Yersinia enterocoliticaに関する研究2

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Studies on Yersinia enterocolitica

II. Relationship between Detection from Swine and Seasonal Incidence, and Regional Distribution of the Organism

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Abstract. The isolation of Yersinia enterocolitica from the cecal contents of swine was dependent on season. The organism was isolated more frequently in winter to early spring than in summer. No relationship could be established between rate of isolation and serogroup distribution of organisms isolated from swine at three abattoirs in Kurayoshi, Hiroshima and Fukuoka. Even if such differences are recognized, more emphasis should be put upon the feed type used on each farm than upon the regional factors. There was no difference in organisms recovered between swine given formula feed and those given garbage.

Human infection with Yersinia enterocolitica occurs more frequently in colder
months of the year [2, 3, 16, 21, 22, 27, 32].

No detailed studies, however, have been
made on animal infection with this organism. Tsubokura et al. [26] and Zen-Yoji
et al. [33] reported that the frequency of
detection of this organism from swine was
higher in the winter months than in the
summer months. No reports, however, have
appeared on the monthly incidence of Y.
enterocolitica infection in swine.

This paper deals with the relationship

between the detection of *Y. enterocolitica* and season, regional distribution or porcine feed.

Materials and Methods

Specimens examined: During a period from March, 1973 to March, 1974, the cecal contents were collected from a total of 2,041 apparently healthy swine at three abattoirs in Kurayoshi, Hiroshima and Fukuoka City, respectively.

Media: As enrichment medium, 1/15 M phosphate buffer solution (PBS), pH 7.6, was used. The SS and MacConkey's agar plates were used for isolation.

190-180-170-160-150-30-20-

Fig. 1. Monthly incidence of Y. enterocolitica

Remarks.

10

□: Specimens tested. ■: Y. enterocolitica.

Sept.

Aug.

Jan.

1974

Dec.

Feb.

Apr.

🖂: Atypical Y. enterocolitica.

July

June

1973

Table 1. O-groups and biotypes of isolates

Oct.

O-group	Biotype	1973 May	June	July	Aug.	Sept.	Oct.	Nov.		1 <i>974</i> Jan.	Feb.			May	Total
3	4						7	1		4		4			16
5	1	2								1	2	5			10
5	2								12	1					13
6	1					1					4	4	1		10
7	1								1	1					2
8	1									2		1			3
10	1													1	1
11	1											1			1
12	1											1			1
12	2						- 1		1	1	1	4	1		9
13	1								2						2
14	1		1					1	2		2	2			8
16	3											1			1
19	1											1			1
U.C.	1								2		2				4
U.C.	3										1	3			4
U.C.	U.C.												1		1
Total		2	1			1	8	2	20	10	12	27	3	1	87

Remarks.

U.C. = Unclassified.

Isolation: Every specimen was suspended in a 1/15 M PBS at a concentration of about 10 percent and the suspension kept at 4°C for about 3 weeks for enrichment. It was then subcultured aerobically

on SS and MacConkey's agar plates at $25\,^{\circ}\mathrm{C}$ for about 40 hours.

Identification and bio- and sero-typing of the isolates: Experiments were performed by the same

Table 2.	Regional distribution	of O-groups
	and biotypes	

Biotype	Kurayoshi	Hiroshima	Fukuoka
4	9	5	2
1	3	6	1
2			13*
1	1	4	5
1	1		1
1	1	1	1
ī	1		
1			1
1			,1
2	6	2	1
1			2
1	1	3	4
3	1		
1	1		
1	2		2
3	1	3	
U.C.			1
	28	24	35
	4 1 2 1 1 1 1 1 2 1 1 3 1 1 3	4 9 1 3 2 1 1 1 1 1 1 1 1 1 2 6 1 1 3 1 1 1 2 5 U.C.	1 3 6 2 1 1 4 1 1 1 1 1 1 1 1 1 1 2 6 2 1 1 1 3 3 1 1 1 2 3 1 3 U.C.

Remarks.

U.C.: Unclassified.

procedure as described in the previous report [25], except that O-antisera 17 to 34 were used additionally in the present study.

