

From the Experimental Station of the Veterinary Institute,  
Skara, Sweden.

BLOOD SERUM AND SYNOVIAL FLUID  
IN BOVINE LAMINITIS AND ARTHRITIS,  
WITH PARTICULAR REFERENCE  
TO THE PROTEIN COMPOSITION\*

By

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ANDERSSON, L. and P. LIBERG: *Blood serum and synovial fluid in bovine laminitis and arthritis, with particular reference to the protein composition.* Acta vet. scand. 1980, 21, 567—577. — In 74 cases of clinical laminitis and in 10 cases of clinical discrete arthritis in adult cattle, the protein picture was changed in blood and synovial fluid. The cases of laminitis were clinically divided into one group without and another with general affection. The changes in serum  $\gamma$ - and  $\alpha$ -globulins indicated a predominantly chronic inflammatory process in the arthritis groups and a more acute process in the laminitis groups. In discrete arthritis, the leukocyte concentration and all protein levels except the relative albumin concentration were increased. In laminitis without general affection no significant changes were found in synovial fluid, while the synovial protein pattern in laminitis with general affection indicated a simultaneous occurrence of arthritis. The combination of laminitis and arthritis might be an expression of different inflammatory reactions to common aetiological, still unidentified factors, indicating the existence of a disease complex which may manifest itself in hoof corium, in joints or in both.

cattle; laminitis; arthritis; hoof diseases; serum proteins; synovial fluid; electrophoresis.

The clinical picture in laminitis has been described by *Nilsson* (1963) and *Maclean* (1965, 1966), among others. Pronounced symptoms are hoof tenderness, stiffness, a stronger pulse in the digital arteries, local venous distension and occasionally hoof deformation.

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The most common symptoms in arthritis are swelling of the joints with filling of the synovial cavity, local joint tenderness, lameness and sometimes fever and loss of appetite (*Greenough et al.* 1972).

The serum protein picture in laminitis in cattle has so far attracted limited interest. *Nilsson* demonstrated lowered albumin concentration and elevated concentrations of  $\alpha_1$ - and  $\gamma$ -globulin, as well as depressed albumin:globulin ratio (A:G). *Liberg* (1977) found in chronic laminitis/polyarthritis an elevated total protein value, depressed albumin concentration and increased values for all individual globulin fractions. Changes similar to those applying to cattle have been described for equine laminitis (*Kirk et al.* 1975).

The protein content of synovial fluid from the hock joint of cattle has been determined by *Kersjes* (1963) and *van Pelt* (1965, 1968, 1973). They found a total protein concentration of 7–12 g/l in normal synovial fluid. *Kersjes* reported relative concentrations of albumin,  $\alpha$ -,  $\beta$ - and  $\gamma$ -globulin of 43, 14, 16 and 27 %, respectively. In aseptic arthritis *van Pelt* (1973) found increased concentrations of total protein, albumin and globulin, whereas the A:G ratio was depressed. In septic arthritis *Kersjes* found elevated total protein concentration, electrophoretically elevated relative globulin concentrations, and reduced albumin concentration.

In normal synovial fluid leukocyte concentrations of  $0.07$ – $0.21 \times 10^9/l$  have been reported. Moderately and appreciably elevated concentrations were found in aseptic and septic arthritis, respectively (*Kersjes, van Pelt* 1968).

The present investigation was carried out with the intention of using, primarily, protein determinations in blood and synovial fluid for further characterization of laminitis and arthritis in cattle.

#### MATERIAL AND METHODS

The material consisted of 84 adult female cattle displaying clinical symptoms of laminitis and/or arthritis. Thirty-nine of these animals had contracted the disease before or in connection with the first calving. Sixty animals were of the Swedish Red & White (SRB) breed, while the remainder were either Swedish Friesians (SLB) or SRB  $\times$  SLB crossbreeds. The laminitis cases were divided clinically into those with and those without signs

of general affection (27 vs. 47 cases). The most important symptoms in laminitis without general affection were tenderness and stiffness in the hooves, abnormal stance, increased digital arterial pulse and local venous distension. In laminitis with general affection these symptoms were more pronounced. Animals so affected showed occasional swellings distally on the legs and in all cases general symptoms such as fever, increased heart rate, shallow and laboured respiration, loss of appetite, and sweating. The onset of the disease was usually precipitate.

