Acta vet. scand. 1979, *20*, 417—428.

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

LISTERIOSIS IN SHEEP

LISTERIA MONOCYTOGENES EXCRETION AND IMMUNOLOGICAL STATE IN SHEEP IN FLOCKS WITH CLINICAL LISTERIOSIS*

By

Hallstein Grønstøl

GRØNSTØL, H.: *Listeriosis in sheep. Listeria monocytogenes excretion and immunological state in sheep in flocks with clinical listeriosis.* Acta vet. scand. 1979, *20*, 417—428. — The excretion of Listeria monocytogenes (Lm) in the faeces and milk, and humoral immunity against Lm, were examined in a sheep flock with outbreaks of listeric encephalitis and in a flock with outbreaks of listeric abortion. The encephalitis flock consisted of 86 ewes and 20 hoggs, the abortion flock of 45 ewes and 3 hoggs, all of them pregnant. Faecal excretion rate in the encephalitis flock varied from about 25 % in the first part of the indoor season to nearly zero 1 month later, to about 30 % 1 month before lambing and about 15 % at lambing. About 15 % of the animals also excreted Lm in the milk. Lm 4 was the dominating serotype.

In the abortion flock about 2/3 of the animals excreted Lm in the faeces and 1/3 in the milk at lambing. All the isolates belonged to serotype 1, which also was isolated from grass silage and strawbedding samples.

In the encephalitis flock ewes with $\geq$ 3 foetuses had a higher excretion rate than the remainder, while no such differences were found in the abortion flock.

Antibody titres against Lm in sera and whey in the encephalitis flock were of the same order as in the healthy flock described in an earlier publication (*Grønstøl* 1979), except that the highest titres were found in the hoggs. Serum titres from the abortion flock after lambing were significantly higher than in the encephalitis flock, while whey titres were of the same order.

Treatment with 2-mercapto-ethanol reduced the titres substantially in sera from the abortion flock, indicating that the antibodies belonged to the IgM-fraction, while only a slight reduction was seen after similar treatment of the whey.

Listeria monocytogenes; sheep; excretion of bacteria in faeces and milk; immunity; encephalitis; abortion.

---

* This work was supported by grants from the Norwegian Agricultural Research Council.

A previous investigation indicated that even in a healthy sheep flock most of the animals may be latent carriers of Listeria monocytogenes (Lm) and may excrete the organism in the faeces and milk (*Grønstøl* 1979). The question naturally arose whether this excretion pattern differs from that in flocks with clinical cases of listeriosis. The present work comprises examinations of a flock with cases of listeric encephalitis and a flock with outbreaks of listeric abortion.

MATERIALS AND METHODS

The flock with outbreaks of listeric encephalitis

The flock consisted of 90 ewes, from 2 to 9 years old, and 30 hoggs, about 8 months old, of the Rygja and Old Norwegian breeds. Only the pregnant animals are included in this report, i.e. 86 ewes and 20 hoggs. The feeding and management were mainly as described by *Waldeland* (1977). The grass silage feeding started on 1st November, and another silo was opened on 15th February. The animals were shorn on 18th and 19th February. They were expected to lamb in April and early May. Shortly before lambing they were transferred from a room with slatted floor where they had been kept during the winter, to individual pens with dry strawbedding. They were let back into the flock 3—5 days after lambing.

Clinical listeriosis had occurred 2 years before the present investigation, when listeric encephalitis was diagnosed in 6 animals. This year, 6 hoggs, 5 gimmers and 2 elder ewes showed clinical symptoms. Ten animals became ill in the period from 25th November to 4th January, and the remaining 3 in the period from 25th February to 26th March (see Fig. 1). Lm serotype 4 was isolated from the brains of the 6 animals which died, and in addition, serotype 1 was isolated from 2 of the 6 brains. Lm serotypes 1 and 4 were also isolated from the cerebrospinal fluid from 1 of the animals which recovered.

Samples of blood, milk and grass silage were examined at the dates recorded in Table 1.

The flock with outbreaks of listeric abortion

The flock comprised initially 46 ewes, from 2 to 11 years old, and 3 hoggs, nearly 1 year old, of the Dala and Old Norwegian breeds. All the animals were pregnant and due to lamb in April

and early May. The feeding and management were mainly as described by *Waldeland*, and the animals were shorn on 20th February. While indoors, they were kept on a very moist strawbedding. Shortly before lambing they were transferred to individual pens with dry strawbedding, and the animals were let back into the flock about 3 days after lambing.

