An Assessment of Yersinia enterocolitica and Its Presence in Foods

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ABSTRACT

Yersinia enterocolitica is one of the few human pathogens that grow at refrigeration temperature for foods, 0-5 C. Typical strains of Y. enterocolitica do not ferment rhamnose. These have been recovered from human infections, various animals, and pig’s feces, but only rarely from foods. The atypical strains tend to utilize rhamnose, raffinose, esculin, salicin, α-methyl glucoside, or Simmon’s citrate. These atypical strains have been recovered from fish, meat, oysters, and water as well as human patients. Inoculated food studies indicate that present recovery methods for Y. enterocolitica need improvement, but its identification is uncomplicated provided that the typical and atypical strains are taken into consideration. The public health implication of its presence in foods cannot be assessed until the incidence and virulence of the food isolates are determined.

Yersinia enterocolitica, Yersinia pestis (the plague bacterium formerly named Pasteurella pestis), and Yersinia pseudotuberculosis are now placed in the same genus in the family Enterobacteriaceae (30). Until recently Y. enterocolitica infections did not receive much attention in the United States, even though sporadic cases and outbreaks were reported as early as 1939 (43). Most of the recent microbiological and clinical studies of Y. enterocolitica were conducted by several European investigators who provided us with much of the present knowledge of this bacterium (27,32,51).

The clinical aspects of Y. enterocolitica infections have been reviewed in considerable detail (5,27,55). Its symptoms are different for various age groups (55). The predominant symptoms in infants are fever and diarrhea, but older children have mesenteric lymphadenitis and ileitis which mimic symptoms of acute appendicitis (55). Adults may experience abdominal pains, acute enteritis, arthritis, and erythema nodosum (55). Septicemia may affect the aged and immune-deficient or immune-suppressed patients (55). Like Salmonella infections, Y. enterocolitica can be systemic and invade tissues outside of the digestive tract; it has been recovered form various abscesses, lesions, and eye infections (1,35).

Most of the Y. enterocolitica infections have involved individual cases or small clusters of people. Three large outbreaks of Y. enterocolitica infections in Japan were reported but sources of infections could not be identified (6,57). In these outbreaks, 931 persons (mostly school children) were infected out of a total population of 2520 students in three elementary schools. Two large outbreaks of Y. enterocolitica infections involving 137 children and one adult out of 831 persons who had gone on outings were reported in Quebec, Canada. Raw milk was implicated as the source of both outbreaks (4). Recently, an outbreak of Y. enterocolitica infections affecting at least 30 persons (mostly children) in the village of Holland Patent, New York, with a population of 600 was reported; chocolate milk was implicated as the source of infection (Morbidity and Mortality Weekly Report 26:53-54, 1977). The clinical picture of Y. enterocolitica infections in the U.S. is still not complete because it is not a notifiable disease and the bacterium is recovered infrequently by clinical laboratories. The reported recovery rates for Y. enterocolitica worldwide range from almost 0 to 1-2% of the fecal specimens; three Belgian hospitals consistently had the highest recovery rates (28,39). The discrepancy of recovery rates must be partly due to different recovery methods employed. It is interesting to note that two Canadian and Belgian Hospitals have reported that the recovery rates for Y. enterocolitica were higher than the rates for Shigella (14,39). For reasons yet unclear, most of the clinical isolates of Y. enterocolitica from Canada (47), Japan (56), and Europe (50) belong to the Nilehn’s biotype 4 serotype 0:3 strain, whereas most of the U.S. clinical strains belong to Nilehn’s biotypes 2 and 3 with many different 0 serotypes (35,53). Some U.S. clinical strains are Y. enterocolitica-related bacteria which utilize Simmon’s citrate, rhamnose, melibiose, and raffinose (1,8).

Recent DNA homology studies have shown that the four biotypes of Y. enterocolitica are a closely related group with 85-100% homology among various strains and thus should be considered a homogenous species (10,31). On the other hand, biochemical characteristics and DNA homology of Y. enterocolitica-related bacteria are different from those of the four biotypes of Y. enterocolitica and in fact consist of three distinct groups of bacteria as determined by DNA homology (10). The DNA of Y. enterocolitica and Y. enterocolitica-related
strains have about 60% homology (10). By comparison, Salmonella and Escherichia coli DNA have 40-60% homology (9).

