Comp Haematol Int (1995) 5:149–157
© 1995 Springer-Verlag London Limited

COMPARATIVE
HAEMATOLOGY
INTERNATIONAL

Original Article

Implication of Clinical Pathology in Assessment of Animal Health and in Animal Production and Meat Inspection

M. J. M. Toussaint, A. M. van Ederen and E. Gruys

Department of Veterinary Pathology, Utrecht University, Utrecht, The Netherlands

Abstract. Clinical, zootechnical and industrial developments of the last decades have led to new ideas on monitoring systems for animal production and meat inspection. Quality assessment systems, integrated monitoring, risk assessment concerning consumer's health and monitoring for infectious animal diseases, are more relevant today than pathomorphological control of individual subjects. Published papers on investigations to assess slaughtered animals by blood variables of classical clinical pathology and by measuring acute phase reactants, are mentioned. Most papers deal with ruminants giving good perspectives for the acute phase proteins in that species. Only limited literature is available regarding acute phase proteins in swine; zinc and iron have been shown to be of little value for general health monitoring in swine.

Preliminary studies on limited numbers of finishing pigs showed good prospects for the development of species specific assays for acute phase proteins for future practical use in the slaughterhouse. Isolation procedures for porcine haptoglobin (HP), serum amyloid A (SAA) and C reactive protein (CRP) are described. It is suggested that for monitoring individual animals, the signal of acute phase variable can be amplified considerably by applying an acute phase index (API) combining positive × negative reactants^{-1}. Calculations on the original data from a former investigation on 233 non-healthy and 21 control cattle gave excellent results for an API. Similar studies are in progress for swine.

Originally presented at the Second European Comparative Clinical Pathology Conference, Dijon

Correspondence and offprint requests to: Prof. E. Gruys, Department of Pathology, Veterinary Faculty, Utrecht University, Yalelaan 1, 3508 TD Utrecht, The Netherlands.

Keywords: Acute phase index; Acute phase proteins; Blood; Cattle; Clinical chemistry; Haematology; Haptoglobin; Isolation procedure; Pig; SAA

Introduction

With the increment of zootechnical possibilities and resulting increases in farm sizes, in most western countries the general health status of the farmed animal has changed and zoonotic diseases such as tuberculosis and trichinellosis are now of minimum prevalance. Consequently, the role of the clinical veterinary profession has changed from applied curative action on sick animals to organised farm guidance, and the function of governmental meat inspection is evolved from one controlling possible carriers of zoonotic agents and hygiene, to additionally encompass another of general quality assurance.

The present paper reviews the developments in the changed veterinary guidance in the meat sector and discusses new possibilities offered by monitoring animal health by blood variables. Our own results in the area of acute phase reactants to assess finishing animals (cattle and pigs at slaughter) will be mentioned and discussed.

Veterinary Guidance in the Meat Sector

Investigations on Quality Assessment Systems

As early as 1960, in the UK a group of swine producers founded a 'Pig Health Control Association', as they wished to use the superior health status of their herds as

a basis for increased trade. The concept of this group was followed in other countries, e.g., in the USA, Denmark, Belgium, Switzerland and Canada (Davies 1992). Monitoring herd health in pigs at slaughter was developed in several countries using different systems, but all concentrating, more or less, on the same major pathological problems: abscesses, mange, atrophic rhinitis, pleuritis, pneumonia, ascariasis, oesophago-gastric ulcers and porcine intestinal adenomatosis (Nilsson 1982; Christensen and Cullinane 1990; Gardner and Hird 1990; Mousing et al. 1990; Liuim and Falk 1991; Elbers et al. 1992; Hurnik et al. 1993, 1994; Mousing and Christensen 1993). Studies to monitor lesions for impact on growth, association with microbiology, or clinical disease episodes during the rearing period at the farm followed (Wertenbroek 1981; Bernardo et al. 1990a,b; Noyes et al. 1990; Falk et al. 1991; Hoie et al. 1991; Hill et al., 1992; Lingaas, 1993; Paisley et al., 1993a,b; Hill et al. 1994; Wallgren et al. 1994). For monitoring porcine infections which may have an impact on growth, several serological studies for different agents on finishing pigs have been published (Elbers et al. 1990; Falk and Lium 1991; Bogh et al. 1994; Ewald et al. 1994).

From 1978 onwards government-guided models were developed throughout the industry in Denmark (Willeberg et al. 1984) and other countries, such as Australia and America (Davies 1992). In The Netherlands 'Integral Chain Control' projects including systemic abattoir herd surveys have been performed in order to attain industrial, collaboratively organised, Integrated Quality Assessment Systems (Tielen and Elbers 1988; Harbers 1991; Harbers et al. 1992; Blocks et al. 1994). In these systems, feed and medication data from the farm accompany the animals when sent to slaughter, whereas the recorded post mortem lesions are used to monitor heard health at the farm (Elbers 1991; Geudeke 1992). Most of these studies concerned pigs and the pork industry. However, similar projects have been developed for veal calves and broiler chickens.

Developments in Meat Inspection Concerning Integrated Quality Assessment Systems

In parallel to technical and health developments, the conveyor speed in meat factories has increased considerably. This includes a new risk, that of missed diagnoses. In the meantime the meat inspection system could no longer give assurance relating to hidden zoonotic agents, toxins and residues, and the changed zootechnical systems induced the risk of contamination with some (new) zoonotic agents, feed additives or prophylactic veterinary drugs. For up-to-date meat inspection, new systems needed to be developed (Berends et al. 1993a), and within the European Union (EU directive 92/117/EEC) integrated quality monitoring methods were proposed and optionally included in national regulations of member states. System guidance by applying hazard analysis of critical control points (HACCP) was also added to the EU directives (93/43/EEC) (Berends and Snijders 1994; Gerigk and Ellerbroek 1994).