Thirteen animals with clinically diagnosed laminitis were slaughtered because of persistent difficulty in moving. Pathological-anatomical investigation confirmed the clinical diagnosis in all cases (*Andersson & Bergman 1980*).

Discrete arthritis was clinically diagnosed in 10 animals. They had a distinct joint swelling with filling of the synovial cavity in fetlock joint or hock joint, local joint tenderness, and lameness. Other findings were fever and diminished appetite.

Blood samples were drawn from all affected animals. Samples of synovial fluid were collected from 37 fetlock joints and five hock joints from altogether 39 animals. Immediately after sampling 3.8 % sodium citrate was added to the synovial fluid in order to prevent coagulation (one part citrate + nine parts synovial fluid).

For reference values blood samples were taken from 154 healthy animals, and synovial samples from 17 fetlock joints and 15 hock joints from 17 healthy animals.

Total protein values were determined by the biuret method in both serum and synovial fluid. Albumin in serum was determined spectrophotometrically (*Doumas et al. 1971*) and in synovial fluid by means of agarose gel electrophoresis ad modum *Carlström & Liberg (1975)*. The latter method was also used for the determination of the various globulin fractions. Total globulin concentration and the A:G ratio were calculated on the basis of the total protein and albumin values.

As hyaluronic acid in synovial fluid gave a blurred fraction pattern, the synovial samples were digested prior to electrophoresis with highly purified hyaluronidase (Leo, Sweden; 0.2 i.u./ml synovia) overnight at room temperature (*Liberg et al. 1977*).

Leukocytes in synovial fluid were counted in a Bürker chamber.

## RESULTS

*Serum protein* (Table 1)

The total protein concentration in laminitis without general affection fell, whereas in laminitis with general affection it remained stable, and it was elevated in discrete arthritis. The globulin concentration was elevated in all diseased groups, while the albumin concentration and A:G ratio were depressed. The absolute  $\alpha_1$ -concentration was significantly elevated in both laminitis groups as also was the absolute  $\alpha_2$ -concentration in laminitis with general affection.

$\beta_2$ - and  $\gamma$ -concentrations were significantly elevated both relatively and absolutely in all groups with the exception of the relative  $\beta_2$ -concentration in laminitis with general affection.

In laminitis with general affection the absolute concentrations of  $\alpha_2$  and  $\beta_1$  were significantly higher, while the relative  $\beta_2$ -con-

Table 1. The serum protein pattern in healthy, laminitic and arthritic cattle.

