Acta vet. scand. 1979, *20*, 492—497.

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

LISTERIOSIS IN SHEEP

ISOLATION OF LISTERIA MONOCYTOGENES FROM GRASS SILAGE*

By

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GRØNSTØL, H.: *Listeriosis in sheep. Isolation of Listeria monocytogenes from grass silage.* Acta vet. scand. 1979, *20*, 492—497. — Two hundred and ninety-one grass silage samples from 113 farms with recent outbreaks of listeriosis were examined for the presence of Listeria monocytogenes (Lm). The frequency of Lm isolations increased with increasing pH. Lm was isolated from 22 % of the samples with pH < 4, from 37 % with pH 4—5 and from 56 % with pH > 5. Formic acid had been used as additive.

A similar investigation was carried out on 32 samples from a farm with no outbreak of listeriosis during the investigation period. Lm was isolated from 9 samples.

Listeria monocytogenes; grass silage; pH.

Several workers have suggested that silage feeding is an important factor in the epidemiology of listeriosis (*Pálsson* 1963, *Gray* 1963, *Krüger* 1963, *Blenden et al.* 1966, *Vanel* 1974) and Listeria monocytogenes (Lm) has been isolated from grass silage (*Pálsson, Dijkstra* 1965, *Ribe* 1965).

The presence of Lm seems to be associated with the pH of the silage. *Irwin* (1968) studied the effect of pH on the growth of Lm in media made of grass silage and found that the growth rate was markedly influenced by the pH of the medium.

The present investigation was undertaken to study the relationship between pH and the presence of Lm in grass silage with formic acid as additive. A comparison was also made between

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

farms with recent outbreaks of listeriosis and a farm with no such outbreak during the investigation period, with regard to isolation of Lm from grass silage samples.

MATERIALS AND METHODS

During a 4-year period 291 grass silage samples were collected from 113 farms with recent outbreaks of listeriosis. The greater part of the silage was made in tower silos, and a small part in pit silos or as clamp silage, all with formic acid as additive.

From this Research Station 32 samples of grass silage, made in tower silos or as clamp silage, with formic acid as additive, were examined during a 2-year period. Listeriosis has never been a problem in the experimental flock belonging to this Research Station (3 cases during the last 14 years), and during the sampling period no case occurred.

The grass silage samples were examined bacteriologically as described by *Grønstøl* (1979).

RESULTS

The grass silage samples from farms with outbreaks of listeriosis were divided into 3 groups according to their pH, and the results from the examination for Lm are recorded in Table 1. The frequency of Lm isolation increased with increasing pH. The Lm isolates were equally divided between serotypes 1 and 4.

Table 1. Isolation of Listeria monocytogenes (Lm) from 291 grass silage samples from 113 farms with recent outbreaks of clinical listeriosis. Formic acid was used as additive.

pH	Number of samples		
	total	Lm isolated	%
<4	101	22	22
4—5	110	41	37
>5	80	45	56
	291	108	37

Table 2 gives the results from the examination of the samples from this Research Station. Lm was isolated from a similar proportion as from the farms with outbreaks of listeriosis.

Table 2. Isolation of Listeria monocytogenes from 32 grass silage samples from a farm with no outbreak of listeriosis during the investigation period. Formic acid was used as additive.

pH	Number of samples	
	total	Lm isolated
<4	13	4
4—5	12	2
>5	7	3
	32	9

DISCUSSION

An increasing number of sheep flocks with outbreaks of listeriosis has been reported in this country during the last 10 years. A change in feeding routine has also taken place during the last 20 years. Grass silage has replaced hay as the main roughage in the indoor season. There seems to be strong circumstantial evidence for an association between silage feeding and listeriosis, but the disease is difficult to produce experimentally by feeding of silage (*Gray* 1963).

Only qualitative methods are available for the isolation of Lm from samples containing a mixed flora, because enrichment step and selective plating media have to be used. Consequently, the number of Lm in each sample could not be determined.

Lm was isolated from a high number of samples from farms with outbreaks of listeriosis. There was apparently a close connection between the presence of Lm and pH of the silage, as the highest proportion of samples which contained Lm was found in the group with pH > 5. *Irwin* (1968) found that Lm was unable to multiply below pH 5.5 in culture media made from grass silage, and *Lehnert* (1964) reported multiplication of Lm down to pH 4.8 on ordinary culture media. At lower pH the bacteria would start dying off.

Gouet et al. (1977) in a model experiment with gnotobiotic silage found that Lm disappeared from the samples within 8 days, and concluded that Lm cannot survive for more than 1—2 weeks in silage of good quality. It is, however, under field conditions difficult to make large amounts of silage without pockets of inferior quality where Lm may survive for some time, or even multiply.

Other microorganisms present in the silage may interfere with the growth of Lm, and some of them may even produce antibiotics acting against Lm.

Errebo Larsen (1969) found that Bacillus spp., moulds and faecal streptococci could inhibit the growth of Lm. These organisms may be present in substantial numbers in silage with pH > 5, and this may explain why Lm was not isolated from an even higher proportion of the samples within this group.

The management of sheep farms makes it almost impossible to obtain silage free from Lm. The farm leys, where the grass is produced, are usually fertilized with sheep dung, and the sheep graze the leys in certain periods of the year. As a large proportion of ewes fed grass silage may excrete Lm in the faeces in periods (*Grønstøl* 1979), the grass and the soil will be contaminated with Lm, as has been found by *Welshimer & Donker-Voet* (1971) and *Weis* (1975). The forage harvester may also bring soil and sheep dung into the silo together with the grass and thus render the silage more suitable for growth of Lm. However, if the silage is prepared in the right way, the pH is lowered rapidly and prevents Lm from multiplying. The top layer is usually of inferior quality because of difficulties in maintaining anaerobic conditions, and should be discarded.

Dijkstra (1965) found Lm in 30 % of grass silage samples made without additives, from 140 farms with outbreaks of listeriosis in cattle. He isolated Lm from only 1 % of samples taken from 100 farms chosen at random. *Ribe* (1965) examined 63 grass silage samples from various parts of this country. About ⅓ came from farms with outbreaks of listeriosis in sheep or goats. Only 3 samples, all of them with pH above 7, were found to contain Lm. Both these workers inoculated extract of the samples subcutaneously on mice as described by *Sandvik & Skogsholm* (1962). The high recovery rate in the present investigation may be a result of better isolation technique and thus represent a better assessment of the actual presence of Lm in grass silage.

Lm was isolated from about ⅓ of the samples from this Research Station, indicating that the experimental flock had been exposed to Lm to a similar extent as the animals on farms with outbreaks of clinical listeriosis. The fact that only 3 cases of listeriosis have occurred in the experimental flock during the

last 14 years and none during the last 3 years, points towards additional factors as important for outbreaks of clinical listeriosis.

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SAMMENDRAG

Listeriose hos sau. Førekomst av Listeria monocytogenes i silofôr.

291 siloprøver frå 113 gardar der det nyleg hadde vore utbrot av listeriose, er blitt undersøkte for Listeria monocytogenes (Lm). Talet på positive prøver auka med stigande pH. Lm blei isolert frå 22 % av prøvene med pH < 4, frå 37 % med pH 4—5 og frå 56 % med pH > 5. Alle prøvene var tilsett maursyre.

32 prøver frå ein gard der det ikkje hadde vore utbrot av listeriose i undersøkingsperioden, blei undersøkte på same måte. Lm blei påvist i 9 prøver.

(Received February 19, 1979).

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