Integrated Monitoring and Risk Assessment

Notwithstanding quality amelioration by the integrated assessment systems, from epidemiological–statistical tables it is evident that in the case of slaughter, all carcasses have to be carefully controlled by routine pathological–anatomical survey in order to reach reliable figures for all recognisable lesions (Wouda et al. 1987). Considering the types of lesions presently encountered, however, it is questionable whether this is still necessary (Hathaway and Richards 1993). Risk assessment and the accceptability of risks to human health appear to represent the modern fundamental approach, allowing for the system of inspection described above to be performed. For some zoonotic agents with low prevalence (such ask *Trichinella spiralis* or *Toxoplasma gondii* in pigs), serological monitoring might be an alternative surveillance system (Berends et al. 1991). Van Knapen (personal communication) has suggested monitoring wild carnivorous predators for trichinellosis instead of surveillance of farmed meat in the slaughterhouse. For more frequently occurring zoonoses such as salmonellosis, serology as well as monitoring of carcass drip fluid might be appropriate.

Infectious Animal Diseases

The risk of spread of infectious animal diseases also became another factor in this assessment, and may possibly be of more importance than the risks for the consumer. International trade agreements have started to ban most monofactorial infectious agents such as porcine and bovine herpes viruses, swine vesicular disease agent and the viruses causing classical and African swine fever. For monitoring such agents, various nationwide serological surveys are to be performed. Assessment by serology of slaughtered animals could give a clear retrospective view on the diseases during the life of the animals.

Assessment of Animal Health by Haematology and Blood Chemistry

Classical Clinical Pathology

In the last decade, there has been further industrialisation of meat plants. Since in robotised conveyor lines, abscesses might infect other carcasses, various blood studies have been performed in order to detect animals with grossly hidden abscesses before further slaughtering. In finishing pigs at the farm and in slaughtered pigs an investigation was carried out to establish whether some blood variables may have predictive value with

regard to inflammatory processes (Odink et al. 1990; Smeets et al. 1990; Elbers et al. 1991). The results revealed different variables (e.g. erythrocyte sedimentation rate (ESR), fibrinogen, albumin, total protein, differentiation of white blood cells) to be valuable tools in identifying groups of finishing pigs with lesions, including animals with abscesses. A striking result was the finding (after meticulous dissection) of several subclinical lesions in groups of 'normal healthy' finishing pigs, where the results of blood variables (including plasma viscosity and retinol) from the same animals appeared to further categorise the severity of these lesions (Visser et al. 1992; Alsemgeest et al. 1992).

These results encouraged the continuation of investigations on blood components as control variables for pigs at slaughter, although the variables used at this time were not reliable enough (as indicated by sensitivity and specificity) to identify positively individual animals as totally healthy or not (Smeets et al. 1990). A need remained for reliable blood variables which could be determined quickly and which had good specificity and sensitivity concerning health status. For example, stress and fatigue due to transport influence meat quality (Maeda et al. 1989; Mori et al. 1990; Klont 1994), and since both exhaustion and acute diseases such as pneumonia, can be associated with catabolism of muscles, both these processes might influence meat quality in terms of dark firm dry (DFD) meat. The relevance of covering these disadvantages with the same blood analysis is evident. In the investigation on healthy finishing pigs (Visser et al. 1992), subjects classified as having more serious lesions, including those with acute fibrinous pneumonia, were found to have a higher meat pH (Berends et al. 1993b).

Acute Phase Reactants as Sensitive Variables for Non-healthy

During infection, inflammation and tissue damage in general, proinflammatory cytokines (IL-1, TNFα, IL-6, IL-8) appear in the circulation. As a result, fever and changes in the hepatic production of blood proteins can be recorded (Heinrich et al. 1990; Kushner 1993). A problem with measuring body temperature is that stress and transport also induce it to rise, and therefore, this variable is not useful for monitoring animals at slaughter. The cytokines are small molecules (<30 kD; Ingenbleek and Young 1994) that disappear rather quickly from the circulation and for that reason they do not present practical options for measuring variables. A better perspective was expected for the acute phase reactants (APR), since these variables form a secondary phenomenon related to the activity of a disease process (Gruys et al. 1993, 1994). They react sensitively and non-specifically to a wide variety of disorders from exhaustion to infection and thus represent good variables for monitoring animals with regard to their health status and the severity of any lesion. Even in longer-lasting diseases, the acute phase reactants maintain their changed concentration as long as the lesions remain active.

Some of the acute phase reactants increase in concentration, and are designated the positive acute phase proteins. They include haptoglobin (HP), serum amyloid A (SAA) and C-reactive protein (CRP). Others decrease in concentration, and are designated the negative acute phase reactants. Plasma zinc and iron and some proteins (albumin, transthyretin (TTR)–retinol-binding protein and cortisol binding globulin) are examples of the latter. As all these proteins have transport functions, their decrease indicates more freely available hormones, and the reactants therefore are called acute booster reactants (Ingenbleek and Young 1994).

Acute phase proteins (APP) mainly originate from the liver. The hepatocytes react upon stimulation of receptors by the cytokines, which are released by inflammatory cells, and upon tissue destruction. They are found to be good, reliable parameters of cellular reactivity (Alsemgeest 1994) since they react fast and can be found in the blood rapidly (from 4 h onwards) after the start of an inflammation or infection, some changing more rapidly than others. These changes in concentration remain as long as the underlying disease process does not heal. As a result of the total changes in blood proteins, blood plasma viscosity increases and the erythrocyte sedimentation rate (ESR) also changes.

In cattle, sheep, dogs and horses acute phase proteins were found to represent sensitive tools for monitoring animals with disease (Gruys et al. 1993, 1994; Alsemgeest 1994; Burton et al. 1994; Horadagoda et al. 1994; Sheffield et al. 1994; Yamamoto et al. 1994; Yamashita et al. 1994) and physical stress (Alsemgeest et al. 1995a). For cows sent to the Internal Medicine Clinic, haptoglobin was found to be a more powerful variable with which to monitor disease in general as far as their combined sensitivity and specificity was concerned than a series of classical clinical chemistry and haematology variables (Alsemgeest 1994). In a small abattoir survey, haptoglobin and SAA appeared to be more related to the severity of the lesions found than plasma zinc and iron concentrations (Guys et al. 1993).

For pigs only a limited number of studies on acute phase reactants have been published (Eurell et al. 1990; Bürger et al. 1992; Lampreave et al. 1994) and reactivity of zinc and iron were also found to be of limited value (reviewed in Gruys et al. 1994). As far as porcine CRP-assays were performed, these were based on cross reactivity with rabbit antihuman CRP; for haptoglobin, a haemoglobin binding assay was used. To bypass possible lack of specificity for the CRP-assay, and haemolytic sera in which haptoglobin cannot be measured by binding assay, it was decided to isolate these proteins and raise specific antisera along the lines published for bovine haptoglobin (Sheffield et al. 1994). Porcine acute phase sera appeared to lack useful cross reactivity with anti-bovine SAA (Alsemgeest 1994). Therefore, porcine acute phase high density lipoprotein (HDL) was isolated to purify the SAA.

