week (week 0) before the manifestation of the first clinical signs of BRD. The ages of the calves at week 0 are presented in Table 1. These calves were initially included in the study to investigate the effects of age on the physiological concentrations of APPs and were blood-sampled weekly without any other manipulation. Calves were housed in two group fences (calves nos. 1–5 in one and the rest in the other) with an automatic milk feeding system (one nipple

for each fence) with milk powder and free access to water, silage and hay. After weaning (approximately at the age of 8 weeks), calves were moved into a group fence with older calves. The first calves were weaned and moved between sampling weeks 4 and 5, after disappearance of the clinical BRD signs. All fences were located in close proximity to each other. The experiment was approved by the local ethics committee for animal experiments.

Blood samples were taken weekly from the jugular vein into plain glass tubes; serum was separated by centrifugation, frozen in portions and stored at –20 °C to await further analysis. Calves were clinically examined in conjunction with each blood sampling. Overall clinical score was calculated according to Hägglund et al. [15] as follows: rectal temperature (°C; 0 = < 39.0, 1 = 39.0–39.5, 2 = 39.6–39.9, 3 = 40.0–40.4, 4 = >40.5), respiratory rate (min⁻¹; 0 = < 40, 1 = 40–49, 2 = 50–59, 3 = 60–69, 4 = 70–79, 5 = >79), nasal discharge (0 = normal, 1 = serous, 2 = mucopurulent or purulent), lung auscultation (0 = no abnormal sound, 1 = wheezing sounds), cough (0 = no cough, 1 = occasional sporadic cough, 2 = more than one spontaneous cough) and demeanour (0 = bright, 1 = mildly depressed, 2 = moderately or severely depressed).

2.2. Analysis of tracheobronchial lavage samples

Tracheobronchial lavage (TBL) samples were taken by the double catheter method by Bengtsson et al. [16] at week 2 and at the end of the experimental period (week 6). The calf was restrained by assistants and a sterile plastic double catheter was inserted through the ventral nose duct into the trachea. The inner catheter was then pushed through the silicone plug of the outer catheter as far as possible into the lung. Isotonic sterile saline solution (30 ml) was injected into the catheter and aspirated immediately. TBL samples of 0.5 ml were transferred into mycoplasma D media [17], and the rest of the sample was used for bacterial examination. TBL samples from three calves at week 6 could not be investigated because of difficulties in performing the lavage procedure. TBL samples were examined for bacterial and mycoplasma

Table 1

Age of calves, bovine respiratory syncytial virus (BRSV) antibody response group, peak clinical score, presence of BRSV and bacterial growth in tracheobronchial lavage (TBL) samples.

Calf no.	Age of calves at week 0 (days)	BRSV antibody response group	BRSV in TBL at week 2	Peak clinical score (week)	Bacterial growth in TBL sample at week 2	Bacterial growth in TBL sample at week 6
1	32	Low	Neg	7 (3)	Pm ^a , Md ^b , Mb ^c	Pm, Md, Mb
2	30	High	Neg	4 (2; 4)	Pm, Md, Mb	Pm, Md
3	27	High	Pos	7 (2)	Pm, Md	Not investigated
4	24	High	Neg	6 (4)	Pm, Psp ^d , Ss ^e , Md, Mb	Md
5	23	Low	Pos	8 (2; 3)	Pm, Fsp ^f , Md, Mb	Pm, Md, Mb
6	21	Low	Pos	4 (3)	Pm, Ss, Ap ^g , Fsp, Md, Mb	Not investigated
7	18	High	Pos	4 (3; 4)	Pm, Md, Mb	Pm, Md, Mb
8	14	Low	Neg	3 (2)	Ss, Md, Mb	Pm, Md, Mb
9	13	Low	Neg	4 (2)	Pm, Mb	Not investigated
10	9	High	Pos	5 (2)	Pm, Fsp, Md	Pm, Md, Mb

^a *Pasteurella multocida*.

^b *Mycoplasma dispar*.

^c *Mycoplasma bovirhinis*.

^d *Pasteurella* sp.

^e *Streptococcus suis*.

^f *Fusobacterium* sp.

