"Q" FEVER AND COXIELLA BURNETII ANTIBODIES

IN EDINBURGH CHILDREN.

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## TABLE OF CONTENTS

**INTRODUCTION** .................................................. 1

The history of "Q" fever ........................................... 2
Distribution of "Q" fever throughout the world .................... 5
Strains of C. burnetii ............................................. 20
Morphology and physical properties ................................ 21
Phase variation of C. burnetii ..................................... 23
Animal pathogenicity ............................................... 24
Clinical features of "Q" fever infection ............................ 28
"Q" fever in children ............................................... 46
The epidemiology of human "Q" fever ............................... 48
Control of "Q" fever infection ..................................... 65
Tests available for diagnosis of infection by C. burnetii ......... 72

**AIM OF THE PRESENT INVESTIGATION** .......................... 86

**MATERIALS AND METHODS** ...................................... 87

Patients, families and animals .................................... 87
Specimens .................................................................. 87
Complement-fixation test .......................................... 88a
Radio-iodine precipitation test .................................... 88b
Intradermal sensitivity test ....................................... 88b
Epidemiological tests ............................................... 90
Isolation of C. burnetii ............................................ 90
Forms, letters and questionnaires .................................. 91

**RESULTS** ............................................................. 98

I. C. burnetii complement-fixing antibodies in Edinburgh children ................................................. 98
Relationship to clinical diagnosis .................................. 99
Relationship to past clinical history ............................... 102
Relationship to age .................................................. 103
Relationship to date of hospital admission ....................... 105

II. Further tests to determine the specificity of the complement-fixing antibodies .................................. 107
Reaction of sera with other antigens .............................. 107
Reaction of sera after removal of heat labile factors .......... 107
Radio-iodine precipitation test ..................................... 109
Relationship to presence of adenovirus antibodies ............. 109

III. Examination of children for other C. burnetii antibodies ......................................................... 110
Intradermal sensitivity test in hospital patients ................ 110
Intradermal sensitivity test in children with complement-fixing C. burnetii antibodies ..................... 110

IV. Significance of C. burnetii antibodies in Edinburgh children ......................................................... 112
Compared with antibodies in adults 1960 - 1967 ................ 112
Compared with antibodies in children 1961 - 1964 ............. 114
V. Epidemiological Studies

Family studies using intradermal sensitivity test and complement-fixation test

Direct contact with animals and their products

Indirect contact with animals and their products

Vaccination with calf lymph

Residential contact with cows and sheep

Milk consumption

Presence of C. burnetii in cows in S.E. Scotland

Presence of C. burnetii in sheep in S.E. Scotland

DISCUSSION

SUMMARY

ACKNOWLEDGEMENTS

REFERENCES
Little boy blue come blow up your horn, the sheep's in the meadow, the cow's in the corn.

Anon.
INTRODUCTION
"Q Fever"

The original report of "Q" fever as a new clinical entity was published by Derrick in 1937 with a very careful and precise description of a series of feverish illnesses that had been occurring since 1933 in the meat workers of Brisbane, Australia (Derrick, 1937). Recognition that he did not have answers to all the problems posed by these outbreaks, led Derrick to assign the name of "Q" (for query) Fever to this clinical entity "until fuller knowledge should allow a better name."

It is proposed to outline here the "fuller knowledge" which has been obtained and show how it has added to the collection of unsolved problems about "Q" fever (Marmion, 1959).
THE HISTORY OF "Q" FEVER.

The occurrence in Brisbane of small epidemics of fever amongst abattoir workers, which could not be diagnosed as typhus, brucellosis or aberrant forms of typhoid or paratyphoid, led Derrick to observe carefully the clinical and epidemiological features of these illnesses. The description of an acute fever accompanied by other symptoms of systemic infection, notably a troublesome and persistent headache, and other more variable signs and symptoms, has become an historical keystone in the study of "Q" fever. (Derrick, 1937). His finding that the disease could be transmitted to guinea-pigs and other laboratory animals made it possible for Burnet and Freeman (1937) to identify a filterable rickettsial organism in smears of the cytoplasmic microcolonies from mouse spleen pulps. Careful observations were made of these tiny pleomorphic organisms and because of their morphology Derrick (1939) placed them in the genus Rickettsia with the specific name burneti - after Burnet.

Burnet and Freeman (1937) prepared an antigen from the organisms and found that it was specifically agglutinated by sera from homologous and heterologous patients although these sera had failed to react with strains of Proteus which were normally agglutinated by convalescent rickettsial antisera.

Apart from reports of mild and subclinical infections amongst laboratory workers, which were diagnosed by the appearance of agglutinating antibodies (Burnet and Freeman, 1939a), very little
more was heard from Australian workers until they started to compare their rickettsiae with those of a group of workers in America.

In 1938 these American workers had started to investigate a filter-passing infectious agent that they had isolated in guinea-pigs from a group of 200 ticks, *Dermacentor andersoni*, collected near Nine Mile Creek, Montana. Guinea-pigs infested by these ticks became feverish and some that died were found at post mortem to have enlarged lymph nodes and spleens. Urine, washed red cells and post mortem splenic tissue transmitted the illness to other guinea-pigs; those that did not die, were found to be immune to later infection; so were white rats, mice, chipmunks and ground squirrels tested in the same way. Blood taken from ninth passage guinea-pigs on the fifth day of their fever was passed through a Berkefeld filter. The filtrate obtained after passing through membranes known to hold back *R. prowazekii* and *R. rickettsii* was found, by titratrian experiments, to be as infective as the original blood sample (Davis and Cox, 1938).

The organism failed to grow in cell free media and was therefore considered different from the organism isolated by Noguchi (1926b) from ticks in Bitter root valley, Montana. However Cox (1939) found that his filter-passing agent survived, unchanged, in cell-free media for long periods and questioned whether Noguchi's transmission experiments had in fact entailed dilution through seven passages. Because of its physical characteristics Cox (1939) also placed his agent in the genus *Rickettsia* and considering it to be a
new species, he called it *R. diaporica* (able to pass a filter).