Materials and Methods

Source of Blood Samples

Sera and heparin-plasma were obtained during exanguination at a University pig slaughter unit. Blood samples were centrifuged (15 min, 1000 *g*); sera and plasma were taken off and stored at −20 °C until use. For the determination of blood cell differentiation, haemoglobin and haematocrit, fresh heparinised blood was used.

Classical Blood Analysis

Determination of classical haematological and blood chemistry variables including iron, was performed according to the methods described by Sloet van Oldruitenborg-Oosterbaan (1990). Albumin (from the plasma protein spectrum) was measured densitometrically using a serum protein electrophoresis kit (Paragon Electrophoresis system, Beckman Instruments Inc., Brea, CA).

Determination of HP and Ceruloplasmin

Haptoglobin (HP) serum concentrations were determined by means of a quantified haemoglobin binding assay, modified after Skinner et al. (1991). For 10 min, 10 μl of sample, standard and a positive control serum were continuously mixed and incubated in triplicate at room temperature, with 90 μl of 0.534 mg/ml swine haemoglobin (Sigma, St Louis, MO) in PBS/EDTA, pH 7.2, on a conventional microtitration plate (Greiner, Alphen a/d Rijn, The Netherlands). Each sample was also incubated with 90 μl PBS/EDTA only (= blank). Then, 150 μl of 0.2 M sodium acetate buffer, pH 4 at 37 °C containing the substrate 1.11 g/ml guaiacol (Sigma, St Louis, MO), was added, immediately followed by the addition of 50 μl of a freshly prepared 0.018% peroxidase solution (37 °C) (Merck, Darmstadt, Germany). Absorbance was immediately read at 490 nm. Absorbance of the blank was subtracted from the mean absorbance of the samples, standards or positive control serum.

Ceruloplasmin in sera was determined from its oxidase-activity by use of *o*-dianiside dihydrochloride, according to Schosinsky et al. (1974).

Sodium Dodecyl Sulphate Polyacrylamid Gel Electrophoresis and Western Blot

SDS-PAGE and Western blot were performed as described by Niewold and Tooten (1990) and Alsemgeest (1994).

Purification of Porcine Haptoglobin (HP)

The purification of porcine HP was performed by a slight modification of the method of Eurell et al. (1990). Initially, a DEAE-Blue column (Bio-Rad Laboratory, Richmond, CA) was used to remove the high amount of albumin in the serum. The sample was eluted with 0.02 M sodium phosphate buffer, pH 7.1, followed by 1.4 M NaCl in 0.02 M sodium phosphate buffer, pH 7.1, to remove the albumin. The V_0-peak was collected and applied to an agarose-globin column (Sigma, St Louis, MO). After elution with 4M NaCl in 0.01M Tris/HCl, pH 7.0, the HP fraction was removed from the column with 1.6 M guanidine in 0.01 M Tris/HCl, pH 7.0. Collected peak fractions were pooled and dialysed against 0.02 M sodium phosphate buffer, pH 7.1, and finally lyophilised. The purification procedure was controlled on SDS-PAGE. With the protein obtained, rabbit antisera were induced by injecting a New Zealand White male rabbit with the protein in complete Freund's adjuvant. Booster inoculations were with incomplete Freund's. After elution of the obtained antiserum on a protein G column (Pharmacia, Uppsala, Sweden), the IgG part was isolated.

Purification of Pig Serum Amyloid A (SAA)

The purification was performed using the cholesteryl hemisuccinate (CH) affinity chromatography method (Niewold and Tooten 1990). Approximaely 40 ml of serum of a diseased pig was mixed with a solution containing 10 ml CH column material (Sigma, St Louis, MO) in 0.9% NaCl for 20 min; the non-bound material was washed through a glass filter. The CH column material was then mixed for 20 min with 100 ml 1 M NaCl on a rollerbank, and the non-specifically bound material was removed through the glassfilter. This step was repeated, and finally the affinity bound material was collected after washing for 20 min with maximally 100 ml 6 M guanidine/HCl, 0.55M Tris/HCl, pH 8.5. This fraction was concentrated on a YM-3 Amicon filter to a final volume of approximately 2 ml. The concentrate was applied to a Superose-12 gel filtration column (Pharmacia, Uppsala, Sweden) and the retarded SAA peak was collected separately. After four runs, the peaks were pooled, concentrated and rechromatographed. The peak fraction collected was dialysed against 10% acid followed by distilled water. Finally the purified protein was lyophilised. The isolated protein fraction was identified by means of molecular weight determination on SDS–urea–PAGE and western blot using anti-bovine SAA (Alsemgeest et al. 1995) and anti-hamster AA (Hol and Gruys 1984) antisera.

Purification of Swine C-reactive Protein (CRP)

The purification of CRP was performed according to Onishi et al. (1994). In short, 40 ml of a suitable serum

sample (as determined with a commercial CRP kit using antihuman CRP (Abbott, Germany)) was eluted over an *O*-phosphorylethanylamine (PEAD)-agarose column (Sigma, St Louis, MO) with a 50 mM Tris/HCl, 150 mM NaCl, 2 mM $CaCl_2$ buffer, pH 8.0. The peak fraction was placed on a Sephacryl-300 HR column (Pharmacia, Uppsala, Sweden) and the CRP fraction was eluted with a 10 mM Tris/HCl, 150 mM NaCl, 2 mM EDTA buffer, pH 8.0. The CRP peak was divided into four separate fractions, according to Onishi et al. (1994), and stored (−20 °C). The presence of CRP in the different fractions was determined on SDS-PAGE, and on dot blots using the human antiserum from the Abbott kit.

Results

Pigs and Blood Variables

According to the gross and microscopic pathological–anatomical diagnosis the animals were divided into different groups. The group defined as 'animals with abscesses and chronical abnormalities' had significantly different values for the acute phase protein haptoglobin (binding assay), ceruloplasmin and albumin as well as for the values of iron (data to be published elsewhere). From these animals, clearly positive examples were chosen for the isolation of the different acute phase proteins.

