Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period

Marie-France Humblet, Hugues Guyot, Benjamin Boudry, Faustin Mbayahi, Christian Hanzen, Frédéric Rollin, Jean-Marie Godeau

Background: Haptoglobin and serum amyloid A are major acute phase proteins in cattle. Dairy cattle often develop pathologic conditions in the peripartum period; acute phase proteins may be useful in their diagnosis. **Objectives:** The purpose of this study was to compare the accuracy of serum haptoglobin (Hp) and serum amyloid A (SAA) concentrations with clinical health status for diagnosing disease during the peripartum period in dairy cattle. **Methods:** Dairy cows from 4 herds were evaluated every 15 days over a 6-month period. Health status was determined by thorough clinical examination. Haptoglobin and SAA concentrations were measured in serum using validated methods and the results were classifed as positive or negative based on defined cutoff points. Disease prevalence, sensitivity, and specificity were compared using clinical examination as the gold standard. **Results:** A total of 1896 samples from 158 cows were analyzed. Significant increases in mean Hp and SAA concentrations were observed in the week following parturition in both primiparous and multiparous cows, although high interindividual variability was observed. Both Hp and SAA had low sensitivity but higher specificity in determining disease status compared with clinical examination. Increased concentrations of Hp and SAA were found in <10% of samples from clinically healthy cows, except in the week after parturition. **Conclusions:** Haptoglobin and serum amyloid A should be used with caution as markers of inflammation in the week after calving. Poor sensitivity in other postpartum periods could be related to the higher incidence of chronic (vs acute) inflammation. Haptoglobin may be appropriate for routine screening, but further work needs to be done to assess its value as an indicator of herd health. (*Vet Clin Pathol.* 2006;35:188–193)

Key Words: Acute phase proteins, cattle, dairy, haptoglobin, serum amyloid A

The acute phase response is a reaction of the animal to disturbances in homeostasis caused by infection, tissue injury, neoplastic growth, or immunologic disorders.[1] When it occurs, the concentrations of serum acute phase proteins (APPs) increase. Haptoglobin (Hp) and serum amyloid A (SAA) are considered major APPs in cattle, since their concentration increases markedly in the acute phase response.[2–4]

The post-partum period is a critical period for dairy cows because of the higher probability of developing pathologic disorders related to calving. Metritis and mastitis are mostly encountered during that period and are responsible for reduced productivity and subsequent economic losses.[5,6] Hp and SAA have been used previously as serum markers of acute inflammation in dairy cows.[2–4] The postpartum period would be an appropriate period to further study the value of these APPs.

The purpose of this study was to measure changes in serum concentrations of Hp and SAA over a 6-month period in dairy cattle, and to compare the acute phase responses in primiparous and multiparous cows. We also determined the sensitivity and specificity of Hp and SAA (as compared with clinical examination findings) to investigate their utility as disease markers.

Materials and Methods

Animals and blood sampling

Two hundred sixteen black and white Holstein dairy cows, 2 to 9 years of age, were included in the study. The cows and heifers belonged to 4 commercial herds in the eastern part of Belgium, characterized mainly by grassland. The experiment took place between mid-October 2001 and mid-April 2002. The criterion for enrollment in the study was all cows and heifers that were 8–7 weeks before parturition (54 animals per herd). Data from cows that were culled and sold from the herds during the study period were later excluded. The animals were stabled in a loose-housing system in 3 herds, and in tie-stall barns in 1 herd. Cows were fed a diet of grass and maize silage as well as concentrate, 2 hours before evaluation and blood sampling. Three peripartum periods were defined and used in the present study: P1, corresponding to the pre-

From the Department of Functional Sciences, Unit of Biochemistry (Humblet, Mbayahi, Godeau), and Department of Clinical Sciences, Unit of Bovine Health Management (Guyot, Boudry, Hanzen, Rollin), Faculty of Veterinary Medicine, University of Liège, 4000 Liège–SartTilman, Belgium. Corresponding author: Marie-France Humblet (mfhumblet@ulg.ac.be).

