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The use of the conglutinating complement fixation test in the diagnosis of human brucellosis

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SUMMARY

The conglutinating complement fixation test was compared with the haemolytic complement fixation test for the detection of brucella antibodies in human sera. Conglutinating-complement-fixation antibodies were detectable during the early stages of acute brucellosis and persisted for approximately 1 year after the infection.

INTRODUCTION

Conglutinin is a globulin present in the serum of normal ruminants which agglutinates red cells that have adsorbed complement and which can also react with complement adsorbed by an antigen-antibody reaction. The conglutinating complement fixation (CCF) test is an indirect method of detecting antibody in serum shown by the decrease in the activity of standardized amounts of conglutinating complement. This is indicated when sensitized sheep red cells do not agglutinate in the presence of conglutinin because the conglutinating complement has been fixed by an antigen-antibody reaction. The CCF test differs from the haemolytic complement fixation (HCF) test in the source of complement as well as the method used to detect its fixation. Whereas the guinea-pig is a satisfactory source of haemolytic complement the horse, pig, and cat are sources of conglutinating complement.

The CCF test has been used in the serological diagnosis of a number of diseases in both man and animals (Hole & Coombs, 1947; Englehard & Carlisle, 1955; Rice, Boulanger, Mackie & Moore, 1952). These various workers indicated that the CCF test was more sensitive than the HCF test in the diagnosis of glanders in horses, contagious pleuropneumonia, Q-fever, and bovine brucellosis. In the present investigation the CCF and HCF tests have been compared in the diagnosis of acute brucellosis in man and in the subsequent detection of brucella antibodies following infection.

METHODS AND MATERIALS

Haemolytic complement fixation (HCF) 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 *Brucella abortus* (PHLS) antigen was diluted 1/60 (this was found to be the optimal concentration

with the method described by Kerr *et al.* 1968). The haemolytic system was standardized and prepared as described by Bradstreet & Taylor (1962). The overnight fixation technique at 4° C. was used with 1·5 haemolytic units of complement, determined by titrating complement in the presence of antigen and normal serum (pooled negative serum). We define one haemolytic unit as the highest dilution of guinea-pig complement to give complete lysis of the sensitized cells in the presence of pooled negative serum and antigen at the dilution used in the test.

Conglutinating complement fixation (CCF) test

Complement. Blood was collected from healthy pigs and allowed to clot before taking off the serum. The serum was stored before use at − 30° C. The titre of the porcine complement (non-haemolytic) was determined with a chessboard titration. A unit of complement was the highest dilution to give complete agglutination of the indicator, sheep red cells; two units of complement were used in the test.

Bovine serum. Fresh bovine serum was heated for 30 min. at 56° C. and stored before use at − 30° C. The bovine serum contributes both conglutinin and natural sheep red cell antibody, although the latter was supplemented by adding sheep cell haemolytic serum i.e. rabbit amboceptor (Rice *et al.* 1952) at one-tenth of the concentration used in the HCF test .The titre of the conglutinin in the bovine serum was determined in the presence of this excess of sheep cell antibody by a chessboard titration similar to that described by Bradstreet & Taylor (1962) for the determination of the optimum sensitizing concentration of haemolysin.

Indicator system. The indicator system was prepared by adding diluted, heated, bovine serum to an equal volume of 2 % suspension of sheep red blood cells and incubating at 37° C. for 30 min. The bovine serum was supplemented with rabbit amboceptor (*vide supra*).

Test. The tests were set up in WHO plastic plates using a four-volume method with veronal buffer as diluent and serum dilutions starting from 1/10. One volume of complement (2 units) was added to each serum dilution followed by antigen at the optimal working concentration (1/60), as determined by a chessboard titration in the presence of positive serum. After adding the antigen the plates were incubated at 37° C. for 1 hr. before adding 1 volume of the indicator red cell system and incubating at 37° C. for a further 30 min. The plates were left at room temperature for 1 hr., and then examined for agglutination and the results recorded. The titre of the serum was expressed as the reciprocal of the highest dilution of serum which inhibits conglutination. When a comparable amount of anti-conglutinating complement activity was observed with and without antigen the reaction was recorded as anticomplementary.

RESULTS

Three hundred sera from patients with a pyrexial illness without detectable brucella antibody when tested with both the standard agglutination and HCF tests were examined with the CCF test. With the exception of four sera which were anticomplimentary all the sera had a CCF titre of < 10.

