BRUCELLA ABORTUS AGGLUTININS IN THE SERA OF PREGNANT WOMEN AND BLOOD DONORS

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The incidence of brucellosis in the human population of England and Wales is still not accurately known, although estimates have been made (Wilson, 1932; Dalrymple-Champneys, 1950). Few persons who acquire *Br. abortus* develop any obvious clinical disease, but the existence of *Br. abortus* agglutinins in blood sera indicates past exposure to the organism.

A survey of human and animal brucellosis made between 1957 and 1959 in the Oxford area (Bothwell, 1960 a, b, c, 1961; McDiarmid, 1960a, b, 1961) included the examination of 3,431 sera samples from pregnant women and of 8,690 samples of sera from blood donors for the presence of *Br. abortus* agglutinins. Both types of sera are readily available; both have limitations for survey purposes. Blood donor serum cannot be regarded as a normal sample compared with a cross-section of the general population, since blood donors are in fact highly-selected healthy persons. The serum from pregnant women also constitutes a selected sample. The incidence of agglutinins in both samples may represent an under-estimate of the incidence of agglutinins in the general population.

The results of both surveys are given, with notes on the investigation of milk supplies consumed by pregnant women with agglutinins to *Br. abortus*.

METHODS

3,431 samples of blood sera from pregnant women, submitted between 1957 and 1959 for Rhesus and Kahn testing, were examined for *Br. abortus* agglutinins at the Oxford Public Health Laboratory,

using the standard Public Health Laboratory Service (PHLS) antigen and reading dilutions of 1/25 to 1/250. The samples were drawn from the population of the northern two-thirds of Oxfordshire, but not from the city of Oxford. The 3,431 samples represented about 90 per cent. of all pregnancies in the area sampled.

The 8,690 samples provided by the Oxford blood transfusion unit were drawn from a wide area in the Oxford region between 1958 and 1959; they were examined at the Agricultural Research Council's Field Station at Compton, using final dilutions of 1/10, 1/20, 1/40, 1/60, etc. WHO/FAO antigen was used and, taking 50 per cent. agglutinations as the end-point, positive reactions were recorded at dilutions of 1/10 and above. Tests for blocking antibodies were not made.

RESULTS

SERA FROM PREGNANT WOMEN

This serum survey made use of routinely-collected ante-natal serum samples; thus only pregnancies lasting more than 3 months are included. To ascertain fully the risk of abortion in the human subject, as reported by Huddleson (1943), Williamson (1944) and Dalrymple-Champneys (1960), it would be desirable to survey a group of women at risk of pregnancy and to follow their conception and early pregnancy history. Most of the pregnant women whose sera gave a positive result were in the age group 20 to 25 (range 17 to 35). The stage of pregnancy at which serum was tested was usually between 3 and 5 months.

Of the 3,431 sera examined, 35 (1·02 per cent.) were positive at a level of 1/25 or greater, as shown in Table I, which also shows the distribution of occupations at the various titres.

TABLE I
RANGE OF *BR. ABORTUS* AGGLUTININ TITRES IN 3,431
SERUM SAMPLES FROM PREGNANT WOMEN (PHLS
ANTIGEN) AND OCCUPATIONAL DISTRIBUTION

Titre	No. of Samples	Occupation	
		Agricultural, Dairying and Veterinary work	Other or Unknown
1/25	12	2	10
1/50	15	5	10
1/100	2	1 (farm domestic)	1
1/125	2	1 (veterinary worker)	1
1/250	1	—	1
1/1000	1	—	1
1/2500	1	1 (farmer)	—
1/5000	1	1 (farmer's wife)	—

Proportion of positive sera 35 out of 3,431 (1·02 per cent.).
Proportion of 1/100 or over 8 out of 3,431 (0·23 per cent.).

Of the 35 positive cases, eleven (31·5 per cent.) had some connexion with occupations concerned with cattle, twenty (57·1 per cent.) had no such connexion, and in four (11·4 per cent.) the occupational background was unknown. The highest titres (1/2500 and 1/5000) were found in a farmer's wife and a woman farmer.

Among cases of clinical brucellosis recorded in England and Wales, 22·8 per cent. have an occupational connexion with farming (PHLS figures for 1957: Wilson, 1961).

