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

# LISTERIOSIS IN SHEEP

## EXPERIMENTAL LISTERIC INFECTION IN SHEEP TREATED WITH VARIOUS IMMUNOSUPPRESSIVA*

By

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GRØNSTØL, H.: *Listeriosis in sheep. Experimental listeric infection in sheep treated with various immunosuppressiva.* Acta vet. scand. 1980, *21*, 415—427. — Four groups of lambs, about 6 months old, were given 4 different immunosuppressiva (prednisolone, cyclophosphamide, niridazole and gestagene hormones), 1 substance to each group. After this treatment the 4 groups together with a 5th group of the same size, were infected with Listeria monocytogenes (Lm).

None of the animals developed clinical listeric encephalitis. The level of antibodies differed between the groups. The group not given any immunosuppressivum reached the highest antibody titres, the group given gestagene hormones had the lowest titres and the other groups ranged somewhere inbetween. All the groups except the group treated with niridazole had a strong delayed hypersensitivity reaction against Lm. The effects of the treatment upon some blood components are also described.

Further studies are necessary to establish the effect of various immunosuppressiva, especially gestagene hormones.

sheep; Listeria monocytogenes; prednisolone; cyclophosphamide; niridazole; gestagene hormones; immunity; blood components.

Sheep seem to be fairly resistant against listeric infections. When a sheep flock is exposed to Listeria monocytogenes (Lm), none or only a few of the animals develop clinical disease (*Grønstøl* 1979 a, b). It seems like resistance-reducing factors have to be present to induce clinical disease, but little is known about this. It had been suggested that sudden temperature changes, a

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change of feed (*Gray & Killinger* 1966), stress and pregnancy (*Grønstøl* 1979b) may be associated with outbreaks.

Several cases of listeric infection have been described in humans treated with immunosuppressiva (*Medoff et al.* 1971, *Gantz et al.* 1975). Such treatment interferes with the immune system and leaves the organism more prone to infection.

The present work was undertaken to study the effect of experimental listeric infection in sheep treated with various immunosuppressiva. The development of humoral immunity (HI) and cell mediated immunity (CMI) and changes in some blood components were examined.

## MATERIALS AND METHODS

### *Animals*

Twenty lambs of the Dala and Rygja breeds, about 6 months old, were used in the experiment. The animals were selected according to their haemoglobin types. They belonged to the experimental flock at this research station, and the feeding regime and management of the flock have been described elsewhere (*Grønstøl* 1979a).

Two weeks before the experiment started, the lambs were taken indoors and divided into 5 groups, each group consisting of 4 animals. The groups were kept in pens with slatted floors in the quarantine department and fed hay and concentrates.

Four of the groups were given immunosuppressiva* as recorded in Table 1. Then 1 animal from each of the 5 groups were put together in 1 pen and kept as uninfected controls during the experiment, in order to judge the toxicity of the substances.

The remaining 15 animals of the 5 groups were each swabbed intranasally with cultures of Lm serotypes 1 and 4 on 3 consecutive days, i. e. on day 0, and on days 1 and 2 post infection (PI). In addition, on day 2 PI they were given about $10^{10}$ bacteria of both serotypes with stomach tube. By this design 3 lambs served as untreated and infected controls, later called group CONTROL.

The animals were weighed once a week during the experiment, and their temperatures were recorded daily.

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* Niridazole was kindly supplied by Ciba-Geigy AG, Basel, Schwitzerland.

Table 1. Scheme for administration of immunosuppressiva to 5 groups, each group consisting of 4 lambs; 1 animal from each group was kept as uninfected control during the experiment.

| Group | Days before infection | Treatment | Amount |
|---|---|---|---|
| PRED | 2 | Prednisolone acetate | 1.5 mg/kg[1] |
| | 2 | Prednisolone | 3.5 „ [2] |
| | 1 | „ | 3.5 „ [2] |
| | 0 | „ | 3.5 „ [2] |
| CY | 2 | Cyclophosphamide | 25 „ [3] |
| NIR | 2 | Niridazole | 30 „ [2] |
| GEST | 2 | Human choriogonadotropin | 3000 i.u.[3] |
| | 2 | Medroxyprogesteroneacetate | 3.5 mg/kg[2] |
| | 1 | „ | 3.5 „ [2] |
| | 0 | „ | 3.5 „ [2] |
| CONTROL | | No treatment | |

[1] intramuscularly.
[2] orally.
[3] intravenously.

