THE USE OF THE MILK RING TEST IN A SURVEY OF THE INCIDENCE OF BOVINE BRUCELLOSIS IN SOUTHERN SCOTLAND

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Since the end of the late war considerable attention has been paid in many countries to the problem of human and animal brucellosis. An expert panel has been set up jointly by the Food and Agricultural Organization and the World Health Organization to consider many aspects of the disease—such as the economic and public health significance, diagnosis, treatment and control (*W.H.O. Tech. Rep. Series*, no. 37, 1951, no. 67, 1953).

An interesting development in these post-war years has been the application of the 'Ring-' or 'Abortus-Bang Ring-Test' ('A.B.R. Test'), now called the 'Milk Ring Test'. This is essentially a rapid agglutination test carried out on whole milk or cream using a suitably stained antigen. The nature of the test is briefly discussed by Bruhn (1948), and in great detail by De Moulin (1951). De Moulin suggests that the ring test does not depend upon the sifting action of rising cream (Schern-Gorlisch Reaction) as stated by Bruhn, but believes that any brucella agglutinins present in milk are adsorbed on to the fat globules. In a positive reaction, the ring is formed by the rising to the surface and settling out of the fat globule-agglutinin-stained brucella antigen complex. The ring test was introduced in 1937 by Fleischhauer (1937, 1938), and its evolution between 1937 and 1944 is described by Bruhn (1948) in a paper from Denmark which indicates that using a suitably prepared antigen the *Brucella abortus* ring test provides a quick, easy and reliable method for diagnosing brucellosis in dairy herds.

Workers in other countries (Norell & Olson, 1943; Bruhn, 1948; Roepke, Clausen & Walsh, 1949; Roepke, Paterson, Driver, Clausen, Olson & Wentworth, 1950; Drimmelen, 1951) have reported on the uses and limitations of the ring test as a means of detecting brucella infection of herds, some countries having adopted it as a screening test in eradication and control schemes. As there were no references to the use of the ring test in this country, and in view of its apparent value, it was decided to undertake a study the objects of which were, first, to examine the relationship of ring-test reactions to blood serum titres and to actual infectivity of milk; secondly, using the ring-test reaction as a criterion, to investigate the extent of brucellosis in dairy herds in southern Scotland; and thirdly, to study the incidence of viable *Br. abortus* in raw milks brought into the City of Edinburgh and County of Midlothian.

METHODS AND MATERIALS

Materials available for examination:

(1) For the purpose of simultaneous blood and milk sampling, material was available from three sources: (*a*) from two large dairy herds with a known history; (*b*) from a small dairy herd with an incomplete history; and (*c*) from slaughterhouse material, i.e. casualty and cast cows with an unknown history as regards brucella infection or vaccination.

(2) Samples were taken from each can of producer milk being received at the main dairies in Edinburgh; these samples were subjected to the ring test only.

(3) Samples of raw milk taken by the sanitary authorities in Edinburgh and Midlothian were examined by means of the ring test. These samples were also examined by guinea-pig inoculation in the Bacteriological Department, Edinburgh University, in the course of routine examination for tuberculous milk; when the guinea-pigs were killed serum was obtained for the agglutination test. In many cases the spleens were also available for cultural examination.

Methods

The ring test was carried out by mixing 0·05 ml. of suitably stained brucella antigen with 1·0 ml. of well-mixed milk contained in a narrow test-tube. The tubes used were of a size to contain 1 ml. of milk in a column 2 cm. high. The test was read after incubating at 37° C. for 40–60 min. Assuming the milk to be normal in composition and physical state the following reactions may be recorded (Ministry of Agriculture and Fisheries Instructions issued with ring-test antigen):

− The cream layer which has settled out is white or nearly so and the column of milk below is deeply coloured according to the colour of the antigen used.

± The cream layer is the same colour or only slightly more coloured than the milk column.

+ The cream layer is distinctly coloured forming a true ring but the milk column remains definitely coloured too.