	Reference material n = 154		Laminitis without general affection n = 47		Laminitis with general affection n = 27		Arthritis n = 10	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Total protein (g/l)	76.7 <sup>a</sup>	4.7	73.6 <sup>b</sup>	8.6	76.0 <sup>abc</sup>	8.3	81.6 <sup>c</sup>	5.1
Albumin (g/l)	44.5 <sup>a</sup>	3.6	36.1 <sup>b</sup>	3.4	37.3 <sup>b</sup>	3.5	36.7 <sup>b</sup>	3.7
Globulin (g/l)	32.2 <sup>a</sup>	5.1	37.4 <sup>b</sup>	8.9	38.7 <sup>bc</sup>	9.4	45.0 <sup>c</sup>	7.5
A:G ratio	1.43 <sup>a</sup>	0.31	1.02 <sup>b</sup>	0.26	1.03 <sup>bc</sup>	0.31	0.83 <sup>c</sup>	0.21
Relative conc. (%)								
$\alpha_1$ -globulin	6.6 <sup>a</sup>	1.9	6.6 <sup>a</sup>	2.6	6.9 <sup>a</sup>	2.3	5.5 <sup>a</sup>	2.0
$\alpha_2$ -globulin	14.5 <sup>a</sup>	2.5	10.9 <sup>bc</sup>	3.1	14.4 <sup>ab</sup>	4.7	12.5 <sup>c</sup>	4.4
Inter- $\alpha$ - $\beta$ -globulin	4.2 <sup>a</sup>	1.5	1.8 <sup>b</sup>	1.6	2.2 <sup>bc</sup>	1.5	3.0 <sup>c</sup>	1.3
$\beta_1$ -globulin	21.3 <sup>a</sup>	2.6	17.7 <sup>b</sup>	4.3	19.6 <sup>b</sup>	5.5	16.3 <sup>b</sup>	6.5
$\beta_2$ -globulin	18.1 <sup>a</sup>	2.6	20.9 <sup>b</sup>	3.9	18.9 <sup>ac</sup>	4.1	19.9 <sup>bc</sup>	4.0
$\gamma$ -globulin	35.3 <sup>a</sup>	5.3	40.6 <sup>b</sup>	9.1	38.1 <sup>b</sup>	11.2	44.4 <sup>b</sup>	9.5
Absolute conc. (g/l)								
$\alpha_1$ -globulin	2.1 <sup>a</sup>	0.6	2.4 <sup>b</sup>	1.0	2.6 <sup>b</sup>	1.0	2.4 <sup>ab</sup>	0.9
$\alpha_2$ -globulin	4.6 <sup>a</sup>	0.6	4.4 <sup>a</sup>	1.2	5.3 <sup>b</sup>	1.1	4.8 <sup>ab</sup>	0.9
Inter- $\alpha$ - $\beta$ -globulin	1.4 <sup>a</sup>	0.5	0.6 <sup>b</sup>	0.5	0.8 <sup>b</sup>	0.5	1.3 <sup>a</sup>	0.5
$\beta_1$ -globulin	6.8 <sup>a</sup>	1.0	6.4 <sup>a</sup>	1.5	7.2 <sup>b</sup>	1.6	7.1 <sup>ab</sup>	2.2
$\beta_2$ -globulin	5.8 <sup>a</sup>	1.3	7.8 <sup>b</sup>	2.4	7.3 <sup>b</sup>	2.4	9.1 <sup>b</sup>	3.0
$\gamma$ -globulin	11.5 <sup>a</sup>	3.2	15.7 <sup>b</sup>	6.4	15.5 <sup>bc</sup>	7.4	20.3 <sup>c</sup>	6.5

Values with different alphabetical codes differ significantly ( $P < 0.05$ ).

centration was significantly lower than in laminitis without general affection.

The arthritis group had significantly higher total protein, total globulin and absolute  $\gamma$ -globulin concentrations and a lower A:G ratio than the laminitis group without general affection.

#### *Protein in synovial fluid* (Tables 2, 3)

No significant differences were found in the protein picture in normal synovial fluid from fetlock joint versus hock joint.

In synovial fluid from fetlock joints of animals displaying laminitis without general affection, the protein picture did not differ to any important degree from that of the reference material. In laminitis with general affection the total protein, total globulin, and absolute  $\beta_2$ - and  $\gamma$ -globulin concentrations were

Table 2. Protein pattern and leukocyte concentration in synovial fluid from the fetlock joint in healthy, laminitic and arthritic cattle.

