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Molecular Epidemiology of *Yersinia enterocolitica* O:3 Infections: Use of Chromosomal DNA Restriction Fragment Length Polymorphisms of rRNA Genes

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***Yersinia enterocolitica* is a major enteric pathogen associated with a wide variety of clinical and immunologic manifestations, including transfusion-associated disease, from which there is a high mortality. Although previously rare in the United States, in the late 1980s *Y. enterocolitica* O:3 emerged as the predominant serotype in the United States, as it has been in Canada, Europe, and Japan. Epidemiologic investigation of this serogroup has been hampered by the limited availability of a phage typing system and the fact that *Y. enterocolitica* harbors few plasmids that are useful as strain markers. We therefore analyzed whole-cell DNA restriction fragment length polymorphisms of rRNA genes (ribotyping) to study a group of 61 (50 human, 11 porcine) *Y. enterocolitica* isolates. Initially, 20 different restriction enzymes were used; *NciI* appeared to give the best discrimination of hybridization banding patterns (ribotypes) within *Y. enterocolitica* O:3. Ribotyping distinguished seven clones among all the study isolates and four clones within *Y. enterocolitica* O:3 (53 isolates studied) and clearly differentiated *Y. enterocolitica* O:3 from *Y. enterocolitica* O:9; O:1,2,3; O:20; and O:5,27. Most serogroup O:3 isolates belonged to two clones, ribotypes I and II, including 23 of 24 *Y. enterocolitica* O:3 (13 human, 11 porcine chitterling) isolates recovered from a recent outbreak of *Y. enterocolitica* in children in Atlanta associated with chitterling preparation and 3 transfusion-associated O:3 isolates from the United States. *Y. enterocolitica* O:3 ribotypes I and II were also isolated in Japan, ribotypes II and IV were isolated in Belgium, and ribotype I was isolated in Canada. Ribotype patterns I and II corresponded to phage types 9b and 8, respectively. Ribotyping was able to distinguish individual strains of *Y. enterocolitica* O:3, but suggests that a limited number of clones have disseminated within the United States and globally. The finding of identical ribotype patterns in chitterling and human specimens from the Atlanta outbreak supports epidemiologic evidence that swine were the source of infection and a major reservoir for *Y. enterocolitica* O:3.**

Yersinia enterocolitica has emerged as a worldwide pathogen associated with a wide variety of clinical and immunologic manifestations (14). Clinical manifestations include enterocolitis, which is the most common presentation and occurs most often in young children and older adults; a pseudoappendicular syndrome, which occurs primarily in older children and young adults; focal infections such as abscess formation; and bacteremia, which is most commonly seen in patients with predisposing conditions such as iron overload states (8). Recently, *Y. enterocolitica* sepsis associated with the transfusion of contaminated erythrocytes has been reported in the United States and several European countries (6, 11, 37). These transfusion-related cases have been associated with a high mortality rate, and the majority of cases have been due to *Y. enterocolitica* serotype O:3. In addition, postinfectious complications of *Y. enterocolitica*, which are thought to be immunologically mediated, include erythema nodosum and reactive arthritis (14, 27).

Y. enterocolitica O:3 has been the serotype most commonly isolated in Canada, Japan, and Europe (13, 16, 24, 38). Before 1978, however, *Y. enterocolitica* O:3 was rarely isolated in the United States. Between 1970 and 1980, only 1 of 100 *Y. enterocolitica* isolates from humans in the United States sent to the Centers for Disease Control (CDC) for identification or confirmation was serotype O:3 (20). Serotype O:8 was the predominant serotype in the United States during that period. However, in the mid to late 1980s, *Y. enterocolitica* O:3 emerged as the predominant serotype in the United States (5, 10, 22). The first cases of *Y. enterocolitica* O:3 in the United States were documented in the New York City area in 1978 and 1979 by Bottone and colleagues (9, 10), and the number of cases of *Y. enterocolitica* O:3 increased in New York in the early 1980s. Reports from CDC (22) and the California Department of Health (5) indicate a dramatic increase in serotype O:3 human isolates in the United States in the mid to late 1980s. A recent outbreak of *Y. enterocolitica* O:3 infection among young children in Atlanta associated with the household preparation of chitterlings (22) and a recent case-control study in Belgium (35) have suggested that swine may be a major reservoir of *Y. enterocolitica* O:3.

