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HEMOLYTIC *ESCHERICHIA COLI* FROM EDEMA DISEASE OF SWINE NEW TO JAPAN

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INTRODUCTION

In Japan, the first outbreak of edema disease of swine was recognized in a district of northern Hokkaido in 1957^{10,18,22}. Since that time, sporadic or enzootic outbreaks of the disease in other parts of the island^{7,23} and northern Honshu (main island)^{13,17} have been reported by several investigators.

It is well known that β -hemolytic *Escherichia coli* has been isolated frequently from pigs suffering from edema disease, though its etiological significance is not yet certified.

Previously, in papers^{10,11} in the writing of which certain of the present authors have participated the isolation has been reported of hemolytic *E. coli* of 2 different O groups (O139 and O2) from the diseased pigs. Thereafter, some investigations on the disease have been carried out.

This paper deals with hemolytic *E. coli* of some O groups isolated from diseased pigs during 4 years from 1957 to 1960, their occurrences in normal animals, epizootiological aspects of the disease in Hokkaido, and some characteristics of the *E. coli*.

MATERIALS AND METHODS

Diseased pigs: A total of 30 pigs with edema disease which had been diagnosed clinically and histo-pathologically were examined bacteriologically, as indicated in table 1. Out

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of them, 17 were autopsied by the present authors in the laboratory or in the field. Specimens of visceral organs and intestinal contents of the remaining 13 cases were submitted from the veterinarians.

Normal pigs and slaughtered animals: Fecal samples were obtained from normal pigs in the edema disease districts and intestinal contents and other specimens from slaughtered pigs, cattle, horses and sheep in Sapporo abattoir.

Isolation of hemolytic *E. coli*: Specimens were cultivated onto 5% sheep blood agar and MacConkey agar. Of each plate, at least 2 cultures of hemolytic and non-hemolytic colonies were subjected to bacteriological and serological screening tests. Then biochemical and serological characteristics of the representative strains were investigated in detail.

Foreign strains of hemolytic and non-hemolytic *E. coli*: A total of 20 strains isolated from edema disease were supplied by Drs. W. H. EWING (U. S. A.), W. J. SOJKA (Great Britain), M. ZELLER (Germany) and Y. RYU (Repub. China).

Biochemical characteristics: Routine methods were employed and the carbohydrate fermentation test was observed for 3 weeks.

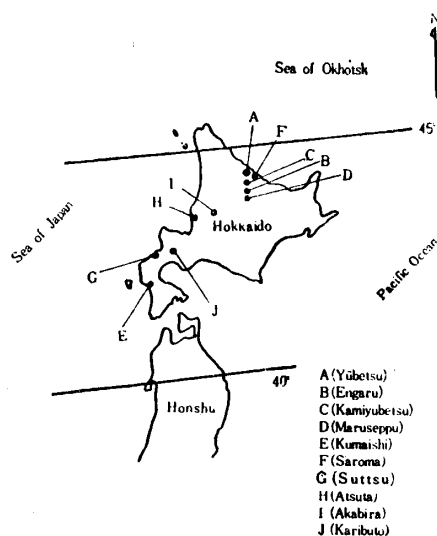
Serological method: O sera were prepared from the following types; O138 and O139 (from Dr. EWING), O6 (from Dr. RYU), O2 and O115. The last 2 serotypes were isolated by the present authors in 1957 and 1959 respectively. In order to screen the isolates one tube method was used with serum dilution of 1:1,000 and the final typing was carried out by SAKAZAKI, one of the junior authors.

RESULTS

1. Outbreak of Edema Disease of Swine in Hokkaido

As indicated in table 1, a total of 296 cases of edema disease were recognized from 1957 to 1960 in Hokkaido. In July of 1957, the outbreak was found in a district of northern Hokkaido and spread enzootically over the district and neighboring regions up to September of the year. It is noticeable that the disease occurred annually in the same district thereafter. The enzootic outbreaks seem to be found generally in coastal districts, as seen in table 1 and figure 1.

FIGURE 1. Localities in which Edema Disease occurred.