_Y. enterocolitica_ -related bacteria were also isolated from human patients with conjunctivitis and urinary tract infections, etc. by Bottone et al. (1,8). Thus, it is possible that _Y. enterocolitica_-related bacteria may cause disease in people with different foci of infection and symptoms than is observed in the typical _Y. enterocolitica_ infections. However, this as well as the public health significance of these microorganisms remain to be documented.

**OCCURRENCE**

_Y. enterocolitica_ and related bacteria have been commonly isolated from a variety of animals, foods, and water sources, but the biotypes were not identified in some of the reports. Among animals, pigs were found to harbor _Y. enterocolitica_ with the same biotypes (4) and serotype (0:3) as the most common clinical strain isolated from humans in Canada, Japan, and Europe (15,48,49, 50,58). For this reason Mollaret (27) considered pigs an important zoonotic source of _Y. enterocolitica_ infections. The bacterium has also been isolated from sick cats (38,46), cattle (20,25), chickens (25), deer and monkeys (33), a beaver, a Canadian goose, a raccoon and a Pekin robin (18), hares (38), and chinchillas (46,50). Recently one case of _Y. enterocolitica_ infection in the U.S. was traced directly to sick puppies (54), and similarly, puppies were implicated in another outbreak which infected 16 of 21 people, with two fatalities (17). The evidence just presented suggests that zoonosis may be an important mode of transmission of _Y. enterocolitica_ and that the reservoir of infection may reside in diseased animals. Presence of _Y. enterocolitica_ in animals suggests, therefore, that it may be present in meats.

In the extensive survey conducted by Leistner et al. (25), feces and meat of chicken, cattle, and pigs were found contaminated with _Y. enterocolitica_. They recovered 35 _Y. enterocolitica_ and 56 related bacteria from 121 samples of chicken meat, 10 _Y. enterocolitica_ and 10 related bacteria from 29 samples of pork, and four _Y. enterocolitica_ and six related bacteria from 37 samples of beef (25). _Y. enterocolitica_ was isolated from 15 of 61 samples of beef in Japan (20). In the U.S., _Y. enterocolitica_ and related bacteria were recovered from 10 of 98 samples of vacuum-packed beef and two of 18 samples of vacuum-packed lamb meat which had been stored at 1-3 C for 21-35 days (19). In the course of testing various enrichment procedures, four native _Y. enterocolitica_ and three related bacteria were isolated from eight pork samples; also, two native _Y. enterocolitica_ and five related bacteria were isolated from 10 samples of oysters (unpublished results). Toma (46) in Canada isolated biotype 1 _Y. enterocolitica_ from four of 17 oyster samples. _Y. enterocolitica_ was recovered from mussels (44), milk (4,42), ice cream (29), banana (29), and fish (21,38).

Both _Y. enterocolitica_ and related bacteria have been isolated from drinking water (3,22,23; Saari, T. N., and T. J. Quan, 1976, Abstr. Am. Soc. Microbiol. C119). The source of infection in one case of _Y. enterocolitica_ septicemia was traced to drinking water obtained from a mountain stream in upper New York State (22). From these data, it can be inferred that certain biotypes of _Y. enterocolitica_ are fairly common in meat, oysters, and water. The public health significance of _Y. enterocolitica_ in foods and water sources must await assessment by epidemiological and virulence studies.

**ISOLATION PROCEDURES**

Isolation methods for enrichment of _Y. enterocolitica_ were developed primarily for clinical and fecal specimens (46,52), and initially some of these methods were also used for testing of food samples (20,25). For instance, cold enrichment with pH 7.6, 0.067 M sodium phosphate buffered saline (PBS) was originally developed for isolation of _Y. pseudotuberculosis_ from clinical material (34), but now is used widely for isolation of _Y. enterocolitica_ from foods (16,20,25,58). In one study with beef, cold enrichment with PBS was superior to enrichment with selenite-F broth plus 40 mg of novobiocin/liter (20). In another study, PBS cold enrichment was used with good results to examine 215 meat samples (25), and cold enrichment has been recommended for the isolation of _Y. enterocolitica_ from foods (16). Preliminary study with inoculated foods indicated that PBS cold enrichment could not recover the two clinical strains of _Y. enterocolitica_ inoculated into meat and oysters, but that the modified MgCl₂ enrichment broth of Wauters (52) was fairly effective for recovery of the inoculated biotype 4 serotype 0:3 strain (24). Because many _Y. enterocolitica_ strains are sensitive to MgCl₂, Wauter's broth (52) failed to recover an inoculated biotype 2 serotype 0:8 strain as well as the native _Y. enterocolitica_ in meat and oysters which were recoverable by other enrichment methods (24). These results indicate that the current enrichment methods may not be adequate for recovery of some clinically important biotypes present in foods and thus must be improved.

