Acute phase protein concentrations in serum and milk from healthy cows, cows with clinical mastitis and cows with extramammary inflammatory conditions

B. H. NIELSEN, S. JACOBSEN, P. H. ANDERSEN, T. A. NIEWOLD, P. M. H. HEEGAARD

The concentrations of the two acute phase proteins, serum amyloid A and haptoglobin, in serum and milk were compared in 10 cows with clinical mastitis, 11 cows with extramammary inflammatory conditions and 10 clinically healthy control cows. The concentrations of both acute phase proteins were higher in the serum and milk of the cows with mastitis than in the cows in the other two groups. Four of the cows with extramammary inflammatory conditions had serum amyloid A concentrations in serum above 100 µg/ml, but negligible concentrations in milk, indicating that a pathogen must be present in the mammary gland for serum amyloid A to accumulate in milk. The acute phase protein concentrations in milk increased significantly with increasing somatic cell count, suggesting that they may be indicators of the severity of an infection.

MASTITIS is the most costly disease affecting the dairy industry (Radostits and others 1994). Its rapid and accurate diagnosis is crucial because it optimises the effect of treatment and minimises the recovery time, thereby reducing production losses and improving the animals' welfare. The diagnosis of mastitis is based predominantly on a clinical examination, measurements of somatic cell count and the cultivation of pathogens from the milk, but the demand for objective and rapidly assessable markers of udder health has increased with the introduction of robotic milking systems. The acute phase proteins have recently been suggested as such indicators of inflammation in the mammary gland (Hirvonen and others 1999a, Eckersall and others 2001).

The acute phase response refers to a group of non-specific host responses to a wide variety of stimuli and it is characterised by changes in the concentrations of a number of hepatically synthesised plasma proteins – the so-called acute phase proteins (Baumann and Gauldie 1994). Serum amyloid A and haptoglobin are the two major acute phase proteins in cattle. They are potentially useful as disease markers owing to their low concentration in normal animals, the rapid increase in their concentration during the acute phase of inflammation and their rapid decrease with the resolution of the disease (Gruys and others 1994, Eckersall 2000).

The concentrations of these two acute phase proteins in bovine serum increase during either spontaneous or induced mastitis (Conner and others 1986, Hirvonen and others 1996, Salonen and others 1996). Eckersall and others (2001) were the first to demonstrate significant increases in their concentrations in the milk of cows with clinical mastitis. Their results suggested that serum amyloid A was produced locally in the mammary gland, potentially making it an early, specific marker of mastitis, which might prove more sensitive than a bacteriological examination and less influenced by the physiological stage of the cow than the somatic cell count or the electrical conductivity of the milk (Sheldrake and others 1983, Biggadike and others 2002). However, it is still not known whether inflammatory processes occurring outside the mammary gland influence its concentration in milk.

The aim of this study was to examine the relationships between the concentrations of the acute phase proteins in the serum and milk of cows with clinical mastitis, cows with miscellaneous inflammatory disorders other than mastitis, and clinically healthy cows.

Veterinary Record (2004) **154,** 361-365

B. H. Nielsen, DVM,
S. Jacobsen, DVM, PhD,
P. H. Andersen, DVM, PhD, DrVetSci,
Department of Clinical Studies, Large Animal Surgery, Royal Veterinary and Agricultural University, Dyrlægevej 48, DK-1870 Frederiksberg C, Copenhagen, Denmark
T. A. Niewold, PhD,
Research Group Animal Physiology and Health, Institute for Animal Science and Health (ID-Lelystad BV), PO Box 65, NL-8200 AB Lelystad, The Netherlands
P. M. H. Heegaard, PhD,
Department of Immunology and Biochemistry, Danish Veterinary Institute, Bülowsvej 27, DK-1790 Copenhagen V, Denmark

MATERIALS AND METHODS

Animals

Samples were collected from 31 Danish red dairy cows from a commercial dairy farm with 260 cows. The cows were lactating, primi- and multiparous, and had calved more than 10 days previously. They were selected on the basis of a general clinical examination and examinations of the udder and milk samples; there were 10 cows with mastitis but free from any other clinical signs of inflammation, 11 cows with extramammary inflammatory disorders and 10 healthy control cows with no clinical signs of inflammation.