Nearly all the positive sera in this series came from

women who lived in rural areas and would have had opportunities to consume raw milk in the recent past, and the eleven women living on farms or otherwise connected with cattle also had opportunities for contact with infected animals.

Definite histories of recent consumption of raw milk were obtained from fifteen of the 35 women with *Br. abortus* agglutinins (twelve drank T.T. milk, and three ungraded milk).

24 (68·6 per cent.) could be classified as rural residents, eight (22·9 per cent.) as living in a small town, and two (5·7 per cent.) as city dwellers; in the remaining case the place of residence was unknown.

Questions about symptoms suggestive of brucellosis in the history of these 35 women revealed only one with a history of "glandular fever". The pregnancies were all uneventful and normal babies resulted, although eight had a titre at or in excess of 1/100. In addition, Dr. J. M. Payne of Compton examined the placenta from twelve of the women, and found no evidence of brucella organisms or lesions suggestive of brucellosis.

In five of these positive cases (14·3 per cent.), a follow-up resulted in the discovery of an infected herd of cows (Table II). This compares with an infection rate of about 5 per cent. in herds in general in this area on one sampling (McDiarmid, 1960b). Four of these five cases, however, came from an area where herd infection is in any case quite high; and three of these lived on a farm. Eight clinical cases of human brucellosis had occurred in this particular area in the previous 8 years.

TABLE II
DETAILS OF *BRUCELLA*-INFECTED HERDS FOUND BY MILK SUPPLY INVESTIGATIONS IN FIVE PREGNANT WOMEN
WITH BLOOD SERUM AGGLUTININS TO *BR. ABORTUS*

Case No.	Residence	Occupation		Titre		Milk Supply and Other Circumstances
		Self	Husband	1st	2nd	
1	Village	Clerical	Factory Worker	1/50	1/10	Present supply pasteurized, but her brother, a farm worker, brought home raw milk which she drank in preference to pasteurized One cow at farm aborted, found positive to <i>Brucella</i> , removed from herd, and slaughtered just before patient's notification Remainder of herd of 57 animals negative
2	Village	Domestic on Farm	Bricklayer	1/100	1/50	Lived 3½ years on farm and drank raw milk, but since marriage, pasteurized milk <i>Brucella</i> isolated by culture from bulk milk at farm. Four infected cows found in herd of forty.
3	Farm	Housewife	Farmer	1/50	1/5000	Milk supply T.T. Had no symptoms of undulant fever <i>Brucella</i> isolated from table jug of milk One cow in herd had aborted and had positive blood titre
4	Farm	Domestic on Farm	Farmer	1/50		Own T.T. herd. History of abortion on farm. Brother in hospital with undulant fever in 1952 Milk consumed by patient positive to <i>Br. abortus</i> . Twelve cows in herd
5	Village	Domestic Worker	Estate Worker Forestry	1/25		Present milk supply consumed for past year T.T. Previous supply pasteurized <i>Brucella</i> isolated from farm milk by culture. Some 10 gallons retailed daily. Two cows positive

FIGURE.—Localization of infected herds, clinical cases of human brucellosis, and blood serum specimens from pregnant women showing agglutinins to *Br. abortus*. The starting point for action to remove brucellosis infection might be either of these three sources.

The Figure shows the localization of infected farms in the area in 1959–60, the clinical cases of human brucellosis there during the previous 8 years, and the pregnant women with agglutinins to *Br. abortus* in 1957–59. Elimination of the disease could be effected by following up clinical cases, persons with positive agglutinins, or infected herds; or all three. Examination of blood sera for *Br. abortus* agglutination as part of the routine testing of blood samples would be useful in cases of pregnant women who live on farms and drink raw milk, so that positive cases could be followed up.

Only 1·02 per cent. of this series of pregnant

women had detectable agglutinins, while of female blood donors 0·87 per cent. were in this category (Table III); this suggests that, irrespective of the

TABLE III
BR. ABORTUS AGGLUTININS IN 8,690 MALE AND FEMALE BLOOD DONORS

Sex	No. Examined	No. Positive	Per cent. Positive 1/10 or Over	Male : Female Ratio of Positives
Male ..	4,345	58	1·33	} 1·5 : 1
Female ..	4,345	38	0·87	
Total ..	8,690	96	1·1	

lack of information about first trimester abortions, *Br. abortus* does not play much part in early abortion in women.

