Q FEVER IN GREAT BRITAIN

EPIDEMIOLOGICAL INFORMATION FROM A SEROLOGICAL SURVEY OF HEALTHY ADULTS IN KENT AND EAST ANGLIA

By B. P. MARMION,* M. G. P. STOKER

Department of Pathology, University of Cambridge

C. B. V. WALKER

Regional Blood Transfusion Centre, Cambridge

AND R. G. CARPENTER

Department of Human Ecology, University of Cambridge

(With 1 Figure in the Text)

INTRODUCTION

The epidemiological findings in sixty-nine sporadic cases of Q fever, which were given in a previous report (Marmion, Stoker, McCoy, Malloch & Moore, 1953), suggested that some of the modes of infection in human Q fever, already recognized or suspected from investigations in other countries, were also to be found here. It was discovered, for example, that some cases of Q fever occurred in persons working in contact with cattle and sheep or with tissues from these animals.

The number of persons having close occupational contact with animals was small however, only eight (11·6 %) falling into the category as defined in the present paper. There remained the larger problem, the occurrence of Q fever in those members of our population who were not overtly exposed to animals as part of their occupation. It was noted that among the latter there were seventeen (24·6 %) persons whose occupation, although not directly concerned with animals, nevertheless led them to make frequent visits to places such as farms or slaughter-houses, and even glue works, etc., where they might be near animals or their tissues and excreta. Others in this group either lived close to farms or regularly met persons from farms or handled objects which had been on farms and in other potentially infectious places.

In addition to these various possibilities of occupational or residential exposure to infection, it also seemed feasible that some patients might have been infected from milk; as some twenty-eight (41 %) of them were consuming raw milk during the period before their illness, and *Rickettsia burneti* was isolated from the milk supplies of ten out of twenty patients who were investigated.

A final assessment of the significance of the epidemiological findings was difficult because of the lack of information on the frequency of these particular

---

* Member of the staff of the Public Health Laboratory Service, seconded to the Department of Pathology, Cambridge.

activities or qualities among the healthy population in the same area as the Q-fever patients.

It was necessary, therefore, to compare and contrast the epidemiological experience of persons infected with *R. burneti* with that of a similar but uninfected group of persons drawn from the same general population. Such a comparison could be made in a number of ways. Bell, Beck & Huebner (1950), for example, in their study of the epidemiology of Q fever in the population of Los Angeles, looked for a higher incidence of antibody in groups which they suspected to be at risk than in others not at risk.

In the present local investigation of Q fever in England, we have compared the epidemiological histories of healthy persons—blood donors—who had evidence of previous exposure to *R. burneti* in the form of specific complement-fixing (CF) antibody in their serum with those of a similar group of donors without such antibody.

It had previously been found (Marmion & Stoker, 1950; Marmion *et al.* 1953) that the sera of blood donors living in places where clinical cases of Q fever had occurred sometimes contained CF antibody to *R. burneti* at low titre. Thus in the survey undertaken in 1950–51 antibody was found in some 3·3 % of donors living in Kent as compared with 0·8 % of donors in certain eastern counties, conveniently referred to as East Anglia, where no cases of Q fever had been detected at that time.

It is well known that blood donors are not a random sample of the population at large. For example, they are taken from a healthy group of persons within a limited range of ages, usually between 18 and 65 years. The selective nature of the blood donor population does not matter when we are considering the positive information derived from comparison of donors with and without antibody and drawn from the same general sample. It may matter, however, when we are considering the limits of the information obtained. It is obvious, for example, that by such comparisons we shall learn nothing of the epidemiology of Q fever in persons aged 10–18 years, which might well be different from that in persons aged 18–65 years. Again, the epidemiology of infection in small specialized groups in the community will escape attention, as the chance of being both a member of such a group and a blood donor in our survey will be small. Lastly, the selective nature of the sample and variations in the composition of groups of blood donors from area to area, arising mainly from different recruiting methods, might invalidate the comparison of the absolute rates of antibody in donors in different geographical areas. Nevertheless, in spite of these limitations we may hope to learn something of the common, and therefore the presumably important ways in which people are infected.

It is the purpose of this paper to describe the results of a comparison of the epidemiological information obtained from a group of Kentish and East Anglian blood donors with Q-fever antibody with that of donors in the same area but without antibody. Variations in the prevalence of Q-fever antibody in whole groups of donors living in various parts of the two areas are also described and their significance considered.

GENERAL CONSIDERATIONS AND METHODS

Specimens of serum have been tested from donors (all members of the general public and not drawn from factory groups or the Armed Forces), who gave blood at twenty-eight centres in towns or villages in all parts of Kent, and at seventy-eight centres in Norfolk, Suffolk, Cambridgeshire, Huntingdonshire, Bedfordshire, Hertfordshire and Essex. The specimens were collected by the staff of the South London Blood Transfusion Centre (B.T.C.), Sutton, the Regional B.T.C., Cambridge, and the North London B.T.C., Edgware.

The blood sample used for the CF test for Q-fever antibody was contained in a small test-tube or bottle filled at the time of the donation. This is an integral part of the transfusion collecting apparatus which is normally used to provide blood for grouping and allied purposes. The residual contents of these tubes, either in the form of clotted blood (South London B.T.C.), or separated serum (North London and Cambs B.T.C.), were posted to us from London or collected by hand in Cambridge. At this stage the samples were identified only by a number.

Method of testing sera for complement fixing antibodies to Rickettsia burneti

A 0·5 ml. volume of a 1/5 dilution of serum was made up by mixing 0·1 ml. of serum with 0·4 ml. of saline using an automatic pipette. This mixture was heated at 60° C. for 30 min. One unit volume, 0·1 ml., of this 1/5 serum dilution was then mixed with similar volumes of the other reagents—complement, *R. burneti* antigen—of the standard complement-fixation test used in this laboratory and described in detail by Stoker, Page & Marmion (1955*b*). In this preliminary 'screen' test the usual controls for specificity and anticomplementary activity were not included. When, 18–24 hr. later, the test was complete, a record was made of the negative sera, i.e. those showing complete haemolysis, and also of those showing 25 % or less lysed cells. The latter sera, which might have fixed complement in the preliminary 'screen' test at 1/5, either because they contained *R. burneti* antibody or because they were anticomplementary or reacting non-specifically, were then retested over a range of dilutions from 1/10 to 1/40 (or higher when necessary) starting again from the stock dilution of 1/5 serum in saline which had been kept at −20° C. in the interim. In this second test a serum control and a dissimilar antigen control, a suspension of murine typhus rickettsiae, were included for each serum. Only those sera which showed 25 % or less haemolysis at 1/10 with the *R. burneti* antigen, and no fixation whatever in the serum or dissimilar antigen controls, were considered to be positive; i.e. to contain specific CF antibody to *R. burneti*. Sera, which fixed complement at a dilution of 1/5 in the original test but did not do so at 1/10 or above, were excluded from further consideration.

