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Title	BACTERIOLOGICAL OBSERVATIONS ON AN OUTBREAK OF OVINE LISTERIOSIS IN SAPPORO
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Citation	獣醫學研究, 1(4), 191-201
Issue Date	1954-01
DOI	https://doi.org/10.14943/jjvr.1.4.191
Doc URL	https://hdl.handle.net/2115/11358
Type	departmental bulletin paper
File Information	KJ00000104929.pdf



BACTERIOLOGICAL OBSERVATIONS ON AN OUTBREAK OF OVINE LISTERIOSIS IN SAPPORO

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I. Introduction

Since 1926 when MURRAY and others¹⁾ described the first isolation of *L. monocytogenes* from spontaneous infections of laboratory animals in England, numerous works have been published on listeriosis not only in sheep but also in cattle, swine, goats, horses, dogs, rodents and poultry as well as human beings. Especially, after this organism was established as responsible for the "circling disease" in sheep by GILL^{2),3)} in New Zealand between 1931-33, listeriosis has become a disease of considerable economic importance in animal husbandry. Consequently, it had attracted many workers' attention from the standpoints of prophylaxis and therapeutics as well as from that of public health.

Recently its occurrence in sheep and cattle has been increasing year by year in the U.S.A., and numerous reports are being published from several States: Illinois⁴⁾, Wisconsin⁵⁾, Michigan⁶⁾, Iowa⁷⁾, Nebraska⁸⁾ and Utah⁹⁾ etc. Now it is obvious that listeriosis appears to be of worldwide distribution and apparently is increasing in frequency.

However, no description of the occurrence of listeriosis has yet been recognized in Japan. In 1948 when equine infectious encephalomyelitis prevailed in Hokkaido, TAJIMA¹⁰⁾ first suggested pathologically the existence of listeriosis in two goats which died in Sapporo under the clinical diagnosis respectively of "circling disease" and of "Japanese B encephalitis". Following this report, in 1951 *L. monocytogenes* was isolated by ASAH¹¹⁾ and others¹¹⁾ from two cases of infected goats in Aomori Prefecture. Their report is the first on the isolation of the causal agent in Japan. In next year, the staff of this laboratory had encountered an outbreak of this disease among sheep in the Hokkaido Agricultural Experiment Station near Sapporo, and afterward some additional cases¹²⁾⁻¹⁵⁾ were reported in various districts of Japan.

Accordingly it may be considered that listeriosis is now increasing in frequency and that it has become a new serious problem for veterinary medicine also in Japan.

The present communication is mostly concerned with some bacteriological and serological observations on this outbreak but also with the biochemical and serological characters of *L. monocytogenes* of different origins.

(This report has already been published at the 34th Meeting of Japanese Society of Veterinary Science on September 21st, 1952).

II. Observations

1. Outline of the occurrence

Hokkaido Agricultural Experiment Station had three flocks of sheep: flock A included 127 old ewes and 147 lambs; flock B, 103 ewes and 106 lambs; flock C 24 rams and 82 lambs. Hitherto, no instance of listeriosis-like maladies had been recognized, nor, recently, had any

Fig. 1. One Case of Ovine Listeriosis Which Shows the Typical Symptoms of the Last Stage



sheep been brought to this Experiment Station. The first case of ovine listeriosis was noticed on May 16th, 1952. During the following short period of 20 days, 21 sheep succumbed one after another as is shown in table 1. All these sheep revealed the encephalitis-like severe symptoms. The general symptoms were dullness, loss of appetite, elevation of body temperature, somnolence, and paralysis of ears and lower lips with salivation. Often the sheep would lean against the fence for support. When forced to move they would tend to circle and in most cases the head and neck turned to one side. The depression was progressive and they proceeded to coma and death (Fig. 1). These symptoms perfectly coincided with those which were presented by many other authors.

