THE DISTRIBUTION OF ENTERIC STREPTOCOCCI

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Comparatively little work has been done to determine the frequency distribution of enteric streptococci in nature. It is generally considered that the enterococci have their origin in the intestinal tract of man and other warm-blooded animals. Horses and cattle have been mentioned by several investigators as sources of these organisms, but the occurrence of fecal streptococci in most warm-blooded animals remains a matter of conjecture. Although enterococci of the *Streptococcus faecalis* and *Streptococcus liquefaciens* types have been shown to occur on plants (Sherman, 1937*a*), their presence on vegetable life and in the soil does not necessarily indicate that these are their natural habitats. The presence of enteric streptococci on plants and in the soil is more apt to be an indication of survival than of growth and reproduction. A knowledge of the frequency distribution of fecal streptococci in animals, together with comparatively simple laboratory methods of isolation and identification, would help to determine the public health and sanitary significance of this group of microorganisms. The finding of fecal streptococci in adequately treated swimming pool water, and in the absence of demonstrable coliform organisms, suggests that they either are more resistant to the chemical treatment (Hajna and Perry, 1943) or occur in greater numbers than is suspected. In either case the enterococci take on an added sanitary significance.

EXPERIMENTAL

The work of Sherman (1937*a*), Snyder and Lichstein (1940), and Hajna and Perry (1943) suggested bacteriological media and laboratory methods of analysis for the demonstration of enterococci. As a consequence of their findings the "SF" medium of Hajna and Perry, employing 0.05 per cent sodium azide as an inhibitor of gram-negative microorganisms, and an incubation temperature of 45.5 C (113.9 F) was used in the initial experiments. The demonstration by Sherman (1937*a*) that enterococci are capable of growth and reproduction in the presence of 6.5 per cent sodium chloride led to its use in later experiments. Fifty-three specimens of human and animal feces and soil were examined for fecal streptococci and coliform bacteria. Thirty specimens were examined for fecal streptococci using "SF" medium alone. The remaining 23 specimens were examined on a comparative basis using "SF" medium and "SF" medium containing 6.5 per cent sodium chloride. The source and number of specimens are shown in table 1.

Fresh specimens were weighed into 125-ml, wide-mouth, ground-glass-stoppered bottles, and sufficient sterile physiological salt solution was added to make an initial dilution of 1:10. Subsequent decimal dilutions of from 1:100 to 1:1,000,000 were prepared for seedings. Parallel inoculations were made of the

well-shaken suspensions into "SF" medium, standard lactose broth, and, in the case of 23 specimens, into "SF" medium containing 6.5 per cent sodium chloride. The inoculated tubes of "SF" and modified "SF" media were incubated in a constant temperature water bath at 45.5 C (113.9 F) for 72 hours. One hundred and sixty-one random temperature recordings, made over a period of 22 days, revealed a minimum temperature of 45.0 C (113.0 F) and a maximum temperature of 45.9 C (114.6 F), with an average temperature of 45.45 C (113.8 F). All sodium azide cultures were examined daily, macroscopically for acid production and microscopically for bacterial growth. At the end of the incubation period tubes containing fecal streptococci, with or without acid production, were

TABLE 1

Source and number of specimens

SOURCE	NUMBER OF SPECIMENS	SOURCE	NUMBER OF SPECIMENS
Human	5	Rabbit	5
Cat	5	Rat	5
Mouse	5	Chicken	6
Guinea pig	5	Flies	3
Dog	6	Monkey	6
		Soil	2

TABLE 2

Selective action of "SF" medium for fecal streptococci

ORGANISMS PRESENT	ACIDITY SODIUM AZIDE BROTH	
	Positive	Negative
Fecal streptococci only	15 (9.9%)	6 (3.9%)
Fecal streptococci and rods	45 (29.8%)	1 (0.7%)
Rods only	11 (7.2%)	
None		72 (47.6%)
Totals	71	79

streaked on sodium azide agar ("SF" medium containing 1.5 per cent agar). These culture plates were then incubated at 37.5 C (99.5 F) for 24 hours and for 48 hours, and colonies showing acid production were fished for taxonomic study.