+ + The cream layer is deeply coloured, the milk column remaining slightly coloured.

+ + + The cream layer is deeply coloured and lies on top of a completely white milk coloum.

The following interpretation is used throughout this paper:

+ + or + + +, positive, indicating the presence in the milk of agglutinins to brucella at high concentrations. +, doubtful, or weak positive, indicating the presence of agglutinins in low concentration only; ± or −, negative, indicating that no brucella agglutinins are present in the milk.

Antigens used. There are two types of antigen in common use—the haematoxylin stained blue antigen and the vitally stained tetrazolium or red antigen (Jepsen & Bruhn, 1951; Wood, 1948; Bendtsen, 1950).

For the sake of consistency the antigen used throughout has been that prepared and supplied by the Veterinary Laboratory of the Ministry of Agriculture and Fisheries. In the preliminary stages, however, and on various occasions since, the

Ministry's antigen has been compared with antigens prepared by Dr Bruhn (haematoxylin), Dr Bendtsen (tetrazolium) and the author (haematoxylin). These comparisons showed the various antigens to be alike in specificity and sensitivity.

In examination of cow and guinea-pig blood serum the Weybridge agglutination test (method I) was used. When guinea-pig spleens were available smears were made and stained by the modified Ziehl-Neelsen method, and cultures were made aerobically and in 10% CO_2 on 5% serum glucose agar containing crystal violet and malachite green. Colonies appearing and resembling those of *Br. abortus* were checked by staining smears by the modified Ziehl-Neelsen method and by slide-agglutination tests with a positive serum.

RESULTS

I. *Factors affecting the ring test*

At the outset certain factors that might be expected to affect the ring test were studied. In the preliminary stages 1164 quarter samples from both infected and non-infected cows were examined to determine whether or not there was variation in ring-test reaction from quarter to quarter; it was established that there was no such variation and thereafter composite milk samples were used throughout the survey.

Table 1. *Effect of stage of lactation on the ring test in brucella-free cows*

Length of time calved (weeks)	Ring test reaction: −	±	+	++ or +++	Total
0–4	6	.	.	.	6
5–20	40	7	.	.	47
21–36	34	9	.	.	43
37–61	11	4	.	.	15
Total no. of cows	91	20	.	.	111

(*a*) *Stage of lactation.* The results in Table 1 show that when applied to the milk of cows whose serum is negative to the agglutination test, the ring test is not seriously affected by the stage of lactation. Further examination of these samples showed also that slight alterations in milk character, as determined by the modified Whiteside test (Murphy and Hanson, 1941), caused no serious interference with the ring-test reaction. This test detects physical changes in milk due to the presence of leucocytes and their products. It is carried out by mixing small volumes of milk and caustic soda on a glass plate and reading immediately. Abnormal milks become viscid or flaky whilst normal milk shows no change. These results also show that in spite of the differences in stage of lactation, and in spite of the presence of a certain amount of mild mastitis, the ring test gave promising results in that it correlated with the blood-test findings (Table 2).

(*b*) *Method of storing milk samples.* The method of storing milk samples was

found to have a more serious effect upon the specificity of ring-test reaction than the factors discussed above.

The use of 0·1 % $HgCl_2$ as a milk preservative has been described (Moore, 1951) and this method of treating the sample was compared with storage at room temperature and in the refrigerator.

It was found that none of these methods was satisfactory if the sample was kept more than 3 days; and that in the case of $HgCl_2$ preservation inconsistent results were obtained even on the second day. In view of the fact that chemically preserved samples were useless for bacteriological examination the use of chemicals was discontinued. In this survey, therefore, all samples were tested in a fresh state or within 36 hr.; soured samples were not used.