The course of the disease was very rapid, and death usually occurred within 2-4 days after the first symptoms appeared. As were reported by many workers, excepting OLSON and others³⁾, no cases of recovery were observed.

At that time, each flock of sheep was pastured in the daytime and kept each in its sheepfold at night. In flock A which was kept in unsanitary environment (dark, moist, gloomy sheepfold) most frequent occurrences were recognized. It is generally comprehended that listeriosis is primarily a disease occurring in winter or early spring and that its occurrence is sporadic. However, the present outbreak in Sapporo was observed in late spring and it was noteworthy that it revealed some epizootic characteristics.

Table. 1. Outline of Ovine Listeriosis Outbreak in Sapporo in 1952

No. of case	Flock	Age	Course of Disease	Termination
1	A	4	May 16-21	Death
2	"	Yearling	" 18-20	"
3	"	"	" 18-20	"
4	"	7	" 18-20	"
5	"	Yearling	" 18-20	"
6	B	3	" 20-22	"
7	A	Yearling	" 21-23	"
8	"	"	" 23-25	"
9	"	"	" 25-28	"
10	"	4	" 25-29	"
11	B	2	" 27-29	"
12	"	2	" 27-29	"
13	A	5	" 27-31	"
14	"	4	" 27-29	"
15	"	5	" 28-30	"
16	C	1	" 30-June 1	"
17	B	3	June 4-10	"
18	"	Yearling	" 5-10	"
19	"	2	" 3-7	"
20	"	2	" 3-8	"
21	"	Yearling	" 5-7	"

2. Isolation of causal agents

Attempts to isolate the causal agents were performed in 8 cases of the 21. *L. monocytogenes* was isolated from all cases. Except 2 cases (Nos. 6 and 7), quite detailed observations

Table 2. Distribution of *L. monocytogenes* in Sheep Body

Materials examined	No. of animal tested									
	6	7	8	9	11	12	13	14		
Main lymph nodes			—			—	—	—		
Eye secretion			—	—	—	—	—	—		
Nasal secretion			—	—	—	—	—	—		
Lung	—	—	—	—	—	—	—	—		
Kidney	—	—	—	—	—	—	—	—		
Spleen	—	—	—	—	—	—	—	—		
Liver	—	—	—	—	—	—	—	++		
Spinal fluid			—		—	—	—	—		
Spinal cord			Lumbar part	++	—	—	—	—		
			Thoracic part	++	—	—	—	—		
			Cervical part	+	+	—	—	—		
Medulla oblongata			++	++	+	++	++	++		
Hinder-brain		Pons	++	++	++	++	+	++	++	
		Cerebrum		—	—	++	—	+	++	++
Mid- and inter-brain		Quadrigenimal body				++	—	—	—	++
		Optic thalamus			+	++	—	++	++	++
End-brain		Caudate nucleus				++	—	—	—	++
		Ammon's horn				++	—	—	—	+
		Olfactory bulb			—	++	—	—	—	—
		Cortical substance of cerebrum			—	+	—	—	—	++
Notes	+	1-49	colonies developed.							
	++	50-100	"							
	++	Over 100	"							

on the bacterial distribution in animal body were carried out (table 2). Although the number of cases observed was too small to determine the distribution of the organisms in the body of field listeriosis, *L. monocytogenes* were generally detected localizing in brain stem such as medulla oblongata, pons, quadrigenimal body, in cerebellum and in optic thalamus. End-brain and spinal cord were less invaded by this organism. The authors could not find *Listeria* organism in any of the visceral organs with the one exception of No. 14, in liver. These results are almost identical with those of other workers.

