Yersinia enterocoliticaに関する研究I

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STUDIES ON *YERSINIA ENTEROCOLITICA*

I. ISOLATION OF *Y. ENTEROCOLITICA* FROM SWINE

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Very few reports have appeared in the literature in Japan on the isolation of *Yersinia enterocolitica*. Recently, ZEN-YOJI and MARUYAMA[36] reported the isolation of 12 strains of *Y. enterocolitica* from the ileum of autopsy cases and the appendix and feces of man in Japan. This was the first report on the isolation of this organism in Japan. In addition, three community outbreaks of *Y. enterocolitica* infection occurred in man[5,37] and much interest in human infections with this organism has since arisen in Japan. No reports, however, have appeared in Japan regarding the isolation of this organism from animal sources.

The authors[29] have previously developed a method for the isolation of *Y. pseudotuberculosis* and *Y. enterocolitica* from feces. In this method, the phosphate buffer solution (PBS) reported by PATERSON and COOK[23] was used as an enrichment medium suitable for these organisms. Additionally, satisfactory results were obtained with MacConkey's agar (for the isolation of *Y. pseudotuberculosis*) and SS agar (for the isolation of *Y. enterocolitica*).

Up to this time the authors have been surveying the occurrence of *Y. pseudotuberculosis* and *Y. enterocolitica* in animal feces or intestinal contents by applying the above-mentioned method. This paper deals with the isolation of *Y. enterocolitica* from swine.

MATERIALS AND METHODS

Specimens examined: During a period from June to August, 1972, the cecal contents were collected from a total of 299 apparently healthy swine at a slaughterhouse in Kurayoshi City, Tottori Prefecture.

Media: M/15 PBS (pH 7.6) was used as enrichment medium. MacConkey's and SS agar plates were used for the isolation.

Isolation: Isolation was made both directly and after enrichment. The cecal contents were cultured aerobically on MacConkey's and SS agar plates at 25°C for 48 hours. A 10 per cent suspension of cecal contents in PBS was allowed to stand at 5°C for 21 days for enrichment culture.

Characterization of isolates: Isolates were characterized by the conventional methods and media used in enterobacteriology.

Resistance to potassium tellurate: Nutrient agar containing 0.1% solution of potassium tellurate at the rate of 0.5% was used. Organisms were streaked on this medium and incubated at 25°C for 48 hours.

Phage susceptibility of isolates: The phages and methods used were the same as described previously[28].

Serological tests: Serological grouping was performed with O-antisera prepared

Table 1. Cultural and Biochemical Characteristics of Isolates

Characteristic		Isolate (13 strains)
SS agar, growth		+
MacConkey's agar, growth		+
Potassium tellurate, resistance		−
Motility	25°C	+
(Semi-solid agar)	37°C	−12 +1
Indole (LIM)		+ 7 −6
Methyl red		+
Voges-Proskauer	25°C	+11 −2
	37°C	−
Urease		+
Hydrogen sulfide (SIM)		−
Citrate, Simmons'		−
Nitrate reduction		+
Malonate		−
Phenylalanine deaminase		−
Lysine decarboxylase		−
Arginine decarboxylase		−
Ornithine decarboxylase		+
Catalase		+
β-galactosidase		+
Acid from glucose		+
gas		−
adonitol		−
arabinose		+
cellobiose		+
dulcitol		−
lactose		x
mannitol		+
melibiose		−
rhamnose		−
salicin		d*
sorbitol		+
sucrose		+
xylose		+9 (+)4
Esculin		−
Phage susceptibility		−

(+): Late positive. x: Late and irregularly positive or negative. *: + in 7 strains, (+) in 3 strains, and − in 3 strains.

against O groups 1 to 16 of *Y. enterocolitica*. These groups had been supplied by Dr. S. WINBLAD, of Lund University in Sweden, and Dr. H. H. MOLLARET, of the Pasteur Institute, Paris.

RESULTS

1. Organism isolated

Out of the 299 specimens, 13 strains of *Y. enterocolitica* were isolated. Of them, 2 strains had been detected by the direct culture and 12 strains (including one positive for

Table 2. Serological Group and Biotype of Isolates

O-group	Biotype	No. of strains
3	4	4*
5	1	4
	3	1
10	1	1
12	3	1
Untypable	1	2

* Lysotype 8, as determined by Dr. H. H. MOLLARET.

the direct culture) by the enrichment culture. No abnormality was recognized in the positive cases.

2. Characteristics of isolates

The characteristics of the isolates are shown in Table 1. All the isolates grew moderately on SS and MacConkey's agar plates at 25°C. Colonies were 0.1~0.3 mm in diameter after 24 hours and increased to about 2 mm in diameter after 48 hours. They became greysh white and translucent. Gram negative. Small rods or coccoid forms and sometimes short filamentous forms were observed. All the strains were motile at 25°C. Of them, 4 strains became motile after several passages through semi-solid medium in a Craigie's tube. All the strains, except one, failed to show motility when incubated at 37°C. Indole production was seen in some strains. The Voges-Proskauer reaction was positive at 25°C for 11 strains, but negative at 37°C for all the strains. All the isolates and the known *Y. enterocolitica* strains failed to grow on potassium tellurate agar, while *Y. pseudotuberculosis* grew on this medium. Fermentation of the carbohydrates was generally prompt, although it took 7 or more days for some strains to produce acid from salicin and xylose. Acid production from lactose was late or irregular. No gas was produced from glucose. No isolates were lysed by any *Y. pseudotuberculosis* phage.