Results

1. Monthly incidence of Y. enterocolitica

A total of 87 strains were isolated from 82 of the 2,041 specimens. The monthly incidence of the organism is shown in Fig. 1. The majority of these strains had been detected in colder months of October to March.

A total of 37 strains of so-called atypical Y. enterocolitica were isolated from 36 specimens. They were isolated also in colder months of December to March. Those designated as atypical Y. enterocolitica strains in the present study had the following characters: To utilize Simmons' citrate at 25°C; to ferment melibiose and/or rhamnose. Of these strains, 23 strains showed negative V.P. reaction at 25 and 37°C and

Table 3. Number of positive specimens in a group

No. of positive specimens in a group	No. of groups
1	23
2	6
3	5
4	3
5	2
11	1
Total	40

Table 4. Rate of detection of Y. enterocolitica by feed type

	Rate of detection				
Feed type	Hiroshima 657*	Fukuoka 560*			
Formula	3.1	6.0			
Garbage	3.0	5.0			

Remarks.

could not be classified serologically. They will be discussed in detail in another report.

2. Monthly incidence of serological groups

Table 1 shows the serological groups of the 87 strains isolated from specimens obtained in the three abattoirs and their monthly distribution. These strains were divided into 12 O-groups, except 9 strains which were unclassified. Although there was no special relationship between the O-group or biotype and its monthly distribution, various O-groups were detected in March.

3. Regional distribution

A total of 28 strains of *Y. enterocolitica* were isolated from 27 of 670 specimens obtained from the Kurayoshi Abattoir. The majority of them were isolated over a period from October to March, especially in March.

A total of 24 strains of the organism were detected from 23 of 686 specimens obtained from the Hiroshima Abattoir. The majority

^{*:} Twelve strains were detected in December.

^{*:} A total number of specimens.

of them were isolated in October, February and March.

A total of 35 strains of the organism were isolated from 32 of 685 specimens obtained from the Fukuoka Abattoir. The majority of them were isolated over a period from December to March.

Atypical Y. enterocolitica was isolated from 21, 14 and 1 specimens in the Kurayoshi, Hiroshima and Fukuoka Abattoirs, respectively.

4. Regional distribution of O-groups

As shown in Table 2, the strains derived from the Kurayoshi and Fukuoka Abattoirs were divided into various O-groups. On the contrary, a limited number of O-groups were seen in the strains derived from the Hiroshima Abattoir.

The most prevalent O-groups were O3 and O12 derived from Kurayoshi, O3 and O5 from Hiroshima, and O5 from Fukuoka. All the strains derived from Kurayoshi and Hiroshima belonged to O5 and were classified into biotype 1, while all the strains, except one, derived from Fukuoka belonged to O5 and were classified into biotype 2.

5. Relationship between hog farm and detection of *Y. enterocolitica*

It was not clear under what circumstances the swine on test had been raised, because the specimens were collected from the abattoirs. Usually, a group of about 20 swine was consigned from each farm. As shown in Table 3, Y. enterocolitica was isolated from a total of 40 groups. Usually, two or more positive specimens were derived from each of 17 of these groups. Exceptionally, 11 positive specimens were found in one group. Two or more positive specimens derived from a single farm belonged to the same O-group, O3 or O5.

6. Difference in the detection of *Y. enterocolitica* between feed types

As shown in Table 4, there was no differ-

ence in the detection of *Y. enterocolitica* between swine given a formula feed and those fed garbage.

Discussion

It is well known that Y. pseudotuberculosis infections occur more frequently in winter [4, 11, 14, 15]. As for Y. enterocolitica, no seasonal variation has been found in any early publication [17]. Many workers, however, reported that Y. enterocolitica infections were most prevalent in autumn and winter [2, 3, 16, 32] or winter and early spring [21, 22, 27]. On the other hand, Rabson and Koornhof [20] reported that the infections appeared most frequently in late summer and early autumn in South Africa. Delorme et al. [8] pointed out that the monthly distribution of the organism showed no such seasonal patterns as described by previous workers, but that a peak of infection occurred in Canada in midsummer.