The antigen used was prepared locally from the Nine Mile strain of *R. burneti*. In the previous survey of blood donors carried out in 1950–51 Henzerling strain antigen was used, but at the beginning of the present work a change was made to the Nine Mile strain, largely because it yielded more antigen. The change was considered safe because the published evidence (Wolfe & Kornfeld, 1949; Strauss &

Sulkin, 1949) did not indicate that there was any difference between the ability of the two antigens to detect specific CF antibody. However, during the course of the present work, it became apparent that the Nine Mile antigen in fact gave a greater proportion of positive reactions with the donor's sera than the Henzerling. For this and other reasons, the results obtained in the present survey are not directly comparable with those obtained in 1950–51. The serological aspects of this difference between the two antigens are discussed in detail elsewhere (Stoker *et al.* 1955*b*).

Choice of donors for the epidemiological inquiry

The results of the serological testing of the donors were broken down to show the positive and negative Q-fever reactors who attended a particular transfusion bleeding session at the town or village centre. All the positive reactors were included, and an equal or slightly larger number of negatives chosen from the remainder by taking those donors whose identifying number ended with the same last two digits as each of a series of numbers coming in sequence in a table of random sampling numbers. The names and addresses of all these donors were obtained, and they were sent a letter explaining the investigation and asking if they would be willing to answer certain epidemiological questions. Donors who agreed to take part were then visited by one of three observers who took an epidemiological history and wrote the answers on a standard record form. The dispatch of letters to donors, the making of appointments and other arrangements were carried out in such a way that the interviewer did not know whether the donor had a positive or negative serum at the time of recording the information.

Duration of the survey period

The epidemiological information obtained from the donor related (with two exceptions) to the 12-year period before the serum was tested, that is from 1942 to 1953.

The choice of this 12-year period was somewhat arbitrary and represented an estimate of the length of time that memories of past events and serum antibody might persist in the donors. Information on the latter point is rather scanty. We have found that three of four workers infected in two of the small laboratory outbreaks of Q fever in Australia still had CF antibody at a titre of 1/40 in their sera some 12 or 15 years after infection.

A re-examination of fifteen of the English cases of Q fever showed that eleven still had CF antibody, at serum titres ranging from 1/10 to 1/160, 4 years after illness. Dr Tonge (1955) has recently re-tested some Queensland (Australia) residents who had Q fever in the past, and finds that, of forty-three of them with little risk of re-exposure to infection, about 50 % still had complement-fixing antibody at 6–10, and 29 % at 11–15 years, after their illness.

Our choice of a 12-year survey period was perhaps not (serologically) unreasonable in the light of all these results.

Moreover, it is probable in any event that the high proportion of persons with antibody found in certain groups of the population, such as farmers, owes as much to the stimulus of repeated re-exposure to infection as to persistence of antibody after a single clinical or subclinical attack.

The questions asked

Analogies from investigations in many other parts of the world (for details see the review by Derrick, 1953), and previous investigations in Great Britain, either already mentioned in the introduction or reviewed by Stoker (1953a), have suggested the main probable modes of spread of *R. burneti* from animals to man. The questions were framed in the light of this experience. In particular, the results of the Californian workers, Bell *et al.* (1950) in Los Angeles, and Clark, Lennette & Romer (1951), in the northern part of the state, were kept in mind to see how far their epidemiological conclusions might be applicable to this country.

Accordingly, questions were asked about the possibility and extent of the donor's contact with cattle, sheep, goats and with other animals or birds; or with excreta or tissues from these creatures. This contact was classified broadly as arising out of any of the donor's present or past jobs (occupational); his (or her) leisure activities; or from the fact that his present house, or another lived in since 1942, was situated within 200 yards of certain potential sources of Q fever, such as a farm building with animals, a slaughterhouse, etc. (residential contact).

Occupational contact was further classified according to intensity of exposure in ways which will become apparent when the results of the survey are described.

Donors were also asked for the names and addresses of the dairy, or dairies, which had supplied their household milk during the period 1942–53, and whether the milk was raw or pasteurized. The appropriate health authority was then asked to provide confirmatory information about the types of milk sold by the dairy during the relevant period, and also to confirm the donor's statement that his house was within 200 yards of one or more potential sources of Q fever specified in the list.

Lastly, general information on age, sex, clinical history, duration of residence in the town or area, the type of domestic animals or birds kept as pets during the period 1942–53; the household water supply and travel or service abroad was noted. The survey period, 1942–53, was partly during the war, but a detailed record was not made of the epidemiological experience of donors during their time in the Armed Forces.

The information was recorded on a standard form which enabled Hollerith machine operators to transfer information to punch cards.

In order to classify the information for punch-card analysis, it was necessary to use some agreed and rigid definitions of qualities and circumstances to which each of the three observers tried to adhere when recording the answers to questions.

Method of analysing the results

The tests of sera from blood donors in the two areas, Kent and East Anglia, give two sorts of information. There is that arising from the complete sample tested at the various centres in each area from which certain comparative conclusions about the prevalence of antibody in the groups as a whole and in the various areas may be drawn, such as, on the variation in the incidence of antibody in donors living in areas 1–11 in Kent (see Table 8). The results of this kind of analysis are given in Part II of the paper.

There is also that information, described in Part I, which arises from the detailed comparison of the epidemiological histories of the two groups of donors: one with and one without Q-fever antibody. The composition of this assembly of donors has been artificially arranged so that about half its members are positive and the other half negative. If such an artificially constructed group is further divided into a series of subgroups with various, different characters, then the number of positives or negatives in each subgroup should be, on an average, approximately equal—provided that there is no association on the one hand, between the particular character on which the total sample is being subdivided and an increased probability of being positive (or negative) on the other. If a departure from the expected number of positives (or negatives) is observed in a particular subgroup there may be an association between its character and the donors' serological state—and this, of course, is what we wish to detect—or the deviation from expectation may merely be due to chance variation in subdividing the sample. The probability that deviations from expectation are in fact due to chance sampling variation, can be estimated by 'χ^2' analysis of the appropriate table. The finding, by this type of analysis, that an association between the serological state of the donors and a particular epidemiological character is unlikely to be due to a sampling effect is not only an essential preliminary step which delineates those epidemiological qualities of the donors which are of importance, but is also a convenient way of describing the data and the results. It has, therefore, been used throughout Part I of the paper. However, this analysis of one character at a time ignores effects due to interrelationships between them. For example, the finding that persons using raw milk have Q-fever antibody more frequently than those using pasteurized milk might in reality be due to the fact that the former were also frequently farm workers exposed to infection in their jobs. A different type of analysis which takes into account such possible interrelationships between the main characters has been made and is given in the Appendix.

