THE RESULTS OF A CHEMICAL, MICROSCOPICAL AND BACTERIOLOGICAL EXAMINATION OF SAMPLES OF LONDON MILKS.

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In view of the importance of a pure milk supply, we considered that it might be of interest to examine chemically, microscopically, and bacteriologically, a number of specimens of milk coming into the Metropolis for which purpose we decided to select samples from the various counties, the milk of which is consigned to London. We found that milk so consigned comes from about twenty-six counties extending from Derby in the North, to Hampshire and Devonshire in the South and South-West, and from Hereford in the West, to Norfolk in the East.

Description of manner of obtaining samples.

The samples were collected on arrival in the early morning at the various railway termini, viz. Euston, St Pancras, Liverpool St., Waterloo, Marylebone, etc., by the inspectors and samplers of several well known Dairy Companies. Two sterilised eight-ounce bottles with new, good sound corks, were used for each specimen, the contents of one bottle being used for the chemical and those of the other for the bacteriological examination, thus obviating any possibility of contamination. The milk contained in a churn was thoroughly roused with a clean plunger; the bottles were immersed, recorked, labelled and marked with the time of collection, date, the particular county in which

it had been produced and the company's name. They were then transmitted without delay to the laboratory, and the examination at once commenced.

The *chemical examination* consisted in determining the specific gravity, the percentage of fat, the non-fatty solids and the total solids, the acidity, and a search for preservatives, viz. formalin, boric acid, and borates.

Microscopically; acid-fast organisms, streptococci, pus-cells, and *débris* were looked for.

Culturally; a search was made for the *Bacillus enteritidis sporogenes* (with subsequent inoculation of typical cultures into guinea-pigs), for the *Bacillus coli* in definite quantities of the sample, and for the *Bacillus diphtheriae* and diphtheroid organisms in the sediment which was finally inoculated into guinea-pigs to test for tubercle and other pathogenic organisms.

Brief description of Methods employed.

1. Determination of specific gravity; the Westphal Balance.
2. Estimation of the fat; Gerber's method.
3. Total solids; calculated by the formula, $T = \cdot 25G + 1 \cdot 2F + \cdot 14$, and the non-fatty solids by difference.
4. The test for formalin employed was the ordinary sulphuric acid reaction, any doubtful ones being controlled by Schiff's method.
5. For boric acid, the turmeric test was used, confirmed by the phenol-phthalein-glycerine method.
6. The acidity was estimated by running into 50 c.c. of the sample $\frac{N}{10}$ sodium hydrate, phenol-phthalein being used as the indicator, each 0·5 c.c. representing 1 degree of acidity: this multiplied by ·009 gives the percentage estimated as lactic acid (Richmond).

For the *microscopical* examination, 100 c.c. of the sample were centrifugalised for 15 minutes at about 1000 revolutions per minute, smears were then made from the deposit, the fat was removed by treating the films with a mixture of equal parts of ether and alcohol. They were then dried and fixed by heat, and examined for acid-fast organisms by the Ziehl-Neelsen method.

For streptococci, the films were stained by Gram's method, (Nicolle's modification with carbol-thionin blue) and for pus-cells and leucocytes with Löffler's methylene blue, and *débris* was looked for in wet specimens.

For the *Bacillus enteritidis sporogenes*, quantities of 1 c.c., 10 c.c. and 20 c.c. were examined. The 1 c.c. was added to a tube of sterile milk, the 10 c.c. and 20 c.c. were placed in sterile test tubes, and all the tubes were heated to 80° C. for 15 mins., and incubated anaerobically at 37° C. for 48 hours, the typical cultures being subsequently inoculated subcutaneously into the abdominal region of a guinea-pig.

For the *Bacillus coli*, quantities of 1 c.c., 0·1 c.c., 0·01 c.c., and 0·001 c.c. were added to tubes of litmus lactose bile salt peptone water and incubated at 42° C. for 48 hours. From the tube containing the least amount which gave acid and gas a loopful was inoculated into 10 c.c. of sterile water, and from this a loopful was smeared upon a slope gelatin tube. This always gave isolated colonies, and from a characteristic colony inoculations were made into broth, peptone water, 1 % lactose peptone water, 1 % glucose peptone water, 1 % mannitol peptone water, 1 % cane sugar peptone water, and 1 % dulcitol peptone water. The gelatin tubes were kept during the whole of the experiments and not one liquefied.

