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LISTERIOSIS IN SHEEP

TICK-BORNE FEVER USED AS A MODEL TO STUDY PREDISPOSING FACTORS*

By

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GRØNSTØL, H. & J. ØVERÅS: *Listeriosis in sheep. Tick-borne fever used as a model to study predisposing factors.* Acta vet. scand. 1980, *21*, 533—545. — Three groups of 6 months old lambs, each group consisting of 5 animals, were infected experimentally with Ehrlichia phagocytophila (Ep), Listeria monocytogenes (Lm) and Ep/Lm, respectively. All the animals had a period with fever and reduced appetite after infection, and these symptoms were most pronounced in the group with the combined infection (Ep/Lm). One animal in group Lm developed listeric meningo-encephalitis.

Lm was isolated from blood samples from both groups infected with Lm during the first week after infection, and from faecal samples during the first 2 weeks. Lm was also isolated from organs from several animals in these 2 groups at post-mortem examination.

Group Ep/Lm developed the highest reciprocal geometrical mean titres and the strongest delayed hypersensitivity reaction against Lm.

After infection, a fall in serum iron and albumin was recorded, and the groups infected with Ep had a substantial fall in neutrophils.

The myeloid:erythroid ratio in the bone marrow tended to decrease in Group Ep/Lm after infection.

An increase in leucocyte counts and total protein content was found in the cerebrospinal fluid in the 2 groups infected with Lm.

The experiment indicates that the blood changes induced by tick-borne fever viz. neutropenia and probably also impaired function of the neutrophils, may predispose for listeric septicaemia, but probably not for listeric meningo-encephalitis.

sheep; Listeria monocytogenes; Ehrlichia phagocytophila; immunity; blood components; bone marrow; cerebrospinal fluid.

A large proportion of healthy sheep seems to be carriers of Listeria monocytogenes (Lm) (*Grønstøl* 1979, 1980 a). This indicates that sheep are fairly resistant against Lm. Factors which

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reduce the resistance are probably of importance for the development of clinical listeriosis (*Seeliger* 1961).

The first author has previously studied experimental listeric infection in sheep treated with various immunosuppressiva (*Grønstøl* 1980 b). Although evidence of reduced resistance was found, none of the animals developed clinical listeric encephalitis.

Tick-borne fever (TBF) is a disease caused by Ehrlichia phagocytophila (Ep). This disease in known to dispose for various infections, such as infections with staphylococci (*Foggie* 1956), louping ill virus (*Gordon et al.* 1962), Pasteurella haemolytica and various streptococci (*Øverås* 1972) and Listeria monocytogenes (septicaemia) (*Grønstøl & Ulvund* 1977).

The present work was undertaken to examine to which extent the blood changes induced by TBF will dispose for clinical listeriosis, especially listeric meningo-encephalitis. The clinical state, development of humoral (HI) and cell mediated immunity (CMI) against Lm, and changes in some blood components were studied. In addition, samples of bone marrow and cerebrospinal fluid (CSF) were also examined.

MATERIALS AND METHODS

Animals

Fifteen lambs of the Dala and Ryggja breeds, about 6 months old, were used in the experiment. They belonged to the experimental flock at this research station, and management and feeding regime have been described elsewhere (*Grønstøl* 1979).

Two weeks before the experiment started, the lambs were divided into 3 groups. Each group consisted of 2 animals with haemoglobin type BB and 3 animals with type AA. They were placed in pens with slatted floors in the quarantine department and fed hay and concentrates. The groups were designed Ep, Lm and Ep/Lm, according to the organisms they were infected with.

On the first day of the experiment (ED 1), 2 of the groups were infected with Ep. The animals were infected intravenously with 5 ml of heparinized blood, taken from an infected sheep shortly after the fever period. The group not infected with Ep and 1 of the infected groups were swabbed intranasally with a culture of Lm on ED 11. The strain* used belonged to serotype 4. The bacteria had been passed 3 times through mice before

* Kindly supplied by prof. E. H. Kampelmacher, Utrecht.

inoculation. The swabbing was repeated on ED 12. On ED 26 the same animals were swabbed once more.

The temperatures were recorded daily and the animals were weighed every fortnight.

The experiment ended on ED 45, when 4 animals from each of the 2 groups infected with Lm were killed and examined post mortem.

Bacteriological examination

Blood samples were taken on the dates recorded in Fig. 1 and examined bacteriologically in the following way: To 10 ml of whole blood was added 5 ml of destilled water, and the haemolysate was incubated at 37°C for 24 h. One loopful of the sample was then plated on Blood agar (Oxoid blood agar base no. 2 with addition of 5 % sheep blood) and Tryptose agar (Oxoid). Colonies and bacteria were identified as described by *Grønstøl & Aspøy* (1977).

