THE PATHOGENICITY OF B. COLI IN RELATION TO THE BACTERIOLOGICAL EXAMINATION OF WATER.

By WILLIAM G. SAVAGE, B.Sc., M.D. (Lond.)

(*Late Lecturer on Bacteriology University College, Cardiff. Medical Officer of Health of Colchester.*)

In this paper the term *B. coli* is used in its most restricted sense, and as implying organisms having all the characters of the typical *B. coli communis*. It is a well-ascertained fact that *B. coli*, even when the term is so restricted, exhibit very varying virulence.

From the point of view of the bacteriological examination of water the question of the virulence of isolated *B. coli* has been raised by some workers to a position of much importance, and the pathogenicity of such bacilli, or of the incubated broth and water, has been advocated as the best criterion of the purity of a drinking-water.

The matter is one of considerable practical importance since it must be admitted that true *B. coli* in water may be derived from quite different sources and so possess different significance. If we could accurately determine their source, or at least divide into two groups those from harmful sources and those from harmless, a great step in the bacteriological examination of water would be attained and it might be possible, and with great confidence, to pass *B. coli* in large numbers in water from one source, while condemning a water from another source with perhaps less *B. coli* but of harmful origin.

It might be thought that the virulence of the isolated *B. coli* would be of service as such an indicator of the source, harmful or harmless. In considering this question I have based my conclusions and deductions in part upon the recorded results of other workers, and in part upon experiments of my own.

Levy and Brun[1] have strongly advocated the importance of the

[1] *Archiv f. Hygiene*, Vol. xxxvi.

https://doi.org/10.1017/S0022172400032927 Published online by Cambridge University Press

virulence test. Their view is, that if genuine *B. coli* can be demonstrated in water it is a proof of the *faecal contamination* of that water.

Such genuine *B. coli* can be distinguished, according to these authors, from what they call the coli-form varieties which occur in quite unobjectionable supplies, by testing their virulence.

They assert that 0·5 to 1·0 c.c. of a 48 hours' broth culture of *B. coli* derived from normal human faeces will if intraperitoneally injected into a guinea-pig kill the animal in 1—3 days, while in diseased conditions a much higher virulence may be exhibited. They further state that virulent coli races gaining access to water will subsist there and maintain their virulence for several weeks, while they have never come across a coli-form isolated from water capable of killing a guinea-pig in doses of 1—2 c.c. when injected intraperitoneally. The method they suggest is to treat 100 c.c. of the water with 1% peptone and 1·5% common salt and incubate at 37° C. for 48 hours. Guinea-pigs are then inoculated with 1—2 c.c. intraperitoneally; mice with 0·2 to 0·5 c.c. subcutaneously; rabbits with 2—3 c.c. intravenously.

With contaminated waters the animals will die, and virulent *B. coli* will be found at autopsy, with or without other species.

Blachstein[1] advocated a similar method, *i.e.* the injection of water after incubation with broth. His paper is however very inconclusive.

Weissenfeld[2] has also investigated this question. He examined 56 good and bad waters. His animal experiments were made either with pure broth cultures of the isolated organism, or, as done by Levy and Brun, and also by Blachstein, with mixed cultures of broth and water. His dose was 1 c.c. of a two days' old culture injected intraperitoneally into a guinea-pig of medium weight.

He found that from the results of the animal experiments no general rule could be enunciated. *B. coli* from, so-called, good waters were pathogenic or non-pathogenic, while with his bad waters both classes were also obtained. He concludes that the isolation of a virulent *B. coli* does not, of necessity, indicate faecal contamination.

His points of identification for his *B. coli* are however very inadequate. The characters he takes are:—vine-leaf surface gelatine colonies; gas in sugar agar stab; bacilli more or less motile, often not motile, decolorised by Gram. He states that he attaches no importance to milk-souring and indol production, while he does not mention

[1] *Annales de l'Institut Pasteur*, 1893, VII. p. 689.

[2] *Zeitschr. f. Hygiene*, 1900, XXXV. p. 78.

acid production or fermentation with different sugars, apart from gas in sugar agar stab cultures. He also classifies his waters into good or bad, but from the details appended this is rather an arbitrary classification. Compared with my results, given below, he obtained a high percentage of virulent *B. coli*. No mention is made, or I have overlooked it, of autopsies on the killed animals and recovery of the inoculated bacillus. He apparently also only examined 1 c.c. and 1 litre of the water.

