EXPERIMENTS ON INFECTION OF COWS WITH TYPHOID BACILLI

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In his report on the outbreak of typhoid fever in August–September 1936 in Bournemouth, Poole and Christchurch, the late Dr W. V. Shaw (1937) established the fact that the raw milk from a particular dealer had been conveying the infection continuously throughout a period of 31 days or thereabouts. In this outbreak there were 718 cases among residents and visitors; of about 518 residents fifty-one died, while the death-rate among visitors is not stated. The general course of the epidemic, with a great fall in the number of fresh cases towards the end of the period, strongly suggested that the infection of the milk was already disappearing before the application of pasteurization brought it to an abrupt stop. Shaw discussed the possible ways in which such continuous contamination of the milk could have been produced and mentioned, in particular, the possibility that cows drinking and wading in typhoid-contaminated water might have been responsible either by direct mechanical transference of typhoid bacilli on the legs, udders and teats or by themselves becoming infected and discharging typhoid bacilli in their dung, urine or milk. The cows on two farms supplying milk to the dealer involved did, in fact, have such opportunities of becoming infected; a stream flowing through their pasture was found to contain abundant typhoid bacilli introduced in the sewage from a house occupied by a profuse faecal typhoid-carrier throughout the summer of 1936 until 10 August, after which he was away till 28 September.

Suspicion that the cows might be suffering from the disease or excreting the bacilli was not aroused until a considerable time after the relevant date. It was decided however to submit the question to some experimental tests, and the results are given in this paper.

EXPERIMENTAL PROCEDURE

The animals under experiment were kept at the Institute in an ordinary loose-box, from which persons unconnected with the work were excluded. The single attendant had been specially inoculated against typhoid. Arrangements were made for collection and sterilization of the milk, faeces and urine, floor washings and uneaten fodder. Slaughtering and post-mortem examination were done in the same box. Sampling of the faeces and milk (about 10 ml. fore-milk from each quarter) was carried out by the attendant under frequent supervision, the samples being at once taken to the Ministry's Laboratories about 3 miles distant.

Particulars of the typhoid cultures used are given below with the results. Isolations from milk and faeces were made on brilliant-green, MacConkey and Wilson-Blair media, all three media being sometimes used for the one sample. The seed material consisted either of 0·1 ml. whole milk or of one or more standard loopsful of gravity cream, i.e. from the cream layer forming after the milk had been standing for 6–18 hr. Repeat platings were frequently made from the milk or cream. For this, the samples were shaken and again allowed to stand at laboratory temperature for 2–6 days. Such repeat tests were usually done when the result of immediate plating was negative or doubtful. Selected colonies were identified when necessary by agglutination. Blood serum was tested for specific 'H' and 'O', and occasionally for Vi agglutinins.

Before inoculating the udder, the gland was milked out. When inoculating the milk cistern, a teat syphon was passed through the teat canal, the culture was mixed with about 10 ml. milk just taken from the quarter and then injected through the teat syphon. After the latter was withdrawn, the base of the teat was manipulated so that some of the inoculum might be forced into the larger milk ducts.

In some experiments the teat sphincter was incised. This was done with a narrow-bladed scalpel which was passed through the teat orifice and which on being withdrawn severed the muscular ring. When the teat orifice was to be scarified, the end of the teat was first cauterized with a hot spatula and the epithelium then scraped away with a knife so as to leave a raw surface.

RESULTS

The first proceeding was to establish that typhoid bacilli could survive in and be easily cultivated from cows' dung after its infection with artificial cultures

* Owing to the sad death of Dr Scott in 1941 as a result of enemy action, and to other misfortunes connected with the war, it has not been possible to compile this paper earlier.

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in the laboratory. The actual period of survival in cows'-dung kept at room temperature exposed to air was not determined but certainly exceeded 7 weeks.

Typhoid bacilli by the mouth

10 *September* 1937. Cow 118 was given a very large dose of *Bact. typhosum* by the mouth, viz. 275,000 millions in ½ gal. of tap water followed by ½ gal. of water containing a little baking soda intended to neutralize stomach acidity. The cow had had no water to drink for 18 hr. but had had cake and hay 5 hr. before the drench. The strain of typhoid bacillus employed was that obtained from the faeces of Mr X, the carrier who was discovered to be the cause of the Bournemouth-Poole outbreak. It had had its virulence enhanced by passage through mice and had then been grown on agar containing ascitic fluid. The dose would correspond very roughly to the ingestion of 4 lb. of Mr X's faeces containing typhoid bacilli in the number found on the one occasion they were examined, i.e. a dose many times greater (*qua* typhoid bacilli) than the cow could possibly have ingested by drinking the sewage effluent concerned with the outbreak.

Cow 118 was feverish and off-feed for 10–15 days after 10 September. Samples of the faeces were taken for examination on 10 September after the drench was given (three specimens), on the three succeeding days (five specimens each), and then twice daily for a month. Samples of her milk were also examined twice daily. All these examinations gave negative results. There was thus no evidence that typhoid bacilli ingested in faeces could pass the barrier of the stomach of this cow. Blood examinations on three occasions (8, 10 and 12 days after infection) showed no evidence of the development of specific agglutinin for the typhoid bacillus, serum tests being negative at 1 : 20.

