

Invasive *Salmonella dublin* Infections Associated With Drinking Raw Milk

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Salmonella dublin is a serotype of *Salmonella* that is host-adapted to cattle and rarely infects people. In one year (1980-1981) we diagnosed five cases of salmonellosis due to *S dublin* at the Veterans Administration Medical Center, San Diego. Four patients had positive blood cultures and one died. A sixth patient, diagnosed in 1978, had a mycotic aortic aneurysm but survived. Compared with nine patients who had *Salmonella* infections due to other serotypes, the *S dublin* patients were older, had a greater number of underlying chronic illnesses and were more seriously ill with their infections. Four of the six *S dublin* cases occurred in association with drinking "certified" raw milk from a commercial dairy.

Two microbiologic features of *S dublin* strains circulating in San Diego were distinctive. They failed to ferment arabinose and could not be grown in a minimal medium using citrate as the sole carbon source. Chronically ill elderly patients should be cautioned against drinking raw milk, an increasingly popular "health food."

Few serotypes of *Salmonella enteritidis* that cause gastroenteritis are adapted to specific hosts; those that are adapted to lower animals seldom cause infections in humans.^{1(p2)} The major exceptions are *Salmonella choleraesuis* and *S enteritidis*, serotype *dublin* (henceforth referred to as *S dublin* for clarity). Whereas the virulence of *S choleraesuis* is well known,² the virulence of *S dublin* is rarely commented on. Farmers and veterinarians, however, are well aware that *S dublin* infection of cattle is a major veterinary problem, and its virulence and potential for invading its natural host are well known.³ There were few reports of human infections from *S dublin*, except occupationally in veterinarians,⁴ until Werner and co-workers⁵ called attention to the problem of *S dublin* in people who drink commercially distributed raw milk. Their report also suggested that *S dublin* is an unusually invasive serotype, which contradicted the impression from previous reports^{6,7} that milk-borne *S dublin* infections are relatively benign.

We report a year's experience with *S dublin* infections at the Veterans Administration Medical Center (VAMC) in San Diego. We wish to emphasize the serious nature of these infections in elderly, chronically ill persons and to call attention to microbiologic characteristics

that permit laboratory diagnosis of *S dublin* before serotyping can be completed.

Methods

From the records of the Microbiology Laboratory at the VAMC we identified all patients who had positive cultures for *Salmonella* sp from September 1980 through September 1981. These patients are the basis of this review.

Cultures of fecal specimens were done by standard methods.⁸ Non-lactose-fermenting bacteria were identified using standard reagents.⁹ Serogrouping and Vi antigen detection were done by slide agglutination using *Salmonella* grouping sera (BBL Microbiology Systems, Cockeysville, Md). *Salmonellae* were serotyped by the California State Department of Health. Antibiotic susceptibility testing was done by the method of Barry and associates.¹⁰

Results

There were 14 cases of *Salmonella* infection diagnosed at this hospital between September 1980 and September 1981, which is an average number for this hospital in a 12-month period. In five cases the infections were caused by *S dublin*. (A sixth case of *S dublin*

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ABBREVIATIONS USED IN TEXT

CFU=colony-forming units
VAMC=Veterans Administration Medical Center

infection diagnosed in 1978 is included in this report because it was so typical of this syndrome.) The other nine cases of *Salmonella* infection were due to five different serotypes of *S enteritidis*; *Salmonella typhimurium* was the only other serotype responsible for more than two cases.

All the *Salmonella* infections were community acquired. Twelve of the patients were men, reflecting the composition of our patient population. The median age of the patients in the *S dublin* cases was 76 (range, 56 to 97 years), whereas the other infections occurred in somewhat younger adults (median age 50; range, 22 to 78 years). Four of the six *S dublin* cases were associated with drinking raw milk. The patient in case 6 (from 1978) drank raw milk on one occasion two weeks before the onset of his illness; the other three patients drank raw milk several times. All the patients purchased raw milk from the same dairy, the principal source of raw milk in southern California. Not all the patients infected with other serotypes were questioned

about raw milk consumption as most were not admitted to hospital and careful dietary histories were not taken. For comparison with the *S dublin* cases, we selected an age-matched control group of 15 patients admitted to hospital with non-*Salmonella* infections, and none had drunk raw milk in the month before admission (χ^2 , $P = .01$).

Some of the clinical features of the 15 cases of salmonellosis are summarized in Table 1. *S dublin* infections were more severe than were the infections caused by other *Salmonella* serotypes. Five of the six *S dublin* cases had bacteremia whereas only one patient in the other group had a positive blood culture. One of the cases of *S dublin* infection (case 5) was fatal. Two patients had evidence of endothelial infections. All but one of the patients who had *S dublin* infection had a serious preexisting chronic disease or were taking drugs that could have contributed to the severity of their infection.

