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PROBLEMS IN THE DIAGNOSIS OF Q FEVER BY COMPLEMENT-FIXATION TESTS *

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SYNOPSIS

For the selection of a suitable antigen concentration for use in routine complement-fixation tests with Q-fever antisera, a rigid system of antigen units is unsatisfactory. The optimum antigen dilution should be judged after inspection of the results of full "chess-board" titrations with a variety of antisera. Non-specific reactions may occur with sera from patients with primary atypical pneumonia or sera which have deteriorated during storage. These may be detected with a typhus antigen or some similar control antigen.

Nine Mile and Henzerling strain antigens, which are widely used for routine diagnosis, were compared in tests with 868 sheep sera, 1055 human blood-donor sera, 66 human convalescent sera, and 20 guinea-pig sera. Considerable discrepancies were found in the results obtained with the two antigens.

Irrespective of antigen concentration, the Nine Mile strain was more sensitive than the Henzerling strain for the detection of Q-fever antibody in human and guinea-pig sera and in some sera from Welsh sheep. With antibody in Kentish sheep sera, on the other hand, the Henzerling antigen was markedly more sensitive than the Nine Mile strain antigen. It is concluded that these two strains cannot be regarded as interchangeable, and the choice of antigen must depend on the geographical area and the species to be tested.

The diagnosis of Q fever depends, in practice, upon the identification of specific antibodies to *Rickettsia (Coxiella) burneti* in sera from naturally infected human beings or animals, or from laboratory animals inoculated

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with materials containing the rickettsia. Burnet & Freeman [6] were the first to detect Q-fever antibodies by means of an agglutination reaction, but soon afterwards Bengston [3] showed that a complement-fixation test could also be employed for this purpose. Agglutination tests or micro-modifications, using slides (Babudieri [1]), or capillary tubes (Luoto [16]), may be used, but for many years the complement-fixation test has been generally accepted as the technique of choice, because it is more economical in antigen, and easier to read and interpret than, at least, the conventional agglutination techniques. Other methods of measuring immunity, which have been reported but not routinely used, involve detection of neutralizing antibody (Burnet & Freeman ; [7] Bengston [4]), opsonin (Victor et al. [27]) conglutinating antibody (Barber [2]), conglutinating complement-fixing antibody (Wolfe & Kornfeld [29]), skin sensitivity (Giroud et al.[10]), and conjunctival sensitivity (Mirri [20]).

In the complement-fixation test, as with most other serological procedures using *R. burneti*, the antigen consists of a purified suspension of rickettsiae made from infected yolk-sacs. Antigens prepared from different strains of *R. burneti* vary widely in their ability to fix complement with Q-fever antibody, however, so that the selection of a satisfactory strain for use in routine complement-fixation tests is a matter of some importance.

The Henzerling strain, isolated from a patient with Q fever in Italy, was shown by Robbins et al.[21] and Topping et al.[26] to be especially suitable in this regard, because it reacted strongly with sera from cases in other localities, and also with sera prepared against itself or against other strains in guinea-pigs. Subsequently, Wolfe & Kornfeld [30] reported that no difference in complement-fixing ability had been found between the Henzerling strain and the Nine Mile strain originally isolated by Davis & Cox [8] from ticks in Montana (see also Strauss & Sulkin [24]). Both these strains have since been widely used for preparing antigens for routine serological diagnosis of Q fever in man and animals.

Recently, Berge & Lennette [5] have carried out a comprehensive study of the behaviour in complement-fixation tests of 11 strains of *R. burneti* from various sources. Human, sheep, and guinea-pig sera were used, and these workers confirmed that antigens from the Henzerling and Nine Mile strains alone reacted well with antisera from all three species, irrespective of the strain used to provoke antibody, the period of time between infection and collection, or the antigen dilution employed in the test. Nevertheless, antibody titres found with Henzerling strain antigen were slightly higher than with Nine Mile strain antigen in tests on pools of sheep and guinea-pig sera.

Part of a programme of epidemiological research carried out in England involved examination of sheep sera for Q-fever antibody. Nine Mile strain antigen was in routine use, but, in view of the slight difference observed

by Berge & Lennette with pools of sheep serum, we decided to test some specimens with Henzerling antigen as well. Marked discrepancies in the results obtained prompted us to carry out a detailed comparison of the two antigens and also to re-evaluate the method of estimation of the optimum dilution of antigen for routine tests. The results obtained and other problems concerned with the detection of Q-fever antibodies by complement-fixation tests are discussed in this paper.

Materials and Methods

Strains of R. burneti

The Nine Mile and Henzerling strains of *R. burneti* were kindly sent by Dr H. R. Cox of the Lederle Laboratories, Pearl River, New York, as lyophilized suspensions of infected yolk-sac. The Christie strain was isolated from a patient with Q fever in London (MacCallum et al.; [17] Stoker [22]). All these strains have undergone numerous passages in the yolk-sacs of fertile hens' eggs.

Antigen preparation

Antigens were prepared by ether extraction and differential centrifugation, using a modification of Plotz's method (Stoker [23]). The final product, as judged by light and electron microscopy, contained little extraneous material. The rickettsial concentration of each batch of antigen, from whatever strain, was adjusted so as to equal that of a standard Nine Mile antigen. Matching was carried out by a simple visual opacity method, or with a photo-electric densitometer.

To find out if antigens matched by these methods contained approximately equal numbers of rickettsiae, direct counts were also made of the organisms in 8 selected antigens using the electron microscope technique described by Williams & Backus.[28] After matching, the numbers of rickettsiae in these antigens were found to lie between 2×10^{13} and 3×10^{13} per ml, but differences of this order did not affect the significance of results obtained in the complement-fixation tests (Stoker & Fiset, unpublished data).

Before matching with the standard, various batches of antigen prepared from the same strain differed very little in rickettsial concentration, but the Nine Mile strain grew more profusely than the Henzerling strain and yielded about five times as many rickettsiae from each gram of original yolk-sac.

