ISOLATION OF *SALMONELLA NEW BRUNSWICK* FROM CHICKENS

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INTRODUCTION

In the previous papers,[1,12] the writers have already reported the isolation of *Salmonella* organisms other than *Salmonella pullorum*, i.e., *Salmonella senftenberg*, *S. thompson* and *S. bareilly* from chickens in Japan for the first time. Successively, studies have been prosecuted on the manner by which these *Salmonella* organisms infect baby chicks and on the presence or disappearance of these organisms in chicks' bodies after infection. At the Symposium on Animal Enterobacteriaceae held by the 35th Meeting of the Japanese Society of Veterinary Science on April, 1953, a part of the results obtained was reported by the senior author.[5] In the course of the continuance of such research, early in June 1954 some other strains of *Salmonella* organism were isolated which had not yet been detected in fowls in Japan. For this reason, the present paper was prepared to furnish some information of these strains.

ISOLATION OF THE *SALMONELLA* ORGANISMS

It is sure that the infection by *Salmonella* organisms other than *S. pullorum*, especially by *S. senftenberg* in newly-hatched chicken, occurs in incubator almost without exception. However, all chicks infected in such a manner do not always die. If they are unfortunately brooded under improper management and sanitation, then some endemic such as bacillary diarrhea rages and causes many deaths among the chicks. On the contrary, if they are reared under good management and sanitation, losses caused by *S. senftenberg* infection are few. Therefore, a group of 60 baby chicks (male White Leghorns) remaining constantly healthy after hatching was selected and fed and managed all in the same way. With purpose of ascertaining how the *Salmonella* organisms, which have entered baby chick bodies in incubator, disappear according to the passage of time after hatching, at regular intervals, 3~5 chickens were picked

TABLE 1. *Distribution of Salmonella Organisms in Apparently Healthy Chicks' Bodies*

DAYS AFTER HATCHING	1 (29/V '54)					2					3					5 (2/VI '54)				
CHICK NO. TESTED	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
MATERIALS EXAMINED																				
Trachea	s	T/S	S	·	S	s	·	S	·	·	B/S	·	·	B	·	·	·	·	·	·
Lung	s	s	T	S	S	S	B	S	·	S	S	·	·	b	·	s	s	·	·	s
Heart Blood	·	·	·	·	S	·	·	·	·	·	B	·	·	B	·	·	·	·	·	·
Liver	·	·	s	·	·	·	s	·	·	·	·	·	·	·	·	b	·	·	·	·
Spleen	·	·	·	·	s	·	·	s	·	·	·	·	·	·	·	·	·	·	·	·
Kidney	·	·	·	·	·	·	·	·	·	·	S	·	·	·	·	·	·	·	·	·
Testicle	·	·	·	·	S	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Egg-yolk	·	·	·	·	·	·	·	·	·	·	·	b	·	·	·	·	·	·	·	·
Esophagus	·	s	S	S	S	T/s	S/b	S	·	S	B/S	B/s	B	B/s	B	·	B	B	·	·
Crop	s	s	S	S	S	s	S	S	S	S	B/S	B/s	s	B/s	B	·	S/b	·	·	·
Pro-ventricu.	·	s	·	·	s	·	·	·	·	·	B/s	·	B	·	·	·	·	·	·	·
Gizzard	·	·	·	·	S	s	·	·	·	·	B/s	·	·	B	·	·	·	·	·	·
Small Intestine	·	·	s	S	·	S	·	·	·	S	B	·	·	S	S	·	·	S	·	·
Caecum	s	S	s	S	S	S	S	S	·	S	B/S	·	B	S	S/b	·	S	·	·	·
Rectum	S	S	S	S	s	S	S	S	S	S	B/S	·	B/S	S/b	S	·	·	·	·	·