^g *Arcanobacterium pyogenes*.

growth as described by Autio et al. [18]. The nested RT-PCR method described by Vilcek et al. [19] was used for detection of BRSV and the RT-PCR method [20] for detection of bovine corona virus (BCV).

2.3. Serum analysis of viral antibodies and acute phase proteins

ELISA kits (SVANOVA Biotech, Uppsala, Sweden) were used to detect IgG₁ antibodies specific to BRSV, parainfluenza virus 3 (PIV-3) and BCV from serum samples according to the manufacturer's instructions. Samples that generated a corrected optical density (COD) value of ≥ 0.2 at 450 nm were regarded as positive. Calves were retrospectively divided into two BRSV IgG₁ antibody response groups based on serum antibody detection: the low-response group with no detectable BRSV IgG₁ before week 4 (serum COD value under the cut-off point) and a COD value < 1.5 at week 6 ($n = 5$) and the high-response group with BRSV IgG₁ response before week 4 and a COD value > 1.5 at week 6 ($n = 5$; Fig. 1).

Serum Hp was determined using the haemoglobin-binding assay described by Makimura and Suzuki [21] with

the modification that tetramethylbenzidine (0.06 mg/ml) was used as a chromogen [22]. Serial dilutions of pooled bovine acute phase serum (lyophilized and stored in aliquots) were used as a standard. The assay was calibrated using a sample with a known Hp concentration provided by a European Union concerted action on standardization of animal APPs (number QLK5-CT-1999-0153). Haemolysis was not visible in any of the samples. SAA was measured with a commercially available ELISA kit (Phase SAA Assay, Tridelta Development Ltd., Maynooth, Co., Kildare, Ireland), according to the manufacturer's instructions. For measuring serum LBP, a commercial multi-species (including cattle) ELISA kit was used (LBP ELISA for various species, Hycult Biotechnology, Uden, The Netherlands). Serum AGP was analysed using a commercial radial immunodiffusion kit for cattle (Bovine AGP, Tridelta Development Ltd., Maynooth, Co., Kildare, Ireland).

2.4. Pulsed-field gel electrophoresis of *Pasteurella multocida* isolates

P. multocida isolates were grown overnight at 37 °C in a brain-heart infusion broth (Difco, Sparks, MD, USA). Cells (0.9 ml) were harvested by centrifugation and washed with 2 ml of PIV buffer (10 mM Tris-HCl, pH 7.5, 1 M NaCl). Cells were resuspended in 0.25 ml of PIV and mixed with an equal volume of 1.8% InCert agarose (Cambrex, Rockland, ME, USA) and then dispensed into plug moulds (Bio-Rad, Richmond, CA, USA). Bacteria in plugs were lysed as described previously [23]. After lysis, plugs were washed in TEN buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, pH 8.0, 50 mM NaCl), and slices of plugs were digested with 10 U Sall restriction enzyme (Roche, Mannheim, Germany). Fragments were separated in 1% SeaKem Gold agarose (Cambrex, Rockland, ME, USA) in $0.5 \times$ TBE by using a CHEF-DR III system (Bio-Rad, Richmond, CA, USA). Ramp time was 1–10 s for 17 h at 6 V/cm, and the temperature was 12 °C. *Salmonella* Braenderup H9812 (XbaI-digested) was used as a molecular marker. Restriction fragment patterns of the isolates were compared visually.

2.5. Statistical analysis

A linear random-intercept model was used for exploring time changes in serum concentrations of APPs during the respiratory disease outbreak. APP changes in all calves during the observation period and differences between BRSV antibody response groups were analysed using contrasts where every time point was compared with the previous time point. Age of calves at the beginning of the observation period and treatment of two calves at week 3 were included as predictors in all models. First-order autoregressive correlation structure (AR1) was used for modelling serial correlations of repeated measurements within calves. Plots of empirical autocorrelation function of the normalized residuals were used to explore adequacy of the AR1 correlation matrix. Model assumptions were verified by scatter and normality plots of standardized residuals, and logarithmic transformation of SAA, LBP and AGP and reciprocal transformation of Hp were used. Age differences between BRSV antibody

