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Serum antibody response in acute brucellosis

By I. D. FARRELL, L. ROBERTSON and P. M. HINCHLIFFE
Public Health Laboratory, Royal Infirmary, Meadow Street, Preston PR1 6PS

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SUMMARY

The serum antibody titres in 62 cases of acute brucellosis are presented. The antibody persisting after infection and the appearance of non-agglutinating anti-human globulin titres were investigated. The criteria for the serological diagnosis of acute and chronic brucellosis are discussed.

INTRODUCTION

The clinical diagnosis of brucellosis is difficult because of the protean nature of the disease, which can vary from an acute febrile illness to a low grade chronic disease, e.g. inflammation of bones and joints, encephalitis and even undefined psychotic illness. In the United Kingdom, where the indigenous organism is *Brucella abortus*, many infections are atypical in that a classical undulant fever is rarely observed.

The incidence of positive blood cultures is disappointingly low and is probably not often as high as the 10–20% estimated by Wilson & Miles (1964) and since culture of biopsy material is seldom attempted a definitive laboratory diagnosis of brucellosis is not often obtained. The laboratory diagnosis is therefore mainly based on the detection and titration of brucella antibodies in the serum.

In this communication the titres of brucella antibodies in the serum of 62 patients with acute brucellosis are reported. They were monitored at 2–3 monthly intervals after the diagnosis, in some cases for more than three years.

MATERIALS AND METHODS

The patients included in this series were suffering from a febrile illness which was considered to be acute brucellosis on clinical, laboratory, and epidemiological evidence. For at least one year after the diagnosis and treatment of the infection sera were examined for the presence of brucella antibodies with the standard agglutination, mercaptoethanol agglutination, complement fixation, and the anti-human globulin tests.

The standard agglutination test

Doubling dilutions of serum were made in 0·5 ml. volumes of phenol saline (0·85% NaCl containing 0·4% phenol) in 50 × 12 mm round-bottomed agglutina-

tion tubes. To each dilution was added an equal volume of *Brucella abortus* antigen (PHLS)* diluted 1/10. Each serum was titrated to at least 1/640 to avoid errors due to prozone phenomena. The tubes were kept at 37° C. for 2 days in a covered water bath and read in indirect light in a viewing box according to the criteria described by Kerr *et al.* (1968). The titre was recorded as the reciprocal of the highest dilution of serum to give partial or complete agglutination.

The mercaptoethanol agglutination test

This test was carried out and read in the same manner as the standard agglutination, except that the serum dilutions were prepared in 0·85% NaCl containing 0·05 M 2-mercaptoethanol.

Anti-human globulin (Coombs) test

The same range of serum dilutions as in the standard agglutination test were prepared in phosphate buffered saline pH 7·2 (PBS) in 50 × 9 mm. round-bottomed tubes in 0·5 ml. volumes. An equal volume of *B. abortus* antigen (PHLS) diluted 1/5 was added to each serum dilution. The tubes were incubated at 37° C. for 24 hr. and any serum dilutions showing either partial or complete agglutination were recorded and removed. The remaining tubes were centrifuged at 2000*g* for 15 m., the supernatant discarded, and the deposit resuspended in PBS. This process of centrifugation and resuspension was repeated three times. After the final washing the cells were resuspended in 0·9 ml. of PBS and to each tube 0·1 ml. of suitably diluted anti-human globulin was added and thoroughly mixed. The final dilution of AHG was that recommended by the manufacturer.† The tubes were kept at 37° C. for a further 24 hr. and examined for agglutination. The test was considered positive when a fourfold or greater difference was recorded between the agglutination titres before and after treatment with AHG.

The complement fixation test

The tests were set up in WHO plastic plates using a four-volume technique with veronal buffer as diluent, and serum dilutions starting at 1/10. The *B. abortus* antigen (PHLS) was diluted 1/60 (this was found to be the optimal concentration using the method described by Kerr *et al.* 1968). The haemolytic system was standardized and prepared as described by Bradstreet & Taylor (1962). Overnight fixation at 4° C. was used with 1·5 haemolytic units of complement. We define one haemolytic unit as the highest dilution of complement to give complete lysis of the sensitized cells in the presence of pooled negative serum and antigen at the dilutions used in the test.

RESULTS

The serum antibody titres in 62 cases of acute brucellosis were measured by the standard agglutination (SA), mercaptoethanol agglutination (ME), and the complement fixation (CF) tests. In the first serum tested at the time of laboratory

* *B. abortus* 'concentrated O suspension' produced by the Standards Laboratory, Colindale.