Samples

Blood samples were taken from a jugular vein into a vacutainer (PrecisionGlide; Becton Dickinson), left to clot for 18 to 20 hours and then centrifuged at 2500 *g* for 15 minutes; serum was separated and stored at –18°C until analysed for serum amyloid A and haptoglobin.

Milk samples were collected in sterile plastic vials. The surface of the teat end was disinfected by wiping it with clean cotton dipped in 95 per cent alcohol. Forestrips were milked out and then 5 to 10 ml were milked into the vial, which was held almost horizontally to avoid bacterial contamination; the samples were taken within three hours of milking. From the cows with mastitis, the milk samples were taken from a quarter with clinical signs of mastitis (quarter 1) and from the diagonally opposed quarter (quarter 2); from the other two groups the milk samples were taken from two diagonally opposed quarters, which were both free from signs of clinical mastitis.

The milk samples were examined by bacteriological cultivation, using standard methods, at a commercial laboratory. The somatic cell counts were assessed semiquantitatively by the California mastitis test (CMT) (Kruuse) according to the manufacturer's instructions. CMT values of 1 and 2, corresponding to a somatic cell count of less than 300,000, were accepted in the cows with extramammary inflammatory conditions and in the control cows. Samples of whole milk were stored at –18°C until they were analysed for serum amyloid A and haptoglobin.

Serum amyloid A assay

The concentrations of serum amyloid A were determined with a commercially available ELISA (Phase Range Serum

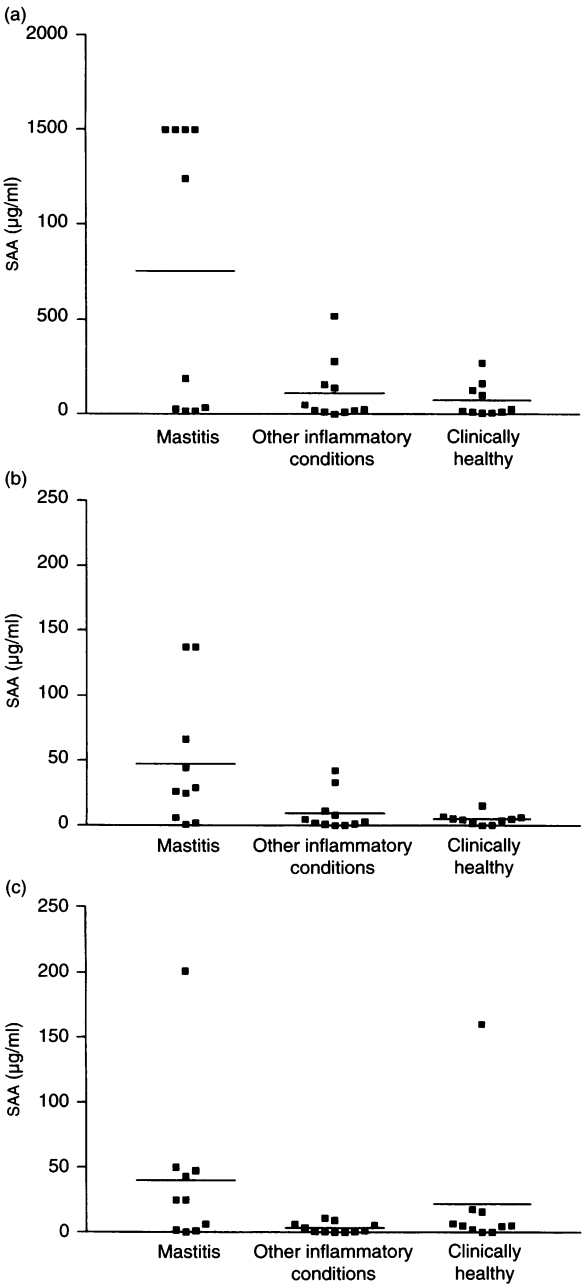

FIG 1: Serum amyloid A (SAA) concentrations in (a) serum, (b) milk from quarter 1, and (c) milk from quarter 2 from 10 cows with mastitis, 11 cows with extramammary inflammation and 10 healthy cows; horizontal bars show the mean values

