Survival of *Yersinia enterocolitica* in Soil and Water

Kazuko TASHIRO, Yoko KUBOKURA, Yukio KATO, Ken-ichi KANEKO, and Masuo OGAWA*

Department of Animal Health, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

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ABSTRACT. *Yersinia enterocolitica* O3, O4, O5A, O5B, O6, O6, 30, O9 and O13 and *Yersinia intermedia* were examined to clarify their survival in natural soil, river water and well water. The O3 strain disappeared most rapidly from soil at both temperature of 4°C and 20°C and from river water at 20°C. Although the O5B and O9 strains disappeared before the O3 strain did from river water at 4°C, the O9 strain survived as long as non-virulent strains did in soil at 4°C. The O3, O5B and O9 strains survived longer at 4°C than at 20°C in soil and river water. Non-virulent strains of O4, O5A and O6 survived longer in well water than in soil and river water. Although the O3 and O5A strains disappeared from supernatant filtered with 100 µm and 5 µm pore size filters, they maintained their viable cell numbers in supernatant of soil filtered with 0.22 µm pore size filter and in the autoclaved supernatant.—**KEY WORDS:** soil, survival, temperature, water, *Yersinia enterocolitica*.

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Ecological studies have been done on *Yersinia enterocolitica* in nature. There are many reports on occurrence of *Y. enterocolitica* in animals. It is now well known that natural reservoirs of the bacteria are mainly dogs and pigs. The bacteria, however, have been isolated from well water samples [5], and water borne outbreaks caused by the bacteria have been reported [3]. The present study was done to know the survival of *Y. enterocolitica* in soil and natural water.

MATERIALS AND METHODS

Soil and water used: Soil, river water and well water were collected from about 15 cm below the surface at a deciduous forest, the Tama river and a well in Fuchu City on April 1983. The soil contained about 10^7 of total viable bacteria per gram: river water, about 10^5 ; and well water, 10^1 at the time of collection.

Bacterial strains used: The bacterial strains used are shown in Table 1. All strains were cultured on Trypticase soy agar (BBL) at 25°C for 48 hours and maintained in equal volumes of calf serum and 10% lactose-water solution at –80°C. Before inoculation, the bacteria were washed with physiological saline solution. In glass flasks, bacteria were inoculated to a concentration of 10^6 viable bacteria per gram of soil or water. The inoculated samples were kept at 20°C and 4°C.

Supernatant of soil: The soil was suspended in four times volumes of sterilized tap water according to the methods of Kubokura *et al.* [8]. The suspension was filtered with 100 µm, 5 µm and 0.22 µm pore size filters (Millipore) according to the methods of Ogawa and Kubokura [10].

Culture methods: Quantitative direct culture and enrichment culture were done. MacConkey agar (Eiken) was used for plate counts. Plate counts were done in duplicate. When the viable bacterial number in soil was under 10^2 and in water or supernatant was under 10^1 , 1 gram or 1 ml of a sample was inoculated in YCC broth (Eiken), and was incubated for 3 weeks at 4°C. Total numbers of viable bacterial cells were counted using Trypticase soy agar (BBL).

RESULTS

Figure 1 shows the survival of *Y. enterocolitica* in soil at 20°C and 4°C. The O3 Tel001 bacteria disappeared most rapidly from the soil at both temperatures. Since the survival of O3 Te673, Te664 and 231–3 strains was as short as the O3 Tel001 strain, the results of these strains were not shown in the Fig. 1. The O4 bacteria survived for 15 weeks at 20°C. At 4°C, the O3 bacteria which disappeared most rapidly from the soil, were followed by O5B and O6 bacteria. Although the results of O6, 30, O13 and *Y. intermedia* bacteria are not shown in Fig. 1, their survival in soil was the same as the O5A bacteria. The O9, O4 and O5A bacteria survived for 35 weeks at least after their viable counts diminished

* CORRESPONDENCE TO: OGAWA, M., Department of Animal Health, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan.

Table 1. The bacterial strains used

Species	Serovar	Biovar ^{a)}	Strain designation	Habitat
<i>Y. enterocolitica</i>	O3	4	Te1001	Swine
	O3	4	Te673	Swine
	O3	4	Te664	Canine
	O3	4	231-3	Canine
	O5B	2	Te19	Human
	O9	2	Te591	Human
	O4	1	Y46	Gray starling
	O5A	1	Te1286	Norway rat
	O6	1	YBK4II	Stream water
	O6, 30	1	Y269	Eastern turtledove
<i>Y. intermedia</i>	O13	1	YSJ4	River water
			YBL-1-1	Pond water

a) According to the scheme of Wauters [12].

Fig. 1. Survival of *Y. enterocolitica* in soil. Viable bacterial counts of O3 Tel001 (●), O5B (■), O9 (▲), O4 (○), O5A (□) and O6 (△) inoculated are plotted. Total number of viable bacteria in the soil (⊙) is the mean of the duplicate experiments. + means the bacteria were recovered by enrichment culture method.

Fig. 2. Survival of *Y. enterocolitica* in river water. See Fig. 1 legend for an explanation of the symbols. Total number of viable bacteria was counted on 0, 1, 2 and 3 weeks with the experiment at 20°C and on 0 through 6 weeks with the experiment at 4°C.

under 10^2 per ml at 4°C. The soil contained about 10^7 viable bacteria. The viable cell numbers of the total viable bacteria were the same during the experiments.

