# THE ACUTE INFLAMMATORY RESPONSE IN FARMED RED DEER (*Cervus elaphus*)

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SUMMARY
The results of measuring haptoglobin, fibrinogen, plasma viscosity and circulating neutrophil numbers in farmed red deer undergoing acute phase reactions are given. The conditions described are naturally occurring *Yersinia pseudotuberculosis* infection, induced *Yersinia pseudotuberculosis* infection, malignant catarrhal fever, physical damage due to yarding and splenectomy. In simple acute phase reactions, neutrophil numbers are raised earliest, but fall back relatively quickly. Although fibrinogen rises more quickly than haptoglobin, the haptoglobin level in health is so low that abnormal levels are generally evident sooner than fibrinogen. Haptoglobin also tends to remain elevated for longer. The complexities in infection are illustrated by the return of fibrinogen and plasma viscosity to normal in a case of malignant catarrhal fever, because fibrinogen was being consumed, while the haptoglobin was clearly elevated for the entire 27 days to termination of the illness by euthanasia. The neutrophils remained within low normal limits throughout. The events which induce changes in these inflammatory markers, and the probable functions in these conditions, are briefly discussed. Aspects of the usefulness of these different markers are considered in the light of these observations.

## INTRODUCTION

In any situation in which cell damage occurs, mammals respond with a wide range of reactions whose complexity and variety is currently the subject of intensive study. One set of reactions is the increased production of a number of proteins. The first report concerning these involved a protein which reacted with the somatic C-polysaccharide of pneumococci, C-reactive protein (Abernethy and Avery, 1941). With the methods available at the time, this was only demonstrable in the sera of patients during the acute phase of infectious diseases, and it therefore became known as an 'acute phase protein', several other proteins having since been classified in this way (Pepys and Baltz, 1983). It has been found that these proteins may also be increased in subacute and chronic inflammatory conditions, so the term 'acute phase proteins' is somewhat misleading. Many of them have a protective function, inactivating proteolytic enzymes and oxygen metabolites derived from neutrophils, which have the potential to damage normal tissue (Laurell C-B.,1985). As the synthesis of these proteins increases, that of albumin and transferrin decreases.

Reports on the use of these proteins as markers of inflammation in a number of species have been published (Conner et al. 1986; - bovine; Cross, Tait and Griffin, 1988 - cervine; Pfeffer et al 1988 - ovine; Pepys, et al., 1989 - equine). In this paper we consider the levels of some inflammation markers in acute phase reactions in yersiniosis, malignant catarrhal fever and non-infective tissue damage in farmed red deer, and how these relate to the disease process.

## MATERIALS AND METHODS

### Laboratory parameters

Fibrinogen, general haematological techniques and plasma viscosity estimations were performed as previously described (Cross, 1987). Serum haptoglobin was measured using the tetraguaiacol method described by Owen et al., (1960), modified for microtitre trays (Jones and Mould, 1984), with red deer haemoglobin as the substrate, the results being expressed as haemoglobin binding capacity (HbBC).

Haptoglobin stability in deer serum was tested using sera with raised haptoglobin left for various times and temperatures as indicated in table 1, then tested against a control serum stored at -20°C using a standard time, ensuring that the optical density of the control gave a similar value on each occasion. The coefficient of variation was found preparing a series of dilutions of sera having similar levels of haptoglobin to those used in the thermal stability tests. (In 2 of the 3 trials the same serum was used).

### Deer

All of the animals were maintained at the Ministry of Agriculture and Fisheries centre at Invermay.

Group A was a group of six 18 month old male red deer from which weekly faecal and serum samples were collected over several weeks. A natural infection with *Yersinia pseudotuberculosis* serotype I took place, as evidenced by the isolation of this organism from the faeces of 5 of the 6 deer. These animals showed no sign of clincal illness at this time.

Group B consisted of 8 male weaner red deer held in isolation and artificially exposed to *Yersinia pseudotuberculosis.*

Group C consisted of 7 rising 2 year old male red deer, 4 of which were controls handled along with 3 others which were splenectomised in mid-September. Pre-yarding tests indicated that one of the controls and one of the test animals already had a mild acute phase reaction in progress prior to yarding, so these 2 were not included in this report.