Haptoglobin Isolation

The DEAE-blue column removed a great deal of the albumin from the serum as shown in Fig. 1. Admitting

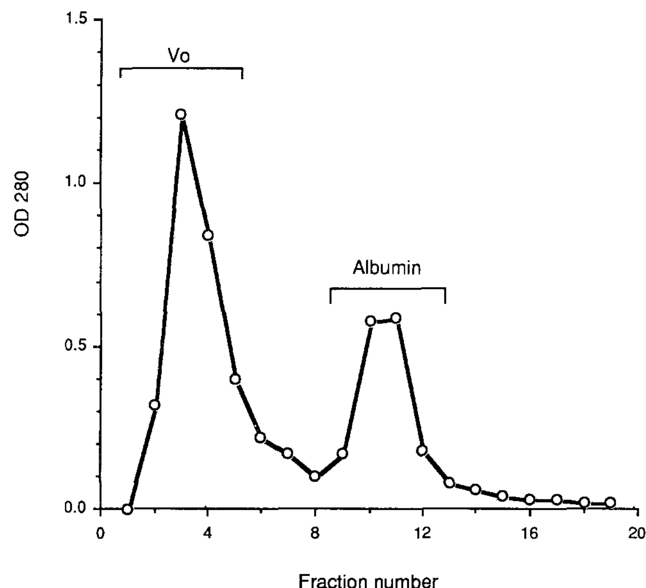

Fig. 1. Purification of pig haptoglobin. Elution pattern of serum on DEAE-blue column. Fractions 1–5 represent the V_0, fractions 9–12 represent albumin.

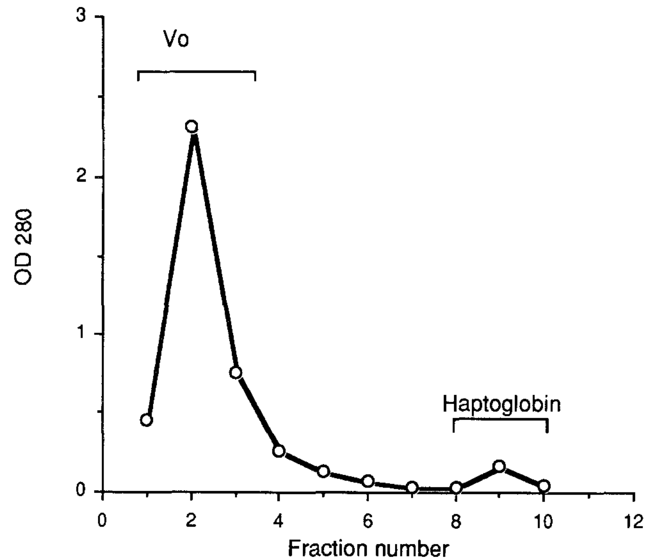

Fig. 2. Purification of pig haptoglobin. Elution pattern of the DEAE V_0 on agarose–globin column. Fractions 9 and 10 represent purified haptoglobin.

the V_0 peak of this column to the agarose–globin column resulted in isolation of a small amount of HP (Fig. 2). The albumin peak fraction of the DEAE-column did indeed contain almost solely albumin, whereas the concentration of albumin in the V_0 peak was drastically diminished as controlled on SDS-PAGE.

The HP obtained was used to immunise a rabbit. The specificity of the antiserum was controlled on Western blot in comparison to the reactivity of rabbit anti-pig HP kindly provided by Lampreave (Zaragoza, Spain). From our antiserum the IgG fraction was purified using a protein G column. The IgG fraction reacted with porcine HP (Fig. 3). The cross-reacting, non-HP pro-

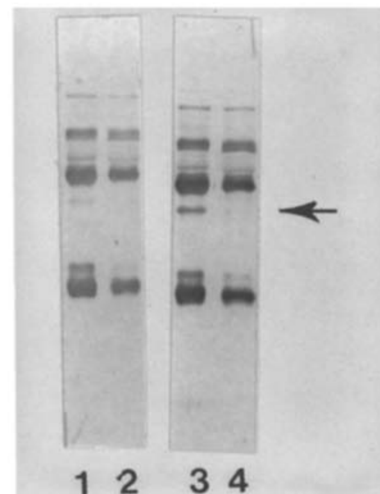

Fig. 3. Western blot of HP-positive and HP-negative pig sera stained with rabbit anti pig HP serum. Lanes 1 and 3: HP-positive serum, lane 2 and 4: HP-negative serum. Lanes 1 and 2 are stained with rabbit anti pig haptoglobin (dilution 1:1000), lanes 3 and 4 are stained with the purified IgG part of the anti pig haptoglobin (dilution 1:2000). Arrow indicates HP.

tein bands could be absorbed totally with commercially available normal pig serum (DAKO A/S, Denmark).

SAA Isolation

After mixing with cholesteryl hemisuccinate and elution on Superose 12, three peaks were obtained (Fig. 4), of which the last one reflected SAA. This peak was collected and rechromatographed. The purified SAA fraction was finally collected, dialysed against 10% acetic acid and distilled water, and lyophilised. Western blot stained with rabbit anti-bovine SAA and rabbit anti-hamster AA revealed definite cross reactivity with the antibovine antiserum, whilst the anti-hamster was negative (Fig. 5).

CRP Isolation

An *O*-phosphorylethylamine (PEA) column was used to select that part of whole serum containing CRP (Fig. 6). The subsequent peak pattern of the selected protein on a Sephacryl 300 HR column is shown in Fig. 7. The

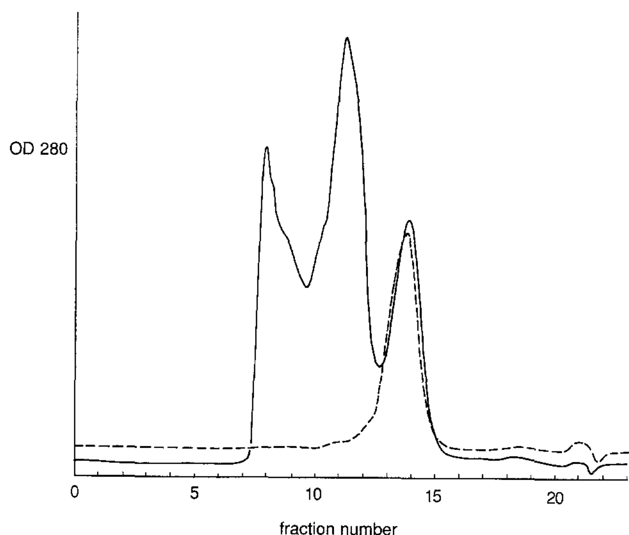

Fig. 4. Purification of pig serum amyloid A. After mixing the serum with cholesteryl hemisuccinate and filtration, the filtrate was eluted on Superose 12 column. The eluent was divided into three fractions of which the last peak represents SAA (____). Rechromatography of this peak provided a pure SAA peak (---).