3. Biological characteristics of isolated organism

There were isolated 9 strains (including strain L 15 that was isolated from a goat in Sapporo on September 17, 1952). They were compared with the known 8 *Listeria* strains which originated from Aomori Prefecture in Japan and from America. All strains demonstrate a narrow zone of hemolysis on sheep blood agar. Morphologically they are Gram-positive rods with probably one flagella. As is indicated in table 3, in respect to sugar fermenting activities and biological characteristics the strains were almost identical with each other. No differences were found among the strains of several sources. Glucose, salicin, melibiose, amygdalin, levulose, mannose, trehalose, dextrin, rhamnose and maltose were fermented by all strains within 24 hours without gas. However, sorbitol fermenting and non-fermenting strains were noticed, and there were some variable differences among the number of days required for the fermentation of glycerol, and lactose. Sucrose was also attacked by all strains excepting one (L 2). As far as examined, arabinose, xylose, raffinose, inulin, inositol, glycogen, mannitol and galactose were never fermented. Gelatin liquefaction, methyl-red reaction, H₂S-production and indol formation were negative and Voges-Proskauer reaction was doubtful excepting 2 American strains (positive).

This organism easily produces R colonies as is seen in *Salmonella* in culture media (Fig. 2)

and filamentous form are recognized in the old subcultures. Fig. 3 indicates the typical uniform shapes at the beginning of isolation and fig. 4 the filamentous or pleomorphic individuals in the old subcultures.

4. Agglutination tests in the flocks

The authors carried out agglutination tests with 71 serum samples obtained from the sheep on the same Experiment Station 54 days after the last occurrence of the disease. Eighteen cases out of 71 sera showed 1:800 or higher titres for *L. monocytogenes*, as is indicated in table 4. Almost all yearlings had the lower titres such as 1:200 or below. On the other hand, in the greater part of animals over one-year-old, higher titres (1:400 or over) were generally found. A second examination 49 days later was performed on the individual sheep which previously has shown comparatively high titres.

From table 5, it will be seen that 12 cases out of 18 sheep show tendency to decline and the others to ascend or to run unchanging in their agglutinin titres. It is supposed that these fact may be interesting: however, the present writers could not draw any conclusions as to the relation between the infection and agglutinin titre in the case of listeriosis.

Table 4. Agglutinin Titre after 50 Days of Epizootic

Age of sheep	Agglutinin titre							Total
	1:25	1:50	1:100	1:200	1:400	1:800	1:1600	
Yearling	2	2	3	3	1			11
1 year				5	15	3	2	25
2 or more		1	3	8	10	10	3	35

Table 5. Rise and Fall of Agglutinin in the Animals which showed high Agglutinin Titres

Flock	No. of individual	54 days after the last occurrence	103 days after the last occurrence
A	23-505	1:800	1:400
	22-505	1:800	1:800
	22-127	1:800	1:400
	25-4	1:1600	1:800
	22-103	1:800	1:400
	23-100	1:800	1:1600
	21-336	1:1600	1:400
	26-344	1:800	1:1600
	25-364	1:800	1:800
B	25-8	1:800	1:200
	24-55	1:800	1:800
	24-54	1:400	1:1600
	25-32	1:400	1:800
	25-55	1:1600	1:800
C	s-32	1:1600	1:400
	s-36	1:1600	1:800
	s-66	1:800	1:400
	s-11	1:800	1:400

5. Antigenic structures of *L. monocytogenes*

With regard to the antigenic structures of *L. monocytogenes*, SEASTONE¹⁶⁾, WEBB and BARBER¹⁷⁾, JULIANELLE and PONS^{18),19)}, PATERSON²⁰⁾, etc. have already reported. However, their opinions

