Yersinia enterocolitica: a review of its role in food hygiene *

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Since Yersinia enterocolitica, *now classified as a member of the Enterobacteriaceae, was recognized as a distinct species in 1964 it has been isolated with increasing frequency from man and animals (including dogs and pigs) and from some human foods.* Y. enterocolitica *infections are now seen as a cause for some concern in both human and veterinary medicine. The organism is commonly found in specimens from swine slaughterhouses and has been isolated from samples of market meat, vacuum-packed beef, mussels, oysters, and ice-cream. It has also been found in nonchlorinated well water used for drinking purposes. Infections in man therefore probably have an alimentary origin. Only 23 human infections were recorded in 1966 but the number increased to over 4000 in 1974. However, reported incidence is affected by growing awareness about the role of the organism in human and animal disease and by intensive laboratory analyses. While knowledge about the geographical distribution of* Y. enterocolitica *is still fragmentary it is clear that infections are very frequent in some parts of the world and probably common but unrecognized in many countries. The most common symptoms of* Y. enterocolitica *infections in man are fever, abdominal pain, and diarrhoea. In the USA most isolations in human infections were made from blood and mesenteric lymph node samples. The pathogenic mechanism is not known. In one experiment involving a human volunteer subject a dose of* 3.5×10^9 *organisms was required to produce an infection. Only recently has some success been obtained in establishing experimental infections in mice, guinea-pigs, rats, and rabbits. Laboratory cultivation techniques for* Y. enterocolitica *are described together with a table of minimal tests for characterizing the organism and two biotyping schema. Little is known about methods for controlling this disease, but environmental hygiene and sanitation with regard to food and water should apply.*

THE STATUS OF THE ORGANISM

The frequency of isolation of *Yersinia enterocolitica* during recent years has increased dramatically, causing much concern. In 1939, Schleifstein & Coleman (*1*) isolated an unidentified microorganism pathogenic for man similar to *Bacterium liguieri* and *Pasteurella pseudotuberculosis*, and they called it *Bacterium enterocoliticum*. In 1949 Hässig et al. (*2*) isolated strains of bacteria which they identified as human *Pasteurella pseudotuberculosis*, partly because of the nature of the pathological changes in their patients. These Hässig strains were studied by Knapp & Thal, who found that they differed biochemically in many respects from *Pasteurella pseudotuberculosis* and concluded that they did not belong to that species (*3*).

In the early 1960s, clinical bacteriologists working in the fields of human and veterinary medicine in various countries obtained a number of new isolates of bacteria which they described as "*Pasteurella pseudotuberculosis*", "*Pasteurella pseudotuberculosis*-like organisms", and "*Pasteurella Y.*" Daniels & Goudzwaard described a similar strain as "*Pasteurella X*" (*4*). In 1964, Frederiksen found *Pasteurella X* and the Hässig strains to be similar to the strain isolated by Schleifstein & Coleman, and proposed a new name for the species—*Yersinia enterocolitica* (*5*). The organism is now considered as

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a member of the family Enterobacteriaceae and this classification is adopted in the latest edition of *Bergey's manual of determinative bacteriology* (*6*).

During the past 10 years increasing evidence has accumulated that *Yersinia enterocolitica* infections are very frequent in some parts of the world, and the infection is probably common but unrecognized in many other countries. Only 23 cases of *Y. enterocolitica* infection were recorded in 1966 but 642 cases were recorded in 1970, over 1000 in 1972, and over 4000 in 1974 (*7*, *8*). The increase in reported isolations is probably a result of greater awareness about this organism and about its potential role in human and animal disease.

FREQUENCY OF ISOLATION

The reported frequency of isolation of *Y. enterocolitica* is greatly influenced by the activities of a relatively few laboratory workers who look for these organisms in routine analyses of enteric specimens. This type of activity has resulted in a total of 108 cases of human *Y. enterocolitica* infections being recognized in one hospital in Montreal, Canada, since 1966 where previously none were reported (*9*).

The first isolate of *Y. enterocolitica* in Czechoslovakia was reported in 1963, but by the end of 1971 positive specimens were obtained from 65 out of 99 districts. Between 1963 and 1971 a total of 845 cases occurred in man (*10*). *Y. enterocolitica* infections are more frequent in children (commonly 3–5 years) than in adults. Esseveld & Goudzwaard (*11*) reported that there were 69 and 59 *Y. enterocolitica* infections in man in the Netherlands in 1970 and 1971, respectively. They reported that *Salmonella* species were isolated from about 10% of the faecal samples examined and *Y. enterocolitica* from about 1%. They also reported that about one-half of the *Y. enterocolitica* infections in humans caused illness without diarrhoea and that samples were not always sent for laboratory examination and therefore concluded that the number of *Y. enterocolitica* infections in man was probably about 20% of the number of *Salmonella* infections. The real number is difficult to estimate because a bacteriological examination is performed in only a small proportion of suspected cases.