In considering the question of virulence of *B. coli* in water supplies it must be remembered that it has no, or but very slight practical importance from the point of view of the possible *direct harmfulness* of such a virulent bacillus. *B. coli* are looked for in water not because they themselves are harmful, actually or potentially, but because they are *indicators* of contamination. In this sense they may be compared to the estimation of, say, chlorine in chemical water analysis, and the question of their direct harmfulness is no more at issue than that of the direct harmfulness of chlorides in water.

Also *B. coli* are not present in *perfectly pure* water and therefore their presence must be looked upon as indicating contamination, but by no means contamination of necessity dangerous. The question of virulent *B. coli* can therefore be narrowed down to the following:—Does the fact that an isolated *B. coli* is pathogenic, when obtained from a water, indicate that the contamination is harmful and of necessity dangerous, and does the fact of its being non-virulent indicate freedom from dangerous pollution? Such a conclusion can be by no means maintained. Sewage and human faecal contamination are the two chief dangers to water supplies and constitute dangerous contamination. For a virulent *B. coli* to be a true indicator of such dangerous contamination it is obvious two conditions must be fulfilled. In the first place *B. coli* from such sources must be virulent, or at least the majority must be virulent, and secondly such virulent *B. coli* must be able to maintain their virulence for at least several weeks after obtaining access to a water supply.

Levy and Brun state that both these conditions obtain, but this is not confirmed by other workers. Thus Lartigau[1] states "general experience abundantly demonstrates that the bacillus (*i.e. B. coli*) is on the whole non-pathogenic as ordinarily found in normal faeces." Lartigau quotes a number of authors whose results confirm this opinion.

[1] *Journ. American Med. Assoc.* April 12th, 1902.

Klecki[1] found the virulence of *B. coli* from the intestinal contents of a normal dog to be in general very variable. Harris[2] found a number of *B. coli* isolated from human faeces and from sewage to be non-virulent to guinea-pigs and rabbits. On the other hand this worker found *B. coli* from abnormal conditions of the intestine to be virulent. Lartigau (*ibid.*) states that alterations from the conditions normally present in the gut soon increase the virulence of the contained *B. coli*, while Sanarelli has shown that the virulence of *B. coli* in the intestines is increased in cases of enteric fever.

On the whole it seems probable that *B. coli* from human faeces and from sewage are in general of relatively low virulence. If that is so the fact that an isolated *B. coli* from water is non-virulent cannot in any way be taken as an indication that it is from a source which is harmless and can be neglected.

Again, the second condition, that virulent *B. coli* will maintain their virulence in water for some time is doubtful, and some experiments recorded below negative it.

There is however another aspect to the problem. It is generally recognised that in inflamed and abnormal conditions of the gut (including enteric fever) the virulence of intestinal *B. coli* is greatly increased. If therefore a virulent *B. coli* is found in a water supply, may it not be an indication of contamination from such sources, and also of fairly *recent* contamination, for it is probable that *B. coli* will lose their virulence gradually in water?

This may possibly be so, but to be able to definitely affirm it we must have a considerably greater knowledge of the virulence of *B. coli* from comparatively harmless sources such as sheep-dung, than we at present possess.

I will now consider the results of my experiments. In all work dealing with *B. coli*, owing to the different interpretations of this term, it is necessary to give the cultural characters of the organisms isolated. These are given in Table I. (p. 392). In Table I *a*. (p. 394) are given the characters of four doubtful or allied organisms which were also examined. It will, I think, be admitted that all organisms included in Table I. are certainly true *B. coli*.

Table I. includes a record of bacteria from 22 different sources. Of these 15 are from water (2 being from sea water), 2 from milk,

[1] *Annales de l'Institut Pasteur*, 1895, IX. p. 710.
[2] *Journ. of Pathology and Bacteriology*, VII. No. 1.

