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## **SALMONELLA TYPES IN ANIMALS IN SAPPORO II**

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### INTRODUCTION

In the preceding paper,<sup>8)</sup> in the preparation of which the junior author participated, a report was made on the incidence of *Salmonella* in 11 animal species including cattle, hogs, dogs, foxes in and around Sapporo during the period from April, 1952 to March, 1954. In this study, only 3 *Salmonella* (2 *S. enteritidis*, 1 *S. typhi-murium*) from the total 76 dogs (68 from small animal clinics, 8 healthy) and also 3 *Salmonella* (*S. enteritidis*, *S. typhi-murium* and *S. cerro*) from the 97 fox organ materials were detected. From the above results, the incidence of *Salmonella* infections in animals in Sapporo seems to be low.

After the publication of this report, the bacteriological survey on *Salmonella* distribution in animals was continued by this laboratory chiefly concerning the fox and the dog. From the results of this later survey (from September, 1954 to December, 1955) pretty high incidence of *S. bareilly* among foxes was especially noteworthy. This organism is one of the usual etiologic agents of the death of newly-hatched chickens and chicks-in-shell in Sapporo<sup>7)</sup>. Pertaining to the recovery of this organism from fox, the present paper is the first report in Japan.

This paper especially deals with the incidence of *Salmonella* in apparently healthy animals in and around Sapporo.

### MATERIALS AND METHODS

For the purpose of this study, apparently healthy dogs, foxes and minks were used. These dogs were all captured stray dogs in Sapporo: they were killed after 3 days feeding in the detention house. The fox and mink which were killed for the purpose of the fur in and around Sapporo, were offered for this study within a short time after death.

Samples of the liver, bile, spleen, kidney, mesenteric lymph node and the caecum contents were taken from every slaughtered individual.

The technic for detection of *Salmonella* was briefly as follows: Except the caecum contents, about 1 or 2 g of the organ materials were scissored as small as possible and

inoculated into yeast-extract-broth. After 48 to 72 hours enrichment cultivation at 37°C, these were transplanted in McCONKEY and B. T. B.-lactose media. Bile was also treated in the same manner. S. S. media were chiefly employed for the caecum contents which were enriched in KAUFFMANN's tetrathionate broth. In addition to the above-mentioned methods, the direct cultivation of the specimens was also conducted. Representative nonlactose fermenting or *Salmonella*-like colonies were transferred to KLIGLER's iron media, S. I. M. semi-solid agar and SIMMONS' citrate agar. All urease negative cultures were then inoculated into further media for biochemical identification as recommended by the Central Laboratory of the Committee of the Animal Enterobacteriaceae in the National Institute of Animal Health, Tokyo. Cultures which showed biochemical reactions characteristic of the genus *Salmonella* were then examined for their antigenic components by O and H factor sera in routine work.

### RESULTS

Incidence of *Salmonella* in each animal species—Of apparently normal animals examined, *Salmonella* was detected in 11 cases out of 355 dogs (3.1%), in 19 cases out of 119 foxes (16.0%) and in none of the minks.

TABLE 1. Incidence of *Salmonella*

ANIMAL SPECIES	NUMBER EXAMINED	SALMONELLA DETECTED	
		No.	%
Dog	355	11	3.1
Fox	119	19	16.0
Mink	44	0	—

Type of *Salmonella* and these distributions in animal bodies are summarized in tables 2 and 3. Except *S. thompson* in 1 dog, *S. typhi-murium* and *enteritidis* were most commonly found in the mesenteric lymph node and in a few cases in the liver and spleen of dogs.

In foxes, it is especially noted that the chiefly encountered type was *S. bareilly*. The localization of the organisms in the fox body is also similar to that in the dog. It may possibly be said that *S. enteritidis* and *S. typhi-murium* were frequently localized in the liver and spleen, and that *S. bareilly* was likely to be localized in mesenteric lymph node or caecum contents. It is also noteworthy that no *Salmonella* was recovered from the bile samples.