Table 2. *Effect of milk changes on ring test reaction*

	Ring test reaction			
	Negative		Positive	
Whiteside number	Neg., ±, or 1+	2+, 3+, or 4+	Neg., ±, 1+	2+, 3+, 4+
Serum negative	65	20	Nil	Nil
Serum positive	Nil	Nil	9	5

Table 3. *Effect on the ring tests of diluting positive milk*

Cow no.	Undiluted sample	Dilution 1/5	1/10	1/20	1/40	1/80	1/160	1/320
1	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +
2*	+ + +	+ + +	+ + +	+ +	+	–	–	–
3	–	–	–	–	–	–	–	–
4	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
5*	+ + +	+ + +	+ +	+ +	–	–	–	–
6	–	–	–	–	–	–	–	–

* Cows no. 2 and 5 had been vaccinated with strain 19, 7 months previously.

(c) *Dilution of positive milk.* The effect of diluting ring test positive milk with negative milk was investigated. Dilutions of various milk samples in the milk of a cow with a negative serum gave the reactions shown in Table 3. These results show that a ring test positive milk will continue to give a positive reaction at high dilutions confirming the reports of Norell & Olson (1943) and Drimmelen (1949, 1951).

As much of the material available for this survey came from slaughterhouse cases, the effect of diluting abnormal secretions in normal creamy negative milk was also investigated. The results, shown in Table 4, confirm the findings of Holm, Eveleth & Rheault (1950), that mastitis secretions and the secretion obtainable from dry cows do not interfere with the ring test when a dilution technique is used. In these fifty samples it will be seen that only in two cases was there disagreement between the agglutination test and the ring test using the dilution technique in the latter.

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Table 4. *Effect of 'dry secretion' and mastitis secretion on the ring test*

	No. of samples	Ring-test reaction: Undiluted sample — Positive	Undiluted sample — Negative	Diluted 1/10 with negative milk — Positive	Diluted 1/10 with negative milk — Negative
Serum negative	35	.	21	1	20
		14	.	.	14
Serum positive	15	5	.	5	.
		.	10	9	1
	50	19	31	15	35

II. *Application of the ring test*

(a) *Herd tests*

In order to assess the value of the test, advantage was taken at an early stage of an opportunity to apply it to two 'clean' herds with a known history.

These herds were self-contained pedigree attested dairy herds with a history of freedom from clinical brucellosis and records of clear blood tests during the past 5 years. The first ring testing was carried out in 1951 in conjunction with blood testing. Both herds were retested in 1953. No positive reactors either to the ring test or to the blood serum agglutination test were disclosed on either occasion. The results are shown in Table 5.

Table 5. *Ring testing of herds* 1 *and* 2, 1951–53

		Serum agglutination test	Ring-test reaction: Negative	Doubtful	Total
Herd 1	1951	Negative	58	4	62
	1953	Negative	58	1	59
Herd 2	1951	Negative	50	4	54
		Doubtful	Nil	1	1
	1953	Negative	66	Nil	66

Table 6. *Ring testing of herd* 3, 1951

Agglutination test	No. of cows	Ring test: Positive	Negative
Negative	13	1*	12
Positive	7	7	Nil
Doubtful	4	1	3
Total	24	9	15

* This cow later became blood-positive.

The ring test was further examined by applying it to a herd which was not self-contained and which had a vague history of infection followed by strain 19 vaccination. The results of this testing in 1951 are shown in Table 6.

In 1953, after considerable culling and introduction of new stock had taken place, the results shown in Table 7 were obtained.

Table 7. *Ring testing of herd* 3, 1953

Agglutination test	No. of cows	Ring test	
		Positive	Negative
Negative	19	Nil	19
Positive	2	2	Nil
Doubtful	4	Nil	4
Total	25	2	23

(*b*) *Abnormal milks—use of dilution technique*

The examination of slaughterhouse material was made difficult by the fact that the udder secretion was often abnormal due to mastitis or to 'drying-off'; in such cases in view of the results in Table 4, the secretion was diluted to give a 1/10 dilution in normal creamy milk which was itself negative to the ring test and was taken from cows negative to the serum agglutination test. The results obtained are shown in Table 8.