Faecal samples were collected on the same dates as the blood samples and examined as described previously (*Grønstøl* 1979).

Organ samples were examined bacteriologically as described by *Grønstøl* (1980 a).

Serological examination

Sera were examined for antibodies on the dates recorded in Table 1, by an indirect haemagglutination method (*Grønstøl* 1979).

Skin test

The animals were tested for delayed hypersensitivity (DHS) against an antigen prepared from Lm, as described by *Grønstøl* (1979), before the experiment started and on ED 26.

Examination of some blood components

Packed cell volume (PCV), haemoglobin, number of red cells, total and differential counts of leucocytes, plasma glucose, potassium and sodium, total serum protein, inorganic phosphorus, calcium, magnesium and iron were determined, and electrophoresis of serum proteins was carried out by methods routinely used in this laboratory (*Øverås* 1969, 1974, *Waldeland* 1977 and *Grønstøl* 1980 b).

Examination of bone marrow and cerebrospinal fluid (CSF)

Bone marrow samples were aspirated from animals in Groups Lm and Ep/Lm on the days recorded in Table 2. The smears were made and stained as described by *Øverås* (1969). The myeloid:erythroid (M:E) ratio was determined.

CSF-samples were taken on ED 42 by occipetal puncture (*Hiepe* 1959), and the cells were counted in a Fuchs-Rosenthal counting chamber. Protein content was determined according to *Meulemans* (1960) and glucose by the method used for blood samples.

Histological examination

Sections from pons, cerebrum, cerebellum, metencephalon, medulla oblongata, lungs, livers and kidneys were taken from the killed animals and stained with haematoxylin-eosin.

RESULTS

Clinical findings

After infection with Ep the animals had elevated temperatures, up to 41 °C for 4—5 days, and those infected with Lm had a similar rise for 3 days after the first infection. Ep was demonstrated in blood smears from all the animals experimentally infected with this organism. During the febrile periods the animals had reduced appetite and looked depressed. The longest distress period was recorded in Group Ep/Lm. During the first 3 weeks of the experiment this group lost weight, and in the period from 1 week before to the 31st day after the experiment started, the animals in this group lost on average 11 g/day, while the animals in Groups Ep and Lm gained 70 and 65 g/day, respectively, during this period.

One of the animals in Group Lm developed symptoms of meningo-encephalitis towards the end of the experiment. Apart from distress during the febrile periods and rhinitis after swabbing with Lm, no other symptoms of disease were seen.

Bacteriological examination

Lm was isolated from blood samples from all the animals in Group Ep/Lm and Group Lm during the first week, and from faeces of all animals in these 2 groups during the first 2 weeks after infection with Lm, but Lm was not isolated from any sample

from group Ep. No significant difference in isolation rate was found between the 2 groups infected with Lm.

Lm was isolated from 2 brains and from 1 pair of lungs in the 4 animals examined in Group Ep/Lm. In Group Lm the bacteria were isolated from the brain of 3 and the lungs of 1 sheep. The isolates belonged to serotype 4.

Serological examination

Reciprocal geometrical mean titres (GMT) against Lm for the 3 groups are listed in Table 1. Group Ep had a GMT of less than 10 throughout the experiment. Group Ep/Lm had a strong increase in titres, an increase which started on ED 15, i.e. 4 days after infection with Lm, and reached its highest value on ED 21. The second infection with Lm, on ED 26, when the titres had started to decline, resulted in a new increase with a peak 9 days later.

Table 1. Reciprocal geometrical mean titres (GMT) for 3 groups of lambs. Each group consisted of 5 animals, and the groups were infected with either Ehrlichia phagocytophila (Ep), Listeria monocytogenes (Lm) or a combination of both (Ep/Lm).

		GMT								
Group	ED	11	15	17	19	21	24	27	34	41
Ep[1]		10	10	10	10	10	10	10	10	10
Lm[2]		10	20	61	106	139	121	121	184	121
Ep[1]/Lm[2]		10	10	17	422	844	844	422	970	211

ED Experimental day.
[1] Infected on ED 1.
[2] Infected on ED 11 and ED 26.

Group Lm had a rise in GMT which started on ED 14 and reached a maximum value on ED 21, i.e. 10 days after infection with Lm. However, the peak was 2.5 units below that of Group Ep/Lm. After reinfection, GMT reached another maximum value 9 days later, but this value was still 2.5 units below the corresponding value for Group Ep/Lm.

