

ブタのサルモネラ保菌について

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SALMONELLA CARRIERS IN SWINE

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The importance of swine as one of major salmonella carrier-animals has been emphasized by many reports. However, their role in the epidemiology for salmonellosis, especially whether the carrier-animals serve as an amplifier for the organisms, or not, still remains obscure, since the emphasis was based simply upon high isolation rate of salmonellae from limited kind of specimens of swine, such as the mesenteric lymph node and/or feces. To clarify the actual role of the swine, it is required to study that how salmonellae are distributed in the digestive organs and affiliated lymph nodes, and whether the organisms do propagation in certain organs during carrier state.

In this experiment, the digestive tracts and their lymph nodes were examined for distribution of salmonellae with special reference to number of organisms present.

MATERIALS AND METHODS

The gastric, duodenal, jejunal, ileal, caecal, colic, and rectal contents or lymph nodes were obtained from a total of 40 apparently healthy swine slaughtered at Yokohama Slaughter House, between May, 1969 and March, 1970. Each animal examined was slaughtered on the different date. As for the jejunum, ileum, caecum, and colon, middle part of each tract and the lymph node located closely to the each part were submitted to this study.

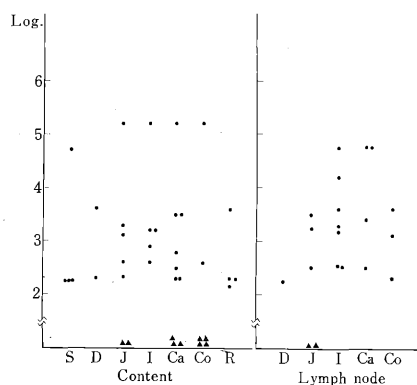
Viable count: Three grams of the gastric, jejunal, caecal, and colic contents, or the jejunal lymph node, and 1 g of the other contents or lymph nodes were homogenized with 27 or 9 ml of PBS to obtain a 1:10 dilution. These homogenates were diluted with PBS up to a 1:1,000. Thus, 3 dilutions containing 0.1, 0.01, or 0.001 g of each specimen per ml were prepared. One ml of each dilution was transferred to a test tube containing 9 ml of Hajna's tetrathionate broth (Eiken), and 5 tubes were prepared for each dilution. The tubes were incubated at 37°C for 24 hrs. After the enrichment, one loopful of the broth in each tube was streaked on desoxycholate-hydrogen sulfide-lactose (DHL) agar plate (Eiken) and incubated at 37°C for 24 hrs. The suspicious colonies were picked up and were identified by physiological, biochemical, and serological properties. The number of salmonellae in the contents or lymph nodes was calculated by means of most probable number (MPN).

Qualitative culture: The culture was done only for the gastric, jejunal, caecal and colic contents or the jejunal lymph node. After taking 8 ml of a 1:10 dilution of each specimen for the quantitative culture (viable count), the rest 22 ml of homogenate containing 2.2 g of the content or lymph node was enriched in 200 ml of Hajna's tetrathionate broth at 37°C for 24 hrs. A loopful of enrichment broth culture was transferred

Table 1. Isolation of salmonellae from the gastro-intestinal tracts and affiliated lymph nodes

Site	Per cent isolated		
	Content		Lymph node
Stomach	4	10%	0
Duodenum	2	5%	1 2%
Jejunum	7	17%	5 12%
Ileum	5	12%	7 17%
Caecum	10	25%	4 10%
Colon	6	15%	3 7%
Rectum	4	10%	0

Fig. 1. Number of organisms isolated. (/100 g)



S: Stomach, D: Duodenum, J: Jejunum, I: Ileum, Ca: Caecum, Co: Colon, R: Rectum,
 ▲: Salmonellae were isolated by qualitative culture only.

on DHL agar and brilliant green agar (Eiken) plates, respectively, and incubated at 37°C for 24 hrs. The suspicious colonies appeared on the agar plates were identified by the methods mentioned above.