Amyloid A Assay; Tridelta), first described by McDonald and others (1991), performed according to the manufacturer's instructions. The serum and milk samples were initially diluted 1:500 and 1:50, respectively, and all the samples, including the standards, were tested in duplicate. Samples with an optical density outside the range of the standard curve were diluted further and re-analysed. The maximum dilution was 1:5000 for the serum samples and 1:2000 for the milk samples, and as the standard curve maximum was 300 ng/ml the maximum serum concentration that could be determined was 1500 µg/ml. The optical densities were read on an automatic plate reader (model 550; Bio-Rad) at 450 nm with a reference at 595 nm.

TABLE 1: Mean (sd) serum amyloid A and median haptoglobin concentrations in samples of serum and milk from 10 cows with mastitis, 11 cows with extramammary inflammatory conditions and 10 clinically healthy cows

	Serum amyloid A (µg/ml)			Haptoglobin (µg/ml)		
	Mastitis	Other inflammatory conditions	Clinically healthy	Mastitis	Other inflammatory conditions	Clinically healthy
Serum	752 (739)	112 (160)	75·0 (90·0)	788	0	0
Quarter 1	47·5 (51·4)	9·8 (14·3)	5·1 (4·4)	110	0	0
Quarter 2	40·2 (59·9)	3·4 (4·0)	21·8 (49·1)	1·0	0	0

Haptoglobin assay

The concentration of haptoglobin in the serum and milk samples was determined by a sandwich ELISA (Godson and others 1996) as described by Heegaard and others (2000), using a pool of bovine serum as standard. The standard was calibrated against a standard serum obtained from EU Concerted Action on standardisation of animal acute phase proteins (QLK5-CT-1999-01532). The lower detection limit of the assay, as defined by the linear range of the standard curve, was 0·05 µg/ml. The serum samples were tested in serial dilutions of 1:100, 1:300 and 1:900, and the milk samples in serial dilutions of 1:10, 1:30 and 1:90, resulting in a lower limit of detection of 50 µg/ml for serum and 0·5 µg/ml for milk.

Statistics

All the statistical analyses were carried out in GraphPad Prism, version 3.02.

To obtain equal variances in the three groups the serum amyloid A data were transformed logarithmically before a one-way analysis of variance was used to compare its concentrations in the three groups and to compare data from cows with different CMT scores. Even after transformation, the haptoglobin data were not normally distributed, and median values and the non-parametric Kruskal-Wallis test were therefore used to compare its concentrations in the three groups and in cows with different CMT scores.

The correlation between the concentrations of serum amyloid A in serum and milk was tested by linear regression and a 95 per cent confidence interval constructed. The assumptions were checked by residual plots and tested for normality.

RESULTS

Clinical examination

The 10 cows with clinical signs of mastitis had changes in the texture, secretions and/or local skin colour and temperature of the affected quarters, increased electrical conductivity of the milk and CMT values over 2. The 11 cows with clinical signs of extramammary inflammatory disease suffered from interdigital phlegmon, purulent metritis and/or periarthritis/bursitis, but there were no abnormalities in the udder on inspection or palpation and no abnormalities in the milk.

None of the 10 control cows showed any clinical signs of inflammatory disease and none had abnormalities in the udder on inspection or palpation, or abnormalities in the milk.

Bacteriological examination of milk samples

In total, 62 milk samples were examined bacteriologically; two samples were excluded because they were contaminated and one was lost during transportation, making the final number 59. The following pathogens were identified in the milk samples from the cows with mastitis: *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Escherichia coli*, yeasts and enterococci/*Streptococcus lactis*/*Streptococcus faecalis*. One cow had bacterio-

