

New Potential Pathogens in Fresh Meat

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Introduction

New potential pathogens of fresh meat that have attracted much attention in recent years include *Yersinia* and *Campylobacter* species. Although their significance in foods has been recognized only recently, *Y. enterocolitica* was first described in the United States in 1939 and *Campylobacter fetus* in 1918. Because of time limitation, our discussion will be focused on the significance of *Y. enterocolitica* in foods with emphasis on meats. Detailed information on the characteristics of this organism, its distribution in man, animals and in the environment, its clinical significance and methods for isolation and identification are presented in recent reviews (2,6,8,10,14,18,26).

In recent years, *Y. enterocolitica* and closely related bacteria have been isolated with increasing frequency from various foods including raw milk, beef, pork, lamb, chicken, turkey, fish, mussels, oysters and vegetables (6,10,14,18). *Yersinia enterocolitica* was first described in 1939 by Schleifstein and Coleman (24). Between 1933 and 1947 only 11 cases of *Y. enterocolitica* infection were reported in the United States (23). By 1966, only 23 cases had been reported throughout the world but more than 5000 cases had been reported by 1975 (28). This rapid increase in reports of *Y. enterocolitica* infections probably resulted from an increased awareness and improved laboratory techniques for its isolation and identification. *Yersinia enterocolitica* is a gram-negative, facultative anaerobic rod-shaped bacterium. It is ubiquitous in the animal environment and in waters of streams and lakes. From the public health viewpoint, one of its most significant characteristics is its ability to grow at refrigeration temperatures. *Yersinia enterocolitica* is comprised of a varied group of organisms representing multiple serotypes, biotypes and phage types. In humans, the most common syndromes of *Y. enterocolitica* infection are gastroenteritis, mesenteric lymphadenitis and terminal ileitis; other symptoms may include polyarthritits, erythema nodosum, septicemia and meningitis. Many of the food and environmental isolates differ in some biochemical characteristics from typical clinical strains re-

sponsible for human yersiniosis. In spite of the apparently frequent presence of *Y. enterocolitica* or related bacteria in foods, few documented outbreaks of foodborne infection have occurred in the United States. One thoroughly documented outbreak which occurred in New York was caused by consumption of contaminated chocolate milk (29). Among meat animals, swine are considered a potential source of clinically significant types of *Y. enterocolitica* (28). The epidemiology of *Y. enterocolitica* infection in humans is still not clear. Contaminated foods, contact with infected animals and contacts among people in an infected family are suggested as possible modes of transmission.

Recovery of *Y. enterocolitica* from Foods

Media and procedures used for the isolation of *Y. enterocolitica* from foods frequently are similar to or modifications of those used for the isolation of enteric bacteria from clinical specimens. Plating media include MacConkey agar, Salmonella-Shigella (SS) agar, lactose sucrose urea agar and desoxycholate citrate agar. Modifications of media for improved isolation of *Y. enterocolitica* include (11) MacConkey agar with Tween 80 (MT) and desoxyribonuclease agar with Tween 80 and sorbitol (DST). Isolation procedures frequently include an enrichment step generally in either (a) phosphate-buffered saline (PBS), phosphate buffer (PB), mannitol broth (MB) or bile enrichment medium (BEM) for various periods (sometimes several weeks) at refrigeration temperature or (b) magnesium chloride-malachite green-carbenicillin medium (MRB), selenite F broth or tetrathionate broth for 2-5 days at 22-25°C. Following enrichment, a loopful of the enrichment media is then streaked onto two or more of the selective media. Lactose-negative isolates are then submitted to a battery of biochemical tests which includes test for: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, motility (at 25 and 35°C), citrate, sucrose, maltose, urease, oxidase, and TSI.