One hundred and fifty-five sera with a HCF titre of $\geqslant 10$ were tested in parallel

Table 1. *A comparison of the CCF and HCF titres with* B. abortus *antigen in* 155 *sera containing brucella antibodies*

	CCF titre											
HFC titre	< 10	10	20	40	80	160	320	640	1280	2560	5120	10,240
< 10		1	4	1	1							
10	18	**11**	4	1	3	1						
20	2	2	**7**	2	1	2						
40	2	1	1	**6**	7	1	1					
80			1	2	**5**	5	0	1				
160				1	1	**3**	2	2				
320					1	2	**2**	5	4	3		
640						3	3	**4**	3	1		
1280								5	**4**	4	0	2
2560											1	
5120											**1**	
10,240												**4**

Titres expressed as the reciprocal of the serum dilution.
HCF, haemolytic complement fixation; CCF, conglutinating complement fixation.

Table 2. *A comparison of the CCF and HCF titres with* B. abortus *antigen in sera from* 30 *cases of acute brucellosis*

	CCF titre											
HCF titre	< 10	10	20	40	80	160	320	640	1280	2560	5120	10,240
< 10												
10		**1**										
20						1						
40					1	1	1					
80								1				
160						**1**		1				
320						2	**1**	2	2	2		
640							3	**1**	1			
1280								3	**1**			1
2560												1
5120												
10,240												**2**

Titres expressed as the reciprocal of the serum dilution.
HCF, haemolytic complement fixation; CCF, conglutinating complement fixation.

with the CCF test. A comparison of the titres are shown in Table 1. In 115/155 (74 %) sera the HCF and CCF were within one doubling dilution of each other. In 29 (18 %) sera the CCF titre was greater than HCF titre by fourfold or more and in 15 (10 %) sera the CCF titre was eightfold or more greater than the HCF titre. In 11 (7 %) sera the HCF titre was greater than the CCF titre by fourfold or more.

The 155 sera included 30 sera from patients with acute brucellosis before treatment with antibiotics (Table 2). In 11 of these sera the CCF titre was greater than the HCF titre by fourfold or more and in the remaining 19 sera the CCF and

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Table 3. *Serological results in seven cases of acute brucellosis*

Case no.	Titre to *B. abortus* antigen SA	ME	HCF	CCF	Comments
1	640	< 20	40	320	Pain in arms and legs, anorexia, nausea, fever, sweating. Animal worker
2*	< 20	< 20	10	10	P.U.O. Farm worker
3	640	160	160	640	P.U.O.
4†	160	< 20	20	160	Drinks untreated milk at week-ends. P.U.O. for 1 week
5	1280	80	320	1280	Pain in back of right thigh. P.U.O.
6	1280	320	320	1280	History of brucellosis 4–6 weeks. Evening temperatures
7	5120	40	320	2560	Anaemia, leucopenia and pyrexial illness with thrombocytopenia

Titres expressed as the reciprocal of the serum dilution.

* Serum examined 21 days later

SA	ME	HCF	CCF
640	320	1280	1280

† Serum examined 10 days later

SA	ME	HCF	CCF
1280	20	160	640

SA, standard agglutination; ME, mercaptoethanol agglutination; HCF, haemolytic complement fixation; CCF, conglutinating complement fixation.

HCF titre agreed within one doubling dilution of each other. In none of these 30 sera was the HCF titre greater than the CCF titre.

The CCF titres in seven cases of brucellosis are compared with the titres in the conventional serological tests in Table 3. The HCF and CCF titres on serial samples of serum submitted from a number of these patients have fallen at a similar rate reaching low titres within one year of the infection.

DISCUSSION

This investigation has demonstrated that the CCF test with porcine serum as the source of conglutinating complement can detect antibodies in human brucellosis. In fact, it appears that the CCF test is more sensitive than the HCF test for the detection of brucella antibodies whilst retaining specificity. These findings are in agreement with those of Rice *et al.* (1952), who examined bovine sera and compared the HCF and the CCF tests. They found that the CCF test was more sensitive than the HCF test for the detection of antibodies in the serum of cattle which were later found to be brucella-infected. They used a CCF test using horse serum as the source of conglutinating complement which has been shown by Englehard & Carlisle (1955) to be extremely sensitive, and they suggested titres < 64 were non-specific.

In this study the CCF test was especially sensitive in detecting antibodies in serum from patients in the early stages of brucellosis. The evidence so far suggests that CCF antibodies persist for approximately one year after the infection. The

test would be a useful supplementary test particularly in persons considered to be in the early stages of brucellosis when conventional serological tests are negative or inconclusive as in cases 2 and 4 in Table 3.

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