THE INCIDENCE OF BRUCELLOSIS CLINICAL AND LATENT AMONG VARIOUS GROUPS OF THE POPULATION

By C. P. BEATTIE

From the Bacteriology Department, University of Edinburgh

Introductory

The object of this paper is to record certain observations on the relative frequency of *Brucella* infection in different occupational classes and to correlate this with the path of infection and the dose of organisms.

A consideration of the paths of infection will show that they can be divided into broad groups which may be correlated with occupations.

Thus we have:

(I) *Ingestion.* Consumption of milk or milk products. To this all members of the population are exposed.

(II) *Contact.* (*a*) Handling of infected animals or meat. To this farm workers, slaughterers and butchers are exposed. (*b*) Assisting in calving, removal of placentas ("cleaning") and other intra-uterine and intravaginal manipulations in cattle. To this veterinary surgeons are mainly exposed.

There is, of course, considerable overlapping. Veterinary surgeons, farm workers, slaughterers and butchers are exposed to infection by ingestion. Some farm workers are exposed to infection by intra-uterine and intravaginal manipulation, while veterinary surgeons are exposed to all types of contact with animals.

Infection will be considered under the headings of "clinical" and "latent". No explanation of the first is required. The presence of antibodies in the blood or the existence of an allergic state in persons who at the time of test show no clinical symptoms of brucellosis and who have not experienced such symptoms in the past are taken as evidence of latent infection past or present. It is a common observation among veterinary workers that after the removal of a placenta from a cow infected with *Br. abortus* they suffer from a rash on their arms. By many this is taken to be a manifestation of allergy. Huddleson & Johnson (1930) term it "erythema brucellum".

Tests for the presence of allergy by the inoculation into the skin of killed *Brucella* or fractions of the organism have been carried out on a large scale in various occupational groups, particularly in the United States. The reactions produced are claimed to be specific.

Complement fixation tests have been used by some and have demonstrated the presence of antibodies in a larger number of cases than have agglutiñation

tests. This is probably because complement fixing antibodies persist longer than do agglutinins (Kristensen, 1931; Sasano *et al.* 1931; Laun & Herde, 1934).

The agglutination test has, however, been most widely used and was the one employed in the observations to be recorded.

The method adopted by the writer was to obtain blood by vein puncture and to test the serum with an antigen prepared according to the recommendation of the Contagious Abortion Committee of the Agricultural Research Council. The tests were incubated at 55° C. for 4 hours and read after they had stood overnight at room temperature or were incubated at 37° C. for 18 hours and then read.

For the purpose of this investigation agglutination in a titre of 1 in 20 or higher was taken as indicating infection. It is realized that objection may be raised that agglutinins found in such a low titre are non-specific or passively acquired. But there is evidence that even in this titre agglutinins for *Br. abortus* are specific and that they are only acquired by active infection and not passively by the consumption of milk containing agglutinins (Carpenter *et al.* 1929; Peterson, 1935). In any case the differences in the frequency of the occurrence of agglutinins in different occupational groups are so great as to be significant.

There are more valid objections to the use of the agglutination test as evidence of infection. It will only detect infection of relatively recent occurrence. Agglutinins for *Brucella* tend to disappear, sometimes in a few months, at other times not for several years. This may happen even in the presence of continued exposure to infection. Some of the veterinary students examined by the writer showed this, and it is a common observation among bacteriologists constantly working with cultures of *Brucella*. Further, agglutinins do not develop in all clinical cases of brucellosis. Burnet (1922) found them absent in 16·6% of cases. They may even be absent when *Brucella* is cultured from the blood (Carpenter, 1926; Gilbert & Dacey, 1932).

BRUCELLOSIS IN COUNTRY AS A WHOLE

The first group to be considered is the population as a whole. Infection among them must, in this country, be mainly by ingestion. The opportunity for infection is great since 20–35% of raw milk contains *Br. abortus* (Wilson & Nutt, 1926; Smith, 1932; Morgan, 1932; Beattie, 1932; Pullinger, 1934). The organism is, of course, killed by efficient pasteurization. In other countries infection has been contracted from cheese and may be acquired from butter. Smith (1934), however, failed to find the organism in these foods in this country.

