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**TUBERCULOUS INFECTION
IN MILK**

**A Report by
THE DEPARTMENT OF HEALTH FOR SCOTLAND**

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PREFACE

The following report deals with an investigation organized by the Department of Health for Scotland with the co-operation of the Local Authorities of Edinburgh, Glasgow, Dundee, and Aberdeen, and with that of the Hannah Dairy Research Institute. The work was aided by a grant from the Empire Marketing Board.

The findings, although they refer in particular to Scottish conditions, have obviously also a more general application. The Medical Research Council are accordingly glad to have the opportunity of publishing the report in this series, to assist in making the information widely available.

MEDICAL RESEARCH COUNCIL,
38 Old Queen Street,
Westminster, S.W.1.

11th October, 1933.

TUBERCULOUS INFECTION IN MILK

A REPORT BY THE DEPARTMENT OF HEALTH FOR SCOTLAND

THIS inquiry was designed to determine the extent of infection in different kinds of milk in selected areas of Scotland. The samples, which amounted to a very appreciable number, included raw milk sampled on arrival in the cities; heat-treated milk; milk as sold to the consumer, and graded milk.

The same rigid technique was observed in each area, and the results show (Chap. 7) the necessity for a standard technique if comparability of results from separate investigations is to be obtained.

The report shows that (1) in the aggregate of the four cities 10 per cent. of the samples of 'raw milk in churns' were infected; (2) bulking of milk increased the infection; (3) heat-treatment reduced it; and (4) the general rate of infection of retailed milk is estimated at a little above 5 per cent. In 714 samples of Certified and Grade A (T.T.) Milks one positive sample was found. These findings are discussed in detail in the body of the report.

The Department recognize that the record cards—see Appendix E—contain information on which, if thought desirable, other and further investigations might be based. They are also aware that some of the data presented in the report relate to controversial issues and that emphasis placed on findings may well depend on individual points of view.

The cost of the inquiry was met partly by the Local Authorities of Aberdeen, Dundee, Edinburgh, and Glasgow, and partly by a grant from the Empire Marketing Board. For the examination of samples in the five centres responsibility was borne in Aberdeen by Dr. J. Smith, City Bacteriologist; in Dundee by Professor W. J. Tulloch of the Bacteriological Department of the University of St. Andrews; in Edinburgh by Professor T. J. Mackie of the Bacteriological Department of the University of Edinburgh and by Mr. A. Gofton, Chief Veterinary Officer of the City; in Glasgow by Dr. W. R. Wiseman, City Bacteriologist; and at the Hannah Dairy Research Institute by Dr. L. Jordan, Veterinary Pathologist. The statistical analysis of the records was carried out by Dr. P. L. McKinlay, Medical Statistician to this Department. The Department desire to express their appreciation of the work done by those named and of the assistance given by the various bodies, the Medical Officers of Health of the four cities, Dr. H. J. Rae, Aberdeen; Dr. W. L. Burgess, Dundee; Dr. J. Guy, Edinburgh; and Dr. A. S. M. Macgregor, Glasgow; the Director of the Hannah Dairy Research Institute, Dr. N. C. Wright; and all others who helped in the investigation.

J. L. BROWNLIE

DEPARTMENT OF HEALTH FOR SCOTLAND,

August, 1933.

TUBERCULOUS INFECTION IN MILK

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CHAPTER I. INTRODUCTORY

THE chief object of this inquiry was to ascertain with exactitude and by uniform methods of examination the extent of infection with tubercle bacilli in various types of milk as consigned to and consumed in the four principal cities of Scotland.

The results of the inquiry will be more clearly understood if the way in which milk reaches the consumer in these cities is realized. The liquid milk consumed in the cities comes from four sources:

- (a) from farms where it is produced and from which it is consigned in churns to distributors in the cities either direct or through creameries in the dairying districts;
- (b) from creameries where it is collected from farms in the surrounding districts and run into large tanks for transport to the wholesale distributors in the cities;
- (c) from producer-retailers, i.e. from the smaller dairy farmers who produce the milk and retail it direct to the consumers; and
- (d) a comparatively small quantity of graded milk (Certified, Grade A (T.T.) or Grade A) arrives in the city in sealed bottles or cans.

Only in the case of Glasgow is any considerable quantity derived from (b) i.e. transported in large tanks from creameries in dairying districts. Milk from producer-retailers forms a considerable proportion of the supply of Edinburgh; in Dundee and Aberdeen and still more in Glasgow the producer-retailer is a less important source of supply. In Edinburgh a considerable proportion of the city's supply is derived from dairy byres within the city boundaries. Almost all the milk except a portion of that consigned from creameries ((b) above) reaches the cities in its raw state.

As regards (a) i.e. milk consigned direct or through creameries from the farm to the wholesale distributor, while each churn contains the milk of more than one cow, it has not undergone bulking on a large scale and is referred to in this report as 'raw milk in churns'.

The term 'raw milk in tanks' has been applied to (b); it represents the bulking of milk from a number of farms.

Almost all the raw milk in tanks and a considerable portion of the raw milk in churns is subjected to a process of heat-treatment before being delivered to the consumer. Very little of the milk supplied by producer-retailers is so treated. This process of heat-treatment is commonly referred to as pasteurization, but the milk is not generally sold as pasteurized—a term which can be legally applied only to milk which has been pasteurized under a licence from the local authority by being retained at a temperature of not less than 145° F. and not more than 150° F. for at least half an hour and immediately passed to an efficiently protected cooler and the temperature reduced to not more than 50° F. It has been thought proper to refer to such milk in this report as 'heat-treated milk'.

The inquiry was designed so as to give an indication of the extent to which the milk at these various stages is infected with tubercle bacilli. The work has been undertaken by the staffs of the four Local Authorities of Aberdeen, Dundee, Edinburgh, and Glasgow, of the Bacteriology Departments of the Universities of Edinburgh and St. Andrews, and of the Hannah Dairy Research Institute, under the general co-ordination of the Department of Health for Scotland; and the expenses of the inquiry have been met partly by the Local Authorities concerned and partly by a grant from the Empire Marketing Board. It is desired to acknowledge the assistance rendered by the Local Authorities, the Universities, and the Institute, who provided facilities for the inquiry, and by the bacteriologists and other members of their staff who carried out the work.

The drafting of the scope of the inquiry and the formulation of the details both of sampling and of biological testing were relegated to a committee representative of the bodies taking part.

The scheme as originally drawn up by this committee is outlined in the *Details of Sampling and Details of Biological Testing*, copies of which were circulated to each of the investigators at the commencement of

Dairy Research Institute was modified as follows: The examination of further samples of dried and condensed milks was stopped forthwith (those already completed or in progress at the time numbered 58), and the work limited to the examination of graded milks and to bulked samples from creameries, the numbers of these to be tested being 750 and 200 respectively. Although the scope of the inquiry had to be curtailed in this manner, it is considered that the curtailment in no way invalidates the results of what still remained a very extensive investigation. In the case of condensed and dried milks the number of samples was inadequate to give significant results.

The results of the inquiry have been included in chapters which roughly correspond with the stages the milk passes through between arrival in the cities and delivery to the consumer. Raw milk is first dealt with, and then follows a chapter on graded milk. The next chapter gives the results of the examination of heat-treated milk, and compares the results obtained by various methods of heat-treatment, including treatment in accordance with the pasteurization standards prescribed in the Milk (Special Designations) Order (Scotland), 1930. Retail milk is the subject of the following chapter, in which the milk is classified according to method of treatment, if any.

The technique followed in this inquiry involved the use of 2 guinea-pigs in connexion with each sample. One was killed and examined at the end of 4 weeks. If the result was positive, the second was immediately killed and examined; if the result was negative, the second was kept for another period of 4 weeks and then killed and examined. Chapter VII compares the results obtained from the examination of guinea-pigs after 4 weeks and 8 weeks respectively, and suggests conclusions to be drawn from this comparison.