Table 1. Fecal consistency and rumen fill scoring.[10,11]

Criteria	Score	Characteristics
Fecal consistency	1	Very liquid feces
	2	Runny feces that do not form a cohesive pile on the floor
	3	Porridge-like fecal consistency, plopping sound as feces hit the floor
	4	Moderate thickening of feces
	5	Firm fecal balls
Rumen fill	1	Para lumbar fossa appears very empty, cow has eaten little or none because of illness
	2	Paralumbar fossa cavitates a hand-width behind the last rib and to a lesser extent under the transverse processes
	3	Paralumbar fossa cavitates less than a hand's width behind the last rib, falls about a hand-width vertically downwards from the trans verse processes, and then bulges out
	4	Paralumbar fossa skin covers the area behind the last rib and arches immediately out below the transverse processes because of an extended rumen
	5	Rumen very distended, nearly obliterates the fossa

partum period (week 8/7 to parturition); P2, the first week following parturition; and P3, the postpartum period from week 2 to week 15 or 16. The protocol was approved by the Ethics Committee of the University of Liège.

Blood samples were obtained every 15 days during the 6-month study. At each visit, animals were blocked in tie-stalls to allow clinical examination and blood sampling. Blood samples were collected from the coccygeal vein into 5-mL Vacutainer tubes (Terumo Europe NV, Leuven, Belgium) that contained no anticoagulant. Sampling was performed by a veterinarian who was careful not to cause hemolysis, which can interfere with the Hp method used in this study.[7] Blood was transported to the laboratory within 3 hours of sampling, so it had time to clot. Serum was separated by centrifugation of samples at 1500*g* for 10 minutes. Part of the serum was used for Hp measurement, which was done 4 to 5 hours after sampling, and the rest was stored frozen at –20°C until assayed for SAA, 2 months later.

Clinical and gynaecologic examination

Clinical and gynaecologic examinations were performed at each blood sampling by 2 experienced veterinarians.[8] The examinations were made 2 hours after feeding, which is the appropriate time to evaluate rumen fill and appetite of the cows. The initial examination consisted of evaluation of behavior and hair coat. A body condition score (BCS) was determined on a scale of 1 (emaciated) to 5 (obese) as previously described, based on palpation of the sharpness and muscle and fat covering the backbones and lumbar processes.[9] Scoring scales of 1 to 5 also were used to evaluate rumen fill and fecal consistency (Table 1).[10,11] Close attention was paid to limbs and feet in order to detect laminitis, swelling, alopecia, or other lesions. Breeders were asked for information regarding milk production by each cow, and somatic cell counts (SCC) were obtained from the Belgian Milk Control Laboratory. The following criteria were taken into account when classifying the cows as healthy or diseased: history of a pathologic process since the last visit, abnormal signs such as lateral recumbency, reduced appetite or anorexia, decreased milk production, milk SCC >300,000 cells/mL more than once, rumen fill <3 or decreased BCS from 1 visit to the next (minimum allowable change of 0.5 points before parturition and 1 point after calving).[12]

The complete clinical examination also included rectal temperature, superficial lymph node inspection and palpation, and evaluation of mucous membranes, which were considered as abnormal if they were pale, icteric, cyanotic, or congested, or if petechiae were noted.[8] Respiratory rate and the type, intensity, and rhythm of respiratory movements were evaluated. Expiratory or inspiratory dyspnea or noises, and mucopurulent, purulent, or hemorrhagic nasal discharge were considered as abnormal, as were abnormal pulmonary sounds or the absence of normal noises as detected by auscultation. Pulse was taken at the facial artery, and was considered as abnormal if >90/min. Cardiac auscultation also was performed. Examination of the digestive system consisted of rumen palpation, auscultation, and percussion for hypermotility, hypomotility, or atony, and tympany. Abdominal wall percussion (combined with auscultation) was performed to detect externally any "ping" or splashing sounds. Transrectal palpation, performed as part of the gynecologic examination, also permitted evaluation of the placement, motility, consistency, sensitivity to pain, or abnormal amount of gas in the abdominal organs and evaluation of internal lymph nodes.