The animals had been healthy and in good condition during the winter, and clinical listeriosis had not occurred in the last 5 years.

The farmer purchased some grass silage which he started to feed to the sheep on 25th March. Two days later several of the animals lost their appetite and looked depressed. One ewe died, and the others recovered gradually. About a week after the introduction of the new silage, a series of abortions in ewes and septicaemia in lambs started.

The 48 sheep had altogether 87 lambs, of which 11 were aborted, 8 died from listeric septicaemia and 3 from other causes. Twelve lambs with septicaemia recovered after treatment. Five of the 7 ewes with triplets, 8 of the 24 ewes with twins and 3 of the 14 ewes with single lambs had offspring which were aborted, died or were ill.

T a b l e 1. Bacteriological and serological examinations in a flock with outbreaks of encephalitis and in a flock with outbreaks of abortion. Sampling dates, number of animals examined and number of grass silage and strawbedding samples examined are given. The varying number of animals on the different sampling dates in flock A was caused by mustering problems.

Date	Flock*	Faeces		Sera		Milk		Silage	Strawbedding
		ewes	hoggs	ewes	hoggs	ewes	hoggs		
Dec. 30	A	77	18	84	20			4	
Feb. 3	A	75	16	86	20				
Feb. 24	A								
Mar. 3	A	86	20						
Lambing	A	83	20	86	20	84	19	4	
Apr. 9	B			16	2			8	4
Lambing	B	45	3			45	3		
May	B	45	3	45	3	43	3		

* Flock A: Encephalitis.
Flock B: Abortion.

Lm serotype 1 was isolated from aborted foetuses and dead lambs.

Samples of faeces, blood, milk, grass silage and strawbedding were examined at the dates recorded in Table 1.

Bacteriological examination

Faeces, milk and grass silage from both flocks were examined as described by *Grønstøl* (1979). Strawbedding was examined in the same way as silage samples.

Serological examination

Sera and whey were examined by an indirect haemagglutination method (*Grønstøl* 1979). Sera and whey from the abortion flock were also examined by the same method after treatment with 2-mercapto-ethanol (2-ME) (*Osebold & Aalund* 1968).

RESULTS

Bacteriological examination

The flock with outbreaks of listeric encephalitis. The excretion pattern for Lm in the faeces is illustrated in Fig. 1. On the first sampling date, when the highest number of clinical cases occurred, 21 % of the ewes and 6 of 20 hoggs excreted Lm in the

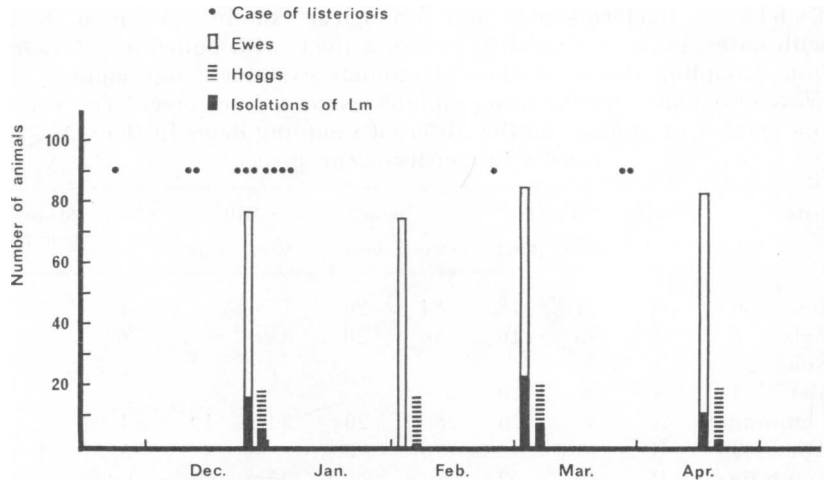

Figure 1. Number of animals which excreted Listeria monocytogenes in the faeces on the various sampling dates, in relation to clinical cases of listeric encephalitis. The varying number of animals on the different sampling dates was caused by mustering difficulties.

