

Brucellosis in Northern Ireland. A serological survey

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(Received 1 November 1968)

INTRODUCTION

The eradication of brucellosis in cattle has been considered a necessary preliminary to the control of disease in man (Bothwell, 1960; Bothwell *et al.* 1963; Anon. 1966), for even when all milk marketed for human consumption is pasteurized, a percentage of the population living in the rural areas will consume unpasteurized milk. In Northern Ireland, this group has been estimated previously as high as 400,000 people or roughly one-fourth of the total population (Kerr & Rankin, 1963).

Britain has no brucellosis eradication programme, but in Northern Ireland a scheme for grade A herds was introduced in 1959 (Milk (Amendment No. 2) Regulations, 1959), which was first widened in scope to include the remaining dairy herds voluntarily (Brucellosis Order, 1960) and then made compulsory in 1963 (Brucellosis Control Order, 1962). By December 1967, 15,527 out of the total of 16,300 dairy herds were certified brucella-free whilst eradication was proceeding in the remainder. In addition, 23,000 herds from a total of 28,000 beef and suckling herds had also reached certified status and 3,500 were under test (Christie, Kerr & McCaughey, 1968).

Pasteurization of all milk supplies except those from Grade A herds has been enforced since 1950 (Milk Act (Northern Ireland), 1950) and brucellosis has been eradicated from these herds since 1961.

During 1967 a survey was carried out in Northern Ireland to determine the prevalence of antibodies to *Brucella abortus* in the population. It was thought that it might give some indication of the initial effect of the eradication programme for bovine brucellosis on the amount of human exposure and disease. The results of the survey are reported in this paper.

METHODS

Sera from the various groups were examined for brucella antibodies by the standard agglutination test, the anti-human globulin (A.H.G.) (Coombs) test, and the complement fixation test.

Serum agglutination test

This was carried out by the method described by Alton & Jones (1967) at serum dilutions from 1/10 to 1/5120. Any sera showing agglutinations at 1/5120 were taken to titre. The antigen used was an agglutinable suspension of *Brucella abortus* Strain 99 standardized to give 50% agglutination with a 1/500 dilution of International Standard Serum.

Anti-human globulin (A.H.G.) (Coombs) test

The method of Wilson & Merrifield (1951), as modified by Kerr, Coghlan, Payne & Robertson (1966*b*) was used. The antigen was the same as described above. Anti-human precipitating rabbit serum (Burroughs-Wellcome) was used at its optimal dilution.

Complement-fixation test

This test was performed as described by Bradstreet & Taylor (1962), by a 4 volume test (unit volume 0.1 ml.) in W.H.O. plastic plates. The short fixation method only was used with 2.0 M.H.D. of complement and the optimal dilution of antigen (heat-killed *Brucella abortus* Strain 99) as determined by a chessboard titration. In each series of tests a known control serum was included.

RESULTS

A total of 1,894 sera were tested. The results are shown in Table 1.

Blood transfusion sera

One thousand and seventy eight sera were obtained from the Northern Ireland Blood Transfusion Service. They originated from a variety of centres, including Belfast, Dungannon, Lisburn and Magherafelt. The majority of donors lived in these centres and thus, whilst all these sera were designated 'urban', a very small proportion of them may have come from rural dwellers. A very small proportion of the sera, never more than 2.7%, showed any evidence of possessing antibodies to *Br. abortus* whichever test was used. The serological titres were always low.

Farmer's lung sera

Two hundred and fifty two sera were included which had been submitted to the Mycology Diagnostic Laboratory with a possible diagnosis of Farmer's Lung. These sera were not necessarily from farmers or farm workers, but it was assumed that the individuals concerned had contact with farms or a rural setting, as only three out of 601 specimens submitted over a period came from Belfast Borough.

The proportion of sera which gave reactions to the serum agglutination test was similar to that of the urban group, but with the A.H.G. and complement-fixation tests this rural group showed a higher proportion of reactions than the urban group.