Hydration status, based on appearance of skin fold, eyes, and mucous membranes, was categorized as 6–7% (slight enophthalmos, slightly increased skin turgor, moist mucous membranes); 8–9% (sunken eyes, increased skin turgor, tacky mucous membranes); or 10–12% (deeply sunken eyes, skin tents, dry mucous membranes, depression).[13] Systematic inspection and palpation of the udder was performed.[8] Milk was sampled for a California Mastitis Test (CMT) when a swollen, warm, or painful udder, abnormal milk, or history of reduced milk production raised the suspicion of mastitis. The CMT results were recorded as 0 (negative), 1 (trace or +), 2 (++), or 3 (+++).[14,15]

Beginning 2 weeks after parturition, each cow was examined by rectal palpation to check uterine involution. A vaginal speculum was used to visually estimate the presence and quantity of abnormal uterine discharge in the vagina.[8] For the purpose of this study, cows with endometritis, metritis, or pyometra were classified as having metritis.[16] Acute puerperal metritis was diagnosed when abnormal vaginal discharge (see Table 2) was observed externally before the 14th day following parturition, with or without general clinical signs such as fever, depression, or anorexia.[17] From the 14th day after parturition onward, the metritis was defined as chronic.[18] The ovaries were examined by transrectal ultrasonography

Table 2. Major clinical signs and pathologic conditions used to determine the disease status of dairy cows.

System	Clinical Sign or Condition
General signs	Fever (>39.5°C), anorexia, recumbancy, reduced milk production
Respiratory	Respiratory rate >40/min, cough, nasal discharge (mucopurulent, purulent, hemorrhagic); dyspnea
Digestive	Rumen impaction, hypomotility (<7 contractions/min), atony, tympany, hypermotility; abomasal displacement or torsion; intestinal tympany, pain, diarrhea, displacement
Locomotive	Lameness, swollen hocks, hoof problems, laminitis (acute or chronic), arthritis
Mammary	Milk clots, California mastitis test result +++, reduced milk production, clinical mastitis (pain, swelling), subclinical mastitis: somatic cell count >300,000 cells/mL more than once
Genital	Placental retention, vaginal or cervical discharge (mucopurulent, purulent, putrid), vulvar tear
Other	Absesses, traumas

using a Concept\MCV portable ultrasound system with a 5–7.5 MHz linear array transducer (Dynamic Imaging Ltd, Livingstone, UK) between 26 and 33 days after the first artificial insemination for an early pregnancy diagnosis.[19]

A cow was considered as diseased if it had at least 1 of the conditions described in Table 2. These critieria were applied serially; only 1 of them was required to categorize the cow as diseased. Otherwise, the animals were categorized as healthy. The health status based on clinical examination was used as the gold standard.

Haptoglobin and serum amyloid A

Serum Hp was measured using a hemoglobin (Hgb) binding capacity method elaborated by Owen et al[20] and adapted to an automated analyzer (Olympus AU 510, Melville, NY, USA). Hp concentration was expressed as milligrams of Hgb bound to Hp per liter. For the purpose of standardizing Hp measurements in Europe, participating laboratories recalibrated their Hp assays using a reference preparation as the primary standard.[21] In our laboratory, Hp results differed by a factor of 1.6 from that of the reference preparation. Subsequently, all Hp results reported in this study have been multiplied by 1.6 for standardization purposes. SAA concentration was determined by means of an ELISA kit (Tridelta Development Ltd, Maynooth, Ireland) and a Multiskan EX analyzer (Labsystems Co, Helsinki, Finland) and expressed as μg/L.

