Temporal changes in serum concentrations of acute phase proteins in newborn dairy calves

Toomas Orro a,b,*, Stine Jacobsen c, Jean-Philippe LePage d, Theo Niewold e, Sakari Alasuutari f, Timo Soveri a

a Department of Clinical Veterinary Sciences, Saari Unit, University of Helsinki, FIN-04920 Saarentaus, Finland
b Department of Animal Health and Environment, Estonian University of Life Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia
c Department of Large Animal Sciences, The Royal Veterinary and Agricultural University, Dyrlægevej 48, DK-1870 Frederiksberg C, Copenhagen, Denmark
d Unit of Animal Health Management, Veterinary School-INRA, BP 40706, 44307 Nantes Cedex 03, France
e Department of Biosystems, Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, B-3001 Heverlee, Belgium
f Suitia Research Farm, University of Helsinki, FIN-02570 Siuntio, Finland

Accepted 6 February 2007

Abstract

Age-dependent changes in blood concentrations of four bovine acute phase proteins (APPs), serum amyloid A (SAA), lipopolysaccharide binding protein (LBP), haptoglobin (Hp) and alpha 1-acid glycoprotein (AGP), were examined using two groups of newborn dairy calves. APP concentrations were monitored for either 3 weeks (Group A, n = 13) or 2 months (Group B, n = 13) after birth. Blood was collected at day 0–1 (Group A only), day 3 and then once or twice weekly until the end of the study. The possible transfer of colostral SAA as a source of SAA in the offspring was investigated by determining SAA isoforms in the serum of calves and in colostrum of their dams.

Serum concentrations of all four APPs were high after birth, and concentrations showed a gradual decrease during the first 3 weeks of life. The lowest concentrations were at 21 days of age, after which concentrations stabilized. The calves synthesized adult SAA isotypes, and circulating SAA was not derived from colostrum. The results indicated that post-partum elevation of APPs is associated with the birth process and/or factors in colostrum and not necessarily with disease-related processes. This stresses the importance of considering a calf’s age when interpreting APP concentrations in serum.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Newborn calf; Serum amyloid A; Lipopolysaccharide binding protein; Haptoglobin; Alpha 1-acid glycoprotein

1. Introduction

Acute phase proteins (APPs) are serum proteins, the hepatic production of which increases during the early reaction of the host to infection or tissue damage – the so-called acute phase response (APR) (Baumann and Gauldie, 1994). During APR, circulating concentrations of APP change substantially and this makes them good candidates for use in veterinary medicine as quantifiable indicators of inflammation or infection.

There are marked differences in APPs and their response profiles between species (Kushner and Mackiewicz, 1987). In bovines, serum proteins that have been recognized as APPs include two major APPs: serum amyloid A (SAA) (Horadagoda et al., 1993) and haptoglobin (Hp) (Eckersall and Conner, 1988). These proteins have been used to evaluate inflammatory conditions in clinical or experimental studies of cattle (Alsemgeest et al., 1994; Heegaard et al.,

* Corresponding author. Address: Department of Animal Health and Environment, Estonian University of Life Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia. Tel.: +372 731 3205; fax: +372 731 3706.
E-mail address: toomas.orro@helsinki.fi (T. Orro).

1090-0233/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2007.02.010

Please cite this article in press as: Orro, T. et al., Temporal changes in serum concentrations of acute phase proteins ..., The Veterinary Journal (2007), doi:10.1016/j.tvjl.2007.02.010
Alpha_1-acid glycoprotein (AGP) is a moderately responding APP in bovines (Conner et al., 1988; Tamura et al., 1989). Lipopolysaccharide binding protein (LBP), an APP in humans and laboratory animals, has also been recognized as an APP in cattle (Horadagoda et al., 1995; Bannerman et al., 2003).

While ample evidence exists of APPs in adults, only a few reports describe APP in neonatal calves. Alsemgeest et al. (1993) observed no changes in concentrations of SAA before and 24 h after birth in four cannulated fetuses, but low SAA and Hp concentrations have been noted in calves sampled within 10 min of parturition (Alsemgeest et al., 1995a). Knowles et al. (2000) reported elevated concentrations of Hp before 9 days of age in some of 14 calves investigated, although Schroedl et al. (2003) described Hp concentrations to be similar at birth and at 1 and 10 days of age. Itoh et al. (1993) reported a rise in fetal and neonatal isoforms of AGP concentrations in fetuses before birth, the highest concentrations at birth and a decrease during the first 3 weeks of life to adult values (Itoh et al., 1993).

