THE SIGNIFICANCE OF STREPTOCOCCI IN WATER SUPPLIES.

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The determination of the presence or absence of *B. coli* in a drinking water is universally regarded as the estimation of most value in determining its safety or as grounds for its condemnation. Valuable as this estimation is it is desirable that, if possible, it should be reinforced and confirmed by the determination of the presence or absence of other organisms. In particular it would be of extreme value and importance if the isolation of other selected organisms could be used to throw light upon two points in regard to which evidence is either lacking or indefinite at the present time. These two matters are the question as to whether ascertained contamination is of human or animal origin and as to the probable date of any contamination shown to be present. The *B. coli* enumeration does throw considerable light upon the latter, but none upon the former point.

It is important to consider to what extent the determination of the streptococcus content of a water is of value in the following connections:

(*a*) To enable an opinion to be given as to the purity of a given water supply and in confirmation of other bacteriological determinations.

(*b*) To supply information as to the actual source of any pollution found to occur, particularly whether human or animal in origin.

(*c*) To throw light upon the interval which has elapsed since such bacterial contamination occurred.

A number of investigations have been carried out in connection with these different aspects of the subject. It has been established that streptococci as a class are abundant in sewage and in excreta, both human and animal. Further a number of workers have failed to find these organisms in water supplies known to be pure while they can be

found, often in large numbers, in water supplies known to be definitely sewage contaminated.

A certain amount of investigation has also been undertaken to study the varieties of streptococci found in waters with the object of ascertaining the strains which are specially associated with excretal contamination, particularly those of human origin. It cannot be said that these researches have yielded data which are available to frame an opinion either as to the recentness of any pollution or its specific source. While it is of value for research purposes and with the hope of ultimate success along these lines, to isolate and investigate the different strains of streptococci in water supplies, the determination of the characters of isolated streptococci gives no information additional to that yielded by the numerical estimation of their presence as a class.

While from a broad and general point of view it may be said that the streptococcus determination has been shown to be of value there is as yet by no means a consensus of opinion as to whether this determination is of sufficient value to make it worth while to carry it out. Indeed many bacteriologists omit the examination altogether, while others who carry it out pay but little attention to the findings when obtained. Very few series of analyses have been published in which streptococcus enumerations have been recorded along with topographical details and the *B. coli* and other bacterial determinations.

In our opinion it is desirable to carefully consider the precise value of this estimation and in this paper we record data dealing with a large series of individual samples. The results deal entirely with the presence of streptococci as a class and not any particular varieties. Some questions dealing with the comparative vitality of streptococci and *B. coli* organisms in natural waters are being investigated, and we hope to publish the results shortly.

Method of Examination and Numerical Determination.

It is a decided drawback to the streptococcus test that none of the available methods of examination are really satisfactory. Two types of method have been recommended—direct and indirect.

The direct method involves the concentration of the water by filtration through porcelain filters, or by other means, and brushing definite fractions of the concentrated water over suitable solid media in Petri dishes. The streptococci are isolated from the plates and if necessary studied in pure culture. This procedure requires a considerable quantity

of the water, is troublesome and time consuming, and the isolation of the individual streptococcus colonies is unsatisfactory. For quantities of 1 c.c. or less, which can be plated direct, this procedure is useful but in our opinion is very unsatisfactory for larger quantities of water. In the indirect method the water is added in varying amounts to liquid media. After incubation the sediment is examined for streptococcus chains, and if necessary these may be isolated on solid media and their characters studied.

Glucose formate broth incubated anaerobically has been recommended as suitable for this purpose, but we have used glucose neutral red broth incubated aerobically. This method has been used by one of us for many years[1]. The exact procedure used is as follows: 0·1 and 1·0 c.c. respectively of the water are added to tubes of glucose neutral red broth while 10 c.c. is added to a tube of the same broth but of double strength. The larger quantity of the water tested, *i.e.* 30 c.c., is mixed with a large tube of the double strength broth. The broth mixtures are incubated at 37° C. for 40–48 hours and are then examined for streptococci in hanging-drop preparations. Only cocci in quite definite chains are taken as evidence of the presence of streptococci, and if none are found several preparations are examined from each tube before a negative result is recorded.

In the rare cases in which doubt arises as to whether streptococci chains are present it may be necessary to centrifugalise and stain the deposit.

Neutral red is preferred to plain neutral broth since the presence of the dye facilitates the detection of the streptococci in the microscopic preparations.

The method is obviously open to several objections of which the two most important are that delicate strains of streptococcus may not develop in the medium selected and these or even ordinary strains may be overgrown by other bacteria, and that the value of the method is too much dependent upon the care and attention of the individual carrying out the examination. It has the very great merit that it is simple and easy to carry out and takes up very little time or material.

