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# Occurrence of *Yersinia enterocolitica* in House Rats

KEN-ICHI KANEKO,[1]* SUKEKAZU HAMADA,[1] YUMI KASAI,[2] AND EIICHI KATO[1]

*Department of Veterinary Public Health[1] and Department of Parasitology,[2] Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan*

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From July 1976 to May 1977, 270 rats (259 *Rattus norvegicus* and *R. rattus*) in Sapporo were examined for the presence of *Yersinia enterocolitica* in house rats. The organism was isolated in 55 rats (54 *R. norvegicus* and 1 *R. rattus*). Isolated strains were determined as O group (O)3, biovar 4; O4, biovar 1; O5A, biovar 1; and O6, biovar 1. The isolation of O3, biovar 4 strains from *R. norvegicus* is the first in the world, as far as we know. The organism was isolated from the duodenum in 3 rats, the jejunum in 7 rats, the ileum in 8 rats, the cecum in 34 rats, the colon in 23 rats, the rectum in 16 rats, and the mesenteric lymph nodes in 5 rats. The organism was not isolated from liver, spleen, and kidneys. Isolation of the organism from the mesenteric lymph nodes was made in 1 out of 2 O3-positive rats, 1 out of 7 O5A-positive ones, and 3 out of 29 O6-positive ones. A high agglutinin titer was recorded in the two O3-positive rats and in one O6-positive animal.

*Yersinia enterocolitica* has received much attention as a causative agent in single cases and mass outbreaks of human yersiniosis. Many workers have isolated the organism from various kinds of animals to clarify its ecological nature. As a result of these isolations (2, 6–8, 14, 16–18, 22), pigs and dogs are accepted as natural reservoirs of the organism.

Although some reports (3, 4, 9, 10, 15, 22) of *Y. enterocolitica* isolation from small rodents have been published, a systematic investigation has not been made of its existence in house rats, which are possible contaminators of human food. In the present study, the authors clarify the significance of house rats as reservoirs of *Y. enterocolitica*.

## MATERIALS AND METHODS

**Specimens examined.** From July 1976 to May 1977, 270 rats (259 *Rattus norvegicus* and 11 *R. rattus*) were trapped alive in one barn, one slaughterhouse, and one zoo in Sapporo, Japan. The contents of the duodenum, jejunum, ileum, cecum, colon, and rectum, and the mesenteric lymph nodes, liver, spleen, and kidneys, were sampled within 10 min after cardiac puncture from each individual anesthetized with ethyl ether in order to isolate *Y. enterocolitica*.

**Age analysis of rats.** This procedure was according to the method of Yabe et al. (21).

**Direct and enrichment culture methods, isolation, identification, biotyping, and serological grouping of *Y. enterocolitica* strain.** These procedures were the same as those described in a previous report (8), except that both salmonella-shigella agar (Eiken Chemical Co.) and MacConkey agar (Eiken Chemical Co.) were used as selective media, and suspected colonies up to 12 on every selective plate were selected.

**Determination of agglutinin titers.** Sera were obtained by cardiac puncture and stocked at −30°C. Out of them, sera obtained from rats which yielded *Y. enterocolitica* were tested with the isolated strains in accordance with the method of Winblad et al. (20), using a microtiter system. The sera were incubated at 56°C for 30 min before the test.

## RESULTS

**Seasonal incidence.** Table 1 shows the monthly incidence of *Y. enterocolitica*-positive rats in three locations. The organism was isolated from 55 out of 270 rats examined. At a barn, incidence of isolations in summer (July and August) was 31.6%; that in autumn (September, October, and November) was 15.8%; that in winter (December, January, and February) was 16.3%; and that in spring (March, April, and May) was 33.3%. Those in spring and in summer were significantly greater than that in autumn ($P < 0.01$). And the former was also greater than that in winter ($P < 0.05$), but the latter was not ($P = 0.10$ to 0.20).

**Age distribution of *Y. enterocolitica*-positive rats (*R. norvegicus*).** Table 2 shows *Y. enterocolitica*-positive rats (*R. norvegicus*) divided into three age groups. The organism was not isolated from 6-month-old and over individuals captured at the zoo. There was no significant difference ($P = 0.10$ to 0.20), however, in the prevalence of *Y. enterocolitica*-positive individuals among the age groups from the zoo.

At the slaughterhouse, O3, biovar 4 strains

TABLE 1. *Monthly incidence of Y. enterocolitica-positive house rats (R. norvegicus and R. rattus) from three locations*

| Location | 1976 | | | | | | 1977 | | | | | Total | %[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | July | August | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | March | April | May | | |
| Barn | 5/12[a] | 7/26 | 2/38(0/1)[b] | 8/25 | 2/13 | 2/3 | 4/31 | 1/9 | 0/1 | 0/5 | 3/3 | 34/166(0/1) | 20.5 |
| Slaughterhouse | | | | | 4/7 | 6/16 | 1/1 | | | | | 11/24 | 45.8 |
| Zoo | | | | | | | | 5/47(0/3) | 5/27(1/7) | 0/6 | | 10/80(1/10) | 12.5 |
| Total (%) | 41.7 | 26.9 | 5.3 | 32.0 | 30.0 | 42.1 | 15.6 | 10.7 | 17.9 | 0.0 | 100.0 | 55/270(1/11) | 20.4 |

[a] Number of positive house rats per number of house rats examined.
[b] The numbers in parentheses indicate black rats (*R. rattus*).
[c] Positive isolation rate in each location.