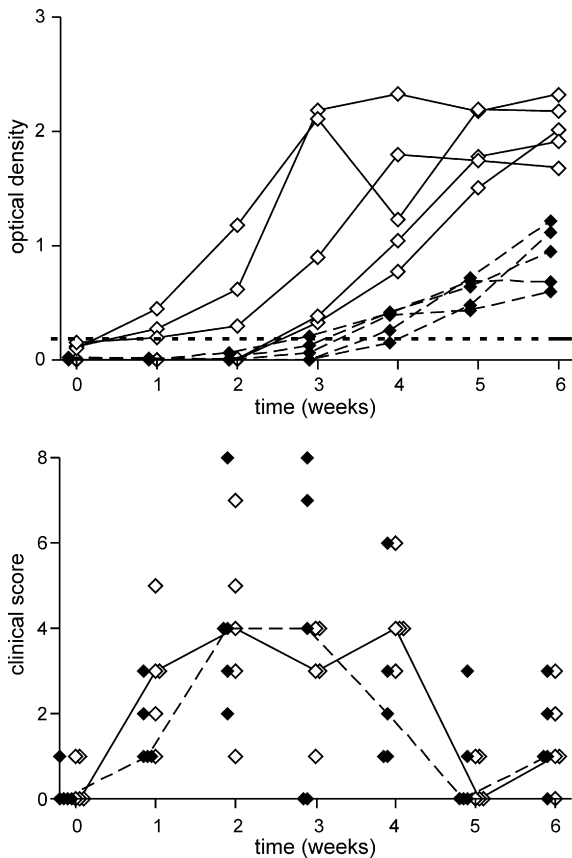


Fig. 1. Bovine respiratory syncytial virus (BRSV) serum IgG₁ corrected optical density values of 10 calves (above) and clinical score (below) of low IgG₁ response (---◆---; $n = 5$) and high IgG₁ response (-◇-; $n = 5$) groups of calves over a 6-week period. Horizontal dotted line represents cut-off value of assay (0.2; optical density figure). Lines connect median values of IgG₁ response groups by weeks (clinical score figure). Statistical differences are given in the text.