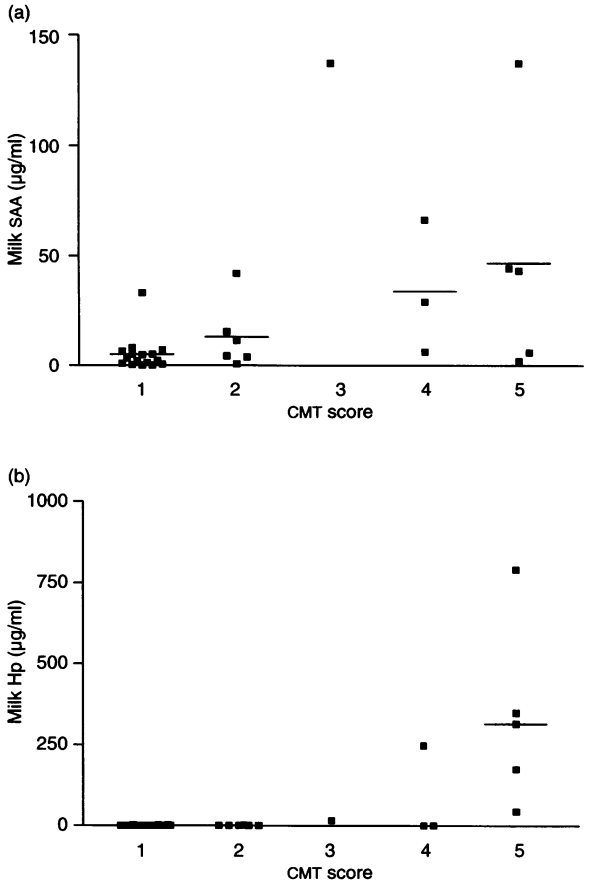

FIG 2: Milk (a) serum amyloid A (SAA) and (b) haptoglobin (Hp) concentrations in the five California Mastitis test (CMT) score groups; the horizontal bars show the mean values in (a) and the median value in (b)

logically positive samples from both quarters, one had sterile samples from both quarters and seven cows had positive samples from one quarter only. One quarter 1 sample was lost and the quarter 2 sample from this cow was sterile.

Of the 21 cows in the other two groups, 13 had sterile samples from both quarters, three had positive samples from both quarters, four had a positive sample from one quarter, and both samples from the other cow were contaminated. The following pathogens were identified in the milk samples: coagulase-negative staphylococci/micrococci, coliforms, enterococci/*S lactis/S faecalis*, and *Staphylococcus aureus*.

Concentrations of serum amyloid A in serum and milk

The concentrations of serum amyloid A in both serum and milk were significantly different between the three groups ($P<0.05$ for serum, $P<0.01$ for quarter 1 and $P<0.05$ for quarter 2), with the mastitis group having the highest concentrations (Table 1, Fig 1). Linear regression analysis revealed that there was no correlation between its concentrations in serum and milk in any of the three groups.

Four of the 11 cows with extramammary inflammatory conditions had serum amyloid A concentrations above 100 μg/ml (range 139·0 to 519·6 μg/ml), but milk samples from them contained only minute amounts, with means of 3·3 and 1·1 μg/ml for quarters 1 and 2, respectively.

The concentration of serum amyloid A in milk increased significantly ($P<0.02$) with increasing CMT score (Fig 2).

Concentrations of haptoglobin in serum and milk

The concentrations of haptoglobin in both serum and milk were significantly different between the three groups ($P<0.02$ for serum, $P<0.01$ for quarter 1 and $P<0.05$ for quarter 2) with the mastitis group having the highest concentrations (Table 1, Fig 3).

