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From the State Veterinary Research Station for Small Ruminants, Høyland, Sandnes, Norway

LISTERIOSIS IN SHEEP

EPERYTHROZOON OVIS INFECTION USED AS A MODEL TO STUDY PREDISPOSING FACTORS*

By

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GRØNSTØL, H. & J. ØVERÅS: *Listeriosis in sheep. Eperythrozoon ovis infection used as a model to study predisposing factors.* Acta vet. scand. 1980, *21*, 523—532. — Three groups of 9 months old lambs, each group consisting of 5 animals, were infected experimentally with Eperythrozoon ovis (Eo), Listeria monocytogenes (Lm) and Eo/Lm, respectively. The animals infected with Eo developed haemolytic anaemia, but otherwise no clinical symptoms were seen. The animals infected with Lm had a period with fever and reduced appetite after infection. These symptoms lasted longer and were more pronounced in the group with the dual infection (Eo/Lm). None of the lambs developed clinical meningo-encephalitis during the experiment.

Group Lm developed the highest reciprocal geometrical mean titres against Lm. No titer rise was found in group Eo, while group Eo/Lm had a slight rise towards the end of the experiment. Group Eo/Lm also had the strongest delayed hypersensitivity reaction against Lm.

After Eo infection, a fall in packed cell volume, haemoglobin, number of red cells, and plasma glucose and an increase in serum iron were recorded. Serum iron dropped and serum copper increased after infection with Lm.

In this experiment the blood changes induced by Eo, i.e. haemolytic anaemia and acidosis, led to a prolonged state of illness in animals infected with Lm, in addition to inhibited development of antibody titres, but not to clinical meningo-encephalitis.

sheep; Eperythrozoon ovis; Listeria monocytogenes; immunity; haemolytic anaemia; acidosis.

Eperythrozoon ovis (Eo) seems to be present at least in parts of the sheep flocks in this country (*Øverås* 1969). An acute infection with Eo leads to haemolytic anaemia (*Øverås* 1969) and

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acidosis (*Sutton* 1977). Both these conditions are suggested as predisposing factors in clinical listeriosis (*Sword* 1966, *Grønstøl* 1980 a), and *Øverås* (1969) described listeric encephalitis in 2 sheep previously infected with Eo, while listeriosis did not otherwise occur in the flock.

The present work was undertaken to examine whether the blood changes induced by an infection with Eo may predispose for clinical listeriosis. The clinical state, excretion of Listeria monocytogenes (Lm), immune response and some blood components were studied.

MATERIALS AND METHODS

Animals

The 15 animals used in this experiment were of the Dala and Ryggja breeds, about 9 months old, and they belonged to the experimental flock at this research station. The management and feeding regime have been described elsewhere (*Grønstøl* 1979).

Ten days before the experiment started, the animals were divided into 3 groups and placed in pens with slatted floors in the quarantine department. Each group consisted of 3 animals of haemoglobin type AA and 2 animals of type BB. They were fed hay and concentrates. Before the experiment started, they were screened for the presence of Eo, antibodies and delayed hypersensitivity (DHS) against Lm.

On the first day of the experiment (ED 1), Group Eo and Group Eo/Lm were inoculated intravenously with 2.5 ml of heparinized blood taken from an animal with a heavy Eo-parasitaemia. On ED 13 the animals in Group Eo/Lm and Lm were given 10^{10} Lm serotypes 1 and 4 by stomach tube and were swabbed intranasally with cultures of these 2 serotypes. The bacteria had previously been passed 3 times through mice. The experiment ended on ED 40.

Throughout the experimental period the body temperatures were recorded daily, and the animals were weighed once a week.

Bacteriological examination

Faecal and blood samples taken on the days recorded in Table 1 were examined bacteriologically as described by *Grønstøl* (1979) and *Grønstøl & Øverås* (1980).

Serological examination

Sera were tested for antibodies against Lm by an indirect haemagglutination method (*Grønstøl* 1979).

Skin test

A skin test was performed before the experiment started and on ED 27 as described by *Grønstøl* (1979).

Examination of some blood components

Blood smears were stained and examined for Eo as described by *Øverås* (1969). Red cell counts and total and differential leucocyte counts were made. Packed cell volume (PCV), whole blood haemoglobin, total serum protein, serum iron, serum copper and plasma glucose were estimated, and electrophoresis of serum proteins were performed by methods routinely used in this laboratory (*Øverås* 1969, 1974, *Waldeland* 1977, *Grønstøl* 1980b).