In addition to antigen made at Cambridge, Nine Mile and Henzerling strain antigens prepared by a similar method at the Lederle Laboratories, and kindly provided by Dr H. R. Cox, were used to check certain results.

Murine typhus antigen was also prepared from infected yolk-sacs at the Lederle Laboratories in a similar way.

Antisera

Specimens of serum from Q-fever patients, healthy human blood-donors, naturally infected sheep and cows, and experimentally infected guinea-pigs were separated from the clot and stored at −20°C. Before testing, the sera were diluted 1 in 5 with physiological saline and inactivated at 60°C for 30 minutes.

Complement, haemolysin, and sheep cells

Normal guinea-pig serum, stored without preservative at −20°C, was used as a source of complement. The haemolytic system consisted of freshly washed 1% sheep cells mixed (and allowed to stand at least 10 minutes at room temperature) with an equal volume of rabbit anti-sheep cell haemolysin (Burroughs Wellcome Co. Ltd.) diluted to contain approximately 5 minimal haemolytic doses. The diluent in all tests consisted of unbuffered 0.9% saline made up daily from stock 9% solution and sterile distilled water. Unless otherwise stated, the unit volume was 0.1 ml.

Complement titration

In the test proper (see below) the antigen, antiserum, and complement were allowed to stand at 4°C overnight before addition of the haemolytic system. Complement was titrated under the same conditions by making ascending dilutions of the guinea-pig serum at 1:20, 1:30, 1:40, etc., and allowing these to stand overnight at 4°C. At the same time, other sets of complement dilutions were allowed to stand in the presence of each antigen at dilutions used in the test proper. Next morning, the haemolytic system was added and the whole incubated at 37°C for 30 minutes. The highest dilution of complement which showed either complete or almost complete haemolysis in the presence of antigen was taken as one " exact " unit (as defined, for example, by Meyer & Eddie [19]) and twice this concentration, i.e., 2 exact units, was used in the test proper. If the presence of antigen lowered the apparent end-point of the complement titration by more than one dilution in the series used, it was judged to be too anticomplementary and was discarded.

Since the complement was very stable it was only necessary to carry out a full complement titration at the beginning of each week, when a new batch of sheep cells arrived. Tests on subsequent days included a complement titration in miniature which confirmed that the correct dose had been used (see below).

The test proper

Ascending twofold dilutions of serum in 0.1 ml volumes, commencing at 1:5 or 1:10, were allowed to stand overnight at 4°C with 0.1 ml of complement diluted to contain 2 exact units, and 0.1 ml of the optimum antigen dilution (see below). Next morning, the haemolytic system was added and the whole incubated at 37°C for 30 minutes. The titre of the antiserum was taken to be the highest dilution of the serum which showed 25% or less haemolysis.

Besides anticomplementary controls of serum (containing serum, saline, and 2 units of complement), each test included anticomplementary controls of each antigen, in the dilutions used, in the presence of 2.0, 1.5, 1.0, 0.75, and 0.5 units of complement. The results were not accepted unless there was complete haemolysis with 2.0 and 1.5 units, complete haemolysis or only a trace of unlysed cells with 1.0 unit, and no haemolysis with 0.5 unit of complement. A standard human Q-fever antiserum was also titrated to check the sensitivity of each test.

Each serum which showed fixation of complement with Q-fever antigen was also tested with a typhus antigen to check specificity, for reasons which will be discussed below.

Sensitivity of the technique

The WHO Expert Committee on Biological Standardization [31] has recently approved and issued a bovine serum as the International Standard for Anti-Q-Fever Serum against which sensitivity of complement-fixation tests carried out in different laboratories can be measured.

A standard technique has also been recommended for use in laboratories collaborating in the WHO Q-fever survey. This technique, which has been described in detail by Kaplan & Hulse,[13] also employs 2 exact units of complement and overnight fixation in the cold. Unlike the test described above, however, magnesium saline diluent, a stronger cell suspension and larger volumes are used, and complement is titrated by allowing it to stand at 37°C for one hour in the presence of antigen before addition of sensitized cells. In our local technique, on the other hand, complement remains at 4°C overnight with antigen before addition of cells, as in the complete test.

Using the WHO technique, the mean titre of the International Standard antiserum, as determined in this and other Q-fever laboratories with Henzerling strain antigen, is 1:128 (with 50% fixation at 1:256). With our usual technique, as described above, different batches of the serum give full fixation at 1:128 or 1:256. Thus our complement-fixation technique is of approximately the same sensitivity as that used in the WHO survey.

Determination of Optimum Antigen Dilution

In order to detect Q-fever antibody by a complement-fixation test, it is obviously necessary to use the rickettsial antigen in a concentration sufficient to react with the antibody. If the antigen concentration is too high, however, it may also be frankly anticomplementary.

Even if not obviously anticomplementary, it may react non-specifically with normal sera or antisera from patients with conditions other than Q fever, such as primary atypical pneumonia. For example, in complement-fixation tests with sera showing high cold haemagglutinin or *Streptococcus MG* agglutinin titres, 1 specimen out of 8 tested reacted strongly with Q-fever antigen diluted 1:16 but not with antigen diluted 1:64.

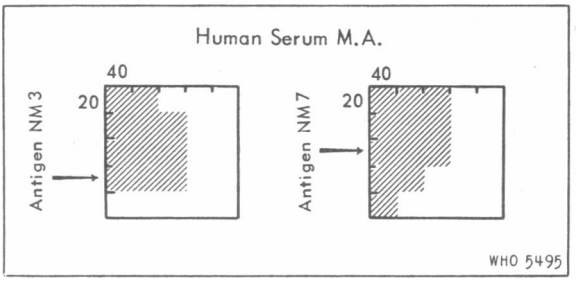
It is, therefore, necessary to choose a suitable antigen concentration, low enough to avoid these non-specific reactions, but high enough to detect Q-fever antibody.