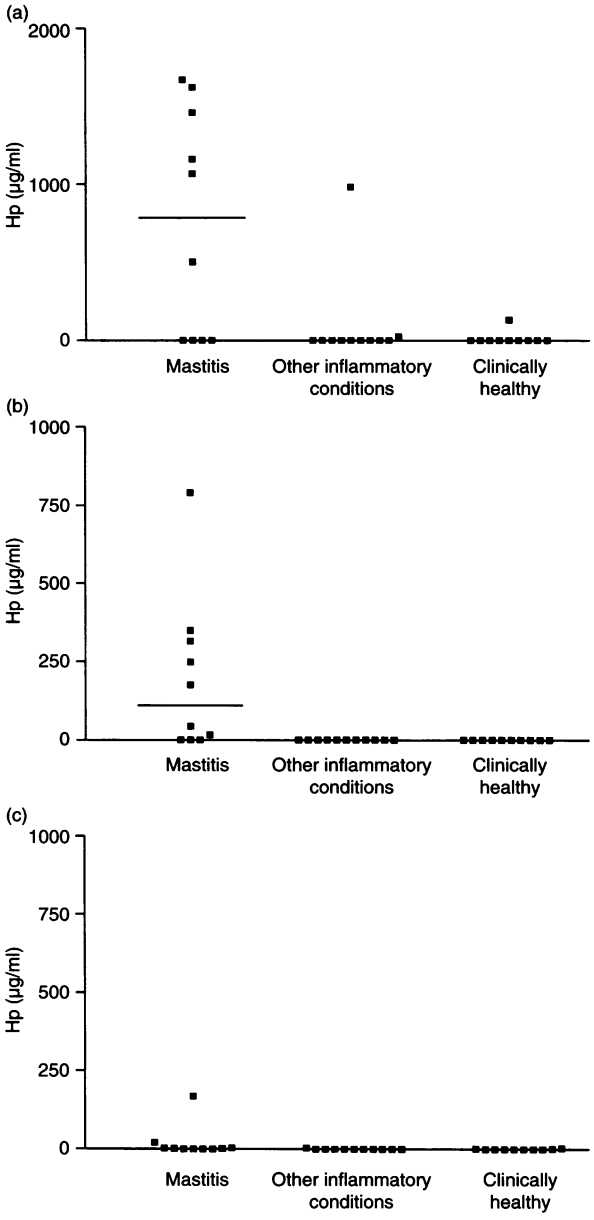

FIG 3: Haptoglobin (Hp) concentrations in (a) serum, (b) milk from quarter 1 and (c) milk from quarter 2 from cows suffering from mastitis, cows with extramammary inflammation and healthy cows; the horizontal bars show the median values

Only two of the 11 cows with extramammary inflammatory disorders had serum haptoglobin concentrations above the detection limit (one cow with endometritis and one with an interdigital phlegmon) (Table 1, Fig 3).

Linear regression analysis showed that there was a significant correlation between the concentrations of haptoglobin in the serum and the milk (quarter 1) of the cows with mastitis ($P<0.05$, $r^2=0.41$).

The concentration of haptoglobin in milk increased significantly ($P<0.01$) with increasing CMT score (Fig 2).

DISCUSSION

The acute phase proteins are non-specific markers of inflammation, the serum concentrations of which increase in response to several infections and inflammatory conditions.

For them to be useful as specific indicators of mastitis it is therefore essential that they accumulate in milk only during episodes of mammary inflammation. The results of this study show that the concentrations of serum amyloid A and haptoglobin were higher in milk from infected quarters and that extramammary inflammatory processes that induced increases in the serum concentration of serum amyloid A were not accompanied by increases in its concentration in milk. An intramammary inflammatory stimulus thus seems to be required for the concentration of serum amyloid A in milk to increase. Whether the same is true for haptoglobin could not be assessed, because only one of the 11 cows with an extramammary inflammation had an increased serum concentration of haptoglobin. This was a surprising finding, because previous studies have observed increases in the concentration of haptoglobin in serum in a variety of inflammatory conditions (Skinner and others 1991, Alsemgeest and others 1994). However, a variable response or a complete lack of a haptoglobin response has been reported in cows with metritis (Smith and others 1998, Hirvonen and others 1999b). The cows with mastitis had lower serum iron and higher fibrinogen concentrations than the cows with extramammary inflammations (data not shown) and it is possible that the cows with mastitis had a more acute or more severe inflammation.