faeces. The lowest number of excretors was found on 3rd February, when none of the ewes and 1 hogg had positive samples. This was in a period when no clinical cases occurred. The highest number of positive samples was found on 3rd March; i.e. 26 % of the ewes and 7 of the hoggs. Milk from 15 % of the ewes and 3 of 20 hoggs contained Lm at lambing. No distinct difference was found between the various age groups, whereas ewes with $\geq$ 3 foetuses had a higher excretion rate in the faeces than the remainder on the last 2 sampling dates. The same pattern was seen for the milk (Table 2), but these differences were not statistically significant (chi-square). Lm was not isolated from 4 samples taken from the first silo on 30th December. Two samples from the new silo on 24th February contained Lm serotype 1, whereas Lm was not isolated from 2 samples taken on 9th April.

Table 2. Excretion rate in the faeces and milk from ewes, according to number of foetuses, in a flock with outbreaks of listeric encephalitis.

Date	Sample	Percentages of excretors		
		$\geq$ 3 foetuses	2 foetuses	1 foetus
Dec. 30	faeces	25	18	31
March 3	„	50	27	23
At lambing	„	20	15	6
„	milk	40	10	11

The flock with outbreaks of listeric abortion. Lm was found in the faeces of 68 % of the 45 ewes and 2 of the 3 hoggs at lambing, and in the milk from 35 % of the ewes. On the next sampling date, 14th May, 2 faeces samples and 2 milk samples from the ewes, but none from the hoggs, contained Lm. The excretion of Lm in the faeces and milk did not seem to be related to age or to number of foetuses. Lm was isolated from 3 of 8 grass silage samples, all of them with pH > 5, and from all the stravbedding samples. All the isolates in this flock belonged to serotype 1.

Serological examination

The flock with outbreaks of listeric encephalitis. The reciprocal geometrical mean titres (GMT) in sera from the ewes and the hoggs are recorded in Table 3. GMT were higher for the hoggs

Table 3. Reciprocal geometrical mean titres (GMT) for the ewes and the hoggs in a flock with outbreaks of listeric encephalitis.

Date	Sample	Number	Animals	GMT
Dec. 30	serum	84	ewes	31
„	„	20	hoggs	65
Feb. 2	„	86	ewes	17
„	„	20	hoggs	31
At lambing	„	86	ewes	18
„	„	20	hoggs	35
„	whey	85	ewes	13
„	„	20	hoggs	17

than for the ewes throughout the investigation period, and highest on the first sampling date, but the differences were not statistically significant (Students t-test). No difference was otherwise found between the various age groups of ewes. Ewes with ≥ 3 foetuses had the lowest GMT. The difference was not statistically significant. GMT in whey ranged between 10 and 20. No distinctive difference was found between the various age groups.

Table 4. Antibodies against Listeria monocytogenes in sera and whey from sheep in a flock with outbreaks of abortion, by an indirect haemagglutination method. The animals were due to lamb in April and early May, and the abortion outbreak started on 2nd April.

Sample	Date	2-ME-treated*	Number of animals		GMT**
			hoggs	ewes	
Serum	Apr. 9	—	0	16	207
„	„	+		16	34
„	May 14	—		16	181
„	„	+		16	20
„	„	—		45	162
„	„	+		45	20
„	„	—	3		640
„	„	+	3		80
Whey	„	—		43	16
„	„	+		43	11
„	„	—	3		25
„	„	+	3		16

* 2-ME: 2-mercaptoethanol.
** GMT: Reciprocal geometrical mean titres.

Table 5. Reciprocal geometrical mean titres (GMT) in sera and whey according to number of foetuses, in a flock with outbreaks of listeric abortion. The examination was performed 1 month after mean lambing time.

		Sera	Whey
Ewes with	3 foetuses	475	22
„ „	2 „	106	15
„ „	1 foetus	214	15

The flock with outbreaks of listeric abortion. GMT of sera and whey both untreated and treated with 2-ME are recorded in Table 4. Highest GMT was found on the first sampling date. On the second sampling date the 3 hoggs had higher GMT than the ewes. GMT was lower in whey than in sera. Treatment with 2-ME reduced GMT in sera substantially while GMT in whey was only slightly reduced. No relationship was found between age and GMT in sera or whey. GMT in sera and whey according to the number of foetuses carried, are shown in Table 5. Ewes with ≥ 3 foetuses had higher GMT in serum than the remainder ($P < 0.01$). The difference in whey was not statistically significant.