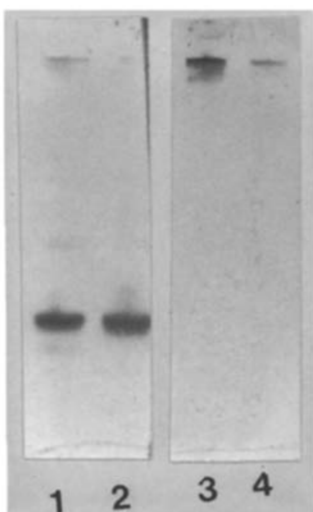

Fig. 5. Western blot of purified serum amyloid A, stained with rabbit anti-bovine SAA and rabbit anti-hamster AA. Lanes 1 and 2 are tested against bovine SAA (dilution 1:500), lanes 3 and 4 are tested against hamster AA (dilution 1:250). Lanes 1 and 3: purified SAA from a pig with chronic abnormalities, lanes 2 and 4: purified SAA from a pig with abscesses and acute pneumonia.

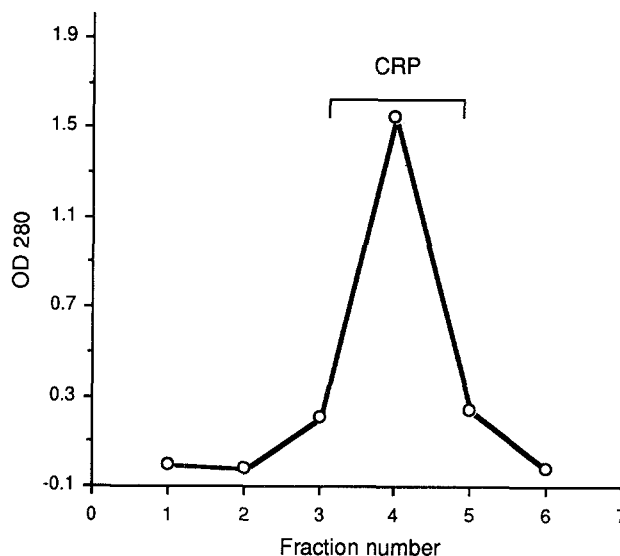

Fig. 6. Purification of pig C-reactive protein. Elution pattern of serum on *O*-phosphorylethanylamine column. Fractions 4 and 5 represent the CRP part.

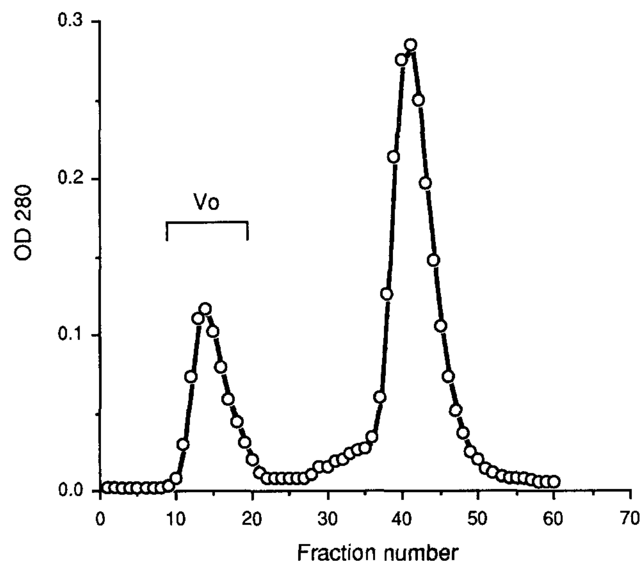

Fig. 7. Purification of pig C-reactive protein. Elution pattern of a PEA-affinity selected protein on Sephacryl 300 HR gel filtration column. Fractions 11–19 represent the V_0, fractions 31–51 were divided into four parts: I = 31–35, II = 36+37, III = 38–46, IV = 47–51.

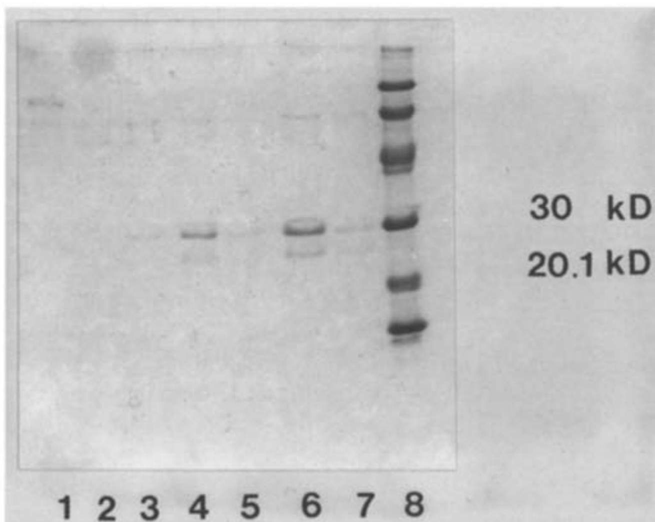

Fig. 8. SDS-PAGE of purified pig C-reactive protein. The V_0 and the four parts of the retarded peak from the Sephacryl 300 HR gel filtration column were analysed. CRP is found in part III. Lane 1: V_0 (3 μl), lane 2: part I (3 μl), lane 3: part II (3 μl), lane 4: part III (2 μl), lane 5; part IV (2 μ), lane 6: part III (5 μl) lane 7: part IV (5 μl), lane 8: low-molecular-weight marker proteins (Pharmacia, Uppsala, Sweden; 2 μl).