Deer D was an 8 month old male red deer, housed indoors as part of a nutrition experiment, which developed malignant catarrhal fever (MCF) following a chronic course lasting 4 weeks.

## RESULTS

The coefficient of variation for the method was 2.6% in one test (n=12) and 3.9% in another (n=20). The sera used for thermal stability testing were tested each day for 5 days. The greatest observed change at -20°C and 4°C were 3.0% and 2.8% respectively. At room temperature there was no change greater than the coefficient of variation until the 4th. day, when the fall reached 5.5%, and was again 5.0% on the 5th. day. At 37°C. the falls on successive days were 4.8%, 5.4%, 13.0% and 13.5% respectively.

The haptoglobin results from the naturally-occurring *Yersinia* infection are shown in Fig. 1. These animals showed no clinical signs of illness. Haptoglobin levels were very low 2 weeks before the organisms was isolated, climbed substantially during the period they were excreting *Yersinia*, and

fell back at a time corresponding to the disappearance of the organism from the faecal specimens. In the deer from which *Yersinia* were not isolated, there was no increase in haptoglobin.

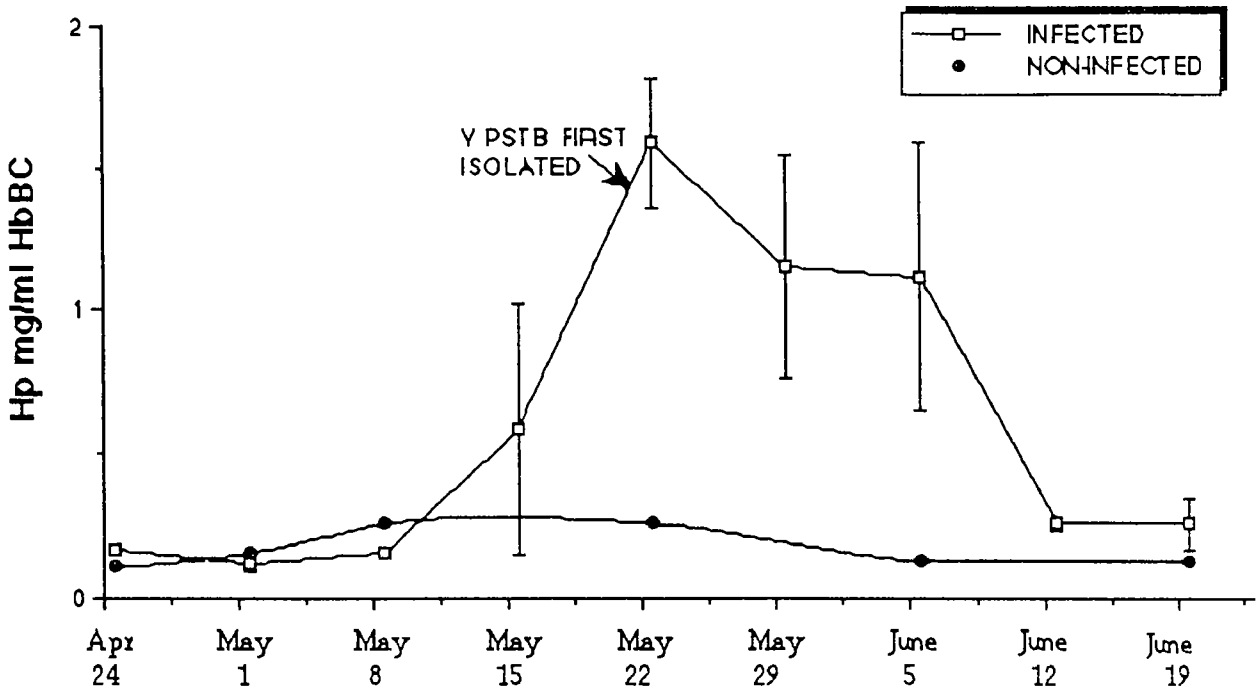


**FIG.1: NATURALLY OCCURRING YERSINIA INFECTION IN FARMED RED DEER.**
(HbBC = haemoglobin binding capacity).

In Group B, in which the disease was taking place under controlled conditions, it was possible to carry out daily estimations of a number of parameters, and the results are shown in Figs. 2a and 2b. The neutrophil count peaked during the second 24 hours, and was well above normal limits (Cross and Griffin, 1989) at this time. It remained elevated into the 4th. day. The haptoglobin did not rise as quickly as the fibrinogen, but was above the upper limit of the reference range between 24 and 48 hours, while the fibrinogens, on average, reached levels modestly above the upper limit of normal somewhat later.

Group C were the yarding/splenectomy group, and the results are shown in Figs. 3a - d. The control animals show slight increases above normal in fibrinogen, plasma viscosity and haptoglobin, while the neutrophils rose, but not above the upper limit of the reference values. In the splenectomised animals the levels were much higher. Once again the neutrophils peaked earliest, followed by fibrinogen then haptoglobin. Plasma viscosity levels reflected fibrinogen most closely, but were back within reference limits between the 5th. and 7th. days, while both splenectomy animals' fibrinogen concentrations only did this between the 7th and 12th days. The haptoglobin of 1 splenectomised animal also reached normal between the 7th and 12th

days, but that of the other remained high until after the 20th. day.

Animal D was a particularly interesting case of MCF, in which haptoglobin, plasma viscosity and fibrinogen were raised at the time of clinical diagnosis, neutrophil levels being normal. However, the fibrinogen returned to within the normal range 14 days after diagnosis, and the plasma viscosity returned to within the normal range on the 7th. day despite the animal's continuing chronic disease state. On the other hand, the haptoglobin level remained well above the reference range throughout the course of the illness, until euthanasia, and the neutrophil count remained at low normal.