	Reference material n = 17		Laminitis without general affection n = 21		Laminitis with general affection n = 9		Arthritis n = 7	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Total protein (g/l)	7.7 <sup>a</sup>	1.7	7.3 <sup>a</sup>	2.4	9.8 <sup>b</sup>	2.5	27.1 <sup>c</sup>	13.2
Albumin (g/l)	6.1 <sup>a</sup>	1.7	5.7 <sup>a</sup>	1.7	6.9 <sup>a</sup>	1.5	9.7 <sup>b</sup>	2.1
Globulin (g/l)	1.5 <sup>a</sup>	0.8	1.7 <sup>a</sup>	0.9	2.9 <sup>b</sup>	1.4	17.4 <sup>c</sup>	11.4
A:G ratio	4.32 <sup>a</sup>	2.08	4.05 <sup>a</sup>	1.71	3.02 <sup>a</sup>	1.60	1.00 <sup>b</sup>	1.02
<b>Relative conc. (%)</b>								
Albumin	78.7 <sup>ab</sup>	7.8	78.1 <sup>a</sup>	6.7	71.8 <sup>b</sup>	9.0	42.8 <sup>c</sup>	17.4
$\alpha$ -globulin	2.1 <sup>a</sup>	1.1	3.4 <sup>b</sup>	2.2	2.9 <sup>ab</sup>	1.9	5.5 <sup>c</sup>	2.2
$\beta_1$ -globulin	4.7 <sup>a</sup>	2.7	5.2 <sup>a</sup>	2.0	5.7 <sup>a</sup>	2.8	8.8 <sup>b</sup>	2.6
$\beta_2$ -globulin	3.7 <sup>ab</sup>	3.1	2.9 <sup>b</sup>	3.1	5.7 <sup>a</sup>	4.0	15.7 <sup>c</sup>	7.5
$\gamma$ -globulin	10.7 <sup>ab</sup>	4.5	10.0 <sup>b</sup>	3.6	13.9 <sup>a</sup>	6.1	26.8 <sup>c</sup>	9.6
<b>Absolute conc. (g/l)</b>								
$\alpha$ -globulin	0.2 <sup>a</sup>	0.1	0.3 <sup>a</sup>	0.3	0.3 <sup>a</sup>	0.2	1.6 <sup>b</sup>	0.8
$\beta_1$ -globulin	0.4 <sup>a</sup>	0.2	0.4 <sup>a</sup>	0.2	0.6 <sup>a</sup>	0.3	2.6 <sup>b</sup>	1.6
$\beta_2$ -globulin	0.3 <sup>a</sup>	0.3	0.3 <sup>a</sup>	0.3	0.6 <sup>b</sup>	0.5	4.8 <sup>c</sup>	3.4
$\gamma$ -globulin	0.8 <sup>a</sup>	0.3	0.7 <sup>a</sup>	0.4	1.4 <sup>b</sup>	0.7	8.1 <sup>c</sup>	6.0
Leukocytes ( $\times 10^9/l$ )	0.06 <sup>a</sup>	0.04	0.17 <sup>ab</sup>	0.32	2.70 <sup>bc</sup>	7.65	1.32 <sup>c</sup>	1.91

Values with different alphabetical codes differ significantly ( $P < 0.05$ ). Because of low concentrations  $\alpha$ -globulins are combined and inter- $\alpha$ - $\beta$ -globulins excluded.

Table 3. Protein pattern and leukocyte concentration in synovial fluid from the hock joint in healthy and arthritic cattle.

	Reference material n = 15		Arthritis n = 5		Probability
	$\bar{x}$	s	$\bar{x}$	s	
Total protein (g/l)	7.0	2.2	15.8	9.8	0.01 > P > 0.001
Albumin (g/l)	5.4	1.8	8.0	3.8	n.s.
Globulin (g/l)	1.6	0.8	7.8	6.1	P < 0.001
A:G ratio	3.73	1.47	1.49	0.65	0.01 > P > 0.001
Relative conc. (%)					
Albumin	76.4	8.3	57.6	10.4	P < 0.001
$\alpha$ -globulin	2.1	0.8	4.6	2.1	P < 0.001
$\beta_1$ -globulin	4.3	1.7	7.1	3.8	0.05 > P > 0.01
$\beta_2$ -globulin	2.7	3.6	6.4	3.9	n.s.
$\gamma$ -globulin	14.1	4.4	23.2	7.3	0.01 > P > 0.001
Absolute conc. (g/l)					
$\alpha$ -globulin	0.2	0.1	0.9	0.9	0.01 > P > 0.001
$\beta_1$ -globulin	0.3	0.2	1.4	1.3	0.01 > P > 0.001
$\beta_2$ -globulin	0.2	0.3	1.3	1.4	0.01 > P > 0.001
$\gamma$ -globulin	1.0	0.4	3.6	2.4	P < 0.001
Leukocytes ( $\times 10^9/l$ )	0.06	0.04	0.33	0.20	P < 0.001