Epidemiologic investigations of *Y. enterocolitica*, and the O:3 serotype in particular, have been hampered by the limited availability of a phage typing system and the fact that *Y. enterocolitica* harbors few plasmids that are useful as epidemic strain markers; most *Y. enterocolitica* isolates contain only a single 40- to 50-MDa virulence plasmid (14, 25). We therefore explored the use of DNA restriction fragment length polymorphisms of rRNA genes (ribotyping) for molecular subtyping of *Y. enterocolitica*. This method uses readily available reagents, supplies, and equipment and

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has proved to be a useful molecular epidemiologic tool in the study of a number of other bacterial pathogens, including *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Campylobacter* species, *Haemophilus influenzae*, *Pseudomonas cepacia*, and *Providencia stuartii* (2, 7, 15, 17, 18, 21, 23, 28, 34). Ribotyping was used in our study in an effort to address a number of questions, including whether isolates from the Atlanta outbreak of *Y. enterocolitica* O:3 were alike and whether chitterling isolates were similar to serotype O:3 isolates recovered from humans. We also explored questions on the origins of *Y. enterocolitica* O:3 in the United States, such as how isolates recovered in the United States compared with those from Canada, Japan, and Europe.

MATERIALS AND METHODS

Bacterial isolates. A total of 61 *Y. enterocolitica* isolates (50 human and 11 porcine) were studied and are listed in Table 1. The isolates studied were obtained from the collection of the *Yersinia* Reference Laboratory at CDC. The porcine isolates were recovered from chitterling specimens purchased in Atlanta but packed at locations throughout the United States. Isolates recovered from patients in Atlanta were collected during an outbreak of *Y. enterocolitica* O:3 infection among children (22).

Serotyping. Serotyping was performed in the *Yersinia* Reference Laboratory at CDC by the slide agglutination test with 12 O-antiserum specimens (O:1,2,3; O:3; O:4; O:5; O:6,30; O:7,13; O:8; O:9; O:13a,13b; O:18; O:20; and O:21).

Plasmid analysis. Plasmid DNA analysis was performed on eight isolates by the method of Birnboim and Doly (4).

Phage typing. Phage typing was performed on selected isolates by the International *Yersinia* Reference Center (Pasteur Institute, Paris, France). Some of the phage typing and plasmid data have been reported previously (22).

DNA preparation, digestion, and separation of fragments. Chromosomal DNA was extracted as described by Pitcher et al. (30) by using guanidium thiocyanate for cell lysis. For this procedure, organisms were grown overnight in L broth at 37°C with aeration. The DNAs of selected isolates were digested with 20 different restriction endonucleases (*Cla*I, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, *Eco*RV, *Bam*HI, *Kpn*I, *Pst*I, *Sal*I, *Sma*I, *Nco*I, *Hin*cII, *Hae*II, *Bgl*II, *Dpn*I, *Ava*I, *Pvu*I, *Hha*I, and *Nci*I) according to the recommendations of the manufacturer. In addition, double digestion with *Hin*cII and *Pvu*II was performed. Enzymes were purchased from New England BioLabs (Beverly, Mass.). *Nci*I (which appeared to give the best discrimination within *Y. enterocolitica* O:3 by analysis of restriction fragment length polymorphisms of rRNA genes) was used to digest DNA from all isolates in the study collection. Two micrograms of DNA was digested with *Nci*I (1 μl, or 16 U) for 2 h at 37°C in a 20-μl reaction mixture; an additional 1 μl (16 U) of restriction enzyme was then added, and the reaction mixture was incubated for 2 h more. For *Nci*I, 2 μl of enzyme buffer (6 mM NaCl, 6 mM Tris hydrochloride, 6 mM $MgCl_2$, and 6 mM β-mercaptoethanol) was used per reaction mixture. Following restriction, samples were heated to 65°C for 10 min and cooled on ice for 5 min, and 5 μl of type II gel loading buffer (31) was added to each sample. The digested DNA was electrophoresed in a 1% agarose horizontal gel at 65 V for 16 h in Tris-acetate buffer (0.04 M Tris-acetate, 0.002 M EDTA [pH 8.1]). After electrophoresis, the gels were stained in ethidium bromide (1 μg/ml) and photographed under UV light. A 1-kb ladder (1 μg) and lambda phage DNA (2 μg; Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were used as molecular mass standards.