## DISCUSSION

During infections in deer the acute phase proteins fibrinogen and haptoglobin are present in the plasma in increased amount. This has been demonstrated here in yersiniosis and malignant catarrhal fever, and also occurs in tuberculosis (Cross, 1987).

While haptoglobin appears to have a number of functions, a major one is likely to be linked to its ability to bind iron, thus making it unavailable for organisms which require iron for multiplication, expression of virulence, etc. The significance of available iron during an infectious process has been recognised for some years (reviewed by Bullen, 1981). In vitro growth of *Yersinia pseudotuberculosis* is enhanced if iron is added to the medium, and iron overload predisposes human dialysis patients to infections involving both *Yersinia enterocolitica* and *Y. pseudotuberculosis* (Boelaert et al. 1987). In view of the correlations noted in human patients, it might be of interest to monitor the levels of iron and iron-binding proteins in groups of deer at risk from *Yersinia* infection.
Eaton et al (1982) showed that an iron-requiring strain of *E. coli* produced lethal infection when injected into the peritoneal cavity of rats along with either stroma-free haemoglobin or ferric ammonium citrate, but that the lethal effect was greatly reduced if the injection consisted of E. coli, free haemoglobin and haptoglobin. This protection may result from the rapid clearance of haemoglobin/haptoglobin complex through the reticulo-endothelial system, and/or protection of the haemoglobin molecule from proteolytic attack by the bacteria, and so preventing the release of the iron.

The mechanisms through which the acute phase reaction takes place are complex and new information is emerging in the scientific press at a tremendous rate. However, the following is a brief summary of the major factors.
Once bacteria gain entry to the tissues, bacterial products such as endotoxin are released into the local tissues. These substances are recognised by a variety of tissue cells, notably tissue macrophages, which respond by producing polypeptides with hormone-like activity, known as cytokines. These cytokines have a multitude of effects on nearly every tissue and organ of the body. However, the main focus of interest here is their effects on bone marrow and liver. One effect is the release of neutrophils from bone marrow

stores (Kampschmidt, 1981). A variety of substances released at the location of the bacteria attract the neutrophils to that site The neutrophils converging on the infection site contain many proteolytic enzymes, which have the capacity to damage normal tissue if released into it in an active form. Anti-proteinases are present in the tissues which inhibit these proteolytic enzymes in an irreversible way, forming the 'antiproteinase shield' (reviewed by Weiss, 1989). Elastase is so effectively contained by this shield that it has a half-life in tissue of just 0.6 msec - though the shield itself is subject to attack by oxidative products of neutrophils.