[1] See *Bacteriological Examination of Water Supplies*, by William G. Savage, 1906, Lewis & Co.

Results obtained from the Examination of different Water Supplies.

The following results are based upon bacteriological analyses of drinking waters made in the Somerset County Laboratory during the last four to five years and carried out by the method described above, which was identical throughout the period of examination.

While a large proportion of the samples were from sources the sanitary circumstances of which were within our personal cognizance this is not the case for many of them and we are on this account precluded from adopting local topographical features as our main basis of classification. Failing this the most profitable method of comparison and classification is to compare the streptococcus findings with the numerical presence of *B. coli* in the same samples, using the sanitary information available to elucidate particular samples.

The data available can be conveniently dealt with in two groups:

I. Comparison of the results in bulk.

II. Consideration of individual supplies and groups of supplies.

I. *Comparison of the results in bulk.*

In view of the different significance to be attached to the presence of streptococci and *B. coli* in different classes of waters it is necessary to consider these classes separately. So few upland surface water samples were available in the present series of analyses that we have decided not to deal with this group and all such analyses have been excluded. The samples included in the present paper are grouped into the two following classes:

A. *Deep water supplies.* This group includes the samples from springs and deep wells.

In regard to these samples it must be taken into consideration that while they must be classed from the point of view of their origin and main source as not derived from the superficial strata many of them at the time of their examination received, or were liable to receive, an admixture of surface water. Indeed the reason why many of these samples were submitted for examination was because surface contamination was suspected and in many cases the analytical results showed that this in fact did occur.

B. *Surface water supplies.* Practically all these samples were obtained from surface (or shallow) wells, including the ordinary draw wells with open mouth and bucket, surface wells with pumps and dip wells.

Here again this statement must be qualified by stating that while all the samples were classed as of this character and so described by the officials who collected them, it is probable that for a few of them the wells although not sunk to any great depth really penetrated the upper impervious layer and their water was derived from the deeper pervious strata beneath.

For purposes of comparison the results are classified on a *B. coli* basis as follows:

B. coli present in	Group A samples	Group B samples
0·1 or 1 c.c.	Evidence of marked contamination.	Evidence of marked contamination.
10 c.c.	Highly suspicious and showing evidence of contamination.	Of suspicious and doubtful quality.
30 c.c.	Ditto.	Passed as showing no definite evidence of contamination.
Absent in 50 c.c.	No evidence of undesirable bacterial contamination.	No evidence of undesirable bacterial contamination.

The characters relied upon for the determination of *B. coli* were gas production in lactose bile salt broth, the character of the growth upon gelatine slope with absence of liquefaction (2 weeks), the production of indol in peptone water and the production of acid and clot in litmus milk within a week.

The findings in 340 samples of Class A and 974 of Class B, grouped according to their *B. coli* and streptococcus determinations, are shown in Tables I and II.

TABLE I.

A. *Deep water supplies.*

	Streptococci present in				
B. coli in	0·1 or 1	10	30 c.c.	Absent from 40 c.c.	Totals
0·1 or 1 c.c.	22	27	12	6	67
10 or 30 c.c.	3	22	35	58	118
Absent from 50 c.c.	1	4	16	134	155
Totals	26	53	63	198	340

B. *Surface supplies.*

	Streptococci present in				
B. coli in	0·1 or 1	10	30 c.c.	Absent from 40 c.c.	Totals
0·1 or 1 c.c.	234	142	39	28	443
10 c.c.	46	88	60	55	249
30 c.c. or absent from 50 c.c.	9	51	63	159	282
Totals	289	281	162	242	974

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TABLE II (*Percentages*).

A. *Deep water supplies.*

B. coli in	Streptococci present in 0·1 or 1		10		30 c.c.		Absent from 40 c.c.	
0·1 or 1 c.c.	84·6	*32·8*	51	*40·3*	19	*17·9*	3	*9*
10 or 30 c.c.	11·5	*2·5*	41·5	*18·6*	55·5	*29·6*	29·3	*49·2*
Absent from 50 c.c.	3·8	*0·6*	7·5	*2·5*	25·4	*10·3*	67·7	*86·5*

B. *Surface supplies.*

B. coli in	Streptococci present in 0·1 or 1		10		30 c.c.		Absent from 40 c.c.	
0·1 or 1 c.c.	81	*52·8*	50·5	*32*	24	*8·8*	11·6	*6·3*
10 c.c.	16	*18·5*	31·3	*35·3*	37	*24·1*	22·7	*22·1*
30 c.c. or absent from 50 c.c.	3	*3·2*	18·2	*18·0*	39	*22·3*	65·7	*56·4*

In calculating percentages nothing is to be gained by working out the percentage of water samples which contain streptococci or *B. coli* respectively in the different amounts. The percentage results are worked out in two ways. In one way (figures in italic type, Table II) each group of *B. coli* prevalence is taken separately and the percentage prevalence of each group of waters on a streptococcus basis is calculated. In the other way (figures in ordinary type, Table II) each group of streptococcus prevalence is taken separately and the percentage prevalence of the waters on a *B. coli* basis is recorded.