RESULTS

Clinical findings

The animals infected with Eo developed haemolytic anaemia with pale mucous membranes, but no other symptoms of disease were seen. The animals in the 2 groups inoculated with Lm became ill after the infection. They appeared dull, had elevated temperatures for 3—4 days (Group Lm) and 7—8 days (Group Eo/Lm). During the febrile period the feed intake was low, and during the first week after infection with Lm the animals in Group Lm lost on average 2.2 kg body weight, while the animals in group Eo/Lm lost on average 2.6 kg.

After the febrile period the animals in Group Lm soon gained weight, and during the whole experimental period the animals in Group Eo and Lm gained 114 g/day and 119 g/day, respectively. The animals in Group Eo/Lm were unthrifty for about 2 weeks and recovered slowly. Their weight gain during the experimental period was on average 14 g/day.

None of the animals developed meningo-encephalitis, and all of them appeared healthy when the experiment ended.

Bacteriological examination

Lm was isolated from blood samples from 2 animals in Group Eo/Lm on ED 15, i.e. 2 days after infection with Lm. The other

blood samples were negative. One week after infection Lm was isolated from 3 of 5 faecal samples from both groups infected with Lm.

Serological examination

Reciprocal geometrical mean titres (GMT) are recorded in Table 1. Group Lm developed high titres with a peak on the 13th day after infection with Lm. No specific titre rise was found in Group Eo, while Group Eo/Lm had a slight rise with the peak towards the end of the experimental period.

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 Eperythrozoon ovis (Eo), Listeria monocytogenes (Lm) or a combination of both (Eo/Lm).

Group	ED —5	3	6	8	10	13	15	17	20	22	24	27	30	40
Lm[2]	13	15	17	26	20	11	23	26	121	211	422	640	485	184
Eo[1]	11	17	30	35	53	30	40	70	53	35	35	26	30	30
Eo[1]/Lm[2]	30	26	40	61	70	30	46	46	35	40	80	139	106	139

ED Experimental day.
[1] Infected on ED 1.
[2] Infected on ED 13.

Skin test

One animal in Group Eo had a strong skin reaction before the experiment started. The other animals had a reaction of less than 0.5 mm. When tested on ED 27, no distinct increase in skin reaction was found in Group Eo. The other 2 groups showed a positive reaction, with an average increase of 2.5 mm in Group Lm and 3.5 in Group Eo/Lm. The difference between these 2 groups was statistically significant (students t-test, $P < 0.05$).

Blood components

All the groups had a fall in PCV (see Fig. 1), haemoglobin and number of red cells. This was most pronounced in Group Eo/Lm. No distinct differences between the groups were found with regard to total serum protein and number of leucocytes.

Plasma glucose values are recorded in Fig. 2. After infection with Eo the values dropped sharply and reached their lowest point on ED 10. No fall was seen in Group Lm.

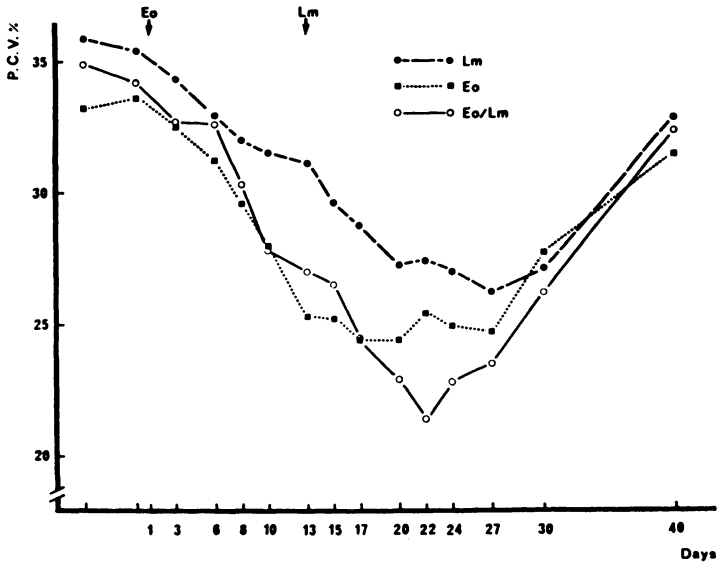

Figure 1. Average packed cell volume (PCV) values in 3 groups of 5 lambs, infected with Eperythrozoon ovis (Eo), Listeria monocytogenes (Lm) or both (Eo/Lm).