For the estimation of the number of organisms present a dilution of 1 in 10,000 was made in sterile water, and 1 c.c., 0·5 c.c., 0·1 c.c. of this dilution was dropped into a sterile Petri dish, and nutrient gelatin of reaction +1·5 (Eyre's scale) added, and incubated at 20° C. for 48 hours.

For *Bacillus diphtheriae* and diphtheroid organisms, loopfuls of the sediment of the centrifugalised milk were inoculated upon blood serum, and examined after incubation in films stained with Löffler's methylene blue, and by Neisser's method.

For tubercle and other pathogenic organisms, 50 c.c. of the milk were centrifugalised for 20 minutes at 1000 revolutions per min., and the upper portion poured off, leaving only 3 c.c., which was thoroughly mixed, and of it 1·5 c.c. was inoculated subcutaneously into one guinea-pig, and the remainder intraperitoneally into a second guinea-pig. Owing to the premature death of some of the animals, duplicate samples from the same source were procured, and the experiment repeated.

The following table (Table 1) sets forth the names of the counties from which the milk was taken, the date of the collection, the specific gravity, and the percentages of the fats, of the non-fatty solids and of the total solids; also the acidity after 24 hours, and the presence or absence of formalin or boric acid, which last are expressed by positive or negative signs.

From Table 1 it will be seen that six of the twenty-six samples (23 per cent.) failed to come up to the Departmental Committee's standards, three in respect of fats, and three in respect of the non-fatty solids. Notwithstanding, the average of all the samples is very good. Preservatives in the form of formalin, boric acid and borates were not detected in any sample.

As regards the acidity, Newman and Swithinbank have suggested as a standard that no milk should have an acidity corresponding to more than 22 c.c. of $\frac{N}{10}$ NaOH for 50 c.c. of the milk at time of sale. It will be seen that seven of the samples exceeded this (26·9 per cent.). The remainder (73·1 per cent.) are well below it. Our test, however, was a rigorous one, the milk being kept for 24 hours before the acidity was tested, whereas actually under normal conditions, all the milk should have been disposed of within 24 hours.

TABLE I.

County	Date	Sp. gr.	Fat	Solids not fat	Total solids	Acidity after 24 hours	Degrees of acidity	Formalin	Boric acid and borates	Remarks
Leicester	1. iii. 06	1032·6	2·95	8·89	11·84	·198	22·0	–	–	1·7 % deficient in fat.
Northampton	,,	1031·6	3·58	8·76	12·32	·172	19·2	–	–	
Derby	2. iii. 06	1032·6	3·08	8·91	11·99	·208	23·2	–	–	
Stafford	,,	1032·5	3·00	8·88	11·88	·189	21·0	–	–	
Essex	5. iii. 06	1034·0	5·30	9·80	15·10	·201	22·4	–	–	
Somerset	8. iii. 06	1031·4	3·62	8·72	12·34	·171	19·0	–	–	
Gloucester	,,	1030·9	3·40	8·54	11·94	·138	15·4	–	–	
Oxford	9. iii. 06	1031·4	2·70	8·67	11·37	·163	18·2	–	–	10 % deficient in fat.
Buckingham	,,	1030·0	3·50	8·35	11·85	·218	24·3	–	–	1·8 % water.
Suffolk	10. iii. 06	1033·7	4·10	9·39	13·49	·170	18·9	–	–	
Hampshire	,,	1030·9	4·10	8·69	12·79	·167	18·6	–	–	
Sussex	15. iii. 06	1032·0	3·84	8·90	12·74	·163	18·2	–	–	
Berkshire	,,	1033·1	3·25	9·07	12·32	·158	17·6	–	–	
Bedford	16. iii. 06	1033·0	3·47	9·09	12·56	·178	19·8	–	–	
Warwick	,,	1033·4	3·85	9·26	13·11	·203	22·6	–	–	
Kent	25. iv. 06	1028·8	4·95	8·34	13·29	·167	18·6	–	–	1·9 % water.
Wiltshire	,,	1032·0	3·56	8·85	12·41	·169	18·8	–	–	
Norfolk	26. iv. 06	1034·5	4·22	9·61	13·83	·172	19·2	–	–	
Cambridge	,,	1032·1	3·67	8·91	12·58	·178	19·8	–	–	
Middlesex	30. iv. 06	1031·4	2·80	8·55	11·35	·180	20·0	–	–	6·7 % deficient in fat.
Surrey	,,	1032·2	3·98	8·98	12·96	·225	25·0	–	–	
Dorset	4. v. 06	1029·8	3·54	8·32	11·86	·151	16·8	–	–	2·2 % water.
Cheshire	,,	1030·9	3·22	8·50	11·72	·169	18·8	–	–	
Devon	11. v. 06	1032·6	4·40	8·97	13·37	·187	20·8	–	–	
Hereford	,,	1032·0	3·80	9·62	13·42	·219	24·4	–	–	
Rutland	,,	1032·8	3·80	8·98	12·78	·206	22·9	–	–	
Average		1032·0	3·68	8·90	12·56	·181	20·2			