Certain broad deductions may be made from these tables.

Absence of streptococci. In two-thirds of the samples from both groups of waters (67·7 and 65·7 per cent. respectively) in which streptococci were absent this absence was associated either with an absence of *B. coli* or their presence in only very small numbers. For the remaining one-third with absence of streptococci *B. coli* were usually present in small numbers only, but this was not invariably the case and this proportion of samples is sufficiently high (3 per cent. in Group A and 11·6 per cent. in Group B) to suggest that by itself absence of streptococci is insufficient to pass a particular sample as satisfactory although a point in favour of its purity.

Consideration of the detailed analyses of the samples with high *B. coli* content but showing no streptococci and a study of the sanitary surroundings (when information was available) showed that in fact all or nearly all these supplies must be considered as contaminated.

It may be that streptococci were really absent from these samples, but it is probable that in some cases, possibly for most of them, the technique was at fault and streptococci present in the water as collected

failed to grow in the culture tubes either from the competition of other organisms or from inherent delicacy.

Streptococci present in comparatively small numbers. When found only in 30 c.c. the findings are fairly in accord with those of *B. coli*, but since in the surface supplies (Group B) the presence of *B. coli* in 30 c.c. is grouped with absence of *B. coli* the streptococcus findings are higher in this group than for Group A.

The presence of streptococci in 10 c.c. was in general associated with samples of bad bacterial quality on a *B. coli* basis. Thus 92·5 per cent. of the deep water samples in which streptococci were present in 10 c.c. contained *B. coli*, while 81·8 per cent. of the surface supplies contained *B. coli* in 10 c.c. or less. All these samples on a *B. coli* basis would be regarded as of bad bacteriological quality.

Streptococci in large numbers (*i.e.* in 0·1 or 1 c.c.). These findings are closely in accord with the *B. coli* results. In only 3 and 3·8 per cent. respectively were abundant streptococci met with in samples which were satisfactory on a *B. coli* basis. These percentages represent one actual sample in Group A and nine in Group B. The details of these ten samples are shown in Table III.

The percentage results may also be considered from the *B. coli* point of view.

Absence of B. coli or presence in only small numbers. Streptococci were only found in 10 c.c. or less in five (*i.e.* 3·1 per cent.) of the deep water samples which were free from *B. coli*, a very close agreement. The details of these five samples are shown in Table III.

The agreement is less close for the surface supplies since 21·2 per cent. of the samples in which *B. coli* was either absent or in 30 c.c. only contained streptococci in 10 c.c. or less. Of these in nine samples (shown in Table III) streptococci were in 0·1 or 1·0 c.c. A closer parallel is however found if the 156 samples free from *B. coli* in 50 c.c. are considered separately. Twenty samples (13 per cent.) contained streptococci in 10 c.c. and only four (2·5 per cent.) in 0·1 or 1 c.c. Assuming a value to the streptococcus determinations these results suggest that the permissible standard of *B. coli* in 30 c.c. for this class of waters is probably often a too lenient one.

Waters decidedly suspicious on a B. coli basis. In 49·2 per cent. of the deep water samples classed as suspicious on a *B. coli* enumeration, streptococci were not found, furnishing further evidence in favour of the view that absence of streptococci is not in itself a reliable criterion of purity. Of the remaining 50·7 per cent. in 48·2 per cent. the streptococcus

and *B. coli* results were the same while in 2·5 per cent. streptococci were very abundant (in 0·1 or 1 c.c.). Of the surface supplies decidedly suspicious on a *B. coli* basis, streptococci were absent in 22·1 per cent., in 10 or 30 c.c. in 59·4 per cent. and very abundant in 18·5 per cent.

Samples showing marked evidence of contamination on a B. coli basis. The tables show that the majority showed abundant streptococci, but sometimes only in 10 c.c., while in 9 per cent. of the deep water samples and 6·3 per cent. of the surface samples no streptococci were found.

TABLE III.