9 *October* 1937. A second attempt was made with the same cow, the only important difference being that for some days her water intake had been reduced, her concentrate ration had been changed by replacing bran and linseed cake with cotton cake, and she had been given chalk and opium powders until the faeces were firm instead of loose. This time the faeces of a typhoid carrier containing large numbers of *Bact. typhosum*, emulsified in water, reinforced with culture of the same strain as in the first experiment, were administered. A rough estimate of the number of bacilli ingested was 125,000 million. Specimens of faeces and milk were examined as in the first experiment until 21 October, all with negative results. Agglutination tests of the blood on 19 October were also negative at 1 : 20.

10 *June* 1938. At 11 a.m. calf 1 was drenched with a mixture of about 300 ml. milk and 110 ml. typhoid culture. The calf was a male, 1 month old, which after weaning had been fed on linseed gruel. The typhoid culture used was Ty. 2 obtained from Dr Felix and was of maximal virulence and Vi content. On the previous day the culture on agar, kept in a thermos flask, had been brought from the Lister Institute to the Ministry's Laboratory, where at 2 p.m. it was heavily seeded (estimated 40 mg.) into 100 ml. 10% ascites broth warmed to 37° C. After 20 hr. incubation, at the time when the culture was given to the calf, the count was about 200 million per ml., which was unexpectedly low.

Next day there was some diarrhoea, and from midday on 11 June to evening of 12 June the body temperature was raised to 104·4–105·2° F., returning to normal within the two following days. By 16 June, however, the calf was very weak and on 18 June it had ceased to feed. On this day it was slaughtered. Up to 17 June samples of freshly passed faeces were collected frequently at different times of the day. Often two or three samples were mixed before culturing. On a single occasion, viz. from a sample collected at 4.30 p.m. on 12 June, *Bact. typhosum* was found, numerous colonies being present. On 16 and 17 June blood samples were taken. No growth was obtained from the clot; the serum gave: 'O' nil at 10, 'H' positive at 40 first day and 160 second day.

Bacteriological examination of material taken post-mortem resulted as follows: no *Bact. typhosum* in spleen, liver, lung or bile; from the ileocaecal gland numerous colonies of *Bact. typhosum*, confirmed by agglutination; from intestine one or two colonies of *Bact. typhosum*, confirmed by agglutination.

25 *June* 1938. At 11.45 a.m. calf 2 was drenched with milk containing 100 ml. ascites broth culture of strain Ty. 2. This culture was of full Vi content and was received from Dr Felix the day before. This calf was also a male, about a month old and weighing 108 lb., and had been hand-fed on milk and gruel.

This animal did not become obviously ill. Its faeces were collected daily as before and blood samples were taken on 28 and 30 June. The only positive results were obtained on the day after drenching when from faecal samples collected at 8 a.m. and 3.30 p.m. numerous *Bact. typhosum* were grown. The calf was destroyed with chloroform on 11 July, but no *Bact. typhosum* was grown on MacConkey and Wilson-Blair media sown from spleen, liver, kidney, bile, lower end of ileum, mesenteric and ileocaecal glands.

Application of typhoid culture to teat orifice

12 *January* 1939. Cow 3, Shorthorn, about mid-lactation. On the previous day the sphincters of the L.F. (left fore) and L.H. (left hind) teats were slit. On 12 January the sphincters were reopened at

10 a.m. and at the same time the orifices of the R.F. (right fore) and R.H. (right hind) teats were scarified. An hour later, the orifices of the four teats were rubbed with typhoid culture (strain X in veal broth) and then dipped into it, at the same time gently manipulating the terminal parts of the teats in imitation of milking. The culture used was prepared as follows: from MacConkey plates, sown from heart blood of a mouse inoculated with strain X, colonies were sown into ascites broth. After 4 hr. incubation, 8 ml. were sown into 800 ml. veal broth which after 18 hr. incubation was used for the cow. Six colonies grown from a loopful of this broth were all Vi positive and gave no trace of 'O' agglutination.

For the first 5 days after inoculation the cow's temperature was rather elevated and for 2 days she was off-feed. The main changes seen were in the L.F. and L.H. quarters which were indurated and painful, the milk being hard to draw and containing clots. There was some all-round reduction in yield. The changes in the L.F. and L.H. were noticeable for a fortnight.

Milk samples were taken twice daily, at 7 a.m. and 5 p.m. *Bact. typhosum* was isolated as follows:

	13 Jan.		14–17 Feb.	18 Feb.	19 Feb.	20–27 Feb.
R.F.	0 (R)	0 (R)	Subsequently all negative			
R.H.	52*	0 (R)				
L.F.	0 (R)	0 (R)				
L.H.	15*	0 (R)	0 0	110 25	20 0	Neg.

* Seeding from three loops of gravity cream. [(R) throughout these tabulated results means a repeat test, usually of gravity cream. The first column of figures under each date represents morning samples and the second column afternoon samples.] The colonies isolated on 18 January (p.m.) and 19 January (a.m.) were all strong Vi colonies, giving no 'O' reaction by direct slide agglutination.