TABLE I. *Cultural characters of the B. coli inoculated.*

No.	Source	Morphology and Motility	Gelatine surface colonies	Gelatine slope	Broth	Litmus milk		Potato	Production of gas		Neutral red reaction Glucose agar shakes	Indol
						Acid	Coagulation		Lactose media	Glucose media		
1	Milk	Short thick bacilli. Sluggish motility	—	Translucent growth. No liquefaction	Uniform turbidity. No scum	+	+ 6 days	Whitish growth	+	+	Not examined	–
2	Milk	,, ,,	—	,, ,,	,, ,,	+	+ 24 hrs.	Yellow brown growth	+	+	+	+
3	A deep well water	Short thick bacilli. Actively motile	—	,, ,,	,, ,,	+	+ 9 days	Yellow growth	+	+	Not examined	+
4	Valves of heart. Case of malignant endocarditis	,, ,,	—	,, ,,	,, ,,	+	+ 7 days	Pale yellow growth	+	+	+	+
5	A pure uncontaminated upland surface stream	Short thick bacilli. No true motility		,, ,,	,, ,,	+	+ 6 days	,,		+	+	+
6	Upland surface reservoir. A pure supply	Short thick bacilli. Sluggish motility		,, ,,	,, ,,	+	+ 2 days		+	+	+	+ (traces)
7	Typhoid excreta	Short bacilli. No true motility		,, ,,	,, ,,	+	+ 3 days	Yellow brown growth	+	+	+	+
8	,, ,,	Short bacilli. Sluggish motility		,, ,,	Uniform turbidity. Scum	+	+ 2 days	,,	+	+	+	+
9	Upland surface reservoir. Contaminated	Short bacilli. No true motility	Quite typical	,, ,,	Uniform turbidity. No scum	+	+ 3 days		+	+	+	+
10	A deep well water. Contaminated	Short bacilli. Motile	,,	,, ,,	Uniform turbidity. Scum	+	+ 3 days	Yellow brown growth	+	+	+	+

11	Spring and upland surface water. Markedly contaminated	Short bacilli. Sluggishly motile	,,	,,	,,	,,	+	+ 3 days	Pale yellow growth	+	+	+	+
12	The same source as No. 11	,, ,,	Not quite typical	,,	,,	,,	+	+ 3 days	White growth	+	+	+	+
13	A contaminated well water	Very short bacilli. Sluggishly motile	Quite typical	,,	,,	,,	+	+ 2 days	White growth	+	+	+	+
14	Sewage	Short bacilli. Fair motility	,,	,,	,,	Uniform turbidity. No scum	+	+ 4 days	Yellow brown growth	+	+	+	+
15	Human excreta "enteritis"	Short bacilli. No true motility	,,	,,	,,	,, ,,	+	+ 24 hrs.	Colourless growth	+	+	+	+
16	Upland surface water	Short bacilli. Sluggish motility	,,	,,	,,	Uniform turbidity. Scum	+	+ 3 days	White growth	+	+	+	+
17	A contaminated well water	,, ,,	Atypical	,,	,,	Uniform turbidity. No scum	+	+ 2 days	Colourless growth	+	+	+	+
18	A deep well water. Contaminated	Short bacilli. No true motility	Quite typical	,,	,,	,, ,,	+	+ 2 days		+	+	+	+
19	Sea water. Slightly sewage contaminated	,, ,,	Atypical	,,	,,	,, ,,	+	+ 4 days	Pale yellow growth	+	+	+	+
20	Sea water. Markedly sewage contaminated	Short bacilli. Motile	Quite typical	,,	,,	,, ,,	+	+ 4 days	,,	+	+	+	+
21	A pure mixed spring and upland surface water	Short bacilli. Sluggishly motile	,,	,,	,,	Uniform turbidity. Thin scum	+	+ 4 days	,,	+	+	+	+
22	A contaminated well water	Short bacilli. No true motility	,,	,,	,,	,, ,,	+	+ 4 days		+	+	+	+

https://doi.org/10.1017/S0022172400032927 Published online by Cambridge University Press

TABLE I *a*. *Organisms allied to B. coli in many of their characters.*

No.	Source	Morphology and Motility	Gelatine surface colonies	Gelatine slope	Broth	Litmus milk		Potato	Gas production		Neutral red reaction Glucose agar shake	Indol (10 days' peptone water)
						Acid	Coagulation		Lactose	Glucose		
a	A mountain stream, uncontaminated except from sheep excreta	Short bacilli. No true motility*		White semi-translucent growth. No liquefaction	Uniform turbidity. No scum	Alkali produced	No coagulation	Yellow-white growth	–	+	+	+ (traces)
b	A pure spring water	Short bacilli. No true motility*		,, ,,	Uniform turbidity. Thin scum	,,	,,	Pale brown growth	–	+	Partial	+ (slight)
c	A pure upland surface water (from reservoir)	Very short bacilli. Showing distinct motility	Typical	,, ,,	Uniform turbidity. No scum	+ Acid produced†	,,	Pale yellow growth	–	+	+ (complete in 48 hrs.)	traces only
d	From body of oyster	Short bacilli. Very sluggish motility	Atypical	,, ,,	,, ,,	Alkali produced‡	,,	Abundant yellow growth	–	+	+	+ (marked)

* After being kept in the Laboratory for some time they both showed sluggish motility.