CHAPTER II. SCHEME OF INVESTIGATION

As already explained, a committee of representatives of the bodies concerned in the inquiry drew up a scheme which was outlined in the *Details of Sampling and Details of Biological Testing*, copies of which were circulated to each of the investigators at the commencement. The details were as follows:

I. DETAILS OF SAMPLING

A. RAW MILK IN CHURNS (excluding graded milk).

1. Samples to be taken on arrival in cities, either at railway stations or at consignees' premises.
2. Samples to be taken from the complete consignment of a farm, churns being sampled by the 'spear' method. The combined 'spearings' to be made into one composite sample from which the test quantity is taken.
3. No samples to be taken from milk which has passed through country creameries, where either bulking or heat-treatment may vitiate the result.
4. The following details to be supplied with each sample: Names and addresses of producer and consignee; place where sampled; date; number of churns from farm and total milk from which sample is taken.

5. Samples to be taken as far as possible to represent the full milk supply coming from the farms into the city.

B. HEAT-TREATED MILK (excluding Grade A Pasteurized).

1. Any method of heat-treatment designed to reduce bacterial contamination to be included.
2. Samples to be taken as convenient and according to the method of treatment. Normally they should be taken either from the cooler direct or from a newly filled bottle.
3. The following details to be supplied with each sample: Name and address of plant; date; type of plant; temperature and time of treatment; method of temperature control.

C. RETAILED MILK.

1. Samples of retailed milk to be taken in order to ascertain the infection of the milk as sold to the public. Random samples to be bought over the counter, either in bottles or from bulk.
2. The following details to be supplied with each sample: Name and address of retailer; date; details of sampling; details of treatment, if known.

D. GRADED MILK.

1. Samples to be taken from the following grades: Certified and Grade A (T.T.).
2. Samples to be taken (*a*) from the mixed milk of each T.T. herd, these samples being taken on the farm, and (*b*) from graded milk samples as bought in the market.
3. The following details to be supplied with each sample:
Farm samples: Name and address of producers, bulk from which sample is taken (total herd, single churn, or bottle), date, grade, name of sampler.
Bought samples: Name and address of retailer and of producer (if known), date, size of bottle or churn, where bottled (i.e. by producer or retailer), grade, name of sampler.

E. OTHER MILK.

Other samples to be undertaken as investigation proceeds, these being dependent on general development of work (includes creamery bulked milk sent to cities in tanks).

GENERAL

All samples in categories A, B, and C should be dealt with by Local Authorities, and all samples under D and E should be in the hands of the Hannah Institute. Local Authorities will co-operate in securing samples for the Hannah Institute where necessary.

It is essential that all samples taken should be random samples: routine samples taken to trace out a source of infection must not be used.

For uniformity in the collection of results, special forms should be used for the details of sampling and of test, and these should be forwarded at six-months' intervals to the Department of Health.

II. DETAILS OF TESTING.

Duplicate samples of 50 c.c. of the well-mixed milk to be centrifuged at 3,000 R.P.M. for $\frac{1}{2}$ hour.

The sediment alone of each tube to be suspended in 3 c.c. of sterile normal saline solution.

Two guinea-pigs to be inoculated, each with 3 c.c. of suspension. Both inoculations to be subcutaneous, and the injections to be made on the inside of left thigh.

The pair of animals inoculated with a particular sample may be kept together in one cage, but separate from other inoculated animals.

One guinea-pig to be killed at the end of 4 weeks, and, if positive, i.e. showing a typical tuberculous condition, the other animal to be killed immediately; if negative, the remaining animal to be kept for 8 weeks from the time of inoculation.

If one guinea-pig dies prematurely of some intercurrent condition, the survivor to be kept for 8 weeks from the time of inoculation.

Premature deaths should be noted, and the cause given where possible.

On post mortem, the presence of tubercle bacilli in lesions to be demonstrated microscopically; where there is any doubt as to the tuberculous nature of the lesions, cultures to be made for organisms other than tubercle bacilli and guinea-pigs to be subinoculated with material from the doubtful lesion.

Note:

It is essential that the technique at each centre should be uniform; and also that the technique for *each type of milk* should be the same. That is to say, no allowance should be made for the possible loss of virulence of the bacilli due to heat-treatment.

At the end of a month a meeting of workers to be held to compare notes, and if necessary modify procedure.

Exact uniformity in the methods adopted (the absence of which has hitherto tended to produce difficulty in comparing published statistics of this nature from various sources), has thus been secured by all workers concerned in the investigation.

At the initiation of the inquiry it was decided that, after one month's experience of the actual working of this programme, a further meeting of the personnel should be held to compare notes, to discuss any difficulties which might have arisen, and, if necessary, to modify the original procedure. At this meeting it was accepted that the sampling details should be altered and for future use the dipper be substituted for the spear method, the dipper itself being used to stir up the milk in the container (which need not then be shaken). It was further agreed that, in order to ensure that there was no precedent tuberculous infection of the test animals, the guinea-pigs should be tuberculin-tested before inoculation. Although doubt was expressed as to the necessity of this addition to the technique (mainly in view of the rarity of this condition in guinea-pigs), it was agreed to apply it.¹ Exact details of the technique to be used—the intradermal inoculation of a depilated area of the abdominal wall of 0.22 c.c. of a 1:1000 dilution of tuberculin (O.T.)—were circulated to each of the bacteriologists.

Specimens of the cards in use for recording the findings of the tests are shown in Appendix E.

¹ This was inadvertently omitted in one part of the Edinburgh Series of tests; but the findings in the extensive tuberculin testing carried out in other areas (the degree of guinea-pig infection being less than 1 in 4,000), are sufficient proof that its omission can scarcely be regarded as a vitiating influence.

CHAPTER III. RAW MILK

PART I. RAW MILK IN CHURNS

The term 'raw milk in churns' as here used applies to milk from source (a) mentioned in Chapter I, i.e. to milk arriving in the cities from the farms where it is produced and whence it is consigned in churns to the distributors either direct or through creameries in the dairying districts. Each churn holds on an average the milk of 3 to 5 cows. The samples examined were, however, not from single churns but were composite samples made up from the churns containing the complete consignment from a farm. The chance of any sample being found infected would presumably be increased in proportion to the number of churns from which the sample was taken. The general findings in relation to this milk for each of the four principal cities and for the aggregate are presented in Table 1.

TABLE 1
Raw Milk in Churns: General Findings.

	<i>Positive.</i>	<i>Negative.</i>	<i>Inconclusive.</i>	<i>Total.</i>	<i>Per cent. positive.</i>
Aberdeen	33	294	7	334	9.9
Dundee	33	413	60	506	6.5
Edinburgh	39	281	16	336	11.6
Glasgow	48	292	18	358	13.4
All areas	153	1,280	101	1,534	10.0

In the whole series there were found 153 positive cases in a total of 1,534 samples examined, these being equivalent to a proportion of roughly 10 per cent. positive. In view of the fact that, of the total, a certain number (101) are assigned to the category 'inconclusive', the incidence of infection based only on tests in which the criteria defined at the commencement of the inquiry were upheld is 10.7 per cent. (Cases classed as inconclusive are those in which the second guinea-pig did not live for the required period of 8 weeks and on autopsy was found non-tubercular.¹) The true incidence of infection thus lies between these two figures (nearer, however, the former than the latter since many inconclusive tests would probably be negative), and we are safe in stating that for these four Scottish cities the average proportion of infected samples of raw milk in churns, the tests being those in which the aforementioned technique are regarded as criteria, is at present in the neighbourhood of 10 per cent.

In the individual cities the highest value is recorded in the samples collected in Glasgow and its environs, where of 358 samples taken, 48 or 13.4 per cent. were found by these tests to be infected with *B. tuberculosis*; or, excluding those classified as inconclusive, 48 out of 340 (i.e. 14.1 per cent.) positive. The lowest figure is recorded in Dundee where, out of a total of 506 samples, or of 446 samples in

¹ A table showing the dates of death of the second guinea-pigs in tests classified as inconclusive is given as Appendix D.

which the results were definite, 33 were found positive, percentages respectively of 6.5 and 7.4. In the remaining areas, Aberdeen and Edinburgh, the incidence of infection is in the former 9.9, and in the latter 11.6 per cent. based on total, and 10.1 and 12.2 based on definite samples.

Volume of milk from which sample is taken in relation to incidence of infection. The crude data requisite for a discussion of this aspect of the problem are collected in Appendix Tables A and B, which show for the 4 areas, individually and combined, the positive, negative, and inconclusive cases tabulated according to (a) the number of churns and (b) the total volume of milk from which the samples were taken.

