THE SEASONAL TRANSITION OF FECAL ISOLATIONS OF ENTEROPATHOGENIC *ESCHERICHIA COLI* FROM HEALTHY MILKING COWS

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INTRODUCTION

In a previous paper (1969), it was reported that some enteropathogenic *Escherichia coli* for humans had been isolated from the feces of cattle with the detection rate of over twenty percent. Also, in that result, it was suggested that the frequency of fecal isolations of enteropathogenic *E. coli* may be influenced by the season. However, as far as we know, there is no report about the seasonal variation of fecal isolations of enteropathogenic *E. coli* from cattle. Therefore, this study was planned and carried out to know the presence and the transition of enteropathogenic *E. coli* in the feces of healthy milking cows over a period of consecutive months.

MATERIALS AND METHODS

Materials : Fecal specimens obtained from a herd of 40 healthy milking cows (31 Holsteins aged 3~13 years, 8 Jerseys aged 2~16 and 1 Brown-Swiss aged 8) in Sapporo were employed. The herd had been pastured in the daytime between the middle of May and the end of September, and especially in July and August all day long excepting milking time. During their grazing in pasture, the cows were fed on hay and silage at half volume, and on three-fifths of the usual volume of heavy feeds.

The fecal specimens were obtained from all cows in a herd, once at the end of each month, between February and November, 1967. The total number of samples was 396. They were sampled soon after evacuation and were transported into the laboratory, as soon as possible.

Methods : A loopful (diameter 3.5 mm) of fecal specimens was directly cultured on MacConkey agar plate for 16~18 hours at 37°C. From each plate, at least 10 colonies which seemed to be *E. coli* were picked up and inoculated onto slant plain agar, and then those strains were examined biochemically and serologically. Biochemical tests were done according to the standard method of the Coli-Aerogenes Subcommittee (1956). Serological typing was done by the following method.

At first, each strain was tested for its agglutinability to the diagnostic OK polyvalent sera-group I, II and III by mixing its live organisms on a slide glass with these antisera.

If this probe agglutination test was positive, that strain was again tested for its agglutinability to the appropriate diagnostic OK monovalent sera on a slide glass, with both its live and boiled antigens being used. The boiled antigen was prepared by boiling the organisms at 100°C for 1 hour. At last, O agglutination test was done in tubes to the appropriate O sera with the bacterial suspension boiled for 1 hour at 100 °C. The agglutinability was estimated after the incubation in a water-bath at 50°C for 16～18 hours.

The above-mentioned agglutination tests were carried out in accordance with the method of EDWARDS & EWING (1962). The diagnostic OK polyvalent and monovalent sera (the former 3 groups, the latter 18 types) used were made by the TOSHIBAKAGAKU CO. The serotypes of OK monovalent sera were as follows:

O26 : K60, O28a, c : K73, O44 : K74, O55 : K59, O86a : K61, O86 : K62, O111 : K58, O112a, c : K66, O119 : K69, O124 : K72, O125 : K70, O126 : K71, O127a : K63, O128 : K67, O136 : K78, O143 : Kx1, O144 : Kx2 and O146 : K89.

The diagnostic O sera were made by us. They were O26, O28a, c, O44, O55, O86, O111, O112a, c, O119, O124, O125, O126, O127a, O128, O136, O143, O144 and O146.

RESULTS

1 Isolations of *Escherichia coli* as a herd

TABLE 1 *Frequency of fecal isolations of enteropathogenic Escherichia coli over the ten month period from February to November, 1967*

MONTH	NO. OF SAMPLFS EXAMINED	NO. OF POSITIVE SAMPLES OF PATHOGENIC *E. COLI*	NO. OF *E. COLI* STRAINS USED FOR SEROTYPING	NO. OF PATHOGENIC *E. COLI* STRAINS
Feb.	40	10 (25.0 %)*1	354	11 (3.1 %)*2
March	40	6 (15.0)	321	19 (5.9)
April	40	11 (27.5)	358	20 (5.6)
May	40	11 (27.5)	356	25 (7.0)
June	40	4 (10.0)	403	4 (1.0)
July	40	0	398	0
Aug.	40	0	359	0
Sept.	40	2 (5.0)	339	2 (0.6)
Oct.	38	7 (18.4)	357	8 (2.2)
Nov.	38	2 (5.3)	362	4 (1.1)
TOTAL	396	53 (13.4)	3,607	93 (2.6)