Recent developments in the recovery of *Y. enterocolitica* from foods include (a) improved recovery of *Y. enterocolitica* from foods by application of a KOH treatment to an aliquot of the enrichment medium just before streaking on the selective agar (MacConkey) medium (1), (b) recovery of *Y. enterocolitica* from milk by enrichment in Butterfield's phosphate buffer for 14 days at 4°C, followed by further enrichment in modified Rappaport broth at 23°C for 5 days (22);

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Reciprocal Meat Conference Proceedings, Volume 33, 1980

plating was done on MT and DST agar, (c) rapid recovery of clinical strains from meats by enrichment in phosphate buffer with added malachite green, carbenicillin and selenite at 22°C for 2-3 days (12), with plating of the enrichment medium on MT, DST and BS agars, (d) development of a new plating medium (CIN agar) formulated on the basis of extensive growth and selective agent studies (21). Cefsulodin-Irgasan-Novobiocin agar contains peptone, yeast extract, mannitol, Na-pyruvate, NaCl, MgSO₄ 7H₂O, agar, Irgasan, bile salts, neutral red, crystal violet, cefsulodin, and novobiocin. *Yersinia enterocolitica* colonies on this medium have a deep red center with an outer translucent zone. Plate incubation is for 24 hr at 32°C or 48 hr at 22°C. Dudley and Shotts (4) reported on a Cellobiose-Arginine-Lysine (CAL) agar for the isolation of *Yersinia enterocolitica*. This medium contains cellobiose, L-arginine, L-lysine, yeast extract, NaCl, Na-desoxycholate, agar and neutral red. *Y. enterocolitica* colonies on this medium have a bright burgundy color or have a pin-point red center. In the CAL agar procedure, plates are incubated for 40 hr at 25°C.

Effect of Extrinsic Factors

In 1976, Hanna et al. (20) isolated *Y. enterocolitica* from 10 of 89 cuts of vacuum packaged beef and from 2 of 30 lamb cuts. The isolates were obtained only from samples stored for 21 to 35 days at 1-3°C and not from samples stored for 0, 7 or 14 days. Growth studies on raw and cooked beef and pork (19) showed that substantial increases in *Y. enterocolitica* counts occurred when inoculated samples were stored at 0-7°C (3-10 days) or at 25°C (within 24 hr). Increases in *Y. enterocolitica* counts on cooked samples stored at 25°C were somewhat greater than those of comparable raw products. Freezing caused large reductions in *Y. enterocolitica* counts when beef cuts inoculated at the surface were stored at -23°C (15). Reductions were greater when the samples were plated on a selective plating medium (bismuth sulfite agar) than on the non-selective tryptic soy agar. Considerable variation was observed in the heat resistance of several strains of *Y. enterocolitica* in skim milk; heating for 3-10 min at 55°C caused large reductions in count. With heating at 60°C, no survivors were detected after 1-3 min (17). Hanna et al. (15) reported that no survivors of *Y. enterocolitica* were detected in beef roasts inoculated with $3.1-3.8 \times 10^6$ cells per g when the final internal temperature in the center of the roast was 60 to 62°C; at 51°C some *Y. enterocolitica* survived. Growth of *Y. enterocolitica* at different pH values was determined at 25°C in brain heart infusion (BHI) broth (15). Growth was better at pH 7 and 8 than at 6 or 9 and little or no growth occurred at pH 5. Stern et al. (27) determined the effect of pH on growth of *Y. enterocolitica* in BHI broth. At 3°C, substantial increases in *Y. enterocolitica* count occurred in 10 days at pH 7.2, only a small increase in count occurred at pH 9. At pH 4.6 and at 9.6 the counts remained the same or decreased slightly. Seelye and Yearbury (25) reported higher levels of *Y. enterocolitica* on vacuum packaged beef of high pH (>6) than on samples of normal pH value. Incorporation of 7% NaCl in BHI broth caused a slight decrease in count over a 10-day period (27). Minor increases in count occurred with

5% NaCl; substantial increases in *Y. enterocolitica* count occurred with 0.5% NaCl in the broth medium.

To compare the growth of *Y. enterocolitica* on beef steaks stored in packages of different oxygen permeability, Hanna et al. (16) inoculated top and bottom round steaks with *Y. enterocolitica* and stored samples at 1-5°C for 21-35 days in vacuum packages and in polyvinyl chloride (PVC) film. *Yersinia enterocolitica* and total aerobic plate counts of steaks were consistently higher in the more oxygen-permeable film (PVC) than on those stored in the vacuum packages. These results indicate that vacuum packaging *per se* did not favor the development of *Y. enterocolitica*.