Thus, from one of the scarified teats *Bact. typhosum* was isolated only once, on the day following inoculation; from one of the slit teats the organism was isolated up to the seventh day with some misses.

Cow 3 was slaughtered on 30 January and cultures were taken on MacConkey, Wilson-Blair and blood agar. Pure cultures of *Staph. aureus* were obtained, but no *Bact. typhosum*.

14 *February* 1939. Cow 987, Shorthorn, calved on 3 January 1939. On the previous day the orifices of the R.F. and R.H. teats were scarified, the L.F. and L.H. had the sphincters slit and the teat orifices slightly scarified. At 11 a.m. on 14 February all four teats were dipped in typhoid culture with gentle manipulation while the tips were immersed. Strain X was again used, the culture being one of 19 hr. in veal digest broth; at the time six colonies derived from it were tested and all were pure Vi, actively motile and gave no 'O' agglutination. There was practically no effect from the inoculations. The body temperature never rose, and the milk was noticed to be a little hard to draw on day 1 only, from the L.F. quarter. Milk samples were taken twice daily as before. *Bact. typhosum* was isolated as follows:

	15 Feb.		16 Feb.		17–24 Feb.
R.F.	0	0 (R)	0	0	All negative
R.H.	20 (R)	0 (R)	1	0	
L.F.	20	∞ (R)	45	11	
L.H.	40 (R)	4 (R)	0	0	

Thus, all cultures taken after the second day were negative, while one of the slit quarters had more *Bact. typhosum* than the others.

Injection of typhoid bacilli into the milk cistern

2 *November* 1937. Cow 118. At 4.45 p.m. the R.F. quarter was injected with 1·0 ml. 20 hr. ascites broth of strain X. This amount of culture contained 1040 million living organisms, of which three-quarters were in the Vi state. Next day the quarter was very tense and painful and the milk from it contained clots. The cow's temperature was 103·4° F. and she was not feeding. On the following day the swelling and tenderness had largely disappeared, the milk appeared more normal and the cow was feeding. The milk flow, however, was diminished for several days. *Bact. typhosum* was isolated on Wilson-Blair medium from the R.F. milk as follows:

3 Nov.	4 Nov.	5 Nov.	6 Nov.	7 Nov.	8 Nov.	9 Nov.	10 Nov.
			Over	Over	Over	Over	
∞ ∞	∞ ∞	∞ ∞	∞ 30	30 40	30 60	100	15 18

11 Nov.	12 Nov.	13 Nov.	14 Nov.	15 Nov.	16 Nov.	17 Nov.	18 Nov.
23 55	4 20	7 5	2 5	2 0	0 0	1 2	1 0

19–20 Nov.	21 Nov.	22–23 Nov.	24 Nov.	25 Nov.	26 Nov.	27 Nov.	28 Nov.–5 Jan.
0 0	1 1	0 0	0 (R) 200 (R)	4 (R) 0 (R)	0 (R) 1	0 (R) 3	0 (R) 0 (R)

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From 3 to 6 November one loopful of gravity cream was plated out, from 7 November onwards three loopsful were spread. Thus, the number of colonies gradually diminished with some fluctuations until 16 November, when the first negative result was obtained. The numbers of colonies during the fortnight of active excretion went from over 100 near the beginning to an average of three during the last 3 days. Subsequent positives, in each case one or two colonies only, were obtained on 17, 18, 21, 26 and 27 November, and, on repeat tests, positive results were also obtained on 24 and 25 November. Thereafter, *i.e. after* a period of 25 days from infection, only negative results were obtained. Throughout this period the milk from the other three-quarters remained normal and typhoid bacilli were never detected in it (cultures made twice daily from each during the whole period). On 24 November, i.e. 22 days from infection, a sample of the cow's blood serum agglutinated *Bact. typhosum*, both flagellar and somatic antigens, to a titre of 160. The same test emulsions were used for this as in the previous negative examinations when the cow was being used for ingestion experiments.

11 *March* 1939. Cow 987, previously used for teat infection experiment on 14 February 1939. At 11.30 a.m., R.F., R.H. and L.H. quarters were inoculated through the teat canal with 1·0 ml. of 16 hr. cultures in broth or ascites broth of strain X. The broth had been sown from colonies on meat digest agar inoculated direct from stock Dorset egg culture, and the strain was in the Vi state. The numbers of organisms injected were: R.F. 3800 millions, R.H. not estimated, L.H. 980 millions. The L.F. quarter was not inoculated. Milk samples were taken twice daily as usual, and *Bact. typhosum* was isolated from gravity cream on Wilson-Blair as follows:

	13 Mar.	14 Mar.	15 Mar.	16 Mar.
R.F.	5 2	0 20	0 50	20 20
R.H.	12 15	10 12	2 200	30 100
L.H.	50 60	6 12	20 300	200 200

	17 Mar.	18 Mar.	19 Mar.
R.F.	1 180	5 2	5 35
R.H.	60 15	25 150	7 30
L.H.	100 30	20 300	8 60

	20 Mar.	21 Mar.	22 Mar.
R.F.	0 30	0 1	0 0
R.H.	8 20	0 20	1 0
L.H.	10 90	70 2	6 10

	23 Mar.	24 Mar.	25–30 Mar.
R.F.	3 (R) 1	0 (R) 0 (R)	All negative
R.H.	2 (R) 0 (R)	0 (R) 0 (R)	
L.H.	∞ (R) 6 (R)	80 (R) 0 (R)	

Thus, the injected quarters continued to excrete *Bact. typhosum* for 13 days. During the first week the numbers grown from a loopful of cream reached 100–300 and then declined. The L.F. quarter never yielded typhoid bacilli. Clinically the udder showed no abnormality, though there was decrease of milk yield by about a gallon daily. On 25 March the cow's blood serum showed 'H' agglutinins to a titre of 320.