Although human infections with *Y. enterocolitica* have now been observed in numerous countries in all parts of the world, knowledge about the geographical distribution of this organism is extremely fragmentary. The reason for this is probably that few laboratories use primary isolation techniques that would detect *Y. enterocolitica*. For reasons that are unexplained, reported incidence rates and distributions of serotypes seem to vary widely, and often abruptly, between neighbouring countries (*12*). For instance, the rate of infection in Belgium is high whereas that in France is low. Even within a given country there are striking regional differences in incidence

CLINICAL ASPECTS AND PATHOGENIC MECHANISMS

The distribution in the body of the sites of infection of *Y. enterocolitica* isolated in the USA during the period 1966–72 is shown in Table 1 (*13*). Most of the isolates were obtained from blood and mesenteric lymph nodes. Clinical symptoms of *Y. enterocolitica* enteritis are shown in Table 2 for outbreaks in Japan (*14*) and the USA (*15*). The most common symptoms were fever, abdominal pain, and diarrhoea.

The pathogenic mechanism of *Y. enterocolitica* is not known. There has been very little research to

Table 1. *Yersinia enterocolitica* isolations in the USA, October 1966 to March 1972, by site of infection [a]

Site	No.
Blood	6
Mesenteric lymph node	5
Stool	4
Eye	2
Abscess, abdominal	2
Abscess, colon	1
Abscess, neck	1
Abscess, spleen	1
Bile	1
Bowel	1
Peritoneal fluid	1
Skin infection	1
Sputum	1
Throat	1
Urine	1

[a] From Weaver & Jordon (*13*).

Table 2. Clinical manifestations of *Yersinia enterocolitica* enteritis

Symptom	Percentage of cases	
	Japan [a]	USA [b]
Fever	61	87
Diarrhoea	36	69
Abdominal pain	76	62
Vomiting	12	56
Pharyngitis	—	31
Headache	60	18
Malaise	33	—

[a] Data from Zen-Yoji et al. (*14*).
[b] Data from Gutman et al. (*15*).

indicate whether the organism is invasive, a toxin producer, or pathogenic in some other way. Although this organism has been isolated from animals, initial attempts to establish experimental infections were unsuccessful (*16*). Only recently has some success been achieved in mice, guinea-pigs, rats, and rabbits (*17–20*).

The pathogenicity of *Y. enterocolitica* appears to depend on the method of cultivation and the site of inoculation (*21*). Various strains may show temperature-dependent differences in pathogenicity and differences with respect to the bactericidal effect of normal serum (*22*). *Y. enterocolitica* infections usually involve the abdominal organs and frequently causes gastrointestinal symptoms.

EPIDEMIOLOGICAL CONSIDERATIONS

The clinical specimens yielding isolates has been a wide variety (Table 1). In addition to infection sites shown in Table 1, *Y. enterocolitica* has also been isolated from animals including dogs (*23*) and pigs (*11*, *24*) and from foodstuffs, such as ice-cream (*25*), mussels (*26*), and oysters (*27*). The organism is commonly found in specimens from swine slaughterhouses and has been isolated from samples of market meat (W. H. Lee, personal communication) and vacuum-packed beef (C. Vanderzant, personal communication). It has also been found in drinking-water (*28*), usually in nonchlorinated well water (*29*, *30*). Most water strains in the USA have been rhamnose-positive and non-typable. Those water strains that are typable are of serotypes not usually associated with human illness (*30*).

Very little is known about how *Y. enterocolitica* spreads; however, in one outbreak in Japan (*31*) a common source, possibly food, was implicated. A food source was also implicated an outbreak in Czechoslovakia in which the food may have been contamined by a food handler (*32*). It is generally felt that the disease probably has a faecal origin. Hospital outbreaks and family and interfamilial outbreaks suggest that transmission through personal contact may occur.

Data for the number of *Y. enterocolitica* organisms required to cause human disease are very limited. A dose of 3.5×10^9 organisms was required to produce an infection in one experiment with a human volunteer subject (*33*).

CHARACTERIZATION OF THE ORGANISM IN FOOD

Y. enterocolitica survives very well in nature, especially at low temperatures. The organism will grow at 4°C, and this unusual characteristic is used in the bacteriological isolation schema.

