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Salmonella Types Isolated from the Gulf of Aarhus Compared with Types from Infected Human Beings, Animals, and Feed Products in Denmark

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A 2-year examination for *Salmonella* was conducted in the gulf of Aarhus, which receives waste water from local industries and from about 100,000 inhabitants. An approximately rectilinear relationship is shown between the most probable number of *Escherichia coli* and species of *Salmonella*. *Salmonella* species can be demonstrated with the same frequency in inlets and outlets of the treatment plants. Data on the distribution of *Salmonella* types in the gulf of Aarhus and in Oeresound outside Copenhagen (1 million inhabitants) in 1966 and 1968 and the distribution in man, animals, and feeding stuff during the period 1960 to 1968 in Denmark as a whole are shown. This indicates that the classical chain of infection (feed stuff-animals-food-man) is without importance in Denmark, and that a great nlumber of the human cases may be due to increasing communication, because severa of the demonstrated types have been found neither in feed stuff nor in animals in this period. We suggest that *E. coli* counts, currently used in examination of waters receiving effluents of streams and sewage treatment plants, should be supplemented at intervals with qualitative *Salmonella* examinations.

The decreasing confidence in the significance of coliforms as an indicator of fecal contamination in recent years (1, 5, 6) and even an incipient doubt as to the sufficiency of *Escherichia coli* counts have led to a demand for specific methods in the demonstration of pathogenic intestinal bacteria and viruses, both in sewage and in the receiving waters, and for quantitative methods of known precision, if possible. There has been, in particular, a great interest in developing methods for qualitative and quantitative determination within the genus *Salmonella*, e.g., Gallagher and Spino (4) have recommended the supplementation of *E. coli* counts with a qualitative *Salmonella* examination.

MATERIALS AND METHODS

The demonstration of *Salmonella* species in this work is done by the qualitative sampling method developed by Moore et al. (9, 10). They demonstrated that a gauze pad, prepared by folding a piece of gauze approximately 4 ft by 6 inches (122 by 15 cm) in size, was able to collect *Salmonella* in a sewage system as well as in polluted waters, when left on the sampling stations for about 48 hr.

On removal from the sampling station, the pad is put directly into a sterile screw-capped bottle and immediately taken to the laboratory, where it is transferred into 300 ml of selective enrichment medium, which inhibits growth of other types of bacteria.

The most frequently used enrichment media are Kauffmann's tetrathionate broth and Leifson's selenite broth with various modifications. A comparative study has demonstrated that tetrathionate broth is superior to selenite broth, because several bacteria (bacilli, coryneforms, fecal streptococci, *Proteus*, *Citrobacter*, *Enterobacter*, *Klebsiella*, and *E. coli*) during their growth in selenite broth, including Stokes and Osborne's (15) modification, are able to produce selenite compounds able to inhibit the growth of some *Salmonella* types, an effect not seen when tetrathionate or Rappaport's (11) medium is used (Gundstrup, Grunnet, and Bonde, Health Lab. Sci., *in press*). In our laboratories, we have always used tetrathionate broth, and the above mentioned investigations indicate that there is no reason to alter this.

In the following examinations, tetrathionate broth with an admixture of 1% alkyl benzene sulfonate (Teepol 515) is used as the enrichment medium. Incubation takes place at 41.5 C (14). From the enrichment medium, subcultures are made daily for 4 days on brilliant green-lactose-saccharose-phenol red-agar plates (BLSF), with an admixture of 0.5% Teepol 515 to prevent *Proteus* swarming. From these plates a