The isolates were differentiated from *Y. pseudotuberculosis* in fermenting cellobiose, sorbitol, and sucrose; in failing to attack melibiose, rhamnose, and esculin; in producing ornithine decarboxylase; and in failing to show susceptibility to any *Y. pseudotuberculosis* phage. From these results the isolates were identified as *Y. enterocolitica*. Additionally, the authors persist that potassium tellurate is available for the differentiation between *Y. enterocolitica* and *Y. pseudotuberculosis*.

2. Serological group and biotype of isolates

Biotyping of the isolates was based on indole production, fermentation of xylose and salicin, and nitrate reduction, as mentioned by NILÉHN[22]. As shown in Table 2, the isolates were divided into four serological groups; that is, groups 3, 5, 10, and 12. The majority of the strains belonged to group 3 and biotype 4, or group 5 and biotype 1.

DISCUSSION

Early in 1939, human infection with *Y. enterocolitica* was reported by SCHLEIFSTEIN and COLEMAN[25]. Then two cases of human infection were described by HÄSSIG et al.[10]. Many papers on the infection of man with this organism have been published, mainly by European investigators[1~4,7,14,17,20,21,24,26,27,30,32~34]. *Y. enterocolitica* was also reported to have been isolated from animals, such as swine[9], chinchilla[6,8], dog[6], hare[8], bush-baby (Galago)[12], and monkey[11,16]. Studies were made on many strains of the

organism isolated from various animal sources, including man[13,15,19,31].

The investigation of this organism has just begun in Japan. ZEN-YOJI and MARUYAMA[36] succeeded in isolating this organism from human sources. Community outbreaks of *Y. enterocolitica* infection in man were reported by ASAKAWA et al.[5] and ZEN-YOJI et al.[37]. The isolation of *Y. enterocolitica* from animal sources mentioned in the present report is the first that was achieved in Japan.

WINBLAD[35] and NILÉHN[22] pointed out that the O-antigen group of *Y. enterocolitica* was closely related to the animal species infected; that is, most strains of human and porcine origin belonged to group 3 and were distinguished from strains derived from other animal species. The predominant serological groups to which a majority of the isolates in this report belonged were groups 3 and 5. Further studies are required on the serological group to which the isolated porcine strains belonged.

The ecology of *Y. enterocolitica* in nature remains obscure. MOLLARET[18] asserted that swine might be the chief reservoir or host for *Y. enterocolitica*. The results obtained from this study suggested that the presence of *Y. enterocolitica* in Japan might not be so rare. The fact that this organism was detected from the intestinal contents of swine will lend support to the presumption that the organism may be a possible cause of meat-borne infection in man. Special care must therefore be taken to prevent pork from being contaminated with *Y. enterocolitica*.

SUMMARY

Surveys were performed on the distribution of *Yersinia enterocolitica* in swine in Tottori Prefecture of Japan. A total of 299 specimens were collected from the cecal contents of apparently healthy swine at a slaughterhouse. As a result, 13 strains of *Y. enterocolitica* were detected. Of them, 4 strains belonged to group 3, five to group 5, one to group 10, one to group 12, and the remaining 2 strains were untypable.

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REFERENCES

1) AHLQVIST, J., AHVONEN, P., RÄSÄNEN, J. A. and WALLGREN, G. R. (1971): *Acta path. microbiol. scand., Sect. A*, **79**, 109～122.
2) AHVONEN, P., SIEVERS, K. and AHO, K. (1969): *Acta rheum. scand.*, **15**, 232～253.
3) AHVONEN, P. (1972): *Ann. clin. Res.*, **4**, 30～38.
4) AHVONEN, P. (1972): *Ann. clin. Res.*, **4**, 39～48.
5) ASAKAWA, Y., AKAHANE, S., NOGUCHI, M., SAKAZAKI, R. and TAMURA, K. (1973): *J. Hyg. Camb.* (in press)
6) BECHT, H. (1962): *Dtsch. tierärztl. Wschr.*, **69**, 626～627.
7) CARLSSON, M. G., RYD, H. and STERNBY, N. H. (1964): *Acta path. microbiol. scand.*, **62**, 128～132.