It is somewhat surprising that clinical brucellosis is not more common. In England and Wales from 1928 to 1935 Dalrymple-Champneys (1935) recorded 255 cases of undulant fever. In Scotland from 1929 to the end of 1935, 108

cases have been reported (Beattie *et al.* 1935, with added unpublished cases). Nor is latent brucellosis common. Sera submitted for the Wassermann reaction have been tested for the presence of agglutinins for *Br. abortus*. Cruickshank & Barbour (1931) found 2·4% to react in a titre of 1 in 20 or higher, Smith (1932) 4·6% and Tulloch (unpublished) 2%.

The reasons for this low incidence of infection may be that, although a large proportion of milk contains *Br. abortus* the concentration of the organism is not high, and that ingestion is not the most dangerous route of infection. Experimental work both in animals and man has shown that a smaller number of organisms will prove infective when they are applied to the skin, especially if abraded, or to a mucous membrane, than when they are given by mouth (Hardy *et al.* 1929; Taylor *et al.* 1934; Morales-Otero, 1933; Bang & Bendixen, 1932).

Brucellosis in farm workers, slaughterers and butchers

This leads to a consideration of the second group—those exposed by contact. First will be discussed persons having contact with cattle or meat while not involving contact with the secretions of the uterus or vagina. Exposed to this risk are farm workers, slaughterers and butchers.

In other countries the incidence of infection among farm workers is high. In the United States Hardy (1930) found that 45% of his series were farm workers. This result is not strictly comparable with conditions in this country for in the region where he worked a larger number of the population are engaged in farming and *Br. suis* is present. *Br. abortus* is the infecting organism in New Zealand and there Maclean (1932) found that 47% of cases of undulant fever occurred in farm workers whose representation in the population was 5%. In Germany two-thirds of the cases of *Br. abortus* infection have been found to occur in people having contact with infected cows or engaged in handling raw milk. In England and Wales, Dalrymple-Champneys (1933) recorded out of a total of 99 cases, 11 in farm workers. This he did not consider an excessive incidence. Beattie *et al.* (1935) obtained similar results. Among 97 cases 10 were in farm workers. They did, however, consider this to be an excessive proportion with regard to the number of farm workers in the population.

Little work has been done to discover the frequency of latent infection among farm workers. In Germany Lentze (1930) found that the sera of 22·8% of 57 farm workers in contact with infected cattle reacted to the agglutination test even when diluted to 1 in 120. One of them had symptoms of undulant fever. He does not state if any of these had assisted in calving or "cleaning". In Denmark, where the infecting organism is *Br. abortus*, Thomsen (1931) found that such infection was not uncommon among chief cattle attendants. These men, however, did assist at births and abortions and will not be considered here. Among other farm workers latent infection was not common. The sera of 20 milkmaids were tested and only one gave a positive

reaction. This result accords with results obtained in Scotland. The sera of 18 farm workers in contact with infected cattle have been examined and in only one case were agglutinins for *Br. abortus* found.

Clinical infection in slaughterhouse workers is common in the United States but is mostly due to *Br. suis*. In this country it is rare. Dalrymple-Champneys (1933) did not find any cases in England and Wales. In Scotland 2 cases are known to have occurred among butchers (Beattie *et al.* 1935).

Evidence of latent infection was found by Dible & Pownall (1932). Agglutinins for *Br. abortus* in a titre of 1 in 40 or higher were found in 12% of slaughterers compared with 2% of controls. Smith (1934) also found the proportion of reacting sera from slaughterhouse workers to be higher than in controls.

The writer has examined the sera of 37 such men and found agglutinins present in 2 in a titre of 1 in 25.

It would, therefore, appear that the incidence of brucellosis among slaughterhouse workers and possibly among farm workers in this country is somewhat greater than the incidence among the population as a whole. These classes are exposed to infection by the most effective route, through the skin and mucous membrane. They are probably not, however, liable to come in contact with a high concentration of the organism. *Br. abortus* has, indeed, been demonstrated in the flesh of slaughtered cattle (Kruger, 1932) and is almost certainly present in the udder or uterus of infected cows. Similar exposure to the American variety of *Br. suis* in pig carcasses would give rise to more instances of infection.

BRUCELLOSIS IN VETERINARIANS

Members of the veterinary profession are exposed, not merely by the most effective route, but also to the maximum concentration of the organism, for it is to be found in greatest numbers in the uterine and vaginal secretions of parturient or aborting cows. They, therefore, run a very special risk of contracting infection when they engage in the removal of placentas or assist in calving.