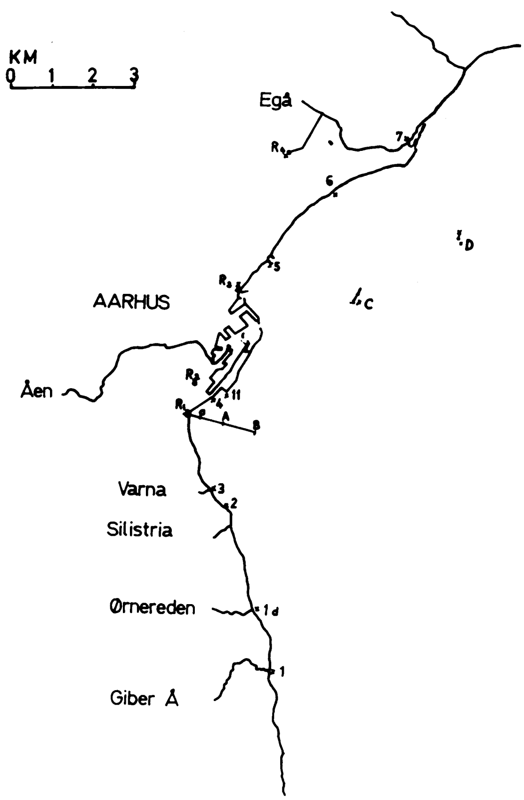

FIG. 1. *Gulf of Aarhus, sampling stations marked.*

suitable number of suspect colonies (normally 8 to 10) are subcultured onto brilliant green-sorbitol-phenol red-agar plates. Organisms fermenting sorbitol on this medium were tested biochemically for ability to ferment glucose and lactose, to reduce nitrate [medium V of Bonde (1)], and to hydrolyze urea. Strains giving typical *Salmonella* reactions by these four screening tests were typed serologically as well as biochemically after the methods of Kauffmann (8). The identification of about 2,000 strains by this technique of *Salmonella* selection has not resulted in any false positive strains, and extra measures, e.g., Gram stain and motility tests, thus seem unnecessary (6). At the beginning, a screening was tried by using slide agglutination in polyvalent *Salmonella* sera. Of the first 1,000 strains examined this way, about 150 proved to be false positives, and therefore this method was abandoned.

Two corresponding samples of water were collected for quantitative demonstration of *E. coli* [most probable number (MPN)], one when the pad was set, and another when it was collected. The highest of the two counts was registered as an estimate of the degree of pollution. Sampling and transport were by the methods of Taylor (16) and the Swedo-Danish Committee 1959–64 (12). Samples were taken in sterile bottles about 30 cm below the water surface and transported quickly (in less than 3 hr) to the laboratory where the samples were immediately inoculated and incubated. The method applied was the multiple-tube technique in which tubes of MacConkey's broth are incubated at 37 and 44 C by Wilson's method IV, supplemented with an indole test (1).

From December 1966 to November 1968, pads for demonstration of *Salmonella* species were collected from the gulf of Aarhus and from sewage outlets draining into it (263 samples). Furthermore, samples were taken from sewage pipes and treatment plants (93 samples). The sampling stations are seen in Fig. 1.

The gulf of Aarhus receives waste water from the town of Aarhus which has about 100,000 inhabitants. The waste water from 75,000 inhabitants is treated by sedimentation only (R_1, R_2, and R_3) and from the rest also biologically (R_4).

RESULTS

The relation between *E. coli* and *Salmonella* at the various sampling localities is shown in Table 1, where it is seen that the content of *Salmonella* seems to increase with increasing number of *E. coli*. Thus 72% of all pads collected when MPN *E. coli* was more than 10,000 contained *Salmonella*. But even at very small MPN *E. coli*, it is still possible to isolate *Salmonella*. Thus 16% of all pads collected when MPN *E. coli* was below 10 contained *Salmonella*.