In Table 2 are given comparisons of the average number of churns and of the average volume of the milk from which the positive and negative (including inconclusive) samples were taken.

TABLE 2

Average number of churns and average volume of milk (in gallons) from which samples were taken.

	Number of churns.		Volume of milk.	
	Positive.	Negative.	Positive.	Negative.
Aberdeen . . .	4.88	3.64	44.62	31.72
Dundee . . .	4.69	3.75	45.68	37.85
Edinburgh . . .	4.87	4.40	45.96	39.82
Glasgow. . . .	8.54	4.82	98.65	42.05
All areas . . .	5.99	4.10	62.14	37.86

These figures show, individually and collectively, higher averages for positive than for negative samples; in other words, that the greater the bulk of milk from which the sample is taken the higher is the proportion of infected samples found. This is shown equally clearly but in a different form in Table 3, wherein are collected the percentage incidence rates of positive cases in samples taken from varying numbers of churns and varying volumes of milk.

TABLE 3

Incidence of infection in relation to (a) number of churns, and (b) volume (in gallons) of milk from which samples were taken.

No. of churns.	Positive.	Total.	Percentage positive.	Volume of milk.	Positive.	Total.	Percentage positive.
1 and 2	27	434	6.2	0—	25	348	7.2
3 „ 4	40	508	7.8	20—	45	540	8.3
5 „ 6	47	339	13.8	40—	36	370	9.7
7 „ 8	23	149	15.4	60—	26	162	16.0
9 „ 10	6	62	9.7	80—	14	63	22.2
11 „ 12	3	14	21.4	100—	1	19	5.3
13+	6	12	50.0	120+	6	22	27.3
Not stated	1	16	6.25	Not stated	..	10	..
Total	153	1,534	10.0	Total	153	1,534	10.0

The above findings might have some relevance in the discussion of two questions: (a) the dissemination of infection by bulking, and (b) inter-local comparisons of the incidence of infection such as previously noted for the present series of four cities. With reference to (b), for example, it is obvious that if gross differences are found in the distribution of samples by volume of milk from which these are taken, part at least of the resulting differences of incidence might be explicable in terms of this factor, and suitable allowance would have to be made for it. In Table 4 are compared the incidence rates previously found with the average number of churns and volume of milk from which the total samples were taken. Although there is no one-to-one correspondence between average bulk and incidence of infection, there is evidence of some degree of association between the two, the heavier bulking of the Edinburgh, and more particularly the Glasgow, samples being perfectly clear. Inquiry as to whether or not the foregoing variations in the volumes of milk in the different cities is to be held explanatory of the differences of incidence of infection can be carried out in two ways; (a) by applying the aggregate results of infection by volume (Table 3) to the numbers of samples in each sub-group of bulking, or (b) by comparison of the incidence in each city for samples taken from more or less similar bulks of milk. To the latter method pertains the objection that with the number of samples available when individual cities are concerned, the subdivision of the total to show incidence rates for milks of varying volumes offers such scope for chance fluctuations that the differences may be inconsistent and subject certainly to large error. Applying each of these methods we find that the difference in the amount of bulking in the individual areas is not to any substantial extent explanatory of the local variations of tuberculous milk infection previously found; in fact, it accounts for only an inappreciable fraction of the observed differences.

TABLE 4

Incidence of infection, average number of churns and volume (in gallons) of milk from which samples were taken.

	<i>Incidence of T.B. in samples.</i>	<i>Average no. of churns from which samples taken.</i>	<i>Average volume of milk from which samples taken.</i>
Aberdeen . .	9.9	3.76	32.99
Dundee . .	6.5	3.81	38.35
Edinburgh . .	11.6	4.45	40.54
Glasgow . .	13.4	5.33	49.81
All areas . .	10.0	4.29	40.29

Seasonal variations of incidence of infection. The statistics for the areas combined are presented in Tables 5 a and b. In the more detailed table showing the incidence in each month of the year considerable fluctuations are found in the proportions of infected samples, but no very defined seasonal trend is manifest. Since part of the variations are undoubtedly due to the small numbers when such detailed

Subdivision is effected, and since a general tendency may be obscured by these fortuitous deviations, the figures are summarized more crudely by quarters in Table 5b. It will be observed that there are somewhat higher proportions recorded in the 4th and 2nd quarters than in the remaining two; but when tested for significance it is found that differences of this order might easily arise by chance. So far as the figures go, we may conclude that there is no difference of any moment in the proportion of samples of raw milk found to be infected with tubercle bacilli in different periods of the year.

TABLE 5a
Seasonal differences of incidence of infection.

	<i>Positive.</i>	<i>Negative.</i>	<i>Inconclusive.</i>	<i>Total.</i>	<i>Percentage positive.</i>
January	11	88	5	104	10.6
February	8	85	19	112	7.1
March	5	56	16	77	6.5
April	14	114	8	136	10.3
May	20	151	3	174	11.5
June	13	96	5	114	11.4
July	5	63	1	69	7.2
August	10	53	3	66	15.2
September	6	119	7	132	4.5
October	32	160	13	205	15.6
November	11	159	11	181	6.1
December	18	136	10	164	11.0
Year	153	1,280	101	1,534	10.0

TABLE 5b
Seasonal differences by quarters.

	<i>Positive.</i>	<i>Total.</i>	<i>Percentage positive.</i>
1st Quarter	21	293	8.19
2nd Quarter	47	424	11.08
3rd Quarter	21	267	7.87
4th Quarter	61	550	11.09
	153	1,534	9.97

PART II. RAW MILK IN TANKS

Special inquiry was made of the amount of tuberculous infection in creamery bulked milk sent to cities in tanks. The bulking of the milk in these cases is very great; in the present series practically all the samples were within a range of 30 to 3,015 gallons, but in one isolated case the volume of milk from which the sample was taken was recorded as 7,276 gallons. The average volume for the total series of 201 samples (in 6 instances this information was not given) was 509 gallons.

In all, 207 tests were made with the following results:

Positive	74
Negative	125
Inconclusive	8
Total	207

Of the total samples, 74 or 37·5 per cent. were found to be infected, the incidence, therefore, being between three and four times the general figures given previously for raw market milk. These findings agree in general with what has already been stated in relation to the influence of bulking on the incidence of infection.

The average volume of milk from which the positive samples were taken is 576 gallons, and of negative samples 469 gallons. The positive samples, that is, tend more frequently to appear in the heavier bulkings.

If the series be roughly subdivided into bulks of increasing magnitude, it is again found that correlation exists between the amount of bulking and the proportion of infected samples found, as is shown in Table 6. The increase is quite definite, although, not absolutely regular.

TABLE 6

<i>Volume of milk.</i>	<i>Positive.</i>	<i>Total.</i>	<i>Percentage positive.</i>
Under 100	2	6	33·3
100—	5	44	11·4
200—	10	45	22·2
300—	23	44	52·3
400—	21	36	58·3
500+	11	26	42·3
Not stated	2	6	33·3
Total	74	207	37·5

A cruder grouping into three categories only, namely, under 200 gallons, 200 and under 400 gallons, and 400 gallons and over, shows that the incidence increases from 14·0 per cent. positives in the first to 37·1 per cent. in the second, and reaches the high figure of over 50 per cent. in milk samples from tanks of over 400 gallons' capacity.

These 207 samples were taken from milk derived from seven different creameries, and that a heavy infection rate is a general feature of this type of milk and is not peculiar to one particular creamery is evidenced (Table 7) by the findings for the individual

TABLE 7

<i>Creamery.</i>	<i>Positive.</i>	<i>Total.</i>	<i>Percentage positive.</i>
A	14	43	32·6
B	12	46	26·1
C	9	24	37·5
D	28	66	42·4
E	10	21	47·6
F	·	1	·
G	1	6	16·7
Total	74	207	37·5

places. The proportions of positive cases, although varying to some extent (e.g. in those with more than 20 samples analysed, from 26·1 to 47·6 per cent.), are extremely high in each of these creameries.