Preparation of Southern blots. Digested and electrophoresed DNA restriction fragments were transferred to a nylon membrane (MSI magnagraph; MSI, Westboro, Mass.) by the method of Southern (33). On completion of the DNA transfer, nylon membranes were rinsed briefly in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), air-dried for 30 min, and then baked for 2 h at 80°C and stored at 4°C until use.

Preparation of labeled probe and hybridization. *Escherichia coli* 16S plus 23S rRNA (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was used as the probe. Hybridization was initially performed with ^{32}P-labeled probe and was recorded by autoradiography as described previously (15, 21). The majority of the hybridization experiments were carried out with a nonradioactive labeling system (Genius System; Boehringer Mannheim Biochemicals) by using digoxigenin-modified dUTP incorporated into the probe, which is made complementary to the rRNA by reverse transcriptase, as described recently (39; instructions of the manufacturer). Labeled fragments were detected by use of an enzyme-labeled monoclonal antibody directed against the modified base according to the instructions of the manufacturer (Genius System; Boehringer Mannheim Biochemicals). No differences in hybridization banding patterns were observed when the radiolabeled probe was used compared with that when the digoxigenin-labeled rRNA probe was used (data not shown).

RESULTS

A total of 61 *Y. enterocolitica* isolates were studied by restriction endonuclease analysis of chromosomal DNA (REAC) and by analysis of DNA restriction fragment length polymorphisms of rRNA genes (ribotyping; Table 1). A total of 27 human isolates from the United States; 10 human isolates from Belgium; 5 human isolates from Canada; 4 human isolates from Japan; and 1 human isolates each from the Republic of Korea, Taiwan, Egypt, and Chile were studied. Thirteen of the U.S. human isolates and 11 porcine chitterling isolates were obtained during an investigation of an outbreak of *Y. enterocolitica* O:3 infection that occurred primarily among infants residing in Atlanta and that was associated with the household preparation of chitterlings (22). A total of 18 of these 24 isolates and an additional 4 transfusion-associated isolates from the United States were also phage typed (Table 1). Phage typing revealed that the *Y. enterocolitica* O:3 isolates were in phage groups 8 and 9b; serotype O:1,2,3 isolates typed to phage group 10z (22).

Chromosomal DNAs of selected *Y. enterocolitica* O:3 isolates were initially digested with 20 different restriction enzymes. *Nci*I appeared to give the best discrimination within the O:3 serotype (data not shown) and was used for all study isolates. Representative REAC patterns are shown in Fig. 1A. Digestion with *Nci*I yielded a good distribution of restriction length fragments. Results of REAC correlated fairly well with the ribotype pattern of each isolate, as illustrated in Table 1. In some instances, however, *Y. enterocolitica* O:3 isolates with the same REAC pattern could be differentiated by ribotyping. For instance, isolates 362-80, 594-81, and 9029-86, which all had REAC pattern B, had three different ribotype patterns (I, II, and IV, respectively). In addition, several isolates had chromosomal digestion patterns which had minor deviations from a particular

TABLE 1. Sources and phenotypic and genotypic characteristics of the *Y. enterocolitica* isolates used in this study[a]