13 *April* 1939. Cow 987. Experiment repeated. Blood taken for serum test at 11 a.m. just before inoculation. A fresh 20 hr. culture in meat digest broth of strain X used, 1·0 ml. being instilled in the usual way into the R.H., L.F. and L.H. quarters. The R.F. was not inoculated. The culture used was plated and the six colonies taken were shown to be Vi positive. From the milk *Bact. typhosum* was isolated as follows:

	13 Apr. (5 p.m.)	14 Apr.	15 Apr.	16 Apr.
R.H.	400	0 (R) 60	3 70	0 50
L.F.	4	4 (R) 0	3 30	0 80
L.H.	20	1 (R) 90	8 50	20 20

	17 Apr.	18 Apr.	19 Apr.	20–21 Apr.
R.H.	1 0	0 0	0 3	All negative
L.F.	0 0	0 0	0 0	
L.H.	10 0	2 0	0 0	

Excretion therefore lasted for 6 days from the R.H., 5 days from the L.H. and 3 days from the L.F. The R.F. never showed typhoid bacilli.

The cow had been very ill the day after inoculation, though the reaction of the udder was not acute.

Cow 987 was slaughtered on 21 April, at which time another blood sample was taken for serum test. This showed 'H' agglutinins to 1280, compared with 80 on 13 April and 320 on 25 March, as above stated. The cow had developed swellings at the back of the right thigh and inside the right axilla. These were chronic abscesses from which *C. pyogenes* was grown. The bile and supramammary glands were negative for *Bact. typhosum*.

26 *April* 1939. Shorthorn cow 876. At 11.30 a.m. the R.F., R.H. and L.H. quarters were injected with milk containing 1·0 ml. 20 hr. broth culture of strain X, diluted 1 : 100 in broth; that is, each quarter received 0·01 ml. of original culture, roughly estimated to contain 30 millions typhoid bacilli. All colonies tested were Vi positive. The L.F. quarter was not injected.

By 5 p.m. on the day of inoculation, the R.F., R.H. and L.H. quarters were swollen and painful, especially the R.F., but the udder was normal on the following day. There was no fever and the cow fed

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well. *Bact. typhosum* was isolated from the milk as follows:

	26 Apr. (5 p.m.)	27 Apr.		28 Apr.		29 Apr.		30 Apr.	
R.F.	70	0	1	4	6	0 (R)	0	0	0
R.H.	100	20	4	2	0	0 (R)	0	0	0
L.H.	30	60	12	0	2	0 (R)	1	2	0

	1–3 May	4 May		5 May		6 May		7–13 May
						Less than		
R.F.	0	0	4	0	0 (R)	0 (R)	50 (R)	0 (R)
R.H.	0	0	0	1	0 (R)	0 (R)	0 (R)	0 (R)
L.H.	0	0	0	0	0 (R)	0 (R)	0 (R)	0 (R)

At this trial excretion of small numbers of organisms and with many apparent misses occurred for 10 days.

17 *May* 1939. Cow 876 was reinoculated into the milk cistern, this time with a much larger dose, viz. 1·0 ml. undiluted 16 hr. meat digest broth culture, strain X. Before sowing the broth, the strain had been passed once through a mouse and then twice subcultured on egg. Six colonies derived from the broth culture were tested and all were Vi positive. The R.H., L.F. and L.H. quarters were inoculated, not the R.F. The cow was bled just before inoculation and the serum showed an 'H' agglutinin titre of 80.

By 5 p.m. on the day of inoculation, the R.H., L.F. and L.H. quarters were swollen, especially the L.H., the milk from which contained clots. The cow's temperature was 106·4° F. and the animal refused to feed. By next day, however, the fever had subsided and the appetite gradually returned.

Plates of Wilson-Blair medium were inoculated from 0·1 ml. whole milk and from the cream, one loop, except 7–14 June when three loops were used. Isolations of *Bact. typhosum* were as follows:

Platings from cream

	18 May		19 May		20 May		21 May		22 May		23 May	
R.H.	0	0	7	2	7	26	0	0	0	0	0	0
L.F.	0	0	70	150	150	68	2	65	5	60	0	250
L.H.	0	15	300	200	40	1	3	300	60	60	300	300

	24 May		25 May		26 May		27 May		28 May		29 May	
R.H.	1	0	Subsequently all negative									
L.F.	35	90	0	55	40	62	150	0	125	0	90	0
L.H.	400	150	250	57	30	78	200	110	200	15	160	32

	30 May		31 May		1 June		2 June		3 June		4 June	
L.F.	100	0	0	31	0	4	40	11	1	50	0	4
L.H.	150	2	150	130	0	100	200	300	80	100	0	4