LABORATORY METHODOLOGY

A suspect food sample is usually added to buffered saline in a proportion of 1 : 9, and this preparation held at 4°C for several weeks. At weekly intervals the suspension is streaked on to plating media. *Y. enterocolitica* tolerates high concentrations of bile salts, and bacteriological media such as SS (salmonella–shigella) agar and MacConkey's agar are good isolation media for this organism. For faecal specimens the cold enrichment procedure may be used, and in addition they may be inoculated directly on to plating media.

Incubation temperatures of 22–29°C have been shown to be optimal. At these temperatures colonies are visible after 24 hours, but plates are usually incubated for 48 hours before colonies are selected for identification. It should be pointed out that the incubation temperature must be below 37°C because *Y. enterocolitica* grows poorly on these selective media at that temperature. Suspect isolates from plating media are screened in triple sugar iron agar (TSI), motility agar, and urea agar. The TSI reactions at 24 hours are acid slant, acid butt, with no gas and no H_2S. Reversion of the slant may occur at 25°C

Table 3. Reactions of *Yersinia enterocolitica* and other closely related bacteria [a]

Tests	*Yersinia enterocolitica*	*Yersinia pestis*	*Yersinia pseudotuberculosis*	*Vibrio cholerae*	*Aeromonas hydrophila*	*Serratia*	*Enterobacter*	*Citrobacter diversus*	*Klebsiella*	*Proteus morganii*	*Proteus rettgeri*	*Providencia*	*Chromobacter violaceum*
Oxidase	−	−	−	+	+	−	−	−	−	−	−	−	−(W+)
Christensen's urea	+	−	+	−	−(+)	−(+)	−(+)	+,−	+,−	+	+	−	−(L+)
Lactose	*	−	−	+(L)	−(+)	−(+)	+(−)	+,−	+(−)	−	−	−	−
Maltose	+(L)	+	+	+	+	+	+(L)	+	+	−	−	−	−
Sucrose	+	−	−	+	+(−)	+	+(−)	+,−	+(−)	−	−(+)	+L(−)	+,−
Simmon's citrate	−	−	−	+(−)	+(−)	+	+	+	+,−	−	+	−	+(L)
Motility, 22°C	+	−	+	+	+	+	+	+	−	+	+	+	+
Motility, 36°C	−	−	−	+	+	+	+	+	−	+	+	+	+
Arginine dihydrolase	−	−	−	−	+	−	−(+)	+	−	−	−	−	+
Lysine decarboxylase	−	−	−	+	−(+)	+	+(−)	−	+,−	−	−	−	−
Ornithine decarboxylase	+	−	−	+	−	+	+(−)	+	−	+	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	+,−	−	−	+	+	+	−

[a] Tests performed at 36°C unless otherwise indicated. L, late; W, weak; ( ), minority of reactions; *, negative with enteric base medium, positive with O-F (Hugh-Leifson) medium.

because of very rapid fermentation of sucrose followed by oxidative degradation of the peptones. *Y. enterocolitica* is non-motile at 37°C but is motile at 25°C. The organism is urea positive.

Minimal tests for the characterization of *Y. enterocolitica* are shown in Table 3. Two biotyping systems have been developed, Wauter's schema (*25*) and Niléhn's schema (*34*). These are set out in Table 4. A serotyping schema of 34 antisera is used for the serological identification of the somatic "O" antigens (*35–38*). Cross-reactions with other genera occur frequently (*39–41*).

A serotyping system utilizing H-antigens has been developed but is not commonly utilized because the H-serotypes are frequently associated with specific O-serotypes and for the most part offer no additional advantage over O-serotyping (*37*).

There are numerous reports in the European literature of *Y. enterocolitica* infection being documented by examination of sera. While some investigators have used an indirect haemagglutination test (*15*, *32*, *42*), most have used an agglutination test similar to that of Winblad et al. (*43*), who used both an autoclaved " O " antigen and a formolized " OH " antigen (*44*).

Some investigators have found nonspecific, positive seroreactions with the " OH " antigen (*45*). In contrast, titres of $\geqslant$1/160 with the " O " antigen appear to be reliable for diagnosis in Europe, where only two serotypes, 3 and 9, account for the majority of cases. This is not the situation in the USA, where multiple serotypes prevail and not just serotype 8 that first appeared to account for most of the cases (*14*). For this reason, serological diagnosis in the USA has been limited to specific outbreaks where an antigen has been prepared from the epidemic strain.