Isolate	Serotype	Phage	Source	REAC pattern	Ribotype	Plasmid (MDa)
2410-89	O:3	9b	ATL pt, stool	A[e]	I	42
2412-89	O:3	9b	ATL pt, stool	A	I	
2413-89	O:3	9b	ATL pt, stool	A	I	42
2416-89	O:3	ND	ATL pt, stool	A	I	
2574-88	O:3	9b	ATL pt, stool	A	I	
2577-88	O:3	9b	ATL pt, stool	A	I	42
2583-88	O:3	9b	ATL pt, stool	A	I	
2401-89	O:3	9b	Brand A chit	A	I	42
C2154	O:3	ND	Brand B chit	A	I	42
C2155	O:3	9b	Brand B chit	A	I	
C2156	O:3	9b	Brand E chit	A	I	
C2262	O:3	9b	Brand C chit	A	I	42
C2264	O:3	9b	Brand C chit	A	I	
C2267	O:3	9b	Brand C chit	A	I	
C2268A	O:3	ND	Brand A chit	A	I	
C3059	O:3	ND	Brand A chit	A	I	
2453-87	O:3	9b	Transfusion (Wisconsin)	A	I	
2608-87	O:3	9b	Transfusion (Texas)	A	I	
2409-88	O:3	8	ATL pt, blood	B	II	
2518-88	O:3	8	Transfusion (Iowa)	B	II	42
2573-88	O:3	8	ATL pt, stool	B	II	42
2575-88	O:3	8	ATL pt, stool	B	II	
2576-88	O:3	8	ATL pt, stool	B	II	
C3063	O:3	ND	Brand D chit	B	II	
2408-89	O:3	9b	ATL pt, stool	A[e]	III	
3084-85	O:3	ND	Florida	A	I	
2426-89	O:3	ND	Florida	A	I	
3541-87	O:3	ND	Utah	A	I	
2495-88	O:3	ND	Pennsylvania	A	I	
2501-89	O:3	ND	New Jersey	A	I	
2442-89	O:3	ND	South Dakota	A	I	
2569-90	O:3	ND	Georgia	A	I	
77-3460	O:3	ND	Canada	A	I	
77-3461	O:3	ND	Canada	A	I	
79-1754	O:3	ND	Canada	A	I	
79-3638	O:3	ND	Canada	A	I	
79-4442	O:3	ND	Canada	A	I	
599-80	O:3	ND	Republic of Korea	B	I	
362-80	O:3	ND	Taiwan	B	I	
594-81	O:3	ND	Egypt	B	II	
2533-87	O:3	ND	Chile	B	II	
2485-87	O:3	ND	Japan	B	I	
2486-87	O:3	ND	Japan	B[e]	II	
2487-87	O:3	ND	Japan	B[e]	II	
2488-87	O:3	ND	Japan	B[e]	II	
9020-86	O:3	ND	Belgium	B	II	
9022-86	O:3	ND	Belgium	B	II	
9023-86	O:3	ND	Belgium	B	II	
9024-86	O:3	ND	Belgium	B[e]	II	
9026-86	O:3	ND	Belgium	B[e]	II	
9028-86	O:3	ND	Belgium	B[e]	II	
9029-86	O:3	ND	Belgium	B	IV	
9031-86	O:3	ND	Belgium	B[e]	II	
2461-89	O:20	ND	Transfusion (Georgia)	C	V	
2403-89	O:1,2,3	10z	ATL pt, stool	B[e]	VI	
C2268B	O:1,2,3	10z	Brand A chit	B[e]	VI	
2455-87	O:1,2,3	10z	Transfusion (Illinois)	D	VI	
2461-87	O:1,2,3	ND	Belgium	D	VI	
2516-89	O:9	ND	Belgium	E	VI	
2415-89	O:5,27	ND	Pennsylvania	F	VII	
2418-89	O:5,27	ND	Pennsylvania	F	VII	

[a] REAC, restriction endonuclease analysis of chromosomal DNA; chit, chitterling; ND, not done; superscript e, strains showing minor deviation from a particular REAC pattern; ATL pt, human isolate from a patient in Atlanta (these isolates were from an outbreak of *Y. enterocolitica* O:3 which has been reported previously [22]); transfusion, isolate from a transfusion-related case of sepsis recovered from the donor, recipient, or transfusion blood bag. All isolates were from human sources except the porcine chitterling isolates. Porcine isolates were recovered from chitterlings purchased in Atlanta but packed in other states, as follows: brand A, Virginia; brand B, Mississippi; brand C, Nebraska; brand D, Texas; Brand E, Michigan.