*1 No. of positive samples / No. of samples examined
*2 No. of pathogenic *E. coli* strains / No. of *E. coli* strains tested

As shown in table 1, 321～403 strains of *E. coli* were isolated every month, and the total number of strains amounted to 3,625 from the 396 cows over the ten month period, from February to November, 1967. Smooth-typed 3,607 isolations were used for the sero-

typing of enteropathogenic *E. coli.*

2 Isolations of enteropathogenic *Escherichia coli* as a herd

As shown in table 1, the strains of enteropathogenic *E. coli* were isolated in months other than July and August. The rates of cows in which enteropathogenic *E. coli* was found each month were as follows: 25% (10/40) in February, 15% (6/40) in March, 27.5% (11/40) both in April and May, 10% (4/40) in June, 5% (2/40) in September, 18.4% (7/38) in October and 5.3% (2/38) in November.

The monthly rates of isolation of enteropathogenic *E. coli* strains to *E. coli* strains were as follows: 3.1% (11/354) in February, 5.9% (19/321) in March, 5.6% (20/358) in April, 7.0% (25/356) in May, 1.0% (4/403) in June, 0.6% (2/339) in September, 2.2% (8/357) in October and 1.1% (4/362) in November.

As the above-mentioned results both in the cows and in the strains showed, the fecal isolations of enteropathogenic *E. coli* were done with relatively high frequency in the spring months-February, March, April and May. But in the summer months-July and August no isolation was carried out, and in the autumn months-September, October and November, the isolations were again done with lower frequency than in the spring.

The total number of strains of enteropathogenic *E. coli* was 93 out of 3,607 *E. coli* strains.

As shown in table 2, the serotypes of these 93 enteropathogenic *E. coli* strains belonged to different 10 O-serotypes; O26, O28a, c, O112a, c, O125, O126, O127a, O128, O136, O144 and O146. Out of 93 strains, 31 strains belonged to O112a, c, 23 strains to O126, 20 strains to O128, 7 strains to O125, 4 strains to O146, 2 strains respectively to O26, O136 and O144, and 1 strain each to O28a, c and O127a.

All strains of O112a, c were concentrically isolated in the spring months, especially in March, April and May. Strains of O126 and O128 were isolated both in the spring and autumn months. Strains of O125, O146, O136 and O144 were isolated in the spring months only.

3 Isolations of enteropathogenic *E. coli* in individual cows

Between February and November, fecal isolations of enteropathogenic *E. coli* were made from 29 out of 40 cows in a herd, and, 93 strains of enteropathogenic *E. coli* were isolated from these 29 cows. The largest number of strains isolated from any cow was 23 strains originated from No. 630-cow as shown in table 3.

In decreasing order of number of strains, 9 strains originated from No. 654-cow, 7 strains from No. 296-cow, 4 strains each from No. M12- and No. 614-cow, 3 strains each from 7 cows, 2 strains each from 8 cows and 1 strain each from 9 cows.