RESULTS

PART I

Comparison of the epidemiological histories of the sample of Kentish and East Anglian donors with and without antibody to Rickettsia burneti

In the following section a detailed analysis of the positive findings is made in respect of blood donors in Kent. Those of East Anglian donors are given in less detail, except where they differ essentially from those in Kent, or where there is some advantage in combining figures from both areas.

General information

Investigation of donors in Kent

Four thousand six hundred and fifty-one donors were tested at the various centres in Kent, and 186 (4·0 %) were found to have serum antibody at a titre of 1/10 or greater. A group of 327 donors was made up by taking positive and

negative donors at each centre, as already described in Methods. Two hundred and eighty-four (87 %) of the 327 donors agreed to take part in the investigation, and 275 were eventually interviewed and their records analysed. The proportions of positive and negative and of male and female among the fifty-two donors who did not agree to take part in the investigation, or who were not visited, were not significantly dissimilar from those in the main sample of 275. Table 1 shows the distribution of donors by serological state and sex. It will be seen that there were slightly more females than males in the sample and that the males more commonly had antibody. Other analyses, not given here, showed that there was no significant difference in the proportion of either positives and negatives, or of males and females seen by each of the two observers who interviewed the donors in Kent.

Investigation of donors in East Anglia

Six thousand three hundred and eighty-six donors were tested in the various eastern counties already described, and of these 163 (2·6 %) were positive. Donors living in Hertfordshire and Essex were not visited, and the investigation of donors in the other counties was undertaken by a third observer different from the two working in Kent. Attention was concentrated mainly on Norfolk and East Suffolk, because most of the positive donors were to be found in these counties. A group of 183 donors was made up by taking positives and negatives, as in Kent, and when these were approached 148 (80 %) agreed to take part. The records of 121 donors were finally analysed. Table 1 shows the distribution of these donors by

Table 1. *Numerical distribution of* 275 *Kentish blood donors and* 121 *East Anglian blood donors by sex and reaction of their sera with* Rickettsia burneti *in the complement-fixation test*

	Kentish donors. Serological state			East Anglian donors. Serological state		
	Positive*	Negative†	Total	Positive*	Negative†	Total
Male	69	58	127	37	39	76
Female	68	80	148	16	29	45
Total	137	138	275	53	68	121

$$\chi_c^2 \text{ (1 degree of freedom)} = 1\cdot6, \qquad \chi_c^2 \text{ (1 degree of freedom)} = 1\cdot48,$$
$$0\cdot3 > P > 0\cdot2 \qquad\qquad 0\cdot3 > P > 0\cdot2$$

* CF antibody to *R. burneti* in donor's serum at dilution of 1/10 or greater.
† No CF antibody to *R. burneti* in donor's serum at dilution of 1/5.
χ_c^2: see Fisher & Yates (1948).

their sex and serological state. It will be seen that in contrast to the Kentish donors the group investigated in East Anglia contained fewer females than males. The sample was also less well balanced in that there were more negatives than positives. Once again, however, there were proportionately more males with antibody than females.

An examination of the age distribution of the sample of donors in Kent and East Anglia revealed that the proportion of persons in each 10-year age group was

almost identical in the two areas. In both, the bulk of the sample was made up of persons between 30 and 60 years old. The incidence of antibody in the various groups was similar at all ages and in both areas, except that East Anglian males aged less than 30 years were less frequently positive.

The numerical distribution of donors by the Registrar General's social classes was also very similar in the two areas, except that there was a slight preponderance in Kent of the category 'not gainfully employed' (mainly housewives).

Because of the similarity in age and social grading, it is concluded that comparisons between the areas may be made with safety.

Occupation, including contact with animals

In this section some evidence is presented on the incidence of Q-fever antibody in Kentish donors with various degrees of occupational contact with animals. This confirms the occupational risk of the disease in this county previously suspected from the investigation of patients with Q fever.

Kent

In the sample of 275 Kentish donors there were twenty-two persons who had contact with animals—cattle, sheep, goats and pigs, or with their tissues or excreta—as an essential and constant part of their present job. This form of exposure is termed 'direct occupational contact'. Eighteen of the group had Q-fever antibody. The group was composed of nine farm workers, one agricultural student, one butcher and slaughterer, one municipal garbage collector who handled slaughterhouse offal, seven persons who handled either raw wool or farmyard manure, a porter who loaded cattle and sheep at a railway goods yard and two housewives who tended goats, pigs or sheep.

There were also twenty-nine persons who had had direct occupational contact in a past job, but were not so exposed at present. This group did not show any striking excess of positives over the general level in the whole sample. Apart from those donors with present direct occupational contact, sixty-three donors had possible exposure which, although not an essential and constant part of their job, arose as the incidental result of it in that they visited potentially infectious places ('active indirect occupational contact'), or received objects, or met people from such places dressed in their working clothes ('passive indirect occupational contact').

The position for these three categories of occupational exposure is shown in detail in Table 2. The χ^2 value for the table relating to Kent suggests that the differences in the proportion of donors with antibody in each of the subgroups is unlikely to be due to chance variations of sampling in subdividing this group of donors (χ^2, with 3 degrees of freedom $= 14\cdot8$, $P < 0\cdot01$). Inspection of the table for Kent reveals that the greatest excess in numbers of donors with antibody over the expected values (i.e. those calculated from the proportion 137/275 (49·8%) of positive donors in whole Kentish sample) is in the two subgroups 'direct occupational contact at present' and 'active indirect occupational contact'. There is also

a corresponding deficiency in the numbers of donors with antibody in the subgroup which did not have any of the three types of occupational contact.

Table 3 shows that there is a direct relation between the duration of direct occupational contact (present and past considered together) and the proportion of Kentish donors with antibody. This relation also held for males and females when considered separately.

Table 2. *Incidence of antibody to* Rickettsia burneti *in groups of Kentish and East Anglian donors with various forms of occupational contact with animals*

Kent. Nature of occupational contact

| Serological state | Direct and present* | Indirect | | None | Total |
		Active†	Passive‡		
Positive O	18	27	10	82	137
E§	11	22	9	95	
Negative	4	17	9	108	138
Total	22	44	19	190	275

East Anglia. Nature of occupational contact

| Serological state | Direct and present | Indirect | | None | Total |
		Active	Passive		
Positive O	13	9	2	29	53
E	12	7	1	32	
Negative	15	8	1	44	68
Total	28	17	3	73	121

* Contact with cattle, sheep, goats or pigs or materials from these animals as an essential part of present job.