For further information on the degree of pollution in the receiving waters, samples were taken of bottom sediments, aquatic plants, and mussels. On sampling stations near the outlets from treatment plants with *E. coli* counts mostly over 10^5

TABLE 1. *Relation between MPN E. coli and Salmonella in sea and rivers*

Sampling stations	No. of samples					
	≤9[a]	10–99	100–999	1,000–9,999	≥10,000	*Salmonella* positive samples
						%
1			1	2 (1)	4	14
1c	2	5	4			0
1d		2	3			0
2		7	7 (2)[b]	5 (4)	2 (2)	38
3		1	5 (1)		1 (1)	29
3a	1				3	0
4			5 (4)	4 (1)	9 (7)	65
5	3	5	16 (4)	12 (9)	2 (1)	37
6	1	2	3	4		0
7	1			2 (2)	17 (16)	90
11	1 (1)					100
a			4 (3)	10 (9)	16 (12)	80
A	2 (1)	15 (9)	17 (11)	3 (3)		69
B	3	21 (8)	6 (1)	3 (2)		33
C	2 (1)	3 (2)	1	3		38
D	9 (1)	3 (1)				18

[a] Levels of MPN of *E. coli*.

[b] Numbers of *Salmonella*-positive pads are expressed parenthetically.

per 100 ml, salmonellas were detected in all three kinds of samples (6). Furthermore gills, gall-bladders, and intestinal contents from 140 fishes were investigated for species of *Salmonella*. In the fishes it was, surprisingly, not possible to find species of *Salmonella* (7).

The number of *Salmonella* isolations from the sewage treatment plants is shown in Table 2. It is seen that species of *Salmonella* have been isolated just as frequently from the outlets as from the inlets, and just as frequently in the biological as in the mechanical treatment plants. This has later on been confirmed by quantitative determinations.

The results from Tables 1 and 2 are presented graphically in Fig. 2, which shows an almost straight-line relationship between qualitative *Salmonella* detection and MPN *E. coli*.

From the area of Aarhus, 1,147 *Salmonella* strains have been typed. As every type is only counted once per sample, the number is reduced to 231 strains representing 24 different serotypes. The most frequently occurring types were *Salmonella senftenberg* (19%), *S. typhimurium* (15%), and *S. paratyphi* (12%); see Table 3 which includes all types isolated.

In Table 4 the *Salmonella* types from the area of Aarhus are compared with similar isolations from the Sound outside Copenhagen (2), and with *Salmonella* isolations from human beings,

TABLE 2. *Results from different treatment plants*

Treatment plants	No. of samples	
	Inlets	Outlets
Mechanical (R_1, R_2, and R_3)	21 (17)[a]	13 (13)
Biological (R_4)	7 (7)	10 (9)

[a] Numbers of *Salmonella*-positive pads are expressed parenthetically.

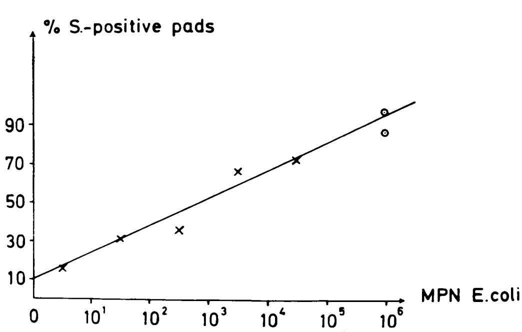

FIG. 2. *Relation between qualitative Salmonella detection and MPN E. coli.*

TABLE 3. *Classification of 231 Salmonella strains isolated from the area of Aarhus*

Classification	No. of samples	Classification	No. of samples
Salmonella senftenberg	44	*S. panama*	3
S. typhimurium	34	*S. blockley*	2
S. paratyphi B	27	*S. heidelberg*	2
S. baildon	21	*S. infantis*	2
S. muenster	18	*S. kentucky*	2
S. enteritidis var. *jena*	16	*S. lomita*	2
S. minnesota	14	*S. bareilly*	1
S. anatum	12	*S. indiana*	1
S. tennessee	10	*S. kottbus*	1
S. newport	8	*S. michigan*	1
S. cubana	3	*S. worthington*	1
S. oranienburg	3	Rough (not typed)	3

animals, and feed stuffs; for animals and feed stuffs, only isolations made by the State Veterinary Serum Laboratory in Copenhagen are included.