A laboratory-acquired illness known as "Nine-Mile" Fever (Dyer, 1939), was the first indication that *R. diaporica* might be pathogenic to man. The aetiology of the acute febrile illness was confused by the fact that the patient had been working with the Australian *R. burnetii* before working with *R. diaporica* and in order to determine which organism was the source of his infection cross immunity studies were carried out in guinea-pigs between strain "X" from the patient, and the two incriminated organisms. Strain "X" was found to have complete cross immunity with both *R. burnetii* and *R. diaporica* and the first link between the organisms had thus been accidentally made (Dyer, 1938). It is interesting to note that strain "X" from the patient, which was later known as the "Dyer" strain, an early American prototype, was found to be antigenically distinct from the "Nine Mile" strain with which the patient had been working (Strauss and Sulkin, 1949), and Stoker (1949) believes that coincidental infection must have occurred from another source. Because of the close antigenic relationship between *R. burnetii* and *R. diaporica*, "American Q Fever" was considered a better name than "Nine-Mile" fever for the illness from which the "Dyer" strain was isolated and other infections at the Rocky Mountain laboratory in Montana which were definitely acquired from *R. diaporica* (Cox, 1940). The name *R. diaporica* was finally dropped and later Philip and Steinhaus suggested that the causative organism of "Q" fever should be given the status of a new genus rather than retain its
position as a subgenus of the rickettsiae. They proposed that it should subsequently be called *Coxiella burneti* - to honour both the American and Australian workers who originally described it (Philip, 1948b). Although Stoker (1954) considered the change unnecessary, this new generic name will be used with the species name *burnetii* (Bergey, 1957) for the causative organism of "Q" fever. The most important features which differentiate *C. burnetii* from the rickettsiae, are its ability to pass bacteriological filters, its greater resistance to chemical agents and heat, and its failure to stimulate production of antibodies to any known strain of *Proteus* (Dyer, 1949) as well as the rarity of a rash in the clinical illness it produces.

**DISTRIBUTION OF "Q" FEVER THROUGHOUT THE WORLD.**

By 1956 "Q" fever had become accepted as a common disease throughout most of the temperate and tropical areas of the world (Tigertt and Benenson, 1956): because it is not possible to differentiate between antibodies produced to the different strains of *C. burnetii*, it is impossible to determine whether "Q" fever infection in any particular country is due to modern diagnosis of an old disease such as "eski hastalik" (the old disease) known to all country people and those associated with animals in Turkey and subsequently found to be the "new" disease "Q" fever (Payzin, 1953) or importation of a new infection as a by-product of improving animal stocks. The pattern of infection alters slightly from country to country, but the typical story in most countries is of infection in occupationally exposed workers such as dairymen, meat packers, farmers and laboratory
workers (Johnson and Kadull, 1966).

AUSTRALASIA:

After the original description of "Q" fever by Derrick (1937) as an illness of abattoir workers, Australian workers searched hard to determine the extent to which the animal kingdom had been infected by the causative organism C. burnetii. They found that a high proportion of small marsupials called bandicoots - Isoodon torosus - especially on Moreton Island, had agglutinating antibodies to the organism and that amongst the infected animals there was a higher proportion infested by the tick - Haemaphysalis humerosa. On a farm, where a human case had occurred, three wild rats and one out of 24 cows had these agglutinins and human agglutinating antibodies were found amongst healthy abattoir and forestry workers, but not amongst soldiers, aborigines or fever patients (Freeman et al., 1940).

By 1942 the importance of infected cows in the spread of human infections had been realised, and it was assumed that human cases occurred either directly from infected animal tissues or from infected ticks infesting such animals (Derrick et al., 1942).

In 1958 a "sheep shearers' epidemic", occurring in old as well as young sheep shearers, suggested that a new infections was being acquired from sheep. It was subsequently found to be "Q" fever (Derrick, 1949), and by 1960 the infection was also found to be endemic amongst kangaroos (Rope et al., 1960) possibly because of the indiscriminate feeding habits of the kangaroo tick Amblyomma triguttatum (Roberts, 1962).
Although it was originally described in and around Brisbane, Queensland, the organism has now been reported from Victoria and New South Wales. In two towns of New South Wales, where 36 cases had recently occurred, 30 per cent. of 381 sera tested, were found to possess complement-fixing antibodies to C. burnetii (Public Health Reports from Australian Virus Laboratories, 1964) and infection in the State was shown, by a study of abattoir workers, to be more concentrated to the north than to the south of Sydney (Hansman et al., 1966).

Despite its widespread distribution in Australia a survey of 1300 sera in New Zealand failed to demonstrate infection there (Fastier, 1954). This has probably been due to the fact that animals being imported are required to have a certificate showing negative results to serological tests for antibodies to C. burnetii but it is surprising that such a simple procedure has kept the country clear in the light of evidence that C. burnetii could be isolated from seronegative cattle and sheep (Salisbury, 1953).

THE AMERICAS

The first endemic clinical cases of "Q" fever in the United States of America were in a man who had been hunting in Bitter Root Valley, Montana, and a student who had been cutting Christmas trees in the woods of Missoula, Montana for the few weeks leading up to his illness. Neither had had direct contact with animals but both had drunk unboiled stream water (Hesdorffer and Duffalo, 1941). Laboratory acquired infections had been reported earlier (Dyer et al.,
1940; Hornibrook and Nelson, 1940) and in 1940 Cox reported that 11 of 27 employees at the Rocky Mountain Laboratory, Montana had agglutinating antibodies at titres between 1:20 and 1:320 (Cox, 1940).

Apart from these occasional sporadic cases and laboratory infections there was little evidence of "Q" fever in North America until April 1947, when a general physician reported an outbreak of fever among his patients in Artesia, California, following the windstorm "Santa Ana" (Young, 1948). This small epidemic led the way for extensive Public Health Service investigations in Southern California where 117 cases were studied in detail (Huebner et al., 1948) and later in Northern California (Clark et al., 1951b).

A serological survey showed that complement-fixing antibodies to C. burnetii were widespread in meat workers from many States (Strauss and Sulkin, 1949). Further studies amongst animal workers in South West Texas, Idaho, Illinois and Ohio confirmed the previous findings (Irons et al., 1949; Stoermer et al., 1959; Ferris et al., 1964; Reed and Schmurrenberger, 1966).

In 1960 after a nation-wide survey of the occurrence of "Q" fever infections in cattle, Luoto (1960) noted that the causative organism was spreading amongst dairy cattle, and the gross environmental contamination so caused would lead to frequent human infection and illness. Earlier studies had shown that cattle were involved in seven States but Luoto demonstrated that by 1960 19, out of the 26 States he tested, had infected dairy herds. Infection rates in these States varied from 1 per cent. in Montana to 98 per cent. in
Southern California and he also suggested that more limited studies in other 24 States indicated that infection was even more widespread. Details of the increase in number of infected herds were convincing in Montana and three other (unnamed) States, but despite a very intensive surveillance of the human population, the incidence of clinical human disease in Montana did not seem to be changing (Luoto, 1960).