One method of determining this concentration is to titrate the antigen in the presence of antibody excess, and thus to determine the highest dilution of antigen which fixes complement with antibody, in other words, the titre of the antigen. This dilution is then said to contain 1 antigen unit, and 2 or 4 units are used in subsequent tests. Such a " straight line " method of titration of the antigen is unsatisfactory because of the great variation in the apparent titre of the same antigen when tested with different individual antisera or even pools of antisera. Thus, 1 antigen titrated with 13 different Q-fever human antisera (using 4 antibody units) showed antigen titres ranging from 1 in 50 to 1 in 400 or more.

A more satisfactory method is to determine the optimum antigen dilution by a full " chess-board " titration in which ascending dilutions of antigen are each tested with ascending dilutions of antiserum. One antigen unit is then taken to be the highest dilution of antigen which gives the highest serum titre. This unit is normally taken as the optimum antigen dilution for subsequent tests, providing there is no " prozone " (i.e., diminution in complement fixation at high serum concentrations).

Even this method is open to difficulty. In the first place, the use of 1 antigen unit (i.e., the highest dilution of antigen giving the highest serum titre) as the optimum antigen dilution may be perfectly satisfactory if there is a gradual decline in apparent antiserum titre with higher antigen dilutions constituting less than 1 unit. If, however, at the next higher antigen dilution no antibody is detectable at all, the so-called optimum chosen may be too close to the limit for safety, because small errors in dilution of the antigen may lead to complete failure to react. Fig. 1 shows the results of titrating 2 Nine Mile (NM) antigens with a standard human convalescent serum. It will be clear that the use of 1 antigen unit (1:80) as the optimum antigen dilution would be satisfactory for NM 7, while for NM 3 one unit would be by definition 1:160, but its use would be

FIG. 1. TITRATION OF TWO BATCHES OF NINE MILE STRAIN ANTIGEN (NM 3 AND NM 7) WITH A HUMAN Q-FEVER ANTISERUM (MA)



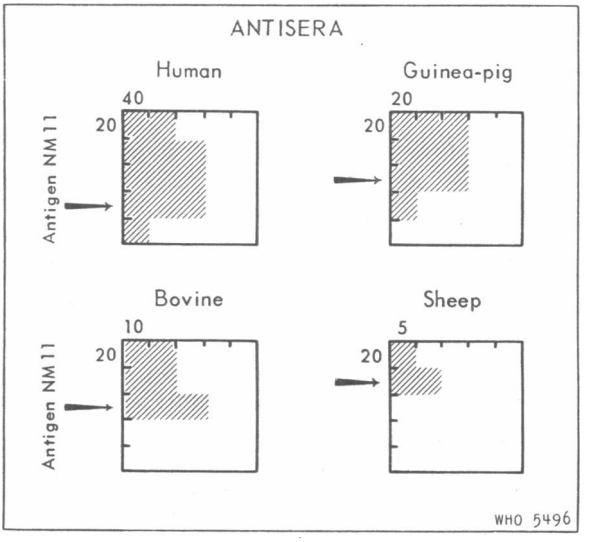
Arrows indicate " one antigen unit ".

This figure and Fig. 2, 3, and 4 show the results of complement-fixation tests as " chess-board titrations ", between ascending doubling dilutions of antiserum, shown horizontally, and ascending doubling dilutions of antigen, shown vertically. The reciprocal of the lowest dilution of antigen and antiserum is given for each titration. The dark area indicates those dilutions at which complement fixation occurred.

open to risk because at the next higher dilution no antibody was detected at all. It would, therefore, be safer to use 2 units (i.e., 1 : 80) as the optimum for this antigen.

Secondly, titrations with antisera from different species may give a different antigen unitage for the same antigen. Fig. 2, for example, shows titrations of a single Nine Mile antigen (Batch NM 11) with sera from a

FIG. 2. TITRATION OF A SINGLE NINE MILE STRAIN ANTIGEN (NM 11) WITH HUMAN, GUINEA-PIG, BOVINE, AND SHEEP SERUM

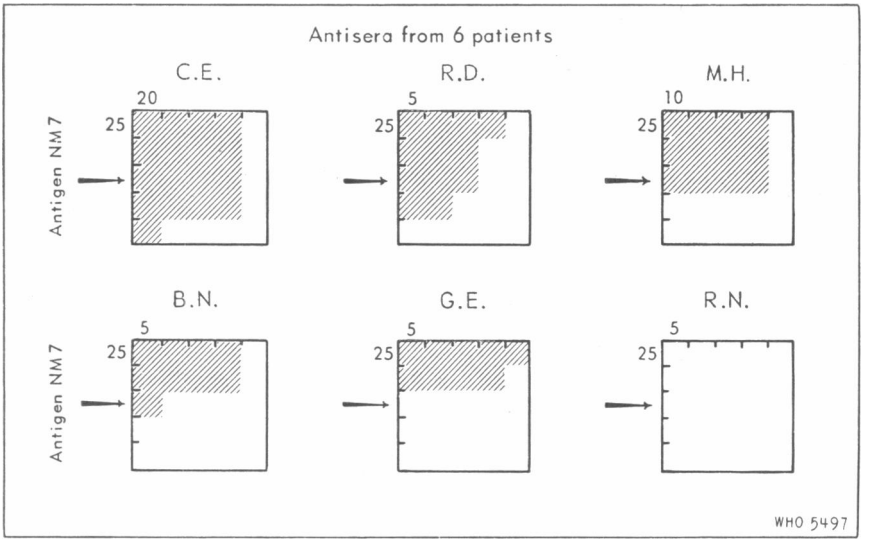


Arrows indicate " one antigen unit ".

human being, guinea-pig, cow, and sheep.[a] Since 1 unit varies from 1:40 to 1:160, it is clearly essential to determine individually the optimum antigen dilution for each species serum to be tested.