CRP peak obtained was divided into four parts and identified on SDS-PAGE. CRP was present mainly in part III (Fig. 8). At control on dot blot, using sheep anti-human CRP (Abbott kit, Germany), this part showed cross-reactivity with the antiserum.

Discussion

As indicated above, the current main interest in animal production and meat inspection is to develop reliable assays. In order to monitor groups of animals as well as individuals, a range of variables are available. The major aim, however, is to identify a reliable variable as a 'stethoscope' for monitoring the individual subject's health status. For ruminants, acute phase proteins appear to represent primary variables in assessing general animal health in groups of clinical patients and of slaughtered animals (Skinner et al. 1991; Gruys et al. 1993; Alsemgeest 1994; Skinner and Roberts 1994).

In the pig, different blood variables (Hb, Ht, cell counts, albumin, HP, ceruloplasmin, fibrinogen, viscosity) have been measured to indicate health of animals at slaughter, as reported by others (Eurell et al. 1990; Odink et al. 1990; Bürger et al. 1992; Visser et al. 1992; Lampreave et al. 1994). From our preliminary data, albumin, HP, ceruloplasmin and iron appeared to show positive reactivity in pigs with lesions. In addition to the literature on acute phase proteins (Gruys et al. 1994), viscosity (Visser et al. 1992) and vitamin A (Alsemgeest et al. 1992) in swine, good results are to be expected when pig-specific assays for acute phase proteins are developed. For this requirement, individual major acute phase proteins of the pig were isolated. For haptoglobin, a specific antiserum was raised in a rabbit, as is shown in Figs 1–3. The purifications for serum amyloid A and C-reactive protein are in progress as is shown in Figs 4–8. The purified SAA from the animal with abscesses and acute pneumonia revealed two protein bands that reacted with the anti-bovine SAA (lane 2 in Fig. 5). Since the purified SAA from the animal with more chronic abnormalities contained only one cross-reacting protein band, it is presumed that more isoforms of pig SAA exist, as is described for other species, e.g., bovine SAA (Alsemgeest et al. 1995b). It is probable that the different isoforms are present during different phases of disease. The described method for isolating canine CRP (Onishi et al. 1994) was also useful for the pig. As can be seen in Fig. 8, protein bands of approximately 24–28 kDa were isolated.

The purified proteins will be used to raise pig-specific antibodies as shown for haptoglobin. These antibodies, in turn, can then be applied to fast, specific and sensitive tests which in the near future will need to be automated.

The best clinical results in human medicine (Ingenbleek and Young 1994), and the most reliable figures in slaughtered individual animals may then be predicted by the calculation of an acute phase index (API). This should be based on:

$$\frac{\text{rapid positive APR} \times \text{slow positive APR}}{\text{slow negative APR} \times \text{rapid negative APR}}$$

For cattle, based on the materials of Alsemgeest (1994, chapter 4A), we calculated an API for 233 clinical patients and 21 healthy control animals from the faculty farm using the formula:

$$\text{Acute Phase Index} = \frac{(\text{haptoglobin (mg/ml)} + 0.1) \times \text{SAA } (\mu\text{g/ml})}{\text{albumin (mg/ml)} \times \alpha\text{-2-macroglobulin (U/ml)}}$$

For the 21 normal cows the calculation revealed mean API + 2SD = 0.0018. Within the 233 clinical patients a group of 21 animals had API values <0.002, and of these, only nine subjects were recorded as having some type of inflammatory process. The sensitivity and specificity combined in Youden's index for bovine API, accounted for the 21 of 233 diseased cattle with values of 0.91, 1 and 0.91, respectively. Therefore, the API was shown to be a much more reliable parameter for monitoring health in cattle than the individual variables (Table 1).

For pigs similarly appropriate results are to be expected, when an API formula is used such as:

$$\frac{\text{CRP or SAA} \times \text{haptoglobin or plasma viscosity}}{\text{albumin} \times \text{vitamin A}}$$

Studies are in progress concerning variables and values of these variables and the calculation of an API for the pig.

*Acknowledgements.*The authors wish to thank Drs SPM Alsemgeest and F Lampreave for kindly offering the raw bovine data and anti-porcine haptoglobin, respectively.

Table 1. Sensitivity, specificity and Youden's index for acute phase proteins and some other blood variables and the calculated acute phase index (API) from the values of these variables (data from Alsemgeest, 1994)

Variable	Units	Mean (SD) Healthy (n=21)	Mean (SD) Non-healthy (n=233)	Sensitivity	Specificity	Youden's index
API	μg/α-2M-U	0.001 (0.0004)	2.9 (4.2)	0.91	1	0.91
HP	mg HbBC/100 ml	0.0 (0.0)	52.3 (53.6)	1	0.70	0.70
SAA	μg/ml	14.0 (5.0)	35.2 (21.8)	0.95	0.65	0.60
CP	U/100 ml	1.4 (0.2)	2.1 (1.3)	0.90	0.69	0.59
α-2MG	U/ml	34.3 (3.1)	26.8 (7.6)	0.95	0.63	0.58
Alb	g/l	35.6 (2.8)	29.1 (7.8)	1	0.19	0.19
TP	g/l	75.3 (3.8)	74.4 (13.5)	1	0.26	0.26
GG	g/l	21.2 (3.5)	23.2 (10.9)	1	0.19	0.19

API, acute phase index; HP, haptoglobin; SAA, serum amyloid A; CP, ceruloplasmin; α-2MG, α-2 macroglobulin; Alb, albumin; TP, total protein; GG, gamma globulin.

References

Alsemgeest SPM (1994) Blood concentrations of acute-phase proteins in cattle as markers for disease. PhD-thesis, Utrecht

Alsemgeest SPM, Odink J, Visser IJR et al. (1992) Measurement of retinol (vitamin A) as an acute-phase parameter in the finishing pig. In: Kimman et al. (eds) Proceedings 12th IPVS congress, The Hague 1992, Royal Netherlands Veterinary Association, p. 398.