† More than three visits a year to farms, slaughterhouses, fertilizer factories, livestock markets, etc., in any job since 1942.

‡ Handles objects contaminated on farms, etc., or meets persons from such environments dressed in working clothes at least once a week in any job since 1942.

Note. These categories are mutually exclusive.

§ 'Expected' number of positive donors in subgroup calculated from proportion of positives in total sample in either Kent or East Anglia; throughout the tables 'E' refers to the 'expected' value and 'O' to that 'observed'.

Table 3. *Incidence of antibody to* Rickettsia burneti *in groups of Kentish donors according to the length of their 'direct occupational contact' in a present or past job*

| Serological state of donor (Kent only) | Duration of present or past occupational contact* in years | | | Total |
	1–5	6–15	16 or more	
Positive O	8	14	10	32
E	11	14	7	
Negative	10	8	1	19
Total	18	22	11	51

An 'exact test' (Freeman & Halton, 1951) gives $P < 0.05$.

* Group composed of twenty-two donors exposed in their present job and twenty-nine donors exposed in a past job, but not so exposed at present.

It seems clear, therefore, that in the Kentish donors there is an association between the possession of antibody and present direct occupational contact with animals or their products, and also with the duration of such contact in a present or past job.

East Anglia

In the sample of 121 donors from this area there were twenty-eight (23 %) who were classified as having direct occupational contact as compared with 22/275 (8 %) among the Kentish donors. Thirteen of these twenty-eight East Anglian donors had Q-fever antibody. There were also twenty donors who had either active or passive indirect occupational contact; eleven of these had antibody. When all the data are considered together, as for Kent (Table 2), the χ^2 value obtained clearly indicates that the differences observed between the subgroups would arise frequently by chance alone, and that there was no evidence of an association between possession of antibody and the various forms of occupational contact ($\chi^2 = 1\cdot6$, $0\cdot5 > P > 0\cdot3$: χ^2, 2 degrees of freedom by combining active and passive indirect contact). Similarly, there was no association between antibody and duration of occupational contact.

This difference between the groups with direct and indirect occupational contact in the two areas might be due, presumably, either to the fact that they were exposed to different species of animal (or their products) or, if exposed to the same species of animal, that these were less frequently infected in East Anglia.

The nature of the animal sources to which persons were exposed in their present job is given in general terms for the two areas in Table 4. It can be seen that the occupationally exposed in Kent had more frequent contact with sheep, or their products, as compared with those in East Anglia.

Table 4. *Number of donors occupationally exposed in their present jobs to various species of domestic animal in Kent and East Anglia*

	Number of donors having contact with cattle and sheep		
Type of animal with which donors had contact*	Kent	East Anglia	Total
Cattle only	13	27	40
Sheep and other animals, including cattle	31	8	39
Total	44	35	79

χ_c^2 (1 degree of freedom) $= 15\cdot8$, $P < 0\cdot01$

* Direct or indirect contact with the whole animal or its untreated products during the period of the donor's *present* job.

The significance of this difference, together with that of some relevant findings in relation to density of stocking with sheep and total antibody rates in the two areas which are given in Part II, will be considered in the discussion.

Indirect contact with animals or materials from them

Apart from the association between the positive serological state and direct occupational contact in the group of Kentish donors, it was found, as already stated, that a greater proportion of donors in Kent with active and, to a less extent, with passive indirect occupational contact had antibody than had those in the *residue* of the sample without such contact (see Table 2). A further analysis of the composition of the former groups of donors showed that antibody was most common among those who visited farms or farmlands to do some work on the agricultural side of the farm. For example, there were eleven persons, nine of whom were positive, with the following occupations: five members of the building trade who worked on farm buildings, four engineers who used dairy or agricultural machinery, and two labourers who dug ditches or operated bull-dozers on farm land.

The remaining thirty-three persons in the group with active indirect occupational contact, of whom eighteen were positive, were not closely concerned with the industry of the place they visited. Among them were such people as village grocers or postmen who delivered goods or mail at farms; or who visited them in connexion with insurance, water rates, or the demonstration of household appliances; or people visiting fertilizer factories and so on.

Other analyses showed that the increased prevalence of antibody in this whole group of forty-four persons was not due to associated factors such as increased frequency of their use of raw milk at home in comparison with the non-occupationally exposed, or to their direct occupational contact in a former job, or to living (but not working) on a farm. Contrariwise, a similar analysis of the small group of nineteen persons classified as having passive indirect occupational contact, which included such persons as village drapers and publicans, village garage mechanics who repaired farm machinery but did not visit farms, etc., revealed that the slightly increased incidence of persons with antibody in this group, as compared with the non-exposed residue of the sample, was due mainly to the inclusion of farmers' wives who might have been exposed in other ways. For this reason, and because of the small numbers, the significance of this form of contact is not established.

Other data, not given here, showed that persons visiting farms or other possibly infectious environments during their leisure also had a significantly higher rate of antibody than that in the sample as a whole.

Residence and residential contact with animals

In the investigations of Bell *et al.* (1950) in Los Angeles, it was found that persons living within one-eighth of a mile of a dairy farm with infected stock more frequently had antibody than those living at a greater distance. In the present investigation, no significantly increased incidence of antibody was found, either among East Anglian or Kentish donors who, during the period 1942 to 1953, had lived within 200 yards either of farm buildings with stock, of a piggery, or pens and fields in which lambing or calving took place, or of fields where cattle and

sheep were grazed. The number of donors in the sample who had lived near glue
works, tanneries, railway yards where stock was handled, live-stock markets or
slaughterhouses (the other places asked about) was too small to permit of valid
conclusions as to the possible epidemiological importance of residence near these
places.

Table 5. *Prevalence of antibody to* Rickettsia burneti *among male and female
Kentish donors who either had or had not lived on a farm at any time*

	Males. Serological state			
	Positive			
Kentish donors who have:	O	E*	Negative	Total
Lived on farm	19	**13**	5	24
Not lived on farm	50	**56**	53	103
	69		58	127

χ_c^2 (1 degree freedom) $= 6\cdot2$, $0\cdot02 > P > 0\cdot01$

	Females. Serological state			
	Positive			
Kentish donors who have:	O	E*	Negative	Total
Lived on farm	24	**20**	20	44
Not lived on farm	44	**48**	60	104
	68		80	148

χ_c^2 (1 degree freedom) $= 1\cdot4$, $0\cdot3 > P > 0\cdot2$

* 'Expected' number of positive donors in subgroup based on proportion of positive males
or females in the total sample of each sex.