Possible factors contributing to changes in APPs in the neonate include neonatal synthesis of APPs in the liver due to birth trauma or intake of colostral inducers such as cytokines, as well as stimulation by microbial or other environmental factors. Increased post-partum APP levels could also be caused by direct transfer of APPs from the colostrum to the calf, similarly to immunoglobulins, as colostrum of healthy cows contains mammary-associated SAA (McDonald et al., 2001).

The objective of the present study was to investigate age-dependent changes in serum concentrations of four bovine APPs (SAA, LBP, Hp and AGP) in neonatal dairy calves by sampling two groups of calves during the first 3 weeks and 2 months after birth, respectively. Serum samples from the calves and colostrum samples from their dams were used to explore passive transfer of colostral SAA and to identify SAA isoforms in calves.

2. Materials and methods

2.1. Animals and sampling

One group of 13 Holstein Friesian calves (7 males, 6 females; Group A), born on the Helsinki University Suita Research Farm, was used for evaluating concentration changes in APPs during the first 3 weeks of life. Calves were raised according to the regular routine of the farm. Within 3 h of birth, calves received colostrum from their dams. Calves were kept in individual pens and fed milk from their dams three times a day for 5 days, after which they were adapted to the automated feeding system with milk powder. At the age of 1–1.5 weeks, they were moved to group fences with an automatic feeding system and free access to water, silage and hay. Calves were raised according to the regular routine of the farm. Within 3 h of birth, calves received colostrum from their dams. Calves were kept in individual pens and fed milk from their dams three times a day for 5 days, after which they were adapted to the automated feeding system with milk powder. At the age of 1–1.5 weeks, they were moved to group fences with an automatic feeding system and free access to water, silage and hay.

Blood was drawn from the jugular vein at the age of 0 or 1 day (median time from birth 18 h, range 4–32 h) and at 3, 7, 10, 14 and 21 days. The second group of 13 Holstein Friesian calves (5 males, 8 females; Group B) born on the same farm and raised similarly was sampled during the first 2 months of life, first at 3 days of age and then weekly. The mean ages of the calves at the time of weekly sampling were 10, 17, 24, 31, 38, 45, 52 and 59 days. Serum was separated by centrifugation and stored in aliquots at −20 °C for further analyses. Aliquots of colostrum from the dams of calves in Group A were also stored at −20 °C. At each sampling point, every calf was examined clinically and rectal temperature was measured.

The need for obstetric assistance was recorded for every calf and graded as spontaneous parturition (no assistance), extraction by one person or forceful extraction by two people.

Disbudding by heat cauterization of calves in Group B was carried out after weekly blood sampling (at age 2–3 weeks) to minimize the effect of the procedure on APP concentrations in blood samples obtained 1 week later. Calves in Group A were disbudded after the experiment.

2.2. Analysis of acute phase proteins

Serum Hp was determined using the haemoglobin binding assay described by Makimura and Suzuki (1982), with the modification of using tetramethylbenzidine (0.06 mg/mL) as a substrate (Alsemgeest et al., 1994). Pooled and lyophilized aliquots of bovine acute phase serum were used to create standard curves. To calibrate the assay, a bovine serum sample with a known Hp concentration provided by the European Commission Concerted Action Project (number QLK5-CT-1999-0153) was used. The range of the standard curve was 0.06–1.16 g/L. The intra- and inter-assay CV% were <12% (mean concentrations of control samples were 0.1 g/L; n = 20 and 0.98 g/L; n = 20) and <11% (mean concentrations of control samples were 0.13 g/L; n = 8 and 0.96 mg/L; n = 8), respectively. Because haemolysis can influence the results, haemolysed (detected by visual examination) samples were removed from the Hp analysis (n = 12).