SUMMARY:

1. The proportion of infected samples in raw milk in churns arriving in the four principal cities of Scotland is found by the methods used in this inquiry to be roughly 10 per cent. The local variations are shown.
2. The incidence of infection increases with the volume of milk from which the sample is taken for examination.
3. The latter finding is only to a small degree explanatory of inter-local variations in incidence of infection.
4. No definite seasonal variations of incidence are found in this inquiry.
5. Raw milk transported in tanks shows a much heavier proportion of infected samples. The average is 37·5 per cent.
6. A high rate is found to apply to each of those creameries from which sufficient samples to give reasonably reliable results have been taken.

CHAPTER IV. GRADED MILKS

The number of samples tested in this group was 714, consisting of 329 samples of Certified milk and 385 samples of Grade A (T.T.) milk. It should be noted that these samples were drawn at random from both producers' and distributors' premises. The findings are given in the accompanying table (Table 8). Only one infected sample

TABLE 8

	<i>Positive.</i>	<i>Inconclusive.</i>	<i>Negative.</i>	<i>Total.</i>
Certified	21	308	329
Grade A (T.T.)	1	4	380	385
Total	1	25	688	714

was found, the infection being shown in both guinea-pigs at the end of the fourth week after inoculation. This sample was taken from the bulked milk of the whole herd, the number of cows in milk at the time of sampling being 22. A special inquiry and report were made by the local investigator, who found that inadequate means were being taken to protect the herd from infection by direct contact with neighbouring infected stock.

SUMMARY:

The fact that only one positive case was found in over 700 tests is sufficient to indicate the high degree of safety in Certified and Grade A (T.T.) milks.

CHAPTER V. HEAT-TREATED MILK

The detailed collection of data in respect of the examination of milk which had been subjected to some form of heat-treatment are relegated to Appendix Table C.

In connexion with heat-treatment, the chief differences of method

and probable differences in effect on bacterial content are with regard to (1) the temperature to which the milk is raised, and (2) the period of time over which the heating process is carried on. The first comparison which falls to be made, therefore, is between samples of milk subjected to heat-treatment by (a) the holding, and (b) the flash methods. These are given in Table 9.

TABLE 9
Comparisons of holding and flash methods.

	<i>Flash.</i>			<i>Holding.</i>		
	<i>Positive.</i>	<i>Total.</i>	<i>Percentage positive.</i>	<i>Positive.</i>	<i>Total.</i>	<i>Percentage positive.</i>
Aberdeen . . .	15	109	13.8	5	232	2.2
Dundee	25	512	4.9
Edinburgh . . .	5	22	22.7	3	311	0.9
Glasgow . . .	4	160	2.5	2	188	1.1
All areas . . .	24	291	8.2	35	1,243	2.8

For the aggregate of cities, the number of infected samples found in 291 tests of milk treated by the flash method is 24, an incidence of 8.2 per cent.; and in those otherwise treated, 35 positive cases in 1,243 samples, an incidence of only 2.8 per cent. The proportion found in the former would, in fact, appear to be scarcely different from the raw milk figure given for the four cities; and inspection of the results in each city shows that this is greatly exceeded in Aberdeen and particularly in Edinburgh¹ (where, however, the percentage is probably unreliable as only 22 such samples were analysed). In Glasgow a much more favourable result than that for Aberdeen is apparent, the positive cases only reaching 2.5 per cent. of the total.

In milk treated by the holding method, local variations are also apparent in the efficacy with which infection is diminished by heat-treatment. In this series the figure found for Dundee is easily the worst, where, of 512 samples, 25 were found positive, a percentage of 4.9. The Glasgow and particularly the Edinburgh samples show a much lower incidence, approximating in each case to 1 per cent., the remaining area, Aberdeen, showing a proportion twice as high, but only half of that recorded in Dundee.²

In comparing the treated milk result with that found for raw milk, it has to be remembered that, without correction in some form, the crude comparison must necessarily be unfair to any judgement of the effects of heat-treatment on the reduction of infection. Specifically, for example, heat-treatment involves bulking to a varying degree, and,

¹ Of the 22 samples of flash-treated milk in this city, 19 were taken from one source, and all of the 5 positive cases arise from this group.

² When a positive result was obtained in heat-treated milk, a special investigation was carried out in Dundee to ascertain that the infecting organism was bovine, and thus to exclude the possibility of contamination from human sources after pasteurization. Of the total (25) positive cases, the dysgonic type of the organism was recovered except in two cases in which the cultures were inadvertently not made and in one in which the growth of the organism was too luxuriant to permit of its being regarded as a typical bovine bacillus and was classified as (?) eugonic.

as shown previously for raw milk statistics, this is accompanied by, and probably is, the direct cause of an increase in the recorded incidence of infection. The capacities of the plant in use in the samples analysed in the present investigation vary from 500 to 2,000 gallons, with an average of 600 gallons. It would appear, therefore, that the incidence rate for raw milk which is more directly comparable with that to be found subsequent to the heating process should be the figure given above for samples taken from tanks, namely 37·5 per cent.

It would appear, therefore, contrary to first impression, that even the flash method of treatment does result in a definite decrease in the proportion of infected samples. The superiority of the holding method is likewise further enhanced by the (presumably) more exact comparison.

Relative merits of various forms of plants. The results in this connexion for the four areas jointly are given in Table 10. In view of the official requirements relating to temperature and time of heating to be observed before milk may be sold as pasteurized milk, it has been deemed advisable to separate the milk samples into two groups, those in which the time and temperature limits (1) were, and (2) were not, observed.

TABLE 10
Results of Different Types of Plant.

	(1)			(2)		
	<i>Positive.</i>	<i>Total.</i>	<i>Percentage positive.</i>	<i>Positive.</i>	<i>Total.</i>	<i>Percentage positive.</i>
A	..	138	..	1	14	7·1
B	8	170	4·7	1	29	3·5
C	..	99
D	..	71
E	21	308	6·8	..	5	..
F	4	217	1·8
G	..	87
H	..	2	7	..
Open cans in boiler	3	..
Sterilized	93	..
Total	33	1,092	3·0	2	151	1·3

Certain significant and disconcerting results emerge from a study of these general figures. It will be observed that the whole of the positive samples in milk pasteurized according to the officially recognized procedure are found in the three types of plants, B, E, and F. In the remaining groups specified it will be observed that, considering the bulking of milk previously mentioned and the infection figure likely to be found (judging from the results quoted in previous chapters) for such quantities of raw milk, the absence of positive cases speaks volumes for the efficacy of the procedure in eliminating tuberculous infection.

In the second half of the table it will be observed that one positive case came from each of the two types of plants, A and B; but reference to Appendix Table C shows that in the former, although

the required temperature of 145° F. was observed, the time of heating fell short of the standard, and that in the latter the temperature was only 130° F. (time of heating omitted). The number of samples included in the second half of Table 10 is insufficient, however, to justify with any pretence of accuracy a judgement as to the differences, if any, between milk treated by methods agreeing and disagreeing with official standards of pasteurization.

The three types of plants from which infected samples were found may be examined now with greater particularity. From the Appendix, wherein the detailed data are presented, it is to be seen that:

1. In plant type B, samples were taken from three different areas, 89 from Aberdeen, 57 from Edinburgh, and 24 from Glasgow, and in *each* of these groups of samples one or more positive cases were found.

2. In plant type E, samples were also taken from three distinct areas, Dundee 208, Edinburgh 93, and Glasgow 7; and the *whole* of the 21 positive cases came from Dundee; and

3. In the case of plant type F, samples were taken in two areas only, Dundee 211 and Glasgow 6; and that the four positive cases came from Dundee.

In the first case the findings are suggestive that it is the type of plant, in the second (especially in view of the time-distribution of infection, explained in the following paragraph), that it is the particular local mechanism or the manner of its operation which is at fault. In the third case specified, the number of samples taken in Glasgow is insufficient to throw any light on the cause.

The case of plant E in Dundee is of further interest. Temporary breakdown or permanent inefficiency of the particular local plant might be the cause—the latter, owing to the relatively large number of positive findings, being the more likely. That this is probably so will be further evidenced by the time-distribution of infection (which is shown below) in the plant from which all the positive cases were obtained:

Plant E. Dundee.

