

## SOURCES OF INFECTION IN UNDULANT FEVER

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IN Great Britain the evidence so far is overwhelmingly in favour of bovine sources of infection in cases of undulant fever. The caprine and porcine types of *Brucella* have not been proved to occur indigenously, and, while the probability of the existence of the caprine type is remote, since comparatively few goats are maintained, the porcine type might quite well occur, as numerous herds of pigs are everywhere present throughout the length and breadth of the land. The bovine type of *Brucella abortus* is cosmopolitan in character, since it may also infect horses and sheep, probably also dogs, chickens, and perhaps other animals. The caprine type of *melitensis* readily infects goats and sheep, but cattle, horses, and swine are only slightly susceptible to it; the porcine type mainly infects swine, though an organism similar in its cultural characteristics has been isolated from cows, horses, and man.

Human cases of undulant fever can be divided into two groups, according to the source of infection: (1) those without, and (2) those with, direct contact with live stock or carcasses. The first group is infected by ingestion or by contact with infected dairy products, particularly raw milk, the second by contact with infected secretions of live animals and infected carcasses.

It has been repeatedly shown that, in this country, raw milk must be a continual potential source of infection, as contagious abortion is a widely prevalent disease (Wilson and Nutt, 1926; Beattie, 1932; Smith, 1932; Morgan, 1932; Priestley, 1932). Authoritative and complete information as to the presence of *Br. abortus* in milk products, however, is not yet available. Lerche (1931) found living *Br. abortus* in one out of four samples of country butter, and that in rennet cheese the organism may remain viable for 3 weeks, but is rapidly killed off by the lactic acid fermentation incidental to the production of hard cheese. Mazé and Césari (1931) report similar results in cheese. Again, Carrieu and Lafenêtre (1932) showed that undulant fever (*melitensis* type) must be conveyed by fresh cheese. In one instance an epidemic was traced to cheese sold by a travelling vendor, for it was possible to tell where he had been peddling his wares by the result amongst his customers. As regards ice-cream, large manufacturers use pasteurised milk, but small retailers or private houses often use raw. Thompson (1933) showed that in ice-cream prepared with raw, naturally infected milk, *Br. abortus* remained viable for at least one month when kept at temperatures below freezing-point.

Several papers have been published showing that men employed in handling domestic animals show a higher incidence of agglutinins for *Br. abortus*

than do normal males. Thus, Dible and Pownall (1932) found that men engaged in slaughtering and dressing meat show a higher incidence of agglutinins for *Br. abortus* than men in similar age groups who are otherwise employed. These workers also found that the greater incidence of agglutinins is particularly noticeable in men who deal with cattle and sheep, and that there was no increased incidence noted in men dealing with pigs. The inference was that, presumably, pigs did not suffer from infection due to *Br. abortus*. Again, Wilson (1932), after examining a series of blood samples collected from ninety-eight veterinarians, found that in sixty-three who were exposed to infection by aborting animals no less than fifteen showed agglutinins in their sera in a dilution of 1/20 or over; whereas, in thirty-five who were not so exposed, only one positive agglutination result was obtained. Wilson concluded that veterinarians, slaughtermen, and farm workers frequently become infected with *Br. abortus*, but in most instances the infection remains latent. Occasionally, however, in a small proportion, clinical symptoms of disease or of typical undulant fever develop.

The literature on animal infections, apart from those in cattle and goats, is comparatively meagre. In horses it has been observed that the serum agglutinated *Br. abortus*, and that the reactors were frequently affected with fistulous withers. This work has been extended, and *Br. abortus* has been isolated from the lesions by Ringard and Hilger (1928), Fitch, Delez, and Boyd (1930), Hultén (1931), van der Hoeden (1931), Magnusson (1932), Hieronymi (1932), Panisset and Delbé (1932), Beijers (1932), and Duff (1933), in America, Sweden, Holland, France, and Great Britain. The bovine type of *Br. abortus* has been isolated mainly from these purulent conditions.

In sheep, where the possibility of infection due to *Br. melitensis* can be excluded, the evidence of invasion by the bovine or porcine types is very scanty. From Germany, however, Meissner and Koser (1931) report that from materials obtained from aborting sheep, *Br. abortus* was isolated from eight out of 232 specimens.