SAA concentrations in serum of calves and in colostrum were measured with a commercially available ELISA kit (Phase SAA kit, Tridelta Development). Intra- and inter-assay CV% were <7% (mean concentrations of control samples were 18.9 mg/L; n = 20 and 89.5 mg/L; n = 19) and <9% (mean concentrations of control samples were 8.9 mg/L; n = 6 and 65.0 mg/L; n = 6), respectively.

Serum LBP concentrations were determined using a commercially available ELISA kit with cross-reactivity to bovine LBP (Bannerman et al., 2003; LBP ELISA for various species, HyCult Biotechnology). Intra- and inter-assay CV% were <9% (mean concentrations of control samples were 21.3 mg/L; n = 10 and 81.2 mg/L; n = 10) and <13% (mean concentrations of control samples were 17.9 mg/L; n = 10 and 91.5 mg/L; n = 10), respectively.

Serum AGP was analysed using a commercial radial immunodiffusion kit for cattle (Bovine AGP, Tridelta Development). The intra-assay CV% was <4% (mean concentrations of control samples were 332 mg/L; n = 10 and 893 mg/L; n = 10).

2.3. Denaturing isoelectric focusing and Western blotting of SAA

Serum samples (at ages of 0 or 1, and 3, 7, 14 and 21 days) from eight calves (randomly selected) from Group A and colostrum samples from their dams were used for denaturing isoelectric focusing (IEF) and Western blotting of SAA isoforms. This was performed as described by Jacobsen et al. (2005). Briefly, samples were diluted in 8 M urea (Amersham Pharmacia) and separated by IEF (PhastGel System, Amersham Pharmacia Biotech) on dried gels (PhastGel Dry IEF, Amersham Pharmacia Biotech) reconstituted with a mixture of 8 M urea and prebubd Ampholine pH 3.5–9.5 (Amersham Pharmacia Biotech), according to the manufacturer’s instructions (Separation Technique File no. 101).

After IEF, semi-dry Western blotting onto a nitrocellulose membrane was performed, and SAA was detected using a biotinylated, monoclonal anti-SAA antibody (Tridelta Development). To determine the apparent pI of the SAA isoforms, serum samples from three of the calves and one colostrum sample were separated as described above on a gel along with standard proteins (Isoelectric Focusing Calibration Kit, Broad pI 3.50–9.30) reconstituted in 8 M urea, Amersham Pharmacia Biotech). The half of the gel that contained the standard proteins was stained with Coomassie Blue (PhastGel Blue R-350, Amersham Pharmacia Biotech), the other half, which contained the samples, was subjected to Western blotting as described above.
2.4. Statistical analysis

If a calf had clinical signs of disease at the time of sampling (signs of respiratory disease, diarrhoea) or a rectal temperature $>39.5\,^\circ$C, the sample was excluded from the analysis at that time point. Non-parametric Wilcoxon signed-rank test was used to evaluate changes in concentrations of APPs after birth. In Group A, every age point was compared with the 21-day sample and with the next sample in the series. In Group B, every age point was first compared with the 59-day sample to identify the age at which the concentrations stabilized. Each sampling time before that time point was then compared with the next sample. Significance was set at $P \leq 0.05$. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS Inc.). All results are presented as means $\pm$ SD.

3. Results

3.1. Clinical condition and obstetric aid

In Group A, one episode of diarrhoea in one calf was recorded (at the age of 7 days). In Group B, six calves had signs of disease at the time of sampling (three calves on one occasion, three calves on two occasions). One calf with a high rectal temperature, one episode of coughing and seven episodes of diarrhoea were recorded. Three calves (two from Group A, one from Group B) needed forceful extraction at delivery, and 10 were born without any assistance (five from both groups). The remaining calves were extracted by one person.