In foxes, there were found 2 cases of double infections by different *Salmonella* types—in animal No. 4, *S. bareilly* from the mesenteric lymph node, spleen and caecum contents, *S. enteritidis* from the liver; in No. 466, *S. bareilly* from the mesenteric lymph node, *S. typhi-murium* from the liver.

The relationship between *Salmonella* types and their frequency in animal bodies examined was simplified in table 3.

TABLE 2. Types of Salmonella and Their Localization in Apparently Healthy Individuals

ANIMAL SPECIES	NO.	MESENTERIC LYMPH NODE	LIVER	SPLEEN	KIDNEY	CAECUM CONTENT	BILE	SALMONELLA TYPES DETECTED
Dog	13	+	+	+	-	-	-	<i>S. enteritidis</i>
	23	+	-	+	+	+	-	<i>S. typhi-murium</i>
	33	+	-	-	-	-	-	"
	39	-	-	-	-	+	-	"
	63	+	+	-	-	-	-	"
	99	+	-	-	-	-	-	<i>S. enteritidis</i>
	121	+	-	-	-	-	-	<i>S. typhi-murium</i>
	143	+	-	-	-	-	-	<i>S. enteritidis</i>
	165	+	-	-	-	-	-	<i>S. thompson</i>
	170	+	+	-	-	-	-	<i>S. typhi-murium</i>
	171	+	-	+	-	-	-	<i>S. enteritidis</i>
Fox	4	+*	+	+*	-	+*	-	{ <i>S. enteritidis</i> and <i>S. bareilly</i> }
	7	+	-	-	-	-	-	<i>S. bareilly</i>
	8	-	-	-	-	+	-	"
	12	+	-	-	-	-	-	"
	13	+	-	-	-	-	-	"
	14	-	-	-	-	+	-	"
	16	+	-	-	-	-	-	"
	17	+	-	-	-	-	-	"
	31	-	+	-	-	-	-	<i>S. enteritidis</i>
	40	-	+	-	-	-	-	<i>S. typhi-murium</i>
	41	-	+	-	-	-	-	"
	47	-	+	+	-	-	-	"
	57	+	-	-	-	+	-	<i>S. bareilly</i>
	407	+	-	-	-	-	-	"
	417	-	-	+	-	-	-	<i>S. enteritidis</i>
	445	+	-	-	-	-	-	<i>S. bareilly</i>
	446	+	-	-	-	-	-	"
466	+*	+	-	-	-	-	{ <i>S. bareilly</i> and <i>S. typhi-murium</i> }	
470	-	+	-	-	-	-	<i>S. typhi-murium</i>	

\* indicates *S. bareilly*

TABLE 3. *Salmonella* Types and the Frequency of Their Discovery in Organ Materials

ANIMAL SPECIES	SALMONELLA TYPE	MESENTERIC LYMPH NODE	LIVER	SPLEEN	KIDNEY	CAECUM CONTENT	BILE	TOTAL
Dog	<i>S. typhi-murium</i>	5	2	1	1	2	0	11
	<i>S. enteritidis</i>	4	1	2	0	0	0	7
	<i>S. thompson</i>	1	0	0	0	0	0	1
Fox	<i>S. bareilly</i>	11	0	1	0	4	0	16
	<i>S. typhi-murium</i>	0	4	2	0	0	0	6
	<i>S. enteritidis</i>	0	2	1	0	0	0	3

All *Salmonella* types recovered had the same biochemical reactions characteristic of the genus *Salmonella*, except that only 1 strain of *S. typhi-murium* which was recovered from the liver of dog No. 63 showed positive fermentation of salicin and sucrose in 5 days. However it had the antigenic components characteristic to *S. typhi-murium*.

The ratio of detection and type distribution of *Salmonella* in different fox feeders seem to be somewhat interesting. These are indicated in table 4.