	<i>Positive.</i>	<i>Total.</i>
January	12
February	17
March	21
April . . .	1	13
May . . .	1	18
June . . .	1	24
July . . .	1	12
August . . .	2	14
September . . .	5	16
October . . .	5	20
November . . .	1	20
December . . .	4	20
Total . . .	21	207

The positive cases were all obtained during the year 1931, and the detailed figures by month of sampling will make evident the spread of the cases over the greater part of the year, not, as would happen

with a temporary lapse of the human or mechanical elements involved in the process, a concentration of positive findings with preceding and succeeding periods of freedom from infection.

The results indicate the need for further study of two matters, (1) the relative efficiency of plants of different design, and (2) the administrative control necessary to secure that reliable plants are properly operated.

SUMMARY:

1. The reduction of tuberculous infection by heat-treatment is shown by the comparison of the percentage of infected samples in the two series. For milk treated by the holding method the incidence found is 2·8 per cent. The corresponding figure for untreated creamery bulked milks is 37·5 per cent.

2. The superiority of the holding over flash methods of heat-treatment in reducing tuberculous infection is shown by the two figures 2·8 and 8·2 per cent. respectively.

3. The merits of several forms of plant in this respect differ. There is no evidence to show to what extent, if any, these differences are due to the human factor. The figures for various plants are given in the text.

4. The special investigation carried out in Dundee seems to exclude for the most part the possibility of these results being vitiated by the infection coming from human sources after the heat-treatment has been carried out.

CHAPTER VI. RETAILED MILK

The examination of samples of milk as exhibited for sale to the consumer yielded the following crude results (Table 11). In 1,494

TABLE 11

Retailed Milk. General Results.

	<i>Positive.</i>	<i>Negative.</i>	<i>Inconclusive.</i>	<i>Total.</i>	<i>Percentage positive.</i>
Aberdeen . . .	27	284	14	325	8·31
Dundee . . .	35	393	47	475	7·37
Edinburgh . . .	48	270	17	335	14·33
Glasgow . . .	38	312	9	359	10·58
All areas . . .	148	1,259	87	1,494	9·91

total samples, 148 or 9·91 per cent. were found to be infected. This proportion differs little from the figure previously given for raw milk samples in the aggregate of these four areas. The incidence found in individual cities varies from 7·4 in Dundee to 14·3 per cent. in Edinburgh. Compared with the raw milk findings there is a certain degree of correspondence in the position of the cities relative to one another, with the outstanding exception of Edinburgh, in which, however, as will be seen later, none of these samples were previously subjected to heat-treatment.

In view of the effect of heat-treatment on the proportion of infected

samples, these crude findings require more detailed investigation according to whether or not previous treatment had been given, and, if so, the nature of that treatment. It is obviously difficult to trace the history of many of these samples; but in Table 12 the results of testing are shown for samples subdivided according to previous treatment in as detailed a manner as is possible from the information given on the cards, where the plant used in heat-treatment is not specified.

TABLE 12

Retailed Milk Results according to previous treatment.

	<i>Positive.</i>	<i>Negative.</i>	<i>Inconclusive.</i>	<i>Total.</i>	<i>Percentage positive.</i>
Untreated . . .	90	619	56	765	11.76
Treated:					
Holding (a) . . .	8	140	7	155	5.16
" (b)	20	..	20	..
Flash . . .	15	182	9	206	7.28
Unknown . . .	5	76	5	86	5.81
Treatment unknown	15	114	4	133	11.28
Treatment not stated	15	108	6	129	11.63
Total . . .	148	1,259	87	1,494	9.91

Roughly half of these samples were known to have been subjected to no form of previous heat-treatment. Of these, 90 or 11.76 per cent. were found infected. In 129 instances no remarks were made as to treatment, and in a further 133 the previous history was definitely stated to be unknown. In each of these two groups the proportions, 11.63 and 11.28 per cent. respectively, are roughly equal to the untreated group (to which they probably in great part actually belong). In 467 cases the milk had previously been subjected to some form of heat-treatment; in 175 by the holding method, in 206 by the flash method, and in the remaining 86 cases the method adopted was not known. In 20 of the 175 instances of holder treatment it was definitely known that the official requirements for pasteurization (145° F. for 30 minutes) were not adhered to. (These are classed as group (b) in Table 12.) The proportion of infected samples in the whole group of heat-treated retail samples was 6.00 per cent.; in those by the holding method, excluding (b), 5.16 per cent., including (b) 4.57 per cent.; in the flash-treated group 7.28 per cent., and those heat-treated by methods unknown 5.81 per cent. The figures found here for holder-treated milk are slightly higher than those given in Chapter V for samples taken at the plant, but the difference is insignificant. As has been explained, the records do not specify the plant used in heat-treatment; reference should, however, be made to page 19, where it is shown that the type of plant used plays an important part in the results obtained. Similarly the figure for retailed milk which has been flash-treated is for all practical purposes equal to that found in samples taken at the place of treatment.

It remains now to present the results of examination for each area according to the previous treatment of the milk. These are shown in Table 13.

TABLE 13

	<i>Aberdeen.</i>					<i>Dundee.</i>					<i>Edinburgh.</i>					<i>Glasgow.</i>				
	+	-	<i>I.</i>	<i>Total.</i>	%+	+	-	<i>I.</i>	<i>Total.</i>	%+	+	-	<i>I.</i>	<i>Total.</i>	%+	+	-	<i>I.</i>	<i>Total.</i>	%+
Untreated	7	56	3	66	10.61	28	295	37	360	7.78	31	137	11	179	17.32	24	131	5	160	15.00
Treated:																				
Holding (<i>a</i>)	3	47	..	50	6.00	4	42	3	49	8.16	1	51	4	56	1.79
Holding (<i>b</i>)	20	..	20	..
Flash	13	137	9	159	8.18	2	45	..	47	4.26
Unknown	3	1	4	..	2	49	4	55	3.51	3	24	..	27	11.11
Treatment unknown	4	41	1	46	8.70	..	2	2	4	..	3	31	1	35	8.57	8	40	..	48	16.67
Treatment not stated	1	5	1	7	14.29	14	102	5	121	11.57	..	1	..	1	..
Total	27	284	14	325	8.31	35	393	47	475	7.37	48	270	17	335	14.33	38	312	9	359	10.58

+ = positive.

- = negative.

I. = inconclusive.

%+ = percentage positive.

The outstanding points revealed here are that none of the Edinburgh samples were known to have been treated and that in those to which it was definitely known that no treatment was given the percentage of infected samples is high. If, however, the two groups, treatment unknown and not stated, are assumed to have been untreated, the Edinburgh percentage differs not at all from the corresponding Glasgow proportion. The percentages found in the treated samples vary widely, the main points being the favourable findings in the Glasgow series and the rather heavy incidence in the Aberdeen and Dundee tests (which, however, are relatively few in number).

It should be added that of the total samples of retailed milk examined in each of the four areas, the proportions of treated and untreated milk samples are obviously not representative of the proportions of treated and untreated milk actually retailed. For instance, of the total tested samples in which the previous history of the milk was known, the proportions of untreated samples are in Aberdeen 23·7, in Dundee 77·6, in Edinburgh 100·0, and in Glasgow 51·6 per cent. As will be seen, however, from Table 14 (see page 25), which shows the estimated total amount of milk retailed daily in each city, together with the amount and corresponding proportion which is heat-treated, the proportions of untreated retailed milk to the total amount of retailed milk (excluding certified and graded milk) are in Aberdeen 22·2, in Dundee 45·3, in Edinburgh 38·3, and in Glasgow 15·4 per cent. With the exception of the figure for Aberdeen, these differ grossly from the proportions of samples of untreated retailed milk examined in the present inquiry. Obviously these differences will affect any conclusion as to the actual proportion of infected samples of milk as sold to the public, and the crude figures in this section do not give an accurate picture of the extent of infection of retailed milk in the cities. An approximate estimate can, however, be made on the basis of these proportions and the previous findings for raw and heat-treated milk by weighting these figures according to the proportions treated and untreated. For example, in Glasgow the infection in raw milk samples was 13·4 per cent.; in milk heat-treated by the holding method 1·1 per cent., and in that treated by the flash method 2·5 per cent. Weighting the figures supplied by the Medical Officer of Health by these percentages, the proportion of infected samples would become 3·21 per cent., contrasting with the figure given in Table 13 of 10·58 per cent. The necessity for some kind of correction is obvious. Unfortunately, however, any correction for other areas must be much more crude in that (1) apart from Glasgow we know only the proportions treated and untreated, but cannot specify of the treated milk how much is treated by the holding and how much by the flash process, and (2) in the figures given for individual cities for heat-treated milk—Table 13—the proportion of these tests carried out on samples treated by the holding and flash methods may not be numerically representative of average conditions. This is evidently true for Glasgow where in the present survey 160 tests were carried out on milk treated by the flash and 188 on milk

TABLE 14

Statement showing for the four large cities in Scotland the proportions of Retailed Milk which are untreated and treated by heat.