BLOOD DONOR SERA

Of 8,690 sera examined from blood donors, 96 (1.1 per cent.) had titres at 1/10 or over and 36 (0.41 per cent.) had titres at 1/20 or over. The proportion of positives in males and females is shown in Table III and the age and sex distribution of positives in Table IV.

TABLE IV
AGE AND SEX DISTRIBUTION OF 96 POSITIVE CASES
AMONG BLOOD DONORS

Sex	Age Group (yrs)					Total
	20-29	30-39	40-49	50-59	Not Known	
Male ..	26	15	6	9	2	58
Female ..	10	12	11	5	0	38
Total ..	36	27	17	14	2	96

The range of titres and the occupational distribution of the positive cases are shown in Table V. The highest titre in the series, 1/160, occurred in a farmer. Of the 96 persons with positive titres, nine (all males) were engaged in farming or allied occupations, 67 were in other kinds of occupation, and in twenty the occupation was unknown. There were 33 persons classifiable as of rural habitation, 33 lived in small towns, and 30 were city dwellers.

TABLE V
RANGE OF *BR. ABORTUS* AGGLUTINATION TITRES
(WHO/FAO ANTIGEN) IN 96 POSITIVE CASES AMONG
BLOOD DONORS, BY OCCUPATION

Positive Titre	Number of Samples			Occupation		
	Male	Female	Total	Farm, Dairy, etc.	Other	Un-known
1/10	40	20	60	3 farming, 3 students of agriculture	40	14
1/20	13	14	27	0	23	4
1/40	4	4	8	1 laboratory worker in dairy 1 student of agriculture	4	2
1/160	1	0	1	1 farmer	0	0
Total	58	38	96	9	67	20

The titres recorded from transfusion sera were mostly low, only 0.41 per cent. having titres at or above 1/20. Unfortunately no direct comparability factor is yet available to allow conversion to the

PHLS antigen scale, and the readings might have been higher if the more sensitive PHLS antigen had been used.

The excess of positives in males (ratio 1.5 : 1) is similar to the sex-ratio in cases of clinical brucellosis, but in the present series the excess occurred entirely in the group with titres of less than 1/20.

The number of persons occupationally connected with farming (nine out of 96, 9.4 per cent.) is lower than for clinical brucellosis, which in Great Britain shows 20 to 30 per cent. of occupational cases. The rural : urban ratio of cases was 2 to 1, corresponding to that in the clinical cases. No significant history or symptoms were recorded in the blood transfusion notes about these donors.

The existence of brucella agglutinins in blood transfusion serum specimens is in itself of some interest, in view of the obvious danger of transmitting the infection by transfusion. Wood (1955) has already recorded such an instance in England; a donor developed brucellosis after blood had been taken, a titre of 1/1280 being recorded; the recipient became unwell 13 weeks after the transfusion, the titre of brucella agglutinins rising to 1/2550. This patient had red cell aplasia and damage to the reticulo-endothelial system, and might have been less able to deal with even a few transfused organisms.

In the U.S.A., Spink and Anderson (1950) found that 302 (18.56 per cent. of 1,627 transfusion blood samples had agglutinins to *Br. abortus*, but only 1.66 per cent. of them at 1/160 or above. Viability of the three species of *Brucella* in transfusion blood was stated to be up to 6 months at 4°C., yet no case of transmission had been recorded from blood transfusions performed at the University of Minnesota. The chance of infection from the small number of organisms transmitted in this way was considered particularly remote for *Br. abortus* but conceivable for the more virulent *Br. melitensis*. Definite transmission by this route has been recorded in Mexico, confirmed by blood culture (Alvarez and Brito, 1944).

COMPARISON OF *Br. abortus* AGGLUTININS WITH INCIDENCE IN OTHER SURVEYS (Table VI, opposite)

The percentages of positive agglutination to *Br. abortus* at over 1/80 found in the present series of pregnant women and of blood donors appear to be much lower than that found in earlier surveys, but differences in the antigen and in the population sampled make conclusions about trends hazardous.