DISCUSSION

Table 1 shows that *Salmonella* can be demonstrated almost everywhere in the gulf of Aarhus, most frequently when the number of *E. coli* is high. From Fig. 2 it can be seen that *E. coli* is a rather satisfactory measure of fecal pollution, for, if *E. coli* is present, *Salmonella* organisms usually can be demonstrated from the same sources. Still a direct demonstration of the pathogenic microorganisms themselves, e.g., *Salmonella*, is preferable. However, it is necessary to have in mind that the excretion of *Salmonella* in some cases is intermittent and the number of *Salmonella* very few. As the demonstration of *Salmonella* is still rather time-consuming, many laboratories hesitate to introduce the *Salmonella* test into the routine. The authors still, like Gallagher and Spino (4), propose that the routine testing of *E. coli* in the receivers be supplemented, at certain intervals, with a qualitative *Salmonella* test.

Table 2 indicates that the treatment plants do not eradicate—perhaps do not even reduce—the number of pathogenic bacteria. This is confirmed by quantitative examinations (6).

The often mentioned classical chain or cycle of infection (feed stuffs-animals-food-man-sewage, Fig. 3) does not seem to be of importance in Denmark any more. Links of this chain can still be found as regards *S. typhimurium*. Even though the first link, feed stuff, seems to be without any importance in maintaining the *Salmonella* level in animals, there is a clear relationship between the occurrence in animals and man. At the same time, *S. typhimurium* is the most frequently occurring

TABLE 4. *List of Salmonella isolations from the gulf of Aarhus, compared with isolations from human beings, animals, and feed stuff*

Salmonella types	Gulf of Aarhus 1966–1968	Oeresound (Copenhagen) 1966–1968	Denmark as a whole 1960–1968		
			Human	Animal	Feed stuff
Salmonella abony		6	4		
S. adelaide		13			
S. amager			1	1	2
S. anatum	12	14	15		1
S. baildon	21		1		
S. bareilly	1		15	1	4
S. bispebjerg			1		
S. blockley	2		29		
S. bonn			1		
S. bovis-morbificans		3	14		
S. braenderup		3	5		
S. brandenburg		14	8		
S. bredeney		15	11	2	
S. bronx			2		
S. bukavu			1		
S. butantan			8		
S. carrau					1
S. cerro			1		9
S. chester		2	1		
S. chicago			7	3	
S. coeln			2		
S. cubana	3	3			29
S. derby		1	2	3	
S. dublin			2	64	
S. eastbourne		2		1	
S. emek		1			
S. enteritidis var. *danysz*			1	1,278	2
S. enteritidis var. *essen*				14	
S. enteritidis var. *jena*	16	14	123	75	
S. escanaba			1		
S. give		17	4		
S. glostrup			1		
S. grumpensis			2		
S. hadar			1		
S. haelsingborg			2		
S. haifa			8		
S. havana			1		
S. heidelberg	2	16	24	1	
S. indiana	1	7	43	10	
S. infantis	2	5	56	7	4
S. isangi				1	
S. java			12		
S. johannesburg					1
S. kaapstad			5		
S. kapemba		3	1		
S. kentucky	2	5	2	8	
S. kingston		2			
S. kottbus	1	2	1		
S. lexington			1		
S. lille				1	1
S. lindenburg		2	11		

