Acta vet. scand. 1980, *21*, 11—17.

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

LISTERIOSIS IN SHEEP

ISOLATION OF LISTERIA MONOCYTOGENES FROM ORGANS OF SLAUGHTERED ANIMALS AND DEAD ANIMALS SUBMITTED FOR POST-MORTEM EXAMINATION*

By

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GRØNSTØL, H.: *Listeriosis in sheep. Isolation of Listeria monocytogenes from organs of slaughtered animals and dead animals submitted for post-mortem examination.* Acta vet. scand. 1980, *21*, 11—17. — The udders from 13 culled ewes and liver, spleen, kidney, lung and brain from 15 lambs, 11 months old, were examined for the presence of Listeria monocytogenes (Lm) at slaughter. Lm was isolated from 1 of 13 udders, from 6 of the 15 brains and from 0—4 of the other organs from each of the 15 lambs.

Internal organs from 68 sheep submitted for post-mortem examination were examined in the same way. Lm was isolated from 25 of these animals. Lm was isolated from the brain of 7 of 9 animals with encephalitis, and from 0—3 of the other 4 organs examined. Lm was also isolated from 10—20 % of the organs from animals with other diagnoses. Altogether 9 of 10 animals with encephalitis and 16 of 58 with other diagnoses (28 %) were found to harbour this organism.

Listeria monocytogenes; organs; latent carriers; sheep.

A large proportion of apparently healthy sheep may excrete Listeria monocytogenes (Lm) in the faeces and milk (*Grønstøl* 1979 a). However, no information is available about the presence of Lm in the udder and internal organs of healthy sheep. The present work concerns bacteriological examination of organs from slaughtered sheep and from sheep brought to this laboratory for post-mortem examination.

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

MATERIALS AND METHODS

Slaughtered sheep

In the autumn 13 ewes, 2—7 years old, were culled from the experimental flock at this research station. The udders were examined bacteriologically at slaughter. In addition, liver, lung, kidney, spleen and brain from 15 hoggs, 11 months old, not pregnant, were examined in the same way at slaughter in March (see Table 1). Ten of the hoggs had been infected by stomach tube and intranasally 2 months previously with Lm serotype 1 and 4. The remaining 5 controls had not been infected.

After the infection, the 10 animals had elevated temperature and reduced appetite for a few days, but later no symptoms of disease were seen.

Sheep submitted for post-mortem examination

Table 2 shows the organs examined from dead animals brought to this laboratory for a post-mortem examination. The diagnoses were based on gross examination, histopathology, microbiology and toxicology. The animals examined were selected at random.

Bacteriological examination

Three g of each organ was removed aseptically and 10 ml of phosphate-buffered saline (PBS) was added. The samples were cut with sterile scissors and treated for 30 s in a homogenizer (Colworth Stomacher 80, A. J. Seward, UAC House, Blackfriars Road, London). The suspension was plated on propolis agar (*Grønstøl & Aspøy* 1977). The plates were incubated at 37°C for 48 h. Lm was identified as reported by *Grønstøl & Aspøy*. The samples were kept at 4°C for up to 1 year and plated on propolis agar once a month.

RESULTS

Table 1 shows the isolation of Lm from organs of the slaughtered animals. Lm was isolated from 1 of the 13 udders, from the brain of 5 of the 10 animals infected experimentally 2 months earlier and from 1 brain of the 5 controls. Lm was also isolated from various organs from both experimentally infected and uninfected animals.

Table 1. Isolation of Listeria monocytogenes (Lm) at slaughter from organs of 13 ewes and 15 hoggs from the experimental flock.

Number of animals	Lm isolated from					
	brain	lung	kidney	liver	spleen	udder
13 ewes	—	—	—	—	—	1
10 hoggs*	5	1	1	0	3	—
5 hoggs	1	0	3	1	1	—

— Not examined.
* Infected orally and intranasally with Lm 2 months previously.

Results from the examination of organs from the diagnostic material are recorded in Table 2. Lm was isolated from the brain of 7 of 9 animals with encephalitis. The 10th brain was not examined bacteriologically. Lm was also isolated from 0—3 of the other 4 organs from these animals.

Table 2. Isolation of Listeria monocytogenes (Lm) from organs of 68 animals brought to the laboratory for post-mortem examination.

Diagnosis	Number of animals		Brain		Lung		Liver		Spleen		Kidney	
	T[1]	P[2]	T	P	T	P	T	P	T	P	T	P
Enterotoxaemia	10	9	9	7	10	3	10	2	10	1	10	2
Pneumonia	9	3	5	1	9	1	9	2	9	2	9	2
Enterotoxaemia	13	3	4	0	13	2	13	2	13	3	13	2
Hepatitis	7	2	0	0	7	2	7	1	7	1	7	1
Braxy	5	1	2	0	5	0	4	0	5	1	5	0
Miscellaneous	24	7	14	2	23	0	24	5	23	2	21	2
	68	25	34	10	67	8	67	12	67	10	65	9

[1] T: Total number of samples or animals.
[2] P: Samples or animals positive for Lm.