As indicated in table 1, out of 30 cases including 3 fecal samples 29 (97%) gave hemolytic *E. coli* cultures. Among these 29 cultures 23 were serotype O139:K82:H1, four O2:Kx1:H1 and two O115:Kx2:-(non-motile) as shown in table 2.

2. Isolation of Hemolytic *E. coli* from the Affected Pigs

As indicated in table 1, out of 30 cases including 3 fecal samples 29 (97%) gave hemolytic *E. coli* cultures. Among these 29 cultures 23 were serotype O139:K82:H1, four O2:Kx1:H1 and two O115:Kx2:-(non-motile) as shown in table 2.

Serotype O139:K82:H1 was isolated from most localities every year. From 3 localities of

TABLE 1. *Outbreaks of Edema Disease of Swine in Hokkaido from 1957 to 1960 and the Numbers and Origins of Diseased Pigs Employed for Bacteriological Examinations*

YEAR	OUTBREAKS IN HOKKAIDO			NAME OF LOCALITY EXAMINED	REGION	FORM OF OUTBREAK	NO. OF CASES EXAMINED			NO. OF DISEASED PIGS
	No. of Deaths	Total Cases of Edema Disease	No. of Localities				Dead	Killed	Total	
1957	40	48	5	A	North Hokkaido	Enzootic	7	0	7	33
				B	"	"	1	0	1	7
				C	"	"	0	1	1	5
1958	78	85	5	A	North Hokkaido	Enzootic	1	0	1	14
				B	"	Sporadic	1	0	1	?
				D	"	Enzootic	2	0	2	?
				E	South Hokkaido	"	5	0	5	68
1959	67	96	6	F	North Hokkaido	Enzootic	2	0	2	25
				E	South Hokkaido	"	0	3	3	47
				G	"	"	1	0	1	10
				H	Centr. Hokkaido	Sporadic	1	1	2	2
1960	53	67	8	I	Centr. Hokkaido	Sporadic	2	1	3	7
				J	South Hokkaido	"	1	0	1	2
Total	238	296					24	6	30	220+

Hemolytic *Escherichia coli* from Edema Disease of Swine

TABLE 2. *Hemolytic Escherichia coli* from Pigs Affected with Edema Disease

YEAR	LOCALITY	NO. OF PIGS EXAMINED	TERMINATION OF THE PIGS	NO. OF PIGS GIVING HEMOLYTIC <i>E. COLI</i>	SEROTYPE OF THE HEMOLYTIC <i>E. COLI</i>
1957	A	7	died	7	O 139: K 82: H 1...6 strains O 2: Kx 1: H 1...1 strain
	B	1	"	1	O 139: K 82: H 1...1 "
	C	1	killed	0	.
1958	A	1	died	1	O 139: K 82: H 1...1 "
	B	1	"	1	O 2: Kx 1: H 1...1 "
	D	2	"	2	"2 strains
	E	{ 4 1	" killed	4 1	O 139: K 82: H 1...4 " "1 strain (mixed with non-hemolytic serotype O 2: Kx 1: H 1)
1959	F	2	died	2	O 139: K 82: H 1...2 strains
	E	3*	killed	3	{ "1 strain O 115: Kx 2: — ...2 strains
	G	1	died	1	O 139: K 82: H 1...1 strain
	H	{ 1 1	" killed	1 1	"1 " (mixed with non-hemolytic serotype O 2: Kx 1: H 1) O 139: K 82: H 1...1 strain
1960	I	{ 2 1	died killed	2 1	"2 strains "1 strain
	J	1	died	1	"1 "
	Total	30		29 (97%)	O 139: K 82: H 1...23 (79%) O 2: Kx 1: H 1...4 (14%) O 115: Kx 2: — 2 (7%)

* Fecal sample only

northern Hokkaido in 1957 and 1958 O 2: Kx 1: H 1 was obtained. However, strains of O 115: Kx 2: — occurred in only one of southern localities in 1959. From the above-described data, it will be seen that the most popular serotype of hemolytic *E. coli* associated with edema disease of swine is O 139 in Hokkaido. Hemolytic *E. coli* organisms from each pig belong usually to the same serotype, although non-hemolytic strains of O 2: Kx 1: H 1 occurred together with O 139 in 2 cases.