	5 June		6 June		7 June		8 June		9 June		10 June	
L.F.	150	100	100	200	150	250	?	>300	>300	3	2	?100
L.H.	150	150	120	150	100	100	?	>300	>300	7	3	?100

	11 June		12 June		13 June		14 June		15 June		16 June	
L.F.	3+	1	20+	0	1	9	150	50	100	100	>200	34
L.H.	0	2	20+	0	0	25	150	100	150	100	>200	15

	17 June		18 June		19 June		20 June		21 June		22 June	
L.F.	150	>300	36	120	18	8	?0	300	200	16	10	100
L.H.	200	>300	18	65	36	42	?0	200	100	3	200	200

	23 June		24 June		25 June		26 June		27 June		28–30 June	
L.F.	125	210	?0	44	100	11	0	0	>300	12	>300	50
L.H.	>200	>300	300	150	150	50	200	0	>300	30	∞	100

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Platings from cream (continued)

	3 July	6 July	10 July	13 July	17 July	20 July
L.F.	50	50	0	0	0	200
L.H.	0	100	0	0	0	400

	24 July	27 July	4 Aug.	5 Aug.	6 Aug.	7 Aug.
L.F.	0	300	97	85 180	1 10	0 1
L.H.	0	300	200	150 >300	6 150	120 >200

	8 Aug.	9 Aug.	10 Aug.
L.F.	1 0	0 1	0
L.H.	>200 11	200 150	150

Platings from milk

	18 May	19 May	20 May	21 May	22 May	23 May
R.H.	150 21	7 1	2 0	0 0	0 0	?1 0
L.F.	>500 134	139 27	14 10	14 25	33 17	1 43
L.H.	400 31	56 7	4 12	40 51	15 23	46 51

	24 May	25 May	26 May	27 May	28 May	29 May
R.H.	Subsequently all negative					
L.F.	16 60	3 42	45 27	48 6	24 16	36 3
L.H.	43 28	30 20	15 44	74 305	82 59	120 47

	30 May	31 May	1 June	2 June	3 June	4 June
L.F.	14 21	8 30	3 2	6 2	3 24	23 36
L.H.	61 138	98 350	350 27	38 108	69 134	118 25

	5 June	6 June	7 June	8 June	9 June	10 June
L.F.	39 65	23 2	96 91	88 103	156 285	134 >300
L.H.	60 198	10 10	178 44	54 69	134 750	225 >300

	11 June	12 June	13 June	14 June	15 June	16 June
L.F.	165 19	3 60	61 36	104 23	40 69	241 38
L.H.	210 38	168 102	37 104	62 80	46 69	170 82

	17 June	18 June	19 June	20 June	21 June	22 June
L.F.	91 >300	98 65	40 59	122 152	145 75	41 28
L.H.	96 220	110 15	156 89	126 103	116 50	24 95

	23 June	24 June	25 June	26 June	27 June	28–30 June
L.F.	17 55	115 1	32 25	49 112	198 108	191 157
L.H.	64 131	181 11	116 35	152 161	241 81	211 218

	3 July	6 July	10 July	13 July	17 July	20 July
L.F.	103	286	172	4	90	309
L.H.	40	216	225	7	44	122

	24 July	27 July	4 Aug.	5 Aug.	6 Aug.	7 Aug.
L.F.	58	350	54	35 98	1 5	2 0
L.H.	14	342	210	156 >300	1 81	33 165

	8 Aug.	9 Aug.	10 Aug.
L.F.	0 1	0 0	0
L.H.	120 9	101 53	12

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Excretion from the inoculated quarters continued for 85 days, i.e. till 10 August, when the cow was slaughtered. The udder remained clinically normal and the cow was in good condition. Eight colonies from each of the four positive plates on 27 July were tested and all were Vi positive. No typhoid bacilli were detected in the R.F. quarter. Blood samples were taken periodically for testing, the results for 'H' agglutinins with strain X antigen being: 13 April and 10 May 80, 14 June 160 (? end-titre), 10 August 1600. With the serum of 14 June, a test for Vi agglutinin by Dr Felix with two strains gave a titre of 20.

On 10 August tests were also made with Vi and 'O' antigens. Results were: Vi negative at 10, 'O' complete at 200.

On 4 August 1939 the cow gave birth to a calf which was unable to stand or suck. It died on 6 August. Blood serum from the calf on 5 August gave 'H' agglutinins to titre of 40.

Agglutination tests with whey from the milk of individual quarters gave the following approximate results:

4 Aug. { 'H' agglutinin titre all quarters 320–640
'O' agglutinin titre R.F. and R.H. 80, L.F. and L.H. 320

5 Aug. { 'H' agglutinin titre all quarters 128–256
'O' agglutinin titre R.F. and R.H. 32, L.F. and L.H. 64 (not end-titre)

9 Aug. { 'H' agglutinin titre all quarters 160
'O' agglutinin titre all quarters < 40

10 Aug. { 'H' agglutinin titre all quarters 160
'O' agglutinin titre all quarters < 40

At post-mortem examination of cow 876 there were no visible lesions. Bacteriological examination resulted as follows. A plate from the right supramammary gland gave one colony of *Bact. typhosum*, that from the left gland was blank. A piece of the L.H. quarter showed typhoid colonies, but none was grown from the R.H. No *Bact. typhosum* was grown from the spleen or the uterus (right horn), but in the latter there were numerous coliforms.