Infection on the American continent outside the United States was first reported from a case of atypical pneumonia in Panama (Cheney and Geib, 1946) and much later from Canada.

A serological survey in Montreal, Quebec, showed that 4.8 per cent. of sera tested had significant titres of complement-fixing antibodies to C. burnetii, although no clinical cases of "Q" Fever had been reported (Pavilanis et al., 1952). The first Canadian case of illness described as "Q" fever was in a 22 year old farmer who was found to have complement-fixing antibodies after a relapse of fever and pneumonia (Marc-Aurele et al., 1956). The antibody titres were never more than 1:32 and because they were only 1:4 three weeks after the second illness, there must be some doubt that his illness had been caused by C. burnetii, although he had certainly been infected at some time. An epidemic of respiratory infection amongst abattoir workers in Princeville, Quebec, confirmed the presence of the organism in Canada (Pavilanis et al., 1958), while a more extensive survey showed that infection was distributed throughout Canada with the exception of the Maritime Provinces (McKeil, 1964). Both Herbert in Alberta (Herbert et al., 1965) and McKeil (1964) in
Eastern Quebec were able to demonstrate significant increases in the number of infected cattle over the previous 5 years, and it was noted that all the 67 Canadian cases which had been reported had occurred in Eastern Quebec, where there was a high incidence of the infection in dairy herds.

In 1955 the first clinical case of "Q" fever was reported from South America (do Valle et al., 1955); since then many reports of cases and epidemiological studies have been published in Brazil and other countries.

The only report from the West Indies has been of a completely negative serological survey in Jamaica (Grant, 1961).

MEDITERRANEAN AREA.

Reports of "Q" fever infections amongst troops in and returning from, Greece and Italy during the 2nd World War (Feinstein et al., 1946; Robbins et al., 1946a; Caughey and Dudgeon, 1947; Caminopetros, 1949) were the first indications of C. burnetii in the Mediterranean area. Explosive outbreaks were reported, involving between 20 and 30 per cent. of the troops in certain billets. The suggestion that this might be a "place infection" was confirmed by the finding that the disease was endemic amongst the native adult population of Pagliana in Italy, while uninfected American troops had very little evidence of past experience with the organism (Robbins et al., 1946a).

Since 1946 cases of endemic infections have been reported
from Turkey (Pavzin, 1953), Algeria (Lacroix et al., 1956; Mimoune et al., 1955), Iran (Rafyi and Maghami, 1954), Egypt (Dirk van Peenen and Reid, 1963), Saudi Arabia (Gelpi, 1966), Israel (Eschar et al., 1966) and Malta (Frazer and Hatch, 1967); although a survey by Maurin (1954) showed that there was no evidence of "Q" fever in Tunisia. Other Mediterranean countries where infection of man, cattle or other animals has been shown to occur include Morocco and Yugoslavia (Kaplan and Hulse, 1953; Berge and Lennette, 1953a).

GREAT BRITAIN.

After the demonstration that outbreaks of "primary atypical pneumonia" amongst troops in Italy had been caused by infection with C. burnetii (Robbins et al., 1946a; Caughey and Dudgeon, 1947), Stoker looked for evidence of a similar aetiology amongst a group of 24 patients with "primary atypical pneumonia" in Great Britain. He found three of them to have complement-fixing antibodies to C. burnetii, two with high titres and one with an unchanging low titre. His most significant finding was that because two of these three patients had never been outside the United Kingdom, there was evidence of indigenous infection in Great Britain (Stoker, 1949).

The first report of an outbreak of "Q" fever in this country soon followed. Harman (1949) reported eight cases associated with the Royal Cancer Hospital. Most of these cases occurred amongst the staff and the source of infection was found to have been a patient who died of bronchopneumonia associated with bulbar palsy. Because
his wife also had a high complement-fixing antibody titre, and had never visited her husband in hospital, it was assumed that he had acquired his infection at home in Kent. Although most of the other cases had had direct contact with this man, either before death or in the post-mortem room, two cases – a hospital physicist and his wife – could not be connected with the others in any way and were presumed to have been infected while on holiday on a farm in South Devon, where there were subsequently found to be seropositive cows (Marmion and Stoker, 1950). Isolation of the "Christie" strain of C. burnetii from the pathologist who performed a post-mortem on the patient referred to above, was convincing proof that the outbreak was "Q" fever and the other cases were diagnosed by complement-fixation tests using the "Henzerling" strain (MacCallum et al., 1949).

The next outbreak reported from South East England occurred in association with the Canterbury School of Art. There was an explosive outbreak of illness, mainly amongst the students, which could not be explained by contaminated milk supplies or any direct animal contact. The possibility that infection may have occurred during the unpacking and disposal of an old case of dusty packing straw, or that a student may have come in from a farm with grossly contaminated clothing, were never proved and exhaustive attempts to isolate the causative organism from dust remaining in the packing case were all negative (Harvey et al., 1951).

Another outbreak in Kent, occurred on a farm where 77 per
 cent. of the adults at risk became infected. The source of infection was again never found despite a very careful search. No new cows, chickens, ducks or other animals had been imported at the time of infection and the only animals to give birth were three sows, who produced two litters and an abortion but were all serologically negative to C. burnetii on repeated retrospective testing. (Stoker and Thompson, 1953).

As well as the three outbreaks described above, 69 sporadic British cases had been collected and described by 1953; of these more than 50 per cent. had occurred in Kent, but this distribution could possibly be accounted for by the more extensive search for cases in that area (Stoker, 1953a). Marmion and his co-workers (1953) reviewed the sporadic cases and found that the clinical features in 43 were of virus pneumonia or "primary atypical pneumonia" while 18 had had fevers of unknown origin. In some the illness was severe and led to debility and death in a few older patients. Two thirds of the cases occurred between April and September and all but 13 had obvious possible sources of infection. Diagnosis depended on the demonstration of a fourfold or greater rise in complement-fixing antibodies to C. burnetii or on a single high titre following an illness suggestive of "Q" fever. Of the cases found in this way, 61 were aged between 20 and 60, and because none were aged less than 10 years, it was thought that children were less at risk.