In 3 cases of No. 630-, No. 642- and No. 650-cow, the isolations of enteropathogenic *E. coli* were made both in the spring and autumn months. The serotypes of 23 strains originated from No. 630-cow were 4 types, O112a, c, O126, O128 and O136. The total number of strains of O126 was 18, O112a, c and O136 2 strains each, and O128 1 strain. The strains of O126 only were isolated both in the spring and autumn months, but the strains of O112a, c, O128 and O136 in the spring months only. In No. 642-cow, 1 strain was isolated

TABLE 2 *Serotypes of enteropathogenic E. coli isolated every month*

MONTH	SEROTYPES IDENTIFIED AND NUMBER OF THEIR STRAINS										TOTAL NUMBER OF STRAINS IDENTIFIED
	O26: K60	O28a,c: K73	O112a,c: K66	O125: K70	O126: K71	O127a: K63	O128: K67	O136: K78	O144: Kx2	O146: K89	
Feb.	·*	·	1	·	1	1	8	·	·	·	11
March	·	·	8	·	6	·	·	·	2	3	19
April	·	·	8	6	3	·	1	2	·	·	20
May	·	·	13	·	8	·	3	·	·	1	25
June	1	·	1	1	·	·	1	·	·	·	4
July	·	·	·	·	·	·	·	·	·	·	0
Aug.	·	·	·	·	·	·	·	·	·	·	0
Sept.	·	·	·	·	·	·	2	·	·	·	2
Oct.	1	·	·	·	2	·	4	·	·	·	8
Nov.	·	·	·	·	3	·	1	·	·	·	4
Total number of strains identified	2	1	31	7	23	1	20	2	2	4	93

* Zero

TABLE 3 *Serotypes and number of strains of enteropathogenic E. coli isolated from each cow over the ten month period from February to November, 1967*

COW NO.	MONTH										TOTAL NUMBER OF STRAINS ISOLATED
	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	
286	・*1	・	・	・	O26 : 1*2	・	・	・	／*3	／	1
289	・	・	・	O112a, c : 1	・	・	・	・	／	／	1
301	・	・	・	・	・	・	・	・	O128 : 2	O128 : 1	3
659	・	・	・	O112a, c : 3	・	・	・	・	・	・	3
M12	・	O144 : 2	O112a, c : 2	・	・	・	・	・	・	・	4
665	O112a, c : 1	・	O112a, c : 1	・	・	・	・	・	・	・	2
614	・	・	O126 : 3	O146 : 1s*4	・	・	・	・	・ s	・	4
296	O128 : 1	・	O125 : 3	O128 : 3	・	・	・ s	・	・ s	・	7
576	O127a : 1	・	O125 : 1s	・	・	・	・	・	・	・	2
B15	O128 : 1	・	・	O112a, c : 2	・	・	・	・	・	・	3
F19	・	・	・	O112a, c : 1	・	・	・	・	・	・	1
630	・	O126 : 6 O112a, c : 2	O136 : 2	O126 : 8	O128 : 1	・	・	・	O126 : 1	O126 : 3	23
612	・	O146 : 2	・	O112a, c : 1	・	・	・	・	・	・	3
670	O128 : 1	・	・	・	・	・	・	・	・	・	1
673	O128 : 1	O146 : 1	・	・	・	・	・	・	・	・	2

*1 Zero
*2 Number of strains isolated
*3 Not done
*4 Suffering from scours

TABLE 3 (continued) *Serotypes and number of strains of enteropathogenic E. coli isolated from each cow over the ten month period from February to November, 1967*

COW NO.	MONTH										TOTAL NUMBER OF STRAINS ISOLATED
	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	
674	·	·	·	·	·	·	·	·	O26 : 1	·	1
669	·	·	·	·	·	·	·	·	O128 : 1	·	1
672	·	·	·	·	·	·	·	O128 : 1	·	·	1
623	O128 : 1	·	·	·	O112a, c : 1	·	·	·	·	·	2
642	·	·	·	·	O125 : 1	·	·	O128 : 1	O128 : 1	·	3
650	·	O112a, c : 1	·	·	·	·	·	·	O126 : 1	·	2
666	O126 : 1 O128 : 1	·	· s	·	·	·	·	·	·	·	2
634	·	·	O112a, c : 3 s	·	·	·	·	·	·	·	3
283	·	·	·	·	·	·	·	·	O28 a, c : 1	·	1
595	O128 : 1	·	·	O112a, c : 1	·	·	·	·	·	·	2
654	· s	O112a, c : 5	O125 : 1	O112a, c : 3	·	·	·	·	·	·	9
660	O128 : 1	·	O112a, c : 2 s	· s	·	·	·	· s	· s	·	3
635	·	· s	O128 : 1 s	·	·	·	·	·	·	·	1
647	·	·	O125 : 1	O112a, c : 1	·	·	·	·	·	·	2
Total number of strains isolated	11	19	20	25	4	0	0	2	8	4	93