A history of being a member of a farming household, as distinct from living in
a house up to 200 yards from a farm or its outbuildings, was associated in Kent
with significantly higher rates of antibody in the appropriate subgroup when com-
pared with the total group of donors (Table 5). The greatest excess of positives
over the expected level was among the male donors, that among females being less.
This suggests that occupational contact with animals rather than residence in
physical proximity to them, is the important factor.

In East Anglia neither the male nor the female donors who lived, or had lived,
on farms showed any increased rate of antibody over the general level. The
finding of a difference in this respect between Kent and East Anglia is in agree-
ment with that already found with the various forms of occupational exposure.

Incidence of antibody to Rickettsia burneti *in donors with
various types of household milk supply*

As already stated in the introduction, the epidemiological significance of raw
milk for the infection of the English Q-fever cases was to some extent uncertain
because of the lack of information on the frequency with which it was used by the
healthy population. In addition to this, people who work with animals on farms

often have a raw household milk supply. It was possible, therefore, that Q fever contracted by exposure at work might be erroneously attributed to raw milk drunk at home.

In this section the results are given of an analysis of the frequency with which Q-fever antibody was found in groups of donors who had been supplied with either pasteurized or raw milk, or both, at their homes during the period 1942–53.

Obtaining accurate information about milk supplies during a relatively long period, i.e. 1942–53, was inevitably difficult because a few donors could not remember the name of the supplying dairy or the type of milk they had had in the past. When this happened, their experience was classified on what was known and they were recorded as having an 'incomplete' or 'unreliable milk history'. Also, some of the donors had been in the Armed Forces for part of the survey period, and it was impracticable to find out the type of milk they had had at each place where they had been stationed. The experience of these donors was classified according to their home milk supply during the period 1942–53, due allowance being made for the period in the services when estimating the length of time they had used a particular type of milk at home during the period. The collection and treatment of the information in this way seems to be justified because the proportion of donors with or without antibody in the groups who had either incomplete or unreliable milk histories or who had been in the Forces, did not differ significantly from the proportions in the sample as a whole. It was also found that the (rather rare) consumption of raw milk at work did not complicate the results obtained in the analysis of household milk supplies.

Serological state and type of milk supplied to home

Table 6 contains details of the number and serological state of the donors who, during the period 1942–53, had either (1) a pasteurized milk supply, or (2) a raw milk supply to their homes during the whole period, or (3) a 'mixed' supply, that is raw milk during the first part of the period and subsequently a change to pasteurized milk or vice versa. If two sorts of milk were supplied at the same time as, for example, raw tuberculin-tested milk together with pasteurized ordinary milk, the supply was counted as raw. It can be seen from Table 6 that both in Kent and East Anglia there was an excess over expectation in the number of positive donors among those who had been supplied at home with raw milk for all or part of the period 1942–53, and a corresponding deficiency of positives among those supplied with pasteurized milk for the whole period. The data given in Table 6 show roughly that there was a relation between the length of time during the period 1942–53 that donors had been supplied with raw milk at home and the incidence of antibody in the appropriate group. A more detailed analysis confirmed this finding and revealed that the relation was most marked with female donors in Kent, but similar in male and female donors in East Anglia. The less pronounced effect in male donors in Kent was presumably due to their more frequent exposure in other ways.

These findings apply only to the use of cows' milk at home. Some twenty-two

(5 %) of the 396 donors in Kent and East Anglia had used goat's milk on one or more occasions during the period, but there was no significant excess of positives among them.

Table 6. *Incidence of antibody to* Rickettsia burneti *in groups of Kentish or East Anglian donors supplied at home with either raw or pasteurized milk or both during the period* 1942–53

(Household milk supply of donors during period 1942–53.)

Serological state of donors	Kent			
	Pasteurized only	Raw only	'Mixed'*	Total
Positive O	41	26	70	137
E†	**53**	**21**	**63**	
Negative	65	17	56	138
	106	43	126	275

χ^2 (2 degrees of freedom) = 8·9, 0·02 > P > 0·01

Serological state of donors	East Anglia			
	Pasteurized only	Raw only	'Mixed'*	Total
Positive O	7	24	22	53
E†	**14**	**20**	**20**	
Negative	24	21	23	68
	31	45	45	121

χ^2 (2 degrees of freedom) = 7·8, 0·05 > P > 0·01

* Raw milk supply for part of the period followed by change to pasteurized supply or vice versa.

† 'Expected' number of positive donors in subgroup calculated from proportion of positives in the total sample in Kent or East Anglia.

Exposure to animals other than cattle, sheep and goats and to miscellaneous birds and insects

There was no significant excess over expectation in the number of donors with antibody among groups in Kent or East Anglia which had kept the following at (or near) their homes between 1942 and 1953; dogs, cats, rabbits, pigs (mostly for fattening), chickens, pigeons or various psittacine birds. Similarly, infestation (generally slight) of the house or outbuildings with mice, rats or mosquitoes, or the use of animal fertilizers (bonemeal, hoof and horn meal, farmyard manure) on the garden was not associated with any significant excess in the number of positives among those in contact. No donor recalled being bitten by a tick during the period, but a few had removed ticks from dogs, cats or ferrets. Once again, however, this group did not show any increase over expectation in its number of positives.

Clinical history and serological state

Distribution of serum titres of CF antibody in male and female donors in Kent and East Anglia

Fig. 1 shows the proportion of donors with serum antibody of various titres, 1/10, 1/20, or 1/40 and greater, among the positive male and female donors in the two areas. In both areas the male donors more frequently had antibody at titres of
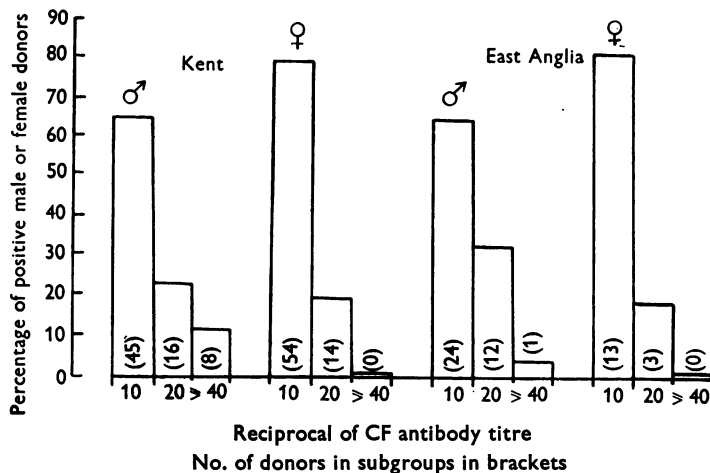

Fig. 1. Proportion of male or female donors, expressed as a percentage of all those with Q-fever antibody, with antiserum titres of 1/10, 1/20 or 1/40 and greater.