TABLE 4. *The Ratio of Detection and Type Distribution of Salmonella in Different Fox Feeders*

FEEDER	NO. TESTED	DETECTED NO. (%)	TYPE	CULTURE
A	54	13 (24.1)	{ <i>S. bareilly</i> <i>S. enteritidis</i>	15 2
B	39	3 (7.7)	{ <i>S. typhi-murium</i> <i>S. enteritidis</i> <i>S. bareilly</i>	2 1 1
C	19	3 (15.8)	<i>S. typhi-murium</i>	4
D	7	0		

In feeder A, the ratio of detection of *Salmonella* was as high as 24.1%. The contamination of the herd was almost entirely with *S. bareilly*. On the contrary only *S. typhi-murium* was detected in feeder C.

#### CONSIDERATION

*Salmonella* distribution in animals has been studied by many workers from the viewpoint of its public health significance, especially in dogs and other domestic animals which have intimate relationship with human beings.<sup>12-14)</sup> It is known that *Salmonella* types and the proportion of positives in different groups or

communities ranged from very low to very high.<sup>3-5)</sup> The present datum (3.1%) on *Salmonella* reservoirs among apparently healthy dogs is almost the same as the previously reported ratio (3.9%)<sup>8)</sup> in diseased dogs in several small animal clinics. No differences in detected ratios of incidence between healthy and diseased dogs was observed in Sapporo. These ratios are pretty low, compared with 11.7% of MURASE et al., 25% of SAKAZAKI et al. in Honshu, 27.6% of GALTON et al. in Florida, so it will be accepted that Sapporo area is comparatively free as far as canine salmonellosis is concerned.

The dogs examined were all apparently healthy and *Salmonella* were in almost all cases recovered only by enrichment method. The question may remain whether these dogs were merely transient carriers. The dog is said to be a potential source of *Salmonella* infection for the human beings. From their survey in private kennels in Michigan, WOLF et al. suggest the possibility that the dogs are merely transient carrier of *Salmonella*. STEELE and other workers<sup>3,4)</sup> are of the opinion that *Salmonella* infection of dogs is of relatively short duration and that they must be exposed frequently.

Pathological and serological investigations were not conducted on the present organ materials, so it is uncertain whether the dogs were actively infected by these *Salmonella* or not.

Also in foxes, the above-stated facts may hold true. *Salmonella* colonies detected were very poor in number and almost all were isolated by enrichment method. A serological survey was carried on the remaining 24 foxes from A feeder which was mostly infested by *Salmonella*. None of the twenty-four indicated positive reactions not only to *S. bareilly*, *S. enteritidis* and *S. typhi-murium* but also to the other groups of *Salmonella*.

There are many reports on the outbreak of salmonellosis in the fox or dog,<sup>1,6,11)</sup> however the present findings will suggest the existence of many cases of healthy and transient state.

In the present report, the authors have insufficient data on type distribution for an exhaustive discussion because the types recovered are poor in variety. However, the detection of *S. bareilly* from fox in such high percentage is noteworthy. It is the first report in Japan, following the report by EDWARDS et al. on 2 outbreaks in the U. S. A. *S. bareilly* is the commonly recognizable organism responsible for the death of newly-hatched chickens and chicks-in-shell in Sapporo, however, the epidemiological significance remains in question.

#### SUMMARY

A survey on *Salmonella* distribution was conducted in apparently healthy animals in and around Sapporo. The data are summarized as follows:

1. *Salmonella* was detected in 11 cases out of 355 (3.1%) dogs in Sapporo. Specimens were serologically typed into 4 *S. enteritidis*, 6 *S. typhi-murium* and 1 *S. thompson*. *S. thompson* from dog is the first finding in Hokkaido.

2. From foxes, total 25 cultures of *Salmonella* were recovered in 19 cases out of 119 (16.0%). Sixteen of them were *S. bareilly*. Furthermore this is the first isolation from fox in Japan. The others are 6 *S. typhi-murium* and 3 *S. enteritidis*. Some cases showed incidence of 2 types.

3. No *Salmonella* was detected from 44 minks.

4. These *Salmonella* from dog and fox were detected from the mesenteric lymph node (70%), liver (30%), caecum contents (30%), spleen (20%) and kidney (3.3%). It is noticeable that no *Salmonella* was detected from the bile samples.

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