There have been considerable changes in the exposure of the general population to raw milk and thus to possible infection with *Br. abortus*—in 1930 less than 50 per cent. of milk was heat-treated

Group		Date Survey	Author	Date of Publication	Area of Survey	No. Tested	Percentage Positive over 1/80
(A) P.U.O. Sera		1929-32 1932 1937	Wade O'Callaghan Menton	1933 1932 1937	England Eire Staffordshire	1,331 416 500	2·1 2·1 7·0
(B) WIDAL Sera		1931 1932 1933-45	O'Callaghan Wilson Soltys	1931 1932 1946	Eire Average of several surveys England	336 1,358 4,925	2·7 3·5 2·7
(C) Wassermann Reaction Sera		1928 1945 1948-53	Harrison and Wilson Soltys Lawy and Hampson	1928 1946 1956	England England England	998 1,392 8,209	0·7 1·1 0·3
(D) Recent Surveys	Pregnant Women	1957-59			Oxfordshire	3,431	0·23
	Blood Donors					8,690	0·41*
	Pregnant Women and Blood Donors	1958-59	Brodigan and Others	1961 1961	Isle of Wight	1,395	3·0
	Psychiatric In-Patients ..					132	4·6

whereas in 1962 this proportion was nearer 95 per cent.—and lower levels of positive agglutinins would therefore be expected. Evidence is available that brucella antibodies persist for a long time, certainly for years; Dalrymple-Champneys (1929) quoted Eyre as stating at least 10 years, and one of us (A.M.) has noted the persistence of agglutinins to *Br. abortus* in a laboratory technician for 8 years. There is, however, no way of knowing whether titres from say 1/10 to 1/80 or even higher represent recently acquired antibodies or merely the persistence of past infection.

agglutination. Schrire (1962), reviewing serologic surveys for brucella agglutinins in South Africa, cited the studies of Coetzee (1956) showing 61·2 per cent. with Coombs technique against 19·4 per cent. using the ordinary test. Use of a blocking antibody test in our surveys might have revealed more cases with agglutinins and a further group of pregnant women would then have become available for observation in regard to abortion and course of pregnancy.

We have referred to the variation in antigens used in England and Wales (as between PHLS and WHO/FAO), and other instances are known in which test material is incubated at 37°C. and not at 52°C., though it is not certain that the temperature used is critical. It is not unlikely that there are variations in the techniques used to detect incomplete antibodies and to obviate prozone difficulties. Since surveys of the incidence of sub-clinical or latent infection, and 95 per cent. of clinical diagnoses of brucellosis, are made on the basis of agglutination tests, the materials and technique of the test should receive attention with a view to standardization. Until such standardization is achieved, we suggest that comparability of survey results might be obtained if samples of serum were tested in parallel with both WHO/FAO antigen and the PHLS antigen.

Coombs, Mourant, and Race (1945a, b) described the detection of incomplete or blocking antibodies to *Brucella* organisms. Wilson and Merrifield (1951) found that the test revealed a further 56·6 per cent. of cases compared with the conventional

SUMMARY

We have analysed the results of *Br. abortus* surveys of 3,431 sera from pregnant women and 8,690 sera from blood donors in the Oxford area between 1957 and 1959. Public Health Laboratory Service (PHLS) antigen was used to test the pregnancy sera, and WHO/FAO antigen for the blood donor sera. 1.02 per cent. of pregnancy sera contained agglutinins to *Br. abortus* at titres of 1/25 or over. 1.33 per cent. of male and 0.87 per cent. of female blood transfusion sera contained agglutinins at titres of 1/10 or over. 0.23 per cent. of pregnancy sera gave a titre of 1/100 or over. 0.41 per cent. of transfusion sera had titres of 1/20 or over.

The presence of *Br. abortus* agglutinins, as detected by the simple tube test, does not appear to be associated with human abortion, but the problem should be studied in a series using a blocking antibody test.

In five out of 35 pregnant women with *Br. abortus* agglutinins, the organism could be isolated from the milk supply. Routine examination of sera from pregnant women for *Br. abortus* agglutinins may frequently reveal infected herds if the positive cases are followed up.

Accurate comparison with the results of earlier surveys is not possible, because of differences in the antigens used and in the samples surveyed, but the current series appear to show much lower *Br. abortus* agglutinin titres than earlier surveys.

Our PHLS antigen study on pregnant women's sera had 0.23 per cent. positives at 1/100 or over, compared with 3 and 5 per cent. at 1/80 and over in two recent surveys in the Isle of Wight. Milk pasteurization in the Oxford area was about 95 per cent., however, and in the Isle of Wight 77 per cent.; the differing levels of antibody in these populations can probably be attributed to differences in the duration of exposure to raw infected milk.

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