Finally, even though antigen titrations do not vary widely with sera from any one species, notable exceptions sometimes occur. Fig. 3 shows titrations of a single antigen (NM 7) with convalescent sera from 6 selected patients, who had clinical Q fever during recognized outbreaks of this disease. The estimated unit of antigen with these sera varies greatly and

FIG. 3. TITRATION OF A SINGLE NINE MILE STRAIN ANTIGEN (NM 7) WITH ANTISERA FROM SIX PATIENTS WITH Q FEVER



Serum RN was positive with Henzerling strain antigen alone.
Arrows indicate " one antigen unit " as determined in previous titrations with a standard human antiserum.

one serum (RN) failed to react with Nine Mile antigen at all, although it did react with low dilutions of Henzerling and Christie strain antigen. Fortunately this type of failure to detect antibody and the low antigen titres given by sera BN and GE are rare. The optimum antigen dilution of NM 7 had been previously judged to be 1:100 and full chess-board titrations on convalescent sera from 26 Q-fever patients showed that only 2 would have been judged negative if the antigen had been used at this dilution, and in 2 more the apparent antibody titre would have been rather low.

[a] Only low-titre antisera from naturally infected sheep were available for standardization of antigens.

We have concluded that the optimum antigen dilution cannot be determined by any rigid rule involving antigen units. We have, therefore, discarded the term " antigen unit ", which in any case leads to confusion with the straight-line titration method. The optimum antigen dilution is determined by inspection of chess-board titrations with a number of sera from different species and taken to be the highest dilution of antigen which will react with the highest dilutions of the various antisera, with a reasonable margin of safety.

Use of Typhus Antigen to Detect Non-specific Reactions

Although Q-fever antigens are highly purified rickettsial suspensions probably containing little host material, non-specific reactions may occur between them and human or animal sera. Past experience has shown that transient positive complement-fixation reactions with *R. burneti* and other antigens are sometimes detectable in a sequence of sera from a patient suffering from " primary atypical pneumonia " of the type stimulating agglutinins to *Streptococcus MG* or human O red-blood cells in the cold. It was thought that this was probably due to a reaction between residual egg material in the antigen and the widely reacting heterologous " antibodies " sometimes found in the sera of such patients and which fix complement with the normal, and particularly with the infected, tissues from a variety of small animals, or with egg tissues, including normal yolk-sac (Thoma et al.;[25] Eaton & Corey [9]). It was also observed that sera which were deteriorating, owing to delays in the post or in storage, fixed complement with a number of antigens including *R. burneti* although the serum controls did not show frank anticomplementary activity. This was suspected to be due to a summation of the anticomplementary effects of antigen and serum analogous to that reported with deteriorating sera and encephalitis virus antigens prepared in the mouse brain (Hammon [11]). Both these non-specific effects can be identified by the use of control antigen.

In the past, a commercial typhus vaccine (Connaught laboratories), which contained rickettsiae and egg material, was used in a dilution which was slightly anticomplementary (Marmion et al.[18]). In the present investigation of blood-donor and sheep sera, a diagnostic murine-typhus antigen was substituted for the typhus vaccine because it seemed that the former was more akin to the *R. burneti* antigen in its state of purification and numbers of rickettsiae.

The summation of anticomplementary effects is well illustrated by complement-fixation tests on batches of sera from healthy blood-donors during an epidemiological study. When batches were received which contained frankly anticomplementary serum specimens, other specimens were found in the same batch which reacted with both Q-fever and typhus

antigens, but which showed no evidence of anticomplementary activity in the serum controls. There was evidence that the presence both of anticomplementary activity and reaction with typhus antigen was linked with conditions of storage and transit. Thus, although tests were usually performed 2 to 4 days after the specimens were taken from the donors, one batch of 62 blood samples (subsequently omitted from the series under study) was by error left, after separation from the clots, for 22 days at 4°C before testing. Sixteen of the 62 sera were frankly anticomplementary in the serum controls, and 11 reacted strongly with Q-fever and typhus antigens and showed slight anticomplementary activity. A further 15 sera, however, reacted with the 2 antigens but showed no obvious anticomplementary activity in the serum controls at all. Thus, without typhus antigen as a control, it might have been concluded that at least 15 of these specimens contained Q-fever antibody.

Typhus antigen or vaccine would not provide a satisfactory control in countries where either epidemic or murine typhus is common and, even in typhus-free countries, widespread use of typhus vaccine might lead occasionally to Q-fever and typhus antibodies in the same individual. Antigen prepared from normal yolk-sacs, or psittacosis antigen, may be used as alternative control antigens, especially as the latter may be needed for diagnosis. Psittacosis antigen, however, is prepared by a different technique from heated egg or mouse tissues and is theoretically a less satisfactory control than another rickettsial suspension purified in the same way as *R. burneti.*

Comparison of Nine Mile and Henzerling Strain Antigens

Sheep sera

Specimens of serum from 552 sheep in an enzootic area in Kent were tested for Q-fever complement-fixing antibodies with Nine Mile and Henzerling strain antigens (see Table I). Eighteen specimens contained antibody at 1/10 or greater with both antigens, but a further 35 were positive with Henzerling antigen alone, and 10 were positive with Nine Mile antigen alone. Almost twice as many reacted with Henzerling (10.0%) as with Nine Mile antigen (5.1%), and when both were positive the antiserum titres were higher with Henzerling antigen.

Specimens from 316 sheep from central Wales were also tested in the same way (Table I). Of these, 1 reacted with both antigens, none with Henzerling alone, and 10 with Nine Mile antigen alone. Thus proportions of Welsh sheep sera reacting with each antigen were the opposite to those obtained with Kentish sera.

Sera from 93 sheep in a single flock from Kent were also examined with Christie antigen. None reacted positively with Nine Mile antigen, but 15 reacted with Henzerling, and 24 with Christie.

TABLE I. RESULTS OF COMPLEMENT-FIXATION TESTS WITH NINE MILE AND HENZERLING STRAIN ANTIGENS ON SHEEP SERA FROM KENT AND WALES

Source	Total tested	Number positive with:				Number positive at 1/10 or above with:			Total
		Nine Mile antigen		Henzerling antigen		Nine Mile antigen alone	Henzerling antigen alone	both antigens	
		1/10 or above	1/20 or above	1/10 or above	1/20 or above				
Kent	552 (100)	28 (5.1)	9 (1.6)	55 (10.0)	32 (5.8)	10	37	18	65
Wales	316 (100)	10 (3.2)	5 (1.6)	1 (0.3)	0	10	0	1	11
Total	868	39	14	56	32	20	37	19	76

Figures in parentheses indicate percentage of total tested.