Table. 6. Serological Differentiation of Somatic Antigen of *L. monocytogenes*

Antigen	Sera/Absorbed with						Type		Source	
	A/B	A/C	B/A	B/C	C/A	C/B	Name	Antigenic Structure	Animal	District
	Factors which will remain after absorption									
	5	4, 5	1	4	1, 3	3				
#8615	400	800	—	200	—	—	A	2, 4, 5	Sheep	America
L 1	400	800	—	200	—	—			"	"
L 2	800	800	—	200	—	—			Cattle	"
L15	400	800	—	200	—	—			Goat	Hokkaido
#L 3	—	800	100	200	400	—	B	1, 2, 4	Cattle	America
#L 4	—	—	200	—	200	1600	C	1, 2, 3	Human	"
L 6	—	—	200	—	400	800			Sheep	Hokkaido
L 7	—	—	200	—	400	800			"	"
L 8	—	—	200	—	400	800			"	"
L 9	—	—	200	—	400	1600			"	"
L11	—	—	200	—	400	800			"	"
L12	—	—	200	—	400	800			"	"
L13	—	—	200	—	400	800			"	"
L14	—	—	200	—	400	800			"	"
511	—	—	200	—	400	800			Goat	Aomori
512	—	—	200	—	400	800	"	"		

Notes: # Indicates the representative strains which were used for the absorbing organisms and preparation of immune sera.

A/B=Type A serum/absorbed with type B antigen, B/A=Type B serum/absorbed with type A, and so on.

Table. 7. Serological Differentiation of Flagellar Antigen of *L. monocytogenes*

Antigen	Sera/absorbed with						Type	
	A/B	A/C	B/A	B/C	C/A	C/B	Name	Structure
	Factors which will remain after absorption							
	d	d	a	—	a, c	c		
#8615	—	320	—	—	—	—	A	b, d
L 1	—	320	—	—	—	—		
L 2	—	160	—	—	—	—		
L15	—	320	160	—	320	80		
#L 3	—	640	—	—	—	—	B	a, b
		160	320	—	640	—		
#L 4	—	—	640	—	1280	160	C	a, b, c
L 6	—	—	160	—	640	160		
L 7	—	—	160	—	640	320		
L 8	—	—	160	—	320	320		
L 9	—	—	320	—	640	320		
L11	—	—	160	—	640	320		
L12	—	—	80	—	640	320		
L13	—	—	320	—	1280	160		
L14	—	—	160	—	640	640		
511	—	—	320	—	320	640		
512	—	—	320	—	320	320		
PATERSON'S Result								
	G/LS	G/J	LS/G	LS/J	J/G	J/LS		
Gibson	640	256	—	40	—	—		B, D
LS-2	—	40	640	—	320	—		A, B
Jungherr	—	—	320	—	640	640		A, B, C

Notes: # Indicates the representative strains which were used for the absorption tests and the preparation of the immune sera.

• Indicates the different agglutinin titres by the colonies examined.

* In this series the agglutinin titres reached to the same degree as in A/C series when the titres were read after standing at room temperature overnight following after incubation at 50°C 2 hours.

do not always agree. WEBB and BARBER¹⁷⁾ did not find any antigenic differences between the strains of several sources. On the other hand, SEASTONE¹⁶⁾, JUNGHERR et al,²¹⁾ JULIANELLE and PONS^{18),19)} reported that they recognized some differences among these strains according to their sources. Especially, JULIANELLE^{18),19)} described two sero-types of *L. monocytogenes*, the one included the strains isolated from rabbit and human being and the other the strains from cattle, sheep and goat. PATERSON classified *L. monocytogenes* into three sero-types according to flagellar antigenic analysis.

Hence, the present writers performed cross agglutination and absorption tests to ascertain whether there can be antigenic differences among the 16 strains originating from America, Aomori and Hokkaido.

The antigens used for the preparation of H sera were prepared as follows: 5 plates of 18-hours-cultured organisms, confirmed S type and having active motility, from 1 per cent glucose agar were suspended in 10 cc of 0.5 per cent formalised saline and after shaking about 5 minutes, were centrifuged at a speed of 3,000 r.p.m. for 30 minutes. The supernatant fluid containing nearly pure flagellar substances was used for H immunogen.

For the O antigen, 18-hours-cultured 1 per cent glucose broth was used after boiling for about 6 hours. Rabbits were immunized by repeated intravenous injections of these antigens.