No.	Source	Organisms per c.c. 37° C.	Organisms per c.c. 21° C.	Per litre *B. coli*	Per litre Streptococci	Remarks
1.	Deep well	220	350	Absent*	1000–10,000	See No. 53, Table IV.
2.	Spring	20	800	,,	100–1000	Some lactose fermenters in 30 c.c., but no *B. coli*. A spring rising through boggy land.
3.	,,	40	2000	,,	,,	Liable to surface contamination from a road.
4.	,,	15	20	,,	,,	A public water supply from springs from Old Red Sandstone. Other examinations satisfactory.
5.	,,	1140	—	,,	,,	A spring about 30 yards from house.
6.	Borehole	2500	—	Over 10,000	Absent*	Sample taken soon after the boring made.
7.	Spring	30	200	1000–10,000	,,	
8.	Deep well	57	170	,,	,,	See No. 51, Table IV. Streptococci found in 500 c.c.
9.	Spring	7	10	,,	,,	Sample taken after passage through old and probably defective pipe.
10.	,,	15	30	,,	,,	See No. 19, Table IV.
11.	,,	350	6000	,,	,,	See No. 29, Table IV.
12.	Shallow well	200	—	Absent	1000–10,000	Atypical organisms, giving no indol and only slightly fermenting lactose, isolated from 30 c.c.
13.	,,	56	—	,,	,,	No true *B. coli*, but very aberrant lactose fermenters.
14.	,,	120	—	,,	,,	
15.	,,	600	—	,,	,,	Very atypical forms isolated from 1 and 10 c.c. Collected after heavy rain. An open draw well about 20 ft deep in Keuper marl and in unsatisfactory position.
16.	,,	240	—	30–100	Over 10,000	A well in a back yard.
17.	,,	320	—	,,	1000–10,000	
18.	,,	600	1200	,,	,,	
19.	,,	145	—	,,	,,	Well with pump but defective cover. Surrounded by cultivated garden land. Also possible contamination from slop drainage.
20.	,,	25	500	,,	,,	Acid and gas in 10 c.c. bile salt broth, but no *B. coli* could be isolated.

* "Absent" means absent from 50 and 40 c.c. respectively.

II. *Consideration of individual supplies and groups of supplies.*

The actual proportion of cases in which the *B. coli* and streptococcus results are widely discrepant has been shown in the tables to be a low one. In Table III the most important of the discrepant individual analyses have been collected together.

Several of the samples in Table III are from supplies which are set out in subsequent tables and are more conveniently dealt with when these are being discussed. It will be noted in a good many of the samples showing abundant streptococci, but no *B. coli* in 50 c.c., that lactose fermenters were present but of types which precluded them being considered as *B. coli* even with a lax interpretation. Information in regard to these supplies was in many cases available and makes it probable that most of them were liable to contamination. For these the streptococcus determinations the more accurately mirror the quality of the supplies.

The significance of streptococci in water can also be studied by considering series of analyses from individual supplies.

A. *Deep water supplies.*

A few supplies have been selected which have been examined on a number of occasions and the local conditions of which are well known to us. The details of the analyses are set out in Table IV.

TABLE IV.

Supply	No.	Date examined	Organisms per c.c. 37° C.	Organisms per c.c. 21° C.	Per litre *B. coli*	Per litre Streptococci	Remarks
A	1	Oct. 24th, 1911	100	500	1000–10,000	1000–10,000	
„	2	Nov. 13th, „	120	950	„	„	
„	3	Nov. 29th, „	60	—	30–100	Absent	
„	4	Jan. 16th, 1912	40	100	100–1000	„	
„	5	May 6th, „	7	35	Absent	„	Streptococci in 100 c.c., but *B. coli* absent in 100 c.c.
„	6	June 4th, „	150	605	Over 10,000	Over 10,000	
„	7	Feb. 5th, 1913	3	20	In 100 c.c. only	Absent	
„	8	Oct. 9th, „	26	53	1000–10,000	100–1000	
B	9	Nov., 1911	—	20	Absent	Absent	
„	10	June, 1912	6	20	30–100	„	
„	11	Dec., „	2	12	Absent	„	
„	12	March, 1914	1	6	„	„	
„	13	Feb., 1915	1	15	„	„	

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TABLE IV—(*continued*).