As the temperature is of considerable importance as regards the rate of acid production the following table (Table 2) shows the maximum and minimum temperatures taken at the Lancet Office and recorded in the *Lancet*, Vol. I. 1906, for the day preceding, the day of, and the day following the collection and examination of every sample. It will be seen that the temperatures on the whole were moderate, the maximum exceeding 60° F. on only five occasions.

TABLE II.

Samples	Date	Shade Maximum	Minimum
	28. ii. 06	46	35
I & II.	1. iii. 06	49	37
III & IV.	2. iii. 06	52	46
	3. iii. 06	49	35
	4. iii. 06	58	37
V.	5. iii. 06	53	36
	6. iii. 06	65	43
	7. iii. 06	67	48
VI & VII.	8. iii. 06	51	47
VIII & IX.	9. iii. 06	50	42
X & XI.	10. iii. 06	47	39
	11. iii. 06	54	41
	14. iii. 06	51	30
XII & XIII.	15. iii. 06	55	35
XIV & XV.	16. iii. 06	57	50
	17. iii. 06	66	47
	24. iv. 06	48	36
XVI & XVII.	25. iv. 06	51	39
XVIII & XIX.	26. iv. 06	49	36
	27. iv. 06	56	35
	29. iv. 06	52	35
XX & XXI.	30. iv. 06	49	37
	1. v. 06	53	37
	3. v. 06	59	47
XXII & XXIII.	4. v. 06	60	50
	5. v. 06	63	45
	10. v. 06	49	46
XXIV, XXV, & XXVI.	11. v. 06	58	47
	12. v. 06	71	50

Table 3 gives the number of micro-organisms per cubic centimetre, the presence or absence of acid-fast organisms, streptococci, leucocytes and pus-cells, and the results of growth on blood serum.

TABLE III.

County	Organisms per c.c.	Acid-fast organisms	Strepto-cocci	Leucocytes and pus-cells	Diphtheria organisms on blood serum cultures	Débris
Leicester	240,000	–	–	Very few	–	1
Northampton	230,000	–	Few	Few	–	2
Derby	1,400,000	–	–	,,	+ (1)	3
Stafford	5,000,000	–	–	,,	– (2)	4
Essex	60,000	–	–	Very few	– (3)	5
Somerset	1,480,000	–	Many	Few	+ (4)	6
Gloucester	56,000	–	–	,,	–	7
Oxford	143,000	–	–	,,	+ (5)	8
Buckingham	7,810,000	–	–	Very few	–	9
Suffolk	96,000	–	–	Few	–	10
Hampshire	50,000	–	–	,,	–	11
Sussex	184,000	–	–	,,	–	12
Berkshire	50,000	–	–	Very few	–	13
Bedford	100,000	–	–	Few	–	14
Warwick	20,000	–	–	Very few	–	15
Kent	8,390,000	–	–	Few	+ (6)	16
Wiltshire	340,000	–	–	,,	–	17
Norfolk	250,000	–	–	,,	–	18
Cambridge	120,000	–	–	,,	– (7)	19
Middlesex	1,830,000	–	–	,,	– (8)	20
Surrey	1,443,000	+	Very many	Many	– (9)	21
Dorset	1,560,000	–	Many	Few	–	22
Cheshire	20,000	–	–	,,	–	23
Devon	880,000	–	–	,,	–	24
Hereford	1,510,000	–	–	,,	–	25
Rutland	2,800,000	–	–	,,	–	26