RESULTS

Of 40 swine examined, 18 (45%) were found to be salmonella carriers. The organisms were isolated more or less from the contents or lymph nodes listed in Table 1. Among the contents, the caecal content showed the highest positivity followed by the jejunal, colic, ileal, rectal, gastric contents. In the lymph nodes, the organisms were isolated most frequently from the ileal lymph node, and less frequently from the jejunal, caecal, and colic lymph nodes. The recovery rate of the organisms from the duodenal content or lymph node was rather low, and no organisms were isolated from the gastric and rectal lymph nodes.

The number of the organisms found in each specimen calculated by means of MPN is shown in Fig. 1. Although most of the specimens showed the numbers within a range

Table 2. Salmonella serotypes isolated

Serotype	Number of cases*
<i>S. typhimurium</i>	9
<i>S. derby</i>	5
<i>S. stanley</i>	4
<i>S. irumu</i>	1
<i>S. anatum</i>	1

* Of 18 cases from which salmonellae isolated, one had 3 serotypes; *S. typhimurium*, *S. anatum* and *S. stanley*.

Table 3. Distribution of salmonellae in the gastro-intestinal tracts and affiliated lymph nodes

Swine No.	Content							Lymph node					Serotypes
	S	D	J	I	Ca	Co	R	D	J	I	Ca	Co	
1	4	3	5	5	5	5	3	—	3	3	3	—	<i>S. stanley</i>
2	—	—	+	2	3	2	—	2	2	3	2	3	<i>S. derby</i>
3	—	—	+ ^b	—	2 ^b	—	2 ^c	—	+	4 ^{ab}	—	—	a: <i>S. typhimurium</i> b: <i>S. anatum</i> c: <i>S. stanley</i>
4	—	—	—	—	+	—	—	—	3	3	4	3	<i>S. derby</i>
5	—	—	—	—	2	—	—	—	—	4	4	—	<i>S. typhimurium</i>
6	2	2	3	3	+	+	—	—	—	—	—	—	<i>S. typhimurium</i>
7	—	—	2	2	2	—	—	—	—	—	—	—	<i>S. stanley</i>
8	—	—	3	3	3	—	—	—	—	—	—	—	<i>S. typhimurium</i>
9	—	—	—	—	+	+	—	—	—	—	—	—	<i>S. derby</i>
10	—	—	—	—	2	+	—	—	—	—	—	—	<i>S. derby</i>
11	—	—	—	—	—	+	2	—	—	—	—	—	<i>S. typhimurium</i>
12	2	—	—	—	—	—	—	—	—	—	—	—	<i>S. derby</i>
13	2	—	—	—	—	—	—	—	—	—	—	—	<i>S. stanley</i>
14	—	—	—	—	—	—	2	—	—	—	—	—	<i>S. typhimurium</i>
15	—	—	2	—	—	—	—	—	—	—	—	—	<i>S. typhimurium</i>
16	—	—	—	—	—	—	—	—	+	—	—	2	<i>S. typhimurium</i>
17	—	—	—	—	—	—	—	—	—	2	—	—	<i>S. irumu</i>
18	—	—	—	—	—	—	—	—	—	2	—	—	<i>S. typhimurium</i>

Figures in the table indicate the exponent of number of salmonellae/100g transformed into logarithm.

+: Salmonellae were detected by qualitative culture only.

S: Stomach, D: Duodenum, J: Jejunum, I: Ileum, Ca: Caecum, Co: Colon, and R: Rectum.

from 10^2 to $10^4/100$ g, the organisms were recovered by the qualitative culture (less than $10^2/100$ g) only from 2 of 7 cases positive for the jejunal content, 3 of 10 cases positive for the caecal content, 4 of 6 cases positive for the colic content, and 2 of 5 cases positive for the jejunal lymph node. None of cases of which showed positive results by the quantitative cultures were found to be negative by the qualitative cultures.