TABLE II. RESULTS OF COMPLEMENT-FIXATION TESTS WITH NINE MILE AND HENZERLING STRAIN ANTIGENS ON SERA FROM HUMAN BLOOD-DONORS IN SOUTH-EAST ENGLAND

Source	Total tested	Number positive with:				Number positive at 1/10 or above with:			Total
		Nine Mile antigen		Henzerling antigen		Nine Mile antigen alone	Henzerling antigen alone	both antigens	
		1/10 or above	1/20 or above	1/10 or above	1/20 or above				
Kent county	471 (100)	25 (5.3)	10 (2.1)	14 (3.0)	6 (1.3)	12	1	13	26
East Anglia (North)	363 (100)	12 (3.3)	3 (0.8)	2 (0.6)	2 (0.6)	10	0	2	12
East Anglia (South)	221 (100)	6 (2.7)	2 (0.9)	3 (1.4)	0 (0)	3	0	3	6
Total	1055	43 (4.1)	15 (1.4)	19 (1.8)	8 (0.8)	25	1	18	44

Figures in parentheses indicate percentage of total tested.

Blood-donor sera

Human sera from 1055 blood donors from various counties in south-east England were also examined with Henzerling and Nine Mile strain antigens. It will be seen from Table II that 18 were positive at 1/10 or above with both antigens, 25 with Nine Mile antigen alone, and 1 with Henzerling antigen alone. Thus, 4.1% reacted with Nine Mile antigen and only 1.8% with Henzerling antigen. There was no obvious difference between different areas in south-east England, and it should be noted that in Kent Nine Mile antigen was more sensitive for human sera, but Henzerling antigen more so for sheep sera.

Differences in titre

Most of the positive sheep and human sera had low titres (1/10 or 1/20) and the differences in numbers reacting with the two antigens might have been due to very small differences within the limits of error of the test. A comparison was therefore made of the actual differences in titre shown with each antigen. The difference was expressed as the ratio of the titre with the Nine Mile antigen to the titre with Henzerling antigen (NM/H ratio). When antibody was detected with only one antigen the titre with the other antigen was assumed to be 1/5, which was one dilution lower than the starting dilution of 1/10. Thus, a serum with a Nine Mile titre of 1/20 and no Henzerling antibody at 1/10 would be given an NM/H ratio of 4.

The frequency distribution of the differences in titre, expressed as ratios, is shown in Table III. It will be seen that, whereas the majority of human sera and Welsh sheep sera showed a higher titre with Nine Mile antigen, most of the Kentish sheep sera reacted more strongly with the Henzerling antigen. Even if sera showing results with two-fold differences are excluded as possibly due to experimental error, 16% of the remaining

TABLE III. FREQUENCY DISTRIBUTION OF SHEEP AND BLOOD-DONOR SERA SHOWING DIFFERENCES IN ANTIBODY TITRE WITH NINE MILE AND HENZERLING ANTIGENS *

Source	Number of specimens showing Nine Mile / Henzerling titre ratio of:								Total
	8	4	2	1	0.5	0.25	0.125	0.0625	
Sheep sera									
Kent	—	—	11	8	22	16	5	3	65
Wales	—	5	5	1	—	—	—	—	11
blood donor sera									
South-East England (including Kent)	2	5	26	9	2	—	—	—	44

* Expressed as ratio of the titre with Nine Mile antigen to the titre with Henzerling antigen

human sera which were positive with any antigen had significantly higher titres with Nine Mile antigen and, likewise, 36% of Kentish sheep sera had higher titres with Henzerling antigen. In fact, 3 sheep sera showed titres with Henzerling antigen which were 16 times those shown with Nine Mile antigen.

Selection of the wrong antigen concentration might have accounted for the low antibody titres even though each batch of antigen had been titrated in " chess-board " fashion with human and sheep antiserum to determine its optimum dilution. To establish that differences in antibody titre were not dependent on antigen concentration, selected single specimens and pools both of human sera and of Kentish and Welsh sheep sera were titrated in dilutions from 1 : 5 in chess-board fashion with ascending dilutions of both antigens in equivalent rickettsial concentrations. Fig. 4 shows that irrespective of antigen concentration the human sera and Welsh sheep sera failed to react with Henzerling antigen (except for a low titre of antibody shown by the Welsh sheep pool) and the Kentish sheep sera failed to react with Nine Mile antigen. In addition, some of the titrations were repeated with Nine Mile and Henzerling strain antigens prepared in the Lederle Laboratories, to make sure that the differences observed were not a unique property of our strains of rickettsiae or method of preparation of antigen. The results did not differ from those obtained with locally prepared antigen.