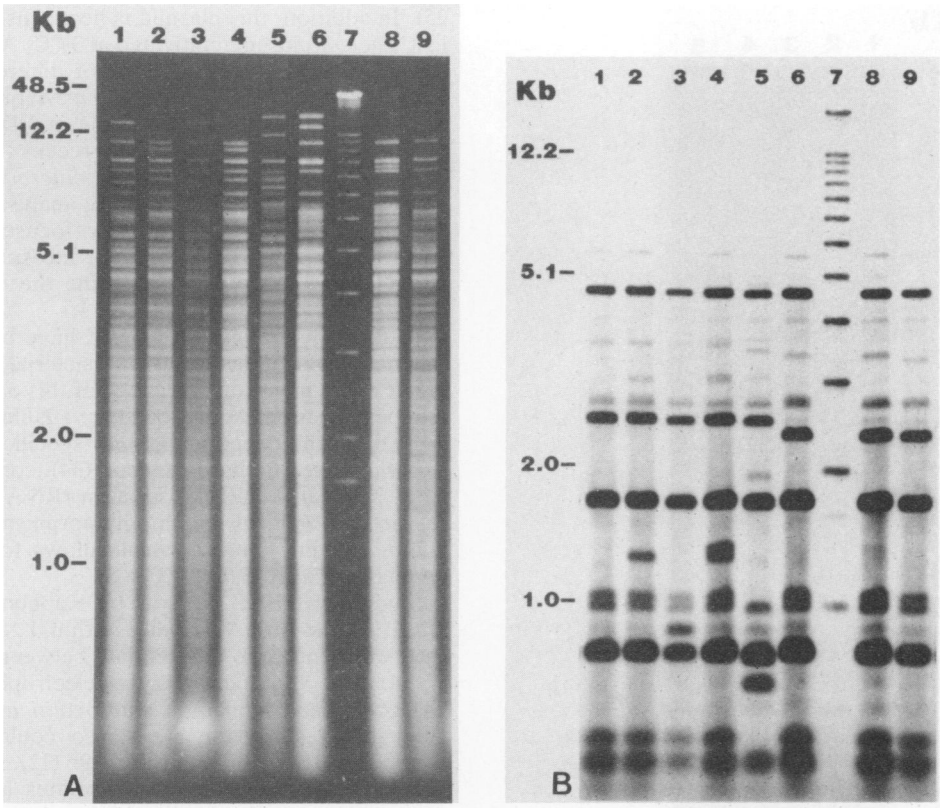

FIG. 1. (A) Restriction endonuclease (*NciI*) digestion of *Y. enterocolitica*. Representative examples of the REAC patterns listed in Table 1 are shown. Lane 1 (isolate C2262), pattern A; lane 2 (2409-89), pattern B; lane 3 (2408-89), pattern A^e; lane 4 (9029-86), pattern B; lane 5 (2461-89), pattern C; lane 6 (2455-87), pattern D; lane 7, molecular mass marker (lambda phage DNA and 1-kb ladder); lane 8 (2516-89), pattern E; lane 9 (2418-89), pattern F. The superscript e indicates strains showing a minor deviation from a particular REAC pattern. (B) Ribotyping of *Y. enterocolitica*. Examples of the ribotype patterns are shown. Lanes correspond to those in panel A. Lane 1 (isolate C2262), pattern I; lane 2 (2409-89), pattern II; lane 3 (2408-89), pattern III; lane 4 (9029-86), pattern IV; lane 5 (2461-89), pattern V; lane 6 (2455-87), pattern VI; lane 7, molecular mass marker (1-kb ladder); lanes 8 (2516-89), pattern VI; and lane 9 (2418-89), pattern VII. Isolates in lanes 1 through 4 are serotype O:3, the isolate in lane 5 is serotype O:20, the isolate in lane 6 is serotype O:1,2,3, the isolate in lane 8 is serotype O:9, and the isolate in lane 9 is serotype O:5,27.