Table 7. *Relation between serological state of Kentish donors and past history of illness during the period 1942–53*

Type of illness during period 1942–53	Males			Females		
	Negative	Positive	Total	Negative	Positive	Total
Pneumonia	2	6	8	4	0	4
Fever lasting longer than 5 days	5	9	14	1	4	5
'Influenza'	8	15	23	15	8	23
All sick donors	15	30	45	20	12	32
All healthy donors	43	39	82	60	56	116
Total (all donors)	58	69	127	80	68	148

Sick and healthy male donors:
χ^2 (1 degree of freedom) $= 3.5$,
$0.1 > P > 0.05$

Sick and healthy female donors:
χ^2 (1 degree of freedom) $= 0.8$,
$0.5 > P > 0.6$

1/20 or greater than had the females. It is known that clinical attacks of Q fever are commoner among males than females, and it seems possible that the larger proportion of male donors with antibody at high titres reflects this tendency. An analysis was made of the medical history of the donors in relation to their serological state; the results for Kent are shown in Table 7. It can be seen from this

table that each illness of the three types simulated by Q fever, i.e. pneumonia, prolonged fever and 'influenza', was commoner among male donors with antibody as compared with the negatives. In the aggregate, 30/69 (43 %) of male donors with antibody had had a suspicious illness compared with 15/58 (25 %) of those who had no antibody. This difference was not statistically significant. Among female donors, on the other hand, illness was more evenly distributed, occurring in 12/68 (17·6 %) of the positive donors and 20/80 (25 %) of the negative during the period 1942–53. In East Anglia, there was no clear association between illness and antibody in either male or female donors.

The question of how many of the donors were ill with Q fever at the time they were first infected will be considered, together with information from other investigations which bears on the problem of the ratio of clinical to subclinical infections, in the final paper of this series.

PART II

Information from the complete sample of blood donors

Table 8 shows the total number of donors tested and the number positive in each of the seven eastern counties and in Kent, those for the latter county being given for each of the eleven areas which are used for the agricultural census. In area 11, the Romney Marsh, only one blood donor was tested, because few donors live in that place. However, because of the large number of sheep there it was desirable to estimate the prevalence of antibody in the local human population. Consequently, a group of 191 persons, composed of 100 healthy volunteers from the general population (see Marmion, Stewart, Richmond, Barber & Stoker, 1954), and ninety-one blood donors from in and around the town of Rye (Sussex), which is on the edge of the Romney Marsh and on the border of Kent, were tested and the results are given in Table 8. From the total figure for Kent (the sum of areas 1 to 10) and that for the eastern counties, it can be seen that the rate for Kent, 4·0 % of donors with *R. burneti* antibody at a serum dilution of 1/10 or above, was still, as in the previous survey in 1950–51, above that for the eastern counties as a whole (2·6 %).

Table 8 also contains some information on the relative number of cattle, sheep and pigs in the various areas of Kent and the eastern counties. It can be seen that the Kentish areas 5 to 11, which are stocked with 48 or more sheep per 100 acres of crops and grass, had in the main, higher proportions of Q fever reactors among the blood donors or volunteers than did those areas (1 to 4 in Kent and the seven eastern counties) with fewer sheep. The coefficients of correlation between, on the one hand, the percentage of blood donors with antibody and, on the other hand, the intensity of stocking with sheep, cattle and pigs in the areas 1–10 of Kent and in the eastern counties give numerical values for this relationship (see Table 9). The results of testing persons living in area 11 have not been used in the calculation of these correlation coefficients because the sample in that area differed from that in the other areas. However, the high proportion of persons with antibody in this area substantiates the general finding.

It was also found, although the data are not given in Table 8, that there was no correlation between the prevalence of antibody in donors and the numbers of chickens, ducks, geese and horses in the various areas of East Anglia or Kent.

Table 8. *Density of stocking with cattle, sheep and pigs in various areas of Kent and East Anglia, and the number tested and proportion of blood donors with Q-fever antibody in these areas*

| Region | Animals/100 acres crops and grass | | | Blood donors and healthy volunteers | | |
	Cattle	Sheep	Pigs	No. tested	No. positive†	% positive
Kent						
Area* 1	14	7	34	1,086	20	1·8
Area* 2	27	15	16	538	25	4·6
Area* 3	16	40	16	525	11	2·1
Area* 4	16	43	22	378	9	2·4
Area* 5	22	61	15	163	4	2·5
Area* 6	16	104	14	347	27	7·8
Area* 7	23	113	14	274	18	6·6
Area* 8	19	48	20	523	36	6·9
Area* 9	27	76	20	426	20	4·7
Area* 10	16	49	25	391	16	4·1
Means‡ and totals						
Areas 1–10	**20**	**56**	**20**	4,651	186	4·0
Romney Marsh and Rye						
Area* 11	14	232	10	191	30	15·7
Eastern Counties						
Norfolk	20	5	12	1,369	35	2·6
Suffolk	17	6	16	1,801	50	2·8
Cambridgeshire (incl. Isle of Ely)	12	5	13	861	10	1·2
Huntingdonshire	15	9	9	225	7	3·1
Bedfordshire	20	9	15	431	13	3·0
Hertfordshire	22	8	13	503	14	2·8
Essex	18	5	13	1,196	34	2·8
Means‡ and totals (Eastern Counties)	**18**	**7**	**13**	6,386	163	2·6
Grand means and total (including Kentish area 11)	—	—	—	11,228	379	3·4

* So-called agricultural census areas. Areas 1–4 include, roughly, that part of Kent adjoining the south-east edge of London up to a line joining Gillingham in the north to Hawkshurst in the south. Areas 5–11 are east and south of this line.

† Sera giving a reaction of at least 75 % fixation of complement when diluted 1/10 and tested with Nine Mile strain antigen.

‡ Means are given in black type.