Five serotypes were isolated (Table 2). *S. typhimurium* was found to be the most dominant serotype followed by *S. derby* and *S. stanley*. Distribution of salmonellae in the gastro-intestinal tracts and affiliated lymph nodes with reference to serotypes and the number of organisms per 100 g is shown in Table 3. Of 18 carriers, 5 cases (27%) harboured the organisms in both the gastro-intestinal contents and lymph nodes, 10 (55%) in the content only, and 3 (16%) in the lymph nodes alone. In 2 cases (swine

Nos. 1 and 2) with *S. stanley* or *S. derby*, the organisms were distributed rather widely in the contents and lymph nodes, and swine No. 1 had relatively high number of the organisms. In case of swine No. 4, *S. derby* was distributed in 4 lymph nodes, while the caecum was only one positive site among the tracts examined. On the contrary to the finding, *S. typhimurium* was detected in almost all of the gastro-intestinal tracts of swine No. 6, but not from the lymph nodes. Three serotypes were isolated from swine No. 3. The jejunal and caecal contents had *S. anatum* and the rectal content showed *S. stanley*. On the other hand, the jejunal lymph node harboured *S. typhimurium* and the ileal lymph node had *S. typhimurium* and *S. anatum*. No particular relationship was observed between the serotypes isolated and the range of distribution with number of the organisms present.

The results of the caecal content which showed the highest positivity was compared with those of the other sites. All the carriers found to be positive in both the contents and lymph nodes, and 5 of 10 animals positive in the contents only, harboured the organisms in the caecal content. The distribution of salmonellae in the caecal content-positive cases was found to be somewhat wider than that in the caecal content-negative cases. When the results of the caecal content was studied with those of the jejunal and ileal lymph nodes, and rectal content, more than 80% of salmonella carriers could be detected.

DISCUSSION

Forty-five per cents of swine examined in this experiment were proved to be salmonella carriers. The incidence is quite high in comparing with that in the past. In between 1952 and 1960, slaughtered swine were almost always free from salmonella and around 1% or less than that of them were only found to be salmonella carriers by the examination on the mesenteric lymph node and/or feces^{1,7,14,16,20}), in this country. In a following period of 1966~1968, the incidence became high and 15 to 20% of slaughtered swine were found as the carriers by the examination on the same specimens^{3,10,11}). Although the rate is lower than that in the present paper, if the various parts of the digestive tracts and affiliated lymph nodes were examined extensively in these surveys just as same as those in the present investigation, the prevalence should be much more high, since the recovery rate of the organisms from the mesenteric lymph node and feces found in this study were almost the same with that in the period of 1966~1968.

The organisms were isolated most frequently from the caecal content followed by the ileal and jejunal contents or lymph nodes, and colic content. Approximately 60% of the carriers harboured the organisms in both the caecal content and some other sites. There was a tendency that distribution of salmonellae in the caecal content-positive cases is rather wide than that negative cases. In the colon, the organisms were isolated from the content rather frequently with low number of the organisms, but less frequently from the colic lymph node. Most of the tracts other than the colon, or lymph nodes harboured 10^2 to 10^4 organisms per 100 g. The amount of salmonellae found in the tracts indicate neither invaded organisms by any means propagate actively in the tracts just resident intestinal flora does nor pass through the tracts simply without propagation. If the amount of salmonellae detected in the contents reflect simply that in the contaminated diet or others, the organisms should distribute more uniformly from the stomach to the rectum without such result as that obtained in the caecal content. In experimental salmonellosis in swine¹⁷), chimpanzees⁴), monkeys^{8,18,19}), rats¹²), mice¹⁵) and guinea-pigs⁹), the *ostium ileo-caeco-colium* or sur-

rounding area of the part was found to be the most important site of localization because a close parallelism between the presence of the organisms and development of lesions in this site was observed by histo-pathological or bacteriological examinations. Therefore, it seemed that salmonellae localized between the middle part of small intestine and the upper part of large intestine, especially in the caecum or the areas close to the caecum, with slight multiplication under the control of host barrier even in the carrier animals. In the lymph nodes, salmonellae may also propagate as well as the lymph nodes act as a filter organ protecting further invasion of the organisms to inside. To clarify the role of the lymph node in case of carrier state, it is required to study the mechanism of invasion of the organisms to the organs; whether the organisms invade the lymph nodes after localization and multiplication in the tracts or whether they invade them directly.