The above workers also compared the serological evidence of past infection with C. burnetii in blood donors - a
selected group aged between 20 and 60 years - in England and Wales. The fact that only 0.78 per cent. of sera tested in East Anglia had antibodies at dilutions of 1:10 or more, compared with 2.96 per cent. in Kent, confirmed the impression that "Q" fever was more common in S.E. England. An estimate that there were 3000 fresh infections annually in Kent, was obtained by extrapolation from the above figure, assuming that complement-fixing antibodies persisted for 10 years. A fairly intensive search had only produced 69 cases in two years so that many cases must have remained undiagnosed, partly because of their subclinical nature and some because of misdiagnosis (Marmion et al., 1953).

In 1953 the true prevalence in Great Britain was hard to establish because very few centres were performing routine serological tests for C. burnetii. At Colindale, antibodies were found in 9 per cent. of cases with "primary atypical pneumonia" (Stoker, 1953) but where these patients were selected from a totally urban area the number with antibodies was less than 2 per cent. (Crofton et al., 1951).

Animal sources of infection were postulated for most of the cases so far described and in 1952 Slavin demonstrated that 2.1 per cent. of British cattle had complement-fixing antibodies to C. burnetii. He also isolated the organism from pooled herd milk supplies in 6.9 per cent. of 2,581 farms in England, 2.0 per cent of 553 farms in Wales but only 0.8 per cent. of 240 Scottish farms (Slavin, 1952).

Because of the difference reported by Marmion, in
numbers of blood donors with complement-fixing antibodies to *C. burnetii* in Kent and East Anglia, a more careful study was made of the differences in animal husbandry between these two localities. The only significant difference that was found was that there were more sheep per acre in Kent and since *C. burnetii* had previously been isolated from these animals (Caminopetres, 1949, Lennette et al., 1949) they examined the sera of sheep sent to the local abattoir. They found that 1.6 per cent. of all these animals had complement-fixing antibodies, while up to 33 per cent. of sheep sera taken from flocks associated with human cases had antibodies at a dilution of 1:10 or greater (Marmion et al., 1954); these findings explained the predominance of sporadic human cases in the spring and summer months, since greater opportunity for sheep/human contact occurred at lambing and shearing. A small outbreak of illness amongst a group of travelling actors in a religious play was studied during the investigation and it was retrospectively considered to have been "Q" fever due to indirect contact with sheep (Marmion et al., 1954; Marmion and Stoker, 1956).

Confirmation of the infectivity of Kent sheep came with Stoker's isolation of three strains of *C. burnetii* from the placentae of three serologically positive sheep in two flocks. The organism was also isolated from a wool tag of one of the positive animals (Stoker et al., 1955).

In 1955 Stoker and Marmion collected ticks, identified as *Haemaphysalis punctata* from sheep in Kent; they divided them
into 25 pools depending on their sex, stage of development and time of collection and found two of the pools to contain viable \textit{C. burnetii}. Because the British distribution of \textit{H. punctata} is limited to areas such as Romney Marsh in Kent, where the sheep are infected, they considered that ticks, infected from the blood or wool of contaminated sheep, may act as intermediaries in the infection of man (Stoker and Marmion, 1955a).

The importance of sheep as a source of human infection in Great Britain was shown by an outbreak of 15 cases in three Welsh villages in the early summer of 1953. All cases were aged 24 years or more and all but two had a history of direct contact with farms or animals, notably with sheep. Many also used raw milk but these supplies were found to be uninfected when tested for \textit{C. burnetii}. The fact that sheep on farms with human cases of "Q" fever had a higher proportion with complement-fixing antibodies to \textit{C. burnetii} than those from a wider area received at the abattoir, suggested that they had been the source of infection (Report Monthly Bull. Minist. Health, 1956).

Evans (1956) found that infection was widespread amongst animals and man in South Wales. Raw milk specimens showed that up to 17.7 per cent. of herds, were producing milk containing \textit{C. burnetii}, which explained why 38.2 per cent. and 5.5 per cent. of 246 specimens of bulked raw and pasteurized milk respectively contained viable organisms. Complement-fixing antibodies were found in 2.7 per cent. of 1,954 sheep and 4.1 per cent. of 1,202 human
blood donors in the area.

One puzzling report of an autumn outbreak, in a suburb of Cardiff, appeared to be related to a plague of mosquitoes. Philip had previously reported experimental ingestion of *C. burnetii*, in America, by the mosquito, *Aedes aegypti*, but had failed to demonstrate transmission of infection (Philip, 1948a) so that the Welsh outbreak was considered to be related either to the autumn calving or the fact that the prevailing wind at the time was from the local marshes (Evans and Baird, 1959). Probably a combination of mosquitoes and dust contaminated by calving were carried by the wind and accounted for the confused story.

A report from Grist in 1956 confirmed Slavin's suggestion that *C. burnetii* was still a rare infection in Scotland. In a total of 676 patients of all ages, whose sera were tested because of pneumonia or fever of unknown origin, only five (less than 1 per cent.) had complement-fixing antibodies to *C. burnetii*. Of these patients three had low unchanging titres but one adult, with "virus pneumonia", had a fourfold rise of antibody titre and one baby of five months, with pneumonia, had a twofold rise from a dilution of 1:64 to 1:128 in the absence of antibodies in its mother. The latter two cases confirm that clinical "Q" fever occurred in Scotland but that the incidence was much lower than would have been demonstrated by such an intensive search in the South East of England (Grist, 1956).

One of the chief features of the cases outlined above, and other outbreaks of "Q" fever in Great Britain, is the number of
patients who have no occupational contact with animals - art school students, hospital staff, religious actors, open prisoners and R.A.F. personnel: it suggested that lack of previous exposure has failed to give immunity in these groups as it would have done to animal workers (Marmion, 1959; Frazer et al., 1960; Holland et al., 1960.) A recent report of "Q" fever infection amongst British naval personnel in Malta G.C. showed that they experienced four times the number of infections as the local Maltese population because they had had a more limited experience with the organism before going to the island (Frazer and Hatch, 1967). In fact Frazer had previously found that only 0.3 per cent. of 2,175 naval personnel from the whole of Britain had antibodies to C. burnetii on recruitment (Frazer et al., 1964).

The British outbreaks and sporadic cases are almost wholly confined to adults. A child of eight was involved in a farm outbreak and one sporadic childhood case has been reported in Scotland. (Grist, 1956; Marmion, 1959).