DISCUSSION

Until lately the epidemiology of listeric infections has been poorly understood (*Gray & Killinger* 1966). Improved media and isolation techniques have enabled us to collect more knowledge about the distribution of Lm. The bacteria seem to be widespread in nature (*Welshimer & Donker-Voet* 1971, *Weis* 1975), and a high frequency of human carriers (*Kampelmacher & Janssen* 1969, *Kampelmacher et al.* 1975) has been found. A high carrier

rate in sheep has been established through examination of milk and faeces (*Grønstøl* 1979 a). In the present investigation organs from animals with and without apparent listeric infections were examined for the presence of Lm. The results showed that Lm may be present in the organs of a large proportion of healthy sheep.

Although the milk from healthy sheep and sheep with recent listeric abortion may contain Lm (*Grønstøl* 1979a, b), the carrier rate of Lm in the udder outside the lactation period is apparently not greater than for other organs. The ewes which excreted Lm in the milk did not show any symptoms of mastitis (*Grønstøl* 1979 a, b), but listeric mastitis may occur in cattle (*Donker-Voet* 1963, *Errebo Larsen & Jensen* 1977, *Bindseil & Errebo Larsen* 1977). The reason why even high numbers of Lm in the milk did not cause mastitis, may be that the bacteria were present in cells, probably macrophages, and thus were unable to invade the udder tissue. A symptomless mastitis, however, cannot be excluded, but results from the present investigation do not indicate a higher carrier rate for the mammary gland than for the other organs examined.

Lm was isolated from the brain from 6 of 15 healthy animals at slaughter. The animals belonged to a flock where a high proportion of the animals had been shown to excrete Lm in the faeces and milk in periods. Ten of the animals had been infected experimentally with Lm 2 months previously, and Lm was isolated from as many as 5 of the 10 brains. Lm was also isolated from the brain from 1 of the uninfected controls. In addition, Lm was isolated from various other organs. This indicates that Lm may stay alive and be tolerated by healthy animals for some time, presumably because it is present intracellularly. The possibility that Lm may survive as L-forms in the animal and revert to S-forms when the conditions are suitable, cannot be excluded. This may be so also for humans and probably explains why listeric infections are a serious problem in patients treated with immunosuppressiva (*Buchner & Schneierson* 1968, *Medoff et al.* 1971, *Niklasson et al.* 1978).

These results may also affect the criteria for the diagnosis listeriosis. The isolation of Lm from the brain after enrichment should be supported by histopathological evidence before a definite diagnosis is made. On the other hand, Lm may be difficult to isolate after prolonged treatment with antibiotics, and in

such cases typical histopathological changes in the brain should be accepted as basis for the diagnosis.

The results from the present investigation also have implications for the meat hygiene. The stress of transport prior to slaughter may lead to bacteriaemia or faecal excretion, with contamination of the carcass as a result (*Grønstøl et al.* 1974, *Elischerova* 1975, *Gitter* 1976). As Lm tolerates freezing and actually grows at refrigeration temperatures, the carcass represents a source of infection for humans. *Bojsen-Møller* (1972) found a higher faecal excretion rate of Lm among slaughter-house workers than in any other occupational group examined. The excretion rate in relation to rural background and direct animal contact has also been examined (*Kampelmacher et al.*), but no differences were found. This result is as expected, provided that the carrier state is induced by ingestion of contaminated meat. Infection through food with induction of a carrier state may also explain why listeriosis suddenly occurs in patients under prolonged treatment with immunosuppressiva, where no apparent route of infection can be found.

As expected, Lm was isolated from a majority of the brains with the histopathological diagnosis encephalitis. From the other organs from these sheep the isolation rate was about the same as from organs of animals dying from other diseases. This supports the conclusion from the examination of the slaughterhouse material, that a proportion of the healthy animals are carriers of Lm. Considering the small amount of tissue in each sample, and that only 1 sample from each organ was examined, it is reasonable to believe that an even higher proportion of the animals were carriers of Lm.

Since the isolation frequency of Lm from organs from animals with listeric encephalitis was of the same order as from animals with other diseases, the results also support the theory that Lm gains access to the brain by migration along the peripheral nerves (*Asahi* 1963, *Charlton* 1977).

It is difficult to judge whether the carrier state is associated with a certain degree of immunity against Lm, or whether the carrier animals are more prone than others to develop clinical listeriosis.

The results indicate, however, that carriers of Lm do not have a better protection against listeric encephalitis than other animals. Although Lm may have been spread through the blood

circulation to the various internal organs, the intracellular localization of the bacteria, or the possible change into L-forms, may have prevented an immune reaction in the host sufficiently strong to have a protective value. However, the material is too small for any firm conclusions to be made.

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SAMMENDRAG

Listeriose hos sau. Funn av Listeria monocytogenes i organ frå slakta og sjølvdaue dyr.

Jur frå 13 utrangerte sauer og lever, milt, nyre, lunge og hjerne frå 15 slakta fjorlam blei undersøkte. Listeria monocytogenes (Lm) blei funnen i 1 jur, 6 hjernar og i 0—4 av dei andre organa.

Indre organ frå 68 sjølvdaua eller avliva sauer blei undersøkte på same måten, og Lm blei isolert frå 25 av desse. Lm blei funnen i hjernen til 7 av 9 dyr med encephalitt og i 0—3 av dei andre organa frå desse dyra. Lm blei og dyrka frå 10—20 % av organa frå dyr med andre diagnosar. I alt blei Lm isolert frå 9 av 10 dyr med encephalitt og frå 16 av 58 dyr med andre diagnosar (28 %).

(Received June 28, 1979).

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