DAYS AFTER HATCHING	7				9					11					15					20					25			30			50		
CHICK NO. TESTED	21	24	25		26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	55	56	57
MATERIALS EXAMINED																																	
Trachea	·	·	·	·	s	s	N	·	·	·	S	·	·	s	·	·	·	·	·	s	·	·	·	s	·	s	b	·	·	s	·	·	·
Lung	S	·	·	s	S	·	·	n	s	s	·	·	S	S	s	·	S	t	·	s	·	·	·	s	·	·	·	S	s	·	·	·	·
Heart Blood	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	s	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Liver	·	·	·	·	·	·	·	·	·	·	s	·	·	·	·	·	·	·	b	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Spleen	·	·	·	·	S	·	·	·	·	·	s	·	·	·	·	·	·	·	·	·	·	·	·	·	n	·	·	·	·	·	·	·	·
Kidney	·	·	·	·	·	·	·	·	·	·	S	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Testicle	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Egg-yolk	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Esophagus	·	B	·	·	B	·	·	B	·	B	B	·	·	B	·	B	·	·	B	B	·	B	T	·	B	B	t	·	·	b	·	t	·
Crop	·	S/b	·	·	·	·	·	·	·	·	·	·	·	·	S	B	·	·	·	·	·	·	·	·	·	·	·	S	·	·	·	·	·
Pro-ventricu.	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Gizzard	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Small Intestine	S	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Caecum	·	S	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	B	·	·	·	·	·	N	·	n	·	·	·	·	·	·	·
Rectum	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	N	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

Notes: S···Direct culture, s···Enrichment culture of *Salmonella senftenberg*.
T··· " " , t··· " " " " *thompson*.
B··· " " , b··· " " " " *bareilly*.
N··· " " , n··· " " " " newly detected *Salmonella* organisms.
All cultures of chick Nos. 22 & 23 (7 day old), 52~54 (40 day old) and 58~60 (60 day old) are negative.

at random from the group and sacrificed to investigate the distribution of *Salmonella* organisms in their bodies.

For the detection of organisms of the *Salmonella* group, small parts from lung, spleen, liver, kidney, testicle, unabsorbed egg-yolk and heart blood were individually cultivated on ENDO agar for direct smears, likewise on plain broth followed by plating on to S. S. agar. Samples of the contents of trachea, esophagus, crop, proventriculus, gizzard, small intestine, caecum and rectum were cultivated directly on S. S. agar, likewise, at the same time on tetrathionate broth and then on smear plates of S. S. agar.

As indicated by the detailed results of cultivation as shown in table 1, in baby chicks less than 3 days old, although they were apparently healthy, *S. senftenberg* was always detected with considerable frequency, and also *S. bareilly* and *S. thompson* were often found. On the other hand, in bodies of chicks sacrificed more than 5 days after hatching, these *Salmonella* organisms were seldom detected. Such findings give support to the writers' previous report that, when large numbers of baby chicks are brooded by poultry raiser in field, the outbreak of a sickness resembled to pullorum disease caused by *S. senftenberg* results in very heavy losses in chicks below the age of 5 days after hatching.

Other findings not directly connected with the main object of the present experiments are also noteworthy. After the frequency of detection of such *Salmonella* organisms as *S. senftenberg* or *S. bareilly* had decreased in each chick body, in a 9 day old healthy baby chick, No. 28, certain *Salmonella* organisms which had never been seen previously, were isolated from the direct culture of trachea and from the enriching culture of lung. Later, the same *Salmonella* organisms as above-mentioned were isolated from the direct smears of rectum contents of a 15 day old chick, No. 40, from the direct culture of caecum contents and from the enriching culture of spleen of a 25 day old chick, No. 46, and also from the enriching culture of caecum contents of a 25 day old chick, No. 48.

In brief, 6 strains of a certain *Salmonella* organism which had never been seen in fowls in Japan were obtained from 4 apparently healthy baby chicks less than 25 days old.

IDENTIFICATION OF THE ISOLATED *SALMONELLA* ORGANISMS

As a result of examining the biochemical properties by routine methods, the above-mentioned 6 strains were first proved to be members of the *Salmonella* group. Accordingly, thereafter they were also examined serologically to determine their positions in the KAUFFMANN-WHITE classification using the typing sera. Table 2 gives a summary of results of the biochemical and antigenic examination of the 6 strains.