REAC pattern (Fig. 2 and Table 1). Because of the difficulty in analyzing restriction digest patterns because of the large number of bands (34), our interpretations and conclusions were based primarily on ribotyping data.

Examination of the probed *NciI* restriction fragments revealed seven ribotypes among the 61 *Y. enterocolitica* isolates studied (Fig. 1B). Ribotyping clearly differentiated *Y. enterocolitica* O:3 from the other serotypes studied, *Y. enterocolitica* O:1,2,3; O:9; O:20; and O:5,27 (Fig. 1B). Ribotyping distinguished four different clones or patterns within *Y. enterocolitica* O:3, designated ribotype patterns I, II, III, and IV. All but two of the serotype O:3 isolates belonged to ribotype patterns I and II. Ribotype I isolates corresponded to phage type 9b, while those of ribotype pattern II corresponded to phage type 8. The *Y. enterocolitica* O:3 isolates collected during the Atlanta outbreak belonged to ribotype groups I and II, with the exception of one patient isolate (2408-89), which was phage type 9b and which belonged to ribotype pattern III. Transfusion-related isolates also belonged to ribotypes I and II. Seven other human isolates from six U.S. states belonged to the ribotype pattern I group (Table 1). Ribotype pattern I was seen in all Canadian *Y. enterocolitica* O:3 isolates, whereas ribotypes I and II were seen in Japanese isolates and ribotype pattern II was seen in seven of eight Belgium serogroup O:3 human isolates. The remaining *Y. enterocolitica* O:3 isolate from Belgium had ribotype pattern IV.

Eight non-O:3 *Y. enterocolitica* isolates were also studied. These isolates yielded three different ribotype patterns (V, VI, and VII), which were clearly distinct from those of serotype O:3 (Fig. 1B). REAC and ribotyping data showed distinct restriction fragment length polymorphism patterns for *Y. enterocolitica* O:20 (patterns C and V, respectively). The single serotype O:9 isolate (2516-89) had the same ribotype pattern as the serotype O:1,2,3 isolates (pattern VI); serotype O:5,27 isolates had a similar but different hybridization banding pattern (ribotype pattern VII). Differences between serotype O:1,2,3; O:9; and O:5,27 isolates were clearly noted by REAC (Fig. 1A), suggesting that REAC may be more sensitive than ribotyping in distinguishing among some non-O:3 *Y. enterocolitica* isolates.

Plasmid analysis was carried out on eight *Y. enterocolitica* O:3 strains collected during the Atlanta outbreak (six isolates with ribotype pattern I, phage 9b, and two isolates with ribotype pattern II, phage 8), as noted in Table 1. All isolates contained a single 42-MDa plasmid (data not shown). Plasmid analysis was unable to discriminate among these *Y. enterocolitica* O:3 isolates.