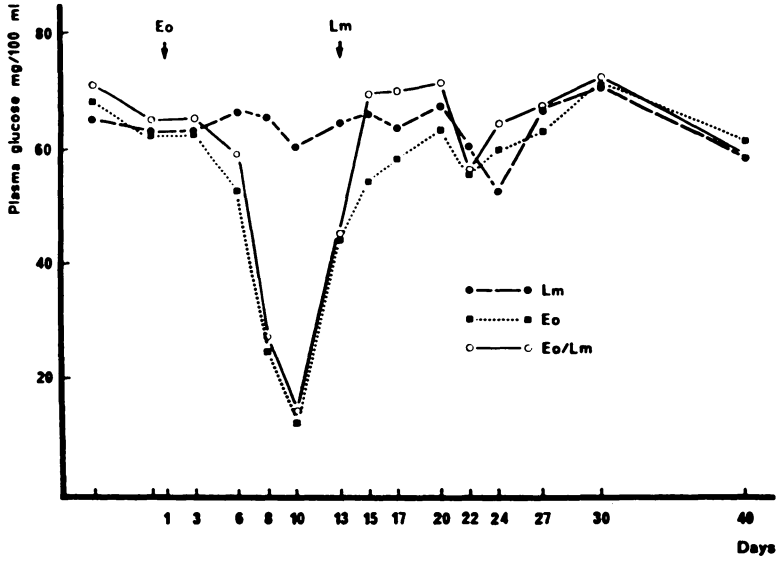

Figure 2. Average plasma glucose values in 3 groups of 5 lambs, infected with Eperythrozoon ovis (Eo), Listeria monocytogenes (Lm) or both (Eo/Lm).

Serum iron increased after infection with Eo, but fell substantially after infection with Lm, as shown in Fig. 3.

The serum copper content is illustrated in Fig. 4. After infection with Lm an increase was seen in both the infected groups. The highest values were recorded in Group Eo/Lm on ED 24.

DISCUSSION

Eo is an organism which parasitizes the erythrocytes of sheep and is apparently common in this country (*Øverås* 1969). An acute infection with Eo causes haemolytic anaemia with subsequent rise in serum iron.

The animals used in this experiment belonged to the experimental flock at this research station and had probably been exposed to Lm previously (*Grønstøl* 1979). The animals in the group with the combined infection (Eo/Lm) reacted with a prolonged period of illness. They had a substantially longer period with fever and unthriftiness than the animals in Group Lm. The blood changes induced by Eo might have rendered the animals more susceptible to an infection with Lm.

The fall in number of red cells was striking. The increased destruction of the red cells resulted in an elevated level of serum iron. Total iron binding capacity was not determined, but the rise in serum iron probably increased the transferrin saturation. An increased transferrin saturation may enhance bacterial growth (*Bullen et al.* 1972). *Sword* (1966) found that the elevated level of serum iron in haemolytic anaemia made mice more susceptible to infection with Lm, and showed that the resistance might be increased by treatment with an iron chelating compound. A high transferrin saturation might have been a virulence factor also in this experiment.

To make iron less available for bacteria is one of the mechanisms used to resist an infection (*Weinberg* 1978). In both groups infected with Lm there was a rapid fall in serum iron shortly after the infection. The previous haemolysis in Group Eo/Lm probably made this mechanism less effective in this group and might have contributed to the prolonged state of illness.

One of the factors responsible for the withdrawal of iron, the leucocyte endogenous mediator (LEM), which is produced by the leucocytes in association with an infection, also has the effect of increasing the production of ceruloplasmin (*Underwood* 1977).

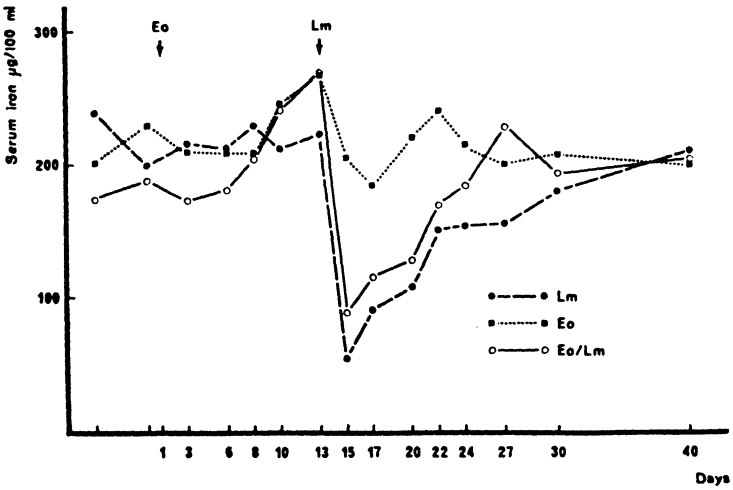

Figure 3. Average serum iron values in 3 groups of 5 lambs, infected with Eperythrozoon ovis (Eo), Listeria monocytogenes (Lm) or both (Eo/Lm).