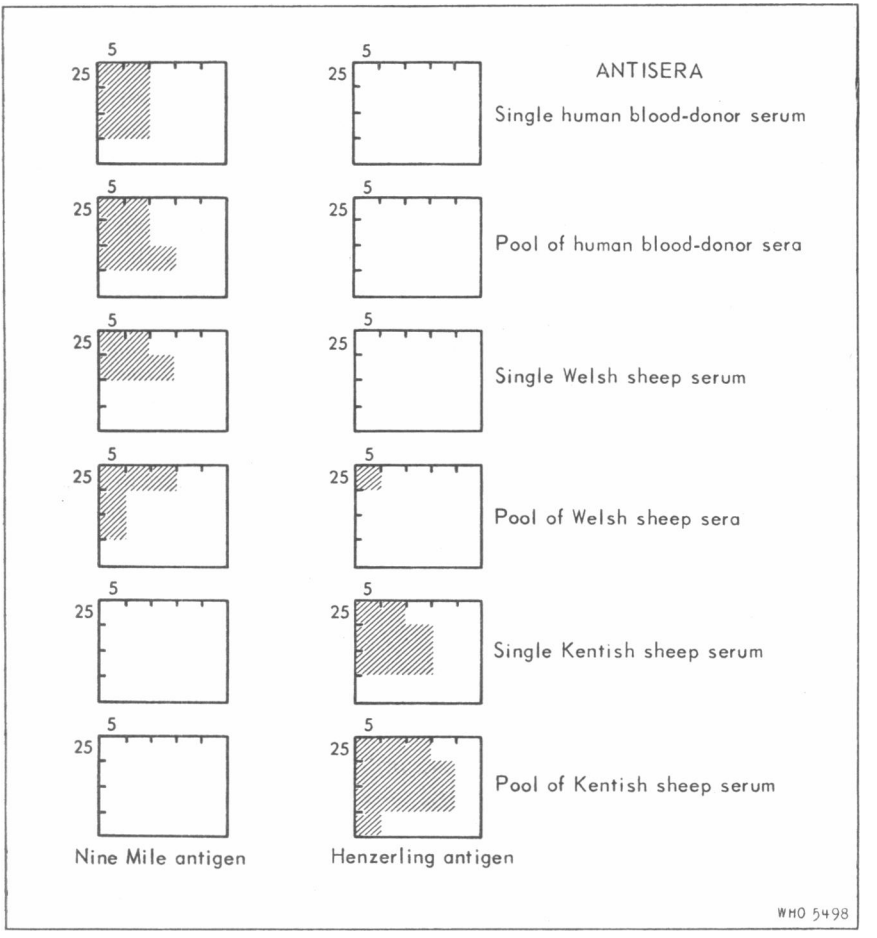
Human convalescent sera

Because of the difference in sensitivity of Nine Mile and Henzerling strain antigens in detecting complement-fixing antibody in human blood-donors and sheep, it was important to compare the antigens for the diagnosis of clinical Q fever in man. Accordingly, complement-fixation tests with Nine Mile and Henzerling and also Christie strain antigens were carried out on 66 specimens of serum taken at various times from 49 persons who had had clinical Q fever. Single samples were used from 46 patients but the remaining 20 specimens were taken at successive times from 3 persons accidentally infected in the laboratory. For these 20 serial specimens, the starting dilution in the tests was 1/5 and for the remainder it was 1/10.

Four specimens collected soon after the onset and five taken over a year after the illness contained no antibody detectable with any antigen. The remaining 57 were positive with one or more antigens, and of these, 56 reacted with Nine Mile, 53 with Henzerling, and 42 with Christie antigen. Thus, Nine Mile antigen failed to detect antibody in one specimen, whereas 4 were negative with Henzerling, and 15 negative with Christie antigens.

In the majority, the Nine Mile antigen gave the highest antibody levels, and the NM/H ratio, determined as before, showed a frequency distribution similar to that seen with positive blood-donor sera and Welsh sheep sera, and quite different from that found with Kentish sheep sera.

FIG. 4. TITRATIONS OF NINE MILE AND HENZERLING STRAIN ANTIGENS WITH INDIVIDUAL SERUM SPECIMENS AND POOLS OF SERA FROM HUMAN BLOOD-DONORS AND FROM WELSH AND KENTISH SHEEP

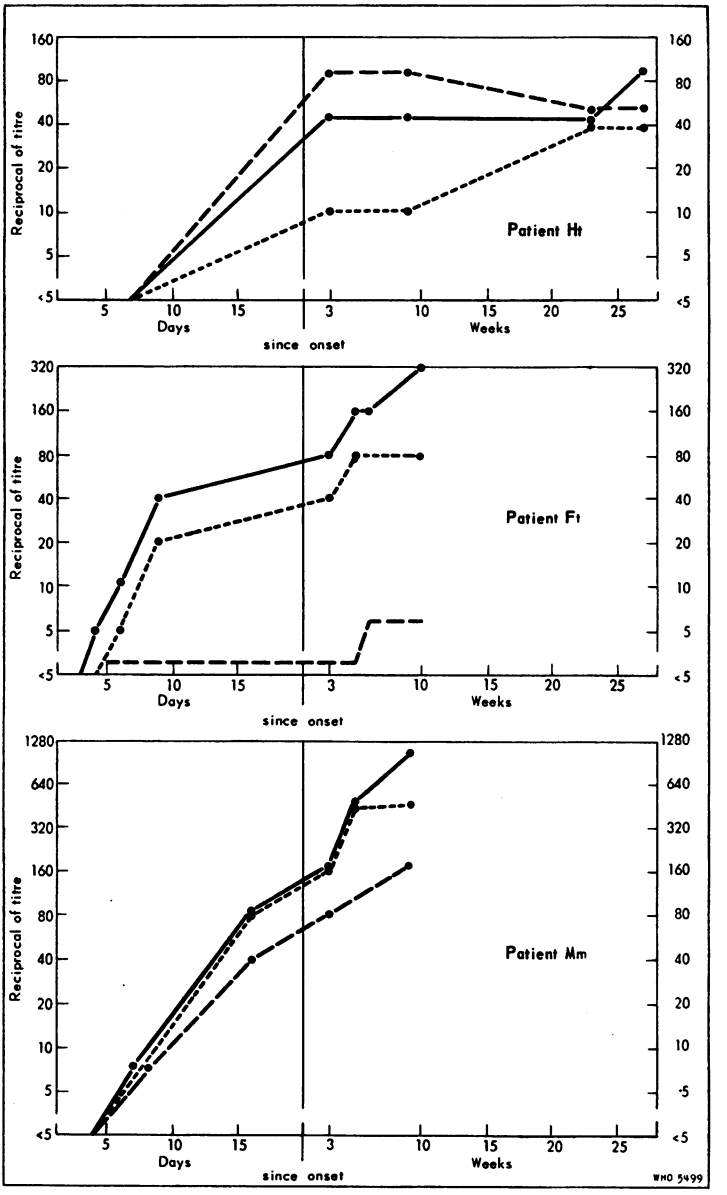


Titres with Christie antigen were usually, but not always, lower than those found with Nine Mile or Henzerling antigens.