Alsemgeest SPM, Lambooy IE, Wierenga HK et al. (1995a) Influence of physical stress on the plasma concentration of serum amyloid-A (SAA) and haptoglobin (HP) in calves. Vet Quart 17:9–12

Alsemgeest SPM, Horadagoda A, Hulskamp-Koch CK et al. (1995b) First evidence for the existence of multiple isoforms of bovine serum amyloid-A (apoSAA) Scand J Immunol 41:407–413

Berends BR, Smeets JFM, Harbers AHM et al. (1991) Investigations with enzyme-linked immunosorbent assays for *Trichinella spiralis* and *Toxoplasma gondii* in Dutch 'Integrated Quality Control for finishing pigs' project. Vet Quart 12:190–198

Berends BR, Snijders JMA (1994) The hazard analysis critical control point approach in meat production (in Dutch). Tijdschr Diergeneeskd 119:360–365

Berends BR, Snijders JMA, van Logtestijn JG (1993a) Efficacy of current EC meat inspection procedures and some proposed revisions with respect of microbiological safety: a critical review. Vet Rec 133:411–415

Berends BR, Smeets JFM, Visser IJR et al. (1993b) An orientation towards blood profile, pathological–anatomical abnormalities and meat quality of apparently healthy slaughter pigs. Fleischwirtschaft 73:757–760

Bernardo TM, Dohoo IR, Donald A et al. (1990a) Ascariasis, respiratory diseases and production indices in selected Prince Edwards Island swine herds. Can J Vet Res 54:267–273

Bernardo TM, Dohoo IR, Donald A et al. (1990b) Effect of ascariasis and respiratory diseases on growth rates in swine. Can J Vet Res 54:278–284

Blocks GHM, Vernooy JCM, Verheijden JHM (1994) Integrated quality control project: relationships between pathological findings detected at the slaughterhouse and information gathered in a veterinary health scheme at pig farms. Vet Quart 16:123–127

Bogh HO, Eriksen L, Lawson LG et al. (1994) Evaluation of an enzyme-linked immunosorbent assay and histamine release test system for the detection of pigs naturally infected with *Ascaris suum*. Prev Vet Med 21:201–204

Bürger W, Fennert EM, Pohle M et al. (1992) C-reactive protein – a characteristic feature of health control in swine. J Vet Med Assoc 39:635–638

Burton SA, Honor DJ, Mackenzie AL et al. (1994) C-reactive protein concentrations in dogs with inflammatory leukograms. Am J Vet Res 55:613–618

Christensen NH, Cullinane LC (1990) Monitoring the health of pigs in New Zealand abbattoirs N Z Vet J 38:136–141

Davies PD (1992) Pig health schemes: initiatives for population health management. Am Assoc Swine Pract 4(3):6–10

Elbers ARW (1991) The use of slaughterhouse information in monitoring systems for herd health control in pigs. PhD-thesis, Utrecht

Elbers ARW, Tielen MJM, Cromwijk WAJ et al. (1990) Sero-epidemiological screening of pig sera collected at the slaughterhouse to detect herds infected with Aujeszky's disease virus, porcine influenza virus and *Actinobacillus (Haemophilus) pleuropneumoniae* in the framework of an Integrated Quality Control (IQC) system. Vet Quart 12:221–230

Elbers ARW, Visser IJR, Odink J et al. (1991) Changes in haematological profiles in blood of apparently healthy slaughter pigs, collected at the farm and at slaughter, in relation to the severity of pathological–anatomical lesions. Vet Quart 13:1–9

Elbers ARW, Tielen MJM, Snijders JMA et al. (1992) Epidemiological studies on lesions in finishing pigs in the Netherlands. I. Prevalence, seasonality and interrelationship. Prev Vet Med 14:217–231

Eurell TE, Hall JC, Bane DP (1990) Purification of swine haptoglobin by affinity chromatography. Can J Vet Res 54:501–503

Ewald C, Heer A, Havenith U (1994) Factors associated with influenza-A virus infections in finishing pigs. Berl Muench Tieraerztl Wschr 107:256–262

Falk K, Lium BM (1991) An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. III. Serological findings and their relationship to pathomorphological and microbiological findings. Acta Vet Scand 32:79–88

Falk K, Hoie S, Lium BM (1991) An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. II. Enzootic pneumonia of pigs: microbiological findings and their relationship to pathomorphology. Acta Vet Scand 32:67–77

Gardner IA, Hird DW (1990) Host determinants of pneumonia in slaughter weight swine. Am J Vet Res 51:1306–1311

Gerigk K, Ellerbroek (1994) Food production and hazard analysis critical control point concept. Dtsch Tieraerztl Wschr 101:270–272

Geudeke MJ (1992) The use of slaughterhouse information in monitoring systems for herd health control in sows (in Dutch). PhD-thesis, Utrecht.

Gruys E, van Ederen AM, Alsemgeest SPM et al. (1993) Acute phase protein values in blood of cattle as indicator of animals with pathological processes. Arch Lebensmittelhyg 44:105–128

Gruys E, Obwolo MJ, Toussaint MJM (1994) Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. *Vet Bull* 64:1009–1018

Harbers AHM (1991) Aspects of meat inspection in an integrated quality control system for slaughter pigs. PhD-Thesis, Utrecht