References to "blood serum cultures":

(1) Somewhat similar to the Klebs-Löffler bacillus, but much too large.
(2) Many yeasts or torulae. (3) A few yeast-cells.
(4) Somewhat like Klebs-Löffler bacillus; not identical.
(5) ,, ,, ,, ,, ,, ,,
(6) Much like Klebs-Löffler bacillus in parallel grouping, polar staining by Löffler's methylene blue, and by Neisser's method; failed to isolate the organism.
(7) Many large capsulated diplococci in deposit.
(8) On blood serum tube many streptococci and leptothrix forms. The indol reaction was intense in a broth culture.
(9) Acid-fast organisms and streptococci present.

References to "débris":

(1) Yeast-cells, cotton fibre, vegetable matter.
(2) ,, ,, ,, ,, ,,
(3) Vegetable matter, hairs. (4) Epithelial cells, vegetable matter.
(5) Wool, vegetable matter. (6) Grit and hairs.
(7) Vegetable matter, cotton fibre. (8) Hairs, epithelial cells.
(9) Cotton fibre. (10) Hair, vegetable matter. (11) Antennae, ? of a fly.
(12) Antennae, ? of a fly. (13) Wool, grit. (14) Grit, cotton fibre.
(15) Straw. (16) Vegetable matter. (17) Vegetable matter.
(18) Grit. (19) Vegetable matter. (20) Hair.
(21) Cotton fibre. (22) Epithelial cells. (23) Yeast-cells.
(24) Grit. (25) Cotton fibre. (26) Cotton fibre.

The above table shows great variation in the total number of organisms per c.c. from 20,000 to 8,390,000; streptococci and leucocytes and pus-cells were scanty with one or two exceptions, and acid-fast bacilli only detected once.

Table 4 shows the production of acid and gas (A & G) in their respective quantities, in the lactose bile salt tubes. (When the sign is bracketed it is to indicate that the change was a slight one.)

TABLE IV.

	Quantity:—1 c.c.	0·1 c.c.	0·01 c.c.	0·001 c.c.
Leicester	A & G	A & G	–	–
Northampton	A & G	A & G	–	–
Derby	A & G	A & G	A & G	A & G
Stafford	A & G	A & G	(A & G)	–
Essex	A & G	A & G	A & G	–
Somerset	A & G	A & G	A & G	–
Gloucester	A & G	A & G	A & G	–
Oxford	A & G	A & G	–	–
Buckingham	A & G	(A & G)	(A & G)	(A & G)
Suffolk	A & G	A & G	A & G	–
Hampshire	A & G	A & G	A & G	(A)
Sussex	A & G	A & G	A & G	–
Berkshire	A & G	A & (G)	–	–
Bedford	A & G	A & G	A & G	–
Warwick	A & G	A & G	–	–
Kent	A & G	A & G	A & G	A & G
Wiltshire	A & G	A	A	A
Norfolk	A & G	A	(A)	–
Cambridge	A & G	A	–	–
Middlesex	A & G	A & G	–	–
Surrey	A & G	A & G	(A)	–
Dorset	A & G	A & G	A & G	A & G
Cheshire	A & G	A & G	A & G	(A)
Devonshire	A & G	A & G	A	–
Hereford	A & G	A & (G)	A & G	A & G
Rutland	A & G	A & (G)	A & (G)	A & (G)

All the twenty-six samples (100 per cent.) therefore contained lactose fermenting (acid and gas) organisms in 1 c.c., 23 (88 per cent.) in 0·1 c.c., 15 (57 per cent.) in 0·01 c.c., and 6 (23 per cent.) in 0·001 c.c.