TABLE 2. *Distinction of Y. enterocolitica-positive rats (R. norvegicus) among three age groups from three locations*

| Location | Age (months) | No. of individuals with: | | | | | | | | Total | No. of individuals examined |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | One O group | | | | Two or three O groups | | | | | |
| | | 0 group 3 Biovar 4 | 4 1 | 5A 1 | 6 1 | 4 5A + 1 1 | 4 6 + 1 1 | 5A 6 + 1 1 | 4 5A 6 + + 1 1 1 | | |
| Barn | Under 3 | | 1 | | 9 | | | | | 10 | 35 |
| | 3 to under 6 | | | 2 | 6 | | | 3 | | 11 | 64 |
| | 6 and over | | 1 | 2 | 2 | | | | | 5 | 39 |
| | Unknown | | | 1 | 6 | | | 1 | | 8 | 28 |
| Slaughterhouse | Under 3 | 1 | | | | | | | | 1 | 1 |
| | 3 to under 6 | | | 1 | | 2 | | 1 | 1 | 5 | 12 |
| | 6 and over | 1 | 2 | | 1 | | 1 | | | 5 | 11 |
| Zoo | Under 3 | | 1 | | 2[a] | | | | | 3 | 19 |
| | 3 to under 6 | | | 1 | 3 | 1 | 1 | 1 | | 7 | 40 |
| | 6 and over | | | | | | | | | | 21 |

[a] This number includes one black rat (*R. rattus*).

were detected in the two rats captured. Out of the rats examined at the barn, 13.9% yielded O6 organism; out of those at the slaughterhouse and the zoo, 4.2 and 6.3%, respectively, yielded it. There was no significant difference, however, in the prevalence of rats with O6 organism among the three locations.

Out of the rats examined at the slaughterhouse, 20.8% yielded two or three O-group organisms; out of those at the zoo and the barn, 3.8 and 2.4%, respectively, yielded two O-group organisms. Prevalence of the rats with two or three O groups at the slaughterhouse significantly differed from that with two O groups at the zoo ($P < 0.05$) and at the barn ($P < 0.01$).

The organism was isolated from three animals by both direct and enrichment culture methods; it was isolated by enrichment culture only from the remaining 52 individuals. The three individuals consisted of two O3 positive and one O6 positive.

**Sex distribution of *Y. enterocolitica*-positive rats.** Out of 122 male and 148 female rats examined, the organism was isolated in 27 males and 28 females.

**Distribution of the organism in rats.** Table 3 shows the organism isolated from various regions in positive cases. The organism was not isolated from liver, spleen, and kidneys. The positive rate was highest in the cecum. From the ileum to the rectum, O4, O5A, and O6 strains were isolated. From the mesenteric lymph nodes, O3, O5A, and O6 strains were also isolated in 1 out of 2 O3-positive cases, 1 out of 7 O5A-positive cases, and 3 out of 29 O6-positive cases. The O6 strains were predominant first in the cecum, second in the colon, and third in the rectum, although they occurred equally in the area from the duodenum to the ileum.

The entire body of each embryo of the nine females from which the organism was not isolated was prepared for bacterial cultures. The organism was not detected by either direct or enrichment culture methods.

**Agglutinin titer in a *Y. enterocolitica*-positive case.** Table 4 shows the agglutinin titers

in 47 *Y. enterocolitica*-positive cases. Out of them, eight yielded two O-group organisms and one yielded three O groups. We calculated the former titer as 2 and the latter as 3 in Table 4 because we determined each titer against each of the two or three O groups in each of the nine rats. The titers in 44 *Y. enterocolitica*-positive cases, including the 9 O-group isolation cases, were no more than 32. The titers in two O3-positive and one O6-positive cases were 1,024, 512, and 512, respectively.

## DISCUSSION

Human yersiniosis has occurred more frequently during the cold season in Europe (5, 12, 13, 19). Isolation of the organism in pigs is also more frequent during cold seasons (16, 22). Kaneko et al. (8) pointed out that there was no seasonal variation in isolation of this organism in dogs. Isolation in rats was more frequent during the warm season in the present study.

Winblad et al. (20) reported that all but one of the 431 blood donors showed an agglutinin titer ≦80 against the organism and that the one showing a titer of 160 had abdominal pain and diarrhea shortly before his visit to a blood bank. Ahvonen (1) suggested that the low agglutinin titer (40 to 80) in humans may be due to a cross-reaction with other bacteria. The low titers (4 to 32) in the present study might occur with the actual infection present because the rats did yield *Y. enterocolitica*. It becomes necessary in the future to clarify whether or not low titers may occur without actual present or past infection because of nonspecific reactors such as normal agglutinin.