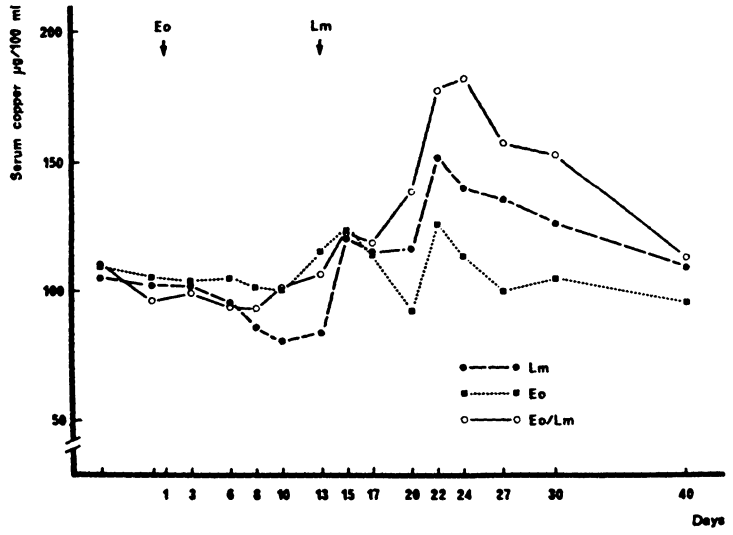

Figure 4. Average serum copper values in 3 groups of 5 lambs, infected with Eperythrozoon ovis (Eo), Listeria monocytogenes (Lm) or both (Eo/Lm).

This is reflected in the increase in serum copper in the present experiment. The group with the longest span of illness, Group Eo/Lm, also had the greatest increase in copper values. A rise in plasma copper in sheep with various infections has also been reported by *McCosker* (1968).

None of the animals showed symptoms of meningo-encephalitis, and after the initial period of unthriftiness no signs of illness were seen.

On the basis of the severity of the illness, the highest titres might have been expected in Group Eo/Lm. However, only a slight development of titres was found in this group, and the maximum value was recorded late after infection with Lm, compared with Group Lm. But Group Eo/Lm had the strongest DHS reaction, significantly stronger than Group Lm. The explanation for these findings may be as follows: Erythrocytes infected with Eo utilize large amounts of glucose (*Sutton* 1976). In the present experiment a profound decrease in plasma glucose was found after infection with Eo, and according to *Sutton* (1977) this leads to a rise in lactic acid in the blood and a state of acidosis. It has been shown that development of antibodies against antigen introduced in periods of acidosis is suppressed in sheep (*Lachmann & Fürll* 1977).

The results from the present experiment ties well in with the results reported by *Lachmann & Fürll.* Little is known, however, about how the cells within the immune system are affected by a state of acidosis, and further work is needed in this field.

In conclusion, the blood changes induced by Eo led to a prolonged state of illness in animals which were later experimentally infected with Lm. Both the haemolytic anaemia and the state of acidosis might have contributed to this condition. Thus the changes induced by Eo may predispose for listeric septicaemia, but probably not for listeric meningo-encephalitis.

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SAMMENDRAG

Listeriose hos sau. Infeksjon med Eperythrozoon ovis brukt som modell til å studera disponerande faktorar.

Tre grupper med 9 månader gamle lam, kvar gruppe på 9 dyr, blei brukte i forsøket. Dei blei infiserte med Eperythrozoon ovis (Eo), Listeria monocytogenes (Lm) og Eo/Lm. Dyr infiserte med Eo fekk hemolytisk anemi, men viste elles ikkje teikn på sjukdom. Dyr infiserte med Lm fekk ein periode med feber og nedsatt matlyst, og desse symptoma var sterkast og varde lengst i gruppa med kombinert infeksjon (Eo/Lm). Ingen av dyra synte teikn på meningo-encephalitt.

Gruppe Lm utvikla dei høgaste geometriske gjennomsnittstitra

mot Lm. Inga titerstiging blei funnen i gruppe Eo, mens gruppe Eo/Lm hadde ei svak titerstiging mot slutten av eksperimentet. Gruppe Eo/Lm hadde sterkast seinka hypersensitivitetsreaksjon.

Dyr infiserte med Eo hadde eit sterkt fall i hematokrit (PCV) og plasma glukose og ein auke i serum jern, serum jern fall etter infeksjon med Lm.

I dette eksperimentet førte blodendringane ved ein Eo-infeksjon (hemolytisk anemi og acidose) til ein alvorleg sjukdomstilstand hos dyr som var infiserte med Lm. Dei førte vidare til ei hemma utvikling av antistofftiter, men ikkje til symptom på meningo-encephalitt.

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