Cutoff points to differentiate diseased and healthy cows during each peripartum period were based on those established in 2 previous studies for Hp and SAA (Table 3).[22] Although plasma Hp concentration in cattle tends to increase to more than 100 mg/L with acute inflammation,[23] it has been suggested that a higher cutoff point should be considered in the week following parturition (P2) because APPs increase physiologically during that period; thus, a cutoff point of 150 mg/L was established for Hp during P2.[23] Hp status during P1 and P3 was interpreted as no inflammation (Hp–), mild inflammation (Hp+), and severe inflammation (Hp++). The cutoff point for SAA in P2 (60,000 μg/L) was based on histograms, such that 97.5% of healthy animals had a concentration of SAA less than or equal to the cutoff point.[24] Sensitivity, specificy, and prevalence of disease were calculated based on the number and percentage of positive and negative samples for each APP using cutoff values and clinical health status as the gold standard.

Table 3. Cutoff points (medical decision limits) used to determine haptoglobin (Hp) and serum amyloid A (SAA) status in each peripartum period.

Peripartum Period	Hp (mg/L)			SAA (μg/L)	
	Hp−	Hp+	Hp++	SAA−	SAA+
P1 + P3	≤30	30–100	>100	≤25,000	≥25,000
P2	<150	>150	—	≤60,000*	>60,000

*Calculated as the SAA concentration of 97.5% of healthy animals, based on clinical status.

Statistical analysis

Data were analyzed with the Statview program (SAS Institute Inc, Cary, NC, USA). To achieve a normally distributed data set, Hp and SAA results were log (ln)-transformed before analysis. Because multiple measurements per animal cannot be regarded as independent units of observation, the association of Hp and SAA concentrations with the time of calving and parity (multiparous versus primiparous) was investigated by ANOVA with repeated measures. The same test was used to compare mean SAA concentrations according to Hp status. When a significant effect of time was observed, the difference between primiparous and multiparous cows during each peripartum period was evaluated with the Scheffe test. The same test was applied to evaluate significant differences between mean SAA concentrations according to Hp status. Differences were considered as significant for $P \leq .05$.

Results

Fifty-eight of the 216 cows were excluded from the study because they were culled or sold before the end of the screening period. A total of 1896 samples from 158 cows were analyzed and statistically evaluated. Mean concentrations of both Hp and SAA peaked significantly during the first week after calving in both diseased and healthy cows ($P < .0001$) (Figure 1). Some cows (n=15) had no increase in Hp or in SAA in the first week. No significant effects of time on Hp and SAA concentrations were observed after the first week after calving.

Healthy primiparous cows (n = 10) had higher mean (±SD) serum Hp (432 ± 426 mg/L) and SAA (136,077 ±