It would be expected that a large number of them should be infected with *Br. abortus*. Yet few develop clinical signs of infection. Of 108 cases of undulant fever recorded in Scotland only one was of a veterinary surgeon. In England and Wales Dalrymple-Champneys (personal communication) has heard of two veterinary surgeons and one veterinary student who have contracted undulant fever out of a total of 314 cases. Kristensen and his co-workers (1929) in Denmark recorded 500 cases of undulant fever, none in a veterinarian. In Germany of 530 cases of the disease during the year 1934, 15 were in veterinary surgeons.

When specific inquiries have been made among groups of veterinarians as to the previous occurrence of an illness having the clinical features of undulant fever more cases have been disclosed. Thus of 49 practising veterinarians in the

United States Huddleson & Johnson (1930) considered 3 had previously had undulant fever. Jordan (1931) found 3 out of 120. Thomsen (1931) in Denmark from among 29 recent graduates found 3 who had a disease of the symptomatology of undulant fever, and among 182 veterinary students the writer found 1 who definitely had undulant fever and 2 who gave a history which combined with a high serum agglutination titre warranted a retrospective diagnosis of undulant fever.

Though few develop clinical brucellosis there is evidence that many develop latent infection.

Rossi (1935) in France examined the sera of 28 veterinarians and found agglutinins to a titre of 1 in 20 or higher present in all but 8. Huddleson & Johnson (1930) in the United States examined the sera of 49 and found 28 or 57% to contain agglutinins in a titre of 1 in 50 or over. Jordan (1931), again in the United States, did not find such a high percentage. The sera of 120 veterinarians was examined and 17 or 14·2% gave a positive reaction in a titre of 1 in 20 or more. In this country Wilson (1932) has conducted a similar examination. Of 63 veterinarians exposed to contact with infected cattle he found 23·8% showed agglutinins in their sera in dilution of 1 in 20 or more. Of 35 who were not so exposed only one had agglutinins in his serum and he was a laboratory worker who handled cultures of *Br. abortus*.

Thomsen (1931) in Denmark examined both veterinary surgeons and veterinary students. He used both agglutination and complement fixation tests, and when the results of both were considered he found that of 65 veterinary surgeons in rural practice for more than a year 94% showed positive reactions. Complement fixation tests gave more positive results than did agglutination tests. His investigation of veterinary students was somewhat similar to that undertaken by the writer, and therefore, for purposes of comparison, the results he obtained with agglutination tests, taking a titre of 1 in 20 as a positive result, will be given.

Group	Total	Number positive	% positive
Veterinary students (none had handled bovine placentas)	8	0	0
Veterinarians 6 months after graduation	18	13	72·2
Veterinarians 1 year after graduation	11	3	27·3
Veterinary surgeons after more than 1 year of practice*	65	17	26·2

* 94% positive when both agglutination and complement fixation tests were considered.

For three years the writer, on behalf of the Scottish Committee on Contagious Bovine Abortion, examined the sera of students attending a veterinary college. In many cases the examination was repeated in subsequent years. The technique employed has already been described. Agglutination in a titre of 1 in 20 or higher was taken as a positive result. For purposes of comparison sera from a group of medical students were tested under similar conditions. Further data concerning the percentage of positive reactions in the population as a whole has already been given.

The results obtained in the different years of study were as follows:

Year of study	Total	Number positive	% positive
1st year	49	5	10·2
2nd year	29	3	10·3
3rd year	54	5	9·3
4th year	26	4	15·4
Total	158	17	10·8
Post graduate	24	14	58·3
Medical students (controls)	96	4	4·2

As was to be expected, a very high proportion of post-graduate students gave a positive result. In the other years the numbers positive were also higher than with medical students serving as controls. This may be accounted for by the fact that many of the veterinary students were brought up on farms while others assisted veterinary surgeons during the college vacation.

All of the post-graduate students had engaged in practice involving contact with cattle and all but one had removed retained placentas or assisted in calving. To this may be attributed the large number of them who showed agglutinins.

In an endeavour to determine further the effect of contact on infection as evidenced by the presence of agglutinins the veterinary students were divided into those who had much and those who had little contact with cattle. This division gave the following results:

	Total	Number positive	% positive
Much contact with cattle	130	30	23·0
Little contact with cattle	52	1	1·9

A further analysis was made of those who had assisted in calving, in removing retained placentas or carried out vaginal examination. This information was not obtained in every case.

	Total	Number positive	% positive
Calving, removal of placentas or vaginal examination	66	23	34·8
No such contact	59	5	8·5

The number of positive results obtained among those who had not engaged in calving or the removal of placentas was small, 5. Of these 5 one had worked for many years with a veterinary surgeon; another lived on a farm; the third worked on a farm for 6 weeks every summer; the fourth had little contact with animals, while the fifth had worked for a year on a farm where contagious abortion was rife and had milked infected cattle. Infection in his case may have been by ingestion or may have been by contact of the hands with infected milk.