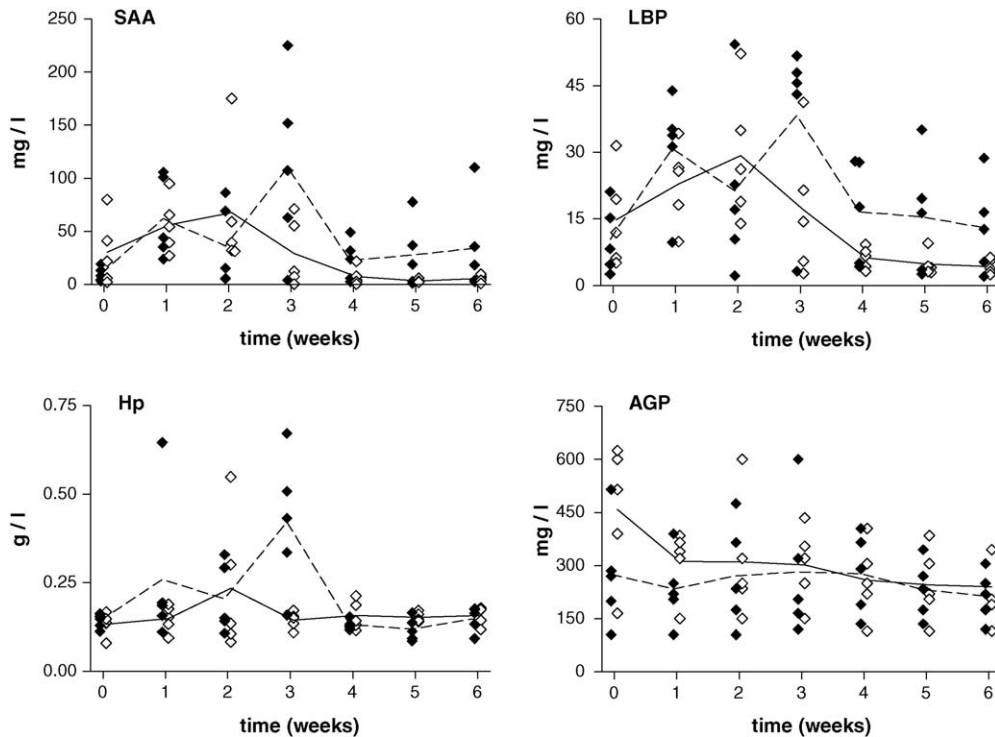


Fig. 2. Serum concentrations of serum amyloid A (SAA), lipopolysaccharide binding protein (LBP), haptoglobin (Hp) and alpha₁-acid glycoprotein (AGP) of low bovine respiratory syncytial virus (BRSV) IgG₁ response (—◆—; $n = 5$) and high BRSV IgG₁ response (—◇—; $n = 5$) groups of calves over a 6-week period. Lines connect mean concentrations of each APP in different IgG₁ response groups by weeks. Statistical differences are given in the text.

response groups at week 0 were analysed using a two-sample Wilcoxon signed-rank test. Statistical analyses were performed using nlme package [24] with R version 2.3.1 [25].

Changes between time points in clinical scores of all calves and differences between BRSV antibody response groups were analysed using a generalized linear mixed model (GLMM) fitted by the GLIMMIX procedure (pdf document “The GLIMMIX Procedure, June 2006” <http://www.sas.com/statistics/doc.html>) with SAS/STAT 9.1 (SAS Institute Inc., Cary, NC, USA) software. Poisson distribution was used for a response variable (clinical score). Calf was included as a random effect, and AR1 correlation structure was used for modelling serial correlations of repeated measurements.

3. Results

Calves presented no signs of respiratory disease or any other disease at week 0 (three calves had a clinical score of 1 at week 0 because of rectal temperature of 39.0–39.5 °C). All calves showed signs of BRD starting at week 1 (Fig. 1). Clinical disease became more pronounced at weeks 2, 3 and 4 (Table 1). Clinical scores of all calves increased from week 0 to 1 ($p < 0.001$) and from week 1 to 2 ($p < 0.01$). Clinical scores decrease from weeks 4 to 5 ($p < 0.001$). No differences were seen in patterns of clinical signs between BRSV antibody response groups. Overall, only mild to moderate clinical respiratory disease was recorded (maximal score 8). Two calves were treated with antibiotics (5

days) based on the clinical evaluation at week 3 (calves nos. 1 and 5, clinical scores of 7 and 8, respectively) (Table 1). These calves had also relatively high Hp values at that time (0.43 and 0.67 g/l, respectively) (Fig. 2).

All calves were seronegative to BRSV at week 0. By week 6, all calves had become seropositive (COD values from 0.6 to 2.3) (Fig. 1). Calves were seronegative to BCV throughout the observation period. Antibodies to the PIV-3 virus were present in all calves at week 0, and these gradually decreased (data not shown).

BRSV was found in five calves in the 2-week TBL samples (Table 1) but not in 6-week TBL samples. No BCV was found in any of the TBL samples investigated.

P. multocida was isolated from nine and six calves in the first and second samplings, respectively, and at least once from every calf (Table 1). The only calf with no *P. multocida* infection at week 2 had an increase of SAA and LBP concentrations from week 0 to 1 (from 13.4 to 23.8 mg/l and from 2.6 to 9.8 mg/l respectively). No change was seen after that (range of 2.7–5.2 mg/l and 2.0–4.3 mg/l in the subsequent samples, respectively) and no Hp response at all. That calf had also the lowest peak of clinical score (Table 1).

All available *P. multocida* isolates ($n = 14$ since isolate of calf no. 3 was not stored for further analysis) had an identical Sall restriction pattern (data not shown). *Mycoplasma dispar* was found in all TBL samples except for one (calf no. 9 at week 2). More bacterial species were isolated in the middle of the BRD outbreak (week 2) than at later stage (week 6) (Table 1).

Mean SAA and LBP concentrations of all calves increased between weeks 0 and 1 ($p < 0.001$) and then decreased between weeks 3 and 4 ($p < 0.01$). Mean Hp concentrations of calves decreased between weeks 3 and 4 ($p < 0.05$) and mean AGP concentrations decreased between weeks 0 and 1 ($p < 0.05$).