Supply	No.	Date examined	Organisms per c.c. 37° C.	Organisms per c.c. 21° C.	Per litre *B. coli*	Per litre Streptococci	Remarks
C	14	Aug., 1911	40	300	100–1000	Absent	
„	15	Nov., „	—	650	1000–10,000	30–100	
„	16	Nov., „	18	350	100–1000	100–1000	
„	17	April, 1912	22	40	Absent	Absent	
„	18	June, „	130	950	Over 10,000	100–1000	
„	19	June, 1913	15	30	1000–10,000	Absent	
„	20	Sept., „	50	500	1000–10,000	1000–10,000	
„	21	June, 1914	7	120	100–1000	30–100	
„	22	Sept., „	12	35	„	100–1000	
„	23	Dec., „	42	1350	„	„	
„	24	June, 1915	4	42	30–100	Absent	
D	25	June, 1912	8	360	100–1000	30–100	
„	26	Aug., „	37	350	30–100	100–1000	
„	27	Sept., „	12	55	„	30–100	
„	28	Nov., „	60	240	Absent	„	
„	29	Oct., 1913	350	6000	1000–10,000	Absent	
E	30	Dec., 1911	200	750	100–1000	30–100	Not collected so as to avoid mixture with surface water.
„	31	April, 1912	9	95	Absent	Absent	Personally collected.
„	32	Dec., 1913	3	32	„	„	
„	33	March 1914	2	9	„	„	
F	34	July, 1911	10	1200	„	30–100	
„	35	July, „	3	—	100–1000	Absent	*B. coli* rather atypical.
„	36	Oct., 1913	9	46	30–100	„	
„	37	Aug. 10th, 1914	11	160	1000–10,000	30–100	
„	38	Aug. 26th, „	2	25	30–100	Absent	
„	39	Sept., „	95	420	1000–10,000	1000–10,000	
„	40	Feb. 11th, 1915	1	2	Absent	Absent	From spring head.
„	41	Feb. 11th, „	170	850	100–1000	Over 10,000	After passage through open agricultural pipes for 2 miles.
G	42	July, 1912	1	—	Absent	Absent	
„	43	May, 1913	820	2500	„	„	
„	44	Aug., 1914	2	4	„	„	
H	45	March, 1913	2	14	30–100	„	
„	46	Nov. 3rd, 1914	400	7500	1000–10,000	Over 10,000	
„	47	Nov. 9th, „	135	1075	„	100–1000	
„	48	Dec. 15th, „	16	312	100–1000	30–100	
J	49	Aug., 1911	20	50	Absent	Absent	
„	50	Jan. 1st, 1912	3	2500	100–1000	„	
„	51	Jan. 24th, „	57	170	1000–10,000	„	
„	52	Feb., „	3	12	Absent	30–100	
„	53	March, „	220	350	„	1000–10,000	
„	54	June, „	2	25	„	Absent	
„	55	Sept., „	2	10	„	„	
„	56	Dec., „	5	70	„	„	
„	57	April, 1913	15	25	„	„	

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TABLE IV—(*continued*).

Supply	No.	Date examined	Organisms per c.c. 37° C.	Organisms per c.c. 21° C.	Per litre *B. coli*	Per litre Streptococci
J	58	Sept., 1913	3	18	Absent	Absent
,,	59	Dec., ,,	2	12	,,	,,
K	60	Jan. 22nd, 1912	110	250	1000–10,000	30–100
,,	61	Feb. 15th, ,,	40	120	30–100	Absent
,,	62	Sept. 9th, 1913	10	250	100–1000	,,
,,	63	June 10th, 1915	5	40	30–100	,,
K_1	64	Feb. 15th, 1912	4	30	Absent	,,
,,	65	Dec. 10th, ,,	72	900	1000–10,000	100–1000
,,	66	June 23rd, 1915	3	12	Absent	Absent
K_2	67	Feb. 15th, 1912	41	120	100–1000	,,
,,	68	Jan. 2nd, 1913	7	35	30–100	30–100
,,	69	Sept. 9th, ,,	5	105	100–1000	Absent
K_3	70	Feb. 15th, 1912	9	80	Absent	,,
,,	71	Jan. 2nd, 1913	18	90	,,	,,
,,	72	June 23rd, 1915	6	26	30–100	,,
K_4	73	Feb. 15th, 1912	3	21	,,	,,
,,	74	June 23rd, 1915	6	35	100–1000	30–100
K_5	75	Aug. 24th, 1914	135	350	Over 10,000	100–1000
,,	76	May 4th, 1915	35	—	100–1000	,,
,,	77	June 23rd, ,,	3	16	30–100	Absent

Addendum to Table IV.

The following particulars as to these sources of supply are necessary to elucidate the analyses.

Supply A. A large and important water supply obtained from a well 36 feet deep with several lateral headings sunk in the Carboniferous Limestone. The supply is liable to considerable intermittent contamination, particularly after heavy rain following a period of dry weather. The limestone is very fissured and the contamination is probably from surface waters through the fissures.

Supply B. Obtained from a well and borehole 130 feet deep in the Carboniferous Limestone. The surroundings are quite satisfactory.

Supply C. A village supply obtained from a spring. Careful inspections and investigations on the spot, together with the bacteriological and chemical analyses made, show that the spring is liable to contamination with surface water. The water supplied is a mixture of (presumably) pure spring water and surface water more or less bacterially contaminated. The analytical results obtained are largely influenced by the rainfall and when this is heavy, with a consequent heavy admixture of surface water to the spring water, the bacteriological results are bad, usually markedly so.

Supply D. From a spring about half-a-mile from the village which it supplies. No local sources of contamination can be found to account for the evidence of pollution nearly uniformly shown by the analyses. We have not however ourselves investigated the surroundings of this supply.