† Antihuman globulin supplied by Burroughs Wellcome & Co.

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Table 1. *Brucella antibodies in the serum of patients at the time of diagnosis of brucellosis* (62 *cases*)

Titre	No. of patients showing stated titre by: Standard agglutination test	2-Mercaptoethanol agglutination test*	Complement fixation test†
< 20	0	2 (4)	4 (7)
20	0	2 (4)	2 (3)
40	0	3 (5)	1 (2)
80	1 (2)	6 (11)	8 (13)
160	1 (2)	8 (14)	7 (12)
320	11 (18)	13 (23)	8 (13)
640	22 (35)	13 (23)	11 (18)
1280	13 (21)	6 (10)	8 (13)
⩾ 2560	14 (23)	4 (7)	11 (18)

* Five sera not tested. † Two sera not tested.
Figures in parentheses are percentages.

Table 2. *Brucella antibodies in the serum of patients approximately one year after infection* (58 *cases*)

Titre	Number of patients showing stated titre by: Standard agglutination test	2-Mercaptoethanol agglutination test*	Complement fixation test
< 20	6 (10)	20 (36)	21 (36)
20	3 (5)	8 (14)	14 (24)
40	17 (29)	12 (21)	10 (17)
80	18 (31)	10 (18)	4 (7)
160	10 (17)	3 (5)	4 (7)
320	4 (7)	3 (5)	3 (5)
640	0	0	1 (2)
1280	0	0	1 (2)
⩾ 2560	0	0	0

* Two sera not tested.
Figures in parentheses are percentages.

diagnosis of the illness, all 62 patients had SA titres of ⩾ 80 and 60/62 (97%) had a titre ⩾ 320. Mercaptoethanol-resistant agglutinins were present in 55/57 (96%) of the sera examined and 50/57 (88%) had a ME titre of ⩾ 80. In the two sera in which mercaptoethanol resistant agglutinins were absent, the SA titre was ⩾ 640; in both patients symptoms were of recent onset. CF antibodies were detected in 56/60 (93%) of the sera examined and 53/60 (88%) had a CF titre of ⩾ 80, a pattern similar to the ME test. In four patients CF antibodies were initially absent but appeared later during the course of the disease.

A further specimen of serum was examined from 58 of the patients approximately one year after the diagnosis of acute brucellosis. By this time 6/58 (10%) sera had a negative SA test and ME resistant agglutinins were absent in 20/56 (36%)

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Table 3. *Brucella antibodies in the serum of patients approximately two years after infection* (34 *cases*)

Titre	No. of patients showing stated titre by: Standard agglutination test	2-Mercaptoethanol agglutination test	Complement fixation test
< 20	5 (15)	20 (59)	15 (44)
20	6 (18)	8 (24)	9 (26)
40	14 (41)	4 (12)	5 (15)
80	6 (18)	1 (3)	4 (12)
160	2 (6)	1 (3)	1 (3)
320	1 (3)	0	0
640	0	0	0
1280	0	0	0
⩾ 2560	0	0	0

Figures in parentheses are percentages.

Table 4. *Brucella antibodies in the serum of patients more than three years after infection* (15 *cases*)

Titre	No. of patients showing stated titre by: Standard agglutination test	2-Mercaptoethanol agglutination test	Complement fixation test
< 20	4 (27)	10 (67)	10 (67)
20	5 (33)	2 (13)	1 (7)
40	0	2 (13)	3 (20)
80	5 (33)	1 (7)	0
160	1 (7)	0	1 (7)
320	0	0	0
640	0	0	0
1280	0	0	0
⩾ 2560	0	0	0

Figures in parentheses are percentages.

sera. There had been a fall in the titres of brucella antibodies in that 26/58 (45 %) sera had an SA titre < 80, 40/56 (71 %) had a ME titre < 80, and 45/58 (78 %) had a CF titre < 80 (Table 2).

Two and three years after the diagnosis the antibody titres continued to fall, particularly the ME-resistant agglutinins and CF antibodies (Tables 3 and 4). Of the 15 patients whose serum was examined three years or more after the infection, 10/15 (67 %) had neither ME resistant agglutinins nor CF antibodies in their serum. The SA titre persisted longer in that 29/34 (85 %) sera contained agglutinins two years after the infection, and 11/15 (73 %) three years after the infection.