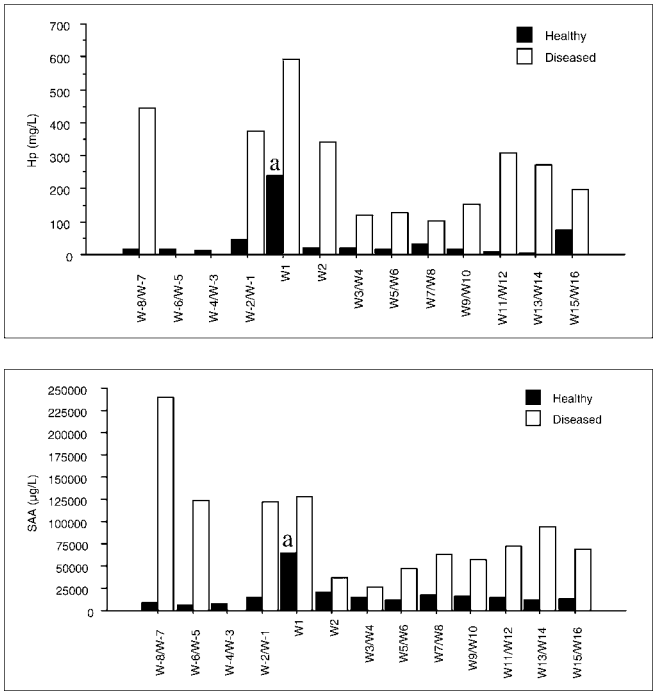

Figure 1. Changes in mean haptoglobin (Hp) (**top**) and serum amyloid A (SAA) (**bottom**) concentrations during the peripartum period in 158 cows, from 8–7 weeks before parturition (W8/W7) until 15–16 weeks after calving (W15/W16). Mean concentrations significantly peaked during the first week after calving, as indicated by the letter ''a'' above the bar plot. Except for weeks 1 and 2, data were obtained at 2-week periods.

75,442 μg/L) concentrations in the week after calving than healthy multiparous cows (198 ± 347 mg/L and 50,974 ± 68,924 μg/L for Hp and SAA respectively) but the differences were not significant.

The clinical abnormalities most frequently noted during the period of study were metritis (n=96, 13 acute and 83 chronic), mastitis (n=41), swollen hocks (n=21), trauma/injury (n=23), respiratory infection (n=16), retained placenta (n=8), milk fever (n=6), lameness (n=16), arthritis (n=2), vulvar lesions and vaginitis (n=6), enteritis or maldigestion (n=4), and abomasal displacement (n=1).

Results based on Hp and SAA status were compared to those obtained by clinical examination (Table 4) and sensitivity and specificity were compared for each peripartum period (Table 5). Because no significant difference in Hp and SAA concentrations was observed in healthy cows between P1 and P3, data from these periods were considered together. For both Hp and SAA, sensitivity was higher and specificity was lower during P2 compared with P1 + P3.

Samples that were SAA+ also were usually Hp++, especially during the week following parturition (Table 6). Mean SAA concentration was 6000 μg/L in Hp– samples (n=1644), 48,000 μg/L in Hp+ samples (n=88) and 125,000 μg/L in Hp++ samples (n=164) ($P < 0001$).

More than 95% of samples from healthy cows were both Hp– and SAA– (Table 7). Only 73% of samples from diseased cows were Hp+/++ and SAA+. In healthy cows, 4.2% of SAA– samples were Hp+/++, and, 48.6% of Hp– samples were SAA+. In diseased animals, 13.9% of samples were SAA– and Hp+ and 24% were SAA+ and Hp–.

Discussion

The significant increase in Hp and SAA concentrations in the first week after calving suggested that parturition is associated with a physiologic acute phase response, as has been described before.[25,26] Other authors reported no significant response in Hp at that time, but their medical decision threshold value was 200 mg/L.[27] In this study, the choice of a higher threshold for SAA in the week following parturition was affected by changes in its serum concentrations throughout the measurement period. Increased maternal SAA concentration has been reported to occur 1 to 4 days after parturition.[28] Hp and SAA status should therefore be used with caution in the days following parturition because it could be difficult to distinguish between the physiologic acute phase response of calving and a pathologic inflammatory process. There is probably important interindividual variability in the physiologic acute phase response because several cows did not have any increase in Hp and SAA concentration at the time of calving. Interindividual variability has indeed already been observed for Hp in a previous study.[29]

Table 4. Diagnosis and prevalence of disease based on serum haptoglobin (Hp) and serum amyloid A (SAA) status as compared to health status based on clinical examination.

Peripartum Period	Health Status	No. (%) of Samples	Hp Status		SAA Status	
			No. (%) Hp–	No. (%) Hp+/++	No. (%) SAA–	No. (%) SAA+
P1 + P3	Healthy	1567 (85.4)	1442 (92.0)	125 (8.0)	1417 (90.4)	150 (9.6)
	Diseased	245 (13.5)	163 (66.5)	82 (33.5)	160 (65.3)	85 (34.7)
	Total	1812	1605	207	1577	235
P2	Healthy	60 (71.4)	35 (58.3)	25 (41.7)	24 (40.0)	36 (60.0)
	Diseased	24 (28.6)	4 (16.7)	20 (83.3)	5 (20.8)	19 (79.2)
	Total	84	39	45	29	55
All combined	Healthy	1627 (85.8)	1477 (90.8)	150 (9.2)	1451 (89.2)	176 (10.8)
	Diseased	269 (14.2)	167 (62.1)	102 (37.9)	165 (61.3)	104 (38.7)
	Total	1896	1644	252	1616	280

Table 5. Sensitivity and specificity of serum haptoglobin and serum amyloid A (SAA) in the diagnosis of disease status, using clinical examination as the gold standard.