The standard lactose broth tubes were incubated at 37.5 C (99.5 F) for 24 and for 48 hours. Tubes in which gas was present at the end of the incubation period were streaked on Levine's eosin methylene blue agar. Following incubation of these culture plates well-isolated coliform colonies were fished for biochemical identification.

RESULTS

A review of the data of the first 30 specimens examined by the "SF" medium revealed that fecal streptococci were present in 26 of the 28 fecal samples. One specimen of mouse feces and one of dog feces failed to show any evidence of

enterococci. The two soil samples failed to produce any evidence of acid production as shown by the bromcresol purple indicator in the medium. Microscopically the tubes from these specimens were negative for any streptococci.

Of the 140 tubes of "SF" medium inoculated from the 28 fecal suspensions, 71 produced evidence of fecal streptococci as indicated by acid production. Fifteen of these tubes (9.9 per cent) contained only fecal streptococci, whereas 45 (29.8 per cent) contained fecal streptococci in combination with rods. Eleven

TABLE 3a

Comparative selective action of "SF" medium and "SF" medium containing 6.5 per cent sodium chloride

ACIDITY BROTH TUBES	MICROSCOPIC GROWTH	MEDIUM							
		"SF" plus 6.5% salt				"SF"			
		Hours				Hours			
		24	48	72	Total	24	48	72	Total
Negative	Negative			45	45			33	33
Negative	Rods only			9	9			4	4
Negative	Fecal strep. only			23	23			18	18
Negative	Fecal strep. and rods			6	6			21	21
Positive	Rods only			1	1	4	1	1	6
Positive	Fecal strep. only	1	16	3	20	2	11	2	15
Positive	Fecal strep. and rods	10	21	3	34	21	18	2	41
Totals		11	37	90	138	27	30	81	138

TABLE 3b

Comparative selective recovery of microorganisms by "SF" medium and "SF" medium containing 6.5 per cent sodium chloride

ORGANISMS PRESENT	ACID REACTION		NO ACID REACTION	
	"SF" plus 6.5% salt	"SF"	"SF" plus 6.5% salt	"SF"
Fecal strep. only	14.5%	10.8%	16.6%	13.0%
Fecal strep. and rods	24.6%	29.7%	4.3%	15.2%
Rods only	0.7%	4.3%	6.5%	2.9%

tubes (7.2 per cent) were false positive, since microscopic examination failed to reveal the presence of streptococci and showed them to contain only rods. In addition to this discrepancy in results 7 tubes (4.6 per cent) in which no acid was produced were found to contain fecal streptococci alone or in combination with rods (table 2).

It thus appeared that bromcresol purple sodium azide broth ("SF" medium) failed as a presumptive medium to reveal the presence of fecal streptococci in approximately 5 per cent and gave a false positive reaction in approximately 7 per cent of the inoculated tubes. It is also of interest that of the 6 tubes which were negative with sodium azide broth (no acid) but positive for fecal streptococci, 4 occurred in the 1:100,000 dilution, 1 in the 1:10,000 dilution, and 1 in

the 1:10 dilution. These results are of numerical significance as will be shown later.

The addition of 6.5 per cent sodium chloride to the "SF" medium had the effect of reducing the number of false positive and negative reactions without any appreciable reduction in the recovery of acid-producing enterococci. Of the 23 specimens examined, both media being used, 21 contained fecal streptococci. One mouse and one rat specimen were negative for enterococci. Of

TABLE 4

Highest dilutions with a positive acid reaction containing either fecal streptococci alone or in combination with rods