Each serum was tested for brucella antibodies with the anti-human globulin (AHG) test. A positive AHG titre was demonstrated in 32/58 (55 %) of the sera examined one year after the infection. This did not appear to decline with time

in that after two years 19/34 (56 %) sera and after three years 11/15 (73 %) sera had a positive AHG titre.

DISCUSSION

Wilson & Miles (1964) considered that a clinical illness developed in only a small proportion of persons infected with *B. abortus*. They were of the opinion that subclinical infection leads to latent immunization, particularly in veterinarians and other persons in close occupational contact with the reservoir of infection. This view has recently been endorsed by Henderson (1973). Therefore any criteria for the serological diagnosis of brucellosis must take into account the possibility of past infection, particularly subclinical and undiagnosed infections.

From the present investigation it is evident that after a brucella infection agglutinins were still present in 85 % of sera two years later whereas CF antibodies were present in 56 % of sera (Table 3). These findings are in agreement with those of Molinelli (1950) in Argentina, who found that brucella agglutinins persisted for four years in 80 % of cases. Dooley (1932), during an outbreak of brucellosis among schoolchildren, observed that, in 9 cases of subclinical brucella infection in which the SA titres were $\geqslant$ 320, agglutinins were detectable in their serum approximately one year after the infection.

In this investigation 62 clinical cases of acute brucellosis were studied, none of whom were veterinarians or persons in continual occupational contact. At the time of diagnosis the SA titre was $\geqslant$ 80 in all cases and in 14 (23 %) the SA titre was $\geqslant$ 2560. It has been suggested by Wilson & Miles (1964) and Henderson (1973) that SA titres $>$ 640 are indicative of active brucella infection. However, it is our experience that a definitive diagnosis of brucellosis in persons in close occupational contact cannot be established on serological findings alone. In a number of instances veterinarians with SA levels $>$ 640 and thought to be suffering from acute brucellosis because of this laboratory finding have been proved, by subsequent clinical and laboratory investigations, to be suffering from other diseases.

It is important to remember that the incubation period of brucellosis can be as long as six months and that the patient may have been ill for many weeks or months before a diagnosis of brucellosis has been considered; in these cases the presenting titres will be high. On the other hand in persons whose symptoms are of recent onset, the presence of low antibody titres does have some significance and in such instances a rising titre in the ME and CF tests may be demonstrable and can be of considerable help in establishing the diagnosis. However, it is those with an SA titre of $>$ 80 together with ME resistant agglutinins and CF antibodies who are most likely to have active brucellosis. It is important to realize that there must be different criteria for the diagnosis in persons at special occupational risk (e.g. veterinarians), compared with those who become infected by consuming infected dairy products.

Acute brucellosis may resolve spontaneously or may progress to the chronic condition if inadequately treated. Chronic brucellosis is a poorly defined clinical state and, when suspected, laboratory confirmation is frequently requested. Kerr *et al.* (1968) described an AHG test based on that of Wilson & Merrifield (1951)

Table 5. *The detection of non-agglutinating antibody in serum after a brucella infection by the anti-human globulin test* (62 *cases*)

Time after infection	Number of cases	AHG test negative	AHG test positive with 4-fold rise	AHG test positive with > 4-fold rise	Total AHG positive
1 year	58	26 (45)	13	19	32 (55)
2 years	34	15 (44)	9	10	19 (56)
⩾ 3 years	15	4 (27)	3	8	11 (73)

Figures in parentheses are percentages.

and advocated its use in the diagnosis of chronic brucellosis. They stated that a positive AHG test when accompanied by CF antibodies and taken in conjunction with clinical evidence may support a diagnosis of chronic brucellosis. In the present investigation the AHG test was positive in over 50% of cases and remained positive for at least three years (Table 5), even though no clinical evidence of brucellosis persisted after the acute infection was treated with antibiotics. In some cases CF antibodies persisted in low titres but in most they were absent.

It appears that the diagnosis of chronic brucellosis rests on the interpretation of clinical symptoms, as the serological picture thought to be indicative of chronic brucellosis may be found in persons after an acute infection with no clinical evidence of chronic disease. The difficulty of diagnosis in both the acute and chronic disease is greatest among those in occupational contact, many of whom will have high antibody titres. A serological test which reliably indicates the presence of active disease has been eagerly sought; but it has not yet been found.

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