Distribution of the hemolytic *E. coli* in 17 pigs, 13 died and 4 killed, which were autopsied in the laboratory of the authors is shown in table 3.

The observations justify the conclusion that intestinal contents frequently gave growths of hemolytic *E. coli* whilst the organisms were isolated seldom from the visceral organs. In one case which died of the disease, the organisms were found only in duodenum. This fact

TABLE 3. *Distribution of Hemolytic E. coli in the Diseased Pigs*

TERMINATION OF PIGS	HEART BLOOD	LUNG	SPLEEN	LIVER	KIDNEY	MESENTERIC LYMPH NODE	STOMACH	DUODENUM	JEJUNUM	ILEUM	CECUM	COLON	RECTUM
Killed	0/3	0/4	0/4	0/4	0/4	0/4	1/4	2/4	1/3	2/3	1/3	2/4	2/2
Died	1/9	2/12	2/12	2/12	1/12	6/12	8/12	11/13	8/9	6/9	6/8	9/13	3/6
Total	1/12	2/16	2/16	2/16	1/16	6/16	9/16	13/17	9/12	8/12	7/11	11/17	5/8

Numerator: No. of positive cultures

Denominator: No. of specimens of pigs examined

seems to indicate that there is no relationship between the termination of the animal and the distribution of hemolytic coli bacilli. The mesenteric lymph nodes of half of the dead cases yielded hemolytic *E. coli*. However, 4 killed cases did not give growths of the bacteria. This may suggest that the organisms invade into the mesenteric lymph nodes after the death of the pigs. On the other hand, it should be emphasized that there was one case which showed clinically and pathologically typical findings of the disease but did not yield hemolytic *E. coli*.

3. Hemolytic *E. coli* in Fecal Samples from Pigs Which Recovered from Edema Disease

In discussing the etiological significance of specific serotypes of hemolytic *E. coli* in edema disease, the occurrence of the organisms in normal pigs should be important. From this viewpoint, the authors employed for cultivation fecal samples of 15 pigs which had recovered from the disease; one strain of O139:K82:H1 was isolated. Unfortunately, the days elapsed before taking of sample since the onset and recovering of the symptoms in this case remain unknown.

4. Hemolytic *E. coli* from Fecal Samples of Normal Pigs in Contact with Animals Suffering from Edema Disease

During 3 years, 1957, 1959 and 1960, 44 fecal samples of normal pigs at 16 farms were examined. Among them 10 (23%) yielded hemolytic *E. coli* and 5 of the 10 were identified as O139:K82:H1. The remaining 5 were of an unknown type. It is noteworthy that 2 strains of the above-noted O139:K82:H1 originated from 2 adult pigs in pens other than those of diseased pigs.

5. Hemolytic *E. coli* from Normal Pigs in the Disease-free Farms of "Edema Disease Districts"

A total of 62 samples from 13 farms free from the disease located in "edema disease districts" were examined. Fifty-one young and 11 adult pigs offered the samples and 18 yielded hemolytic *E. coli* cultures. Among these cultures, 6 belonged to serotype O139:K82:H1, 2 to that of O115:Kx2:—, 2 to O2:K?:H?, and 8 to unknown types. The serotype O115

occurred only in E locality in 1959 where the serotype had been detected from the diseased pigs.

6. "Edema Disease Serotypes" (O groups 2,115,138 and 139) of Hemolytic *E. coli* in Slaughtered Animals

The authors obtained 41 cultures of hemolytic *E. coli* chiefly from the intestinal contents of 15 pigs, 22 cattle, 3 horses and one sheep. However, they could not find the above-noted serotypes among those cultures.

7. Serotypes of Foreign Strains of *E. coli* Isolated from Edema Disease

Apart from 2 standard strains of O138 and O139 supplied by Dr. EWING, five British strains were divided into one O138, one O139 and 3 untypable. Among 8 German strains, 7 hemolytic *E. coli* were typed as O139 whilst one non-hemolytic remained untypable. It was noticeable that 2 hemolytic strains out of 5 Formosan cultures belonged to O6 group, 2 non-hemolytic to O7, whilst the remaining non-hemolytic was untypable.