The higher concentrations of acute phase proteins in the milk from infected quarters could be due either to their production within the gland or to their leakage across the blood-milk barrier as a result of its disruption by the inflammation, or to a combination of these mechanisms. It has been shown that the permeability of the blood-milk barrier increases during episodes of mastitis allowing serum proteins to gain access to the milk (Shuster and others 1997). However, the demonstration of serum amyloid A gene expression at several extrahepatic sites and of several cell types that can synthesise the protein (Marhaug and others 1997, Vreugdenhil and others 1999), together with the recent identification of a unique isoform of the protein in normal bovine colostrum (McDonald and others 2001), suggests that serum amyloid A may be synthesised locally during episodes of mastitis. In a study of clinical mastitis, Eckersall and others (2001) observed that the concentrations of serum amyloid A in milk and serum were not correlated and they suggested that this might indicate that it was produced locally in the inflamed mammary gland. The results of this study agree; whereas the concentrations of haptoglobin in milk and serum were correlated, there was no correlation in the case of serum amyloid A.

The extent to which acute phase proteins cross from blood to milk (and vice versa) is not known. The fact that there was an increase in the serum concentration of haptoglobin only in the cows with mastitis might suggest that haptoglobin produced by the mammary tissue gains access to the blood serum. However, the lack of a haptoglobin response by the cows with extramammary inflammation may be explained by a number of other factors.

It has been shown that in some cases acute phase proteins appear in the milk of cows with clinical mastitis even before the somatic cell count starts to rise (Hogarth and others 2002). In the present study, the milk concentrations of the acute phase proteins increased significantly with increasing CMT score, indicating that they may be valuable as an indication of severity. The diagnostic potential of measurements of acute phase proteins in the serum of cattle is well documented (Conner and others 1986, Alsemgeest and others 1994, Karreman and others 2000), and the increases observed in the concentrations of serum amyloid A and haptoglobin in the serum and milk of cows with acute mastitis are in line with several previous studies (Conner and others 1986, Hirvonen and others 1996, Salonen and others 1996, Eckersall and others 2001). Four of the cows with mastitis had very low serum concentrations of both acute phase proteins, despite having clinical signs of mastitis and high somatic cell counts. However, three of them had high concentrations of serum amyloid A in their milk and two had high concentrations of haptoglobin. This apparent discrepancy between the clinical findings and the serum and milk concentrations of the acute phase proteins in these four cows may have been due to variations in the duration or the severity of the udder infection.

The results of this study suggest that measurements of the concentrations of acute phase proteins in milk may be useful in the online monitoring of udder health in robotic milking systems.

ACKNOWLEDGEMENTS

The authors are grateful to Pharmacia Animal Health, Copenhagen, Denmark, and the Memorial Foundation of Søren Chr. Sørensen and his wife for supporting the project financially; the staff at Assendrup Hovedgaard, Haslev, Denmark, are thanked for their valuable help and cooperation with the sampling.

References

ALSEMGEEST, S. P. M., KALSBEEK, H. C., WENSING, T., KOEMAN, J. P., VAN EDEREN, A. M. & GRUYS, E. (1994) Concentrations of serum amyloid A (SAA) and haptoglobin (Hp) as parameters of inflammatory diseases in cattle. *Veterinary Quarterly* **16,** 21-23

BAUMANN, H. & GAULDIE, J. (1994) The acute phase response. *Immunology Today* **15,** 74-80

BIGGADIKE, H. J., OHNSTAD, I., LAVEN, R. A. & HILLERTON, J. E. (2002) Evaluation of measurements of the conductivity of quarter milk samples for the early diagnosis of mastitis. *Veterinary Record* **150,** 655-658

CONNER, J. G., ECKERSALL, P. D., DOHERTY, M. & DOUGLAS, T. A. (1986) Acute phase response and mastitis in the cow. *Research in Veterinary Science* **41,** 126-128

ECKERSALL, P. D. (2000) Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. *Revue de Médicine Vétérinaire* **151,** 577-584

ECKERSALL, P. D., YOUNG, F. J., MCCOMB, C., HOGARTH, C. J., SAFI, S., WEBER, A., MCDONALD, T., NOLAN, A. M. & FITZPATRICK, J. L. (2001) Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Veterinary Record* **148,** 35-41

GODSON, D. L., CAMPOS, M., ATTAH-POKU, S. K., REDMOND, M. J., CORDEIRO, D. M., SETHI, M. S., HARLAND, R. J. & BABIUK, L. A. (1996) Serum haptoglobin as an indicator of the acute phase response in bovine respiratory disease. *Veterinary Immunology and Immunopathology* **51,** 277-292