To find out if the differences in serum titres were due to antigen concentration, samples from 18 patients were retested with twice the estimated optimum concentration of Nine Mile and Henzerling antigens. In 5 samples the difference in titre with the two antigens was reduced, in 6 it was increased, and in 7 the difference remained the same.

The antibody response, as measured by the 3 antigens, sometimes varied markedly from patient to patient, as illustrated by Fig. 5, which shows successive antibody levels in 3 persons infected in the laboratory.

FIG. 5. COMPLEMENT-FIXING ANTIBODY TITRES AS DETERMINED WITH NINE MILE, HENZERLING, AND CHRISTIE STRAIN ANTIGENS, AT SUCCESSIVE INTERVALS AFTER ONSET OF SYMPTOMS, IN THREE PATIENTS WHO DEVELOPED Q FEVER AS A RESULT OF LABORATORY INFECTION



Titre with Nine Mile antigen
Titre with Henzerling antigen
Titre with Christie antigen

TABLE IV. RESULTS OF COMPLEMENT-FIXATION TESTS WITH NINE MILE, HENZERLING, AND CHRISTIE STRAIN ANTIGENS ON SERA FROM PATIENTS IN TWO OUTBREAKS

	Weeks after onset	Reciprocal of complement-fixing titre		
		Nine Mile strain	Henzerling strain	Christie strain
Kentish outbreak				
Case 1	3	80	80	40
2	3	40	10	20
3	4	40	10	40
4	4	20	80	320
5	4	20	80	320
6	4	<10	20	160
7	4	40	40	80
8	4	20	20	<10
9	4	80	80	80
Welsh outbreak				
Case 1	5	160	40	<10
2	6	160	80	10
3	6	20	<10	<10
4	6	10	<10	<10
5	8	160	80	<10
6	10	40	10	<10
7	11	80	20	10
8	12	640	160	80
9	12	160	40	20
10	12	20	10	<10

Patients Ht and Mm were unvaccinated but Ft had received two doses of Henzerling strain vaccine 38 and 31 days before the onset of symptoms, and this presumably accounts for the very early appearance of antibody on the 4th day.

It has not been possible to relate different types of antibody response to particular sources of infection, but sera from two outbreaks showed certain interesting features. Nine specimens were from an outbreak in Kent described by Harvey et al.,[12] and 10 specimens were from an outbreak in Wales possibly originating from sheep. Table IV gives the antibody titres obtained. It will be seen that the Welsh sera reacted well with Nine

Mile, and poorly or not at all with Christie antigen. Most of the Kent sera, on the other hand, reacted better with Christie antigen than Nine Mile. The Kentish sera had been stored longer than those from Wales, but evidence from other specimens does not suggest that storage enhances antibody to Christie antigen. The Kentish sera were also taken earlier after onset than the Welsh sera but the difference is small. It is possible that the different patterns of antibody response in the two outbreaks were caused by differences in the infecting strains of *R. burneti.*

In the general series of human cases, specimens which showed large discrepancies in antibody titre with given antigens had one obvious factor in common, namely, they were all taken within a year of illness. Table V shows the frequency distribution of NM/H and NM/Christie ratios (determined as before) related to the time after the onset of the disease, when the specimen was taken. NM/H ratios of 2 and NM/C ratios of 2 and 4 occurred even up to 12 years after the illness, but all the greater discrepancies were within the first year. The apparent approximation of antibody level which occurs after a year is not due to a general fall in antibody titre which

TABLE V. COMPLEMENT-FIXATION TESTS ON SERA TAKEN AT INTERVALS AFTER ONSET FROM PATIENTS WITH Q FEVER *

Sera taken	Number of specimens showing Nine Mile/Henzerling titre ratio of :							Total
	8 or more	4	2	1	0.5	0.25	0.125 or less	
less than 1 year after onset	0	12	17	13	0	2	0	44
one year or more after onset	0	0	8	5	0	0	0	13
Total	0	12	25	18	0	2	0	57
Sera taken	Number of specimens showing Nine Mile/Christie titre ratio of :							Total
	8 or more	4	2	1	0.5	0.25	0.125 or less	
less than 1 year after onset	14	6	11	5	4	0	2	42
one year or more after onset	0	5	6	4	0	0	0	15
Total	14	11	17	9	4	0	2	57

* Frequency distribution of specimens showing differences in antibody titre with Nine Mile, Henzerling, and Christie strain antigens, expressed as ratios of titres with 2 antigens.

acts by reducing the extent of possible differences. Ten of the 15 specimens taken over a year after onset in fact had titres of 1/40 or above with Nine Mile antigen.

Guinea-pig sera

Specimens of serum from 20 guinea-pigs taken 6 weeks after inoculation with cows' milk containing *R. burneti* were also tested with Nine Mile and Henzerling strain antigens. Eighteen showed higher titres with the Nine Mile strain (NM/H ratios of 2 to 8), in one the titres were equal, and in one a low titre against Henzerling strain antigen alone was found.

Absorption tests

The differences in antiserum titres detectable with Nine Mile and Henzerling antigens suggested that the antibodies might have been produced in response to distinct antigenic structures. However, human convalescent serum which reacted equally well with Henzerling and Nine Mile antigen was absorbed with either antigen, and all complement-fixing antibody to both was removed. Even sheep antibody which reacted with Henzerling and not with Nine Mile antigen could be partially removed by absorption with Nine Mile antigen. These absorption tests showed no evidence of antigens specific to either strain, but there remains the possibility of quantitative differences between common antigenic components.

Discussion

Complement-fixing antigens prepared from the Nine Mile and Henzerling strains of *R. burneti* are widely used for detection of Q-fever antibodies and they have been regarded as more or less interchangeable. Nevertheless, Kitaoka & Takano[14,15] found that, although the Nine Mile strain was slightly more sensitive than the Henzerling strain in complement-fixation tests with guinea-pig sera, the Henzerling strain gave the higher titres with camel sera. Gordon Smith in Malaya has also found that one ox serum reacted to a dilution of 1/512 or higher with Nine Mile antigen but was negative at 1/8 with Henzerling antigen (personal communication, 1954).