The two cases of endothelial infection are presented below in more detail.

Reports of Cases

CASE 5. An 85-year-old woman with Paget's disease and asymptomatic calcific aortic stenosis was found to have chronic lymphocytic leukemia in 1979. In Sep-

TABLE 1.—Comparison of 15 Patients Infected With *Salmonella dublin* and Other Serotypes of *Salmonella enteritidis*, 1980-1981

Case	Age, Sex	Gastrointestinal Symptoms	Source of (+) Cultures	Temperature >38°C (100°F)	Underlying Illnesses	Therapy	Outcome
<i>S dublin</i>							
1	80, ♂	Diarrhea	Feces	No	None	None	Improved
2	86, ♂	None	Blood	Yes	Splenectomy, PUD, vagotomy and pyloroplasty	Sulfamethoxazole-trimethoprim	Improved
3	56, ♂	Nausea, vomiting	Blood, Feces	Yes	COPD	Sulfamethoxazole-trimethoprim	Improved
4	97, ♀	Nausea, vomiting, diarrhea	Blood, Feces	No	Organic brain syndrome, PUD	Sulfamethoxazole-trimethoprim	Improved
5	85, ♀	Diarrhea	Blood, Feces, Urine	Yes	Chronic lymphocytic leukemia, calcific aortic stenosis, Paget's disease	Sulfamethoxazole-trimethoprim, chloramphenicol	Died
6* . . .	73, ♂	None	Blood, aorta	Yes	Rheumatoid arthritis, diverticulitis	Ampicillin, surgical treatment	Improved
<i>S enteritidis</i> (all others)							
7	56, ♂	Diarrhea	Feces	Yes	Ulcerative colitis	None	Improved
8	22, ♂	Nausea, vomiting, diarrhea	Feces	No	None	None	Improved
9	33, ♂	Diarrhea	Feces	Yes	None	None	Improved
10	56, ♂	Diarrhea	Feces	Yes	None	None	Improved
11	29, ♂	Nausea, vomiting, diarrhea	Feces	Yes	None	Sulfamethoxazole-trimethoprim	Improved
12	34, ♂	Diarrhea	Feces	Yes	None	None	Improved
13	61, ♂	Diarrhea	Feces	No	Postgastrectomy diarrhea	None	Improved
14	63, ♂	Diarrhea	Feces	No	Diabetes mellitus	None	Improved
15	78, ♂	Diarrhea	Feces	Yes	Pulmonary fibrosis, PUD, antrectomy	Sulfamethoxazole-trimethoprim	Improved

PUD=peptic ulcer disease, COPD=chronic obstructive pulmonary disease

*Admitted to hospital and diagnosed in 1978. This case is included because of its typicality.

tember 1981 she was treated for the first time with cyclophosphamide and prednisone three times a day. A week later diarrhea, fever and chills developed and she had a syncopal episode. When she was brought to hospital her temperature was 37.6°C (99.6°F), pulse rate 90 and blood pressure 86/0 mm of mercury. She had no lymphadenopathy but her spleen was enlarged. There were bibasilar rales, her heart was enlarged and she had a midsystolic ejection murmur that radiated into her carotid arteries. Her hemoglobin level on admission was 7.4 grams per dl and her leukocyte count had fallen from 75,000 per μ l (pretreatment) to 5,400 per μ l, with 84 percent mature lymphocytes. She was treated initially with packed red cells, saline, cefazolin sodium and gentamicin sulfate. Pretreatment blood, urine and stool cultures all grew *S dublin*. The initial blood culture had more than 500 colony-forming units (CFU) per ml, determined from a pour plate of her blood. Blood cultures done 24 hours later were still positive, but there was less than 1 CFU per ml. Therapy was changed to trimethoprim and sulfamethoxazole given intravenously. A thoracentesis yielded clear fluid that had 5,600 leukocytes (98 percent lymphocytes), 2.6 grams per dl of protein and on culture grew a single colony of *S dublin*.

On the seventh hospital day the patient became unresponsive and was found to have third-degree heart block and pulmonary edema. A pacemaker was placed, which initially improved her heart failure. After a blood culture done that day again grew *S dublin*, treatment was changed to administration of chloramphenicol. A diagnosis of endocarditis was considered, but the patient and her family refused any further diagnostic studies. Despite treatment with digitalis and diuretics, progressive left ventricular failure developed and she died on the 17th hospital day. Permission to do an autopsy was refused.