DISCUSSION

When low titres of complement-fixing antibody in the sera of healthy persons are taken as evidence of past exposure to *R. burneti*, as in the present survey, some comment is required on the specificity of the test. Our complement-fixation test was more sensitive than that employed by Bell *et al.* (1950) in Los Angeles, but of

approximately the same sensitivity as that of Clark *et al.* (1951) in North California, and also that recommended by the World Health Organization (Kaplan & Hulse, 1953). The specificity of the low serum titres could be demonstrated, presumably, in two ways: first, by showing that such levels of antibody are more frequently to be found in persons known to have had Q fever in the past, as compared with the population at large; and secondly, by the logical exclusion of causes of technical error known to complicate complement-fixation reactions in general. On the first count we have, in fact, more frequently observed reactions at the chosen levels in persons who had had Q fever in the past. On the second count error might be due to non-specific reactions either between sera and substances

Table 9. *Correlations of the proportion of blood donors with Q-fever antibody to the intensity of stocking with cattle, sheep and pigs in agricultural census areas 1–10 in Kent and for seven counties in East Anglia*

	Region	
	Kent	Eastern Counties
	No. of agricultural areas or counties	
Correlations of percentage positive with density of	10	7
Cattle	+ 0·25	+ 0·63
Sheep	+ 0·67*	+ 0·58
Pigs	− 0·47	− 0·07

* Significant at 5 % level.

other than *R. burneti* in the antigen, or to the summation of latent anticomplementary activity of sera, rickettsiae and yolk-sac material. It is considered that the inclusion in the test of a control antigen prepared from murine typhus rickettsiae considerably reduces, if not eliminates, the possibility of non-specific reactions being mistaken for specific. Similar evidence can be obtained by the use of antigens prepared from strains of *R. burneti* which are in the early stages of egg adaptation, and which fail to fix complement with homologous antibody (Stoker, 1953 *b*). Such antigens, although closely similar to the Nine Mile strain antigen in the numbers of rickettsiae they contain, in residual egg content and in the method of preparation, invariably fail to react with the low titre antisera from the blood donors. This suggests that the reaction with the sera is highly specific inasmuch as it depends on the particular antigenic state of the Nine Mile antigen.

The results of the survey show that persons in Kent, who are in constant occupational contact with cattle and sheep or materials from them, and those who, by virtue of their occupation or leisure activities, make visits to farms, slaughterhouses, etc., have a greater risk of infection, as judged by their more frequent possession of antibody, than those without such contacts. Similarly, in both Kent and East Anglia those persons which a household supply of raw cow's milk appear to have a greater risk of infection than those with a supply of pasteurized milk.

In contrast to the findings of Bell *et al.* (1950) we did not find that residence

within a short distance of farms was of significance. However, there are probably few, if any, situations in Great Britain which are closely comparable to those in Los Angeles where urban dwellers live close to heavily infected herds of dairy cattle concentrated in small areas from which bovine dejecta can be disseminated in dust.

Among the other possibly important negative findings there was no association between infection and the keeping of cats, dogs, poultry or psittacine birds, or with the infestation of the home or its surroundings with mice or rats. This suggests that, in spite of the few reports of the natural infection of pigeons and geese (Babudieri & Moscovici, 1952) and mice (Pérez Gallardo, Clavero & Hernandez, 1952), *R. burneti* is not behaving, at least in Kent and East Anglia, like the psittacosis viruses or *R. mooseri* (for example, see Saint, Drummond & Thorburn, 1954) in causing infection in the home.

It will be evident that interrelation between the effects of various forms of occupational exposure and of raw milk supply to the home could render difficult the identification of any one of these possible modes of infection as primary. It could be argued, for example, that users of pasteurized milk in Kent less frequently have antibody than users of raw milk in the same county, because the former are urban dwellers, whereas the latter come mainly from areas of Kent and from population groups, such as country dwellers, in which the use of raw milk is common, and that exposure in fact occurs as the result of associated occupational or leisure activities.

The possibility of such complicating interrelations has been in mind throughout the analysis of the data, and is dealt with specifically in the Appendix. In respect of the particular example given, it may be stated in simpler terms that the effects in the various groups presumed to be exposed by occupation, or by the use of raw milk at home, occur in all areas of Kent, not merely when the far rural areas and the suburban fringe of London are compared. Further, the finding that raw milk users in both Kent and East Anglia are more frequently at risk than users of pasteurized milk, whereas the occupationally exposed are at risk only in Kent, also suggests the separate identity of effects due to milk and occupation.

The difference between those occupationally exposed to animals in the two areas seems to be related to the presence of larger numbers of sheep in Kent than in East Anglia (Tables 4 and 8). Infection of Kentish sheep has already been shown on serological grounds, and some preliminary evidence that they act as a source of infection for man in one area of Kent has been provided (Marmion *et al.* 1954). Recently also their infection has been confirmed with the epidemiologically important demonstration that they excrete the organism from the genital tract, as do sheep in other countries (Stoker, Brown, Kett, Collings & Marmion, 1955*a*).

In addition to sheep, cattle are known to be infected in both East Anglia and Kent, but more commonly in the latter county (Marmion *et al.* 1953). The finding that those with occupational contact are at risk only in Kent would be well explained by the view that an increased risk of infection exists in this county owing to the presence there not only of infected sheep, but also of cattle which are more frequently infected than those in East Anglia.

In East Anglia, on the other hand, where sheep are few, and where the cattle, although present in numbers similar to those in Kent (see Table 8), are relatively rarely infected, it might be expected that an effect would not be detectable among the small numbers of those with occupational contact in our sample of East Anglian donors.

However, it is understandable that an effect due to the use of raw milk in the home would be detected among the East Anglian donors because milk from a small number of infected herds of cattle can reach a relatively large number of people.

Lastly, it must be emphasized that we have measured the differences in exposure of the various groups of donors in terms of their acquisition of antibody, which might be quite a different measurement from the acquisition of a clinical attack of Q fever. It is feasible that different methods of exposure might lead to different proportions of clinical to subclinical infections. The fate of those ingesting milk containing *R. burneti* (and its attendant antibody in the whey) might be different in this respect from that of those inhaling an infective aerosol from lambing sheep. The information in our present survey is insufficient to determine whether illness which might have been Q fever was equally distributed among donors exposed to infection in different ways. However, other investigations (to be published) have been carried out in two areas of Kent, and these show that attacks of Q fever can certainly result from milk-borne infection, and from lambing sheep.

SUMMARY

1. Eleven thousand two hundred and twenty-eight sera from persons, mostly blood donors, living in Kent and seven counties in East Anglia, have been tested and 379 (3·4 %) found to have complement-fixing antibody to *Rickettsia burneti* at a serum titre of 1/10 or greater.

2. A group of 396 donors in Kent and East Anglia, composed of 190 donors with antibody, and 206 donors from those without antibody to serve as controls, were interviewed, and an epidemiological history was obtained on the possible ways in which they had been exposed to *R. burneti* during the period 1942–53.

3. A comparison of the epidemiological histories of the positive and negative donors showed that donors in Kent who had constant occupational exposure to cattle and sheep including unprocessed materials from them, or who had had a raw milk supply to their homes for all or part of the period 1942–53, more frequently had antibody than those donors without such qualities. In East Anglia the positive serological state among donors was associated only with the use of raw milk and not with occupational exposure to animals. There were suggestive findings among Kentish donors that those who visited farms or other potentially infectious places, either during the course of their job or during their leisure, also experienced greater risk of infection.