Peripartum Period	Haptoglobin		SAA	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
P1 + P3	33.5	92.0	34.7	90.4
P2	83.3	58.3	79.2	60.0
All combined	37.9	90.8	38.7	89.2

Healthy primiparous cows had slightly higher Hp and SAA values in the week following parturition. This observation could suggest a more intense physiologic response during the first calving compared with later calvings. Damage to the uterus, vagina and vulva may be more severe during first parturition.

Hp and SAA status, using the defined cutoffs, both had low sensitivity and thus had a poor ability to identify animals with pathologic processes. Low sensitivity could be partly explained by the fact that the disease encountered most often during the postpartum period was chronic metritis. Thus, no positive response would be expected in either Hp or SAA because both APPs are markers of acute but not chronic disease.[30] On the other hand, except during the first week after calving, specificity was relatively high such that normal APP concentrations could more accurately be used to identify healthy animals.

The percentage of Hp+ and SAA+ samples was similar in healthy animals, but nevertheless, positive samples originated from different cows. A possible explanation for Hp+ or SAA+ samples in animals that were classified as healthy during the third period (P3) could be the presence of subclinical uterine infection, without any visible abnormal discharge or general signs (gynaecologic examination was only performed from 2 weeks after parturition onwards). Results from studies dealing with metritis in dairy cows diverge: some authors have observed a Hp response in cows with acute metritis, whereas others have concluded that metritis is not characterized by an acute phase response.[31,32]

Negative Hp and SAA status was in good agreement with the assessment of health status by clinical examination for healthy cows. On the other hand, agreement in identifying samples from diseased cows was slightly lower, and Hp and SAA status did not always agree in assessing disease. In samples from diseased cows, a higher percentage of samples was SAA+/Hp– than SAA–/Hp+. A difference in the kinetics of the 2 APPs could be responsible for disagreements between Hp and SAA status in diseased cows. SAA concentration increases earlier in the acute phase response than does Hp, so a cow at an early stage of inflammation could have increased SAA concentration while Hp is still at a physiologic level.[33] On the other hand, because Hp has a longer half-life than SAA, Hp+/SAA– samples from diseased cows could indicate a later stage of acute inflammation, in which SAA already returned to normal values while Hp concentrations were still elevated.[34] Individual differences in APP responses probably exist, as mentioned before for Hp.[29] A high percentage of false positive results based on SAA status was suspected because samples from many healthy animals were SAA+/Hp–. In routine analysis, Hp would thus be more appropriate than SAA for detecting disease. Furthermore, because the half-life of Hp is longer, the risk of false negative results is reduced compared to SAA.[33]

One of the aims of this study was to assess the ability of Hp and SAA to identify diseased animals in dairy herds under field conditions. Hp and SAA were in good agreement in the identification of healthy animals, but their ability to identify animals with pathologic processes (sensitivity) was low, probably because of the high number of cows with chronic metritis (vs. acute inflammation) in this study. Because Hp is more likely to be used as a routine parameter of disease, herds could be classified on the basis of their Hp status in order to evaluate the overall sanitary status of the herd. It is still necessary to determine what level of disease prevalence could be considered acceptable. There is still a lot to be done from this perspective in epidemiologic studies, but it should be remembered that the physiologic status of a cow, especially at parturition, can have an influence on serum Hp concentration.

Table 6. Number and percentage of samples positive for serum amyloid A (SAA) based on haptoglobin (Hp) status.