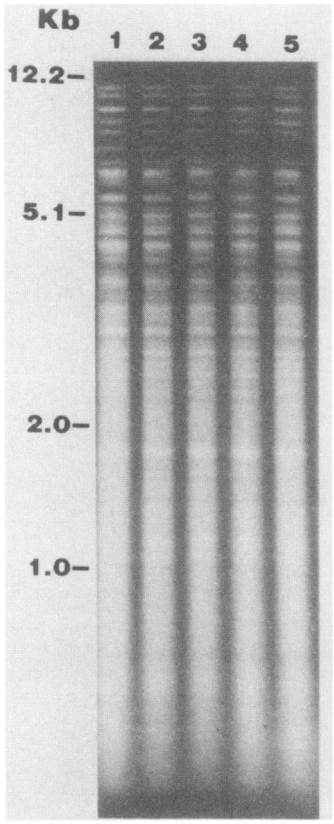

FIG. 2. Restriction endonuclease (*Nci*I) digestion of *Y. enterocolitica* O:3. Examples of REAC pattern B and isolates with minor variations from this pattern are shown. Lanes 1 and 2, isolates with REAC pattern B; lanes 3 through 5, isolates with REAC patterns with minor deviation from REAC pattern B (denoted B^c in Table 1). All isolates had the same hybridization pattern, ribotype II. Lane 1, isolate 9022-86; lane 2, isolate 9023-86; lane 3, isolate 9024-86; lane 4, isolate 9026-86; lane 5, isolate 9028-86.

DISCUSSION

Previously, *Y. enterocolitica* strain differentiation has relied on phenotypic characteristics such as biochemical profiles (biotyping), antibiotic resistance patterns, serotyping, and phage typing (19, 26, 41). Phage typing of *Y. enterocolitica*, however, is not widely available (no centers in the United States, for instance, have the capability to do phage typing), and reagents are not commercially available. These phenotypic methods have additional disadvantages since they rely on characteristics which may not be stably expressed or sensitive enough to distinguish each strain within a species (34, 40). Recent advances in DNA technology have provided methods based on genotypic characterizations, which lessen the dependence on phenotypic determinations. Such techniques include plasmid analysis, REAC, ribotyping, and pulsed-field gel electrophoresis (1, 34, 39, 40). In addition, non-DNA-based techniques, such as multilocus enzyme electrophoresis analysis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, have been used to study the molecular epidemiology of bacterial infections (18, 32).

Plasmid analysis, however, has not been a particularly useful molecular epidemiologic tool in the study of *Y. enterocolitica* infections because most pathogenic isolates contain only a single 40- to 50-MDa virulence plasmid (20, 25). In addition, this plasmid is heat sensitive and is usually lost when strains are cultivated at 37°C. As illustrated in this study, plasmid analysis could not differentiate among isolates which were different by phage typing and ribotyping. Restriction enzyme analysis of plasmid DNA has been used to differentiate strains (25), but a recent study found little or no variation of patterns within *Y. enterocolitica* O:3 isolates determined by restriction enzyme analysis of plasmid DNA (19). In addition, plasmid analysis focuses on extrachromosomal DNA and, thus, does not measure stably inherited strain differences which determine the relatedness of isolates.

Patterns determined by REAC have been used successfully in investigations of several bacterial infections (36, 40). However, a major disadvantage of this approach is that the number of chromosomal bands available for analysis is so large that the identification of specific bands from one experiment to another is difficult (34), complicating comparisons between isolates. Use of an rRNA probe to highlight restriction fragment length polymorphisms yields a hybridization banding pattern which allows for a much simpler comparison of isolates.

Studies of REAC patterns by Kapperud et al. (19) with *Hae*III found little variability within *Y. enterocolitica* O:3; they were unable to differentiate between phage type 8 and 9b isolates. Multilocus enzyme electrophoresis analysis of 34 porcine and human *Y. enterocolitica* O:3 isolates from geographically diverse areas also could not differentiate between phage type groups 8 and 9b (12). Multilocus enzyme analysis data showed that all isolates had essentially the same allelic profile, leading the authors to conclude that isolates in the O:3 serogroup are clonal (12).

Our data suggest that ribotyping is the most useful molecular epidemiologic technique reported thus far for subtyping *Y. enterocolitica* O:3. Ribotyping clearly differentiated *Y. enterocolitica* O:3 from other serotypes tested, including the antigenically related serotype O:1,2,3. Four different ribotype patterns or clones were demonstrated within serotype O:3. However, 51 of 53 *Y. enterocolitica* O:3 isolates had ribotype pattern I or II. Thus, although ribotyping was able to distinguish individual strains of *Y. enterocolitica* O:3, our data suggest that a limited number of clones have disseminated within the United States and globally.