4. In general, these findings of infection from occupational exposure, from visits to infectious localities, or from the use of raw milk in the home, confirmed the tentative epidemiological conclusions drawn from a previous investigation of patients with Q fever. It was not found in this survey, however, that residence

near dairy farms, receipt of objects from potentially infectious localities, or contact with persons from such localities was associated with a special risk of infection.

5. No association was found between the donors' serological state and the keeping of dogs, cats, poultry, pigs, rabbits, psittacine birds in the vicinity of the home or its infestation by mice, rats or mosquitoes.

REFERENCES

BABUDIERI, B. & MOSCOVICI, C. (1952). *Nature, Lond.*, **169**, 195.

BELL, J. A., BECK, M. D. & HUEBNER, R. J. (1950). *J. Amer. med. Ass.* **142**, 868.

CLARK, W. H., LENNETTE, E. H. & ROMER, M. S. (1951). *Amer. J. Hyg.* **54**, 319.

DERRICK, E. H. (1953). *Med. J. Aust.* i, 245.

FISHER, R. A. & YATES, F. (1948). *Statistical tables for Biological, Agricultural and Medical Research*. Edinburgh: Oliver & Boyd.

FREEMAN, G. H. & HALTON, J. H. (1951). *Biometrika*, **38**, 141.

KAPLAN, M. M. & HULSE, E. C. (1953). *W.H.O. Monograph series*, no. 19, p. 175.

MARMION, B. P., STEWART, J., RICHMOND, P., BARBER, H. & STOKER, M. G. P. (1954). *Lancet*, i, 1288.

MARMION, B. P. & STOKER, M. G. P. (1950). *Lancet*, ii, 611.

MARMION, B. P., STOKER, M. G. P., McCOY, J. H., MALLOCH, R. A. & MOORE, B. (1953). *Lancet*, i, 503.

PÉREZ GALLARDO, F., CLAVERO, G., HERNANDEZ, S. (1952). *Rev. Sanid. Hig. publ., Madr.*, **26**, 81.

SAINT, E. G., DRUMMOND, A. F. & THORBURN, I. O. (1954). *Med. J. Aust.* ii, 731.

STOKER, M. G. P. (1953a). *Brit. med. Bull.* **9**, 231.

STOKER, M. G. P. (1953b). *J. Hyg., Camb.*, **51**, 311.

STOKER, M. G. P., BROWN, R. D., KETT, F. J. L., COLLINGS, P. C. & MARMION, B. P. (1955a). *J. Hyg., Camb.*, **53**, 313.

STOKER, M. G. P., PAGE, Z. & MARMION, B. P. (1955b). *Bull. World Hlth. Org.* **13**, 807.

STRAUSS, E. & SULKIN, S. E. (1949). *Amer. J. Publ. Hlth.* **39**, 1041.

TONGE, J. (1955). Personal communication.

WOLFE, D. M. & KORNFELD, L. (1949). *J. Immunol.* **61**, 297.

(MS. received for publication 6. X. 55)

APPENDIX

AN ACCOUNT OF AN ANALYSIS OF THE DATA RELATING TO THE POSITIVE BLOOD DONORS AND THEIR SELECTED NEGATIVE CONTROLS WHEN SIMULTANEOUSLY CLASSIFIED IN TWO WAYS

BY R. G. CARPENTER

The problem of analysing the material obtained from the questionnaires is a typical one of multiple classifications in which the different factors may be interrelated. These may be disentangled by adopting some model, and fitting constants for the different factors, which enables us to examine one factor after taking account of the effect of others. The method is well described by Kempthorne (Kempthorne, 1952). However, if many factors are involved the computing becomes very heavy, and it is not practical to fit constants for every factor considered in this paper, nor are the data sufficient. Analysis was therefore confined to what appeared, from preliminary analysis, to be the most important factors, namely occupational contact with animals and household milk supply.

A simple additive model was adopted, the percentage of positives in any subclass being regarded as the sum of an overall average plus an effect relating to the degree of occupational contact and another relating to the type of milk supply. The residual error was weighted according to the numbers in the subclass. The data for Kent were broken down into three groups of areas relating to the density of sheep, but inspection did not suggest any important interaction of effects (i.e. occupational contact and milk supply) with areas. The East Anglian data were analysed separately, but as the estimates of residual variance were almost identical, these were subsequently pooled to compare Kent with East Anglia. The levels of occupational contact considered were 'direct' and 'indirect' (active and passive together). The effects are assessed by considering the increase in the percentage of positives in these groups compared with the group with no history of contact. Similarly, a raw and 'mixed' milk supply was compared with a pasteurized supply. These groups have already been described in detail (see pp. 125–128 and 130–131).

Results for the group of positive blood donors and their selected negative controls that were analysed

Occupational contact after milk

Kent. Contact seems a definite factor after possible effects of milk are considered for $0 \cdot 01 > P > 0 \cdot 001$. The estimated increases in the percentage of positives are: direct occupational contact, $+ 34 \cdot 0 \pm 9 \cdot 76 \%$; indirect occupational contact, $+ 12 \cdot 8 \pm 6 \cdot 27 \%$.

East Anglia. The effect of occupational contact is not significant. The estimated increases due to contact, after the effects of milk had been taken out are: direct contact, $+ 1 \cdot 6 \pm 7 \cdot 48 \%$; indirect contact, $+ 12 \cdot 0 \pm 8 \cdot 17 \%$.

Comparison of these findings

An overall comparison of these findings is not significant, but $P < 0.07$. But while the effects of indirect exposure are very similar, the effects of direct exposure differ significantly, a 't' test giving $P < 0.025$.

Milk after contact

In Kent the effects of milk after the effects of contact have been taken into account are not established, but $P < 0.06$. In East Anglia the effects of milk, after taking account of possible effects of contact, are significant, $0.05 > P > 0.01$. Nevertheless, the effects of milk, after the differing effects of contact in the two regions have been taken into account, are very similar. An overall comparison shows that, were both the effects of a raw and a mixed milk supply the same in the two areas, differences in the apparent effects of milk, as large as those observed or larger, would be expected in 30 % of such surveys by chance alone.

The overall effect of milk, after the different effects of contact have been eliminated, are significant as $0.01 > P > 0.001$. Raw and 'mixed' milk supplies cause a similar increase in the percentage of positives: raw, $+ 17.9 \pm 5.94$ %: mixed, $+ 17.1 \pm 4.79$ %.

REFERENCE

KEMPTHORNE, O. (1952). *Design and Analysis of Experiments*. John Wiley Inc. New York.