Table 8. *Ring testing of slaughterhouse material*

Agglutination test	No. of cows	Ring-test reaction*		
		Positive	Doubtful	Negative
Negative	108	2	1	105
Positive	27	23	0	4
Doubtful	12	3	1	8
Total	147	28	2	117

* Abnormal udder secretions were diluted to give a 1/10 dilution in normal creamy negative milk.

(*c*) *Testing of bulk (herd) milks*

Having confirmed the efficacy of the ring-test reactions in individual animals it was decided to apply the test to bulk milks, on producers' samples received in cans at the larger dairies in Edinburgh and on samples of raw milk taken at random by the sanitary authorities.

The ring test was applied to the greater part of the raw milk coming into Edinburgh over the period 1952–3 by sampling the individual cans of each producer; this involved the sampling of some 2200 cans as they arrived in lorries from the country. The results are, however, expressed in Table 9 in terms of the ring-test reaction of the bulked supply from each producer; the table also includes the samples taken by the sanitary authorities.

Table 9. *Ring testing of bulked milk samples*

Source of sample	No. of samples	% positive to ring test
Dairy no. 1	112	49·1
Dairy no. 2	126	45·2
Dairy no. 3	132	63·6
Sanitary authority	256	63·6
Total	626	57·34

If the individual can tests on producer's raw milk are considered, the percentage of samples positive to the ring-test reaction differs somewhat from that shown in Table 9 in that 957/2183 samples or 43·8 % were positive.

(*d*) *Brucella-infected milk supplies*

The final object of this investigation was to ascertain the present incidence of brucella-infected milks in this area, and also to see whether or not the ring-test reaction of a milk sample bore any relation to presence of infection. This work was carried out on sanitary authorities' samples which had been sent for routine guinea-pig inoculation in the course of tuberculosis control. Results tabulated in Table 10 show that over 10 % of the milk samples contained viable brucella organisms. It is worth noting that on three occasions brucella organisms were recovered from the spleens of guinea-pigs whose bloods were negative to the agglutination test and were recorded as 'guinea-pig negative' in the table.

The relative numbers of the various Special Designations samples available show an expected bias as special attention is paid by the sanitary authorities to 'Standard' and 'Non-designated' milks (Table 11).

Table 10. *Brucella infection of milk samples*

	Ring-test reaction				
	Positive: 167		Negative: 89		Total
Guinea pig serum Agglutination test	Negative	Positive	Negative	Positive	
No. of samples	140	27	89	Nil	256

Table 11. *Brucella infection of milk samples—grades of milk examined*, 1952–3

Grade:	Certified	'T.T.'	'Standard'	Non-designated	Total
Ring-test positive	1	4	18	50	73
Ring-test negative	—	5	3	32	40
Guinea-pig positive	—	1	1	10	12
Guinea-pig negative	1	8	20	72	101
Total samples	1	9	21	82	113

The claim of Jones (1943) to have demonstrated a maximum incidence of brucella infections in milk in January and a minimum in August was not confirmed during the limited period of this survey. (Table 12.)

DISCUSSION

Testing of individual animals

The preliminary testing carried out on herds 1 and 2, where the individual cows were free of *Brucella abortus* infection as indicated by the Weybridge serum agglutination test, showed that the ring test when applied to milks of normal character was remarkably accurate. The results of the ring-testing carried out on herd 3 and on the slaughterhouse material are less easily interpreted (Tables 7 and 8). In the latter case there was, of course, no possibility of carrying out retests;

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consequently it is not known what might have happened to the twelve samples in which the serum agglutination test was doubtful (Table 8). In the case of herd 3, the four doubtful blood reactors remained so after retesting; it may be assumed, in view of the herd history at this stage, that they were vaccinated animals.

Table 12. *Percentage milk samples containing viable brucella*

(The figures in brackets show the numbers of infected samples over the total number of samples examined by guinea-pig inoculation each month. The other figures represent the percentages of infected milk samples.)