8. Biochemical Characteristics of "Edema Disease Serotypes" of Hemolytic *E. coli*

As previously stated, O139:K82:H1 is the most common "edema disease serotype" while O2:Kx1:H1 and O115Kx2:— are rarely isolated from the diseased pigs in Hokkaido. Almost all strains of these types are β -hemolytic on 5% sheep blood agar. A non-hemolytic strain of O139 was found in one case in 1957. Moreover, non-hemolytic O2:Kx1:H1 occurred on 2 affected pigs together with O139 hemolytic and was also detected in a fecal sample of a normal pig in contact with a diseased animal.

Biochemical characteristics of sixty-nine strains of hemolytic *E. coli* (O139, O2, O115, and other types) and 22 of non-hemolytic were investigated. Serotype of the majority of the latter was not clearly ascertained. From this investigation, it was found that all of 43 hemolytic strains of O139 and O2 isolated both in 1957 and 1958 showed stronger activity against sucrose compared with the non-hemolytic strains from normal pigs, while 3 of 14 hemolytic strains of O139 and 4 of 5 strains of O115 which were isolated in 1959 did not attack this carbohydrate. These facts suggest that there may be some year-by-year variations of ability to ferment sucrose in strains of the specific serotypes. Excepting this point, no distinct difference of characteristics was found among serotypes of the isolates or between the hemolytic and the non-hemolytic strains. Marked biochemical difference between the present isolates and those 20 foreign strains was not found. Moreover, none of the strains examined showed production of urease which finding differs from the statement of QUINCHON et al.

9. Pathogenicity of the Isolated Strains

Necrotizing power: Necrotizing power was tested according to the method of NAMIOKA et al. A dose of 0.1 ml of each broth culture of 6 hemolytic strains including 5 of O139 and one of O2 serotype was injected intradermally into depilated rabbit back. As the control, a non-hemolytic strain from normal pig was employed. On the next day, all indicated positive reaction, though the controlled strain showed weak reaction.

Mouse virulence: One dose each of 0.5 ml of overnight broth culture of 10 hemolytic strains (serotypes of O2 and O139) and 4 non-hemolytic (O2, O139 and 2 of undetermined serotype) was injected intraperitoneally into each of three to five mice. In similar manner, 2 hemolytic and 41 non-hemolytic strains from slaughtered pigs were applied.

Hemolytic strains from edema diseased or normal pigs killed mice generally within a few hours. On the contrary, in cases of the non-hemolytic strains, deaths occurred after overnight or a part of animals survived. The doses of 0.05 ml of broth culture of hemolytic strains yielded generally similar results. Subcutaneous inoculations of hemolytic strains did not result in rapid death of the mice and sterile filtrate of the broth culture or killed culture had no virulence for the mice. Therefore, the rapid death of the mice seems to be due to living cells of hemolytic coli regardless of O antigen or of source of the strains.

Rabbit pathogenicity test: According to the procedure of DE et al. and NAMIOKA et al. one ml each of overnight peptone water culture of 4 hemolytic (one strain of O2 and 3 of O139) and one non-hemolytic strain (O2) of *E. coli* from edema disease was inoculated into the separated small intestine of rabbit. In addition, pathogenicity of 2 strains (hemolytic and non-hemolytic) from normal pigs were tested. On the next day the intestine into which the culture was inoculated was subjected to pathological examination.

Among the strains tested, 5 strains from edema disease and one hemolytic strain of unknown type from normal pig showed marked macroscopical changes. On the contrary, non-hemolytic strain of unknown type from a normal animal indicated only weak changes.

From the above-described tests, strains of "edema disease serotypes" appear pathogenic. However, it is obvious that the pathogenicities are not specific to the strains of "edema disease serotypes".