*Bacteria*

The bacteria used in this experiment were isolated from brains of sheep which had died from listeric encephalitis. They had been passed 3 times through mice before inoculation.

*Serological examination*

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

*Skin test*

The animals were tested for delayed hypersensitivity (DHS) against Lm by a skin test (*Grønstøl* 1979a).

*Examination of some blood components*

Total serum protein, serum iron, plasma sodium and potassium were estimated and the serum protein pattern was determined by electrophoresis. Plasma glucose was estimated by the gluco-rapid test, according to the manufacturer's manual (Med-Kjemi, Høn, Norway). Packed cell volume, haemoglobin, red cell counts, total and differential leucocyte counts were also de-

termined. The methods are given by *Øverås* (1969, 1974) and *Waldeland* (1977).

## RESULTS

### *Clinical findings*

On day 2 PI, all the experimentally infected animals, except group CY, had a temperature rise up to 41°C. In group CY, temperatures above 40.5°C were not recorded. All the infected animals developed rhinitis, and the feed intake was reduced. This condition lasted for about 5 days.

On day 4 PI the fleece started to fall off the sheep treated with cyclophosphamide. On days 9 and 10 PI 1 of the animals in group GEST had periods with convulsions, twitching at the mouth and ataxia. Otherwise no symptoms were seen neither in the experimentally infected animals nor in the uninfected controls.

The average weight in the infected groups was reduced during the first 1—2 weeks, but later all except the animals treated with cyclophosphamide gained weight.

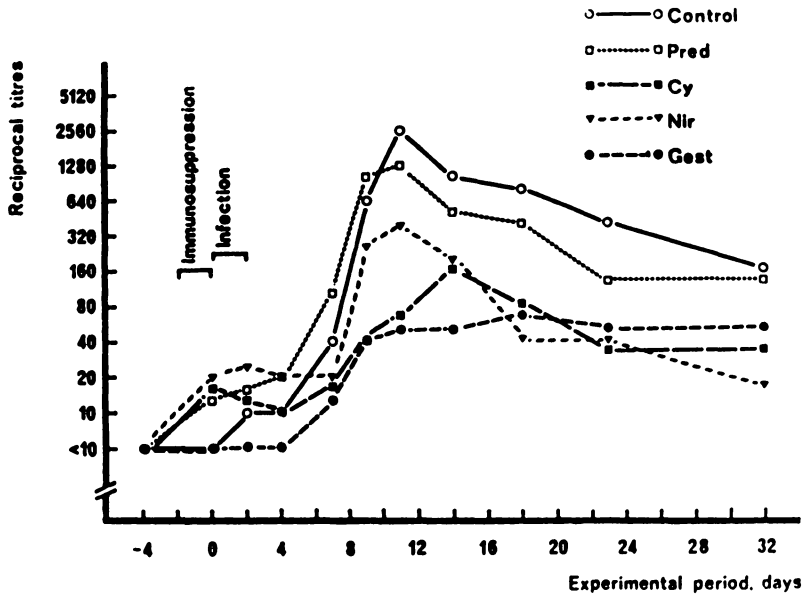


Figure 1. Reciprocal geometrical mean titres (GMT) in 5 groups of lambs (1 untreated group and 4 groups treated with various immunosuppressiva) experimentally infected with Listeria monocytogenes (Lm). Each group consisted of 3 animals. Symbols as in Table 1.

*Serological examination*

The results from the serological examination are shown in Fig. 1. The highest reciprocal geometrical mean titres (GMT) were found in group CONTROL and the lowest in group GEST, while GMT for the other groups ranged somewhere inbetween. In group CY and group GEST the titres reached their peaks on day 14 PI and 18 PI, respectively, whereas in the other groups the highest GMT were found on day 11 PI. The titres decreased more rapidly in group NIR than in the other groups.

The non-infected animals did not develop any titres against Lm, except for the animal treated with gestagene hormones. This lamb reached a maximum titre of 40 on day 0, and had titres of this magnitude throughout the experiment.

*Skin test*

Before the experiment started, the reaction to the skin test was less than 1 mm for all the animals. When the test was performed on day 10 PI, all the infected groups except group NIR had a strong reaction, as recorded in Table 2. On day 40 PI the reaction was still positive for all groups, but weakest in group CY.