From the calf the contents of the abomasum and of several loops of intestine were examined with negative result.

Experiments involving two methods of infection

6 *January* 1938. Cow 118. Previously used on 2 November 1937 when R.F. milk cistern was inoculated.

At 5 p.m. the R.H. and L.F. milk cisterns were inoculated with 1·0 ml. *Bact. typhosum* suspension, and the L.H. teat orifice was dipped in a suspension of *Bact. typhosum* after it had been scarified. The suspension used for R.H. and L.F. consisted of a 20 hr. ascites agar culture of *Bact. typhosum*, dispersed in ox-serum broth to make about 3000 million per 1·0 ml. The suspension for the L.H. was very much thicker. The strain used was 'Warlingham M.H.', freshly isolated from the blood of a case in the Croydon typhoid outbreak of 1937–8. Isolations on Wilson-Blair of *Bact. typhosum* from the milk were as follows:

	7 Jan.	8 Jan.	9 Jan.	10 Jan.
R.H.	300 100	100 120	56 60	45 9
L.F.	250 100	100 —	150 200	40 100
L.H.	100 1	0 (R) 0 (R)	Subsequently all negative	

	11 Jan.	12 Jan.	13 Jan.	14 Jan.
R.H.	8 60	1 15	22 100	4 (R) 1
L.F.	100 90	100 80	100 100	100 90

	15 Jan.	16 Jan.	17 Jan.	18 Jan.
R.H.	0 1 (R)	Subsequently all negative		
L.F.	50 100	90 0 (R)	50 150	40 100

	19 Jan.	20 Jan.	21 Jan.	22 Jan.
L.F.	60 50	35 30	17 50	8 19

	23 Jan.	24 Jan.	25 Jan.	26 Jan.
L.F.	6 11	7 38	2 (R) 0 (R)	0 (R) 30 (R)

	27 Jan.	28 Jan.	29 Jan.	30 Jan.
L.F.	15 (R) 0 (R)	2 0 (R)	2 7	0 (R) 0 (R)

	31 Jan.	1 Feb.	2 Feb.	3–11 Feb.
L.F.	0 (R) 0 (R)	0 (R) 0	0 40	All negative

Thus, the organisms failed to pass the sphincter barrier of the L.H. quarter. From the R.H. they were excreted for 9 days, but from the L.F. in generally far higher numbers and as long as 27 days with a few blanks. The R.H. quarter was chronically infected with *Staph. aureus* and giving little milk, and this may have contributed to the more rapid disappearance of the organism.

The cow was slaughtered on 15 February 1938, cultures for *Bact. typhosum* from bile, bone-marrow and udder were negative.

5 *August* 1938. Shorthorn cow, 'Redwater', in fifth month of lactation.

At 11 a.m. the R.F. milk cistern was injected with 1·0 ml. typhoid culture mixed as usual with milk from the same quarter. The teat orifices of the R.H.

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and L.H. were dipped in the culture with some manipulation of the teat for a few seconds, the sphincter of the R.H. having been slit the day before. The L.F. was untouched. The culture used was of strain Ty. 2, Vi-rich, grown in 10 % ascites veal broth at 37° C. for 17 hr.

By 5 p.m. on day of inoculation the temperature rose to 106·3° F. but had fallen to normal by the following day. For the first 2 days the cow was not feeding well. Changes were seen only in the R.F. quarter which was acutely inflamed for about a week, with clots in the milk.

Before taking the milk samples on the day of inoculation the teats were washed in Jeyes's fluid and then in water. Isolations of *Bact. typhosum* on Wilson-Blair plates sown with one standard loop of gravity cream were as follows:

	5 Aug. (5 p.m.)	6 Aug.	7 Aug.	8 Aug.	9 Aug.	10 Aug.	11–12 Aug.
R.F.	50	150 (R) 0 (R)	100	0 (R) 6	29 0 (R)	1 0 (R)	Negative
R.H.	50	Subsequently negative					
L.H.	Negative throughout						

Excretion of *Bact. typhosum* thus continued for 5 days from the quarter inoculated in the milk cistern, but the organisms failed to pass the sphincters of the other two teats inoculated.

22 *September* 1938. Experiment repeated on the same cow. The R.F. and R.H. quarters were injected through the teat canal with 1·0 ml. typhoid culture. The L.F. and L.H. teats had their sphincters incised the day before and their orifices were also scarified; inoculation was by dipping the ends of the teats in undiluted typhoid culture with some massage for about 2 min. The culture, strain X, had been passed once through a mouse, then from the heart blood on to blood agar, thence a Vi colony subcultured twice in 10 % ascites veal broth, the second subculture being used for inoculation after 18 hr. growth at 37° C.