	Jan.	Feb.	Mar.	Apr.	May	June
1951	.	.	.	.	.	.
1952	(3/21) 14	(0/12) 0	(1/15) 7	(2/13) 15	(2/9) 22	(1/16) 6
1953	(2/4) 50	(0/6) 0	(0/6) 0	(2/13) 15	.	.
	July	Aug.	Sept.	Oct.	Nov.	Dec.
1951	.	(2/18) 11	(4/16) 25	(2/12) 17	(3/26) 12	(0/14) 0
1952	(1/14) 7	(1/9) 11	(1/15) 7	(0/3) 0	(0/21) 0	(2/14) 14
1953	.	.	.	.	.	.

Table 13. *Summarized results of tests on individual animals*

Source	Serum agglutination test	No. of samples	Ring test		
			Negative	Doubtful	Positive
Herds 1–3, 1953	Negative	252	248	2	2
Slaughterhouse	Positive	29	4	—	25
	Doubtful	16	7	1	8
	Total	297	259	3	35

The collective results of tests on individual animals in herds 1–3 in 1953 and of tests on slaughterhouse material are summarized in Table 13. These results can be considered in two ways, either by ignoring or by including samples doubtful to the serum-agglutination test.

Using the serum-agglutination test as the criterion, and considering only the samples which were negative or positive to that test, it can be said that the ring test agreed with the blood test in 97·2 % of 281 cases. In 0·7 % of these cases the ring test itself gave a doubtful reaction (Table 13).

Bendsten (1952) summarized the results of other workers when testing individual cows; a modification of his table including his own figures is given in Table 14. In some instances (Sjollema, Schaaf & Sluis, 1949) a long interval elapsed between blood and milk tests, in others the milk under test showed poor creaming properties and thus failed to produce a positive ring-test reaction, even though it came from a reacting animal (Bruhn, 1944).

In order to compare the results in this survey with those shown in Table 14, it is necessary to classify the doubtful reactors as either positive or negative. If, following the method adopted by Norell & Olson (1943), the doubtful reactors to either test are classified as positive the results of the present survey can be tabulated as in Table 15. This classification of doubtful reactors results, of course, in judgement of the ring test under the least favourable conditions, as in reality

the majority of these doubtful blood reactors would have become negative on retesting. Under these strict conditions, the results shown in Table 15 indicate that the ring test disagrees with the blood test in 6·7 % of 297 cases.

Table 14. *Summarized results of tests on individual animals; continental workers*

Workers	Blood positive. Ring test: Positive	Negative	%	Blood negative. Ring test: Positive	%	Negative	Total	% disagreement
Norell & Olson, Sweden, 1943	111	19	4·2	16	3·5	307	453	7·7
Bruhn, Denmark, 1944	122	24	4·1	1	0·2	440	587	4·3
Christiansen, Denmark, 1948	649	81	2·0	376	9·5	2682	3952	11·5
Sjollema *et al.* Holland, 1949	14	26	3·9	12	1·8	612	664	5·7
Bendtsen, Denmark, 1952	565	198	2·6	60	0·8	6645	7468	3·4

Table 15. *Summarized results of tests on individual animals; reclassification of doubtful reactors*

	Number of samples	Serum positive. Ring test: Positive	Negative	Serum negative. Ring test: Positive	Negative		Doubtful reactions: Occurring as: Ring test	Blood	Recorded here as: Ring test	Blood
Herd no. 1, 1953	59	—	—	1	58	1	Doubtful	Negative	Positive	Negative
Herd no. 2, 1953	66	—	—	—	66		—	—	—	—
Herd no. 3, 1953	25	2	4	—	19	4	Negative	Doubtful	Negative	Positive
Slaughterhouse material	147	27	12	3	105	3	Positive	Doubtful	Positive	Positive
						1	Doubtful	Doubtful		
						8	Negative	Doubtful	Negative	Positive
						1	Doubtful	Negative	Positive	Negative
	297	29	17	3	248					