Peripartum Period	No. (%) of Samples SAA+/Hp–	No. (%) of Samples SAA+/Hp+	No. (%) of Samples SAA+/Hp++
P1 + P3	105/1605 (6.5)	40/82 (48.8)	86/125 (68.8)
P2	5/39 (12.8)	4/6 (66.7)	34/39 (87.2)
All combined	111/1644 (6.8)	44/88 (50.0)	120/164 (73.2)

Table 7. Number and percentage of samples based on serum amyloid A (SAA) status, healthy/diseased status based on clinical examination, and haptoglobin (Hp) status.

Hp Status	SAA–		SAA+	
	Healthy n=1451 (%)	Diseased n=165 (%)	Healthy n=175 (%)	Diseased n=104 (%)
Hp–	1392 (95.9)	142 (86.1)	85 (48.6)	25 (24.0)
Hp+	36 (2.5)	8 (4.8)	31 (17.7)	13 (12.5)
Hp++	24 (1.7)	15 (9.1)	59 (33.7)	66 (63.5)
Hp+/++	60 (4.2)	23 (13.9)	90 (51.4)	79 (76.0)

Acknowledgments

The authors would like to thank Daniel Burtin for the data encoding and Kim-Thu Phan and Lisette Trzpiot for their technical assistance in completing this work. This study was supported by the Belgian Federal Ministry of Agriculture.

References

1. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J.* 1990;265:621–636.
2. Conner JG, Eckersall PD, Doherty M, Douglas TA. Acute phase response and mastitis in the cow. *Res Vet Sci.* 1986;41:126–128.