Systemic reactions to the inoculations were much as on the previous occasion though rather less severe. Visible alterations lasting for a few days were seen in the R.F. and R.H. quarters. Five days after inoculation the L.H. sphincter was still inoperative, and the ends of the L.F. and L.H. teats were noted to be very painful. Platings of the milk for *Bact. typhosum* gave the following results:

	22 Sept. (5 p.m.)	23 Sept.	24 Sept.
R.F.	+R	Num. (R) Num. (R)	Num. (R) 0 (R)
R.H.	+R	Num. (R) Num. (R)	4 +
L.F.	+R	Num. (R) 0 (R)	Subsequently all negative
L.H.	+R	Num. (R) 0 (R)	Subsequently all negative

	25 Sept.	26 Sept.	27 Sept.	28 Sept.–3 Oct.
R.F.	Num. (R) Num. (R)	Num. (R) Few (R)	Num. (R) 0 (R)	Negative
R.H.	Subsequently all negative			

Thus the organisms failed to become established in the L.F. and L.H. quarters, from the R.H. quarter there was excretion for 2 days only, from the R.F. typhoid bacilli were excreted for 5 days in large numbers and not at all subsequently.

After 3 October an experiment was carried out to see if the udder could be infected with *Str. agalactiae*. The ends of the teats were scarified and cultures of the organism applied, with the result that all quarters quite readily became infected. The cow was slaughtered on 8 November 1938, when cultures taken from the supramammary glands failed to show *Bact. typhosum*.

DISCUSSION

There appear to be few recorded attempts at infecting cows with typhoid bacilli or well-authenticated cases in which the organism has been isolated from their tissues. In a case described by Levy & Jacobsthal (1902), typhoid bacilli were isolated from a large splenic abscess in an apparently normal cow brought to slaughter; there were also several smaller abscesses in the liver. No cultures were made from the blood or mammary tissue but cultures from muscle proved sterile. This was claimed to be the first time *Bact. typhosum* had been isolated from natural infection in cattle.

In our work two attempts to infect an adult bovine using an enormous quantity of virulent typhoid culture by the mouth failed and typhoid organisms were never found in the faeces or milk. On the other hand, when a large dose of culture of high Vi content was swallowed by two young calves, one of them can be said to have taken the infection. When large numbers of virulent typhoid bacilli were introduced into the mammary gland through the teat canal, the organism could be recovered from the milk for a variable period of days. The longest time was 85 days (cow 876), when the animal was slaughtered, and at post-mortem typhoid organisms were recovered from the tissue of one-quarter and one udder lymph gland. Most commonly excretion lasted for only

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5–10 days. Excretion was generally not continuous, or at least there were many occasions when typhoid bacilli could not be isolated from the fresh or even from the stored cream. Had more repeats been done from the stored samples, the gaps might have been fewer.

These results might have more meaning if it could be shown that typhoid bacilli are capable of passing into the teat canal when applied to the teat orifice. However, nothing more than a transient infection was set up when large numbers of typhoid bacilli were massaged on to the teat orifice, even when this had been grossly injured beforehand by severing the sphincter.

Agglutination tests with the blood of some of the cows injected intracisternally also suggest an actual infection. For instance, cow 987 14 days after a first injection gave an 'H' titre of 320; this declined within the month but 8 days after a second injection the 'H' titre had again risen to 1280. Cow 876 was given a large dose of culture into the cisterns of three udder quarters. The 'H' titre of the blood serum before infection was 80, 28 days later the 'H' titre was 160 and the Vi titre 20, while 80–85 days after the injection the 'H' titre had risen to 1600 and the 'O' titre was then at least 200. Possibly, a previous small dose of culture into the milk cisterns of this animal contributed to the high titres noted after the second large dose.

In this connexion reference may be made to the reactions of normal cattle blood to typhoid antigens. It is known that the blood serum of normal cattle often contains 'H' and 'O' agglutinins for *Bact. typhosum*. Gibson (1932) obtained titres of 32–64 with 'O' antigen of *Bact. typhosum* and similar titres with suspensions composed almost entirely of flagellar antigen, the relative preponderance of the two types of agglutinin varying in different samples of serum. Jordan (1937) out of 339 tests with 293 serum samples from cattle obtained 30 % positive at 1 : 40 and 7 % at 1 : 100–1 : 200, using live suspensions of three strains grown on 24 hr. agar slants.

We may contrast the above with what happens when a natural udder pathogen, *Str. agalactiae*, is employed. When this organism is injected into the milk sinus, the usual result is an infection of the quarter with enduring excretion in the milk. Although repeated application of *Str. agalactiae* to sound teats, as by milking with hands intentionally contaminated with the organism, frequently fails to infect (e.g. Seelemann & Siemonsen, 1932; Klimmer & Haupt, 1933), the organism usually passes the sphincter and leads to permanent infection when the orifice has been slightly damaged (Bendixen, 1934). The vulnerability of the udder to *Str. pyogenes* from man is similarly explained.