There were no differences in initial APP concentrations or age between BRSV antibody response groups before the outbreak of BRD. In general, APP patterns changed differently in the antibody response groups. Calves with low antibody response had more elevated concentrations at week 3 (Fig. 2). Mean SAA concentration increased in the low antibody group between weeks 2 and 3 ($p < 0.001$) compared with the high antibody group, where the mean concentration decreased. Mean LBP concentrations decreased between weeks 1 and 2 ($p < 0.05$) and increased between weeks 2 and 3 ($p < 0.001$) in low antibody group compared with the high-response group, where the mean concentrations increased and decreased, respectively. SAA and LBP concentrations of the same three calves from the low antibody group remained elevated to the end of the study (weeks 4, 5 and 6) (Fig. 2). Similar differences in changing patterns between antibody response groups were seen also in Hp concentrations. In the low antibody group Hp concentrations increased between weeks 2 and 3 ($p < 0.05$) and decreased between weeks 3 and 4 ($p < 0.01$) compared with the high-response group, where no other clear changes occurred except two high values at week 2 (Fig. 2).

4. Discussion

Changes of APP concentrations in this study will give a holistic view of possible time sequences and host–pathogen interactions during a spontaneous BRD outbreak in dairy calves. The initial cause of the enzootic pneumonia outbreak was most probably BRSV. None of the calves had maternal antibodies against BRSV before the outbreak, as IgG₁ antibodies are transferred via colostrum [26]. All calves developed a marked increase in antibodies by the end of the study period. BRSV was found in TBL samples of half of the calves at week 2, indicating a key role of the virus infection in the outbreak. Calves were exposed to the BRS virus probably around the time that the first samples were obtained. Four days before the week 0 sampling, one older calf was brought back from the veterinary clinic after receiving operation on an umbilical hernia. This calf showed clinical signs of BRD on arrival and was treated with antibiotics.

Respiratory disease caused elevation of APPs in the majority of calves. Increased values of sensitive APPs (SAA and LBP) on weeks 1 and 3, and the highest concentrations of Hp on week 3 also emphasize the two-stage nature of the inflammatory response. The first inflammatory reaction, reflected as an increase in SAA and LBP concentrations at week 1, is probably a response to the BRS virus. The subsequent more pronounced elevation of SAA, LBP and especially Hp is likely a sign of a secondary bacterial infection. Heegaard et al. [2] reported a biphasic response of SAA and Hp after BRSV challenge in one calf, possibly caused by secondary infection with *Pasteurella avium*,

which was found at necropsy. In the same study, high SAA responder calves to the virus challenge had a subsequent Hp rise, but medium and low SAA responders did not show a Hp response. This suggests that Hp response requires more profound tissue damage, which is more likely to occur in bacterial infection. This is supported also by a field study of Humblet et al. [12], where Hp was found to be a good marker to detect calves with BRD that requiring antibacterial and anti-inflammatory treatment. In our study, the two calves treated with antibiotics at week 3 both had higher Hp concentrations at that time. A synergistic effect of BRSV and bacteria was observed in the present study. There were more bacterial species involved in microbial growth in TBL samples taken around the middle of the outbreak than in samples taken after clinical manifestation (Table 1). *P. multocida* was most likely responsible for the more enhanced responses of APPs in the later stages of BRD. The only calf that had no *P. multocida* infection at week 2 had a minor increase of SAA and LBP concentrations (at week 1), no increase of Hp concentrations and the lowest clinical response (at week 2). In our previous study [27], *P. multocida* infection was related to high concentrations of all APPs investigated (SAA, Hp, LBP, AGP, fibrinogen) in calves with BRD.

Knowledge about the genetic diversity of *P. multocida* strains in bovine respiratory infections is very limited. All calves in this study were infected with *P. multocida* at week 2 or week 6, and at least five on both occasions. We genotyped all isolates with Sall restriction analysis, which efficiently differentiates between *P. multocida* strains from the bovine respiratory tract (T. Pohjanvirta, Personal communication). All isolates had an identical Sall banding pattern, indicating that one infective strain had persisted throughout the study. Such predominant strains have been described previously and suggested to have increased capacity to cause disease [28]. *P. multocida* infection after clinical disease at week 6 seems to have little effect on the inflammatory response, supporting the findings of Allen et al. [29]. They reported recolonization of *P. multocida* in the lower respiratory tract of calves suffering from BRD after treatment with antibiotics.