Table 2. Skin testing of 1 untreated control group and 4 groups treated with various immunosuppressiva, after infection (PI) of all 5 groups with Listeria monocytogenes. Average increase in skin thickness (mm). Symbols as in Table 1.

| Group | Day 10 PI | Day 40 PI |
|---|---|---|
| PRED | 4.8 | 3.5 |
| CY | 4.0 | 1.8 |
| NIR | 1.8 | 3.7 |
| GEST | 5.0 | 2.5 |
| CONTROL | 5.5 | 3.8 |

The uninfected controls had a skin reaction of less than 1 mm on both testing dates, except for the animal treated with gestagene hormones. This lamb had a skin reaction of 2 mm on day 40 PI.

*Blood components*

After the experimental infection there was a decrease in packed cell volume, haemoglobin content and number of red

cells. In group CY, the lowest point was reached on day 7 PI, for the other infected groups about day 13 PI. The uninfected animals also had similar, but much less pronounced falls.

Plasma potassium also fell in the infected groups, while no definite pattern could be found for sodium.

Plasma glucose reached a peak in all the infected groups except group PRED, on day 2 PI. Treatment with prednisolone resulted in a rise in plasma glucose before the experimental infection started.

The serum iron content fell sharply on day 2 PI in all the infected groups except group CY, which had an increase as shown in Fig. 2. No changes were found in the uninfected controls.

Total serum protein rose in group CY from day 7 PI, a rise which was due to an increase in the α- and β-globulins. A similar pattern was found in group CONTROL, but this group also had an increase in the γ-fraction. All the groups had a decrease in the albumin/globulin ratio after infection with Lm. No specific changes were seen in the uninfected animals.

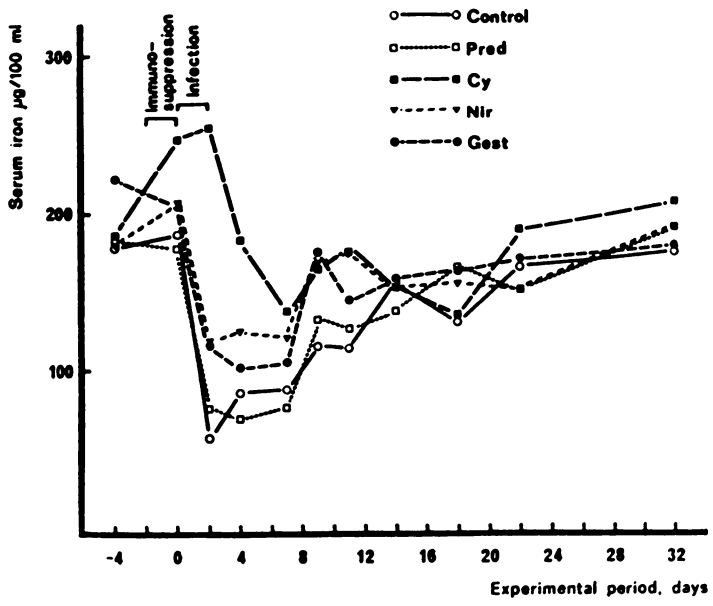


Figure 2. Average serum iron values in 5 groups of lamb, each group consisting of 3 animals, after treatment with immunosuppressiva (as described in Table 1) and infection with Listeria monocytogenes (Lm).

The leucocytes were influenced both by the infection with Lm and by the treatment with immunosuppressiva. In group CONTROL, where no immunosuppressivum had been used, infection with Lm led to a fall in lymphocytes and a short-lasting increase in neutrophils, but the values were back to normal within 1 week.

Treatment with prednisolone led to a strong increase in neutrophils, an increase which continued on day 1 PI. At the same time there was a reduction in the number of lymphocytes. A similar pattern was seen in the uninfected lamb treated with prednisolone.

Treatment with cyclophosphamide led to a rise in the number of lymphocytes, followed by a reduction after infection with Lm. Immediately after infection there also was a reduction in number of neutrophils, and a strong increase was recorded from day 4 PI. A similar pattern was seen in the uninfected animal treated with cyclophosphamide.

All the animals treated with niridazole had lymphocyte and neutrophil counts largely on the same level throughout the experimental period.