Many investigators have speculated that swine may act as a source of infection in man [1, 9, 17, 20, 21, 26, 31, 33]. In Japan, it became clear that *Y. enterocolitica* was present even in apparently healthy swine, and that the detection of the organism was more frequent in winter than in summer [26, 33].

The reason why *Y. enterocolitica* is most prevalent in the winter months is unknown. Niléhn [18] and Carter and Collins [7] reported that this organism was more pathogenic when grown at 25°C than when grown at 37°C. Gutman et al. [10] suggested that the organism might remain as a source of infection in the field in the winter months, because *Y. enterocolitica* could replicate at such a low temperature as 4°C. Paterson and Cook [19] mentioned that when the ambient temperature was 5°C or below, *Y. pseudotuberculosis* survived in feces for several days.

In order to clarify this subject, it is necessary to study this organism under the circumstances where swine are raised. In addition, experiments are needed to determine whether the organism is transient or multipliable in the alimentary tract of swine.

Atypical Y. enterocolitica strains have been isolated frequently from man, animals and water [5, 6, 13, 24, 26, 28–31]. It will require further studies to determine whether they should belong to Y. enterocolitica or any other species. In the present paper, the authors distinguished atypical Y. enterocolitica from Y. enterocolitica proper, because attempts were made recently to reexamine the taxonomy of this organism [12, 23, 31].

Previously, the authors inferred that the frequency of Y. enterocolitica isolation might be related to the variation in feeding practice of swine [26]. This inference was verified by the present study. When two or more swine harbored this organism in a group, O-groups O3 and O5 were detected rather frequently from this group. It is not clear whether O-groups O3 and O5 are readily transferred from swine to swine or from one contaminated farm to another.

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References

- [1] Ahvonen, P., and Rossi, T. (1970). Familial occurrence of Yersinia enterocolitica infection and acute arthritis. Acta paediatr. scand., 206 (Suppl.), 121-122.
- [2] Ahvonen, P. (1973). Yersinia enterocolitica infections in Finland. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 133-134.
- [3] Arvastson, B., Damgaard, K., and Winblad, S. (1971). Clinical symptoms of infection with Yersinia enterocolitica. Scand. J. infect. Dis.,

- 3, 37-40.
- [4] Borg, K. (1968). Silvatic pseudotuberculosis in Scandinavia. Symp. Series immunobiol. Standard. 9,, 129-132.
- [5] Bottone, E. J., Chester, B., Malowany, M. S., and Allerhand, J. (1974). Unusual Yersinia enterocolitica isolates not associated with mesenteric lymphadenitis. Appl. Microbiol., 27, 858–861.
- [6] Botzler, R. G., Wetzler, T. F., and Cowan, A. B. (1968). Yersinia enterocolitica and Yersinia-like organisms isolated from frogs and snails. Bull. Wildlife Dis. Assoc., 4, 110-115.
- [7] Carter, P. B., and Collins, F. M. (1974). Experimental Yersinia enterocolitica infection in mice: Kinetics of growth. Infect. Immunity, 9, 851–857.
- [8] Delorme, J., Laverdière, M., Martineau, B., and Lafleur, L. (1974). Yersiniosis in children. Canad. med. Assoc. J., 110, 281-284.
- [9] Esseveld, H., and Goudzwaard, C. (1973). On the epidemiology of Y. enterocolitica infections: Pigs as the source of infections in man. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 99-101.
- [10] Gutman, L. T., Ottesen, E. A., Quan, T. J., Noce, P. S., and Katz, S. L. (1973). An interfamilial outbreak of Yersinia enterocolitica enteritis. New Engl. J. Med., 288, 1372-1377.
- [11] Knapp, W. (1958). Mesenteric adenitis due to *Pasteurella pseudotuberculosis* in young people. New Engl. J. Med., **259**, 776-778.
- [12] Knapp, W., and Thal, E. (1973). Differentiation of Yersinia enterocolitica by biochemical reactions. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 10-16.
- [13] Lassen, J. (1972). Yersinia enterocolitica in drinking-water. Scand. J. infect. Dis., 4, 125– 127.
- [14] Mair, N. S. (1968). Pasteurella pseudotuberculosis infection in Great Britain 1959-1966. Symp. Series immunobiol. Standard., 9, 121-128.
- [15] Mollaret, H. H. (1960). L'adénite mésentérique aiguë à *Pasteurella pseudotuberculosis* (Bacille de Malassez et Vignal) à propos de 30 observations. II. Étude épidémiologique. *Presse Méd.*, **68**, 1447-1450.
- [16] Mollaret, H. H. (1871). L'infection humaine à Yersinia enterocolitica en 1970, à la lumière de 642 cas récents. Aspects cliniques et perspectives épidémiologiques. Path.-Biol., 19, 189-205.
- [17] Niléhn, B. (1969). Studies on Yersinia en-