OTHER AREAS OF THE WORLD

Clinical cases and outbreaks of "Q" fever have been reported from Europe. One of the first of these outbreaks, in Switzerland, was believed to have been associated with the unpacking of a machine in straw from the United States of America (Wegman, 1948), but the subsequent diagnosis of 975 sporadic cases and the demonstration of antibodies in many others showed that Switzerland itself was a highly endemic area (Bungdorfer, 1951; Rossier, 1962).
The disease in Portugal was reviewed by Fonseca, who believed that infection by ingestion of infected milk was very uncommon but that infection by the tick *Hyaloloma rufipes* may occur (Fonseca et al., 1949) and in Czechoslovakia, Syrucek and Raska, (1956) showed infection amongst domestic fowls. In Austria, Bulgaria, Cyprus, Hungary, Rumania, San Marino, Spain and Yugoslavia infection is also known to be present and reports of the degree of infection in these and other countries were reviewed in 1955. At that time the following European Countries were noted to be free of infection – Denmark, Finland, Iceland, Ireland, the Netherlands, Norway, Poland and Sweden, (Kaplan and Bertagna, 1955), but since then Ireland has experienced epidemic infection amongst livestock following the importation of infected sheep to a single flock (Connolly, 1967).

Infections in India, Ceylon and Japan have all been reported (Kalra and Taneja, 1954; Takano et al., 1954), while Eastern Siberia, Central Asia and the Caucasus have been considered to be natural reservoirs of infection (Zdrodovskij, 1964) and a few cases of "primary atypical pneumonia" due to "Q" fever have been reported from China (Chang et al., 1951).

African surveys have been concentrated mainly in French Equatorial Africa and the Belgian Congo, testing for agglutinating and intradermal allergic antibodies. These antibodies have been found in native Africans and Europeans, especially butchers, shepherds and veterinarians, and also in cattle, sheep, goats, donkeys and dogs (Jadin and Giroud, 1950; Giroud et al., 1951; Giroud et al.,
1952; Porte and Capponi, 1954). Human cases of illness have also been described in Southern Rhodesia (Gelfand and Berney, 1953) and South Africa (Gear et al., 1950).

**Strains of C. burnetii**

Strains of *C. burnetii* isolated in different parts of the world have been shown to have close immunological relationships with each other. (Dyer, 1939; 1939; Burnet and Freeman, 1939b; Bengtson, 1941b; Topping et al., 1946). The Australian strain was the first to be studied and was followed by Strain "X" (later known as the "Dyer" strain) and the "Nine Mile" strain in America. A classical Italian strain was called "Henzerling" after the 339th Infantryman from whom the organism was isolated. (Robbins et al., 1946b) and the "Balkan grippe agent" isolated in Greece by Caminopetros (1949) was later identified as *C. burnetii* by its morphology and growth characteristics. (The Commission on Acute Respiratory Diseases, 1946a and b).

The strains vary in specificity and virulence, and some are considered to be almost avirulent to man and laboratory animals. (Eschar et al., 1966). Only two British strains have been studied in any detail: "Christie", isolated from a patient and "Ml" from raw milk which were both demonstrated to have reciprocal cross immunity with the "Henzerling" strain. (Stoker, 1950).

"Nine Mile" and "Henzerling" are the most commonly used strains for producing antigens to be used in serological tests.
MORPHOLOGY AND PHYSICAL PROPERTIES

Animal infectivity, even after filtration through a 0.7μ filter, allowed Burnet and Freeman (1937) to postulate a "virus" aetiology for the cases of "Q" fever they were investigating for Derrick. Davis and Cox (1938) allowed nine guinea-pig passages to occur before they demonstrated that the organism they had isolated from the tick D. andersoni could readily be passed through a Berkefeld W. filter, which normally holds back bacteria and the rickettsiae of typhus and Rocky Mountain spotted fever.

The first demonstrations of a micro-organism in "Q" fever were the cytoplasmic microcolonies shown on a haematoxylin and eosin stained section of infected mouse spleen. Smears of these colonies allowed many tiny intra and extracellular rods and cocci to be seen, after staining them blue with Castenada's stain or reddish purple with Geimsa stain. (Burnet and Freeman, 1937). Cox, (1938) using Geimsa stained preparations of guinea-pig spleen, described lanceolate rods of dimensions .25μ X .5μ, bipolar rods of .25μ X 1μ and diplobaccilary forms of .25μ X 1.5μ with occasional segmented filamentous chains. The deep sharp staining observed with Geimsa and Machiavello's stains, compared with the rather faint shadows on Gram and Loeffler stains, suggested to Brainerd (1955) that the organism was a rickettsia although, unlike the prototype of the species, R. prowazekii, it could be demonstrated extracellularly.

Cox's attempts to repeat Noguchi's (1926a) apparent cultivation of the organism in cell-free media were unsuccessful, but he was able to demonstrate that it survived with unaltered titre in
cell-free leptospira medium for 109 days at 20°C. and dilution through six subcultures (Cox, 1939). Resistance to heating at 63°C. for 30 minutes differentiated \textit{C. burnetii} from the rickettsiae because the latter were inactivated by 50°C. for only 15 minutes (Ransome and Huebner, 1951). The importance of this remarkable resistance to heat in the pasteurisation of milk has been considered by many workers.

As well as being resistant to storage, dessication and heating, \textit{C. burnetii} was found to be resistant to treatment with formalin in concentrations of less than one per cent. for periods of less than three days, whereas the rickettsiae were destroyed within 24 hours (Ransome and Huebner, 1951). Its resistance to formalin had been unexpected and was probably responsible for many of the laboratory outbreaks (Huebner, 1947) and illnesses following early vaccination (Smadel et al., 1948). A comparison of various chemical disinfectants showed that only one per cent. lysol at 37°C. for three hours gave complete inactivation. Ether was found to be partially effective but it was recommended that ether-treated vaccines should be required to undergo several blind egg passages before use in man (Malloch and Stoker, 1952).

\textit{C. burnetii} possesses remarkably potent antigenic properties, which are stable and resist autoclaving, extraction with non-aqueous organic solvents, and treatment with trichloracetic acid (Lackman et al., 1964).

Unlike most of the rickettsial group, it fails to stimulate production of antibodies to any known strain of \textit{Proteus} (Burnet and
Freeman, 1937) but this property cannot be used in the diagnosis of "Q" fever in laboratory workers who have been vaccinated against typhus or Rocky Mountain spotted fever (Dyer, 1938).

