SALMONELLA TYPES IN ANIMALS IN SAPPORO

Kazuo OSAMURA, Tadashi AOKI and Kiheiji SHIMIZU
Laboratory of Veterinary Hygiene and Microbiology,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan
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INTRODUCTION

In 1951, the Committee of Enterobacteriaceae in Animals was organized in Japan and it took up the project of learning the occurrence and distribution of *Salmonella* in this country. This laboratory participated in the work of the Committee; the study herein reported was started in April, 1952.

The early work of this laboratory, before *Salmonella* typing was established, was carried out mostly on the etiological relationship of *Salmonella* to the paratyphoid among domestic animals. Former Professor KASAI et al. first isolated *S. abortus-equi* in Hokkaido from the outbreak of infectious abortion in mares. WATANABE recovered *S. aertrycke* from cases of paratyphoid in foxes and dogs in the vicinity of Sapporo. Then NISHIMURA observed that paratyphoid infections were often found in foxes, racoon-dogs, dogs, fitches, cats and guinea pigs and that the etiological agents were mostly *S. typhimurium*, the rest being *S. enteritidis* var. *chaco* and *S. enteritidis* var. *essen*. HAMADA reported the isolation of *S. senftenberg* and *S. thompson* besides *S. pullorum* from dead-in-shell and dead baby chicks. ONO et al. added the isolation of *S. bareilly* in the further bacteriological observations of these diseases.

MURASE et al. inspected 534 stray dogs in Tokyo and isolated 11 types, 63 strains of *Salmonella*. One-third of them were *S. enteritidis*.

The present study was undertaken to determine the incidence of *Salmonella* in the several species of animals in and around Sapporo during the period from April, 1952 to March, 1954.

MATERIALS AND METHODS

As is indicated in table 1, the animal species tested are cattle, hogs, dogs, foxes and several other small animal species including 2 imported minks. Detailed descriptions of the materials for cultivation are also listed in table 1.

The disease cases in these animals were mostly of unknown causes, however, the dogs were generally suffering from distemper, distemper-like diseases and gastroenteritis.

Generally, these materials were directly cultivated on B. T. B. lactose agar. S. S. media were mainly employed for the intestinal contents, feces and bile samples after enriching in KAUFFMANN's media.

TABLE 1. *General Description of Materials and Results*

SPECIES OF ANIMAL	NUMBER TESTED		MATERIALS FOR CULTIVATION	OCCURRENCE OF	
	Healthy	Diseased		*Salmonella*	Bethesda-paracolon
Cattle	·	1	I	0	1
	100	·	E, G, H, J, K, L	1 (1 %)	0
Hog	683	·	"	1 (0.15%)	2
Dog	·	68	A, B, C, D, E, F, G, H	3 (3.9 %)	0
	8	·	"	0	0
	·	235	I	0 (0 %)	0
	20	·	I	0	0
Sheep	8	·	A, B, C, D, E, F, G, H	0	0
Fox	95	·	B, C, E, G, M	3 (3 %)	0
	·	2	A, B, C, D, E, F, G, H	0	1
Cat	·	3	"	1	0
Mink	·	2	"	1	0
Rat	3	·	"	1	0
Guinea pig	·	2	"	0	0
Rabbit	·	2	"	0	0
Nutria	·	1	"	0	0
Total	1,233			11	4

Letters in the third column indicate:

A···Lung
B···Liver
C···Spleen
D···Kidney
E···Mesenteric lymph node
F···Duodenum contents
G···Caecum contents
H···Rectum contents
I···Feces
J···Tonsil
K···Submaxillary lymph node
L···Retropharyngeal lymph node
M···Bile

Mink: Two minks were imported from U.S.A. and they died in the plane on the way from Utah to Haneda.

RESULTS

Salmonella was encountered in 3 dogs and foxes and in 1 each: cow, hog, cat, mink and rat. Bethesda group bacilli were isolated from 2 hogs, 1 cow and 1 fox. The materials and the biochemical properties of the isolated organisms are shown in table 2.