El-Zawahry and Rowley (5) reported on the radiation resistance of *Y. enterocolitica*. Their data indicate that *Y. enterocolitica* cells are among the most radiation sensitive of the foodborne bacteria. D values for 3 strains of *Y. enterocolitica* irradiated at 25°C in trypticase soy broth ranged from 9.7 to 11.8 krad. A dose of 200 krad at 5-25°C would have reduced *Y. enterocolitica* in meat by 10 log cycles.

Pathogenicity

Perhaps the most exciting aspect of research on this organism has been in the area of pathogenicity of various strains of *Y. enterocolitica* (3,7,13,30). Zink et al. (30) examined a group of strains associated with an outbreak of enteric disease in New York for heat-labile toxin (LT), heat-stable toxin (ST) production, tissue invasiveness (Sereny test) and plasmid DNA content. The group of strains included those of symptomatic and asymptomatic persons, from the incriminated chocolate milk, from dairy cattle and from water collected from a dairy farm. No strains produced LT whereas ST production was common. Only strains of serotype 0:8 were Sereny-test positive. All invasive strains contained a 41×10^6 - MW plasmid, but this plasmid was not found in non-invasive strains. Isolates derived from strain 54 after curing with ethidium bromide no longer contained plasmid DNA and were no longer tissue-invasive. More recent information (D. L. Zink, personal communication) indicates that this plasmid is associated with the VW antigen complex. Strains which produce the VW complex can be detected by placing them on magnesium oxalate agar. This medium inhibits growth at 37°C but not at 25°C. Strains without the 41×10^6 MW plasmid did not show this inhibition at 37°C. A recent report by Laird and Cavanaugh (9) indicates that virulent strains of *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* autoagglutinate in tissue culture media when grown at 36°C. Avirulent strains did not have this property. Virulence was tested by oral feeding of adult mice and was defined as the ability of a strain to induce diarrhea or to produce a fatal systemic infection or both.

In summary, rapid progress is being made in two important areas, which are: (a) isolation and identification of *Y. enterocolitica* from various foods and (b) development of model systems and tests to determine pathogenicity. New information undoubtedly will contribute to a better understanding of the role of *Y. enterocolitica* in foodborne illness and hopefully will reduce the number of disease outbreaks caused by this organism.

Discussion

D. Bartholomew, *Land O'Lakes*: On the toxin it (yersinia) produced, is this what caused the food illness?

C. Vanderzant: No, it is a highly invasive organism, it is invasive to the gastrointestinal tract. If you have a typical yersiniosis, it will start off through the lymphoid tissues surrounding the mesenteric lymph nodes and then in the terminal ileum it will have a rather severe infection so it invades the gastrointestinal tract if you have a typical human yersiniosis. Now on the other hand, I want to make it clear, that in addition to the typical human yersiniosis that, for example, Dr. Bottone at the Mt. Sinai Hospital in New York has found particularly in compromised patients many other symptoms, as you may call it, of yersinia infections: septicemia, liver infections, urinary infections, and so on. So yersinia can manifest themselves in many different ways.

D. Bartholomew: It must be a viable organism?

C. Vanderzant: Oh yes. At the present time, we know very little, if any, about toxins. If they exist.

D. Bartholomew: I also had a question about *Streptococcus bacillus*. I know in times past it had been implicated in some food illnesses. Has anything been done on this? I haven't read anything about as to why they had implicated it.

C. Vanderzant: *Streptococcus bacillus*, of course, is a very controversial organism. It has ranged all the way from use as a starter culture in cheese manufacture to human illnesses. In past AMSA meetings, I think the hottest debate usually occurred when we touched on *Streptococcus bacillus* and then we immediately got to enormous disagreements as to what constituted the pathogenicity and then to humans. But that is an extremely controversial area. It still has a tremendous cloud over it, or blanket of confusion. Your question, of course, raises all kinds of interesting things about why do we have all these reports on polyarthritis in Europe for example, and we have no reports, or very few reports of that, in the United States and Canada. Why do we have the differences in strains and in Europe primarily three and nine and in Canada three and in the United States 0:8. I think we should do a better job of looking for them. I think we will find three and nine in the United States also.

A. Nour, *Cornell University*: I realized in one of the slides, when you compare vacuum package against PVC, there is a slight drop in the vacuum packaged product. Would you just elaborate?