**PHASE VARIATION OF C. BURNETII**

During diagnostic tests for "Q" fever, discrepancies were noted in the complement-fixing ability of different strains of *C. burnetii*. The weakly reacting "Dyer" strain only fixed complement with antibodies produced in guinea-pigs about nine to 10 weeks after infection, although the "Henzerling" strain reacted with guinea-pig sera collected three weeks after infection and Robbins postulated that there must be two specific antigen/antibody systems involved. It has been found that most strains contain the antigen which reacts with late convalescent antibodies, but that only a few strains, including "Henzerling" and "Nine Mile", could be used to measure the early convalescent antibodies produced after three weeks in the guinea-pig (Robbins et al., 1946b; Topping et al., 1946; Stoker, 1949; Berge and Lennette, 1953b).

The possibility that a change in antigenic structure occurred during egg adaptation was suggested by the fact that neither of the two British strains studied by Stoker (1950) would react with British antisera until they had undergone several yolk-sac passages. Later studies showed that strains with only a few yolk-sac passages also failed to fix complement with homologous or heterologous human "Q" fever antisera although they reacted like the classical strains.
after a number of yolk-sac passages (Stoker and Fiset, 1956). Stoker termed this adaptive process "phase variation"; antigens prepared from early yolk-sac passages are considered to be in "phase 1" and only react with late guinea-pig convalescent antisera, while egg-adapted strains are in "phase 2" and react well with both early and late convalescent antisera, although the yolk-sac content of organisms is no different from those in "phase 1". Animal passage of antigen in "phase 2" causes it to revert to "phase 1" and it requires several yolk-sac passages to reconvert it to "phase 2".

It has been found that most human cases of "Q" fever never produce late convalescent antibodies to the complement-fixing antigen in "phase 1". The development of these antibodies after human infection and their significance in chronic "Q" fever infection is discussed in relation to "Q" fever endocarditis.

**ANIMAL PATHOGENICITY**

**Culture in Guinea-Pigs.**

Derrick's (1937) historical recognition of "Q" fever as a clinical entity was confirmed by the transmission of a feverish illness to guinea-pigs inoculated with blood from a patient with fever. The guinea-pigs were usually between 2000 and 4000 grams in weight and were inoculated with citrated blood or urine from the patients. (Derrick later advocated the use of blood clot, to avoid possible inoculation of antibodies with the organisms). The incubation period in guinea-pigs was found to have a mean of 10.5 days, varying between
eight and 14 days and was dependent on the dose of inoculum. The onset of fever, to 40°C or more, was acute and it lasted for one to eight days before its sudden fall. Associated symptoms included anorexia, limtness and failure to gain weight. The mortality in 190 guinea-pigs, infected by Derrick, was nil and he observed occasional inapparent infections which were only diagnosed by passage of tissues or subsequent immunity. Guinea-pigs sacrificed during their fever showed no scrotal reaction, but some had petechiae in the wall of the caecum and all showed marked increase in the weight of the spleen. The American "Dyer" and "Panama" strains were found to be more pathogenic to guinea-pigs than the Australian strain (Burnet and Freeman, 1939b; Topping et al., 1946) and Israeli strains could not be isolated in these animals because of their lack of virulence (Eschar et al., 1966).

Transmission of the fever to other guinea-pigs could be achieved by inoculation of an emulsion of blood or post mortem splenic material. Subsequent inoculation of convalescent guinea-pigs with further infected material failed to produce a febrile reaction, so that ultimate diagnosis rested not only on production of the characteristic fever, but on transmission to a second guinea-pig or development of immunity to later challenge with infected material. Demonstration of organisms in splenic smears gave an additional conclusive method of diagnosis. This was demonstrated by Cox (1938) when working with his filter-passing agent.

Challenge immunity tests of guinea-pigs became redundant with the advent of serological tests. Demonstration of antibodies
to C. burnetii was considered proof that the organism had caused infection in the guinea-pig under test. The appearance of antibodies was proportional to the size of the inoculum and varied between 10 and 40 days after infection (Babudieri, 1953b).

Babudieri recommended Seitz filtration of inocula, such as pus and faeces, to prevent the production of pyogenic abscesses at the inoculation site. Inoculations of blood, milk, urine, faeces, pus, or sputum were given by the intraperitoneal, the subcutaneous or the intramuscular routes, and the inoculum was between one ml. and four ml. in volume (Babudieri, 1953a; The Commission on Acute Respiratory Diseases, 1946c).

It is important to note that guinea-pigs did not appear to excrete organisms in their stools, so that guinea-pig to guinea-pig infection in cages was very uncommon (Huebner, 1947). However, after artificial infection, they were found to harbour C. burnetii in the placenta and foetus early in pregnancy and again in large numbers late in pregnancy and at parturition, but there was a total lack of organisms at these sites during the mid-period (Abinanti, 1957).

Culture in other laboratory animals.

Mice:

White mice have been used for experimental work ever since Burnet and Freeman first described rickettsial organisms in infected mouse spleens. They were used for the first cross immunity tests (Burnet and Freeman, 1937) and later for a reintroduction of the same technique in the mouse neutralisation test (Abinanti and
Marmion, 1957). Sidwell also used mice to demonstrate that whole body irradiation, multiple cortisone injections and parturition all produced a reactivation of latent infection in the tissues of white mice, which had been inoculated 3 months earlier (Sidwell et al., 1946b; Sidwell et al., 1964a; Sidwell and Gebhardt, 1966). Less material is required for infecting mice but infection has been found to spread from mouse to mouse under experimental conditions (Babudieri, 1953b) and they have not been widely used for diagnostic isolation.

**Other Animals:**

Rats and rabbits are both less sensitive to *C. burnetii* than guinea-pigs and mice; monkeys and hamsters have been found to be equally sensitive, but are less readily available and not often used, (Derrick, 1937; Burnet and Freeman, 1937; Babudieri, 1953b).

**Growth in Embryonated Hen's Eggs**

Despite the great susceptibility of guinea-pigs and mice to infection with *C. burnetii*, it is useful to have a non-mammalian method of cultivation. Attempts by Cox (1939) to culture the organism in cell-free leptospira medium were unsuccessful, although the organism was found to survive dilution through six subcultures and to remain with unaltered concentration for 109 days at 28°C. Continuation of the search for a culture medium revealed that chick embryo tissue, particularly minced yolk-sac in human ascitic fluid, maintained an infective titre of between $1 \times 10^{-7}$ and $1 \times 10^{-8}$ of
C. burnetii through 38 consecutive transfers, and that inoculation of embryonated eggs produced high concentrations of the organism in the yolk-sac, even after 50 passages (Cox and Bell, 1939).