Because of low concentrations  $\alpha$ -globulins are combined and inter- $\alpha$ - $\beta$ -globulins excluded.

elevated. In arthritis all protein concentrations in synovial fluid from the fetlock joint were affected to a very significant degree. The relative albumin concentration and A:G ratio were depressed, while the other proteins were considerably elevated, particularly in the case of  $\beta_2$  and  $\gamma$ . Compared to laminitis without general affection, laminitis with general affection demonstrated significantly higher total protein, total globulin,  $\beta_2$ - and  $\gamma$ -globulin concentrations, but lower relative albumin concentration. In comparison with both laminitis groups, the arthritis group had higher concentrations of all globulins.

The protein picture in synovial fluid from the hock joint of arthritic animals tallied in principle with the synovial fluid from the fetlock joint. No investigations of synovial fluid from the hock joint were performed in the laminitis groups.

*Leukocytes in synovial fluid* (Tables 2, 3)

The average leukocyte concentration in normal synovial fluid both from fetlock joint and hock joint was  $0.06 \times 10^9/l$ . Laminitis with general affection, like arthritis, gave significantly elevated leukocyte counts.

The leukocyte concentration in arthritis was significantly higher than in laminitis without general affection, while that in laminitis with general affection had a wide range and did not differ significantly from the other diseased groups.

## DISCUSSION

Laminitis is patho-anatomically a sharply defined disease process, localized primarily to the hoof corium. The clinical picture is more diffuse. It may include symptoms not only from the hooves alone, but also from other parts of the extremities. General symptoms may be present. Laminitis may appear as an independent disease but also concomitantly with other disease processes such as retentio secundinarum, endometritis, mastitis and ketosis (*Rosenberger 1978*).

In the present investigation laminitis was an independent disease. Retentio secundinarum had earlier occurred in five cases and mastitis in three. In the animals with arthritis, retentio secundinarum was reported in one case and endometritis in another. The serum protein changes found were considered to be related primarily to the symptoms of laminitis or arthritis in these animals too. The serum protein picture both in laminitis and arthritis clearly denotes the presence of an inflammatory reaction. The arthritis group showed increased total protein, which was not the case in laminitis. On the contrary, total protein was even lower than normal in laminitis without general affection. The rise in serum  $\gamma$ -globulin and  $\beta_2$ -globulin in all disease groups must be seen as an expression of an immune response. The particularly heavy increase in  $\gamma$ -globulin and the simultaneous absence of increase in  $\alpha$ -globulin in the arthritis group are evidence of a predominantly chronic inflammatory process. The conditions in the laminitis groups are more difficult to interpret. The increase in  $\alpha$ -globulin and the less pronounced rise of  $\gamma$ -globulin than in the arthritis group, may denote more acute inflammatory processes.

The observed differences in the serum protein picture between the laminitis groups cannot be ascribed any clinical significance. The protein changes may be considered as correlated with the common symptoms and not dependent on the general affection found in one of the groups.

The laboratory results argue against an affection of the joints in laminitis without general affection. In contrast, changes of synovial fluid especially in  $\beta_2$ - and  $\gamma$ -globulins were found in laminitis with general affection. These changes and the fact that seven of nine animals displayed diffuse swellings distally on the legs indicate the simultaneous presence of arthritis.