Skin test

Before the experiment started, all the animals had a skin reaction of less than 0.5 mm. On ED 26, i.e. 15 days after infec-

tion with Lm, the average reaction was less than 0.5 mm in Group Ep, 3.7 mm in Group Ep/Lm and 2.9 mm in Group Lm. The difference between the last 2 groups was not statistically significant (students t-test).

Blood components

PCV (Fig. 1), haemoglobin content and number of red cells fell in all groups during the first 4 weeks of the experiment, and the lowest values were found in Group Ep/Lm.

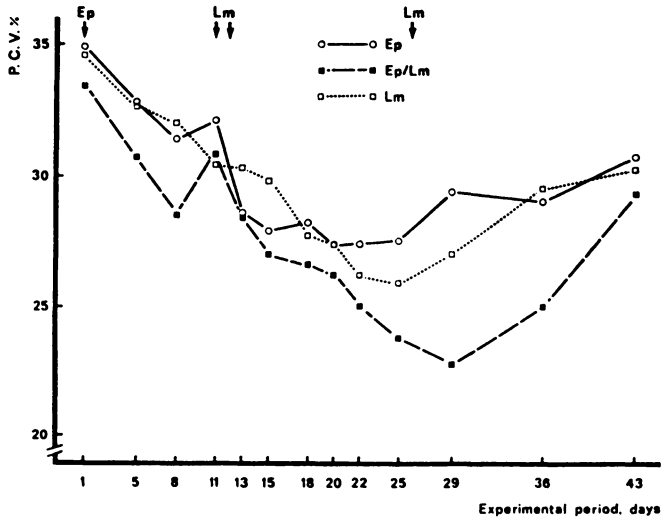

Figure 1. Average packed cell volume (PCV) values in 3 groups of 5 lambs, infected with Ehrlichia phagocytophila (Ep), Listeria monocytogenes (Lm), or both (Ep/Lm).

No significant differences were found between the groups with regard to potassium, sodium, inorganic phosphorus and glucose.

Infection with Ep resulted in a fall in serum iron. The difference between the 2 Ep infected groups and Group Lm was statistically significant ($P < 0.001$). After infection with Lm, a similar fall was recorded in Groups Lm and Ep/Lm, as shown in Fig. 2, and the difference between these groups and the remaining group was statistically significant ($P < 0.001$).

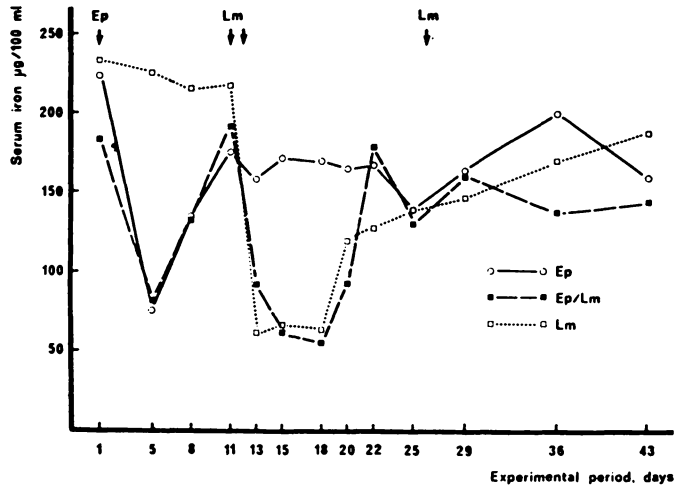

Figure 2. Average serum iron values in 3 groups of 5 lambs, infected with Ehrlichia phagocytophila (Ep) Listeria monocytogenes (Lm), or both (Ep/Lm).

Albumin and γ-globulin fractions are shown in Fig. 3. The Groups Ep and Lm had a fall in albumin to less than 3 g/100 ml and a ratio albumin:globulin (A:G) which fell from 1.1 to 0.7 and 0.8. Group Ep/Lm had a fall in albumin to less than 2.5 g/100 ml and in A:G to 0.5. An increase in the γ-globulin fraction to nearly 3 g/100 ml was also found in this group.

After infection with Ep the number of neutrophils was reduced to about 500 cells/µl. After infection with Lm the number of neutrophils rose rapidly.

Bone marrow and CSF

As can be seen from Table 2, the M:E ratio tended to decrease in Group Ep and increase in Group Ep/Lm.

The number of leucocytes and content of glucose and protein in CSF on ED 42, are shown in Table 3. In the 2 groups infected with Lm, the leucocyte counts were increased in most of the animals, while the counts in Group Ep were within normal limits. The protein content was also elevated in most animals in the 2 groups infected with Lm.