SAMPLE NUMBER	"SF" PLUS 6.5% SALT		"SF"	
	Fecal strep. alone	Fecal strep. and rods	Fecal strep. alone	Fecal strep. and rods
31	10^{-4}		10^{-5}	
32	10^{-3}		10^{-5}	
33	Negative	Negative		10^{-1}
34		10^{-1}		10^{-1}
35	10^{-6}			10^{-2}
36	10^{-3}		10^{-3}	
37		10^{-1}		10^{-2}
38	10^{-4}			10^{-4}
39	Negative	Negative	Negative	Negative
40	Negative	Negative	10^{-4}	
41	10^{-5}			10^{-2}
42		10^{-1}		10^{-2}
43	10^{-6}		10^{-3}	
44		10^{-2}	10^{-5}	
45	Negative	Negative	Negative	Negative
46		10^{-1}		10^{-2}
47	10^{-2}			10^{-2}
48		10^{-3}		10^{-3}
49	10^{-3}		10^{-3}	
50	10^{-2}			10^{-3}
51		10^{-2}	10^{-3}	
52	10^{-4}			10^{-3}
53		10^{-2}	10^{-2}	

the 138 tubes of each medium inoculated, 62 tubes (44.8 per cent) of "SF" and 55 tubes (39.8 per cent) of modified "SF" showed acid production. Among the positive cultures in both media, 3.7 per cent more tubes of the modified "SF" than of the original medium contained fecal streptococci without any interfering bacilli. Of greater significance is the marked reduction in the number of cultures giving a false positive reaction from 4.3 per cent to 0.7 per cent.

Both media gave a high percentage of tubes containing enterococci alone or in combination with rods without any macroscopic evidence of acid production. Thirty-nine "SF" tubes (28.2 per cent) and 29 modified "SF" tubes (20.9 per cent) failed to show any evidence of acidity yet contained either pure cultures of enterococci or mixtures of enterococci and bacilli at the end of the 72-hour incubation period (table 3a and b).

A comparison of the two media on the basis of the presumptive (acid) reaction reveals that in the modified "SF" broth 11 specimens (47.8 per cent) contained pure cultures of fecal streptococci in dilutions ranging from 1:100 to 1:1,000,000. In the "SF" medium 9 specimens (39.1 per cent) contained these organisms in

TABLE 5

Comparative incidence of enterococci and Escherichia coli

SOURCE	SAMPLE NUMBER	HIGHEST DILUTION SHOWING		SOURCE	SAMPLE NUMBER	HIGHEST DILUTION SHOWING	
		Enterocci	*E. coli*			Enterococci	*E. coli*
Human	1	10^{-3}	10^{-5}	Rabbit	11	Negative	10^{-2}
	12	10^{-3}	10^{-5}		19	10^{-1}	10^{-3}
	51	10^{-3}	10^{-4}		26	10^{-1}	10^{-5}
	52	10^{-4}	Negative		33	10^{-1}	Negative
	53	10^{-2}	10^{-4}		46	10^{-2}	10^{-6}
Cat	2	10^{-2}	10^{-2}	Rat	14	10^{-2}	10^{-5}
	8	10^{-3}	10^{-5}		22	10^{-1}	10^{-5}
	18	10^{-3}	10^{-5}		23	10^{-1}	10^{-5}
	31	10^{-5}	10^{-7}		37	10^{-2}	10^{-6}
	40	10^{-4}	10^{-7}		39	10^{-2}	10^{-3}
Mouse	3	10^{-1}	10^{-5}	Chicken	17	10^{-4}	10^{-5}
	10	10^{-3}	10^{-5}		24	10^{-5}	10^{-5}
	15	10^{-1}	10^{-5}		25	10^{-5}	10^{-5}
	38	10^{-4}	10^{-6}		30	10^{-5}	10^{-3}
	45	10^{-2}	10^{-3}		32	10^{-3}	10^{-3}
					44	10^{-5}	10^{-6}
Guinea pig	4	10^{-1}	10^{-5}	Flies	20	10^{-4}	10^{-4}
	9	10^{-2}	10^{-5}		27	10^{-5}	10^{-4}
	13	10^{-2}	10^{-2}		28	10^{-2}	Negative
	34	10^{-1}	10^{-4}				
	42	10^{-2}	10^{-2}				
Dog	7	10^{-3}	10^{-5}	Monkey	36	10^{-3}	10^{-6}
	16	Negative	10^{-5}		41	10^{-5}	10^{-5}
	21	10^{-2}	10^{-2}		47	10^{-2}	Negative
	29	10^{-3}	10^{-3}		48	10^{-3}	Negative
	35	10^{-6}	10^{-6}		49	10^{-3}	10^{-2}
	43	10^{-2}	10^{-3}		50	10^{-3}	10^{-4}
Soil	5	Negative	Negative				
	6	Negative	Negative				