Veterinary surgeons

There were 125 veterinary surgeons in private practice in Northern Ireland in 1967, of whom 118 gave blood for examination. They were predominantly engaged

Table 1. Serological titres of the different groups investigated

Group	Total no. in group	Standard agglutinations titre			Anti-human globulin titre			Complement-fixation titre		
		≥ 1/10	≥ 1/20	≥ 1/40	≥ 1/10	≥ 1/20	≥ 1/40	≥ 1/4	≥ 1/8	≥ 1/16
Blood transfusion sera (urban)	1078	23 (2.1)	2 (0.2)	0	29 (2.7)	4 (0.4)	1 (0.1)	2 (0.2)	2 (0.2)	0
Farmer's lung sera (rural)	252	6 (2.4)	1 (0.4)	1	19 (7.5)	9 (3.6)	3 (1.2)	6 (2.4)	5 (2.0)	4 (1.6)
Vets in private practice	118	34 (28.8)	20 (16.9)	8 (6.8)	110 (93.2)	107 (90.7)	92 (78.0)	84 (71.2)	78 (66.1)	55 (46.6)
Vets in Ministry practice	78	7 (9.0)	5 (6.4)	0	50 (64.1)	44 (56.4)	26 (33.3)	29 (37.2)	25 (32.1)	18 (23.1)
Non-qualified Ministry helpers	26	4 (15.4)	4 (15.4)	2 (7.7)	8 (30.8)	6 (23.1)	5 (19.2)	6 (23.1)	5 (19.2)	5 (19.2)
Artificial insemination workers	7	0	0	0	0	0	0	0	0	0
Abattoir workers	243	22 (9.1)	13 (5.3)	8 (3.3)	35 (14.4)	30 (12.3)	8 (3.3)	20 (8.2)	7 (2.9)	0
Dairy farmers or farm workers	92	20 (21.7)	14 (15.2)	4 (4.3)	40 (43.5)	32 (34.8)	25 (27.2)	16 (17.4)	9 (9.8)	6 (6.5)
Total	1894	116 (6.1)	60 (3.2)	23 (1.2)	291 (15.4)	232 (12.2)	160 (8.4)	163 (8.6)	131 (6.9)	79 (4.2)

Figures in parentheses are percentages of totals for each group. ≥ 1/10 includes figures for ≥ 1/20 etc.

Table 2. Serological titres related to occupational exposure to cattle

Group	Total no. in group	Standard agglutination titre			Anti-human globulin titre			Complement-fixation titre		
		≥ 1/10	≥ 1/20	≥ 1/40	≥ 1/10	≥ 1/20	≥ 1/40	≥ 1/4	≥ 1/8	≥ 1/16
No definite exposure to cattle	1330	29 (2.2)	4 (0.3)	1 (0.1)	48 (3.6)	13 (1.0)	4 (0.3)	8 (0.6)	7 (0.5)	4 (0.3)
Definite occupational exposure to cattle	564	87 (15.4)	56 (9.9)	11 (3.9)	243 (43.1)	219 (38.8)	156 (27.7)	155 (27.5)	124 (22.0)	75 (13.3)
Total	1894	116 (6.1)	60 (3.3)	12 (0.6)	291 (15.4)	232 (12.2)	160 (8.4)	163 (8.6)	131 (6.9)	79 (4.2)

Figures in parentheses are percentages of totals for each group. ≥ 1/10 includes figures for ≥ 1/20 etc.

in 'large animal' practice and spent between 50 and 75 % of their time with cattle. The Ministry of Agriculture employed a further 108 veterinary surgeons during this period; 78 sera were obtained from this group. Of these, approximately one fourth worked in an administrative capacity and two worked in artificial insemination centres, but the majority were employed in bovine tuberculosis and brucellosis eradication programmes or carried out meat inspection duties. Owing to the different nature of their employment, these two groups were considered separately.

The proportion in each group showing reactions to the serological tests was higher than in either the urban or rural groups. Also a greater proportion of veterinary surgeons in private practice had positive tests than those in Ministry employment. All three tests showed these differences, but the A.H.G. and complement-fixation tests gave many more positive results than the standard agglutination test. Many of the sera were positive in the higher dilutions by the A.H.G. and complement-fixation tests.

Ministry of Agriculture non-veterinary assistants

The Ministry of Agriculture also employ non-veterinary field staff to assist in eradication and meat inspection. Their work is mainly clerical but they do have some practical duties. Twenty-six sera were examined from this group. Their tests showed some evidence of exposure to brucella antigen, but the number was small and detailed comparison not possible.

Artificial insemination workers

The workers from one of the Ministry of Agriculture Artificial Insemination Plants were also submitted to serological investigation. There were only seven sera and all of them were negative to all three tests. In addition, the results of the Ministry veterinary surgeons include two qualified veterinary surgeons working in artificial insemination. Their tests were entirely negative.