3.2. Changes in concentrations of APPs during the first 3 weeks (Group A)

The results are shown in Fig. 1. Mean concentrations of SAA and AGP from all time points were higher than 21-day concentrations. After birth, mean serum concentrations of SAA increased quickly, being highest at the age of 7 days ($112.0 \pm 30.0\,\text{mg/L}$), and decreased after 10 days of age. The two youngest calves at the first sampling had the lowest SAA values (7.4 mg/L and 6.4 mg/L at 4 h and 6 h from birth, respectively). The same calves had the lowest and the third lowest LBP concentrations (4.5 mg/L and 13.8 mg/L, respectively). Two calves requiring forceful extraction had the highest SAA values (134.7 mg/L and 129.2 mg/L) in the first sample (sampling times from birth of 11 h and 32 h, respectively). Concentrations of AGP were highest in the first sample ($1318.5 \pm 503.6\,\text{mg/L}$), then decreased gradually throughout the observation period. Serum concentrations of LBP and Hp were more stable. The highest and lowest mean concentrations for both proteins occurred at 10 and 21 days, respectively. Three calves had very high Hp concentrations (0.49 g/L and 0.38 g/L at day 10 in two calves, and 0.72 g/L at day 14 in the third calf).

![Fig. 1](https://example.com/figure1.png)

Fig. 1. Mean ($\pm$SD) serum concentrations of serum amyloid A (SAA), lipopolysaccharide binding protein (LBP), haptoglobin (Hp) and alpha-1-acid glycoprotein (AGP) in calves sampled during a 3-week period after birth (Group A; $n = 13$). (*) Significant difference ($P \leq 0.05$) from the last sampling (21 days of age). (#) Significant difference ($P \leq 0.05$) from the next sampling.

Please cite this article in press as: Orro, T. et al., Temporal changes in serum concentrations of acute phase proteins ..., The Veterinary Journal (2007), doi:10.1016/j.tvjl.2007.02.010
3.3. Changes in concentrations of APPs during the first 2 months (Group B)

The results are shown in Fig. 2. The highest mean serum concentrations of all APPs occurred in the first sample taken 3 days after birth. From day 24 onwards APP levels remained stable. Mean concentrations of SAA and AGP decreased relatively more during that period than other APPs (75% and 73%, respectively). Although mean concentrations of LBP were higher in the first three samples than in the last sample, the decreases in concentrations from 3 to 24 days of age were relatively smaller (40% decrease). Con-

Fig. 2. Mean (±SD) serum concentrations of serum amyloid A (SAA), lipopolysaccharide binding protein (LBP), haptoglobin (Hp) and alpha 1-acid glycoprotein (AGP) in calves sampled during a 2-month period after birth (Group B; n = 13). (*) Significant difference (P ≤ 0.05) from the last sampling (mean age 59 days). (#) Significant difference (P ≤ 0.05) from the next sampling.

Fig. 3. Serum amyloid A isoforms by denaturing isoelectric focusing and Western blotting of serum samples from eight calves (panels a–h; sampling age 1, 3, 7, 14 and 21 days) and a colostrum sample from their dams (c).
centrations of Hp at the second sampling were even lower than those at the last one, and concentrations remained low (<0.2 g/L) throughout the observation period.

3.4. Concentrations of SAA in colostrum and isoforms of SAA in serum and colostrum

The mean SAA concentration in the colostrum of the eight cows investigated was 25.8 ± 26.8 mg/L. All colostrum samples contained one or more alkaline SAA bands (pI values >9.3; Fig. 3). Four colostrum samples also contained an isoform with an apparent pI of 6.8 (Fig. 3, panels a–d). None of the serum samples obtained from the calves contained alkaline isoforms. Two isoforms with a pI of 6.8 and 6.2 were found in the serum of all eight calves. Additional isoforms (pI 5.8 and 7.4) were found in two calves (Fig. 3, panels d and g).

4. Discussion

Temporal changes occurred in all bovine APPs within the first 3 weeks of life. In Group A, all APPs were elevated after birth and showed a decrease over the 3-week study period. These results were corroborated by findings in Group B. The changes were most pronounced in concentrations of SAA and AGP, and, in general, concentrations were higher within 2 weeks of birth than later in all proteins. Stabilization of APP concentrations occurred after 3 weeks of age.