8) DANIËLS, J. J. H. M. (1963): *Zbl. Vet.-Med.*, **10,** 413～417.
9) DICKINSON, A. B. and MOCQUOT, G. (1961): *J. appl. Bact.*, **24,** 252～284.
10) HÄSSIG, A., KARRER, J. and PUSTERLA, F. (1949): *Schweiz. med. Wschr.*, **79,** 971～973.
11) MCCLURE, H. M., WEAVER, R. E. and KAUFMANN, A. F. (1971): *Lab. Anim. Sci.*, **21,** 376～382.
12) MAIR, N. S., SCHUBERT, F. K. and HARBOURNE, J. F. (1970): *Vet. Rec.*, **86,** 69～71.
13) MOLLARET, H. H. and CHEVALIER, A. (1964): *Ann. Inst. Pasteur*, **107,** 121～127.
14) MOLLARET, H. H. and DESTOMBES, P. (1964): *Presse méd.*, **72,** 2913～2915.
15) MOLLARET, H. H. and LUCAS, A, (1965): *Ann. Inst. Pasteur*, **108,** 121～125.
16) MOLLARET, H. H., GIORGI, W., MATERA, A., PESTANA DE CASTRO, A. F. and GUILLON, J. C. (1970): *Rec. Méd. vét.*, **146,** 919～924.
17) MOLLARET, H. H., OMLAND, T., HENRIKSEN, S. D., BAEROE, P. R., RYKNER, G. and SCAVIZZI, M. (1971): *Presse méd.*, **79,** 345～348.
18) MOLLARET, H. H. (1971): *Path.-Biol.*, **19,** 189～205.
19) NILÉHN, B. (1967): *Acta path. microbiol. scand.*, **69,** 83～91.
20) NILÉHN, B. and SJÖSTRÖM, B. (1967): *Acta path. microbiol. scand.*, **71,** 612～628.
21) NILÉHN, B., SJÖSTRÖM, B., DAMGAARD, K. and KINDMARK, C. (1968) *Acta path. microbiol. scad.*, **74,** 101～113.
22) NILÉHN, B. (1969): *Acta path. microbiol. scand.*, Suppl., No. 206, 1～48.
23) PATERSON, J. S. and COOK, R. (1963): *J. Path. Bact.*, **85,** 241～242.
24) RUSU, V., HOTNOG, E., ANDRONESCU, C. and DUMITRIU, M. (1968): *Symp. Series Immunobiol. Standard.*, **9,** 99～102.
25) SCHLEIFSTEIN, J. I. and COLEMAN, M. B. (1939): *N.Y. State J. Med.*, **39,** 1749～1753.
26) SEDGWICK, A. K. and TILTON, R. C. (1971): *Appl. Microbiol.*, **21,** 383～384.
27) SJÖSTRÖM, B. and NILEHN, B. (1968): *Symp. Series Immunobiol. Standard.*, **9,** 319～322.
28) TSUBOKURA, M., ITAGAKI, K., KAWAMURA, K., SASAKI, T. and NAGAI, T. (1971): *Jap. J. vet. Sci.*, **33,** 137～144.
29) TSUBOKURA, M., ITAGAKI, K. and KIYOTANI, K. (1973): *Jap. J. vet. Sci.*, **35,** 33～40 (in Japanese with English summary).
30) WAUTERS, G. (1968): *Symp. Series Immunobiol. Standard.*, **9,** 323～326.
31) WETZLER, T. F. and HUBBERT, W. T. (1968): *Symp. Series Immunobiol. Standard.*, **9,** 343～356.
32) WINBLAD, S., NILEHN, B. and JONSSON, M. (1966): *Acta path. microbiol. scand.*, **67,** 537～541.
33) WINBLAD, S., NILEHN, B. and STERNBY, N. H. (1966): *Brit. med. J.*, **2,** 1363～1366.
34) WINBLAD, S. (1968): *Symp. Series Immunobiol. Standard.*, **9,** 133～136.
35) WINBLAD, S. (1968): *Acta path. microbiol. scand.*, Suppl., No. 187, 115 (Proc. XVth Scand. Congr. Path. Microbiol.).
36) ZEN-YOJI, H. and MARUYAMA, T. (1972): *Japan. J. Microbiol.*, **16,** 493～500.
37) ZEN-YOJI, H., MARUYAMA, T., SAKAI, S., KIMURA, S., MIZUNO, S. and MOMOSE, T. (1973): *Japan. J. Microbiol.*, 17, 220～222.

Yersinia enterocolitica に関する研究

I. ブタからの分離

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(昭和48年3月14日受付)

わが国の動物における *Yersinia enterocolitica* の分布を明らかにする調査の一つとして，見かけ上健康なブタの盲腸内容について，検索を行なった．1972年6月から8月の間に，鳥取県倉吉市の屠場で集めた標本299例のうち，13例より *Y. enterocolitica* を検出した．

分離菌株は，血清学的には4つの群に所属していた．すなわち，3群 (4株)，5群 (5株)，10群 (1株)．12群 (1株) である．なお1～16群のいずれにも属さない2株があった．

わが国の動物における *Y. enterocolitica* の分布は，これまで不明であったが，この菌はけっしてまれな存在ではないように思われた．ヒトへの感染源として，ブタの保菌に注目する必要があろう．