Retailled Milk.

(Estimated daily consumption in gallons)

	<i>Untreated.</i>				<i>Treated.</i>				<i>Estimated totals. Gallons daily.</i>
	<i>Certified.</i>	<i>Grade A (T.T.)</i>	<i>Grade A.</i>	<i>Ordinary.</i>	<i>Holder.</i>	<i>Flash.</i>	<i>Sterilized.</i>	<i>Not stated.</i>	
Aberdeen		312½ (2·5%)	..	2,706 (21·5%)	9,499½ (76%)	12,518
Dundee		140 (1·5%)	..	4,100 (44·5%)	360 (4%)	4,600 (50%)	9,200
Edinburgh	290 (1%)	742 (3%)	81	9,322 (37%)	15,011 (59%)	25,446
Glasgow	10,000 (15%)	45,000 (70%)	10,000 (15%)	65,000

treated by the holding method, whereas actually in the milk as retailed which has been treated the flash and holding methods are in the ratio of 10:45. As a tentative correction, however, we may take the appropriate raw milk figure for each area and the corresponding figure for treated milk (irrespective of method of heat-treatment), and weight these figures by the proportions of such milk actually retailed. The estimated proportions of infected samples in retailed milk are: Aberdeen 6·79, Dundee 5·43, Edinburgh 5·92, and Glasgow 3·50 per cent. positives. The general figure for the four cities together based on the raw milk finding of 10·0 per cent. positive, on the treated of 3·8 per cent. positive, and the proportion of untreated retailed milk as 23·6 per cent. of the total and of treated as 76·4 per cent. is estimated as 5·26 per cent. compared with the crude figure of 9·91 per cent. It will be apparent that the estimates given above are open to objection because of the fact that the treated milk figures for each area may be unreliable. If we take the general figures for the proportion of infected samples in raw and treated milks as 10·0 and 3·8 per cent. (i.e. the figures for the four cities combined), and weight these by the several areal proportions of treated and untreated milk as retailed, we obtain these estimated figures of infection: Aberdeen 5·15, Dundee 6·41, Edinburgh 6·14, and Glasgow 4·74 per cent. It will be noted that the order of the four cities does not correspond to that found above, and it is unfortunate that the retailed samples tested were not more random in nature, or that there was not greater detail available to indicate more clearly the nature of treatment of the milk in the figures available. It is clear, however, that the proportion of infected samples in milk as sold to the public is very much less than would appear from Table 13 and is probably little, if at all, in excess of 5 per cent. For the city of Glasgow, where there is more detail available, the estimate found is only 3·2 per cent., since such a large proportion of the milk is effectively heat-treated before being retailed to the public.

SUMMARY:

1. The general figures for retailed milk samples do not differ widely from the raw milk results previously set forth. The general rate of infection of samples taken in the four cities together is roughly 10 per cent.
2. As the numbers of samples taken were not representative of the proportions of untreated and treated milks actually retailed, an attempt has been made to correct for this factor, and it is estimated that the general rate of infection of the total quantity of milk retailed in the four cities is considerably lower than 10 per cent. and probably little more than 5 per cent.
3. The proportion of infected samples is lower in the heat-treated than in the untreated milks.
4. The local variations are given, but these are influenced by the differences in the proportions treated and untreated in the several areas.

CHAPTER VII. USE OF SECOND GUINEA-PIG IN TESTS

Proportion of positive cases in which infection is only found in the second guinea-pig. The method of testing outlined in Chapter II permits of an estimate being made of the number of positive cases which would have escaped detection if only one guinea-pig had been used and if the period of observation had been four instead of eight weeks. For this purpose the positive tests have been divided into three groups:

- (a) those in which the second guinea-pig alone was infected, the first guinea-pig surviving till the 28th day but being free from infection on autopsy;
- (b) those in which infection is only found in the second animal, but in which the first died (of some condition other than tuberculosis), before the expiry of the required 28 days' period; and
- (c) those in which infection was discovered in both pigs.

Table 15 shows the positive tests for each of the main categories of milk subdivided in this manner. It will be observed that of the total positive cases 43 are assigned to class *b*. Of the remaining 391 cases 141, or 36·1 per cent., are class *a*. In other words, over a third of the positive cases were discovered only in the second guinea-pig.

TABLE 15

Number and percentage of the positive cases in which only the second guinea-pig is infected.

	<i>a.</i>	<i>b.</i>	<i>c.</i>	<i>Total.</i>	<i>a expressed as percentage of a + c.</i>
Raw (in Churns)	44	14	95	153	31·7
Raw (in Tanks)	4	14	56	74	6·7
Heat-treated (flash)	13	2	9	24	59·1
„ (holding)	22	..	13	35	62·8
Retailed (untreated)	33	10	47	90	41·3
„ (heat-treated)	15	1	12	28	55·5
„ (unknown or not stated)	10	2	18	30	35·7
Total	141	43	250	434	36·1

If the figures for the various categories of milk are compared, it will be seen that of the total positive cases the proportion revealed only in the second guinea-pig is substantially greater in heat-treated than in raw milk in churns. In the retailed milk groups the difference between the proportions for heat-treated milk and untreated milk is not, however, significant. There is no great difference between the results in the two methods of heat-treatment in this respect. It is obvious that the under-estimate of the incidence of infection by a technique involving a shorter period of observation of the inoculated animals will be very much more serious in the case of heat-treated milk than in that of raw, and will consequently tend to magnify the benefits likely to accrue (relative to tuberculous infection) by heat-treatment. For example, if the incidence of tubercle is compared in

raw and treated milk, the relative differences are grossly magnified by measuring incidence from results of single guinea-pig inoculation, the period of observation being only four weeks. Thus: With two guinea-pigs and an eight weeks' period, the incidence in raw milk in churns was 10.0, in holder treated 2.8, i.e. the incidence is reduced to 29 per cent. of the original raw milk value; with one guinea-pig and four weeks' period, the respective figures are 6.2 and 1.04 per cent., i.e. the incidence in treated milk is reduced to 17 per cent. of the original value. Or, if we use for more accurate comparative purposes the figures of raw milk obtained from milk in tanks, the relative differences are these:

2 guinea-pigs 8 weeks; raw 35.7%, treated 2.8%; reduction to 7.8% of original.
 1 „ 4 „ „ 27.0%, „ 1.04%; „ 3.9% „

The positive tests for raw milk in churns have been further subdivided and Table 16 shows the numbers in the three groups in each area. It will be observed that of the total positive cases 14 are assigned to class *b*. Of the remaining 139 cases 44, or 31.7 per cent., are class *a*. This proportion varies rather widely in the individual cities, from 60.0 per cent. in Aberdeen to 17.4 per cent. in Glasgow.

TABLE 16.

Raw Milk in Churns. Number and percentage of the positive cases in which only the second guinea-pig is infected.

	<i>a.</i>	<i>b.</i>	<i>c.</i>	Total.	<i>a</i> expressed as percentage of <i>a</i> + <i>c.</i>
Aberdeen . .	18	3	12	33	60.0
Dundee . .	10	3	20	33	33.3
Edinburgh . .	8	6	25	39	24.2
Glasgow . .	8	2	38	48	17.4
All areas . .	44	14	95	153	31.7

The findings recorded in the above Table have a bearing and an obvious importance in any discussions of the recorded incidence of tuberculous infection of the milk supply of an area. They demonstrate clearly how wide are the differences in the apparent incidence of infection which are likely to arise solely from differences in the technique which is adopted for demonstrating the presence of *B. tuberculosis*. For example, Table 17 shows that the position of the

TABLE 17

Raw Milk in Churns. Contrast of incidence with different techniques.