It is generally regarded as that the stomach plays an important role as a barrier for oral invasion of enteropathogenic organisms because of its high acidity. In this experiment, however, salmonellae were isolated from the gastric content of 4 of 18 carriers with $10^2\sim 10^4$ organisms per 100 g. In the 4 swine, *S. typhimurium* or *S. stanley* was distributed widely in the tracts of 2 animals, while *S. stanley* or *S. derby* was found in the gastric content only in the rest animals. Although more extensive studies are required to demonstrate the role of the stomach in case of the carrier, these facts may suggest that the stomach also serve as one of the sites of localization for salmonellae in swine carriers.

In the routine survey for salmonella carriers among slaughtered swine, the mesenteric lymph node and/or feces are examined most commonly. Some others isolated the organisms frequently from the caecal content, gall bladder, or portal lymph node^{2,5,6,13}. On the basis of the results obtained in this experiment, however, the caecum was regarded as the most important site chosen for the survey, and the caecum with the ileal and jejunal lymph nodes, and the rectal content should be examined at least for the detection of the carriers as high proportion as possible.

SUMMARY

In an attempt to learn the role of swine carriers in the epidemiology of salmonellosis, the distribution of salmonellae in the digestive tracts and affiliated lymph nodes with special reference to number of the organisms present were studied. Of 40 apparently healthy slaughtered swine examined, 18 animals (45%) were found to be salmonella carriers. Five of them harboured the organisms in both the contents of the digestive tracts and affiliated lymph nodes, 10 in the contents only, and the rest in the lymph nodes alone. In the contents, salmonellae were isolated most frequently from the caecal content followed by the jejunal, colic, ileal, gastric, rectal, and duodenal contents in the order listed. As for the lymph nodes, the organisms were recovered most frequently from the ileal lymph nodes, but less frequently from the jejunal, caecal, colic, and duodenal lymph nodes. No organisms were isolated from the gastric and rectal lymph nodes. Most of the specimens, except those from the colic content, harboured $10^2\sim 10^4/100$ g organisms. In cases of the colic content, most of them had less than $10^2/100$ g salmonella organisms. There was a tendency that the swine carriers with positive results in the caecum showed somewhat wider range of distribution of the organisms than those with negative results in the caecum. Five serotypes were detected and *S. typhimurium* was isolated most frequently. No particular relationship was observed between the serotypes isolated and the range of the distribution with number of the organisms present.

On the basis of the results obtained, it is suggested that the organisms propagate slightly in between the middle part of the small intestine and the upper part of the large

intestine of the carrier-animals. The caecum was found to be the most important site for salmonellae in the swine carriers under this experimental condition and the tract was regarded as the essential site to be chosen for the survey of salmonella carriers among swine.

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ブタのサルモネラ保菌について

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保菌豚のサルモネラ症の疫学における役割を知るための基礎的研究として、保菌豚の消化管内容および附属リンパ節におけるサルモネラの分布を定量的に検討した。

消化管内容では、盲腸におけるサルモネラの検出率をもっとも高く、ついで空腸、結腸、回腸、胃、直腸、十二指腸の順であった。

リンパ節では、回腸からの本菌の検出率をもっとも高く、ついで空腸、盲腸、結腸、十二指腸の順であった。胃リンパ節および直腸リンパ節は陰性であった。

各部位におけるサルモネラの菌量は、ほとんどのが $10^2 \sim 10^4/100 \text{ g}$ の範囲にあった。但し、

結腸内容では、定性培養 ($10^2/100 \text{ g}$ 以下) のみで検出される場合が多かった。

盲腸内容陽性例における分布域は、陰性例におけるそれよりも広い傾向を示した。

5 菌型が検出され、*S. typhimurium* の検出率をもっとも高かった。検出された菌型と分布域、ならびに菌量の間には、特に関係は認められなかった。

以上の成績を総合して考えると、サルモネラは、ブタの小腸中部より大腸上部に至る間で、多少増殖しながら保菌されているものと推測された。また、盲腸は特に重要な保菌部位であり、ブタの保菌調査には不可欠の器官と認められた。