Salmonella types	Gulf of Aarhus 1966–1968	Oeresound (Copenhagen) 1966–1668	Denmark as a whole 1960–1968		
			Human	Animal	Feed stuff
S. litchfield			1		
S. liverpool		3			
S. lomita	2	6		1	1
S. london			1		
S. madelia					3
S. manchester			1		
S. manhattan			3		
S. matadi			1		
S. meleagridis		8			
S. michigan	1		1		
S. mikawasima			3		
S. minnesota	14	1	3		
S. montevideo		2	33	1	67
S. muenchen		7	27	2	
S. muenster	18	1		1	
S. napoli			3		
S. ndolo		1	1		
S. nessziona		1	2		
S. newbrunswick			4		
S. newington			5		
S. newport	8	1	115	4	
S. ohio			1		1
S. oranienburg	3	7	21	4	2
S. orion		2			
S. oslo			1		
S. panama	3	8	15		2
S. paratyphi B	27	11	126		
S. poona				1	
S. pretoria		1			
S. reading		4	4		
S. richmond			1		
S. saintpaul		5	9		
S. sandiego		1	2		
S. sanjuan			1		
S. schwarzengrund			3		
S. senegal			1		
S. senftenberg	44	3	1	2	40
S. souza					6
S. stanley			5		
S. stanleyville			1		
S. takoradi		1			
S. taksony				1	10
S. tennessee	10		14	2	1
S. thomasville			1		
S. thompson		4	18		
S. tuebingen					5
S. typhi			56		
S. typhimurium	34	44	2,951	1.048	12
S. vejle			1	1	3
S. virchow		1	1		
S. weltevreden			3		
S. westerstede		15	2		
S. worthington	1	3	4		
S. not typed (rough)	3	1			

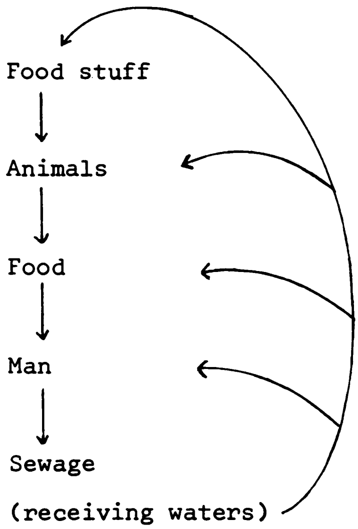

FIG. 3. *Salmonella cycle.*

Salmonella type in the sea, lakes, and water courses. In the sewage-(foodstuff)-animal pathway, wild animals, especially sea gulls and rats in great numbers foraging in the sewage-outlets and on dumps, are often involved, as described by Brest Nielsen (3), who showed that 1 to 2% of sea gulls shot were infected with *Salmonella*. At the same time, sea gulls are often seen in great numbers among the domestic aquatic fowls, which are known as the most constantly infected of the domestic animals, and among which, on the basis of several years' experience, the *Salmonella* infection is very difficult to eradicate. The circle of infection may also be closed as shown by Schaal (14), who reports an enzootic salmonellosis in a great cattleherd caused by drinking from a polluted brook.

The first link in the chain of infection, the feed stuff, is of little importance in Denmark (Table 3). Of 22 various *Salmonella* types demonstrated in feed stuff in an 8-year period, only 3 have been demonstrated frequently: *S. cubana*, *S. montevideo*, and *S. senftenberg*. The last two have been demonstrated once each in animals during this period, whereas *S. cubana* has not been demonstrated in animals at all. This fact is undoubtedly mainly the result of strict veterinary legislation aiming at an interruption of the sequence in its first link by demanding resterilization of imported feed stuff of animal origin and by demanding a proper sterilization of Danish animal products sent to the rendering plants for destruction. When *S. montevideo* is demonstrated 33 times in man in the same period, the infection must have another source, and the most probable cause is increasing international communication. This increasing communication must, moreover, be the source of several of the types demonstrated in man, as many of them have neither been demonstrated in feed stuffs nor in animals.

The demonstration of *S. paratyphi* B in particular calls for attention. Whereas *S. typhimurium* was demonstrated nearly 3,000 times in man from 1960 to 1968, and more than 1,000 times in animals, *S. paratyphi* B was only demonstrated 126 times in man during the same period. Still, the two types occur with almost the same frequency in the receiving waters. In Denmark as a whole, the number of new infected cases of *S. paratyphi* B has, at least since the twenties, been rather constant (about 15 per year). In the area of Aarhus, six *paratyphi* B excreters are at present registered. This fact does not correspond to the frequent findings in the gulf. It might be due to a particular resistance (ability to survive in natural waters) of *S. paratyphi* B or to the existence of unknown carriers or individuals with inapparent infection.

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