TABLE 2. *Results of Biochemical and Antigenic Examination of 6 Strains of Newly Isolated Salmonella Organisms*

NO. OF STRAINS		1	2	3	4	5	6
DATE OF ISOLATION		7/VI'54	9/VI'54	13/VI'54	23/VI'54	25/VI'54	25/VI'54
NO. OF CHICKS		28	28	40	46	46	48
ORGAN FROM WHICH ORGANISMS WERE ISOLATED		Trachea	Lung	Rectum	Caecum	Spleen	Caecum
METHOD OF CULTIVATION		Dir.	Enri.	Dir.	Dir.	Enri.	Enri.
KRUMWIEDE MEDIUM	Slant	−	−	−	−	−	−
	Stick	⊕*	⊕	⊕	⊕	⊕	⊕
S. I. M. MEDI.	H_2S, Motili.	+	+	+	+	+	+
	Indole	−	−	−	−	−	−
SIMMON'S CITRATE MEDI.		+	+	+	+	+	+
UREASE		−	−	−	−	−	−
GRAM-STAINING		−	−	−	−	−	−
LITMUS MILK		faint red→ blue	faint red→ blue	faint red→ blue	faint red→ blue	faint red→ blue	faint red→ blue
V. P.		−	−	−	−	−	−
M. R.		+	+	+	+	+	+
GLUCOSE		⊕*	⊕	⊕	⊕	⊕	⊕
LACTOSE, SUCROSE		−	−	−	−	−	−
SLIDE TEST H·Sera O·Sera	A,B,C,D-Gr.	−	−	−	−	−	−
	E-Gr.	++	++	++	++	++	++
	S. senftenberg	+	+	+	+	+	+
	g·s·t, y, k	−	−	−	−	−	−
	1·5	+	+	+	+	+	+
TUBE TEST O·Sera	*S. senftenberg*	1:600	1:600	1:600	1:1200±	1:600	1:1200±
	S. london	1:3200±	1:3200±	1:3200±	1:3200±	1:3200±	1:3200±
TUBE TEST H·Sera	g·s·t	1:50−	1:50−	1:50−	1:50−	1:50−	1:50−
	1·5	1:400±	1:400±	1:400±	1:200	1:200	1:400±

Notes: * +···Acid formation.
○···Gas "

In table 2, it can be seen that all of the 6 strains were typically agglutinated with diagnostic O immune serum of E group in slide test, and in tube test they showed the O-agglutination titre 1:600~1200± with the serum of *S. senftenberg* (somatic antigens 1·3·19) and O-titre 1:3200± with the serum of *S. london* (somatic antigens 3·10). On the other hand, in H-agglutination all strains showed negative results with specific H sera

of *S. senftenberg* (flagellar antigens g·s·t), *S. bareilly* (flagel. antigen y) and *S. thompson* (flagel. antigen k), but they were agglutinated with non-specific H serum of *S. thompson* (flagel. antigen 1. 5) both in slide and tube test.

From the results described above, it was presumed that the newly isolated *Salmonella* organisms belong to *Salmonella* E group and possess non-specific flagellar antigen 1 or 5. As this laboratory unfortunately possesses no sera of another type, samples of all these strains were forwarded to the Committee of Animal Enterobacteriaceae at the Government Experimental Station for Animal Hygiene for more exact identification. All 6 strains were identified as *S. new brunswick* (antigenic formula 3 · 15 : 1 · v : 1 · 7).

The authors wish to express their thanks to the Comittee of Animal Enterobacteriaceae for the type identification.