Comment. This woman's immune status was compromised by both the leukemia and the therapy. She presented in shock with an overwhelming *Salmonella* bacteremia. The heavy bacteremia suggested an intravascular focus of infection^{1(p60)} that later became apparent when heart block and progressive heart failure developed. *Salmonella* endocarditis is too uncommon to be certain whether combined medical and surgical therapy is always required, but it appears that, as in this case, medical therapy is often unsuccessful.¹¹

CASE 6. A 73-year-old man with long-standing rheumatoid arthritis was taking prednisone, 10 mg a day, when he was admitted to hospital in Palm Springs, California, in May 1978 with fever, chills and abdominal pain. Blood cultures grew *S dublin*, but fecal and urine cultures did not. He was treated with ampicillin for 12 days and discharged afebrile. A week later a low-grade fever developed, and two weeks after that left lower quadrant abdominal pain prompted him to come to the VAMC. His spleen was just palpable and he had signs suggesting sigmoid diverticular perforation. His blood culture again grew *S dublin* but stool cultures were still negative. An abdominal sonogram

TABLE 2.—Biochemical Differentiation of *Salmonella dublin* From Other Group D *Salmonella* Species

Test	<i>S dublin</i> * (percent)	<i>Salmonella enteritidis</i> † (percent)	<i>Salmonella typhi</i> ‡ (percent)
Hydrogen sulfide (KIA)	+(100)‡	+(98)	w(94)
Citrate (Simmons)	-(0)	+(99)	-(0)
Ornithine decarboxylase	+(100)	+(99)	-(0)
Gas (glucose)	+(100)	+(100)	-(0)
Arabinose	-(0)	+(99)	-(0)
Rhamnose	+(100)	+(95)	-(0)
Trehalose	+(100)	+(100)	+(100)

KIA = Kligler's Iron Agar.

*Based on 14 strains found in San Diego County.

†Modified from Edwards and Ewing.⁹

‡+ = Positive reaction, w = weakly positive reaction, — = negative reaction.

showed a left lower quadrant mass and gallstones, but no aortic aneurysm was seen. An aortogram, however, showed an eccentric 3-cm abdominal aortic aneurysm below the renal arteries. At operation he was found to have a perforated colonic diverticulum with an abscess that did not contain *S dublin*. A colostomy was done and treatment with ampicillin, clindamycin and gentamicin was continued. Ten days later his aortic aneurysm was excised, and an aortofemoral graft was placed. Culture of a specimen of the aneurysm grew *S dublin*. Intravenous administration of ampicillin was continued for an additional six weeks, followed by oral administration of ampicillin. Three years later, the patient continues to take ampicillin and remains free of any signs of *Salmonella* aortitis.

Comment. This elderly man presented originally with *S dublin* bacteremia without an apparent source. The possibility of a mycotic aneurysm was not considered at that time. His second admission to hospital was prompted by diverticulitis, which was probably unrelated to the salmonellosis. Despite the diverticulitis, the search for a mycotic aneurysm was pursued vigorously because of the original presentation with *Salmonella* bacteremia in the absence of gastroenteritis.¹² An abdominal sonogram did not show the aneurysm, but an aortogram did.

The nine cases of salmonellosis due to serotypes other than *dublin* were typical examples of uncomplicated gastroenteritis in adults (Table 1). The age distribution and the number with underlying illnesses reflect our patient population. Only one patient required admission to hospital because of the infection (case 15) and he was also bacteremic. Interestingly, he had had a partial gastrectomy for a peptic ulcer and was taking corticosteroids, two factors that predispose to serious *Salmonella* infections.¹³

Bacteriology

All the strains of *S dublin* differed from typical strains of *S enteritidis* in that they failed to ferment arabinose and would not grow on Simmons' citrate media (Table 2).⁹ All strains agglutinated with group D antiserum, and one gave a positive Vi reaction. The isolates from cases 1 through 5 were resistant to ampicillin, carbenicillin,

tetracycline and kanamycin. The isolate from case 6 was sensitive to these antibiotics but otherwise resembled the other five strains. Eight additional strains of *S dublin* found at other hospitals in San Diego had the same biochemical features as our six isolates.