The isolation of a *Brucella* organism from swine was first reported by Traum (1914). This work has been adequately confirmed in the U.S.A. by Good and Smith (1916), Doyle and Spray (1920), Connaway (1922), Boak and Carpenter (1930), and James and Graham (1930). From Switzerland Nagel (1931) presents a report of an epidemic of abortion, affecting seventy out of 110 sows, and involving ten human beings. Thomsen (1931), however, maintains that the disease is comparatively rare in Europe, except in Hungary; while Lockhart (1932) in America states that in the middle west *Brucella* infection in swine is prevalent, but there is little relationship between the disease of cattle and that of swine on the same farms.

As regards infection in other domestic animals, Planz and Huddleson (1931) isolated the porcine type of *Br. abortus* from the pus obtained from an abscess in a dog, while Gilman and Burnet (1931) examined the sera from a number of fowls but found no evidence of widespread disease, although experiments by

Huddleson and Emmel (1929) show that these birds are definitely susceptible to infection.

#### METHODS

In order to determine the presence of *Br. abortus* in milk and milk products, guinea-pig inoculation has been used throughout. The method employed for mixed milk has already been described (Smith, 1932). For milk from individual cows, 50 c.c. of the samples was centrifuged for 30 min. at 3000 r.p.m. The cream and the supernatant milk was discarded, and the deposit resuspended in 6 c.c. of salt solution, 3 c.c. being inoculated subcutaneously into two guinea-pigs. The first animal was killed after a period of 1 month, and the second after 2 months. The blood was collected and serum agglutination tests were carried out, and so also cultural examination of the spleen and liver.

To examine the butter samples for *Br. abortus*, these were placed in containers in a water bath at 41° C. After a period the material liquefied, and then, with previously warmed syringes, 5 c.c. was injected subcutaneously into each of two guinea-pigs. The animals were then treated as for milk. Margarine was prepared for inoculation by the same method. The problem of how to emulsify cheese in order that it might be made suitable for animal inoculation was solved by first passing the cheese through a fine tissue mincer, thereafter adding saline to make a thin paste, and again injecting 5 c.c. into each of two guinea-pigs. Ice-cream was allowed to melt, and then 5 c.c. of the liquid was inoculated.

In an attempt to demonstrate the presence of the porcine type of *Br. abortus*, tissues from sows—uterus, bladder, and spleen—were emulsified in saline, and thereafter 5 c.c. of the emulsion was inoculated into each of two guinea-pigs, one animal being killed after 1 month and the second after 2 months. Further, with a view to obtaining evidence of infection, a series of agglutination tests have been carried out, using the sera of swine and sheep. The agglutination tests were carried out by the usual macroscopic methods.

#### RESULTS

##### *Brucella abortus in milk and milk products*

(1) *Br. abortus in milk samples from individual cows.* Milk samples were obtained from a herd of eighty cows which had been vaccinated 1–2 years previously with living *abortus* vaccine. Nineteen animals or 23·75 per cent. gave a positive whey agglutination test in a dilution of 1/25 or more, but only ten or 12·5 per cent. showed *Br. abortus* in the milk. In a further series of tests made with milk samples from individual cows sent to the laboratory for examination for tubercle bacilli, a smaller percentage of positive results was obtained. Thus, from 103 samples, *Br. abortus* was recovered from seven or 6·7 per cent.

In the first series of samples, all those which by guinea-pig inoculation showed the presence of *Br. abortus* previously gave positive whey agglutination

tests in a dilution of 1/200 or greater. In the second series, one sample which gave a negative whey agglutination test showed the presence of *Br. abortus*, but all the other six samples again agglutinated *Br. abortus* to a dilution of 1/200 or greater. In all milk samples, from a total 183 cows, seventeen or 9.2 per cent. showed the presence of *Br. abortus*.

(2) *Presence of Br. abortus in butter samples.* Butter samples were obtained from various sources. Thus, fifty-one samples were obtained in the local market, and consisted entirely of butter prepared on individual farms. Fifteen samples of English dairy butter, five from colonial, and eight from foreign sources were similarly examined. Guinea-pig inoculation failed to show the presence of *Br. abortus* in any of the seventy-nine samples, but five out of fifty-one locally prepared samples contained tubercle bacilli.

(3) *Margarine samples.* Milk is employed in margarine manufacture for the purpose of giving this product a butter flavour, and to act as an emulsifying agent. The treatment of the milk is essentially that of separation of cream, and then pasteurisation. Thereafter the pasteurised milk is inoculated with specific micro-organisms to induce acidity and aroma. Thirty-nine samples of margarine in which milk had been incorporated in its preparation were examined, and all, as would be expected, gave negative results.