The inoculation of fertile hen's eggs was first described as a method of growing the rickettsiae of Rocky Mountain spotted fever and typhus. The same technique has been used for the isolation of C. burnetii. Yolk-sacs of five or six day old fertile eggs, under incubation at 39°C, were inoculated with 0.5 ml. of the infectious material - defibrinated heart blood from an infected guinea-pig on the third or fourth day of its fever - and reincubated at 35°C. Passage of the agent to other eggs was achieved by using a 10 per cent. suspension of yolk-sac in normal saline as the infectious material. The yolk-sacs of all passages were found to be the most highly infective component of the fertile egg, both by direct smear and by guinea-pig sensitivity tests. Growth in yolk-sacs of embryonated eggs did not alter the morphology of the organisms (Cox and Bell, 1939).

The use of embryonated eggs has been invaluable for the production of C. burnetii antigen - in varying degrees of purity - for the numerous serological tests used in the diagnosis of human infection.

**CLINICAL FEATURES OF "Q" FEVER INFECTION**

Man is the only animal known to have a recognisable clinical illness attributable to C. burnetii. Infections occur in two forms: epidemic, where a group of highly susceptible individuals
are simultaneously exposed to the organism, as in the outbreaks amongst troops (Robbins et al., 1946a; Fraser et al., 1960) or a large dose of virulent *C. burnetii* suddenly contaminates the atmosphere (Stoker and Thomson, 1953); or endemic, where isolated and sporadic cases occur in an area with a high proportion of inapparent infections amongst the animal population (Stoker and Marmion, 1955b). Only the most severe of the latter cases are usually diagnosed so that comparative studies of clinical features are difficult (Stoker, 1954).

**SUBCLINICAL INFECTION:**

"The interaction between any infectious agent and the human body varies from an overwhelming of body defences by the parasite, and subsequent death of the patient, to a response which is so efficient that no signs of infection appear, except a subsequent immunological response" (Therapeutic notes; 1967). That infection with *C. burnetii* fits this pattern has been shown by many workers. Burnet himself was one of the first laboratory workers to be shown to have developed agglutinating antibodies to *C. burnetii* in the absence of known infection (Burnet and Freeman, 1939a). Bengtson (1943a) later demonstrated complement-fixing antibodies in American laboratory workers who had no history of "Q" fever but whose immunological picture may have been confused by repeated vaccination with tick material against Rocky Mountain spotted fever. Isolation of *C. burnetii* from a patient and demonstration of a specific antibody rise in the absence of clinical illness was conclusive proof
that asymptomatic infections do occur (Wagstaff, et al., 1965).

It is still not clear exactly how many infections with the organism fit into this category because results of attempts to calculate the proportion of subclinical infections vary in different studies (Freeman et al., 1940; Bell et al., 1950; Slavin, 1952; Evans, 1956). In an explosive outbreak amongst art students, it was possible to show that subclinical infections were probably three times as common as overt illness (Harvey et al., 1951), while another outbreak in Kent showed only 10 per cent. to have subclinical infection (Stoker, 1954). Studies on the development of immunity to C. burnetii amongst prisoners drinking raw milk, showed that there was no clinical evidence of infection amongst the 35 per cent. who developed antibodies (Benson et al., 1963).

Experimental infections in mental patients suggested that the illness was dependant on the dose and route of infection — inhalation producing the largest number of illnesses while the intradermal route produced only immunity with inapparent infection (Blanc et al., 1948). Other workers have ascribed this phenomenon of subclinical infection to strain variation and Eschar assumed that the Israeli strains had low virulence for man (Eschar et al., 1966). It has also been shown that the time of onset and length of fever are directly proportional to the infecting dose of organisms (Tigertt et al., 1961).

Retrospective antibody studies on large numbers of sera have not been able to give comparable results, because of the
variation in persistence of agglutinating and complement-fixing antibodies. (Freeman et al., 1940; Stoker et al., 1955). The finding that there was no significant sex difference in British blood donors with complement-fixing antibodies, despite clinical cases being far more common in men, suggested that more subclinical infections occurred in women (Stoker, 1953a). It is hoped that the radio-iodine precipitation test (Tabert and Lackman, 1965) will be able to give a more complete picture of the relationship of subclinical to clinical infections.

**ACUTE "Q" FEVER**

**Incubation Period**

Derrick originally noted that the incubation period between contact with *C. burnetii* and clinical manifestations of "Q" fever was 15 days or less (Derrick, 1937). Later workers compared the results of numerous epidemiological surveys. Some stated that the incubation period varied between 14 and 26 days with a mean of 19 days (Robbins et al., 1946a; MacCallum et al., 1949) while others considered the outside limits to be 10 to 30 days (Beck et al., 1949). Human experimental evidence showed that the incubation period was directly proportional to the infecting dose (Tigertt et al., 1961) and that in guinea-pigs the time was also reduced by intraperitoneal inoculation (Tigertt and Benenson, 1956).

**Fever**

The most common single symptom reported in clinical cases of "Q" fever is a high fever, notable for its acute onset. Derrick
(1937) described it in his classical account of the illness and it has subsequently been noted in all outbreaks of the disease. Stoker and Clark noted fever to be present in all cases they studied (Stoker and Thomson, 1953; Clark et al., 1951d) but in a series reported by Johnson and Kadull (1966), only 35 out of 50 patients had elevated temperatures. The maximum temperature may be as high as 105°F (40.6°C) (Derrick, 1937; Johnson and Kadull, 1966) and fall either by crisis after about a week or by more gradual defervescence in which case convalescence may be very prolonged (Derrick, 1937). In a group of 69 untreated patients the fever was noted to last for periods of five to 20 days with a mean of 17 (Marmion et al., 1953).

Most authors report the occurrence of non-specific clinical features associated with fever: malaise, chilly sensations, night sweats, headache, limb and back pains, weakness and anorexia (Derrick, 1937; Dyer, 1938; Beck et al., 1949; Bell et al., 1950; Clark et al., 1951d; Marmion et al., 1953; Stoker, 1954; Johnson and Kadull, 1966). Beisel (1966) noted that catabolic changes and nitrogen loss were associated with infection and that they may both persist well into convalescence.

Relapses of "Q" fever have been reported (Beck et al., 1949; Marmion et al., 1953; Bertrand and Roux, 1955) but the presence of stable complement-fixing antibodies at a titre of only 1:25 makes the first report of relapsing "Q" fever slightly dubious (Gear et al., 1950).