in June, September and October. Moreover, in June O125, and, in September and October O128 were detected, that is, the serotype in spring was different from the one in autumn. In No. 650-cow, 1 strain of O112a, c was isolated in March and 1 strain of O126 in October.

There were 21 cows from which enteropathogenic *E. coli* was isolated in spring only, and 5 cows in autumn only. In the former 21 cows, there was no cow in which the same serotype had been continuously detected for 2 or more months. The number of cows in which the same serotype was detected in 2 months was only 3 (Nos. 665, 296 & 654), and, those serotypes were O112a, c in No. 665- and No. 654-cow, and O128 in No. 296-cow.

In the 5 cows from which enteropathogenic *E. coli* was isolated in the autumn season only, there was 1 cow (No. 301) in which the same serotype (O128) had been detected over a period of 2 months. In 2 cows (Nos. 630 & 666), 2 serotypes were detected from the same sample once only.

As shown in the above-mentioned results, it was ascertained that, though they are apparently healthy milking cows, some of them discharge the enteropathogenic *E. coli* in their feces. However, there was no cow in which enteropathogenic *E. coli* had been continuously detected every month from February to November. In 3 cows only, enteropathogenic *E. coli* had been isolated both in the spring and autumn season, not in the summer. On the other hand, in 5 cows, enteropathogenic *E. coli* had been isolated in the autumn season only, and, in 21 cows, in the spring season only. However, the serotypes of isolated strains were not always the same every month. In only 3 cows (Nos. 301, 630 & 642), the same serotype (O126 in No. 630-cow, O128 in No. 301- and No. 642-cow) had been continuously detected for 2 months. In only 4 cows (Nos. 665, 296, 630 & 654), the same serotype (O112a, c in No. 665- and No. 654-cow, O128 in No. 296-cow and O126 in No. 630-cow) had been intermittently detected twice or more over the ten month period.

The number of strains isolated from an individual cow per month was under 8, and most of them were under 3. Namely, it seemed that the number itself of strains of enteropathogenic *E. coli* isolated from apparently healthy cows was very few.

4 The relationship between scours and isolation of enteropathogenic *E. coli*

When fecal specimens were sampled in a herd, some of the cows had been scouring. In these 14 scouring fecal samples, enteropathogenic *E. coli* was isolated from only 5 samples originated from 5 different cows. The number of organisms detected in these feces was under 3 in all. Therefore, in these cases, it is not presumed that enteropathogenic *E. coli* was a direct cause of scours. The serotypes of strains isolated from these feces were as follows: O146, O125 and O128 from 1 each, and, O112a, c from 2.

Discussion

The seasonal variation of fecal isolations of enteropathogenic *E. coli* from a herd of 40 healthy milking cows was investigated over the ten month period from February to November, and, the following results were obtained: the fecal isolations of enteropathogenic *E. coli* were made with higher frequency in the spring season than in the autumn season, and, in the summer season no strain of enteropathogenic *E. coli* was isolated from any cow. In our previous report (1969),

it was reported that the enteropathogenic *E. coli* from the feces of slaughtered calves or cattle had been isolated more frequently in early winter than in early summer. So, it is considered that the data obtained in this study supported the results of our previous report.

On the other hand, the Public Health Laboratory Service and the Society of Medical Officers of Health (1965) reported that the isolations of enteropathogenic *E. coli* from the feces of healthy children under 4 years old had not been affected by the season in a year. But, MAIN (1959) reported that the highest percentage of positive cultures of enteropathogenic *E. coli* from household pets (dogs and cats) had been obtained during the summer months. That is to say, the above-mentioned results are different according to the author. As these differences may originate from the differences of human or animal species and food or feed of the object investigated, further investigation about these points is required to clarify the differences of seasonal frequency of fecal isolations of enteropathogenic *E. coli* from animal species.