Treatment with gestagene hormones led to an increase in number of lymphocytes, with a fall shortly after infection. In the uninfected animal the high level was maintained throughout the experiment. After infection group GEST also had a peak in number of neutrophils, and another peak was seen on day 11 PI. The uninfected had a low number of neutrophils throughout the experiment.

## DISCUSSION

The animals belonged to a flock where a large proportion had been found to excrete Lm in the faeces and milk in periods (*Grønstøl* 1979a). They had therefore most likely been exposed to Lm before the experiment started, and some of them might even have been latent carriers. If sheep with no previous exposure to Lm had been used, the results might have been different, but the experimental animals were probably representative for sheep under farm conditions in this country.

Each group consisted of a small number of animals, and the results must be interpreted with this in mind.

The substances used in this experiment were chosen because of their immunosuppressive properties, and they were dosed in

amounts and at times known to give a suppressive effect. The corticosteroids have both an immunosuppressive and an anti-inflammatory effect. The immunosuppressive effect varies from species to species (*Bach* 1975). Mouse, rat and rabbit belong to the corticosteroid-sensitive species, man and guinea-pig belong to the corticosteroid-resistant species (*Claman* 1972).

Cyclophosphamide is an alkylating agent commonly used as immunosuppressivum. In sheep the drug is also used for chemical defleecing (*McIntosh et al.* 1971). The drug was given 2 days before infection (*Tripathy & Mackaness* 1969), and 25 mg/kg was used as dose because higher doses have been found to be toxic for sheep (*McIntosh et al.*).

Niridazole has been used to treat schistosomiasis. This substance suppresses CMI (*Mahmoud et al.* 1975), but has virtually no effect on HI (*Pelley et al.* 1975).

One of the effects of gestagene hormones is probably to protect the foetus from rejection (*Anon.* 1976). The hormones used in this experiment, medoxyprogesterone and human chorio-gonadotropin, have immunosuppressive effect (*Munroe* 1971, *Han* 1975).

Treatment with the 4 immunosuppressiva before the experimental infection seemed to have no obvious effect on the clinical symptoms. It is noticeable, however, that 1 lamb in group GEST showed symptoms from the central nervous system. A higher dose of the hormones over a longer period might have induced a more pronounced effect.

The lambs in group CY had only a slight temperature rise. The weight loss in these animals was probably due to a toxic effect of the drug, earlier reported by *McIntosh et al.*, as a similar weight loss was also recorded in the uninfected lamb treated with the same drug.

On day 4 PI the animals in group CONTROL started to develop antibody titres against Lm, and GMT reached a peak on day 11 PI. GMT declined steadily during the rest of the experimental period.

Corticosteroids are potent suppressors of antibody responses in corticosteroid-sensitive species (*Bach*). In the present experiment antibody titres in group PRED started to rise on day 4 PI and reached a peak on day 11 PI. Later in the experimental period GMT for this group were 1—2 units below GMT for group CONTROL. This is in accordance with *Dukor & Dietrich* (1967)

who found that late antibody titres were more readily suppressed than early antibody titres (day 4—10) by corticosteroids. In the present experiment the early antibody titres were not suppressed at all in group PRED, indicating that sheep belong to the corticosteroid-resistant species. But timing of the drug administration is crucial. The strongest effect has been found when the drug is given 2—4 days before infection (*Elliott & Sinclair* 1968).

In group CY the antibody titres rose slowly from day 7 PI and reached a peak on day 14 PI, but the peak was 4 units below maximum GMT in group CONTROL. This suppression in development of antibody titres has also been found in mice and humans and seems to be dose dependent (*Bach* 1975). *Dukor & Dietrich* (1968) showed that cyclophosphamide was a less effective suppressor of late antibody responses than of early responses, in contrast to prednisolone, and this ties well in with the findings in this experiment.

Development of antibody titres in group NIR followed the pattern in group CONTROL, but GMT were 2—5 units below the corresponding GMT for group CONTROL throughout the experiment. This may reflect a suppression of helper T-cells, as the production of antibodies against Lm probably is T-cell dependent. *Pelley et al.* found that some effect on the primary HI response may be seen after treatment with niridazole, whereas the secondary response was unimpaired.