Harbers AHM, Snijders JMA, Smeets JFM et al. (1992) Use of

information from pig finishing herds for meat inspection purposes. Vet Quart 14:41–45
Hathaway SC, Richards MS (1993) Determination of the performance attributes of post-mortem meat inspection procedures. Prev Vet Med 16:119–131
Heinrich PC, Castell JV, Andus T (1990) Interleukin-6 and the acute phase response. Biochem J 265:621–636
Hill MA, Scheidt AB, Telaw RE et al. (1992) Association between growth indicators and volume of lesions in lungs from pigs at slaughter. Am J Vet Res 53:2221–2223
Hill MA, Scheidt AB, Teclaw RF et al. (1994) Relationship between the indicators of performance and the weight of pneumonic lesions from pigs at slaughter. Res Vet Sci 56:240–244
Hoie S, Falk K, Lium BM (1991) An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. IV. Bacteriological findings in chronic pneumonic lesions. Acta Vet Scand 32:395–402
Hol PR and Gruys E (1984) Amyloid A proteins in different species. Appl Pathol 2:316–327
Horadagoda A, Eckersall PD, Hodgson JC et al. (1994) Immediate responses in serum TNFα and acute phase protein concentrations to infection with *Pasteurella haemolytica* A1 in calves. Res Vet Sci 57:129–132
Hurnik D, Hanna PE, Dohoo IR (1993) Evaluation of rapid gross visual appraisal of swine lungs at slaughter as a diagnostic screen for enzootic pneumonia. Can J Vet Res 57:37–41
Hurnik D, Dohoo IR, Bate LA (1994) Types of farm management as risk factors for swine respiratory disease. Prev Vet Med 20:147–157
Ingenbleek Y, Young V (1994) Transthyretin (prealbumin) in heath and disease: nutritional implications. Annu Rev Nutr 14:495–533
Klont RE (1994) Effects of preslaughter stress factors on muscle metabolism and meat quality. PhD-thesis, Utrecht
Kushner I (1993) Regulation of the acute phase response by cytokines. Perspect Biol Med 36:611–622
Lampreave F, Gonzalez-Ramon N, Marinez-Ayensa S et al. (1994) Characterization of the acute phase serum protein response in pigs. Electrophoresis 15:672–676
Lingaas F (1993) Epidemiological and genetical studies on diseases and blood parameters in pigs. PhD-thesis, Lobo Grafisk, Oslo.
Lium BM, Falk K (1991) An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. I. Prevalence and morphological description of gross lung lesions. Acta Vet Scand 32:55–65
Maeda H, Mori C, Kurokawa M et al. (1989) Production of dark firm dry meat in slaughtered pigs with so-called liver degeneration characterized by yellowish discoloration and high lipid contents. Jpn J Vet Sci 51:925–933
Mori C, Maeda H, Yuasa A (1990) Relationship between liver degeneration and the production of dark firm dry meat in slaughtered pigs. Jpn J Vet Sci 52:613–620
Mousing J, Christensen G (1993) Pathological lesions in the right and left porcine lung: evaluation of an alternative method for scoring pneumonic lesions based on right lung examination. Acta Vet Scand 34:151–158
Mousing J, Lybye H, Barford K et al. (1990) chronic pleuritis in pigs for slaughter: an epidemiological study of infectious and rearing system-related risk factors. Prev Vet Med 9:107–119
Niewold TA, Tooten PCJ (1990) Purification and characterization of hamster serum amyloid A protein (SAA) by cholesteryl hemisuccinate affinity chromatography. Scand J Immunol 31:389–396
Nilsson O (1982) Ascariasis in the pig. Acta Vet Scand Supp 79.
Noyes EP, Feeney DA, Pijoan C (1990) Comparison of the effect of pneumonia detected during lifetime with pneumonia detected at slaughter on growth in swine. J Am Vet Med Assoc 197:1025–1029
Odink J, Smeets JFM, Visser IJR et al. (1990) Hematological and clinicochemical profiles of healthy swine and swine with inflammatory processes. J Anim Sci 68:163–170
Onishi T, Shimizu T, Kajikawa T (1994) Simple and efficient purifications of C-reactive protein from canine serum. J Vet Med Sci 56:417–419
Paisley LG, Vraa-Andersen L, Dybkjaer L et al. (1993a) An epidemiologic and economic study of respiratory diseases in two conventional Danish swine herds. I: Prevalence of respiratory lesions at slaughter and their effects on growth. Acta Vet Scand 34:319–329
Paisley LG, Vraa-Andersen L, Dybkjaer L et al. (1993b) An epidemiologic and economic study of respiratory diseases in two conventional Danish swine herds. II: Association between lesions present at slaughter and mean daily gains during specific intervals of the growth period. Acta Vet Scand 34:331–344
Schosinsky KH, Lehman HP, Beeler MF (1974) Measurement of ceruloplasmine from its oxidase activity in serum by use of *o*-dianisidine dihydrochloride. Clin Chem 20:1556–1562
Sheffield CL, Kamps-Holtzapple C, DeLoach JR et al. (1994) Production and characterization of a monoclonal antibody against bovine haptoglobin and its use in an ELISA. Vet Immunol Immunopathol 42:171–183
Skinner JG, Roberts L (1994) Haptoglobin as an indicator of infection in sheep. Vet Rec 134:326–327
Skinner JG, Brown RAL, Roberts L (1991) Bovine haptoglobin in clinically defined field conditions. Vet Rec 16:147–149
Sloet van Oldruitenborg-Oosterbaan MM (1990) Heartrate and blood lactate in exercising horses. PhD-thesis, Utrecht
Smeets JFM, Odink J, Visser IJR et al. (1990) Haematology and blood-chemistry for predicting abscesses and other abnormalities in slaughtered pigs. Vet Quart 12:146–151
Tielen MJM, Elbers AR (1988) Experiences with integrated chain control (ICC) for pig production in the Netherlands. In: Ekesbo J (ed) Environment and animal health. Proc of the 6th International Congress on Animal Hygiene Vol I. June 1988, Västergötlands Tryckeri, Skara, pp 227–231
Visser IJR, Odink J, Smeets JFM et al. (1992) Relationship between pathological findings and values of haematological and blood-chemistry variables in apparently healthy finishing pigs at slaughter. J Vet Med B 39:123–131
Wallgren P, Beskow P, Fellstrom C et al. (1994) Porcine lung lesions at slaughter and their correlation to the incidence of infections by *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* during the rearing period. J Vet Med B 41:441–452
Wertenbroek ACJM (1981) Field studies in the incidence to damage to the liver caused by migrating *Ascaris* larvae in baconers and its treatment (in Dutch). Tijdschr Diergeneeskd 106:662–670
Willeberg P, Gerbola MA, Kirkegaard Petersen B et al. (1984) The Danish pig health scheme: nation-wide computer-based abattoir surveillance and follow-up at the herd level. Prev Vet Med 3:79–91
Wouda W, van den Broek MJM, Snijders JMA et al. (1987) Endocarditis and meat inspection in finishing pigs (in Dutch). Tijdschr Diergeneeskd 112:1226–1235, 1236–1242
Yamamoto S, Shida T, Okimura T et al. (1994) Determination of C-reactive protein in serum and plasma from healthy dogs and dogs with pneumonia by ELISA and slide reversed passive latex agglutination test. Vet Quart 16:74–77
Yamashita K, Fujinaga T, Miyamoto T et al. (1994) Canine acute phase response: relationship between serum cytokine activity and acute phase protein in dogs. J Vet Med Sci 56:487–492