DISCUSSION

The excretion pattern of Lm in a healthy sheep flock where clinical listeriosis had not occurred for 3 years, has been described earlier by the author (*Grønstøl* 1979). None of 106 ewes and 2 of 10 hoggs excreted Lm in the faeces when the indoor season started. No excretors were found 2 months later, in mid-pregnancy, while a large proportion of the animals excreted Lm in the faeces and milk at lambing.

In the present investigation Lm was isolated from the faeces of about 25 % of the animals in early and late pregnancy, whereas Lm was isolated from none of the 86 ewes and 1 of the 20 hoggs in mid-pregnancy in the flock with outbreaks of listeric encephalitis. For obvious reasons no samples were examined until lambing in the abortion flock, and then Lm was isolated from the faeces of about two thirds of the animals and from the milk of about one third.

Several factors may influence the excretion pattern. Exposure to Lm through the feed both in the investigation period and earlier may be of importance. A subclinical infection with Lm

earlier in life might have induced a certain degree of local and systematic immunity, with a reduction in excretion rate as a result.

An outbreak of listeric encephalitis occurred 2 years previously in the encephalitis flock, and animals older than 2 years probably had been through a clinical or subclinical infection at that time. This is supported by the fact that 11 of the 13 animals with clinical symptoms were less than 2 years old. The increased local and systemic immunity induced by the infection before and during the first part of the investigation, probably caused the steady excretion rate and the absence of an excretion peak at lambing.

In the abortion flock the first symptoms were seen 2—3 days after the introduction of the new silage, and the first case of abortion occurred 1 week later. No cases of listeriosis had occurred in this flock during the last 5 years, and consequently the degree of immunity against Lm probably was low. The top layer in a silo is usually of inferior quality, with a high pH level which favours multiplication of Lm. When such silage is suddenly introduced, a diarrhoea may be the result. The diarrhoea may be due to an inadequate dietetic quality of the silage, or to a large number of Lm present in the feed. When Lm is ingested, the bacteria penetrate the intestinal epithelium cells, multiply and reach the blood system (*Racz et al.* 1972). The bacteria can further reach the uterus and cause abortion. The invasion of Lm probably induces an inflammation reaction which leads to increased local immunity. In the encephalitis flock a high degree of local immunity was probably established at the time of lambing, while the degree of local immunity probably was low in the abortion flock at that time, resulting in a high excretion rate.

Various forms of stress may also influence the excretion rate. In the encephalitis flock, the silage feeding started in the mating season. The animals were kept indoors during the nights, but were let out during the days and thus exposed to changing weather conditions. In January and the first part of February the feeding regime and management were fairly constant, and this was probably the reason why practically no excretors were found on 2nd February.

In the middle of February the animals were shorn. The shearing process itself represents a stress condition for the animals (*Kilgour & de Langen* 1970) and the defleecing left them more

vulnerable to cold stress. These factors, together with the opening of a new silo and the advanced pregnancy were probably responsible for the increase in excretion rate on 3rd March. The immunity in the gut may temporarily have been weakened, but was later sufficiently strong to prevent a further increase at lambing.

The number of foetuses seemed to be of importance for the excretion rate in the encephalitis flock, in contrast to the abortion flock and the healthy flock described earlier (*Grønstøl* 1979). This was probably a reflection of a longstanding exposure to Lm in the encephalitis flock, combined with the effect of immunosuppressive substances, such as progesterone (*Munroe* 1971). *Emady et al.* (1974) found that the concentration of progesterone in sheep plasma increased with advancing pregnancy and number of foetuses. This may explain the high proportion of excretors among ewes with ≥ 3 foetuses towards the end of the gestation period.

The silage may also be of importance in other ways than just as a vehicle for Lm. *Grønstøl* (to be published) found a higher excretion rate, reduced numbers of lymphocytes in the peripheral blood and an increased degree of delayed hypersensitivity against Lm in sheep fed grass silage, compared with ewes fed hay.