(4) *Cheese samples.* Cheese is perhaps the oldest of all milk products. At the present time, however, although there are still numerous varieties, most is now made in factories rather than on farms. For the purposes of this investigation, samples of Cheshire, Cheddar (red and white), Double Gloucester, Bel Paese, Convalli, Camembert, Wenslet, Roquefort, Gouda, Stilton, Edam, Gorgonzola, Chilvern, Parmesan, and locally prepared Scotch cheese were examined. Twenty-three samples were made in England, six were colonial in their origin, twenty-eight foreign, and six local. Examination of all sixty-three samples failed to show the presence of *Br. abortus*, but two out of the six locally prepared cheeses were found to contain tubercle bacilli.

(5) *Ice-cream samples.* It was intended that a fairly large series of ice-cream samples should be examined, but difficulties were encountered. Some of the large dairying concerns use nothing but efficiently pasteurised milk for the preparation of this product. The examination of milk samples had shown that it would be useless to examine ice-cream, prepared from milk so treated, for *Br. abortus*. Samples (about fifty) were therefore obtained from Italian ice-cream vendors, but the results for twenty-one only are available, and all these failed to show *Br. abortus*. Many of the results were vitiated by the fact that the ice-cream was contaminated with various anaerobes, which produced in the inoculated animals intense anaerobic infections. Attempts were made to overcome this by first treating the animals with a polyvalent antitoxin prepared by mixing monovalent antitoxins, but without success. All ice-cream samples failed to show the presence of *Br. abortus*.

*Evidence of infection as a result of contact with animals and carcasses*

In order to obtain evidence of possible infections in men whose daily labours bring them into contact with live stock and carcasses, a number of sera were tested for agglutinins for *Br. abortus*. With the assistance of the Veterinary Officer, Mr Jas. McAllan, B.Sc., M.R.C.V.S., who acted also as detention officer at the main slaughterhouse, and with the co-operation of the slaughterhouse and meat mart officials, it was possible to obtain blood from practically every man employed in the slaughtering and meat trade business.

The results are set out in Table I. Out of a total of 106 sera examined,

Table I. *Agglutinins in the sera of men employed in slaughtering and allied trades.*

Total number of sera examined	...	...	106
No. of sera from actual slaughtermen	...	...	68
No. positive in dilution of 1/25 or more	...	...	11
% positive in dilution of 1/25 or more	...	...	16.1
No. of sera from workers in associated trades	...	...	38
No. positive in dilution of 1/25 or more	...	...	4
% positive in dilution of 1/25 or more	...	...	10.5

fifteen agglutinated *Br. abortus* to a dilution of 1/25 or more; eleven positive reactions occurred in sera from actual slaughtermen, and four in sera from men employed in the meat trade. From the slaughtermen, five sera agglutinated *Br. abortus* to a dilution of 1/25, five to 1/50, and one to 1/100; from the men working with carcasses, hides, and offal, three agglutinated *Br. abortus* to a dilution of 1/25, and one to 1/50. It was found that fifty-five men were employed in killing cattle, sheep, and pigs, ten in killing cattle and sheep, and three in killing cattle only; all the eleven with agglutinins of 1/25 or more in their sera killed all three types of animals.

When the relationship of the length of time employed to the agglutinins was investigated (Table II), it was found that in slaughtermen the positive

Table II. *No. of years employed in trade in relation to agglutinins.*

	Years				
	0-5	6-10	11-20	21-30	31 +
<b>SLAUGHTERMEN:</b>					
No. of negative sera	17	7	20	5	19
No. of sera positive 1/25 or more	0	0	3	3	5
<b>MEN IN ALLIED TRADES:</b>					
No. of negative sera	9	8	7	8	6
No. of sera positive 1/25 or more	2	1	0	0	1

reactions occurred in men who had been thus employed for 11 years or more, whereas in the men handling hides, carcasses, and offal, three out of four positive reactions occurred in men who had been employed for less than 10 years. Furthermore, when the relationship of agglutinins in age groups in the abattoir workers was compared with the agglutinins in the sera of normal individuals in the same age groups, this showed a greater incidence in the slaughtermen and other workers than amongst the normal males. Thus, 16.1 per cent. of sera from slaughtermen and 10.5 per cent. of sera from men

working in allied trades agglutinated *Br. abortus* to a dilution of 1/25 or more, as compared with only 6·8 per cent. of sera from normal men.

When the men who showed agglutinins in the blood were questioned as to a history of previous illness, no evidence was obtained that any of them had suffered from an illness suggesting undulant fever. It is presumed, therefore, that latent infections must have occurred in some to give rise to the higher incidence of agglutinins.