3. Skinner JG, Brown RA, Roberts L. Bovine haptoglobin response in clinically defined field conditions. *Vet Rec.* 1991;28:147–149.
4. Alsemgeest SPM, Kalsbeek HC, Wensing TH, 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;16:21–23.
5. Rajala PJ, Gröhn. Effects of dystocia, retained placenta, and metritis on milk yield in dairy cows. *J Dairy Sci.* 1998;81:3172–3178.
6. Nielen M, Schukken YK, Brand A, Deluyker HA, Maatje K. Detection of subclinical mastitis from on-line milking parlor. *J Dairy Sci.* 1995;78: 1039–1049.
7. Makimura S., Suzuki N. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory diseases. *Jpn J Vet Sci.* 1982;44:15–21.
8. Rosenberger G, ed. *Die Klinische Untersuchung des Rindes.* 3rd ed. Berlin, Germany: Verlag Paul Parey; 1990.
9. Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. *J Dairy Sci.* 1989;72: 68–78.
10. Zaaijer D. Contrôle des effets de la nutrition sur les caractéristiques de la vache [Control of the effects of nutrition on the cow's characteristics]. In: *Proceedings of the Journée d'Etude de la Société Belge Francophone de Buiatrie,* Liège, Belgium; 2001:27–30.
11. Skidmore AL, Brand A, Sniffen CJ. Monitoring milk production: defining preset targets and execution. In: Brand A, Noordhuizen JPTM, Schukken YH, eds. *Herd Health and Production Management in Dairy Practice.* Wageningen, The Netherlands: Wageningen Press; 1996: 249–250.
12. Skidmore AL, Peeters KA, Sniffen CJ, Brand A. Monitoring dry period management. In: Brand A, Noordhuizen JPTM, Schukken YH, eds. *Herd Health and Production Management in Dairy Practice.* Wageningen, The Netherlands: Wageningen Press; 1996:171–221.
13. Roussel AJ. Fluid therapy in mature cattle. *Vet Clin North Am Food Anim Pract.* 1990;61:111–123.
14. Schalm OW, Noorlander DO. Experiments and observations leading to development of the California Mastitis Test. *J Am Vet Med Assoc.* 1957;130:199–204.
15. Barnum DA, Newbould FHS. The use of the California Mastitis Test for the detection of bovine mastitis. *Can Vet J.* 1961;3:83–90.
16. Lewis G. Uterine health and disorders. *J Dairy Sci.* 1997;80:984–994.
17. Derivaux J. La retention placentaire et les affections utérines du postpartum [Retained placenta and postpartum uterine infections]. In: Constantin A, Meissonnier E, ed. *L'Utérus de la Vache: Anatomie, Physiologie et Pathologie.* Maisons-Alfort, France: Editions Société Française de Buiatrie; 1981:329–343.
18. LeBlanc SJ, Duffield TF, Leslie KE, et al. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J Dairy Sci.* 2002;85:2223–2236.
19. Pieterse MC, Szenci O, Willemse AH, Bajcsy CSA, Dieleman SJ, Taverne MAM. Early pregnancy diagnosis in cattle by means of linear-array real-time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. *Theriogenology.* 1990;33:697–707.
20. Owen JA, Better FC, Hoban J. A simple method for the determination of serum haptoglobins. *J Clin Pathol.* 1960;13:163–164.
21. Eckersall PD. Co-ordination, harmonisation and standardization of measurement of bovine and porcine acute phase protein in blood; reference preparations for animal protein assays (final report). *Concerted Action Number.* 1999;QLK5-CT-1999-0153:20–21.
22. Humblet M-F. Validation de l'Haptoglobine sérique en tant que marqueur de l'inflammation aiguë dans l'espèce bovine [Validation of serum haptoglobin as an acute phase protein in cattle]. PhD Thesis. Press of the Faculty of Veterinary Medicine, University of Liège, Liège, Belgium, 2005.
23. Panndorf H, Richter H, Dittrich B. Haptoglobin in domestic mammals. V. Plasma haptoglobin level in cattle under pathological conditions. *Arch Exp Veterinarmed.* 1976;30:193–202.
24. Tietz NW. Establishment and use of reference values. In: *Fundamentals of Clinical Chemistry.* 3rd ed. Philadelphia, PA: WB Saunders; 1987: 202–209.
25. Koets AP, De Schwartz N, Tooten P. Release of proinflammatory cytokines related to luteolysis and the periparturient acute phase response in prostaglandin-induced parturition in cows. *Theriogenology* 1998;49:797–812.
26. Uchida E, Katoh N, Takahashi K. Appearance of haptoglobin in serum from cows at parturition. *J Vet Med Sci.* 1993;55:893–894.
27. Skinner JG. Haptoglobin—a useful marker for detecting periparturient infection in automated metabolic profile screening. In: *Proceedings of the Vth Congress of the International Society for Animal Clinical Biochemistry,* 1992, Parma, Italy; p 133.
28. Alsemgeest SPM, Taverne MAM, Boosman R, Van Der Weyden BC, Gruys E. Peripartum acute-phase protein serum amyloid A concentration in plasma of cows and fetuses. *Am J Vet Res.* 1993;54:165–167.
29. Jacobsen S, Andersen PH., Toelboell T, Heegaard PMH. Dose dependency and individual variability of the lipopolysaccharide-induced bovine acute phase protein response. *J Dairy Sci.* 2004;87: 3330–3339.
30. Horadagoda NU, Knox KWG, Gibbs HA, et al. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Vet Rec.* 1999;144:437–441.
31. Smith BI, Donovan GA, Risco CA, Young CR, Stanker LH. Serum haptoglobin concentrations in Holstein dairy cattle with toxic puerperal metritis. *Vet Rec.* 1998;142:83–85.
32. Hirvonen J, Huszenicza G, Kulcsar M, Pyorala S. Acute-phase response in dairy cows with acute postpartum metritis. *Theriogenology* 1999;51:1071–1083.
33. Jahoor F, Sivakumar B, Delrosario M, Frazer EM. Isolation of acute-phase proteins from plasma for determination of fractional synthesis rate by a stable isotope tracer technique. *Anal Biochem.* 1996;236: 95–100.
34. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med.* 1999;340:448–454.