GMT increased slowly after infection in group GEST. On day 11 PI, when group CONTROL had reached a GMT of 2540, the GMT for this group was 50. The highest value, 64, was reached on day 18 PI. This indicates that the hormones used probably are strong suppressors of HI. *Grønstøl* (1979a, b) found that antibody titres in sheep were reduced during the gestation period, and that animals with 3 foetuses or more had a stronger reduction than the remainder. *Emady et al.* (1974) showed that the blood concentration of progesterone increased during the gestation period, and also with increasing number of foetuses. The present experiment indicates that the reduced development of antibodies probably was caused by the gestagene hormones.

Virtually no depression of DHS was seen in group PRED on day 10 PI or on day 40 PI. *North* (1971) found that cortisone suppressed CMI induced by Lm in mice, but this result may not be directly applicable to sheep.

Group CY showed a reduced DHS reaction on both testing

days, and on day 40 PI the reaction was only half of that recorded in group CONTROL. *Willers & Sluis* (1975) found that high doses of cyclophosphamide (300—350 mg/kg) almost completely eliminated the B-lymphocytes in mice, but also reduced the T-lymphocytes substantially. Consequently, the T-lymphocytes may have been partly suppressed by this drug also in this experiment.

Niridazole is a selective suppressor of CMI (*Mahmoud et al., Webster et al.* 1975), and the result from the present experiment ties well in with these findings.

No reduction in DHS reaction was found on day 10 PI in group GEST, but on day 40 PI the average skin reaction was about 1 mm less than in group CONTROL, indicating some effect also on CMI.

The uninfected animals showed no increase in HI or CMI against Lm, except for the lamb treated with gestagene hormones. The moderate increase in antibody titres and DHS recorded in this animal may have been caused by an activation of a latent carrier state, induced by the treatment with gestagene hormones.

The blood components in the groups treated with immunosuppressiva followed largely the pattern in the group CONTROL. The increase in plasma glucose after treatment with prednisolone and a fall in serum iron in all the infected groups were as expected, except for group CY, where the expected fall was absent. The increase in γ-globulin seemed to be associated with an increase in antibody titres.

The variation in number of lymphocytes and neutrophils was clearly influenced by the drugs used. In the corticosteroid-sensitive species relatively small doses of corticosteroids lead to lymphocytopenia (*Bach*). But corticosteroid-resistant species are also affected, and this may explain the decrease in lymphocyte numbers in group PRED.

Treatment with cyclophosphamide led to a reduction in number of neutrophils after infection with Lm. This is in agreement with *McGregor & Koster* (1971) who found that cyclophosphamide has an inhibitory effect upon production of granulocytes.

The results from the experiment showed that all the drugs had some effect on development of HI against Lm and this effect was most pronounced in the animals treated with gestagene hormones. Niridazole, and to some extent cyclophosphamide and

gestagene hormones, had an inhibitory effect upon DHS against Lm. Some of the blood components were also affected, but none of the animals developed typical listeric encephalitis.

Immunosuppressiva seem to be a useful tool to study resistance and development of immunity to infections. Such work has to a large extent been carried out in small laboratory animals, but the results may not be valid for other species. The present results indicate that further studies with larger animal groups have to be carried out to learn more about the reactions in sheep.

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## SAMMENDRAG

*Listeriose hos sau. Eksperimentell infeksjon med Listeria monocytogenes hos sauer behandla med ulika immunosuppressiva.*

Fire grupper av 6 månader gamle lam blei behandla med 4 ulike immunosuppressiva (prednisolon, cyclophosphamate, niridazol, gestagene hormon). Etter behandling blei dei 4 gruppene saman med ei tilsvarande femte ubehandla gruppe infiserte med Listeria monocytogenes (Lm).

Ingen av dyra fekk typisk listeria-encefalitt. Gruppene utvikla ulike nivå av antistoff mot Lm. Mest antistoff blei funne i den gruppa som ikkje hadde fått immunosuppressiva, og minst i den gruppa som hadde fått gestagene hormon. Nivået i dei andre gruppene låg ein stad mellom desse. Alle gruppene utanom den gruppa som hadde fått niridazol viste ein sterk seinka hypersensitivitetsreaksjon mot Lm. Eit oversyn over verknaden på ulike blodkomponentar er og gjeve.

Forsøket synte at vidare studier trengst for å finna meir ut om korleis ulike immunosuppressiva, og særleg då gestagene hormon, verkar på immuniteten mot mikroorganismar.

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