† The milk tubes became acid in 24 hrs. and did not subsequently become alkaline (kept for one month).

‡ Some preliminary acid production, distinctly alkaline after one week.

3 from excreta, and 1 each from sewage and a case of malignant endocarditis.

The inoculations were made mainly into guinea-pigs and mice, the results being given in Table II. It will be noticed that in most cases the dose inoculated was very large both for guinea-pigs and mice. To ensure the maximum effect the inoculation was made intraperitoneally. Table II. shows that even when these massive doses were inoculated intraperitoneally into guinea-pigs the results were negative for most of the injected organisms. The guinea-pigs used were of approximately equal weight (270–320 g.).

The results may be further classified as follows:—

Source	Virulent to guinea-pigs	Non-virulent to guinea-pigs
Pure water	1	2
Suspicious water	0	3
Contaminated water	3	6
Sewage or excreta	0	3
Valves of heart (Malignant Endocarditis)	0	1
	4	15

The figures are not large but, as far as they go, they show that $\frac{1}{3}$ of the *B. coli* from both pure and contaminated sources were virulent, while it is significant that all 3 organisms from excreta or sewage were non-virulent.

It certainly was not true for these waters that a virulent *B. coli* indicated a bad water and a non-virulent a good water.

It should be added that the distinction between pure, suspicious, and contaminated waters was based not upon a single examination, but upon an intimate knowledge of the waters in question, both from the point of view of their liability to pollution and from the figures of bacteriological and chemical analyses made every three months for at least $2\frac{1}{2}$ years. It is not thought necessary to give particulars of these waters.

It is of interest to note that the water supply from which bacteria 12 and 13 were obtained should on the only two occasions on which the virulence was tested, have yielded *B. coli* both of which were distinctly pathogenic. This water showed marked evidence of contamination. Thus the sample from which No. 11 was isolated contained 330 and 2600 organisms per c.c. growing on agar (37° C.) and gelatine (22° C.) plates respectively, while *B. coli* was readily isolated from 0·5 c.c. of the water.

TABLE II. *Inoculation experiments.*

No. of organism	Source	Character of source	Guinea-pig inoculations			Mouse inoculations		
			Dose	Method of inoculation	Result	Dose	Method of inoculation	Result
3	A deep well water	A suspicious water	1·5 c.c. 24 hrs. broth culture	Intra-peritoneal	No effect			
5	Upland surface stream	Pure uncontaminated water	2 c.c. 3 days broth culture	,,	,,			
6	Upland surface water	,, ,,	,, ,,	,,	,,			
9	,, ,,	A contaminated water	Standard dose *	,,	,,	1 c.c. whey from coagulated milk 4 days old	Intra-peritoneal	Death in about 24 hrs.
10	A deep well water	,, ,,	,, ,,	,,	,,	,, ,,	,,	Death in about 30–40 hrs.
11	Mixed spring and upland surface water, in reservoir	Markedly contaminated	,, ,,	,,	Dead in less than 20 hrs.	,, ,,	,,	Death in less than 16 hrs.
			A second guinea-pig inoculated with same dose	,,	Dead in about 50 hrs.			
12	Same source as 11, but examined three months later and isolated from a fresh sample	,, ,,	Standard dose	,,	Dead in about 48 hrs.			
13	A well water	,, ,,	,, ,,	,,	No effect	,, ,,	,,	Dead in 20 hrs., repeated many times with the same result
16	Upland surface water	A suspicious water	,, ,,	,,	,,			
17	A well water	,, ,,	,, ,,	,,	,,			

18	Deep well water	Contaminated	,, ,,	,,	,,	1 c.c. old broth culture	,,	Death in less than 20 hrs.
19	Sea water	Contaminated with sewage, but not markedly	,, ,,	,,	Dead in about 40 hrs.			
20	,, ,,	Markedly sewage contaminated	,, ,,	,,	No effect			
21	Mixed spring and upland surface water	A pure water	,, ,,	,,	Dead in about 22 hrs.			
22	Well water	Contaminated	,, ,,	,,	Local tissue necrosis. Recovery			
4	Malignant endocarditis (valves)	—	1·5 c.c. broth culture (3 days)	,,	No effect			
7	Typhoid excreta	—	2 c.c. broth culture (3 days)	,,	,,			
8	,, ,,					0·5 c.c. broth culture (3 days)	Subcutaneous	No effect
14	Sewage	—	Standard dose	,,	No effect	,, ,,	Intraperitoneal	Dead in less than 20 hrs.
15	Human excreta, "enteritis"	—	,, ,,	,,	,,			
a	Upland surface water	Contaminated	2 c.c. of 1 week's broth culture	,,	,,			
b	A pure spring water	A pure water	Standard dose	,,	,,			
c	Upland surface water	A pure water	,, ,,	,,	,,	0·5 c.c. broth (1 week old)	,,	,,
d	Oyster	—	,, ,,	,,	Dead in about 50 hrs.			