Interestingly, a more pronounced APP response, which was seen in later stages of the BRD outbreak, was connected with calves' lower specific IgG₁ response to the initial BRSV infection. Calves with marked antibody production had lower concentration of APPs at week 3 and no APP response after clinical BRD. These differences in APP response patterns could be explained by different onset of infection. However, calves from both groups had similar SAA, LBP and clinical responses at week 1, and BRS virus findings at week 2 were comparable (2 from low-response and 3 from high-response groups; Table 1). There was a similar distribution of antibody response groups calves between the two fences, and one nipple of the automatic feeder in the fence possibly assisted the quick spread of the virus. Because this was not a controlled infection, we cannot confirm that all calves received the virus infection at a similar dosage and at the same time, but our results suggest that the initial onset of infection probably happened over a relatively short timeframe. Also, comparable bacterial growth was

detected in the calves of both groups (Table 1); thus, the triggering agents for secondary infection were available. Neither immaturity of the immune system of younger calves (similar age distribution in groups) nor the presence of maternal antibodies against BRSV (no detectable IgG₁ at week 0) can explain this. One possible explanation is that calves with lower humoral immune response to the BRSV were unable to efficiently remove virus despite inflammatory response and were more susceptible to subsequent secondary bacterial infections. Calves with good antibody production, by contrast, were more efficiently able to mount a protective inflammatory response to clear the virus and prevent secondary bacterial infections or the viral antibodies were able to protect the respiratory epithelium against bacterial adherence. The mechanisms by which viruses can alter host susceptibility to a secondary bacterial infection by altering environmental conditions and suppressing the respiratory defence systems are numerous and complex [30]. One of these mechanisms is enhanced adherence of bacteria to virus-infected respiratory cells due to viral glycoprotein expression. Bartelt and Duncan [31] suggested that a protein receptor is needed for adherence of bacteria because treatment of cells with trypsin reduced bacteria binding. Glycoprotein G has been found to increase *Neisseria meningitidis* binding in RSV-infected human epithelial cells [32], and a weak antibody response to RSV infection has been noted in children because of their low production of protective antibodies against glycoproteins [33]. A study in which antibodies to human influenza A virus protected cells from streptococcal adherence [34] further supports the hypothesis that early and sufficient production of antibodies to the virus may have protected calves from exacerbated bacterial infection. However, T-cell responses [35] along with the production of specific IgG₂, IgA and virus-neutralizing antibodies [36] also have important roles in immune response to BRSV infection, and we cannot argue solely in favour of specific IgG₁ production since we have not measured other immune functions.

The present study shows that SAA and LBP are sensitive markers of respiratory infection. In our previous study, where we used calves from the same farm in exactly the same conditions, we show how age affects concentrations of APPs [37]. Generally, concentrations of SAA, LBP and AGP were at the highest during the first 2 weeks of life, decreased afterwards and then stabilized around at 3–4 weeks. This age effect explains the high values of SAA, LBP and AGP at week 0 and the decreasing change in concentrations of AGP between week 0 and 1. SAA and LBP concentrations increased in the same time, indicating the presence of inflammatory stimulus and not the effect of age. We also controlled age along with treatment of two calves in our statistical models, to avoid possible confounder effect.

In conclusion, our results indicate that APPs can be used as a tool in clinical research for studying different aspects of host–pathogen interaction in respiratory diseases of calves under field conditions. They are more sensitive markers than evaluation of clinical response, especially when clinical disease is not very severe, as in this study. An

early increase in response to viral infection and a marked rise in the secondary bacterial infection even when only mild or moderate clinical signs of BRD are present, make SAA and LBP the best candidates for this purpose. In the same time, parallel measuring of the Hp response can be valuable to improve the evaluation of the severity of inflammation.