Abattoir workers

Abattoir and meat packing plants in Northern Ireland employed 1005 people in 15 different centres in 1967. From these 243 sera were obtained.

This group showed an incidence of reactions considerably higher than the rural and urban groups with all three tests. Except with the complement-fixation test, many of the sera were positive in the higher dilutions.

Dairy farmers or farm workers

Ninety-two sera were obtained from farmers or farm workers engaged in dairy farming in Northern Ireland; these were divided between Counties Armagh, Down, Fermanagh and Londonderry. No samples were available from Counties Antrim or Tyrone.

The serum agglutination test in this group gave a proportion of reactions similar to but rather lower than those of the veterinary surgeons in private practice. There was also a considerable number of reactions with the other tests, some of which persisted at higher dilutions.

Occupational exposure

The influence of occupation in Northern Ireland on the prevalence of serologically positive tests for brucella antibody can be seen from Table 2.

Sera of persons in Northern Ireland with no known exposure to cattle, whether living in urban or rural settings, showed a very low incidence of serological positives for *Br. abortus* when measured by the standard agglutination test. In fact, only one out of 1330 sera in this category had a titre as high as 1/80. Those working in occupations bringing them into contact with cattle showed a much greater proportion of positives. The amount of exposure and subsequent antibody production seemed to vary with occupation. Veterinary surgeons in private practice contributed most to this group, but those employed by the Ministry in field work, by abattoirs or in dairy farming were also very much at risk.

More positives were obtained with either the A.H.G. test or the complement-fixation test than with the serum agglutination test in groups which were occupationally exposed. These tests were often positive at high dilutions.

DISCUSSION

In the only previous serological survey for brucellosis in Northern Ireland, Murdock (1944), using the standard agglutination test, found 6.5% of 2073 sera submitted by blood donors with a titre of 1/40 or greater against *Br. abortus*. He considered part of his sample to be rural. In this present study, even including 252 sera from what was thought to be a rural and probably a symptomatic group, only 0.1% of 1330 sera had a titre as high as 1/40. This suggests that there is now a smaller proportion of persons with brucella antibodies in Northern Ireland, but the lack of standardization in the tests and in the materials used for the tests makes exact comparisons between such surveys difficult.

Murdock estimated that, if the proportion of positive results in his series was representative of the whole province, the total number expected to show specific agglutinins against *Br. abortus* would have been about 81,000. From this present study the comparable figure would be 1500, though the population has risen by approximately 250,000 to one and a half million.

Recent brucellosis studies in England on blood transfusion sera give conflicting reports. In the Isle of Wight, Brodigan, McDiarmid, Mann & Skone (1961) found about 17% to have brucella antibodies, with 5% showing a titre of at least 1/80. However, Bartram *et al.* (1963) in a large series from the Oxford area, found only 1.1% positive, with 0.4% at higher titres. Both of these surveys were carried out between 1957 and 1959 with the serum agglutination test. Bartram *et al.* (1963) suggested that different exposure to raw, potentially infected milk might account for the wide variation between the findings of these two surveys. In 1959–1960, 95% of milk was pasteurized in the Oxford area, but only 77.3% in the Isle of Wight. Also, Brodigan *et al.* (1961) had found positive titres in 70% of sera from 27 persons known to be drinking raw milk infected with brucella organisms.

Murdock believed that the exposure encountered in his survey was milk-borne,

and it has long been held that ingestion of raw infected milk is the principal method of transmission of brucellosis in Great Britain (Dalrymple-Champneys, 1960; Bothwell, 1963; Parry, 1966). The brucellosis eradication in cattle, together with the amount of pasteurization of milk and the special regulations pertaining to milk retailed raw in Northern Ireland may explain why Murdock's figures differ from this present survey; but this fails to explain why, despite the differences in milk regulations and brucellosis legislation, the figures for the Oxford survey and the present Northern Ireland one are similar.

There are several factors which may contribute to this. Firstly, the surveys may not be comparable; the difficulties relating to laboratory standardization have already been mentioned. Secondly, as far as the general population is concerned, milk may be the vehicle of infection and the amount of unpasteurized milk consumed may be similar in the two areas. Thirdly, a proportion of normal individuals may possess antibodies which agglutinate *Brucella abortus* at low titres. Lastly, serological titres may persist for long periods, even up to 10 years, despite the absence of continuing exposure to infection (Dalrymple-Champneys, 1929; Bartram *et al.*, 1963; McDevitt, 1968). Thus it may be too early to assess the effects of the brucellosis eradication programme by this means.