In this study, when the cows were kept in a cow-shed and were fed on a large amount of hay, silage and heavy feeds, the fecal isolations of enteropathogenic *E. coli* were made with the most frequency, with the highest rate in the spring. But, when the cows had been especially fed on a large amount of fresh grass in pasture in the summer season, the frequency of fecal isolations of enteropathogenic *E. coli* decreased or went down to zero. By this result, it is estimated that the seasonal frequency of fecal isolations of enteropathogenic *E. coli* from cows may be affected by their feeds, their environment and so on.

In this study, some strains of enteropathogenic *E. coli* were isolated from the feces of healthy milking cows, but among 40 cows, there was only one cow from which isolations of enteropathogenic *E. coli* were continuously made twice or more both in the spring months and in the autumn months. Moreover, even in this cow, the strain of the same serotype was not always isolated every month and the number of organisms was very few—under 8 in all cases.

KAUFFMANN & PERCH (1943) and WALLICK & STUART (1943) reported that the human intestinal becteria flora are not stable and are continuously changing. SEARS et al. (1956) reported that *E. coli* strains in healthy men and dogs can be divided into residents or transients, and, transients are various and few in number and persist in their host for a few days only. A similar relationship is known about *E. coli* flora in the intestines of horses (1956) and calves (1960). Therefore, in healthy milking cows also, some enteropathogenic *E. coli* in the intestinal canal may exist as transient flora rather than as resident flora.

In this study, even in scours, the fecal isolations of enteropathogenic *E. coli* were not always made in all cases, that is to say, enteropathogenic *E. coli* was

isolated from a few cases only, and, the number of organisms was very few.

In the present study, the serotype O112a, c, O126 and O128 were especially dominant among 93 strains isolated. This tendency coincided with the one in cows in our previous report (1969).

Summary

The fecal isolations of enteropathogenic *E. coli* for humans in a herd of 40 healthy milking cows were tested every month between February and November, 1967. The results obtained are summarized as follows:

1) The enteropathogenic *E. coli* was isolated from the feces of 29 out of 40 healthy milking cows, with no isolations in the summer months.

In only 1 cow, the organism was continuously detected twice or more both in the spring months and in the autumn months. In this case, the dominant serotype was O126, but the same serotype was not always detected every month.

2) Out of the 29 positive cows, 21 were positive in the spring months only. In these cows also, the same serotype was not always detected every month.

3) In 25 positive cows, the frequency of isolation of the organism was less than two times, and, in 3 cows three times and in only 1 cow six times.

The number of cows in which the same serotype was detected twice over a period of consecutive months was only 3. The number of cows in which the same serotype was intermittently detected twice was only 4.

4) In all 29 positive cows, the number of the organisms isolated in 1 sample was under 8, and in most of the positive samples it was very few—under 3.

5) There was no case in which the enteropathogenic *E. coli* has proved a direct cause of scouring.

6) The serotypes of 93 strains isolated were divided into 10 different serotypes: O26, O28a, c, O112a, c, O125, O126, O127a, O128, O136, O144 and O146. Among them, O112a, c, O126 and O128 were dominant, and, O112a, c was concentrically detected in the spring months only, and, O126 and O128 were detected both in the spring and autumn months.

7) The serotypes detected twice over a period of months were O128 and O126, and, the former in 2 cases and the latter in 1 case.

The serotypes detected twice intermittently over the ten month period were O112a, c, O128 and O126. The O112a, c was isolated from 2 cases, and, the O128 and the O126 from 1 case each.

8) In the present study, the enteropathogenic *E. coli* was isolated with higher frequency in the spring months than in the autumn months, and, the dominant serotypes detected were O112a, c, O126 and O128 in a herd. These results were in accord with the results obtained in our previous report.

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