Table III. *Age in relation to agglutinins.*

	Age groups				
	15-20	21-30	31-40	41-50	51+
(1) SLAUGHTERMEN:					
No. of men	11	24	14	7	12
No. of sera positive 1/25 or more	0	3	3	2	3
% positive		15·8		31·5	
(2) OTHER WORKERS:					
No. of men	4	11	9	8	6
No. of sera positive 1/25 or more	1	1	1	0	1
% positive		10·5			
(3) NORMAL MEN:					
No. of men	34	99	61	48	62
No. of sera positive 1/25 or more	2	9	4	4	2
% positive	5·8	9·0	6·5	8·3	3·2
		8·1		5·4	

*Evidence of Brucella abortus infections in sheep and swine*

Bovine infections due to *Br. abortus* are extremely prevalent, but little evidence has been produced so far by the veterinary profession in this country to show whether or not sheep and swine are also naturally infected. So far as can be ascertained from veterinary research workers, there is little evidence of enzootic abortion in either sheep or swine.

Samples of blood were therefore obtained from animals killed in the slaughterhouse. From sheep, 170 samples were obtained from hogs, two from withers, fifteen from ewes, seven from tups, and five from lambs. All sera gave negative agglutination tests against *Br. abortus*, the lowest dilution of the sera to be tested being 1/25. Similarly, from swine, 194 samples of blood were obtained from half-grown fat pigs (male and female), twenty-one from sows, and four from boars. Only two samples from the half-grown animals agglutinated *Br. abortus* to a dilution of 1/25. Thus, only 0·9 per cent. of sera showed agglutinins, and then only to a titre of 1/25, which can scarcely be regarded as significant.

Furthermore, in order to attempt to isolate the porcine type of *Br. abortus*, specimens of uterus, bladder, and spleen were obtained from forty sows as slaughtered. In some instances the animals were slaughtered on account of illness at the termination of their pregnancy. Guinea-pig inoculation of emulsions of the tissues proved negative for *Br. abortus*, but five (12·5 per cent.) of the animals became infected with *B. tuberculosis*. Presumably, therefore, had the

porcine type of *Br. abortus* been present in the tissues, their presence would have been detected as reliably as that of *B. tuberculosis*.

#### DISCUSSION

The percentage of positive results in the milk samples from individual cows again emphasises the prevalence of *Br. abortus* in the milk supply. In fact it is surprising that more than 30 per cent. of the bulk supply do not reveal *Br. abortus*. Also, although only some 10 per cent. of cows excrete *Br. abortus* along with their milk, it must be the case that a considerable number of animals harbour the organisms in organs other than the udder, and that in some instances probably urine, faeces, and uterine secretions contain the organism when it is absent from the milk.

In so far as milk products are concerned, the evidence presented shows that these cannot play an important part in the dissemination of undulant fever. In samples of butter and cheese coming from abroad, one would not have been surprised at negative results, since a considerable interval of time elapses between the actual preparation of the product and its distribution for consumption by human beings. It is also worthy of note, however, that all locally prepared samples of butter and cheese failed to yield *Br. abortus*, although living tubercle bacilli were evidently present in some.

As regards infections by direct contact with infected animals and carcasses, the evidence of the agglutination tests indicates that some latent infections do occur, but, so far, among slaughtermen no serious illness has been encountered. Indirect evidence of the presence of *Br. abortus* in sheep and pigs has not been obtained, since the men employed slaughter cattle as well as some other type of domestic animal.

The agglutination tests on serum obtained from pigs and sheep, and the examination of organs obtained from pigs, produced no evidence whatsoever that these animals are subject to infections with *Br. abortus*. In fact all available evidence goes to show that the porcine type of *Br. abortus* does not occur in this country. This probably explains why there are relatively fewer cases of undulant fever in this country than, for instance, in America.

#### SUMMARY

1. Samples of milk from 183 cows were examined, and seventeen (9·2 per cent.) yielded *Br. abortus*.
2. Two hundred and two samples of butter, margarine, cheese, and ice-cream failed to yield *Br. abortus*, although samples of locally prepared butter and cheese both contained tubercle bacilli.
3. Examination of the serum from slaughtermen and men working in allied trades showed some evidence of latent infection, but no indication of much real illness due to *Br. abortus*.
4. Examination of the sera of sheep failed to show any evidence that these animals become infected with *Br. abortus*.

5. The examination of sera and organs from pigs failed to show any evidence that the porcine type of *Br. abortus* occurs.

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(MS. received for publication 30. II. 1934.—Ed.)