The examination was repeated in succeeding years in 32 students. Seven of them at first gave a negative result but later after further practical work reacted positively. They had all helped in delivery or in the removal of placentas. One whose serum in 1934 was shown to have agglutinins to a titre

of 1 in 400 was found to be negative in 1935 and in 1936. The titre of another fell from 1 in 320 to 1 in 40. Twenty-one gave negative results in every test. Of these 13 had engaged in calving or "cleaning". Three were positive in every test.

In many cases the agglutination titre was high. It is commonly stated that a titre of 1 in 100 or over indicates undulant fever. A dilution of 1 in 100 was not used, but if 1 in 80 be considered 16 reacted to that titre or higher. Three of these had previously suffered from an illness having the symptomatology of undulant fever.

The numbers reacting in the various titres were as follows:

1 in 20	7	1 in 160	7
1 in 40	8	1 in 320	4
1 in 80	2	1 in 640	3

A number of the students gave a history of having a rash on their arms after calving or "cleaning," but this occurrence could not be correlated with the appearance of agglutinins.

Discussion

The population of Great Britain is exposed to the risk of infection with *Br. abortus* by the consumption of milk containing the organism. A large proportion of the milk supply contains *Br. abortus*. It is, therefore, surprising that so few cases of clinical brucellosis occur. There are many possible reasons for this. *Br. abortus* is probably of low virulence for man (Thomsen, 1931; Morales-Otero, 1933). Correlated with its relatively low virulence it may not be present in milk in sufficient concentration to produce disease. Moreover, the alimentary tract is not the most effective route of infection. The evidence at present available does not point to latent infection being common. Examination of sera submitted for the Wassermann reaction has given percentages of from 2 to 5 showing agglutinins for *Br. abortus* in dilutions of 1 in 20 or higher. It is possible, however, that this is an underestimate and that agglutinins may have been present in more but in the course of time have disappeared.

It would be expected that infection should be more common among persons having contact with cattle. In some countries farm workers and slaughterhouse workers do develop clinical brucellosis to a greater extent than the rest of the population. In Britain the evidence is indefinite but it would appear that they are somewhat more prone to the disease. The results of a small number of agglutination tests carried out in Denmark and in this country indicate that in these countries latent infection is not markedly more frequent among farm workers who are engaged only in milking, feeding and grooming. Elsewhere different results have been obtained.

Slaughterhouse workers in Britain, in contrast to those in the United

States, are not often attacked by clinical brucellosis. It would appear that they contract latent infection more frequently than do others.

These classes, although they are exposed to infection by the most effective route, i.e. through the skin and mucous membrane, are probable not exposed to a high concentration of the organism.

Members of the veterinary profession are exposed by the same route. They come in contact, however, when engaged in calving or "cleaning", with the greatest natural concentration of the organism. Few of them develop clinical brucellosis, but agglutination tests show that, at some time or another, many have contracted latent infection. For the reasons already indicated agglutination tests will fail to detect all who have been infected. Probably all who engage in cattle practice are infected at some time, generally soon after commencing such practice.

This adds to the difficulties of the diagnosis of undulant fever. In members of the veterinary profession a single positive agglutination reaction is not in itself sufficient. It should be repeated and a rising titre looked for. Confirmatory tests should also be carried out, the most valuable of which is blood culture.

SUMMARY

1. The population as a whole is exposed to *Brucella* infection by the consumption of infected milk. This does not appear to be a very potent source of infection as relatively few clinical cases of brucellosis occur, nor are many latent infections discovered by the agglutination reaction.

2. Those exposed to contact with infected animals or meat, as are farm workers, slaughterers and butchers, run a greater risk of contracting infection. The risk is not, however, markedly increased when the contact is limited to the handling of carcasses or meat, milking or ordinary animal husbandry.

3. Members of the veterinary profession, who assist in calving and remove placentas from infected cows are exposed to the greatest risk of infection. They come in contact with the greatest natural concentration of *Br. abortus* through the most dangerous route, the skin. Clinical brucellosis is not common among them, but many contract latent infection. 58·3% of post-graduate and 10·8% of undergraduate veterinary students were found to have agglutinins for *Br. abortus* in their serum. This should be borne in mind when a diagnosis of undulant fever is considered in a member of the veterinary profession.

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