A phage typing system has been developed and has been used to distinguish between European and Canadian sources of serotype 0:3 strains (*46*, *47*).

CONTROL MEASURES

Very little work has been carried out on methods of controlling this disease. This area of knowledge cannot be developed further until the means through which *Y. enterocolitica* is spread are understood. *Y. enterocolitica* is widespread in nature in both living and non-living systems. Therefore, general techniques of environmental hygiene and sanitation food and water should apply in controlling disease caused by this organism.

Table 4. Biotype schema for *Yersinia enterocolitica*

Tests [a]	Biotypes									
	Wauter's [b]	1	2		3		4		5	
	Niléhn's [c]	1	2		3		4		5	
Salacin		+	—		—		—		—	
Esculin		+	—		—		—		—	
Lecithinase	+		—		—		—		—	
Indole	+	+	+	+	—	—	—	—	—	—
Lactose (O-F)	+	+	+	+	+	+	—	—	—	—
Xylose	+	+	+	+	+	+	—	—	—	—
Nitrate	+	+	+	+	+	+	+	+	—	—
Trehalose	+	+	+	+	+	+	+	+	—	—
β-galactosidase	+	+	+	+	+	+	+	+	—	—
Ornithine decarboxylase	+	+	+	+	+	+	+	+	—	—
Voges-Proskauer		+		+		+[d]		+		—
Sorbose		+		+		+		+		—[d]
Sorbitol		+		+		+		+		—
Sucrose		+		+		+		+		—[d]

[a] Blank spaces in the table indicate tests that are not carried out in the indicated schema.

[b] For Wauter's schema biochemicals are incubated at 25°C, except indole, which is incubated at 29°C.

[c] For Niléhn's schema, biochemicals are incubated at 37°C, except lactose (O-F), ornithine decarboxylase, Voges-Proskauer, β-galactosidase, and sucrose, which are incubated at 25°C.

[d] Reactions may vary for specific strains. For practical purposes, results should be recorded after 7 days even though the authors may have incubated the tests for longer periods.

RÉSUMÉ

YERSINIA ENTEROCOLITICA: SON RÔLE EN HYGIÈNE ALIMENTAIRE

C'est en 1974 qu'on a reconnu *Yersinia enterocolitica* comme une espèce distincte, classée maintenant parmi les Entérobactériaceae. Depuis lors, ce germe a été isolé avec une fréquence croissante de l'homme et des animaux (y compris le chien et le porc) ainsi que de certains aliments humains. Les infections à *Y. enterocolitica* sont maintenant considérées comme assez préoccupantes en médecine humaine et vétérinaire. Ce micro-organisme est souvent trouvé dans des spécimens provenant d'abattoirs de porcs et il a été isolé à partir d'échantillons de viande sur des marchés, de bœuf emballé sous vide, de moules, d'huîtres et de crème glacée. On l'a découvert également dans de l'eau de puits non chlorée utilisée pour la boisson. Chez l'homme, l'infection est donc probablement d'origine alimentaire. En 1966, on avait enregistré 23 infections humaines seulement mais le nombre en est passé à 4000 en 1974. Dans l'interprétation de cet accroissement, il faut néanmoins tenir compte du fait que l'on est plus attentif au rôle pathogène de ce germe chez l'homme et les animaux, et qu'il fait l'objet de recherches intensives au laboratoire. La connaissance de la distribution géographique de *Y. enterocolitica* est encore fragmentaire, mais il est clair que les infections sont très fréquentes dans certaines parties du monde, et probablement courantes mais méconnues dans bien des pays. Les symptômes les plus communs des infections à *Y. enterocolitica* chez l'homme sont: fièvre, douleurs abdominales et diarrhée. Aux Etats-Unis d'Amérique, la plupart des isolements obtenus au cours d'infections humaines l'ont été à partir d'échantillons de sang et de ganglions lymphatiques mésentériques. La pathogénie de la maladie n'est pas connue. Dans une épreuve portant sur un volontaire, il a fallu une dose de $3,5 \times 10^9$ germes pour produire une infection. Ce n'est que récemment qu'on est parvenu à établir des infections expérimentales chez la souris, le cobaye, le rat et le

lapin. Le présent article décrit les techniques de culture de *Y. enterocolitica* au laboratoire et présente un tableau des épreuves minimales permettant de caractériser le micro-organisme, de même que deux schémas de classification en biotypes. On sait peu de chose des méthodes de lutte contre la maladie, mais les mesures d'hygiène du milieu et d'assainissement en ce qui concerne les aliments et l'eau sont indubitablement applicables.

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