pure culture and in dilutions of from 1:100 to 1:10,000. Fecal streptococci associated with bacilli were found in 8 specimens (52.2 per cent) cultured in modified "SF" broth as compared with 12 specimens (60.9 per cent) in the unmodified medium (table 4).

Forty-six (90 per cent) of the 51 fecal specimens examined for coliform organisms contained *Escherichia coli* in dilutions ranging from 1:100 to

1:10,000,000. Five specimens which did not contain *E. coli* did contain other coliform types. Only 2 of the 51 specimens were negative for enterococci, the remaining 49 samples contained these organisms in dilutions of from 1:10 to 1:1,000,000 (table 5). Among the specimens containing both *E. coli* and enterococci, 8 (15.7 per cent) contained enterococci in greater numbers than *E. coli*, 11 (21.6 per cent) contained these organisms in equal numbers, and 32 (62.7 per cent) contained greater numbers of *E. coli* than of fecal streptococci (table 6).

DISCUSSION

Although it has been suspected that the enterocci are rather generally distributed in the intestinal tract of warm-blooded animals, earlier work has been confined to a very few animal species. This study demonstrates that fecal streptococci are common in the excreta of 10 animal species and that they occur in

TABLE 6

Numerical occurrence of Escherichia coli and enterococci

SPECIMENS IN WHICH	NUMBER OF SAMPLES
E. coli less than enterococci	8 (15.7%)
E. coli equal enterococci	11 (21.6%)
E. coli greater than enterococci by 1 dilution	7 (13.8%)
E. coli greater than enterococci by 2 dilutions	11 (21.6%)
E. coli greater than enterococci by 3 dilutions	5 (9.8%)
E. coli greater than enterococci by 4 dilutions	8 (15.6%)
E. coli greater than enterococci by 5 dilutions	1 (1.9%)
Total	51

significant numbers. Though generally outnumbered by *E. coli*, the resistance of enterococci to chemical agents and possibly to other environmental factors make them of sanitary significance as indices of fecal contamination and pollution.

"SF" medium is of great value in the detection of fecal streptococci in natural sources, but it is deficient in that false positive (acid) reactions occur and in that fecal streptococci may be present in the absence of a positive (acid) reaction. Modification of the "SF" medium by the addition of 6.5 per cent sodium chloride results in the elimination of most false positives but does not appreciably influence the occurrence of false negative reactions. Studies should be undertaken looking toward the development of a modified medium with which greater recovery of enterococci can be obtained without any sacrifice in specificity.

SUMMARY

Fifty-one fecal specimens and two soil samples were examined for enterococci and coliform organisms. The two soil samples were negative for both enterococci and *Escherichia coli*. Forty-nine specimens representing 10 animals contained enterococci in from one-tenth to one one-millionth of a gram of feces. *E. coli*

was present in 46 specimens and was recovered in dilutions ranging from 1:100 to 1:10,000,000.

Acidity was produced in 71 tubes of "SF" broth, of which 60 contained fecal streptococci alone or in combination with rods. Eleven tubes were false positive (acid), containing bacilli but no streptococci. Four and six-tenths per cent of the inoculated tubes were false negative (no acid), containing fecal streptococci without evidence of acid production.

The addition of 6.5 per cent sodium chloride to the "SF" medium reduced the number of false positive reactions from 4.3 per cent to 0.7 per cent, but did not appreciably influence the occurrence of false negative reactions.

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