Agglutination tests were carried out after the method used with the *Salmonella* group. From tables 6 and 7 (showing the results of agglutination reactions), it may be considered that *Listeria* strains consist of 3 different O types according to cross absorption tests. As to the flagellar antigens they seem to go parallel with O.

The authors provisionally named these types A, B and C. It is suggested that these types will have a common O factor "2", and moreover, type A and C seem to have the type specific factors represented by "1, 3" and "4, 5" respectively. However, type B will have the "1, 4" factors which are partially common with type A and C. Pertaining to the flagellar antigen, it is supposed that types A, B and C have the factors "b, d", "a, b" and "a, b, c" respectively. In strain L3 (type B) the factors common with the other two types were recognized and consequently, just as the diphasic *Salmonella* types, it revealed the different H agglutination reactions resembling the reaction of type A or type C. The same phenomenon was observed in strain L15. However, these findings may require further investigations. Moreover, A/B serum (type A serum that was absorbed with type B organisms) did not react to type A antigens, as is shown in table 7, at the end of incubation (50°C for 2 hours) but it agglutinated type A bacteria after the tube was left standing at room temperature over-night following the incubation.

From the above-described results, the writers will conclude that there are three different sero-types in *L. monocytogenes* and that these types have no connection with the source of isolation as far as examined. Our results are almost identical with PATERSON'S work²⁰⁾, except for two points as seen in the table 7.

Owing to the lack of strains from fowl and rodents and to the small number of strains employed, further trials on these points will be desirable. Consequently, as PATERSON suggests, the writers suppose that other types may be found upon further examinations.

6. Artificial inoculation experiments

In spite of interest and the importance associated with prophylaxis and control of this disease the modes of natural infection of this organisms are not yet clarified. However, GILL suggested that *Oesterus ovis* which occasionally carry this organism, may be responsible for natural infection by injuring the nostrils of sheep. PALLASKE²²⁾ also supported this possibility from his experimental and field observations.

On the other hand, JULIANELLE²³⁾ is of the opinion that there is per oral infection.

Recently, ASASHI and others^{21,25)} carried out experimental works on the natural infection modes in goats and mice, and from their bacteriological and pathological findings, emphasized the possibility of natural infection modes via trigeminal nerves through the injured nasal and oral mucous membrane.

The writers, employing 13 goats and 3 sheep, performed oral and nasal administration with small doses of the organisms after injuring or not injuring their mucous membranes (table 8). However, from the clinical, bacteriological and pathological standpoints, these examinations had showed only negative results except one goat which showed the temporary bacteriemia in company with the elevation of body temperature (40.2°C) 13 days after nasal administration. On the 7th day after the appearance of *L. monocytogenes* in blood stream, this animal died but the organisms could not be detected from any part of the body. Moreover, one sheep which received the per oral administration of the organisms after injuring the oral mucous membrane, died on the 15th day after administration, without showing any symptoms of listeriosis but *L. monocytogenes* were recovered from submaxillary lymph nodes. However, the histo-pathological signs of these two cases had also showed negative.

Table 8. Inoculation Experiments

Animals No.	Route	Dosis	Date	Termination	*Recovering of <i>L. monocytogenes</i> from animals						
Goat	Per oral	1 cc of the brain emulsion (5 million bacilli)	Jun. 13	Jul. 5, died	Negative						
				Aug. 26, died	"						
				Jul. 18, died	"						
				Jul. 7, died	"						
	10	2 cc of the brain emulsion (10 million bacilli)	Jul. 2	Sept. 29, died	"						
				Aug. 11, died	"						
	5	Intranasal	0.5 cc of the broth culture	Jun. 13	Aug. 7, killed	"					
					" died	"					
					Aug. 12, died	"					
					Sept. 17, died	"					
	11	0.1 cc of the broth culture	Jul. 1, died	Jul. 9, died	Jul. 17, died	#Positive (from the blood stream)					
						Negative					
						"					
	Sheep	First Administration (Sept. 22)		Second Administration (Nov. 12)		Termination	Recovering of <i>L. monocytogenes</i>				
		Route	Dosis	Route	Dosis						
1		Intranasal (after injuring)	0.5 cc of broth culture	Intranasal and per oral (after injuring)	2 cc of broth culture	Dec. 2, killed	Negative				
								2	Intranasal (not injured)	Dec. 2, killed	Positive (Submaxillary lymph nodes)