Ribotyping has been used previously to differentiate among various *Yersinia* species by using *Eco*RI and *Hae*III (29). However, the choice of a restriction enzyme is critical when trying to differentiate within serotype O:3. *Eco*RI and *Hae*III, as well as most of the 20 restriction enzymes we tested, could not discriminate between isolates. Both our data and those of Andersen and Saunders (3) suggest that *Nci*I yields the most discriminating hybridization banding patterns for *Y. enterocolitica* O:3. Andersen and Saunders (3) reported five ribotype patterns among 37 serotype O:3 human and porcine isolates, but they presented no phage typing data.

Interestingly, in our study there was a correlation between genotypic and phenotypic techniques, because ribotype patterns I and II corresponded to phage type groups 9b and 8, respectively. Ribotype pattern I was seen in isolates from Canada and the United States, as well as isolates from Japan, Korea, and Taiwan. Previously, phage type 9b was known as the "Canadian phage type" because it was initially thought to be present only in Canada (38). It was later identified in the United States in New York (9, 10), and now this clone, as identified by ribotyping, appears to be widely disseminated. Ribotype pattern II isolates from the United

States corresponded with phage group 8; those isolates have previously been referred to as the "European and Japanese phage group" (38). Ribotype pattern II isolates also appear to be widely disseminated, because isolates of this ribotype were found in the United States, Belgium, Egypt, Chile, and Japan. Two other ribotype patterns, III and IV, were seen, but only in a single isolate each.

Swine have been suggested as a major reservoir of *Y. enterocolitica* O:3. European investigators have shown that pigs can be healthy carriers of *Y. enterocolitica* O:3, probably as a pharyngeal commensal organism, and this serotype has been isolated from European, Canadian, and Japanese swine, including porcine tonsils, tongue, throat, intestines, feces, and mesenteric lymph nodes (13, 16, 24, 35). In addition, a case-control study in Belgium associated yersiniosis with the consumption of raw pork (35). Our findings of identical genotypic ribotype patterns in chitterling and human isolates from the Atlanta outbreak of *Y. enterocolitica* O:3 infections in young children associated with the household preparation of chitterlings lends further support to the epidemiologic evidence that swine were the source of infection in that outbreak and a major reservoir for *Y. enterocolitica* O:3 and probably serotype O:1,2,3 as well.

The current predominance of serotype O:3 in the United States, where it had been so rare previously, suggests that the *Y. enterocolitica* O:3 clones may have been introduced into the swine population in the late 1970s and 1980s. The importation of large numbers of Canadian swine, both live hogs for slaughter and others for breeding, into the United States may explain how isolates of ribotype pattern I were introduced. Although the importation of European swine into the United States has been much less than that from Canada, this practice may have led to the introduction of ribotype pattern II isolates, which correspond to phage type 8. The finding of phage type 8 isolates in the United States occurred almost 10 years after the documentation of phage type 9b isolates in New York.

Concomitant with the increased incidence of *Y. enterocolitica* O:3 in the United States has been the report of transfusion-associated sepsis caused by erythrocytes contaminated with *Y. enterocolitica* (37). The majority of cases were due to serotype O:3 and were associated with a very high rate of mortality (five of seven cases). Donors appeared to have been asymptomatically bacteremic at the time of blood donation; refrigerated storage of blood for several weeks allowed *Y. enterocolitica*, which can grow at low temperatures, to multiply to high levels (10^7 to 10^8 CFU/ml) prior to transfusion (37). Ribotyping of three U.S. transfusion-associated *Y. enterocolitica* O:3 isolates revealed ribotype patterns I and II in geographically diverse areas, again suggesting wide dissemination of these two *Y. enterocolitica* O:3 clones within the United States.

In summary, ribotyping was able to distinguish individual strains of *Y. enterocolitica* O:3 but suggests that there are a limited number of clones which have disseminated within the United States and globally. The finding of identical hybridization banding patterns from chitterling and humans specimens from the Atlanta outbreak supports epidemiologic evidence that swine are the source of infection and a major reservoir for *Y. enterocolitica* O:3 and probably O:1,2,3 as well.

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