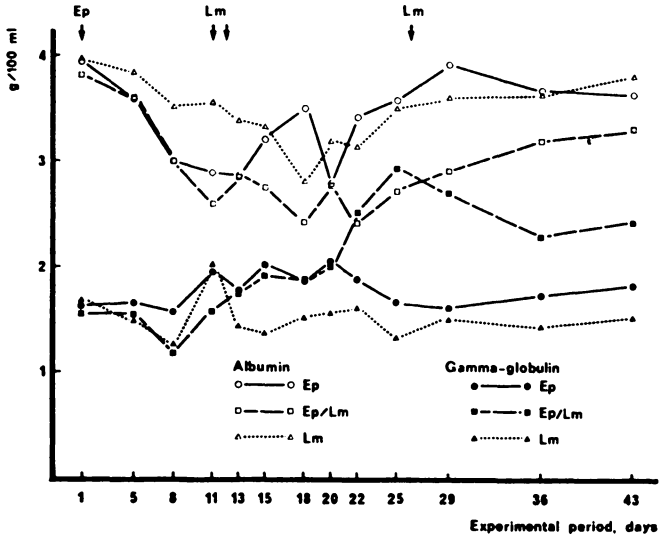

Figure 3. Average serum albumin and gamma-globulin values in 3 groups of 5 lambs, infected with Ehrlichia phagocytophila (Ep), Listeria monocytogenes (Lm), or both (Ep/Lm).

Table 2. Myeloid:erythroid ratio recorded in bone marrow smears from lambs infected with Ehrlichia phagocytophila (Ep) and Listeria monocytogenes (Lm).

Group	Animal no.	ED 1	ED 10	ED 19
Ep	1	0.7	0.4	0.5
	2	0.9	0.8	0.4
	3	0.5	0.6	0.3
	4	1.2	1.6	0.5
	5	1.0	—	0.7
Ep/Lm	6	1.2	0.6	1.9
	7	1.1	0.7	1.1
	8	1.0	0.4	2.1
	9	0.7	0.9	5.2
	10	0.8	0.9	2.6

ED — Experimental day.

Table 3. Average cerebrospinal fluid (CSF) values and range of leucocytes, glucose and protein in the experimental groups on ED 42.

Group	Leucocytes/mm³	Glucose mg/100 ml	Protein mg/100 ml
Ep	2 (1—3)	45.0 (42—49)	37.2 (24—58)
Ep/Lm	18 (1—42)	44.0 (39—48)	73.8 (45—136)
Lm	103 (8—450)	36.6 (2 —49)	133.4 (56—243)
Lm*	16 (8—24)	45.3 (41—49)	106.0 (56—208)

* One animal with clinical listeric meningo-encephalitis was excluded from the group. This animal had the following values: Leucocytes 450, glucose 2, total protein 243.

Histological findings

Histological examination revealed infiltrations of cells in the hepatic triads, cell infiltrations in the meninges of the animal which had shown nervous symptoms, cuffing, microglia proliferation and a slight demyelinization in some of the animals in the 2 groups examined.

DISCUSSION

TBF has been shown to reduce resistance to certain infections (*Foggie* 1956, *Øverås* 1972). The exact mechanism behind this is not known, but so far 2 effects of TBF have been suggested as disposing factors. *Taylor et al.* (1941) demonstrated that neutropenia is a characteristic feature in sheep with TBF, and this will most likely reduce the resistance to several infections. In addition, *Foster & Cameron* (1970) found that diapedesis of Ep-infected neutrophils may be inhibited. Consequently, a period with impaired function of these cells seems to precede and partly accompany the neutropenic phase of an Ep infection. Because of these effects TBF was thought to be a useful model to study the importance of these cells in the resistance to infections with Lm.

The main defence against Lm seems to be CMI (*Mackaness* 1962), but unspecific resistance may be of importance during the first stages of infection. None of the animals had a specific immunity against Lm when the experiment started, judged by the serological examination and the skin tests. However, they could have been exposed to Lm previously, because a large pro-

portion of the sheep in the flock had been found to excrete Lm in periods (*Grønstøl* 1979).

Animals in the 2 groups infected with Lm had bacteriaemia for some days and excreted Lm in the faeces during the first 2 weeks after infection. But none of them developed fatal septicaemia.

The lambs in Group Ep/Lm lost weight during the first 3 weeks of the experiment, but none of them showed symptoms from the central nervous system (CNS). They had a temperature rise for a few days after infection with Lm, but recovered without further symptoms. In Group Lm, 1 of the animals showed symptoms of meningo-encephalitis towards the end of the experimental period. The histological examination confirmed the clinical diagnosis.