Serologically, as is shown in table 3, these *Salmonella* were identified as belonging to 5 types, namely *S. enteritidis* (5), *S. typhimurium* (3), *S. thompson* (1), *S. amager* (1) and *S. cerro* (1). *S. enteritidis* was detected from each 1 healthy cow and fox, 2 dogs, and from 1 cat suffering from gastroenteritis. *S. typhi-*

TABLE. 2. *Biochemical Characteristics of the Isolated Organisms*

STRAIN NO.	SOURCE	H_2S	INDOLE	V. P.	M. R.	UREASE	MOTILITY	GELATINE	SUGAR FERMENTATION: Ammonium citrate	Salicin	Adonitol	Dulcitol	Arabinose	Trehalose	Sucrose	Lactose	Glucose
B 1	Cattle, Caecum contents	+	−	−	+	−	+	−	+	−	−	+	−	+	−	−	+
B 2*	" Feces	+	−	−	+	−	+	−	+	−	−	+	+	+	−	$+_{10}$	+
P 1	Hog, Caecum contents	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
P 2*	" "	+	−	−	+	−	+	−	+	−	−	+	+	+	−	$+_{10}$	+
P 3*	" Rectum contents	+	−	−	+	−	+	−	+	−	−	+	+	+	−	$+_{10}$	+
D 1	Dog, "	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
D 2	" Liver	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
D 3	" Mesenteric l. n.**	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
F 1	Fox, Liver	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
F 2	" Bile	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
F 3	" Caecum contents	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
F 4*	" Liver	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
C 1	Cat, Caecum contents	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
M 1	Mink, Mesenteric l. n.**	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+
R 1	Rat, Caecum contents	+	−	−	+	−	+	−	+	−	−	+	+	+	−	−	+

* indicates Bethesda-paracolon group organisms. ** indicates lymph node.

TABLE 3. *Antigenic Formulas and Serological Identifications of the Isolated Organisms*

STRAIN NO.	ANTIGENIC STRUCTURES: *Salmonella* O	*Salmonella* H Phase 1	*Salmonella* H Phase 2	*Bethesda* O	*Bethesda* H	IDENTIFICATION
B 1	1, 9, 12	g·m	—	—	—	*S. enteritidis*
B 2	—	—	—	—	—	Perhaps Bethesda
P 1	6, 7	k	1·5	—	—	*S. thompson*
P 2	—	—	—	8	19	Bethesda
P 3	—	—	—	8	22	"
D 1	1, 9, 12	g·m	—	—	—	*S. enteritidis*
D 2	1, 9, 12	g·m	—	—	—	"
D 3	1, 4, 5, 12	i	1·2	—	—	*S. typhimurium*
F 1	1, 9, 12	g·m	—	—	—	*S. enteritidis*
F 2	1, 4, 5, 12	i	1·2	—	—	*S. typhimurium*
F 3	18	$z_4 \cdot z_{23}$	—	—	—	*S. cerro*
F 4	—	—	—	5	8·9	Bethesda
C 1	1, 9, 12	g·m	—	—	—	*S. enteritidis*
M 1	3, 10	y	1·2	—	—	*S. amager*
R 1	1, 4, 5, 12	i	1·2	—	—	*S. typhimurium*

murium from 1 dog having gastroenteritis, 1 healthy fox and from 1 apparently normal rat, *S. thompson* from 1 healthy hog, *S. cerro* from 1 healthy fox and *S. amager* from 1 mink which died for some unknown cause during air transportation.

TABLE 4. *Localities of the Isolated Salmonella Organisms in Each Animal*

STRAIN NO.	SALMONELLA TYPE										
	S. enteritidis					*S. typhi-murium*			*S. thompson*	*S. amager*	*S. cerro*
	C 1	B 1	F 1	D 1	D 2	R 1	D 3	F 2	P 1	M 1	F 3
Lung	−	·	−	−	−	−	−	−	·	+	·
Liver	−	·	+	−	+	−	−	−	·	+	−
Spleen	−	·	+	−	−	−	−	−	·	+	−
Kidney	−	·	−	−	−	−	−	·	·	+	·
Mesenteric*	−	−	·	−	−	·	+	−	−	+	−
Submaxillary*	·	−	·	·	·	·	·	·	−	·	·
Retropharyngeal*	·	−	·	·	·	·	·	·	−	·	·
Tonsil	·	−	·	·	·	·	·	·	−	·	·
Bile	·	·	·	·	·	·	·	+	·	·	−
Caecum contents	+	+	·	−	−	+	−	−	+	·	+
Rectum "	−	−	+	+	−	+	−	·	−	·	·
Duodenum "	·	·	+	−	−	+	−	·	·	·	·

* indicates lymph node.

The locality of the isolated organisms in each animal body are listed in table 4. The caecum and rectum contents seem most often carry *Salmonella* organisms. These results do not coincide with the data from dogs in Tokyo by MURASE et al. which indicated that about 85% of the isolated *Salmonella* were detected from the mesenteric lymph nodes. The antigenic formulas were carefully investigated by SAKAZAKI and others in Government Experimental Station for Animal Hygiene in Tokyo.