Figure 2 shows the survival of *Y. enterocolitica* in river water. The O3 Tel001 bacteria disappeared most rapidly from the river water 4 weeks after inoculation, followed by O9 bacteria at 20°C. The O5B and O9 bacteria disappeared most rapidly at 4°C 9 weeks after inoculation. The O3, O4, O5A and O6 bacteria disappeared by 16 weeks. The O3, O5B and O9 bacteria survived for 5 to 8 weeks after their viable counts diminished under 10^1 per ml at 4°C. The river water contained total viable bacteria with the number of about 10^5 before inoculation of 10^6 *Y. enterocolitica*. The number of the total viable bacteria was 10^6 at early stage of the experiment due to the inoculum of 10^6 *Y. enterocolitica*. It was maintained at 10^{4-5} after *Y. enterocolitica* decreased under 10^4 .

Figure 3 shows the survival of *Y. enterocolitica* in well water. The O3 Tel001 bacteria disappeared most rapidly from well water followed by the O5B and O4 bacteria at 20°C. The O3 and O5B bacteria

Fig. 3. Survival of *Y. enterocolitica* in well water. See Fig. 1 legend for an explanation of the symbols.

also disappeared within 13 weeks at 4°C. The O5A, O6 and O9 bacteria, however, maintained their bacterial cell number for at least 13 weeks at both temperatures of 20°C and 4°C. Well water contained 10 viable bacteria per ml at the beginning of the experiment. These bacteria grew to 10^6 or 10^5 per ml 2 or 4 weeks after inoculation with *Yersinia* at 20°C or 4°C.

Figure 4 shows the survival of *Y. enterocolitica* in filtered supernatant of soil. At 20°C, the O3 Tel001 bacteria disappeared from supernatant filtered through filter papers of 100 μm and 5 μm within 2 and 5 weeks, respectively. The O5A bacteria, however, survived for 5 weeks in the supernatant filtered through the 100 μm , and 8 weeks through 5 μm . At 4°C, survival time of these bacteria was prolonged in the supernatant through these filters. In the supernatant filtered through 0.22 μm pore size filter, the O3 and O5A bacteria maintained their population 10 weeks after inoculation at both temperatures of 20°C and 4°C. The same results were obtained in the soil, river water and well water which were autoclaved. In the supernatant filtered

Fig. 4. Survival of *Y. enterocolitica* in soil supernatant filtered. Viable bacterial counts of O3 are plotted in the supernatant filtered with 100 μm (●), 5 μm (■) and 0.22 μm (▲); that of O5A, with 100 μm (○), 5 μm (□) and 0.22 μm (△).

through 100 μm pore size, many protozoans of flagellate, ciliate and amoeba which prey on bacteria were observed. In the supernatant filtered through 5 μm pore size, however, protozoan was not present.

DISCUSSION

In the present study, human enteric pathogens of *Y. enterocolitica* O3, O5B and O9 survived longer at 4°C than at 20°C. This might account for the fact that the isolation of the bacteria from animals and human yersiniosis occur more frequently in cold months [4, 9]. Furthermore, the O3 and O5B could not survive in soil and river water very well. While

the non-virulent strains of O4, O5A and O6, and *Y. intermedia* could survive for a long time. Previous reports demonstrated that most of the strains isolated from water were non-virulent serovars [1, 2, 5]. The O9 strain survived in soil and in well water at 4°C for a long time. Since Botzler *et al.* [1] reported the isolation of serovar O9 strain from pond water, long survival of the bacteria in soil and well water might contribute to the isolation from water.

Ogawa and Kubokura [10] pointed out that there were three different substances of protozoa, bacteria and something less than 0.22 μm in diameter which are involved in the clearance with 2 weeks, 3 to 6 weeks and 8 weeks after addition of *Escherichia coli*, respectively. Since the most quick clearance was observed with the O3 and O5B strains 4 weeks after inoculation, clearance of *Y. enterocolitica* might be due to bacteria in the present study. The human enteric pathogens of O3, O5B and O9 strains were cleared by 9 weeks at 20°C in soil and river water which contained 10^5 or 10^6 viable bacteria per ml. However, their clearance in well water which contained only 10 viable bacteria was not observed for 13 weeks at 20°C. Furthermore, viable bacterial cell number of the O3 strain was maintained in the supernatant filtered through 0.22 μm pore size filter. These facts would suggest that the clearance of the human enteric pathogen *Y. enterocolitica* might be due to the bacteria of which habitat is soil and river water. All strains used survived longer in soil and in river water at 4°C than at 20°C. This fact might be due to the lower activities of competitive bacteria in low temperatures.

Although non-virulent serovars of *Y. enterocolitica* and *Y. intermedia* survived in the river water only for 16 weeks, they survived in soil for 50 weeks and over. Furthermore, even decrease of their viable cell numbers was not observed in well water. These facts suggested that there were very few microorganisms which contribute to the clearance of non-virulent *Y. enterocolitica*. At 4°C, non-virulent strains survived for 50 weeks in soil and for 18 weeks in river water. On the other hand, virulent strains of O3 and O5B diminished their bacterial counts to 10 per ml gradually in soil and more quickly in river water. Future investigations are warranted to know what kind of substance might be involved in these phenomena.

Non-virulent *Y. enterocolitica* strains are frequently isolated from free-living mammals but virulent ones are not detected in these animals [6, 7,

11]. Although the food habit of free-living mammals varies, they need water and contact with soil directly or indirectly. It is of particular interest in the ecological viewpoint to know whether or not the prolonged survival of non-virulent *Y. enterocolitica* in soil and well water might cause the high occurrence of these bacteria in free-living mammals.

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