- terocolitica with special reference to bacterial diagnosis and occurrence in human acute enteric disease. Acta path. microbiol. Scand., Suppl. 206, 1–48.
- [18] Niléhn, B. (1973). The relationship of incubation temperature to serum bacterial effect, pathogenicity and in vivo survival of Yersinia enterocolitica. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 85-92.
- [19] Paterson, J. S., and Cook, R. (1963). A method for recovery of Pasteurella pseudotuberculosis from faeces. J. Path. Bact., 85, 241-242.
- [20] Rabson, A. R., and Koornhof, H. J. (1973). Yersinia enterocolitica infections in South Africa. Contribut. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 102-105.
- [21] Rakovský, J., Paučková, V., and Aldová, E. (1973). Human Yersinia enterocolitica infections in Czechoslovakia. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 93-98.
- [22] Rusu, V., Lucinescu, St., Stanescu, C., Totescu, E., and Muscan, A. (1973). Clinical and epidemiological aspects of the human Yersinia enterocolitica infections in Romania. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 126-127.
- [23] Stevens, M., and Mair, N. S. (1973). A numerical taxonomic study of Yersinia enterocolitica strains. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 17-22.
- [24] Toma, S., and Lafleur, L. (1974). Survey on the incidence of *Yersinia enterocolitica* infection in Canada. *Appl. Microbiol.*, 28, 469–473.
- [25] Tsubokura, M., Otsuki, K., and Itagaki, K. (1973). Studies on Yersinia enterocolitica. I. Isolation of Y. enterocolitica from swine. Jap.

- J. vet. Sci., 35, 419-424.
- [26] Tsubokura, M., Otsuki, K., Fukuda, T., Hira-yama, N., Sanekata, T., Takahashi, K., and Kubota, M. (1974). Isolation of Yersinia enterocolitica from swine. J. Japan. vet. med. Assoc., 27, 278-281 (in Japanese with English summary).
- [27] Vandepitte, J., Wauters, G., and Isebaert, A. (1973). Epidemiology of Yersinia enterocolitica infections in Belgium. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 111-119.
- [28] Wauters, G. (1973). Correlation between ecology, biochemical behaviour and antigenic properties of Yersinia enterocolitica. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 38–41.
- [29] Weaver, R. E., and Jordan, J. G. (1973). Recent human isolates of Yersinia enterocolitica in the United States. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 120-125.
- [30] Wetzler, T. F., and Hubbert, W. T. (1968). Yersinia enterocolitica in North America. Symp. Series immunobiol. Standard., 9, 343–356.
- [31] Winblad, S. (1973). Studies on the O-serotypes of Yersinia enterocolitica. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 27–37.
- [32] Winblad, S. (1973). The clinical panorama of human Yersiniosis enterocolitica. Contrib. Microbiol. Immunol., Yersinia, Pasteurella and Francisella, 2, 129–132.
- [33] Zen-Yoji, H., Sakai, S., Maruyama, T., and Yanagawa, Y. (1974). Isolation of Yersinia enterocolitica and Yersinia pseudotuberculosis from swine, cattle and rats at an abattoir. Japan. J. Microbiol., 18, 103-105.