CONSIDERATIONS

S. new brunswick was isolated from a baby chick, the origin of which was unknown, by BEAUDETTE in 1936 for the first time in the world and was identified by EDWARDS as a new *Salmonella* type. As this organism was isolated at New Brunswick, N. J., U. S. A., it has been thereafter referred to as the new brunswick type. Afterwards, in the animal kingdom, this organism was isolated from the contents of intestine of a diseased chicken by MALIMAN et al. (1942), Michigan, from the mesenteric lymph glands of apparently normal hogs slaughtered for market by RUBIN et al. (1942), from fowls, hogs and a ruminant by EDWARDS and BRUNER (1943), Kentucky, from poults in California by HINSHAW et al. (1944), from the samples of spray-dried whole egg powder by SOLOWEY et al. (1947), Philadelphia, and from turkeys, hogs, chickens and sheep by EDWARDS et al. (1948). Taking into consideration these reports, it will be realized that, since *S. new brunswick* was first reported by EDWARDS in 1937, all strains of this organism isolated from the animal kingdom were detected in America only and many of them have reference to poultry.

On the other hand, there exist reports of isolation of *S. new brunswick* from human beings. The earliest one is that of KAUFFMANN in Denmark in 1938, which is the next year after EDWARDS had made the original report about this organism. He reported the incidence of this type from feces of a 51 year old woman suffering from acute gastro-enteritis for 1 week, and also from feces of a patient who had returned home from a tropical district and whose condition was diagnosed as a suspected case of malaria. Two reports by TOPLEY and WILSON (1946), and EDWARDS et al. (1948) followed. The former reported about food poisoning caused by *S. new brunswick* in England, and, the latter isolated this organism from the feces of each of 3 patients affected with enteric fever or gastro-enteritis and from the feces of an asymptomatic carrier in America. The published reports indicate strongly that *S. new brunswick* is capable of inciting gastro-enteritis or enteric fever in man.

These above-mentioned reports, so far as the writers can determine, reveal

conclusively that many strains of S. *new brunswick* originating from animals are confined largely to poultry and in America only, while strains of S. *new brunswick* originating from human beings have been isolated merely in 3 such countries as Denmark, England and America.

With respect to the reports of S. *new brunswick* in our country, this organism was first isolated in 1949 by NAKAYA et al. (1952) from the stools of 3 patients in the Yokohama National Hospital who were affected with food poisoning after eating raw tunny meat. Again by the same men, a strain of *Salmonella* group isolated from feces of a healthy food-handler in Niigata Prefecture in 1951 was identified as S. *new brunswick*. Next, this organism was detected from feces of a 73 year old healthy man by OHHASHI et al. (1953) in Tochigi Prefecture. As mentioned above, 3 reports of S. *new brunswick* in our country made in recent years are confined to human beings only.

At this time, in the course of the writers' studies on poultry salmonellosis, 6 strains of S. *new brunswick* were accidentally isolated from trachea and lung, or, spleen and contents of caeca, or contents of rectum of 4 apparently healthy young chickens.

This particular type of *Salmonella* organism originating from animals (poultry) was reported here for the first time in Japan, but as yet has not been incriminated in the causation of disease in animal, the original isolations having been made from normal animal carriers. However, it is highly probable that sooner or later this type will appear in animals here and there in Japan, an opinion based upon this report. Furthermore, it goes without saying that much work remains to be done in the investigation of natural reservoirs of infection for this group of organisms. Additionally this report, together with the writers' previous reports regarding the first isolation of S. *senftenberg*, S. *thompson* and S. *bareilly* from baby chicks in Japan, and the report by IWAMORI and SHIMAKURA of the first detection of S. *montevideo* from a 50 day old chick in Gifu Prefecture,— these reports explain clearly that not merely S. *pullorum* but some other types of *Salmonella* organism are or may be associated with an outbreak of chicken salmonellosis in Japan. It seems appropriate to point out that all of these species isolated from chickens have been found in human infections in our or in foreign countries and therefore these organisms are of interest both for an epizootiological and epidemiological standpoint.

SUMMARY

Salmonella new brunswick was isolated from 4 apparently healthy chicks less than 25 days old. This is considered to be the first instance in which *S. new brunswick* has been isolated from animals (avian species) other than human beings in Japan.

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