Differences in neonatal and adult AGP isoforms (Itoh et al., 1993) and a rise in AGP concentration already in fetal stages have been reported in calves and piglets (Stone and Maurer, 1987; Itoh et al., 1993). This indicates that neonatal AGP is probably fetally regulated. High neonatal serum concentration of AGP is not necessarily related to the activation of APR by some external stimulus, which is supported by the continued gradual decrease after birth. The pattern of changes in concentrations of SAA immediately after birth differed from that of AGP (Fig. 1), suggesting the presence of some external stimulatory factor for SAA at birth or soon after. The lowest SAA concentrations were in calves sampled within a few hours of birth, but then levels increased quickly. The low SAA levels immediately after birth we are in accordance with the results of Alsemgeest et al. (1993, 1995a). However, for their studies, the follow-up period ended at 24 h after birth.

Concentrations of LBP changed in a pattern similar to SAA, which further supports the presence of some external stimulatory factor for synthesis of APPs. However, relative rise of LBP were smaller in both groups and increase of LBP was slower in Group A. Higher Hp concentrations occurred within 3 days of birth, the concentrations stayed low and relative rise was very small. This difference from the other APPs may be explained by different regulatory mechanisms for hepatic synthesis (Alsemgeest et al., 1996) and stimulatory factors being insufficient to cause Hp response (Werling et al., 1996).

Colostrum intake is the external factor most likely to cause the temporal changes in APPs of newborn calves. Isoforms of SAA in serum of calves corresponded to those earlier reported in adult cattle, including exceptional isoforms with pI 5.8 and with pI 7.4 in two calves (Alsemgeest et al., 1995b; Jacobsen et al., 2005).

The SAA isoforms in the colostrum samples had highly alkaline apparent pI values (pH >9.3), similar to those previously demonstrated in colostrum and mastitic milk (McDonald et al., 2001; Jacobsen et al., 2005). Colostral SAA isoforms were not found in any of the serum samples obtained from the calves. This finding indicates that SAA was not transported across the neonatal intestine or alternatively, that levels of uptake were very low or highly alkaline colostral SAA isoforms were selectively removed from circulation. Although there was no evidence for direct transfer of SAA, inflammatory mediators present in colostrum may have crossed the neonatal intestine and stimulated the hepatic production of APPs. Earlier studies have shown that colostrum contains high quantities of pro-inflammatory cytokines, such as interleukin (IL)-β, IL-6 and tumour necrosis factor (TNF)-α (Goto et al., 1997; Hagiwara et al., 2000), which are known to be the main inducers of hepatic production of APPs (Baumann and Gauldie, 1994). Concentrations of pro-inflammatory cytokines in the serum of calves increase immediately after colostrum intake and then decrease gradually, being almost undetectable around 3–4 weeks of life (Yamanaka et al., 2003).

Physical stress or trauma during parturition may induce additional increases in levels of APPs (Marchini et al., 2000), as demonstrated by the highest SAA concentrations in two calves needing forceful extraction in Group A. As only three calves (2 in Group A, 1 in Group B) needed forceful extraction, they probably had only a minimal effect on the age-dependent changes in APPs seen at the group level.

Although we removed all samples from the study if signs of disease were recorded, concentrations of APPs may still have been influenced by the presence of subclinical infections or other disease processes not evident at the time of sampling. This might have been the case in three calves in Group A that showed very high Hp concentrations at the second week of life. Presence of a subclinical disease explains also higher SAA and LBP concentrations at second week in Group A than in Group B. No further indication of disease-related mechanisms was observed, indicating that changes in APPs were mainly related to post-partum physiological adaptation of newborn calves.

The results of this study indicate that the temporal changes of APP concentrations in newborn dairy calves are associated with the birth process and/or some inductive factors in colostrum, and not necessarily with disease processes. Relatively high SAA and AGP concentrations after birth stress the importance of considering the age of the calf when using these proteins as disease markers.
Because APPs and SAA, in particular (Urieli-Shoval et al., 2000), play an important role in the host’s innate immune response, it is tempting to speculate that temporal changes after birth also indicate a key role of APPs in defence mechanisms of newborn calves. This conjecture remains to be addressed in future studies.

Acknowledgements

This study was supported by the Archimedes Foundation in Estonia, the Mercedes Zacharias Foundation in Finland and the Danish Agricultural and Veterinary Research Council.

References


Please cite this article in press as: Orro, T. et al., Temporal changes in serum concentrations of acute phase proteins ..., The Veterinary Journal (2007), doi:10.1016/j.tvjl.2007.02.010