The results of this survey suggest that brucellosis or certainly the acquisition of brucella antibodies in Northern Ireland is now mainly an occupational hazard, as in America (Spink, 1956). However, it is worth remembering that people, occupationally exposed to infected animals, with the exception of abattoir workers, are also more likely to have access to raw milk (Barrett & Rickards, 1953; Bothwell, 1963). Close proximity to cattle is not the only factor as the Ministry veterinarians and their helpers, whose duties relate mostly to sampling blood, milk or vaginal mucus, seem less exposed than veterinary surgeons in private practice or dairy farmers. The major hazard appears to be the intimate contact with material infected by *Br. abortus* that bovine 'midwifery' involves, and, although veterinary surgeons in private practice are most at risk (Huddleson & Johnson, 1930; Kerr, Coghlan, Payne & Robertson, 1966*a*), farmers and farm workers may also acquire infection in this way.

Handling infected carcasses is a real hazard, as is shown by American experience in the meat packing industry (Spink, 1956). Brucellosis eradication has increased the number of infected animals being slaughtered, and, if the farmer claims that his condemned cow is pregnant, which entitles him to increased compensation, then careful examination is required. Adequate safety precautions at this point are, therefore, necessary and justify more attention.

Various authors in previous surveys have commented on the greater sensitivity of the A.H.G. test in the detection of 'incomplete' brucella antibodies (Ferris, Stevenson & Lewis, 1953; Coetzee, 1956; Zoutendyk, 1958; Schrire, 1962). The results of this present study would confirm this. In the occupationally exposed groups, the A.H.G. and complement-fixation tests revealed many more positives than the direct agglutination test; this was not so with the groups of blood transfusion and farmer's lung sera so far as the complement-fixation test was concerned. More sensitive tests might be clinically useful, but it is worth remembering that a

positive serological test in a patient does not necessarily prove a diagnosis of active brucellosis.

Enthusiasm for these more sensitive tests may be reflected in the number of cases of brucellosis notified to the Ministry of Health and Social Services in Northern Ireland. Whereas 63 cases were reported between 1949 and 1959 (Bothwell, 1963), and 20 cases were notified from 1960–1966, 44 cases were notified in 1967 alone (T. T. Baird, pers. comm.) at a time when, despite the brucellosis eradication programme, interest was being renewed in the A. H. G. test as a diagnostic tool in human brucellosis (Kerr *et al.* 1966*b*) and facilities for carrying it out were becoming available in the province.

Although it may be too early to assess the effects of the eradication of bovine brucellosis in the human population by serological methods, this survey does provide a basis from which the future effects may be estimated.

SUMMARY

A serological survey for brucella antibodies has been carried out in Northern Ireland in an attempt to assess the effects of the brucellosis eradication programme in cattle which has been in progress since 1959. One thousand eight hundred and ninety four sera from blood donors, from farmer's lung tests, and from various groups occupationally exposed to cattle were examined by the serum agglutination test, the anti-human globulin (A.H.G.) (Coombs) test and the complement-fixation test.

The incidence of brucellosis in Northern Ireland is now less than it was 20 years ago, but is similar to that reported in a recent survey in England. It now appears that the principal method of transmission of brucellosis in the province is by occupational exposure rather than by drinking infected milk. Though it may be too early to make a serological assessment of the effects on human infections of brucellosis eradication in cattle, this survey provides a basis for future evaluation.

We thank Prof. O. L. Wade, Prof. P. C. Elmes and Dr W. R. Kerr for their encouragement and helpful advice; the Medical Officers of Health in Antrim, Armagh, Down and Fermanagh, Londonderry, and Tyrone, and in Belfast and Londonderry County Borough for help in obtaining blood samples; Dr M. C. Huth of the Northern Ireland Blood Transfusion Service, for sera; Dr D. W. R. Mackenzie and Miss H. E. Cairns for sera from the Mycology Diagnostic Laboratory; Dr J. H. Connolly for sera from the Virus Reference Laboratory, and for helpful advice; the farm workers and veterinary surgeons for volunteering blood samples; the technical staff of the Veterinary Research Laboratories for carrying out the tests.

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