Synovial protein and leukocyte concentrations at the levels found in arthritis and laminitis with general affection have been observed previously in aseptic arthritis (*Kersjes 1963, van Pelt 1973*). Bacteriological investigations were not carried out in the present study. However, the observed moderate increases in leukocyte and protein levels indicate aseptic joint processes. The leukocyte concentration in laminitis with general affection did not differ significantly from that found in arthritis. Leukocyte levels in arthritis were elevated in six cases of seven ( $> \bar{x} + 2s$ ), whereas in laminitis with general affection only four of nine values were elevated. Therefore markedly deviating individual values and a wide standard deviation within the groups might hide a real difference between them.

The designations 'with' and 'without' general affection in laminitis reflect primarily the intensity of the disease and not its duration. Laminitis with general affection can, in most cases, be regarded as synonymous with acute laminitis. Also the cases without general affection may usually represent acute — though less intense — inflammatory processes. A type of the disease with still lower intensity is described by *Peterse (1980)* as sub-clinical laminitis, in which the only clinical signs were slight chronic changes of laminitis such as concave wall and blood spots in the sole horn.

The aetiological factors of the inflammatory reaction in laminitis are not known. It has been intimated that a release of histamine is involved (*Åkerblom 1934, Nilsson 1963, Maclean 1966*). Hence the disease has been considered as local manifestation of an atopic reaction. More recent investigations (*Eyre et al. 1973*) have suggested that histamine plays only a subordinate role in hypersensitivity reactions in cattle. Other bio-



logically active substances such as lactic acid and certain endotoxins have been associated with the origin of laminitis in horses (Garner *et al.* 1977, Moore *et al.* 1979). This may also have relevance in cattle. Thus Rosenberger does not exclude a hypersensitivity reaction attributable to toxic substances from the uterus or the alimentary tract as an aetiological factor in laminitis. Particular attention has been directed to the adjustment of diet at the onset of lactation, the period when most cases of laminitis occur (Andersson 1980).

Rosenberger states that arthritis may arise post partum as a hypersensitivity reaction or as metastasis from inflammatory processes — for instance in the uterus. In the present investigation nine of the 10 cows with discrete arthritis fell ill within two months of calving. The present cases of arthritis were considered aseptic. A hypersensitivity reaction cannot be excluded as an aetiological factor either in arthritis.

In conclusion the material investigated proved to consist partly of animals affected solely by laminitis or solely by arthritis, partly of animals affected by a combination of the two. The different clinical manifestations might be an expression of different reactions to a common aetiology, indicating the existence of a disease complex which manifests itself in hoof corium, in joints or in both.

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#### SAMMANFATTNING

*Blodserum och ledvätska vid fång och artrit hos nötkreatur med särskild hänsyn till förändringar i proteinbilden.*

Hos 84 vuxna nötkreatur med de kliniska diagnoserna fång (74 fall) och fristående artrit (10 fall) påvisades förändringar dels i blodproteinbilden, dels i proteinbild och leukocytkoncentration i ledvätska. Fångfallen grupperades i sådana utan respektive med allmänpåverkan. Totalglobulinet var förhöjt och albuminet sänkt i blodserum i alla sjukdomsgrupperna. Totalglobulinökningen orsakades i

första hand av förhöjt  $\gamma$ -globulin i alla grupperna.  $\alpha$ -globulinökningar förekom endast i fånggrupperna. Vid fristående artrit var den relativa albuminkoncentrationen och A:G-kvoten sänkta i ledvätskan, medan övriga proteiner och leukocytkoncentrationen var förhöjda. Vid fång utan allmänpåverkan förelåg inga väsentliga förändringar i ledvätska från kotleden. Vid fång med allmänpåverkan var totalprotein-, totalglobulin-, absoluta  $\gamma$ -globulin- och leukocytkoncentrationerna förhöjda. Dessa förändringar talar för samtidig förekomst av artrit i denna grupp. Kombinationen av fång och artrit skulle kunna vara uttryck för olika reaktioner på en gemensam etiologi och indikera förekomsten av ett sjukdomskomplex, som manifesterar sig i klövläderhud, i leder eller i bådadera.

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