Testing of bulk milks

Investigations carried out in this survey (Table 3) show that positive milks continue to give positive reactions even when highly diluted in negative milks, so that the examination of can samples which is unlikely to involve the examination of the bulked milk of more than ten cows can be considered a safe procedure. The claim of Drimmelen (1951) that the ring test can be used to distinguish between vaccinated and infected animals by adopting the dilution technique was not completely confirmed in this survey. Although the number of animals vaccinated experimentally was too small to justify detailed reporting of results, in two cases cows vaccinated with strain 19 as adults remained strong positive reactors to the ring test for about 9 months, and in one case for over 2 years. Both animals were negative to the serum agglutination test before vaccination. On the other hand, four animals vaccinated as calves failed to react to either test once they came into milk.

Moore (1951) states that non-specific positive ring-test reactions may result if cows are tested within 14 days after calving or if their milk yield drops below

1 gallon per day. No cows in the survey were blood-tested unless they had been calved at least 1 month, but in the case of cows in late lactation it was found that the results of a ring test carried out using the dilution technique gave results consistent with those of the blood test. It would, however, be advisable, if applying the test in a routine manner to individual animals, to exclude recently calved or 'drying-off' animals. In bulk samples the milk of such animals will have little or no effect on the ring test.

It seems, therefore, that the ring test applied to individual animals is a useful means of detecting animals whose serum will show at least 50 % agglutination to *Br. abortus* at dilutions of 1/40 or over using the Weybridge agglutination test.

It is not possible, at this stage, to say whether such animals are infected or merely vaccinated; this will probably be the case as long as the indiscriminate use of strain 19 vaccine continues.

It was not possible in this survey to carry out extensive blood testing of herds whose bulk milk samples had been ring tested. Much work of this nature has, however, been carried out in America (Roepke *et al.*, 1949, 1950; Gilman, 1950) and in Scandinavia (Norell & Olson, 1943; Bruhn, 1948; Bendtsen, 1952), the results showing rather better agreement between blood and milk tests on a herd basis than in the case of individual animal testing. This, of course, is to be expected, as individual variations in milk character due to various factors will be of less significance in bulk milks. Certain errors ascribed to the ring test are in some cases due to sampling techniques. For example, a whole herd may be blood tested, but only animals in milk may be ring tested, or a considerable period may elapse between the application of the two tests, allowing animals to 'clear' or new infections to develop.

The results of herd testing by other workers are shown below:

Agreement between bulk ring tests and blood sampling of herd

Norell & Olson (1943)	94·7 %
Seit & Leth Jørgensen (1944)	96·2 %
Christiansen (1948)	93·2 %
Roepke *et al.* (1950)	96·2 %

Roepke *et al.* (1950), in their paper on the brucella ring test, concluded that, in a dairying area, one country-wide ring test would locate 50–60 % of infected herds and that two tests carried out at 6 month intervals would reveal 75–80 % of infected herds, an infected herd being a herd containing one or more animals giving positive serum agglutination tests. These workers point out that though the ring-test positive herds would have to be retested by blood examination a considerable saving of time and labour would be effected. Gilman (1950) drew attention to the fact that in parts of America, as in this country, the strain 19 vaccination scheme may well confuse the interpretation of the ring test.

It is therefore difficult to draw firm conclusions from the results of the ring-test survey applied to producers' raw milks coming into the Edinburgh area from many regions of South Scotland. It is possible, however, to conclude that a high percentage, 196/370 or 53 %, of the dairy herds concerned will contain a number of milking cows whose blood sera are positive to the Weybridge *Br. abortus* agglutina-

tion test. Whether these are infected or vaccinated herds it is, at this stage, impossible to say.

In order to throw further light on this question the incidence of brucella-infected milks has also been considered.

Brucella-infected milks

Beattie (1932), using guinea-pig inoculation methods, examined milk sold in Edinburgh. He compared diagnosis of infection in the guinea-pig by culture of the spleen with diagnosis by the serum agglutination test, and concluded that the latter was sufficiently reliable and was economical in time and labour. He showed that about 34·9 % of 'retailed' milk samples were infected with viable brucella organisms.