Lm was isolated from a small proportion of the milk samples in the encephalitis flock compared with the abortion flock or the healthy flock. The milk from ewes which aborted contained apparently large numbers of Lm, which could be isolated by cultivating the milk directly on blood agar plates. Some of the lambs, which were fostered under these ewes, became ill, presumably because they ingested large numbers of Lm through the milk. This was a striking feature in the abortion flock, not found in the encephalitis flock or in the healthy flock.

Lm isolated from the abortion flock belonged all to serotype 1. In the encephalitis flock the bacteria belonged mainly to serotype 4. An interesting question is whether there is any association between the serotype and the course of the infection, but no conclusions can be drawn from this material.

GMT in sera from the encephalitis flock was similar to those found in a healthy flock (*Grønstøl* 1979), except for the hoggs which had the highest GMT on the first sampling date. This is an indication of an infection before the investigation started. As the younger animals probably had the lowest degree of immunity

against Lm before the indoor season, they would also be most seriously affected and develop the highest GMT. This is supported by the finding that 11 of 13 animals with clinical symptoms were less than 2 years old.

GMT in sera from the abortion flock was much higher than from the encephalitis flock. This result probably reflects the difference in pathogenesis. In listeric encephalitis the bacteria seem to enter through intact epithelial cells, devitalized epithelial cells or small wounds in the mucosa, and later move along nerve branches to the brain (*Asahi* 1963, *Charlton & Garcia* 1977). Abortion is usually proceeded by a bacteriaemia (*Gray & Killinger* 1966) which may lead to a strong humoral response. As expected, GMT was higher on the first sampling date than on the second. Treatment with 2-ME reduced GMT in sera substantially, indicating that antibodies against Lm determined by indirect haemagglutination are within the IgM-fraction.

The titres seem to be sufficiently high for the method to be used as a diagnostic tool in outbreaks of abortion. Only listeric infections seem to give titres of that order. Infections with bacteria such as Staphylococcus aureus, which may give strong cross reactions in the agglutination test, seem to give titres < 80 (*Grønstøl*, unpublished).

GMT in whey in the 2 flocks was of the same order as in the healthy flock (*Grønstøl* 1979), and treatment with 2-ME seemed to make little difference, indicating that the main part of the whey antibodies determined by this method does not belong to the IgM-fraction. This is in accordance with *Lascelles & McDowell* (1974) who found that IgM was the predominating immunoglobulin fraction in colostrum and milk later in the lactation period.

Grønstøl (1979) in an investigation of a healthy flock found that ewes with ≥ 3 foetuses had lower GMT in serum than the remainder, and this result was also found in the present investigation of the encephalitis flock. In the abortion flock, however, ewes with triplets had higher GMT both in serum and in whey than the remainder. A possible explanation may be that progesterone has a suppressive effect upon humoral immunity. The ewes were infected when the progesterone concentration was at its highest, i.e. 2—3 weeks before parturition (*Cox* 1975). The day after parturition or abortion the concentration will fall to normal values (*Carter et al.* 1976), and the immunosuppressive effect will disappear, as has also been shown in women (*Pasca*

& Pejtsik 1977). Because of the reduced degree of humoral immunity ewes with $\geq$ 3 foetuses probably had a more serious infection than the remainder, with development of higher antibody titres.

In the abortion flock there also seemed to be an association between the number of foetuses the ewes carried and the frequency of illness in the offspring. Of 7 ewes with triplets, 5 aborted or gave birth to lambs which later became ill. This ratio was higher than for the remainder, but the difference was not statistically significant.

From the present investigations and the investigation carried out in a healthy flock (*Grønstøl* 1979), it may be concluded that the excretion pattern of Lm in the faeces and milk may vary from flock to flock. An infection just before or early in the indoor season seems to lead to a steady excretion rate throughout the housing period, while a slight antigen exposure during the winter, with or without a clinical infection in late pregnancy, seems to give a strong increase in excretion rate at lambing.

GMT in sera in the healthy flock and the encephalitis flock was of the same order. The serological method used is thus of little value for the diagnosis of listeric encephalitis, but may be useful for the diagnosis of listeric abortion.

REFERENCES

Asahi, O.: Pathogenesis of listeric encephalitis: Invasion of nerve fibers by Listeria monocytogenes. *In* Second Symp. on Listeric Infection, ed. by M. L. Gray, Bozemann 1963, 99—108.