This paper reports marked differences in the complement-fixing titres of human and sheep sera given by the two antigens. In specimens from over a third of serologically positive Kentish sheep, the antibody titre with Henzerling was at least four times that with Nine Mile antigen, but in a smaller number of Welsh sheep sera, the proportions were reversed. Most human antisera showed higher titres with Nine Mile antigen: 21% of specimens from Q-fever patients and 16% of positive sera from blood donors having Nine Mile antibody at titres at least 4 times those shown with Henzerling antigen. Antigen prepared from the local Christie strain

reacted better than either of the classical antigens with the few sheep sera tested. With human sera, on the other hand, the results were erratic and in two outbreaks the antibody response to the Christie strain differed markedly.

The tests with human convalescent sera showed that large discrepancies in results with different antigens only occurred during the first year after infection. This is in agreement with the observations of Berge & Lennette [5] who reported that late convalescent sera from humans reacted better than early convalescent sera when tested with poor complement-fixing antigens made from strains such as the Konitzer or Ralph. As Berge & Lennette suggest, it may eventually be possible to determine the length of time that has elapsed since infection by the ratio of titres obtained in tests with suitable antigens. That the earliest serum specimens to be taken should show greater specificity would, in any case, be in accord with general immunological principles.

Apparent differences in behaviour of strains in complement-fixation tests might also be caused by differences in the number of yolk-sac passages undergone by the strains at the time of testing, because of the variation which occurs during egg adaptation (Stoker [23]). The 3 strains used in the present investigation had all undergone numerous yolk-sac passages, however, and it was considered unlikely that they would be subject to this type of variation.

It should be emphasized that the results given apply to complement-fixation tests alone. A few experiments suggest that the agglutination reaction and other serological procedures do not necessarily show the differences between the Nine Mile and Henzerling strain antigens, which are observed in complement-fixation tests.

It would appear that, at least in parts of England, Nine Mile antigen is more sensitive than Henzerling antigen for the detection of Q-fever complement-fixing antibodies in humans. In practice, the choice of antigen would certainly affect the results of serological surveys designed to estimate the proportion of individuals with serological evidence of infection, but for the diagnosis of clinical Q fever, where a rise in antibody is more important than the absolute titre, antigen from either strain might reasonably be used. The results with sheep sera suggest that the antigen of choice may vary from one part of the country to another. Thus it might be impossible to compare serological rates of infection in these animals in different areas, because there may be no one antigen which has a wide enough range of specificity to react equally well with antibodies against different local strains.

RÉSUMÉ

Le diagnostic de la fièvre Q est fondé sur la mise en évidence des anticorps spécifiques dans les sérums humains ou animaux. On choisit généralement à cette fin la réaction de fixation du complément, de préférence aux tests d'agglutination qui demandent de plus

grandes quantités d'antigène et sont plus difficiles à lire et à interpréter. L'antigène utilisé pour l'épreuve de fixation du complément est constitué par une suspension purifiée de *Rickettsia burneti* provenant d'une culture sur sac vitellin d'œuf de poule fécondé. Le pouvoir fixateur du complément varie d'une souche de rickettsie à l'autre. Aussi le choix de l'antigène à employer pour les épreuves systématiques a-t-il une grande importance. La souche Nine Mile, provenant de tiques des Montagnes Rocheuses, et la souche Henzerling, isolée d'un malade en Italie, ont été couramment utilisées jusqu'à maintenant dans les réactions de fixation du complément; elles étaient considérées comme interchangeables. En effet, l'étude comparée de onze souches de *R. burneti* avait démontré que seules ces deux souches réagissaient bien avec les sérums d'homme, de mouton et de cobaye. Cependant, des titrages comparatifs, effectués au cours de l'enquête épidémiologique sur la répartition de la fièvre Q en Angleterre, devaient révéler des différences de sensibilité entre ces deux antigènes, assez marquées pour justifier une étude approfondie. C'est à cette question que la plus grande partie de cet article est consacrée.

Les essais comparatifs ont porté sur 868 sérums de mouton, 1055 sérums de donneurs de sang, 66 sérums de convalescents et 20 sérums de cobaye. Quelle que soit la concentration de l'antigène, la souche Nine Mile s'est montrée plus sensible que la souche Henzerling, s'il s'agit de déceler les anticorps dans les sérums humains et les sérums de cobaye et dans quelques sérums de mouton du Pays de Galles. Avec les sérums de moutons du Kent, en revanche, l'antigène Henzerling était plus sensible que le Nine Mile. Il faut souligner que ce n'est que dans le test de fixation du complément que ces différences se sont manifestées. Dans les épreuves d'agglutination, les deux antigènes se sont comportés de façon semblable. En outre, les divergences n'ont été très fortes, pour les sérums humains, qu'au cours de la première année suivant l'infection. Un fait déjà connu se vérifie ici encore: les sérums de convalescents tardifs réagissent mieux avec les antigènes que les sérums de convalescents récents.

Les auteurs concluent de leur étude que, dans certaines parties de l'Angleterre du moins, l'antigène Nine Mile est plus sensible que l'antigène Henzerling, s'il s'agit de déceler les anticorps anti-fièvre Q chez l'homme. Pratiquement, dans les enquêtes épidémiologiques, le choix de l'antigène affectera certainement les résultats des épreuves de fixation du complément destinées à dépister les sujets infectés. Mais, dans le diagnostic clinique — où l'élévation du titre a plus d'importance que sa valeur absolue — les deux souches pourront être utilisées avec une certaine sécurité. D'autre part, en raison des divergences entre les deux antigènes qui viennent d'être signalées, il est possible que la comparaison du taux d'infection chez les moutons provenant de zones diverses perde toute valeur, car il ne semble pas qu'il existe actuellement d'antigène ayant une marge de spécificité assez étendue pour réagir également bien avec les anticorps correspondant à des souches locales variées de *R. burneti.*

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