Since Beattie's investigation there have been determined attempts to eliminate brucellosis from our dairy herds, culminating in the present strain 19 vaccination programme. It might, therefore, have been expected that by now infection of our milk supplies would have been practically eliminated. The results reported here, however, and the work of others show that this is not so.

The figure of 10·5 % infected samples reported in this survey is no doubt an underestimation as only guinea-pigs with sera completely agglutinating at dilutions of 1/80 have been regarded as positive, and on three occasions the serum has been found to be negative when the spleen culture has been positive. As spleens were not always available for examination, it is not known what significance this last phenomenon may have in routine animal inoculation experiments to detect brucellosis. The phenomenon has also been recorded by Beattie (1932), Pullinger (1934) and Doyle (1935). The efficiency of guinea-pig inoculation as a means of detecting *Br. abortus* is not high; Doyle (1935) isolated *Br. abortus* from only 63 % of guinea-pigs inoculated with known infected material, while Drimmelen (1951) states that cultural and biological tests detect only 60 % of infected animals.

It should be noted that in no case was infection found in a ring-test negative milk sample (Table 10).

It does not appear that brucella infection of raw milk supplies is confined to southern Scotland. Smith (1932) reported that at least 28 % of all milk supplies derived from individual farms in the north-east of Scotland contained *Br. abortus*. In a further paper Smith (1951) questions whether there has been any real decrease in the number of dairy farms affected with contagious abortion. He most pertinently states: 'Furthermore, many dairy farmers have their animals inoculated against contagious abortion with an attenuated living vaccine but there is no evidence to show whether or not this helps to reduce the possibility of an infected milk supply'.

The occurrence of *Br. abortus* in milk supplies is confirmed by reports from other parts of the country, the Medical Officer of Health for Sheffield (1951), for instance, reporting that 11·7 % of some 435 samples of raw milk yielded *Br. abortus*. From Hertfordshire, Stringer (1951) reported in 1949–50 that in his district *Br. abortus* was found in 3·28 % of milk samples from 'T.T.' herds, in 8·08 % from 'accredited' and in 9·3 % from 'non-designated' herds.

Dalrymple-Champneys (1948) and Smith (1951) discuss a series of collected cases of human brucellosis in England and Wales and north-east Scotland respectively. According to Dalrymple-Champneys (1950), if diagnosis were more efficient some 1300 new cases per annum would be reported in England and Wales, and according to Smith (1951) 160 cases per annum in Scotland. Both these authors attach importance to the milk supply as an agent in causing human brucella infection (Dalrymple-Champneys, 1948, 1950, 1953; Smith, 1951). In view of the apparent frequency with which *Br. abortus* can be recovered from milk samples by a comparatively inefficient technique, one can only conclude that there is a need to investigate more fully the efficacy of strain 19 vaccination in reducing the incidence of brucella infection in milk.

SUMMARY

A report is made of a total of 438 simultaneous blood serum and milk ring tests, and an attempt made to evaluate the milk ring test as a diagnostic aid in individual animals.

The ring test has been used to test the bulk milk from some 370 herds providing the greater part of the raw milk supply for Edinburgh.

The incidence of viable *Br. abortus* in raw milk samples taken in the Edinburgh area has been studied together with the ring test reaction of such samples.

The survey has shown that:

(1) The ring test is correct in some 93 % of cases in detecting cows whose blood serum will be found positive to the brucella agglutination test.

(2) The ring test, if carefully carried out, is a simple time-saving and accurate screening test for detecting herds possibly infected with brucella.

(3) A high proportion of herd milks (53 %) giving a positive reaction to the ring test is disclosed, and the probable effect of vaccination with strain 19 in giving rise to this figure is noted.

(4) The relatively high incidence (at least 10·5 %) of *Br. abortus* in Edinburgh milk supplies is noted.

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