Various enteric plating media such as deoxycholate citrate, eosin methylene blue, lysine sucrose urea (32), MacConkey, _Shigella_ _Salmonella_ (SS) and YL agars (T. Saari, personal communication) have been used for isolation of _Y. enterocolitica_ in clinical work. Some of these media are inhibitory to the more sensitive strains of _Y. enterocolitica_ (8,13). However, MacConkey and SS agars adjusted to pH 7.4 gradually have gained acceptance (14,16,25). One of the problems encountered with SS, YL, or MacConkey agars in examining foods is that it is difficult to distinguish the lactose-negative _Y. enterocolitica_ colonies from many other food-borne lactose-negative bacteria on these agars. Toma (46) and Wauters (52) reported that _Y. enterocolitica_ colonies could be recognized on SS agar when the colonies were
examination under a dissecting microscope with oblique illumination. Recently, Vanderzant (personal communication) found that *Y. enterocolitica* formed typical black colonies on bismuth sulfite (BS) agar after incubation for 2-3 days at 25°C, and that BS agar was useful for the isolation of *Y. enterocolitica* from meats. Random selection of colonies reduces the sensitivity of the isolation procedure and also makes the procedure tedious. Part of this problem can be resolved by adding a fermentable sugar like sucrose to the DHL agar (Eiken, Japan) (25) or mannitol to YM agar (T. Saari, personal communication). Some strains of *Y. enterocolitica* have lipase and lecinthinase activity but lack nuclease activity (13, 51). These properties were utilized to aid in the recognition of *Y. enterocolitica* colonies by addition of Tween 80 to MacConkey agar, and sorbitol and Tween 80 to "DNase" agar (24). *Y. enterocolitica* forms typical colonies on the above agars after 48 h of incubation at 25°C and generally can be distinguished from other bacterial colonies with some practice (24). Identification of *Y. enterocolitica* is not difficult provided that allowance is made for the five biotypes and the various related bacteria. These characteristics have been described (1,8,13,32). The biotypes and biochemical reactions of *Y. enterocolitica* isolates should continue to be reported until the taxonomy of this species becomes clear.

**VIRULENCE TESTS**

Unlike the other two *Yersinia* species, *Y. enterocolitica* was not considered to be virulent to laboratory animals (2) until recently, when two strains of *Y. enterocolitica* isolated from humans were found to be pathogenic to mice (12,35). Alonso et al. (2) found that only five, possibly six, of 4,500 "old" strain of *Y. enterocolitica* in the Pasteur Institute collection were pathogenic to normal mice, but three of the "non-virulent" strains could infect immuno-deficient athymic nude mice. Rabson et al. (36) found that mice treated with ferric ammonium citrate were also susceptible to *Y. enterocolitica* infections. The virulence of *Y. enterocolitica* has been tested with germ-free mice (7), HeLa tissue culture (40), gerbils (35), monkeys (26), rabbits (37,40), rabbit ileal loop test (41), and the Sereny test using guinea pigs (45). However, the adequacy of the above tests is not yet assured. A reliable procedure must be sought for determining the virulence of *Y. enterocolitica* because it is essential for investigating the pathogenic potential of food and water isolates.

**SURVIVAL**

Resistance of *Y. enterocolitica* to heating, drying, NaCl, or fresh and salt water has not been determined. All three species of *Yersinia* grow at about 0-4°C (11,25,34) and the number of *Y. enterocolitica* in chicken broilers decreased only slightly after freezing for 90 days at -18°C (25).

Without question, *Y. enterocolitica* grows at recommended food refrigeration temperatures below 5°C, but it is not known whether it can compete with other bacteria that also grow well at low temperatures. In one study large numbers of *Y. enterocolitica* were isolated from vacuum-packed meats where such packaging would be expected to suppress the outgrowth of common spoilage bacteria (19). However, the true incidence of this microorganism in refrigerated foods and its role as a food-borne pathogen must await further investigation.

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