* *Standard dose* is made by adding the scrapings of an agar sloped culture 24 hours old to 5 c.c. of a 5–6 days old broth culture of the same organism. The whole enriched 5 c.c. is injected.

The pure water from which the virulent *B. coli* was isolated consists partly of spring and partly of upland surface water from the hill sides. It is collected directly into a reservoir.

It is a very pure water and it is rare to find *B. coli* even in as much as 50 c.c. In this particular sample *B. coli* was found in 40 c.c., but not in smaller amounts, while the numbers of organisms per c.c. were 3 at 37° C., and 145 at 22° C.

The results of inoculations on mice are of some interest.

Five *B. coli* from waters and one from sewage were all pathogenic to mice when injected intraperitoneally in large doses. Several mice, as controls, were injected with 1 c.c. of sterile milk intraperitoneally and showed no permanent ill effects, so the inoculation results must be ascribed to the organisms injected. In every case, mouse, guinea-pig, or rabbit, an autopsy was made on the animals killed by inoculation, and the *B. coli* recovered from the spleen. All the *B. coli* used seemed to have sufficient virulence to kill mice when injected intraperitoneally. For the two *B. coli* isolated from milk, rabbits were used for testing the virulence:—

No. 1 inoculated intraperitoneally in a dose of 5 c.c. of a 24 hours' broth culture, killed the animal in less than 24 hours, and the same bacillus was recovered from the internal organs.

No. 2, with an equal dose injected intraperitoneally, produced no effect.

A few experiments on altering the virulence of *B. coli* were performed. With bacillus No. 11 two parallel inoculations were made.

Exp. A. Sterile tap-water in a flask was inoculated with this organism and the flask then kept outside the laboratory, in the open air, for 12 days. Subcultures were then made on to an agar slope and into glucose broth. Both subcultures were grown for 5 days, then 3 c.c. of the broth culture plus the growth scraped from the agar slope were inoculated intraperitoneally into a guinea-pig. Animal ill for the first 24 hours but subsequently recovered completely.

Exp. B. (Control.) The organism was grown in glucose broth for 12 days at 37° C. Then subcultures were made on an agar slope and into glucose broth as in Exp. A. After 5 days' growth the same dose was inoculated into a guinea-pig of equal weight. Animal dead in less than 17 hours. Bacilli recovered from the spleen. Here a diminution of virulence resulted from 12 days' growth in water.

Bacillus No. 21 when first inoculated a few days after its isolation, the standard dose killed a guinea-pig (wt. 270 g.) in 22 hours. After being grown in sterile water (plus 2 drops of sterile broth) at room temperature for two weeks, 6 c.c. of a 5 days' old broth, plus scraping from an agar slope failed to kill and showed no effect upon a guinea-pig (wt. 450 g.). For this inoculation, unfortunately, an equal sized

guinea-pig was not available and the dose was not proportionately increased so the result is not conclusive, but as in the preceding experiment an apparent loss of virulence by growth in water is shown.

Several experiments were made to try and raise the virulence of these organisms. Thionot and Masselin[1] state "the virulence of this bacillus varies with different growths, but any growth may have its virulence greatly augmented by passing the bacillus by intrapleural injection through a series of guinea-pigs or rabbits."

It was not found possible to take *B. coli* non-virulent for guinea-pigs but virulent for mice, and make them virulent for guinea-pigs by passage through mice.

Thus *B. coli* No. 9 was passed through 3 mice by intraperitoneal injection, but then still failed to kill a guinea-pig.

No. 13 was passed through 5 mice by intraperitoneal injection. It was then capable of killing mice in doses of 0·5 c.c. by *subcutaneous* inoculation. After two further passages by subcutaneous inoculation it was still unable to kill a guinea-pig, even when the large 'standard dose' was used.

Conclusions.

These experiments lend no support to the view that the pathogenicity of isolated *B. coli* is of help in determining the potency for evil of the water examined. Virulence as a property of *B. coli* is, I believe, a very variable character and one which can be readily lost, and with greater difficulty acquired, and the view advanced by some writers, *e.g.* Harris (*ibid.*), that toxicity is a specific distinguishing character seems to be without foundation.

[1] Text-book, p. 272.