The combined infection clearly aggravated the stress on the animals. In addition to the weight loss, the lambs in Group Ep/Lm had the greatest fall in serum iron and albumin values. Serum iron may drop during periods of stress (*Underwood* 1977). The fall in PCV, haemoglobin and number of red cells was most pronounced in Group Ep/Lm and was probably caused by a depressed formation of red cells in the bone marrow, as also indicated by the increased M:E ratio. The calculated mean volume of the red cells (MCV) did not increase during the anaemic period, a fact that also supports this hypothesis. The haemolysin produced by Lm may have contributed to this effect.

Group Ep/Lm also had a fall in albumin to less than 2.5 g/100 ml. This is in accordance with the findings of *Grønstøl & Ulvund* (1977) who found an albumin content of 2.37 g/100 ml in a spontaneous case of combined infection. The low albumin values might be caused by a reduced production of albumin in the liver or/and loss of albumin through the kidneys and the gastrointestinal tract.

This group also had an increase in γ-globulin, up to nearly 3 g/100 ml, and a low A:G.

The HI response was clearly higher in Group Ep/Lm than in Group Lm, while Group Ep had no HI reaction against Lm. Group Ep/Lm also had the strongest DHS reaction against Lm. Both these results indicate that the effect of the Lm infection was stronger in Group Ep/Lm than in Group Lm, and that Ep may predispose for listeric septicaemia.

Group Ep had CSF values which were within the limits found in healthy animals by *Frankfurter* (1962) and *Lippmann* (1969). Group Ep/Lm had slightly elevated leucocyte counts and total protein content. The animals in Group Lm had values on the same level, except for the animal with meningo-encephalitis. This lamb had a marked increase in number of leucocytes and protein content, and a strong decrease in glucose content.

This indicates that even though the animals had not shown any symptoms from the CNS, their brains might have been exposed to Lm. It is also possible that some of the animals might have carried Lm in their brains already before the experiment started. *Grønstøl* (1980 a) isolated Lm from brains of healthy sheep at slaughter and from brains of sheep with various diseases without any histological evidence for inflammation of the brain. In the present experiment Lm was also isolated from some of the brains, and it is noticeable that the histological examination revealed slight lesions which might have been induced by Lm.

The immune system may therefore be able to prevent clinical meningo-encephalitis, but not to get rid of the bacteria. What mechanisms the bacteria use to escape the immune system are not known, but conversion to L-forms and immunological tolerance have been suggested (*Pease* 1967).

The fact that 1 animal in Group Lm developed listeric meningo-encephalitis while none of the animals in Group Ep/Lm showed symptoms from CNS, tend to exclude neutropenia and impaired function of the neutrophils as predisposing factors for listeric meningo-encephalitis.

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SAMMENDRAG

Listeriose hos sau. Sjodogg (tick-borne fever) brukt som modell til å studera disponerande faktorar.

Tre grupper med 6 månader gamle lam, kvar gruppe på 5 dyr, blei brukte i forsøket. Dei blei infiserte med Ehrlichia phagocytophila (Ep), Listeria monocytogenes (Lm) og Ep/Lm. Alle dyra fekk feber og nedsatt matlyst etter infeksjonen, og desse symptoma var sterkast i gruppa med kombinert infeksjon (Ep/Lm). Eitt lam i gruppe Lm fekk listeria meningo-encephalitt.

I den fyrste veka etter infeksjon med Lm blei bakteriane isolerte frå blodprøver frå dyr i begge dei Lm infiserte gruppene, og frå faecesprøver i dei to fyrste vekene. Dessutan blei Lm isolert frå organ frå fleire dyr i desse gruppene etter avliving.

Gruppe Ep/Lm hadde dei høgaste geometriske gjennomsnittstitra og den sterkaste seinka hypersensitiviteten mot Lm.

Dyra hadde eit tydeleg fall i serum jern og albumin etter infeksjonen, og gruppe Ep hadde eit stort fall i neutrofile leukocyttar.

Tilhøvet myeloide:erythroide celler i beinmargen såg ut til å minka i gruppe Ep og auka i gruppe Ep/Lm etter infeksjonen.

Talet på leukocyttar og proteinmengda auka i cerebrospinalvæska etter infeksjon med Lm.

Det ser ut til at dei endringane i blodet som sjodogg (tick-borne fever) fører til, kan disponera for listeria septikemi, men truleg ikkje for listeria meningo-encephalitt.

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