The results of the experiments reported in this paper and of the facts just mentioned may be considered in the light of the findings of Pullinger & Kemp (1938) on the fate of typhoid bacilli when added directly to milk. These authors found that *Bact. typhosum* readily multiplied in raw cow's milk, even when it was absolutely fresh, on storage at 15 and at 18° C., as well as in heat-treated milk. In the raw milk significant increases occurred within 24 hr., whether the inocula were small (36–316 organisms) or larger (2500–31,800). Rather surprisingly, they also ascertained that growth was more rapid with strains recently isolated from man than with old stock cultures. This work confirms the much earlier observation of Eyre (1904) that small numbers of *Bact. typhosum* added to milk can multiply a hundred-fold within 24 hr., and it is also in line with the numerous positives obtained in the present work with stored samples when the fresh samples were negative.

The work of Eyre and of Pullinger emphasizes that the chance introduction of even small numbers of typhoid bacilli into milk may be fraught with great danger to the public health and also suggests there is no need to envisage the cow as an active agent in bringing about the contamination. Quite the contrary is the case with milk-borne *Streptococcus* epidemics. Here there is good evidence that at least some outbreaks, and especially widespread ones, are due to an actual infection of the udder with *Str. pyogenes* from man; in such cases the milk as it is drawn from the gland may contain very large numbers of these organisms for weeks or months, although the infected quarters may show no gross abnormality. Pullinger & Kemp (1937), who summarize some of the evidence just referred to, have shown that *Str. pyogenes* when added to fresh raw milk begins to multiply slowly only after 48–72 hr. of storage at 18–22° C. During the earlier part of this period the numbers of streptococci may even fall; this was so with two strains, freshly isolated respectively from man and from the udder of a naturally-infected cow. In similar experiments with commercially pasteurized milk and with raw graded milk bottled for distribution, the milk soured too rapidly for multiplication to take place after artificial contamination. The results were the same whether the milk was heavily or lightly inoculated. We are thus left with the conviction that, whereas in milk-borne *Streptococcus* outbreaks it is necessary to look for a cow or cows that may be responsible, in similar outbreaks of enteric fever the object of search need not extend beyond the human carrier.

[*Concluding note* (*F.C.M.*). Dr Shaw on a review of the evidence at the time of the Bournemouth outbreak was inclined to think that an infection of the cows might be responsible for the continuous contamination of the milk. He also concluded that Mrs A—the wife of one of the producers involved—contributed to this contamination from the time

she became infected with typhoid until she was removed to hospital. Now that the experiments reported in this paper have failed to alter the current view that cows do not actively disseminate typhoid and now that further facts regarding the multiplication of typhoid bacilli in raw milk have been published, some reconsideration of the problem may be permissible. Dr Shaw gives reasons for his belief that the well water, which was the only source of supply to the dwellings in the immediate neighbourhood, was very unlikely to be at fault. But it was definitely shown that communication was possible between the stream, proved to contain human sewage, and the well, and further that organisms of human intestinal origin were present in this well water not only in 1934 but also in September and November 1936. It seems therefore that the contamination of the milk produced by A may have been due to: (*a*) the well water, which was used for all dairy purposes and also for washing the udders of the cows before they were milked; (*b*) the contributions of Mrs A—under the conditions outlined by Dr Shaw. To be mentioned are the existence of a pail closet a few yards from A's dairy, the activities of flies, and the fact that Mrs A—before she was removed to hospital—was being nursed by her husband who at the same time was milking the cows and attending to the dairy utensils; (*c*) surface contamination of the udders and teats of A's cows by the stream water (see also Savage, 1912). In Dr Shaw's opinion this would not in all probability be an everyday event.

Once typhoid bacilli had found their way into the milk produced by A—and perhaps to some extent that produced by his neighbour C—in one or more of the ways mentioned, contamination of the great bulk of the dealer's milk was inevitable, and before it was all consumed there must often have been ample time for appreciable multiplication of the typhoid organisms.]

SUMMARY

1. A cow given by the mouth very large doses of virulent typhoid bacilli (275 and 125 thousand millions) on two occasions at an interval of 29 days failed to show signs of infection or to pass the bacilli in faeces or milk.

2. Of two-month-old calves, dosed with about 100 ml. ascites broth culture of typhoid mixed with milk, one became infected and typhoid bacilli were grown from the intestine and ileocaecal gland. Both, however, passed typhoid bacilli in the faeces on the first or second day after dosing.

3. When large numbers of typhoid bacilli (say, 1–3 thousand millions) were instilled into the udder through the teat canal, excretion of the bacilli in the milk usually ceased within 10 days (fourteen quarters of four cows). In two quarters of one of these cows excretion continued for 25 and 27 days, and in two quarters of another cow for at least 85 days. The procedure usually caused an acute mastitis lasting for a few days, and the 'H' and 'O' agglutinin titre of the blood rose.

4. When large numbers of typhoid bacilli were applied to the teat orifice, even when this had been grossly injured beforehand by slitting the sphincter, there was as a rule only a very transient infection. In eleven cases out of twelve, either the organism could not be found in the milk or was found for 1 day only; in the remaining case there was intermittent excretion for 7 days.

5. The strain of typhoid used for most of the above experiments was of high Vi content and was isolated from the carrier involved in the Bournemouth-Poole epidemic of 1936.

6. These experiments do not support the view that milk-borne typhoid outbreaks are brought about by an active infection of the cow and suggest that the main object of the epidemiologist must still be the location of the human carrier.

7. The chances of typhoid bacilli growing in the milk after its withdrawal from the cow are stressed.

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