10. Some Epizootiological Considerations on the Outbreaks of Edema Disease in Hokkaido

Epizootiological observations on the first outbreak of edema disease in northern Hokkaido (outbreaks in A, B and C localities, in 1957) were reported by MIURA, one of the present authors, and SONODA et al. MIURA, in his report, pointed out that the disease spread probably along the traffic network and he concluded that the above-described outbreaks revealed an enzootic form such as observed in several contagious disease. It should be noted that his conclusion was obtained from the outbreaks of edema disease under distinct circumstances of the Japanese farm areas where pig growing is generally in a small scale. In fact, 48 pigs belonging to 34 growers suffered from edema disease within two months (July 15 to September 17) in northern Hokkaido in 1957. That is, more than 50% of the diseased pigs were kept in farms which grew only one to three pigs.

From table 1 and figure 1, it will be seen that during the period of 3 years from 1957 to 1959, enzootic outbreaks of edema disease were recognized generally in coastal or semi-coastal districts such as A, B, C, E, F, and G. Regarding this point, MIURA stated that there were some predisposing factors such as rapid increase in number of growing piglets and inadequate feeding facilitating the occurrence of this disease in these districts where pig growing was developing rapidly.

DISCUSSION

From the present investigations, it is obvious that the serotype of O139:K82:H1 was isolated most frequently from edema disease in Japan, whilst serotypes O2:Kx1:H1 and O115:Kx2:— were occasionally. Moreover, it is noticeable that

none of the strains of serotypes O138 and O141 was found in this country. In several countries, such as U.S.A.^{4,5)}, Canada^{8,9)}, Great Britain²¹⁾ and Ireland⁴⁾, serotype O138 or O141, or both occur frequently with O139. On the other hand, as described previously, all German hemolytic *E. coli* strains belonged to O139. Therefore, this type of the bacteria appears to be dominant in Germany as in Switzerland⁶⁾.

These facts may indicate that there are some differences of distribution of the specific serotypes of hemolytic *E. coli* between some countries and others.

Regarding serotypes other than O138, O139 and O141, SOJKA et al. (1960) described a number of types including serotype O2:K1 from edema disease, while EWING et al. found several hemolytic strains (O5, O8, O46, O75 etc.) including new somatic antigens. Moreover, occasional occurrence of strains having somatic antigens of O6, O8, O15, O45, O78 and O121 have been described by some investigators^{6,8,9)}.

At the present time, it is generally accepted that specific serotypes of hemolytic *E. coli* strains relating to edema disease are isolated from other diseases^{15,16,19,21)} and from normal pigs^{1,2,20,21,23)}. Thus SOJKA et al. (1960) state that these serotypes are widespread in the environment of pigs in Great Britain.

In the present studies, normal pigs in contact with edema disease and those kept in the districts having previous history of the disease yielded "edema disease serotypes" with high frequencies such as 11% and 13% respectively. From these observations, it may be concluded that specific serotypes are prevalent among pigs in "edema disease districts" in this country, though the frequency of their occurrence is lower than in Canada²⁾.

Recently BUXTON and THOMLINSON attempted a new approach to clarify the etiology of edema disease from allergic reaction. Also in their opinion, specific serotypes of hemolytic *E. coli* seem to be an important factor in the etiology of edema disease as in another theory supposing enterotoxemia caused by rapid multiplication of hemolytic *E. coli* in the intestinal tract. Thus, both the above-mentioned theories of the etiology of edema disease premise predisposing factors such as changes of diet or environment which permit an enormous multiplication of the organisms. It is an interesting fact that in Hokkaido massive outbreaks of edema disease were encountered frequently in the districts where pig growing was developing rapidly and poor management of animals was common.

SUMMARY

Since 1957, occurrences of edema disease of swine, new to Japan, have been found in Hokkaido. In the present study, a total of 30 cases of diseased pigs which died or were killed were examined bacteriologically. Among them, 29 (97%) showed

dominant growth of hemolytic *E. coli*. Out of 29 positive cases, 23 yielded cultures of serotype O139:K82:H1, four O2:Kx1:H1, and two O115:Kx2:-- (non-motile). These specific serotypes were found in one of 15 pigs which had recovered from edema disease, 5 of 44 normal pigs in contact with diseased animals and 8 of 62 normal pigs kept in "edema disease districts". Biochemical characteristics and pathogenicity of the organisms for mouse and rabbit were described.

From the epizootiological aspects of edema disease in Hokkaido, the authors would emphasize the fact that the disease may occur enzootically in some definite environments.

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