Moreover, 4 strains of Bethesda-paracolon (formerly so called) organisms were also isolated. Two of them were detected from each rectum and caecum contents of the different individual of hogs; the antigenic formulas were determined by SAKAZAKI as 8 : 19 and 8 : 22 respectively and seem to be new ones. One from the liver of the diseased fox was serologically identified, the antigenic formula 5 : 8, 9, however the organism derived from cattle was biochemically identified to be Bethesda-paracolon group, although serologically it was not possible to identify it with the known Bethesda organism.

CONSIDERATIONS

At the beginning of this experiment, a considerably larger number of isolations of *Salmonella* cultures were expected especially in dogs and hogs. However, contrary to the authors' expectation, only 1 *Salmonella* was isolated from 683 hog materials (0.15%) and also only 3 from 76 dog organ materials (3.9%). In this country, SAKAZAKI has already recognized *Salmonella* in 4 cases out of 94 apparently healthy hogs (4.3%) in Mie Prefecture and it is said that in a certain group of hogs, a larger number of *Salmonella* could be isolated but not in another group. It seems to depend upon the districts where the hogs are raised whether the ratio of detection is high or low. The Public Health Laboratory of this Faculty made the same study on the materials of hog in Hokkaido but failed to isolate *Salmonella* in almost the same number of samples as the authors'.

On the other hand, according to the recent reports, FOURNIER et al. detected *Salmonella* from the mesenteric lymph node of 35 cases out of 360 head of hog in Saigon (9.6%) and CRANEVELD et al. also in 25 cases out of 150 head of healthy hog in Indonesia (16.7%). These ratios of detection are considerably high. This fact may possibly be due to the difference of the food and locality where they are raised, also due to the grade of *Salmonella* incidences, although the detection techniques are of course the most important. Such bases for widely differing results are proven by the work of MURASE et al. on the dog materials, viz., in Tokyo about 13% of dogs are harboring *Salmonella*, however the dogs from Minamitama and Nishitama, the suburbs of Tokyo, were affected in only about 1%. The ratio of detection goes parallel with the human populations, namely it corresponds to the grade of the contamination of food-water supply.

In the other animals such as cattle, dogs and foxes, the detection ratio is also comparatively low. In the fecal samples from 255 dogs, no *Salmonella* was isolated, although 3 *Salmonella* types were detected from the organ materials from the total of 76 autopsied dogs (3.9%). These low incidences may be due to the detection techniques employed because in this case, the authors did not conduct the enriching cultivations on the organ materials other than feces and intestinal contents.

In the present study, the most interesting points were the isolations of *S. cerro* and *S. amager*. These were both the first isolations in Japan. Especially with regard to *S. amager*, it was for the first time isolated from the feces of a person suffering from gastroenteritis in Copenhagen in 1939; so far as the authors are aware, the present data are the first report on the isolation from animal.

EDWARDS et al. made a large scale expriment on *Salmonella* distribution in

U.S.A. and 12,331 cultures were obtained from 47 species of animals including man. These included a great many *Salmonella* types, however *S. amager* was not recognized. The mink from which *Salmonella amager* was isolated, arrived here from U.S.A., dead by unknown cause during the air transportation and the organism was detected from each of the organs examined (Table 4). Accordingly it is very reasonable to suppose that the animal was a carrier of *S. amager* in U.S.A. from before and that the sepsis may have resulted under the abnormal conditions of air transport. Thus, uncommon types of *Salmonella* may occasionally introduced to other previously clean areas.

S. cerro was originally isolated by HORMAECHE et al. in the mesenteric lymph nodes of hog in Uruguay; since that time it has sometimes been isolated by several workers from turkeys, chicken, hogs, dogs and man. The present isolation from fox seems to be the first one.

The pathogenicity of Bethesda group of paracolon is not established yet. However, it should be noted that a cow was suffering from diarrhoea when Bethesda group organism was isolated from fecal sample. The fox also died from encephalitis-like disease but the etiological relationship of this organism is not clear because it was only isolated from liver sample in very small numbers.

SUMMARY

Some investigations on *Salmonella* distribution in several animal species in and around Sapporo have been performed. Data are summarized as follows:

1. Eleven Strains of *Salmonella* organism in total were isolated from 1,233 cases of animals mainly including cattle, hog, dog, fox etc.

2. *Salmonella* types found were *S. enteritidis* (5) from cattle, dogs, fox and cat, *S. typhimurium* (3) from dog, fox and rat, one each *S. thompson* from hog, *S. cerro* from fox and *S. amager* from mink.

3. The isoiation of *Salmonella cerro* and *Salmonella amager* were the first to be made in Japan.

4. Besides *Salmonella*, 4 Bethesda-paracolon group organisms were also isolated from cow, hogs and fox.

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