	4 weeks; 1 guinea-pig.	8 weeks; 2 guinea-pigs.
Aberdeen . .	3.6	9.9
Dundee . .	3.9	6.5
Edinburgh . .	7.4	11.6
Glasgow . .	10.6	13.4
All areas . .	6.2	10

four cities relative to the frequency of infected raw milk samples is altered appreciably if the rates are calculated on the positive cases discovered by adopting a four weeks' period and one guinea-pig instead of the more extended procedure actually used.

With the more trustworthy procedure the position of the cities from lowest to highest is Dundee, Aberdeen, Edinburgh, and Glasgow, whereas with the less rigid, the order becomes Aberdeen, Dundee, Edinburgh, and Glasgow. More particularly, however, the relative magnitude of the differences is obviously also entirely altered, by the less rigid technique the rate in Glasgow, for example, being 2.9 times, by the more trustworthy only 1.35 times as high as that found in Aberdeen. There is, therefore, in this series, at least, an approximation of the incidence rates in the individual cities when the more advisable laboratory procedure is adopted. The even greater discrepancies that would arise if one area uses a 4 weeks', another an 8 weeks', test period can also be gauged from the statistics collected in Table 17.

In certain areas, for routine public health purposes, the procedure has been to use only one guinea-pig which, after inoculation, was retained for only 3-4 weeks. This procedure has its advantages for a local authority looking for the source of infection of milk, as there is less likelihood of a cow having been disposed of from a herd 4 weeks after the sample was taken than 8 weeks after. But the fact that, of the positive cases revealed in this inquiry roughly one-third have been discovered only in the second guinea-pig is evidence sufficient (*a*) to show that the real infection rate may be grossly understated by the short method of search for the organism, and (*b*) to invalidate completely any geographical comparison of the incidence of infection so long as such wide discrepancies in the technique adopted as are known in this country are operative. At the same time it affords strong support for the general adoption of uniform technical methods in this and other common laboratory procedures.

SUMMARY:

1. Over one-third of the positive cases would have been missed by a technique in which only one guinea-pig kept for a period of four weeks was used.
2. No accurate comparison between local incidences of infection can be made unless a common procedure has been used.

GENERAL SUMMARY

The principal results of this collaborate investigation may briefly be summarized as follows:

1. The extent of infection of samples of raw milk in churns, ascertained by the technique outlined, is in the aggregate of the four principal Scottish cities 10 per cent.
2. As would be anticipated, the degree of infection increases with the amount of bulking to which the milk is subjected; and in a series of over 200 samples of creamery bulked milks, wherein the average

volume of milk from which the samples were taken reaches 500 gallons, more than one-third of the samples were found infected with tubercle bacilli.

3. A marked reduction of infection results from heat-treatment. The superiority of the holding over the flash methods has been elicited, and the relative efficiency of different plants examined.

4. It is estimated that the general rate of infection of milk retailed in the four cities is a little above 5 per cent.

5. The examination of a large number of samples of Certified and Grade A (T.T.) milks has shown the safety of these in respect of tuberculous infection.

6. The necessity for a standard method of examination if comparisons between different areas are to be made has been clearly demonstrated.

APPENDIX TABLE A

Raw Milk in Churns. Infections in relation to number of churns from which sample is taken.

Number of churns.	Aberdeen.				Dundee.				Edinburgh.				Glasgow.				All areas.			
	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.
1	1	34	..	35	4	82	12	98	1	18	2	21	3	23	..	26	9	157	14	180
2	8	71	2	81	3	73	12	88	3	42	1	46	4	31	4	39	18	217	19	254
3	4	50	3	57	4	50	11	65	7	43	2	52	5	47	2	54	20	190	18	228
4	3	54	2	59	4	69	7	80	4	61	6	71	9	59	2	70	20	243	17	280
5	5	39	..	44	9	57	6	72	13	38	3	54	3	38	1	42	30	172	10	212
6	3	19	..	22	1	24	4	29	6	31	2	39	7	30	..	37	17	104	6	127
7	2	10	..	12	2	14	4	20	..	17	..	17	6	19	3	28	10	60	7	77
8	3	5	..	8	2	21	1	24	4	15	..	19	4	15	2	21	13	56	3	72
9	2	10	..	12	1	9	1	11	..	7	..	7	..	7	..	7	3	33	1	37
10	1	1	1	6	2	9	..	5	..	5	1	9	..	10	3	20	2	25
11	1	1	..	2	..	2	..	2	..	1	..	1	1	4	..	5	2	8	..	10
12	..	1	..	1	1	1	..	2	1	1	1	2	1	4
13					1	1	..	2	1	1	2	1	..	3
14					..	1	..	1	..	1	..	1	2	..	2
15				
16					1	1	2	..	1	1	2	..	1	1	2
17				
18					1	1	1	1	1	1
55					3	..	1	4	3	..	1	4	3	..	1	4
N.S.					1	4	..	5	..	1	..	1	..	9	1	10	1	14	1	16
Total	33	294	7	334	33	413	60	506	39	281	16	336	48	292	18	358	153	1,280	101	1,534

+ = positive.

- = negative.

I. = inconclusive.

APPENDIX TABLE B

Raw Milk in Churns. Infections in relation to volume of milk from which sample is taken.

Volume of milk (gallons).	Aberdeen.				Dundee.				Edinburgh.				Glasgow.				All areas.			
	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.
-5	..	6	..	6	..	11	1	12	..	9	..	9	..	4	..	4	..	30	1	31
5-	..	28	..	28	2	26	5	33	1	14	2	17	2	6	1	9	5	74	8	87
10-	1	32	1	34	2	37	6	45	..	15	..	15	1	21	1	23	4	105	8	117
15-	4	28	..	32	5	26	7	38	3	19	1	23	4	16	..	20	16	89	8	113
20-	8	38	3	49	1	54	10	65	4	19	5	28	2	22	2	26	15	133	20	168
25-	..	24	1	25	1	12	5	18	3	25	..	28	4	14	2	20	8	75	8	91
30-	1	30	1	32	4	51	6	61	4	32	1	37	4	41	..	45	13	154	8	175
35-	1	17	..	18	3	29	3	35	1	24	..	25	4	24	..	28	9	94	3	106
40-	5	30	1	36	1	36	3	40	2	29	3	34	1	28	1	30	9	123	8	140
45-	1	12	..	13	..	21	3	24	7	15	1	23	1	28	1	30	9	76	5	90
50-	2	16	..	18	4	26	1	31	5	18	2	25	1	13	2	16	12	73	5	90
55-	2	6	..	8	1	14	1	16	1	10	..	11	2	12	1	15	6	42	2	50
60-	1	8	..	9	2	16	..	18	3	12	1	16	4	12	2	18	10	48	3	61
65-	1	2	..	3	..	10	2	12	1	8	..	9	3	10	..	13	5	30	2	37
70-	1	3	..	4	1	5	3	9	1	9	..	10	4	12	1	17	7	29	4	40
75-	1	2	..	3	..	8	..	8	1	7	..	8	2	3	..	5	4	20	..	24
80-	..	4	..	4	3	8	..	11	1	4	..	5	2	6	..	8	6	22	..	28
85-	1	3	..	4	1	4	..	5	..	1	..	1	1	3	..	4	3	11	..	14
90-	1	3	..	4	..	3	1	4	..	2	..	2	1	2	..	3	2	10	1	13
95-	1	1	1	1	..	2	..	2	..	2	1	2	..	3	3	5	..	8
100-	1	1	..	2	..	3	1	4	1	4	1	6
105-	2	..	2	2	..	2
110-	..	1	..	1	..	4	..	4	..	2	..	2	..	3	..	3	..	10	..	10
115-	1	..	1	1	..	1
120-	1	1	2	..	2	..	2	2	2	..	3	3	6
125-	1	..	1	..	2	..	2	3	..	3
130-	1	1	..	1	..	1	1	1	2
135-
140-	1	..	1	1	..	1
145-	1	1	1	1
150-	1	2	..	3	1	2	..	3
155-	1	..	1	..	1	..	1
160-
165-	1	1	1	1	1
..
825-	3	..	1	4	3	..	1	..	4
N.S.	2	..	2	7	1	8	..	9	1	10
Total	33	294	7	334	33	413	60	506	39	281	16	336	48	292	18	358	153	1,280	101	1,534

+ = positive. - = negative. I. = inconclusive.