Carter, J. L., C. L. Chen & S. M. Dennis: Serum levels of progesterone, estradiol and hydrocortisone in ewes after abortion due to Listeria monocytogenes type 5. Amer. J. vet. Res. 1976, *37*, 1071—1073.

Charlton, K. M. & M. M. Garcia: Spontaneous listeric encephalitis and neuritis in sheep. Light microscopic studies. Vet. Path. 1977, *14*, 297—313.

Cox, R. I.: The endocrinologic changes of gestation and parturition in sheep. Adv. vet. Sci. comp. Med. 1975, *19*, 287—305.

Emady, M. J., J. C. Hadley, D. E. Noakes & G. A. Arthur: Progesterone level in the peripheral blood of pregnant ewes. Vet. Rec. 1974, *95*, 168—169.

Gray, M. L. & A. H. Killinger: Listeria monocytogenes and listeric infections. Bacteriol. Rev. 1966, *30*, 309—382.

Grønstøl, H.: Listeriosis in sheep. Listeria monocytogenes excretion and immunological state in healthy sheep. Acta vet. scand. 1979, *20*, 168—179.

Kilgour, R. & H. de Langen: Stress in sheep resulting from management practices. Proc. N. Z. Soc. Anim. Prod. 1970, *30*, 65—76.

Lascelles, A. K. & G. H. McDowell: Localized humoral immunity with particular reference to ruminants. Transplant. Rev. 1974, *19*, 170—208.

Munroe, J. S.: Progesteroids as immunosuppressive agents. J. reticuloendoth. Soc. 1971, *9*, 361—375.

Osebold, J. W. & O. Aalund: Interpretation of serum agglutinating antibodies to Listeria monocytogenes by immunoglobulin differentiation. J. infect. Dis. 1968, *118*, 139—148.

Pasca, A. S. & B. Pejtsik: Impairment of immunity during pregnancy and antiviral effect of amniotic fluid. Lancet 1977, 330—331.

Racz, P., K. Tenner & E. Merö: Experimental listeria enteritis. 1. An electron microscopic study of the epithelial phase in experimental listeria infection. Lab. Invest. 1972, *26*, 694—700.

Waldeland, H.: Toxoplasmosis in sheep. Epidemiological studies in flocks with reproductive loss from toxoplasmosis. Acta vet. scand. 1977, *18*, 91—97.

SAMMENDRAG

Listeriose hos sau. Utskiljing av og immunitet mot Listeria monocytogenes i sauflokkar med utbrot av klinisk listeriose.

Utskiljing av Listeria monocytogenes (Lm) i faeces og mjølk, og utvikling av immunitet mot denne bakterien, blei undersøkt i ein saueflokk med utbrot av listeria-encephalitt og ein flokk med utbrot av listeria-abort. Av dei 86 sauene og 20 årslamma, alle drektige, i encephalitt-flokken, skilde omlag 25 % ut bakterien i faeces tidleg i innefôringsperioden. Ein månad seinare var det berre eitt årslam som skilde ut Lm, 2 månader seinare omlag 30 %, og ved lamming blei Lm funnen i faeces og mjølk frå omlag 15 % av dyra. Storparten av dei isolerte stammene tilhøyrde serotype 4. Fleire utskiljarar blei funne mellom sauer med $\geq$ 3 foster enn mellom dei andre dyra i flokken.

I abortflokken skilde 2/3 av dyra ut Lm i faeces og 1/3 i mjølka ved lamning. Alle dei isolerte bakteriane tilhøyrde serotype 1, og denne serotypen blei og isolert frå siloprøver og talleprøver.

Antistoffnivået i sera og mjølk i encephalitt-flokken var omlag det same som i den friske flokken som var undersøkt tidlegare (*Grønstøl* 1979), men årslamma hadde høgast titer. I abort-flokken derimot var titra etter lamming tydeleg høgare enn i desse to flokkane. Antistofftitra i sera frå abort-flokken blei tydeleg reduserte etter behandling med 2-mercapto-ethanol, og dette tyder på at antistoffa høyrer til IgM-fraksjonen av immunglobulinene, mens berre ein svak reduksjon av antistofftitra i mjølk blei funne etter same behandlinga.

(Received January 9, 1979).

Reprints may be requested from: Hallstein Grønstøl, the State Veterinary Research Station for Small Ruminants, Postbox 248, 4301 Sandnes, Norway.