Supply E. A large supply obtained from a spring which rises at the junction of the Old Red Sandstone and the Lower Limestone Shales. The surroundings are satisfactory and show no likelihood of contamination.

Supply F. This supply is derived from a spring and after Nov., 1912, also from a borehole both in the Dolomitic Conglomerate. The surroundings of the spring and borehole are satisfactory and properly protected, but the water is conveyed by earthenware pipes for a distance of several miles before it is again collected and transmitted to the reservoirs of the town supplied. The majority of the samples are from the water as distributed and after contamination has taken place through the faulty pipes.

Supply G. A considerable supply from a well and borehole in the Trias, about 290 feet deep.

Supply H. Obtained from a spring from the Dolomitic Conglomerate. The surroundings of the spring are very unsatisfactory and the water is rapidly discoloured after rain.

Supply J. Obtained from a well 31 feet deep sunk in the Carboniferous Limestone. At one time the analyses were unsatisfactory as shown in the table and this was traced to contamination from surface waters along the sides of the well. Quite satisfactory analytical results have been obtained after this matter was remedied.

Supply K. Water derived from five springs (K_1 to K_5) issuing from Devonian rock collected under the soil and passing to a water main which runs along the valley. The pipes are so arranged that the individual springs can be added or cut off as required. Considerable alterations and improvements have taken place during the period covered by the analyses and unsatisfactory agricultural pipes have been replaced by proper iron pipes. In general the surroundings are satisfactory although liable to some contamination from sheep grazing over the gathering area, but the means of collecting the water at the time the early samples were collected were not good. The samples K are from the mixed waters K_1, K_2, etc. from the individual springs.

Many of these supplies have been selected for this table because they have been from time to time, or continuously, contaminated by surface water or from other undesirable sources and it is of interest to note how far such contamination is shown by both the *B. coli* and streptococcus enumerations.

Table IV shows that with a few exceptions the correspondence is a close one. For supplies A and B the agreement is very close. Most of the samples for supply C agree, but on several occasions streptococci were not detected when *B. coli* were abundant. None of the samples showed abundant streptococci with absent or relatively few *B. coli*.

Supplies E, G and H agree closely as regards these two determinations, but for D and F a few of the samples showed considerable differences. Supply J is of great interest since in the early part of the year 1912 the *B. coli* findings (but not the streptococcus estimations) showed marked contamination. This was traced to contamination from surface water

along the sides of the well. Steps were taken to prevent this and later analyses were quite satisfactory. Sample No. 53 was taken very soon after the alterations had been made and although *B. coli* were absent streptococci were abundant. The high 21° or 37° counts confirm the streptococcus findings and probably here the streptococcus count was more reliable than the absence of the *B. coli* and was of decided utility.

The results for supplies K mostly agree but as in other cases in several instances absence of streptococci was associated with presence of *B. coli* in small and in three cases in considerable numbers.

B. *Surface water supplies.*

Almost all the samples were from shallow wells mostly with a pump, but many were open draw wells, while a few were dip wells.

As a rule each well was examined not more than once, or at the most twice, so that it is not possible to submit series of analyses from individual supplies. Very variable results are liable to be obtained from surface water samples taken on different occasions since the local conditions vary from time to time while, in particular, much depends upon the extent of the previous rainfall.

Table V is of interest from the point of view of comparing the *B. coli* and streptococcus results of surface wells, all from the same parish, re-examined after a long interval. Between the examinations some steps had been taken to improve the condition of the wells, while all the later samples (examined May, 1915) were collected after a long period of dry weather.

TABLE V.

No.	Date examined	No. of organisms at 37° C.	Per litre		Remarks
			B. coli	Streptococci	
1.	July, 1914	10	1000–10,000	Absent	A well about 8 ft deep with pump; position unsatisfactory.
1 *a*.	May, 1915	3	Absent	,,	
2.	August, 1914	25	100–1000	100–1000	Well with pump. Leaky w.c. and drains near well at time of first examination. Position also unsatisfactory in other ways. Drainage put right between the examinations.
2 *a*.	May, 1915	4	Absent	Absent	
3.	August, 1913	120	,,	1000–10,000	Open draw well with defective walls. Position rather unsatisfactory.
3 *a*.	May, 1915	24	,,	Absent	
4.	March, 1914	170	1000–10,000	100–100	Well with pump.
4 *a*.	May, 1915	12	30–100	Absent	
5.	August, 1913	42	100–1000	30–100	Well with pump. Pump in scullery of the dwelling house. Well not opened, but position and surroundings unsatisfactory.
5 *a*.	May, 1915	25	1000–10,000	100–1000	
6.	May, 1913	105	,,	,,	Open draw well about 60 ft deep. 6 ft from house and 60 from privy. Liable to surface contamination.
6 *a*.	May, 1915	20	Absent	,,	