C. Vanderzant: Well, one of the slides shows a little bit more of valleys and mountains than some of the others and the reason for that, I think, was in part of the configuration or photography of the steaks themselves. In one instance we had a very difficult time sampling the surfaces and the other set of steaks we had a very smooth surface. I mention that in a paper that I published, I wasn't too happy. I would have liked to see a smoother curve there. But I attribute this primarily to experimental procedure, rather than speculate on something else. Yes, I am aware of that.

D. Fung, *Kansas State University*: How do you prevent contamination of the laboratory when you prepared the meat for vacuum packaging of yersinia enterocolitica? I was worried about that when I was trying to do experiments like that.

C. Vanderzant: Well, there are two things you have to do.

In the first place, you have to serotype and biotype even the control samples because you are never sure when you start off an experiment that you may not have natural yersinia there. So, what you have to do in the laboratory is to select strains that you can identify, very easily, from the rest and that can be done and done very easily. There is enough difference in biochemical types and serotypes and phage types that you can do that. Secondly, we use laminar flow cabinets, we do all the packaging under, you might say, rather primitive conditions, we use Millipore filters and everything else. We pull all the air through a disinfecting bath and so on. So, I agree with you. You don't go downstairs and run an experiment like that with a commercial vacuum packaging machine. This would give you nightmares, from the standpoint of trying to disinfect the equipment. Yes, I agree with you. We have thought about that for days. Even when we do the experiments in ovens with thermocouples, these thermocouples are not going to be used for anything anymore after they have been in the meat with yersinia—because I love my name on papers, but not in morbidity and mortality reports.

References

1. Aulisio, C. C. G., I. J. Mehlman and A. C. Sanders. 1980. Alkali method for rapid recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from foods. *Appl. Envir. Microbiol.* 39:135-140.
2. Bottone, E. J. 1977. *Yersinia enterocolitica*. A panoramic view of a charismatic microorganism. *CRC Crit. Rev. Microbiol.* 5:211-241.
3. Damare, J. M., R. E. Smith, M. E. Harris, R. W. Johnston and W. H. Lee. 1980. Detection of the virulence associated plasmid in *Yersinia enterocolitica* isolated from foods. *Proc. 1980 Ann. Meetings Amer. Soc. Microbiol.* p. 189 (P-2).
4. Dudley, M. V. and E. B. Shotts, Jr. 1979. Medium for isolation of *Yersinia enterocolitica*. *J. Clin. Microbiol.* 10:180-183.
5. El-Zawahry, Y. and D. B. Rowley. 1979. Radiation resistance and injury of *Yersinia enterocolitica*. *Appl. Envir. Microbiol.* 37:50-54.
6. Feeley, J. C., W. H. Lee and G. K. Morris. 1976. *Yersinia enterocolitica*. In: *Compendium of methods for the microbiological examination of foods*. M. L. Speck (ed.). APHA, Washington, D.C.
7. Forney, E. D. and I. J. Mehlman. 1980. A rapid method for recognition of invasive potential of *Yersinia enterocolitica*. *Proc. 1980 Ann. Meetings Amer. Soc. Microbiol.* p. 189 (P-3).
8. Highsmith, A. K., J. C. Feeley and G. K. Morris. 1976. Isolation of *Yersinia enterocolitica* from water. In: *Bacterial indicators/health hazards associated with water*. A. W. Hoadley and B. J. Dutka (eds.). ASTM, Philadelphia, Pa.
9. Laird, W. J. and D. C. Cavanaugh. 1980. Correlation of autoagglutination and virulence of *Yersinia*. *J. Clin. Microbiol.* 11:430-432.
10. Lee, W. H. 1977. An assessment of *Yersinia enterocolitica* and its presence in foods. *J. Food Prot.* 40:486-489.
11. Lee, W. H. 1977. Two plating media modified with Tween 80 for isolating *Yersinia enterocolitica*. *Appl. Envir. Microbiol.* 33:215-216.
12. Lee, W. H., M. E. Harris, D. McClain, R. E. Smith and R. W. Johnston. 1980. Two modified selenite media for the recovery of *Yersinia enterocolitica* from meats. *Appl. Envir. Microbiol.* 39:205-209.
13. Lee, W. H., R. E. Smith, J. M. Damare, M. E. Harris and R. W. Johnston. 1980. Evaluation of virulence test procedures for *Yersinia enterocolitica* isolated from foods. *Proc. 1980 Ann. Meetings Amer. Soc. Microbiol.* p. 189. (P-1).