APPENDIX TABLE C

Heat-treated milk.

Method of Heat-treatment.	Aberdeen.				Dundee.				Edinburgh.				Glasgow.				All areas.			
	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.	+	-	I.	Total.
A	..	114	..	114									..	23	1	24	..	137	1	138
A (1)													..	1	1	2	..	1	1	2
A (2)													1	10	1	12	1	10	1	12
B	4	82	3	89					3	52	2	57	1	23	..	24	8	157	5	170
B (1)	1	28	..	29													1	28	..	29
C									..	88	2	90	..	9	..	9	..	97	2	99
D									..	66	5	71					..	66	5	71
E					21	167	20	208	..	89	4	93	..	6	1	7	21	262	25	308
E (1)													..	1	..	1	..	1	..	1
E (2)													..	1	..	1	..	1	..	1
E (3)													..	3	..	3	..	3	..	3
F					4	187	20	211					..	6	..	6	4	193	20	217
G													..	85	2	87	..	85	2	87
H													..	2	..	2	..	2	..	2
H (1)													..	3	..	3	..	3	..	3
H (2)													..	3	..	3	..	3	..	3
H (3)													1	1	1	1
Open cans immersed in boiler													..	1	..	1	..	1	..	1
(1)													..	1	..	1	..	1	..	1
(2)													..	1	..	1	..	1	..	1
(3)													..	1	..	1	..	1	..	1
Sterilized					..	86	7	93									..	86	7	93
Flash treated	15	93	1	109					5	17	..	22	4	150	6	160	24	260	7	291
Total	20	317	4	341	25	440	47	512	8	312	13	333	6	329	13	348	59	1,398	77	1,534

A (1) Temp. 145; time 13 mins.

.. (2) .. 145; .. 20 ..

B (1) .. 130; .. not stated

H (1) .. 144; .. 30 mins.

.. (2) .. 145; .. 15 ..

.. (3) .. 150; .. 10 ..

E (1) Temp. 140; time 30 mins.

.. (2) .. 143; .. 30 ..

.. (3) .. 145; .. 20 ..

Open cans (1) .. 150; .. 20 ..

.. (2) .. 150; .. 30 ..

.. (3) Temp. and time not stated

+ = positive.

- = negative.

I. = inconclusive.

APPENDIX TABLE D

Dates of death of guinea-pigs in tests classified as inconclusive.

	-7	7-	14-	21-	28-	35-	42-	49-	Total.
<i>Raw Milk in Churns.</i>									
Aberdeen	1	2	..	1	3	..	7
Dundee	2	6	8	21	12	9	2	60
Edinburgh . . .	1	4	..	2	..	3	4	2	16
Glasgow	3	..	1	3	3	4	4	18
All areas . . .	1	9	7	13	24	19	20	8	101
<i>Retailed Milk.</i>									
Aberdeen	2	1	4	2	2	3	14
Dundee	3	5	9	9	7	13	1	47
Edinburgh	5	2	3	2	3	2	17
Glasgow	4	2	3	..	9
All areas	3	12	12	20	13	21	6	87
<i>Heat-treated Milk.</i>									
Aberdeen	2	1	1	..	4
Dundee . . .	2	2	4	6	8	10	12	3	47
Edinburgh	1	2	1	1	3	2	3	13
Glasgow	1	..	2	2	6	2	13
All areas . . .	2	3	7	7	13	16	21	8	77
Certified . . .	1	..	1	..	6	5	8	..	21
Grade A (T.T.)	1	2	1	..	4
Total . . .	1	..	1	..	7	7	9	..	25
Raw Milk in Tanks . . .	1	2	2	2	..	1	8

**INQUIRY INTO THE TUBERCULOUS INFECTION OF THE
MILK SUPPLY OF SCOTLAND**

GROUP B. HEAT-TREATED MILK

Centre.....

Sample Number.....

Name and Address of
Pasteurizing Plant:

Whether sampled from (a) Cooler, (b) Churn, (c) Bottle. Date
(Delete what does not apply)

Details of Process.

- (i) Type of Pasteurizer
- (ii) Capacity of Pasteurizer
- (iii) Temperature and Time of Heating
- (iv) Method of Temperature Control
- (v) Were temperatures checked by Sampling Officer?

Remarks:

Signature of Sampling Officer.....

**RESULT OF BIOLOGICAL TEST Positive
Negative**

DETAILS OF BIOLOGICAL TEST

Guinea-pig No.	Guinea-pig No.
Date of inoculation	Date of inoculation
Date of autopsy: died	Date of autopsy: died
killed	killed
<u>Number of days after inoculation</u>	Number of days after inoculation.....

INQUIRY INTO THE TUBERCULOUS INFECTION OF THE MILK SUPPLY OF SCOTLAND

GROUP C. RETAILED MILK

Centre.....

Sample Number.....

Name and Address of Retailer:

Whether sampled from (a) Churn, (b) Bottle. Date (Delete what does not apply)

Previous treatment, if any (If unknown, say so)

Remarks:

Signature of Sampling Officer.....

RESULT OF BIOLOGICAL TEST Positive Negative

DETAILS OF BIOLOGICAL TEST

Guinea-pig No. Date of inoculation Date of autopsy: died killed Number of days after inoculation

Guinea-pig No. Date of inoculation Date of autopsy: died killed Number of days after inoculation

AUTOPSY

Typical tuberculous lesions?

AUTOPSY

Typical tuberculous lesions?

DISTRIBUTION:

Local? Lymphatic Glands (regional? abdominal? thoracic?) Spleen? Liver? Lungs? Other areas? Acid-fast bacilli demonstrated in

DISTRIBUTION:

Local? Lymphatic Glands (regional? abdominal? thoracic?) Spleen? Liver? Lungs? Other areas? Acid-fast bacilli demonstrated in

DOUBTFUL LESIONS:

Features Results of culture Guinea-pig inoculation

DOUBTFUL LESIONS:

Features Results of culture Guinea-pig inoculation

DIED OF INTERCURRENT INFECTION:

Features and nature of condition

DIED OF INTERCURRENT INFECTION:

Features and nature of condition

Signature of Bacteriologist.....

INQUIRY INTO THE TUBERCULOUS INFECTION OF THE MILK SUPPLY OF SCOTLAND

GROUP D. GRADED MILK

Centre.....

Sample Number.....

Name and Address of
Producer:

Name and Address of
Retailer:

Where sampled? Date

Bulk from which } (a) Total herd, (b) Single churn, (c) Bottle.
sample is taken } (Delete what does not apply)

For farm samples, state number of cows in milk

Remarks:

Signature of Sampling Officer.....

RESULT OF BIOLOGICAL TEST Positive Negative

DETAILS OF BIOLOGICAL TEST

Guinea-pig No.	Guinea-pig No.
Date of inoculation	Date of inoculation
Date of autopsy: died	Date of autopsy: died
killed	killed
Number of days after inoculation	Number of days after inoculation

AUTOPSY

Typical tuberculous lesions?

DISTRIBUTION:

Local? Lymphatic Glands { regional?
 { abdominal?
 { thoracic?

Spleen? Liver? Lungs?

Other areas?

Acid-fast bacilli
demonstrated in

DOUBTFUL LESIONS:

Features

Results of culture

Guinea-pig inoculation

DIED OF INTERCURRENT INFECTION:

Features and nature of condition

AUTOPSY

Typical tuberculous lesions?

DISTRIBUTION:

Local? Lymphatic Glands { regional?
 { abdominal?
 { thoracic?

Spleen? Liver? Lungs?

Other areas?

Acid-fast bacilli
demonstrated in

DOUBTFUL LESIONS:

Features

Results of culture

Guinea-pig inoculation

DIED OF INTERCURRENT INFECTION:

Features and nature of condition

Signature of Bacteriologist.....

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