Table 5 gives the attributes, *i.e.* indol in peptone water, acid and gas in :

1%	lactose	peptone	water
„	„	glucose	„
„	„	mannitol	„
„	„	cane-sugar	„
„	„	dulcitol	„

of the organisms isolated from the lactose bile salt tubes.

TABLE V.

County	Peptone Water	In peptone water					Organisms probably isolated, and from what amount
		Lactose	Glucose	Mannite	Cane sugar	Dulcite	
Leicester	in	A & G	A & G	A & G	–	A & G	*B. coli* ·1 c.c.
Northampton	(in)	A & G	A & G	A & G	–	A & G	„ ·1 c.c.
Derby	–	A & G	A & G	A & G	–	–	*B. ac. lactici.*
Stafford	–	A & G	A & G	A & G	A & G	A & G	*B. coli* ·1 c.c.
Essex	in	A & G	A & G	A & G	A & G	–	*B. lact. aerog.*
Somerset	–	A & G	A & G	A & G	(A & G)	(A) & G	*B. coli* ·01 c.c.
Gloucester	–	A & G	A & G	A & G	–	(A) & G	„ ·01 c.c.
Oxford	–	A & G	A & G	A & G	–	A & G	„ ·1 c.c.
Buckingham	in	A & G	A & G	A & G	A	A & G	„ ·1 c.c.
Suffolk	–	A & G	A & G	A & G	–	–	*B. ac. lactici.*
Hampshire	in	A & G	A & G	A & G	–	–	„ „
Sussex	in	A & (G)	A & G	A & G	A & G	–	*B. lact. aerog.*
Berkshire	in	A	A	A & G	A & G	A & G	?
Bedford	(in)	A & G	A & G	A & G	–	–	*B. ac. lactici.*
Warwick	in	(A)	A	A	(A)	A & G	?
Kent	in	A	A	A & G	A & G	A & G	?
Wiltshire	–	A & G	A & G	A & G	–	A & G	*B. coli* 1 c.c.
Norfolk	(in)	(A)	A	–	(A)	–	?
Cambridge	–	A & G	A & G	A & G	–	A & G	*B. coli* 1 c.c.
Middlesex	(in)	–	A	–	A	–	?
Surrey	–	A & G	A & G	A & G	–	A & (G)	*B. coli* ·1 c.c.
Dorset	in	A & G	A & (G)	A & G	A & G	–	*B. lact. aerog.*
Cheshire	in	A & G	A & G	A & G	(A)	A & G	*B. coli* ·01 c.c.
Devonshire	–	A & G	A & G	A & G	–	–	*B. ac. lactici.*
Hereford	–	A & G	A & G	A	(A)	A & G	*B. coli* ·001 c.c.
Rutland	–	A & G	A & G	A & G	A & G	–	*B. lact. aerog.*

The diagnosis of the organisms from the fermentation tests in Table 4 is based on MacConkey's researches[1]. According to him the *B. coli* ferments dulcitol and mannitol, it may or may not ferment cane-sugar; the *B. lactis aerogenes* ferments mannitol and cane-sugar, but not dulcitol; the *B. acidi lactici* ferments mannitol but not dulcitol nor

[1] MacConkey (1905.) *Journal of Hygiene*, Vol. v. p. 333.

cane-sugar. All the organisms were bacilli, not staining by Gram's method (Nicolle's modification with carbol-thionin blue). No gelatin culture showed liquefaction. The *B. coli* was therefore definitely found in twelve of the samples (46 per cent.) and the *B. lactis aerogenes* in 4 (15·4 per cent.).

B. coli was found three times (11·5 per cent.) in not less than 1 c.c., four times (15·4 per cent.) in 0·1 c.c., twice (8 per cent.) in 0·01 c.c., and three times (11·5 per cent.) in 0·001 c.c.

Table 6 shows the results of the *B. enteritidis sporogenes* test. The contents of the tubes containing the smallest amount showing the characteristic change were inoculated into guinea-pigs.