14. Lee, W. H., C. Vanderzant and N. J. Stern. 1980. The occurrence of *Yersinia enterocolitica* in foods. In: *Yersinia enterocolitica* - a multifaceted human pathogen. E. J. Bottone (ed.). CRC Press (In Press).
15. Hanna, M. O., J. C. Stewart, Z. L. Carpenter and C. Vanderzant. 1977. Effect of heating, freezing and pH on *Yersinia enterocolitica* - like organisms from meat. *J. Food Prot.* 40:689-692.
16. Hanna, M. O., J. C. Stewart, Z. L. Carpenter and C. Vanderzant. 1977. Effect of packaging methods on the development of *Yersinia enterocolitica* on beef steaks. *J. Food Safety* 1:29-37.
17. Hanna, M. O., J. C. Stewart, Z. L. Carpenter and C. Vanderzant. 1977. Heat resistance of *Yersinia enterocolitica* in skim milk. *J. Food Sci.* 42:1134-1136.
18. Hanna, M. O., J. C. Stewart, Z. L. Carpenter, D. L. Zink and C. Vanderzant. 1979. Isolation and characteristics of *Yersinia enterocolitica* - like bacteria from meats. *Contr. Microbiol. Immunol.* 5:234-242.
19. Hanna, M. O., J. C. Stewart, D. L. Zink, Z. L. Carpenter and C. Vanderzant. 1977. Development of *Yersinia enterocolitica* on raw and cooked beef and pork at different temperatures. *J. Food Sci.* 42:1180-1184.
20. Hanna, M. O., D. L. Zink, Z. L. Carpenter and C. Vanderzant. 1976. *Yersinia enterocolitica* - like organisms from vacuum packaged beef and lamb. *J. Food Sci.* 41:1254-1256.
21. Schiemann, D. A. 1979. Synthesis of a selective agar medium for *Yersinia enterocolitica*. *Can. J. Microbiol.* 25:1298-1304.
22. Schiemann, D. A. and S. Toma. 1978. Isolation of *Yersinia enterocolitica* from raw milk. *Appl. Envir. Microbiol.* 35:54-58.
23. Schleifstein, J. L. and M. Clark. 1947. Interesting observations (*Bacterium enterocoliticum*), Annual Rpt. Albany Div. Health and Res. N.Y. State Dept. Health, pp. 71-73.
24. Schleifstein, J. L. and M. B. Coleman. 1939. An unidentified microorganism resembling *B. lignieri* and *Pasteurella pseudotuberculosis* and pathogenic for man. N.Y. State J. Med. 39:1749-1753.
25. Seelye, R. J. and B. J. Yearbury. 1979. Isolation of *Yersinia enterocolitica* - resembling organisms and *Alteromonas putrefaciens* from vacuum-packed chilled beef cuts. *J. Appl. Bacteriol.* 46:493-499.
26. Stern, N. J. and M. D. Pierson. 1979. *Yersinia enterocolitica*: A review of the psychrotrophic water and foodborne pathogen. *J. Food Sci.* 44:1736-1742.
27. Stern, N. J., M. D. Pierson and A. W. Kotula. 1980. Effects of pH and sodium chloride on *Yersinia enterocolitica* growth at room and refrigeration temperatures. *J. Food Sci.* 45:64-67.
28. Toma, S. and V. R. Deidrick. 1975. Isolation of *Yersinia enterocolitica* from swine. *J. Clin. Microbiol.* 2:478-481.
29. United States Department of Health, Education and Welfare. 1977. *Yersinia enterocolitica* outbreak - New York. *Morbidity and Mortality Weekly Report*, 26:53-54.
30. Zink, D. L., J. C. Feeley, J. G. Wells, C. Vanderzant, J. C. Vickery, W. D. Roof and G. A. O'Donovan. 1980. Plasmid - mediated tissue invasiveness in *Yersinia enterocolitica*. *Nature* 283:224-226.