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References

- [1] Baumann H, Gauldie J. The acute phase response. *Immunol Today* 1994;15:74–80.
- [2] Heegaard PM, Godson DL, Toussaint MJ, Toornehooj K, Larsen LE, Viuff B, et al. The acute phase response of haptoglobin and serum amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. *Vet Immunol Immunopathol* 2000;77:151–9.
- [3] Gånheim C, Hulten C, Carlsson U, Kindahl H, Niskanen R, Persson Waller K. The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or *Mannheimia haemolytica*. *J Vet Med B Infect Dis Vet Public Health* 2003;50:183–90.
- [4] Grell SN, Tjørnehoj K, Larsen LE, Heegaard PM. Marked induction of IL-6, haptoglobin and IFN γ following experimental BRSV infection in young calves. *Vet Immunol Immunopathol* 2005;103:235–45.
- [5] Schroedl W, Fuerll B, Reinhold P, Krueger M, Schuett C. A novel acute phase marker in cattle: lipopolysaccharide binding protein (LBP). *J Endotox Res* 2001;7:49–52.
- [6] Dowling A, Hodgson JC, Dagleish MP, Eckersall PD, Sales J. Pathophysiological and immune cell responses in calves prior to and following lung challenge with formalin-killed *Pasteurella multocida* biotype A:3 and protection studies involving subsequent homologous live challenge. *Vet Immunol Immunopathol* 2004;100:197–207.
- [7] Godson DL, Campos M, Attah-Poku SK, Redmond MJ, Cordeiro DM, Sethi MS, et al. Serum haptoglobin as an indicator of the acute phase response in bovine respiratory disease. *Vet Immunol Immunopathol* 1996;51:277–92.
- [8] Wittum TE, Young CR, Stanker LH, Griffin DD, Perino LJ, Littledike ET. Haptoglobin response to clinical respiratory tract disease in feedlot cattle. *Am J Vet Res* 1996;57:646–9.
- [9] Young CR, Wittum TE, Stanker LH, Perino LJ, Griffin DD, Littledike ET. Serum haptoglobin concentrations in a population of feedlot cattle. *Am J Vet Res* 1996;57:138–41.
- [10] Carter JN, Meredith GL, Montelongo M, Gill DR, Krehbiel CR, Payton ME, et al. Relationship of vitamin E supplementation and antimicrobial treatment with acute-phase protein responses in cattle affected by naturally acquired respiratory tract disease. *Am J Vet Res* 2002;63:1111–7.
- [11] Berry BA, Confer AW, Krehbiel CR, Gill DR, Smith RA, Montelongo M. Effects of dietary energy and starch concentrations for newly received feedlot calves. II. Acute-phase protein response. *J Anim Sci* 2004;82:845–50.
- [12] Humblet MF, Coghe J, Lekeux P, Godeau JM. Acute phase proteins assessment for an early selection of treatments in growing calves suffering from bronchopneumonia under field conditions. *Res Vet Sci* 2004;77:41–7.
- [13] Larsen LE. Bovine respiratory syncytial virus (BRSV): a review. *Acta Vet Scand* 2000;41:1–24.
- [14] Larsen LE, Tjørnehoj K, Viuff B, Jensen NE, Uttenenthal A. Diagnosis of enzootic pneumonia in Danish cattle: reverse transcription-polymerase chain reaction assay for detection of bovine respiratory syncytial virus in naturally and experimentally infected cattle. *J Vet Diagn Invest* 1999;11:416–22.
- [15] Hägglund S, Hu KF, Larsen LE, Hakhverdyan M, Valarcher JF, Taylor G, et al. Bovine respiratory syncytial virus ISCOMs—protection in the presence of maternal antibodies. *Vaccine* 2004;23:646–55.