GRUYS, E., OBWOLO, M. J. & TOUSSAINT, M. J. M. (1994) Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. *Veterinary Bulletin* **64,** 1009-1018

HEEGAARD, P. M. H., GODSON, D. L., TOUSSAINT, M. J. M., TJØRNEHØJ, K., LARSEN, L. E., VIUFF, B. & RØNSHOLT, L. (2000) The acute phase response of haptoglobin and serum amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. *Veterinary Immunology and Immunopathology* **77,** 151-159

HIRVONEN, J., EKLUND, K., TEPPO, A. M., HUSZENICZA, G., KULSCÁR, M., SALONIEMI, H. & PYÖRÄLÄ, S. (1999a) Acute phase response in dairy cows with experimentally induced *Escherichia coli* mastitis. *Acta Veterinaria Scandinavica* **40,** 35-46

HIRVONEN, J., HUSZENICZA, G., KULSCÀR, M. & PYÖRÄLÄ, S. (1999b) Acute-phase response in dairy cows with acute postpartum metritis. *Theriogenology* **51,** 1071-1083

HIRVONEN, J., PYÖRÄLÄ, S. & JOUSIMIES-SOMER, H. (1996) Acute phase response in heifers with experimentally induced mastitis. *Journal of Dairy Research* **63,** 351-360

HOGARTH, C. J., FITZPATRICK, J. L., NOLAN, A. M. & ECKERSALL, P. D. (2002) The acute phase response in bovine mastitis. Proceedings of the XXII World Buiatrics Congress. Hannover, Germany, August 18 to 23, 2002. p 121

KARREMAN, H. J., WENTINK, G. H. & WENSING, T. (2000) Using serum amyloid A to screen dairy cows for sub-clinical inflammation. *Veterinary Quarterly* **22,** 175-178

MCDONALD, T. L., LARSON, M. A., MACK, D. R. & WEBER, A. (2001) Elevated extrahepatic expression and secretion of mammary-associated

serum amyloid A 3 (M-SAA3) into colostrum. *Veterinary Immunology and Immunopathology* **83,** 203-211
MCDONALD, T. L., WEBER, A. & SMITH, J. W. (1991) A monoclonal antibody sandwich immunoassay for serum amyloid A (SAA) protein. *Journal of Immunological Methods* **144,** 149-155
MARHAUG, G., HACKETT, B. & DOWTON, S. B. (1997) Serum amyloid A expression in rabbit, mink and mouse. *Clinical and Experimental Immunology* **107,** 425-434
RADOSTITS, O. M., BLOOD, D. C. & GAY, C. C. (1994) Mastits. In Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 8th edn. London, Baillière Tindall. pp 563-701
SALONEN, M., HIRVONEN, J., PYÖRÄLÄ, S., SANKARI, S. & SANDHOLM, M. (1996) Quantitative determination of bovine serum haptoglobin in experimentally induced *Escherichia coli* mastitis. *Research in Veterinary Science* **60,** 88-91
SHELDRAKE, R. F., HOARE, R. J. T. & MCGREGOR, G. D. (1983) Lactation stage, parity, and infection affecting somatic cells, electrical conductivity, and serum albumin in milk. *Journal of Dairy Science* **66,** 542-547
SHUSTER, D. E., KEHRLI, M. E., Jr, RAINARD, P. & PAAPE, M. J. (1997) Complement fragment C5a and inflammatory cytokines in neutrophil recruitment during intramammary infection with *Escherichia coli*. *Infection and Immunity* **65,** 3286-3292
SKINNER, J. G., BROWN, R. A. L. & ROBERTS, L. (1991) Bovine haptoglobin response in clinically defined field conditions. *Veterinary Record* **128,** 147-149
SMITH, B. I., DONOVAN, G. A., RISCO, C. A., YOUNG, C. R. & STANKER, L. H. (1998) Serum haptoglobin concentrations in Holstein dairy cattle with toxic puerperal metritis. *Veterinary Record* **142,** 83-85
VREUGDENHIL, A. C. E., DENTENER, M. A., SNOEK, A. M. P., GREVE, J-W. M. & BUURMAN, W. A. (1999) Lipopolysaccharide binding protein and serum amyloid A secretion by human intestinal epithelial cells during the acute phase response. *Journal of Immunology* **163,** 2792-2798