Discussion

The principal difference between the *S dublin* infections and the other *S enteritidis* infections in this series was the severity of the former. The invasiveness of *S dublin* was manifested by a high incidence of bacteremia (5/6), metastatic infections (2/6) and death (1/6). Five patients were sick enough to require antibiotic therapy and admission to hospital. In contrast, none of the other *Salmonella* infections were complicated by bacteremia and only one patient was admitted to hospital and received an antimicrobial agent. In fact, the second group of patients fits the usual pattern of *Salmonella gastroenteritis*,¹³ whereas *S dublin* infections resembled the bacteremic syndrome, which is more commonly associated with *S choleraesuis* infections.^{1(p2)} Our experience thus confirms the report of Werner and associates,⁵ who found that *S dublin* was more likely to cause bacteremia and metastatic infection than were other serotypes of *S enteritidis*. In their series of 113 cases of human *S dublin* infections diagnosed in California between 1971 and 1975, 50 percent had positive blood cultures, 17 percent died and many had metastatic infections. In that series there was a disproportionate number of cases in patients older than 50 years of age, and in them the case fatality rate was 43 percent, which suggests that this disease is particularly virulent in the elderly. In our series all the *S dublin* infections occurred in patients older than 50 years, which cannot be explained entirely by the age of our patient population as four out of nine of the patients with other *Salmonella* infections were younger than 50 years of age. Elderly people are probably predisposed to *S dublin* by virtue of their underlying diseases rather than by increased exposure.

In this series, three patients were taking prednisone, two were taking antacids and one had leukemia. These same conditions decrease resistance to all salmonellae,¹³ including *S choleraesuis*.² However, both *S dublin* and *S choleraesuis* are more likely to present as sepsis or with metastatic infections than are other nontyphoid salmonellae, even in the absence of predisposing illnesses.^{1(p2),2,6,14} Some strains of *S dublin* have a Vi antigen that is a virulence factor for *Salmonella typhi*,¹⁵ but Vi antigen is unlikely to be the main virulence determinant for *S dublin* since only one of our six isolates had a Vi antigen. There is some evidence that not all strains of *S dublin* are equally pathogenic in cattle,¹⁶ but no virulence mechanism has been identified in that host either.

When we reviewed the bacteriology of our *S dublin* strains, we found that all were citrate negative (Simmons' citrate agar) and did not ferment arabinose. We also tested eight other isolates of *S dublin* made in San Diego County in 1981 and all had those reactions.

Using conventional media, *S dublin* could be recognized as group D, citrate- and arabinose-negative strains of *S enteritidis* (Table 2). This pattern was not apparent using the API-20E system (Analytab Products Inc, New York) in that the citrate and arabinose reactions were variable. Arabinose fermentation has been reported to be a variable feature of *S dublin*.¹⁷ Most human isolates in England¹⁶ and all of our strains were arabinose negative. All of our recently isolated strains were resistant to ampicillin, carbenicillin, kanamycin and tetracycline. The strain from 1978 was biochemically similar to the later isolates but was sensitive to all antibiotics. In Europe the prevalence of antibiotic-resistant *S dublin* has been increasing for the past decade.¹⁸

S dublin infections are uncommon in humans. In 1979 only 53 cases were reported in the United States (24 from California) out of 31,123 cases of salmonellosis.¹⁹ The epidemiology of *S dublin* suggests that most cases are acquired from drinking contaminated milk.⁵⁻⁷ Four out of six of our patients drank raw milk. *S dublin* is frequently isolated from cattle but only rarely from other animals or feed. The association with raw milk therefore is not surprising. Because the five cases in 1980 through 1981 occurred sporadically during the year, the contamination of milk must be an ongoing but intermittent problem. This could be related to the biology of this infection in cattle because some cows become chronic, if not lifetime, carriers of *S dublin*. The presence of carriers in a herd increases the likelihood of animal-to-animal transmission, thereby perpetuating the problem.²⁰ Samples of certified raw milk are tested for coliform contamination, which should detect bovine fecal contamination. Unfortunately, this testing cannot eliminate the risk of *S dublin* since stress, including abortion, can reactivate latent *S dublin* infection, and in these circumstances *S dublin* may be excreted directly into the milk.²¹ Even small numbers of *S dublin* introduced in this way could multiply in milk that was not adequately refrigerated at all times.

It is ironic that our patients, and presumably most others who drink raw milk, chose this product because they believed it to be healthier than pasteurized milk. Furthermore, they believed the raw milk was safe to drink because it was "certified" and sold with the approval of various government agencies. I will not comment on the validity of the nutritional claims that are made for raw milk but it is important to reiterate that heat treatment (pasteurization) is the only reliable way to ensure that milk is not contaminated by *Salmonella*.²²

In view of the virulence of *S dublin* infection for elderly people with underlying chronic diseases, physicians should be aware of the risk of drinking raw milk and advise their patients accordingly. It would also seem appropriate for a warning notice to be placed on all containers of raw milk that are sold commercially.

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