TABLE VI.

County	1 c.c.	10 c.c.	20 c.c.	Remarks	
Leicester	–	+	+	Animal negative.	(10 c.c.)
Northampton	–	+	+	,, typical.	(10 c.c.)
Derby	+	+	+	,, negative.	(1 c.c.)
Stafford	–	+	+	,, ,,	(10 c.c.)
Essex	–	+	+	,, ,,	(10 c.c.)
Somerset	–	+	+	,, typical.	(10 c.c.)
Gloucester	–	–	+	,, ,,	(20 c.c.)
Oxford	–	–	–	Cultures not typical.	
Buckingham	–	–	+	Animal typical.	(20 c.c.)
Suffolk	–	–	–	Cultures not typical.	
Hampshire	–	+	+	Animal typical.	(10 c.c.)
Sussex	–	–	+	,, negative.	(20 c.c.)
Berkshire	+	+	+	,, typical.	(1 c.c.)
Bedford	–	–	–	Cultures not typical.	
Warwick	–	–	+	Animal typical.	(20 c.c.)
Kent	–	+	+	,, ,,	(10 c.c.)
Wiltshire	–	–	–	Cultures not typical.	
Norfolk	–	+	+	Animal typical.	(10 c.c.)
Cambridge	+	+	+	,, negative.	(1 c.c.)
Middlesex	–	+	+	,, ,,	(10 c.c.)
Surrey	–	+	+	,, typical.	(10 c.c.)
Dorset	–	+	+	,, ,,	(10 c.c.)
Cheshire	–	–	+	Tube broken, no inoculation.	
Devon	–	–	–	Cultures not typical.	
Hereford	–	+	+	Animal typical.	(10 c.c.)
Rutland	–	+	+	,, negative	(10 c.c.)

From Table 6 it will be seen that in one instance the tube was broken and no inoculation could therefore be made. Of the 26 samples 21 (80·8 per cent.) showed the enteritidis change in 20 c.c. or less. Excluding the broken tube, of the 20 samples showing the enteritidis change and tested by inoculation 12 (60 per cent.) gave a positive

result on the animal. Therefore, one-fifth of the number showing the enteritidis change culturally proved not to be infected with the enteritidis by animal inoculation.

As regards tuberculosis, only one sample, Surrey, out of the 26 (about 4 per cent.) gave definite evidence of tubercle bacilli. The inoculated guinea-pig died with typical tubercle and acid-fast bacilli were found in the milk, which contained an excess of streptococci and leucocytes and pus-cells. The cow was subsequently identified on the farm, and the further use of its milk stopped.

Although we have not alluded in this paper to the work of others on the bacterial content of milk, etc., we are by no means unmindful of the numerous valuable contributions which have been made on the subject. We would refer particularly to Houston's report[1] which contains a summary of previous work.

Conclusions.

As regards the general results of the examination it may be said:

1. There is no correlation between poor milk and its content of total bacteria, *B. coli* or *B. enteritidis sporogenes.*

2. There is no correlation between the content of *B. coli* and of *B. enteritidis sporogenes.*

3. The total number of organisms was below 2,000,000 per c.c. in 22 out of the 26 samples (85 per cent.) and below 1,000,000 in 16 of the samples (61·5 per cent.).

4. *B. coli* was found in 46 per cent. of the samples, in a quantity of milk not exceeding 1 c.c.

5. *B. enteritidis sporogenes* was found in 60 per cent. in a quantity of milk not exceeding 20 c.c.

6. Preservatives in the form of formalin, or boric acid, or borates, were not detected in any sample.

7. The acidity on the whole is well below Newman's standard.

8. *B. tuberculosis* was not so frequent as might have been expected from the results of other investigators.

In conclusion we desire to express our best thanks to the various companies, etc., who supplied us with samples, and particularly to the company supplying the Surrey milk. The latter, when we found acid-fast bacilli, etc., in the sample, gave us every facility for examining the herd and we were thus able to identify the infected animal.

[1] Houston, A. C. "The Bacteriological Examination of Milk." *Report No.* 933 *to the London County Council.*