* Cultivations were made from the lung, liver, spleen, kidney, heart blood, brain, spinal cord, digestive organs, tonsil, nasal and eye secretions, and main lymph nodes which can be obtained.

Post-mortem cultivations showed negative.

III. Discussion

From the viewpoint of epidemiology, our present observations seem to be somewhat interesting for the severe occurrence of the epidemic during a short period. Such instances are comparatively rare except as reported by OLSON and others⁹⁾ and by BIESTER and others⁷⁾.

Although these organisms produce several types of diseases in animal species, encephalitis seemed the most familiar type in sheep from previous reports. Accordingly, the bacterial distribution in animal body is generally restricted to the brain. However more careful bacteriological examinations are desirable.

The present writers found one field case in which the organisms were located in the liver and brain in spite of the small number of inspections. However, OLSON and others⁵⁾ reported many cases in which the organisms were detected from the liver, spleen and occasionally from the heart, and especially in one peracute case, they isolated the organisms from the heart muscles, liver and spleen. GRAY and others⁶⁾ also reported similar cases. Moreover, the authors observed the temporary bacteriemia in one goat which received intranasal inoculation with small dosis of these organisms. On the other hand, PATERSON²⁶⁾ succeeded in the isolation of the *L. monocytogenes* from 3 foetuses out of 4 examined in 16 cases of abortion in sheep, which were free from *V. fetus* and *S. abortus ovis*.

From these facts, it is evident that the listeriosis in sheep involved several types of disease. Therefore it may be certain that the natural infection modes will be considerably complicated. Accordingly, ASAHI and others' considerations on the infection modes via trigeminal nerves through the injured mucous membrane and the other infection modes via oral and intranasal, which are suggested by some workers, may be considered as possible infection Routes. At any rate it is desired to carry out more detailed examination for ascertaining the modes of infection in this disease.

On the other hand, OLSON and others⁸⁾ already reported inapparent infection from their clinical (elevation of body temperature) and hematological (neutrophilic leucocytosis) observations. The authors tried to find the inapparent infection and recognized the rise and fall of the agglutinin titre in the same individuals in accordance with the days elapsed after the outbreak. This suggested bacterial influence. However, obviously, no definite conclusion on the existence of inapparent infections can be based on the serological procedures alone.

Our strains showed almost the same response to fermentive activities and other biochemical characteristics. Although some authors^{11),17),27)} described the galactose and raffinose fermenting activities of *L. monocytogenes*, such activities were not recognized by the writers.

There are found 3 distinctly different sero-types in our strains. However, more detailed examinations are desirable, because the antigenic structures of this organism are the foundation of serological diagnosis and vaccination (if possible). Hitherto, experiments on this problem have been comparatively rare and these data reported are every contradictory to each other. The authors' data on the antigenic structures of H and O are almost identical with those of PATERSON. However, strains which were tested are not only few but also they do not include the strains from rodents and fowl. Accordingly, it is supposed that there may be exist other sero-types and it must be left to future investigations to solve this problem.

IV. Summary

(1) An outbreak of ovine listeriosis which was experienced in Sapporo in 1952 and some bacteriological investigations on the *L. monocytogenes* were described.

(2) Twenty-one head out of 589 sheep were affected during the short period of 20 days. The first case was recognized on May 16th, 1952.

(3) Eight cases of patients were bacteriologically investigated and *L. monocytogenes* were recovered from every case. The specimens were detected from distinctly localized parts of the body, brain stem, cerebellum and optic thalamus.