*Y. enterocolitica* was not isolated from liver, spleen, and kidneys in all of the rats. Because a high agglutinin titer did not develop and because O4 and O5A organisms could not be isolated from the mesenteric lymph nodes, with the exception of the intestinal contents, in all but one case, the organisms may be weak in their ability to penetrate into other organs from intestinal lumen. The O4 and O5A organisms may be transient in rats because they occurred almost equally in the intestinal tract and because the numbers of them were less in the cecum or colon than numbers of O6 organism.

Kapperud (9, 10) and Zen-Yoji et al. (22) reported that O6 strains were the most prevalent in small rodents. In the present study, these were isolated from 38 out of 55 *Y. enterocolitica*-positive rats. Because O6 strains were in order of predominance, first in the cecum, second in the colon, and third in the rectum, the O6 organism may reside in the cecum and may flow to the colon and rectum with the cecal contents. That the O6 organism occurred equally in all three age groups suggests that it may not develop effective immunity in rats. Furthermore, the strains were detected without inducing a high agglutinin titer in all but one of the cases.

TABLE 3. *Isolation of Y. enterocolitica from body regions in positive cases (R. norvegicus)*

| O group | No. of cases in which *Y. enterocolitica* was isolated from: | | | | | | |
|---|---|---|---|---|---|---|---|
| | Duodenum | Jejunum | Ileum | Cecum | Colon | Rectum | Mln[a] |
| 3 | | | | 2 | 2 | 2 | 1 |
| 4 | 1 | | 2 | 2 | 4 | 1 | |
| 5A | | 1 | 3 | 4 | 2 | 1 | 1 |
| 6 | 2 | 4 | 3 | 23[b] | 13 | 8 | 3 |
| 4+5A | | 2 | | 1 | 1 | | |
| 4+6 | | | | | | 1 | |
| 5A+6 | | | | 2 | 1 | 3 | |

[a] Mesenteric lymph nodes.
[b] This number includes one black rat (*R. rattus*).

TABLE 4. *Agglutinin titer against the O antigen of the isolate in positive cases*

| O group | No. of individuals showing titer of: | | | | | | | | Total[a] |
|---|---|---|---|---|---|---|---|---|---|
| | <4 | 4 | 8 | 16 | 32 | (64, 128, 256) | 512 | 1,024 | |
| 3 | | | | | | – | 1 | 1 | 2 |
| 4 | 5 | | | 3 | 1 | – | | | 9 |
| 5A | 11 | 2 | 1 | | | – | | | 14 |
| 6 | 20 | 5 | 3 | 2[b] | 1 | – | 1 | | 32 |

[a] Total includes eight rats respectively calculated as 2 and one rat calculated as 3.
[b] This number includes one black rat (*R. rattus*).

According to the above facts, the O6 organism may become resident in rats. On the other hand, it is important to clarify whether or not O6 organism invades not only the lumen of the cecum but also the mucosal epithelium, since it was isolated from the mesenteric lymph nodes in three cases.

Zen-Yoji et al. (22) reported that *Y. enterocolitica* was isolated from 35.2% of the rats examined at a slaughterhouse. The prevalence of positive rats at the slaughterhouse was significantly higher than that at other locations. The prevalence of rats with two or three O groups was also higher at the slaughterhouse than at other locations. Based on these facts, it is estimated that the slaughterhouse rats were more frequently exposed to *Y. enterocolitica* invasion than were rats at other locations.

The O3, biovar 4 strain is the most common in human yersinioses. This strain was isolated from two Norway rats (*R. norvegicus*) during this investigation. We found that the two O3-positive rats recorded a high agglutinin titer and that the organism invaded the mesenteric lymph nodes in one of them. Consequently, it may be said that the O3, biovar 4 organism may be pathogenic in rats. Pokorná and Aldová (11) isolated the same organism from one black rat (*R. rattus*), although it had not been isolated in small rodents before, thus necessitating determination of the rat's role as a reservoir of this organism.

Zen-Yoji et al. (22) reported that the swine carrier rate of the O3, biovar 4 organism was 6.8 or 7.9% in the cecal contents. Aldová et al. (3) isolated the O3 organism from black rats in pig houses. In the present study the O3, biovar 4 organism was isolated from the two rats captured at a slaughterhouse in which several hundred pigs were slaughtered in a day. The relationship between pigs and rats in the ecology of *Y. enterocolitica*, therefore, warrants future investigation.

Kaneko et al. (8) isolated O3 organism from 16, O5A organism from 2, O5B organism from 5, O6 organism from 1, and O9 organism from 2 out of 451 dogs examined in the same district of Sapporo in which the organisms could, theoretically, invade rats as well as dogs. That O5B and O9 organisms detected previously in dogs were not found in rats examined from the same district may be due to differences in their host susceptibilities.

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