Clinicopathological findings in sheep from Sardinia showing neurological signs of disease

C. LIGIOS, A. VIGLIETTI, P. CARTA, G. DEXTER, U. AGRIMI, M. M. SIMMONS

Histopathological and bacteriological examinations were performed on 178 brains from Sardinian sheep which were showing neurological signs. The sheep represented the total number of sheep with neurological syndromes submitted for diagnostic investigations over a three-year period in Sardinia. Scrapie was detected in 57 cases, cerebrocortical necrosis in 25, intoxication by a typical Mediterranean plant (*Cistus species*) was suspected in 25, coenurosis was detected in 11 cases, *Listeria monocytogenes* in eight cases and focal symmetrical encephalomalacia in six cases. Non-suppurative inflammatory changes were observed in three of the brains and suppurative changes were noted in two. Lesions restricted to the spinal cord were found in three cases. In the remaining 38 cases there were no significant neuropathological changes.

Veterinary Record (2004) **154,** 365-370

C. Ligios, DVM,
A. Viglietti, DVM,
P. Carta, DVM,
Istituto Zooprofilattico Sperimentale della Sardegna, via Duca degli Abruzzi 8, 07100 Sassari, Italy
G. Dexter, HNC,
M. M. Simmons, BVMS, MVM, PhD, MRCVS,
Pathology Department, VLA – Weybridge, New Haw, Addlestone, Surrey KT15 3NB
U. Agrimi, DVM,
Laboratorio di Medicina Veterinaria, Istituto Superiore di Sanità, Rome, Italy

THE importance of knowing more about nervous diseases in sheep has been emphasised over the past few years by the emergence of bovine spongiform encephalopathy (BSE), which may have come from scrapie in sheep through feeding cattle with meat and bone meal containing the scrapie agent (Wilesmith and others 1991). Scrapie and BSE belong to the family of transmissible spongiform encephalopathies (TSEs), and there is evidence that the agent of BSE is indistinguishable from the agent of variant Creutzfeld-Jacob disease (Bruce and others 1997). So far, scrapie is the only neurodegenerative disease of the TSE group that has been found in sheep and goats. However, the possibility that sheep could be harbouring the BSE agent has aroused great concern about the implications for human health of ovine TSEs. Indeed, BSE can be transmitted experimentally to sheep (Foster and others 1993), and preliminary studies indicate that the clinical and neuropathological signs of experimental oral BSE infection in sheep are not readily distinguishable from these described in natural scrapie (Ligios and others 2002).

Since 1998, the health authorities of the EU (Decision 98/272/EC) have imposed surveillance planning for TSEs on each member state. For this reason, the clinical and pathological signs of all nervous diseases must be assessed, particularly in small ruminants. Owing to their minor economic importance, nervous diseases in these species have been less well investigated than those in cattle, and there is a lack of clearly defined clinical parameters for many neurological disorders. However, such neuropathological investigations have great economic implications for Sardinia, where there are about 3,500,000 sheep of the Sarda breed, the most widespread and important dairy breed of sheep in Italy.

Nevertheless, there have been few surveys of neuropathological disease in Sarda sheep, and they have focused mostly on slaughtered sheep, in which there is a low incidence of clinical nervous diseases (Guarda and others 1979, Cerruti-Sola and others 1981).

This paper reports the diagnoses recorded during three years of diagnostic work in native Sardinian sheep showing neurological signs.

MATERIALS AND METHODS

The brains of 178 sheep, three months to seven years of age, from 126 farms, were examined between 1998 and 2001; they were collected from all sheep clinically suspected of having neurological disorders and submitted for diagnostic investigations during the period 1998 to 2001 to the Istituto Zooprofilattico Sperimentale of Sardinia. Some of the sheep arrived alive at the laboratory and were euthanased with a