(4) Bacteriological and serological studies were performed on isolated strains and the other known *Listeria* strains originating from other districts of Japan and America. Three

sero-types were recognized, however, in biochemical characteristics they were almost identical with each other.

(5) Artificial inoculation experiments by per oral and intranasal instillation with small dosis of the organisms after injuring or not injuring these mucous membranes, indicated negative results except for one case which showed temporary fever and bacteriemia.

The authors would like to express their thanks to Prof. YAMAGIWA the Laboratory of Veterinary Pathology of this University and Dr. TAJIMA, a member of the Nippon Institute of Biological Science, who provided the pathological findings and many other kindly advices, and to the members of the (Department of Animal Husbandry), Hokkaido Agricultural Experiment Station for their willingly supplying of materials.

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札幌に於ける緬羊リステリア症流行例に関する細菌學的研究

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本邦に於ては 1950 年(昭和 25 年)田島¹⁰⁾により初めてリステリア症の存在が指摘されて以來、漸く本症に對する關心が拂われ、最近各地に於て散發的な若干の發生報告¹¹⁾⁻¹⁵⁾がなされつつある現狀である。著者等は 1952 年(昭和 27 年)5 月札幌近郊の 1 牧場の羊群に著明な腦炎様症狀を呈し急激に死の轉歸をとる流行病の發生に遭遇し、細菌學的檢索の結果、本症は緬羊のリステリア症であることを確認した。本例は短期間に於ける相當數の集團發生例であり、文献上甚だ稀有のものと考えられるので、ここにその發生の概要を報告するとともに、細菌學的血清學的檢索並びに 2, 3 の地域及び數種動物由來の *L. monocytogenes* の生化學的・血清學的諸性狀に就き若干の檢討を行つた。これらの成績を要約すれば、次の如くである。

(1) 5 月 16 日より 6 月 5 日に至る約 20 日間に 589 頭中 21 頭が罹患し、何れも 2~5 日の経過で斃死した。主な症狀は呆然佇立、食慾の廢絶、沈鬱、体温上昇、耳及び下唇の麻痺、旋回、倦怠、起立不能等である。一般に環境衛生の不良な羊舎に多發する傾向が認められた(表 1, 圖 1)。

(2) 8 例に就き細菌學的檢索を行つたところ、全例から主に延髓・腦橋・小腦等に局限して原因菌が證明されたが、鼻汁・眼賦・臟器・淋巴節等からの檢出は不成功に了つた(1 例のみ肝から檢

出)。これらは何れもグラム陽性の短桿菌で從來の記載の *L. monocytogenes* に完全に一致した(表 2)。

(3) 國內系及び米國系の菌株 17 株を用い、本菌屬の血清學的・生化學的性狀の比較檢討を行つた結果、凝集反應(O 及び H)により A, B, C の 3 群に型別することが出來た。然し分離場所及び分離動物種との間に特別の關係を認め得なかつた。又血清學的菌型とは無關係に生化學的性狀に於ては何れもほぼ同一の態度を示すが、sucrose 及び sorbitol の分解態度に若干の差異を認めた(表 3, 6, 7)。

(4) 本症の場合不顯性感染が存在するや否やを血清學的に檢討してみた。即ち流行終熄後 2 回に亘り同一個体につき凝集價をしらべた結果、個体により凝集價の上昇するものは或いは下降するものを認め得たが、確實に不顯性感染の事實を裏書するが如き成績を得ることは出來なかつた(表 4, 5)。

(5) 本症の自然感染経路追及の目的で、山羊及び羊に對する微量菌の經口並びに經鼻感染試験を實施したが、經口投與後 1 時的に体温上昇と菌血症を呈した山羊 1 例(死後の菌檢索及び病理組織學的檢索は陰性)以外は特記すべき所見が得られなかつた。