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Table VI is given as a good example of a series of samples from surface wells which were all in the same parish and sunk in the same geological strata. Forty-seven different wells were bacteriologically examined, two samples being from the same well. The parish has a population of 550 and is situated in central Somerset on a slight elevation of Keuper marl, the low-lying land round being covered with alluvium and recent deposits. When the samples were collected the parish depended entirely upon surface wells for its water supply. The wells were sunk in the marl, usually to a depth of 20 to 30 ft, and yielded but limited supplies of water. Many of them were inspected by one of us. All these were of the usual type with sides of loose stone not made impervious in any way and not set in cement. Some were open draw wells but most had pumps, many of the latter with faulty coverings to the wells. The majority were in close proximity to the houses they supplied and a good many were in yards or outhouses, or in some cases under the floors of the kitchens or wash-houses. On inspection many could be condemned outright, a few were in reasonably satisfactory positions while the remainder were in positions which rendered the pollution of their contents likely to result but for which bacteriological examinations were necessary to say if it occurred.

In general the streptococcus and *B. coli* results agree closely. Samples Nos. 8 and 9 were markedly contaminated on a *B. coli* basis, but no streptococci were found. We have not examined well No. 8, but the well from which Sample No. 9 was obtained was in a shed, with a proper cement floor, by the side of the house. The well was covered over and provided with a pump and there were no drains near or other obvious sources of contamination apart from the close proximity to the house.

In five samples, *i.e.* Nos. 14, 15, 16, 17 and 18, streptococci were present only in small numbers while *B. coli* were very abundant. Sample No. 14 is from the same well as Sample No. 27, in which fairly numerous streptococci were found. Sample No. 16 was obtained from a well with a pump, but the position was one liable to marked pollution and the well was condemned on this ground alone. No. 17 was from a well with a pump by the side of the house. The ground round was dirty and there was considerable probability of contamination. No. 18 was a draw well showing no direct evidence of sources of contamination apart from the open mouth. Samples Nos. 18 and 19 were from wells which inspection showed were unsatisfactory as regards their position and surroundings and the streptococcus results are probably more reliable than the *B. coli* findings. In No. 20 lactose fermenters were present

TABLE VI.

No.	Organisms per c.c. at 37° C.	Per litre	
		B. coli	Streptococci
1.	55	Absent	Absent
2	40	,,	,,
3.	65	,,	,,
4.	8	30–100*	,,
5.	18	,,	,,
6.	70	,,	,,
7.	23	,,	,,
8	450	1000–10,000	,,
9.	260	,,	,,
10.	20	100–1000*	30–100
11.	1800	,,	,,
12.	95	,,	,,
13.	650	,,	,,
14.	220	1000–10,000	,,
15.	12	,,	,,
16.	450	,, *	,,
17.	400	Over 10,000	,,
18.	1120	,, *	,,
19.	220	Absent	100–1000
20.	180	,,	,,
21.	140	30–100	,,
22.	710	,,	,,
23.	200	100–1000*	,,
24.	320	,,	,,
25.	320	,,	,,
26.	125	,, *	,,
27.	85	,,	,,
28.	7	1000–10,000	,,
29.	640	,,	,,
30.	300	,, *	,,
31.	45	,,	,,
32.	700	,,	,,
33.	1200	,,	,,
34.	1200	,,	,,
35.	650	,, *	,,
36.	260	,,	,,
37.	1700	Over 10,000	,,
38.	90	,,	,,
39.	700	,,	,,
40.	200	1000–10,000	1000–10,000
41.	720	,, *	,,
42.	880	,,	,,
43.	270	,,	,,
44.	450	Over 10,000	,,
45.	4000	,,	,,
46.	520	,,	Over 10,000
47.	50,000	,,	,,
48.	520	,,	,,

* The isolated *B. coli* were indol negative, otherwise typical.

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in 10 and 1 c.c., but they were not classed as *B. coli*, failing to clot milk or produce indol, while the growth on gelatine slope was of unusual type. Probably a further sample would have shown numerous typical *B. coli*.

The majority of the samples in Table VI show evidence of contamination. In contrast to this the second series of wells shown in Table VII may be considered. The samples were all collected from the wells in

TABLE VII.