- [16] Bengtsson B, Franklin A, Gånheim C, Jacobson S-O, Persson M, Virling S. A simple field method for tracheo-bronchial lavage in calves. In: Proceedings of the 20th World Buiatrics Congress; 1998.p. 1127.
- [17] Friis NF, Krogh HV. Isolation of mycoplasmas from Danish cattle. Nord Vet Med 1983;35:74–81.
- [18] Autio T, Pohjanvirta T, Holopainen R, Rikula U, Pentikäinen J, Huovilainen A, et al. Etiology of respiratory disease in non-vaccinated, non-medicated calves in rearing herds. Vet Microbiol 2007;119: 256–65.
- [19] Vilcek S, Elvander M, Ballagi-Pordany A, Belak S. Development of nested PCR assays for detection of bovine respiratory syncytial virus in clinical samples. J Clin Microbiol 1994;32:2225–31.
- [20] Tsunemitsu H, Smith DR, Saif LJ. Experimental inoculation of adult dairy cows with bovine coronavirus and detection of coronavirus in feces by RT-PCR. Arch Virol 1999;144:167–75.
- [21] Makimura S, Suzuki N. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory diseases. J Vet Med Sci 1982;44:15–21.
- [22] Alsemgeest SPM, Kalsbeek HC, Wensing T, Koeman JP, van Ederen AM, Gruys E. Concentrations of serum amyloid-A (SAA) and haptoglobin (Hp) as parameters of inflammatory diseases in cattle. Vet Q 1994;16:21–3.
- [23] Cameron DN, Khambaty FM, Wachsmuth IK, Tauxe RV, Barrett TJ. Molecular characterization of *Vibrio cholerae* O1 strains by pulsed-field gel electrophoresis. J Clin Microbiol 1994;32:1685–90.
- [24] Pinheiro J, Bates D, DebRoy S, Sarkar D. nlme: linear and nonlinear mixed effects models. R package version 3.1-73; 2006. URL <http://www.R-project.org>.
- [25] R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; 2006. ISBN 3-900051-07-0, <http://www.R-project.org>.
- [26] Kimman TG, Westenbrink F, Schreuder BEC, Straver PJ. Local systemic antibody response to bovine respiratory syncytial virus infection and reinfection in calves with and without maternal antibodies. J Clin Microbiol 1987;25:1097–106.
- [27] Nikunen S, Härtel H, Orro T, Neuvonen E, Tanskanen R, Kivelä S-L, et al. Association of bovine respiratory disease with clinical status and acute phase proteins in calves. Comp Immunol Microbiol Infect Dis 2007;30:143–51.
- [28] Davies RL, MacCorquodale Reilly R. Characterisation of bovine strains of *Pasteurella multocida* and comparison with isolates of avian, ovine and porcine origin. Vet Microbiol 2004;99:145–58.
- [29] Allen JW, Viel L, Bateman KG, Rosendal S. Changes in the bacterial flora of the upper and lower respiratory tracts and bronchoalveolar lavage differential cell counts in feedlot calves treated for respiratory diseases. Can J Vet Res 1992;56:177–83.
- [30] Babiuk LA, Lawman MJ, Ohmann HB. Viral–bacterial synergistic interaction in respiratory disease. Adv Virus Res 1988;35:219–49.
- [31] Bartelt MA, Duncan JL. Adherence of group A streptococci to human epithelial cells. Infect Immun 1978;20:200–8.
- [32] Raza MW, Blackwell CC, Ogilvie MM, Saadi AT, Stewart J, Elton RA, et al. Evidence for the role of glycoprotein G of respiratory syncytial virus in binding of *Neisseria meningitidis* to HEp-2 cells. FEMS Immunol Med Microbiol 1994;10:25–30.
- [33] Murphy BR, Alling DW, Snyder MH, Walsh EE, Prince GA, Chanock RM, et al. Effect of age and preexisting antibody on serum antibody response of infants and children to the F and G glycoproteins during respiratory syncytial virus infection. J Clin Microbiol 1986;24: 894–8.
- [34] Sanford BA, Shelokov A, Ramsay MA. Bacterial adherence to virus-infected cells: a cell culture model of bacterial superinfection. J Infect Dis 1978;137:176–81.
- [35] Taylor G, Thomas LH, Wyld SG, Furze J, Sopp P, Howard CJ. Role of T-lymphocyte subsets in recovery from respiratory syncytial virus infection in calves. J Virol 1995;69:6658–64.
- [36] Grell SN, Riber U, Tjørnehoj K, Larsen LE, Heegaard PM. Age-dependent differences in cytokine and antibody responses after experimental RSV infection in a bovine model. Vaccine 2005;23:3412–23.
- [37] Orro T, Jacobsen S, LePage J-P, Niewold T, Alasuutari S, Soveri T. Temporal changes in serum concentrations of acute phase proteins in newborn dairy calves. Vet J 2008;176:182–7.