Cytokines not only promote neutrophil release from the bone marrow, they also stimulate the liver to produce a wide range of proteins including these anti-proteinases which limit the damaging effect neutrophil enzymes can have on normal tissues Other proteins whose production by the liver is promoted in this way include haptoglobin, one function of which has already been discussed, and fibrinogen. The cytokines have another effect which may be linked to the increased fibrinogen production. They induce tissue thromboplastin formation by endothelial cells, (Bevilacqua et al. 1984). Since tissue thromboplastin initiates fibrin formation from fibrinogen, this could be significant in promoting the deposition of fibrin in the vicinity of the infection site. Hepatic albumin production is decreased by the same messengers that increase the formation of haptoglobin, fibrinogen and other proteins.

The observations in deer described in the results section of this paper are generally explicable in terms of the acute phase response as seen in other species. In all 4 groups A,B,C and D, one function of haptoglobin increase appears to be to prevent bacteria from utilising iron from iron-containing proteins that may be released as a result of tissue damage. Increased fibrinogen production with deposition of fibrin around the infection site probably assists in the containment of the infection in groups A and B, while the increase in release of neutrophils makes these cells more available to perform their normal functions in tissues. The controls in group C indicate that minor trauma during yarding can raise the level of some acute phase proteins. Among the splenectomised deer in group C, one operation was relatively difficult, and it was considered likely that direct physical trauma to the pancreas had occurred. In this animal, acute phase markers remained outside the reference values for a substantially longer period than in the less traumatised deer.

The choice of suitable acute phase proteins to detect and monitor inflammation in deer depends on several factors. The blood level of cells and proteins is clearly the complex resultant of production and release rates, breakdown and removal. It has been shown that haptoglobin and fibrinogen increases are stimulated by different messenger molecules (Andus et al. 1988, Geiger et al. 1988). The results in yersiniosis illustrate that the most rapidly rising acute phase protein (fibrinogen) may not show abnormal results as quickly as haptoglobin, because the levels in health are relatively high.

Neutrophil counts appear to be the most sensitive early indicator, but may fall back to normal levels relatively quickly. Plasma viscosity is a good 'all-round' measure of acute phase proteins, but may be relatively insensitive since values were within the normal range in the splenectomised deer on the 7th day, when both fibrinogen and haptoglobin were still clearly raised. Sutherland (1987) has shown that fibrinogen may be consumed at an abnormally high rate in MCF , and this is likely to be the reason that in case D the plasma fibrinogen and plasma viscosity returned to normal while the animal was still very ill. It has been suggested that a sensible routine approach would be to choose a 'short half-life' and a 'long half-life' acute phase protein as markers of inflammation, with C-reactive protein and alpha-1-antichymotrypsin being proposed for human patients (Calvin et al. 1988). The potential usefulness of haptoglobin as an acute phase marker in deer lies in the fact that, in health, levels are low to absent. This means that a modest increase is immediately apparent, whereas a modest increase in fibrinogen, for example, is far less obvious. A similar finding has been reported in cattle (Conner and Eckersall, 1986). In the diagnosis of inflammatory states in deer and cattle, haptoglobin occupies a place analogous to that of C-reactive protein in humans. A further advantage is that haptoglobin is relatively stable as a component of serum, very little change being seen over 96 hours at refrigerator temperature (and even at room temperature) - assuming the serum has been separated.

Few acute phase protein levels have been reported in red deer, and there may be more useful markers than the ones reported here. We have some suggestion of another such substance being raised in deer with tuberculosis, as the plasma viscosity may be moderately raised while the haptoglobin and fibrinogen are well within the normal ranges. Further work is needed to investigate this possibility.

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