No.	Organisms per c.c. at 37° C.	Per litre	
		B. coli	Streptococci
1.	5	Absent*	Absent*
2.	5	,,	,,
3.	2	,,	,,
4.	8	,,	,,
5.	3	,,	,,
6.	22	,,	,,
7.	2	,,	,,
8.	8	,,	,,
9.	7	,,	,,
10.	6	,,	,,
11.	12	,,	,,
12.	8	Absent (atypical 30–100)	,,
13.	5	Absent (atypical 30–100)	,,
14.	7	30–100	,,
15.	110	100–1000†	,,
16.	60	,,	,,
17.	2500	Over 10,000	,,
18.	7	Absent	30–100
19.	15	,,	,,
20.	8	,,	,,
21.	88	100–1000	,,
22.	16	100–1000†	,,
23.	15	1000–10,000	,,
24.	200	Absent	100–1000
25.	500	30–100	,,
26.	15	,,	,,
27.	55	,,	,,
28.	50	100–1000	,,
29.	35	,,	,,
30.	35	1000–10,000	,,
31.	95	,,	,,
32.	300	Over 10,000	,,
33.	150	100–1000	1000–10,000
34.	800	1000–10,000	,,
35.	640	Over 10,000	,,
36.	520	,,	,,

* "Absent" = absent from 50 or 40 c.c. respectively. † *B. coli* indol negative.

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a small country town of about 4600 population, provided with a pure water supply from springs but which also contained a considerable number of surface wells which the occupiers preferred to use to the town supply. The essential object for the collection of the samples was to ascertain if these wells should be closed. Almost all had pumps provided.

In general the *B. coli* and streptococcus results show a large measure of agreement the greatest differences being met with in Samples Nos. 15, 16, 17, 23, 24 and 25.

A third series of analyses of the water of surface wells from another parish is shown in Table VIII. They are reproduced here as the sanitary surroundings of every well were carefully investigated by us on more than one occasion. The parish is a somewhat scattered one and the wells are at very different levels. Some are 50 or more feet deep, the level of the subsoil water being at least 30 ft from the surface when the samples were taken, while others are in low-lying parts, the water level being but a foot or so from the ground level and sunk in the alluvium. In winter the latter are usually flooded.

All the wells were of defective construction and all in such unsatisfactory surroundings that sanitary inspection alone was sufficient to condemn them.

TABLE VIII.

No.	Organisms per c.c. at 37° C.	Per litre		Remarks
		B. coli	Streptococci	
1.	50	1000–10,000	Absent	*B. coli* indol negative. A rather deep well with pump.
2.	80	30–100	30–100	Pump.
3.	50	100–1000	,,	,,
4.	10	1000–10,000	,,	Draw well.
5.	400	Absent	100–1000	,,
6.	50	30–100	,,	A dip well fed by a superficial spring but liable to gross surface contamination.
7.	320	100–1000	,,	Pump.
8.	200	,,	,,	Draw well.
9.	550	,,	,,	,,
10.	140	1000–10,000	,,	,,
11.	30	100–1000	1000–10,000	,,
12.	1200	,,	,,	,,
13.	320	,,	,,	Pump.
14.	330	1000–10,000	,,	,,
15.	120	Over 10,000	,,	,,
16.	480	1000–10,000	Over 10,000	Draw well.
17.	5000	Over 10,000	,,	,,
18.	350	,,	,,	Pump.
19.	150	,,	,,	,,

It is of interest to note that the streptococcus as well as the *B. coli* findings in nearly every case confirm the results obtained from the inspection of the wells and their surroundings. Only in No. 1 were streptococci not found. This sample was from a well in the higher parts of the parish and on inspection considerably less liable to contamination than most of the others. The *B. coli* content was higher than would have been expected from the inspection. In No. 5 no *B. coli* were found, but some very slow lactose fermenters were isolated. Probably a further sample would have shown *B. coli*. An insanitary privy was near this open draw well and the well was situated immediately at the back of the house. On sanitary grounds the well would be condemned at once. This opinion is confirmed by the high 37° C. count and the abundant streptococci.

The results obtained from the examination of the wells of a number of other parishes have been tabulated and critically examined, but as the evidence they furnish is all on the lines of the above it is unnecessary to reproduce them and multiply the number of tables.

Conclusions.

The results of the analyses studied in bulk and detailed consideration of individual supplies yield results which are essentially the same.

The absence of streptococci (at least with the methods used) is of less significance than their presence. Absence of streptococci, even from a considerable bulk of the water, cannot be accepted, to the same extent as absence of *B. coli*, as reliable evidence of the freedom from serious contamination of the water at the time the particular sample was collected, although it is a point in favour of its purity.

Standards for permissible number of streptococci broadly correspond to similar numerical standards for *B. coli*, but are of less significance and reliability.

While we do not know enough about the varying vitality and distribution of streptococci to say whether the presence of certain strains may or may not be disregarded as evidence of excretal contamination it is, in general, reliable to assume that streptococci in large numbers are only present in waters from unsatisfactory sources.

We are decidedly of opinion that the streptococcus estimation, carried out by the simple method described, is of undoubted value as evidence for or against excretal contamination.

We think further that if the reliability of the method could be improved the value of the streptococcus enumeration would be enhanced and even more clearly demonstrable.