**Headache**

"Severe", "intense", or "raging" headaches have been a
notable feature in most clinical reports since Derrick (1937) described them in eight out of the nine cases he studied. Gallacher (1961) noted that headache occurred in 90 per cent. of cases, was usually frontal and was occasionally accompanied by mucedal rigidity, although Harman (1949) had remarked on the absence of neck stiffness. Harrell (1953) noted the associated photophobia and also that the headaches were remarkably resistant to analgesics. One outbreak of "Q" fever in the suburbs of Cardiff was noted to be remarkable for the absence of complaints of severe headaches although the other clinical features followed those in most series. (Evans and Baird, 1959).

**Respiratory System**

Derrick (1937) observed that a few of his patients complained of cough, but an outbreak of pneumonitis due to C. burnetii (Hornibrook and Nelson, 1940) and the epidemics of possible "atypical pneumonia" among troops indicated that the respiratory system is commonly involved during "Q" fever infection (Caughey and Dudgeon, 1947; Feinstein et al., 1946; Robbins and Ragan, 1946). Experimental infections by the respiratory route were found to give more severe clinical illness than by other routes (Blanc et al., 1948) and radiographic pulmonary signs developed in 50 per cent. (Tigertt and Benenson, 1956). The actual number of patients in any series with respiratory signs and symptoms depends on the group examined. Of 16 cases in a British outbreak only five had clinical respiratory signs (Stoker and Thomson, 1953); however, in a series of 50 patients
who had all had chest X-rays taken, 56 per cent. were found to have radiological evidence of pneumonitis (Johnson and Kadull, 1966) while Gallacher's (1961) review of the literature noted that radiological patchy infiltration of the lungs had been observed in 34 per cent. of cases by Clark and 84 per cent. of cases by Beck, (Beck et al., 1949; Clark et al., 1951b).

In an outbreak of "atypical pneumonia" due to C. burnetii, at Camp Patrick Henry, 90 per cent. of hospitalised patients had positive X-rays. In these cases evidence of peri-bronchitic and alveolar infiltration in the outer two-thirds of the lung fields were the most prominent features, (Feinstein et al., 1946).

Upper respiratory signs have been observed (Clark et al., 1951b; Gallacher, 1961; Johnson and Kadull, 1966) but are uncommon, and epistaxis was noted in three of Derrick's (1937) original cases. Cough is fairly common, but usually unproductive or only productive of small amounts of mucoid sputum (Harman, 1949; Gallacher, 1961; Johnson and Kadull, 1966). Pleuritic pain is less common but has been reported by Johnson and Kadull (1966) and in association with haemoptysis by Manderson (1949) and pleural effusion by Beck (Beck et al., 1949). Signs of consolidation or pleural friction are occasionally found but fine papery crepitations are the most common respiratory sign (Gallacher, 1961; Feinstein et al., 1946).

**Cardiovascular System**

The pulse in "Q" fever is remarkably normal, even in cases of high fever (Derrick, 1937; Dyer, 1938; Harman, 1949), and other cardiovascular symptoms and signs are uncommon in the acute illness.
Pericarditis, first described by Ludwig (1956a), has subsequently been reported by other workers (Armand et al., 1960; Stephan and Saliba, 1963) and myocarditis has been described in two cases, although no details were given of the diagnostic titres of \textit{C. burnetii} (Tapie et al., 1960). In a review of 1,000 cardiovascular patients at \textit{post mortem}, Nicolau (1963) isolated \textit{C. burnetii} in the blood or organs of 70 cases with coronary artery disease, arteritis or thrombophlebitis, and demonstrated microcolonies in the vessel walls of fifteen patients. Gallacher (1961) considered phlebothrombosis and thrombophlebitis to be "not uncommon" and to be responsible for the cases of recurrent fever with leg pain and oedema recorded by Beck (Beck et al., 1949). Marmion and Harvey (1956) reported a death due to pulmonary embolism from a case of probable "Q" fever and arteritis has been reported in a man of 42 (Baylon et al., 1952) as well as gangrene due to "Q" fever arteritis by Gallacher (1961).

"Q" fever endocarditis will be considered under chronic "Q" fever.

\textbf{Gastro-Intestinal System}

The occurrence of anorexia has already been mentioned in relation to fever. Nausea and vomiting have been reported to occur in up to 25 per cent. of cases (Clark et al., 1951b; Stoker, 1954), diarrhoea less frequently and pancreatitis, oesophagitis and intestinal haemorrhage on rare occasions (Gallacher, 1961).

Derrick (1937) was the first to report jaundice, lasting 17 days, in a severe case of "Q" fever. Since then it has been
reported by many workers, usually in association with tender hepatomegaly (Beck et al., 1949; Clark et al., 1951b; Ludwig, 1956b; Fraser et al., 1960; Evans, 1963; Eschar et al., 1966). Picchi diagnosed three cases of granulomatous hepatitis associated with "Q" fever by liver biopsy (Picchi et al., 1960). Abnormal liver function tests were found in association with jaundice by Eschar (Eschar et al., 1966) while careful studies on six consecutive cases of "Q" fever showed that the major hepatic abnormality occurred in the cephalocholesterol flocculation and the thymol turbidity tests (Gerstl et al., 1956) while the zinc sulphate turbidity test remained normal (Gallacher, 1961). Needle biopsies in four of these patients showed three with focal hepatocellular damage and infiltrating eosinophils and monocytes, while the fourth, a convalescent patient, had mild fatty infiltration of the parenchyma without focal lesions (Gerstl et al., 1956).

**Central Nervous System**

Severe cases of "Q" fever have commonly been associated with severe prostration, delirium, apprehension and even coma (Harman, 1949; Clark et al., 1951b; Gillet et al., 1963). These symptoms have been considered to suggest that cerebral inflammation can be caused by *C. burnetii* (Harrell, 1953); cases of toxic psychosis (Beck et al., 1949), encephalitis (Porte and Capponi, 1954; Lelong et al., 1955) and aseptic meningitis (Germer and Schaubter, 1953; Eschar et al., 1966) can probably all be included in this
Central nervous system involvement in "Q" fever has also been shown in the rare reports of other neurological syndromes: "Nerves" and numbness of the hand reported by Derrick (1937); Severe dysarthria and ataxia by Marmion (Marmion et al., 1953) the Guillain-Barré syndrome by Alajouanine (Alajouanine et al., 1960); Parkinsonism by Gallacher (1961), and, if taken seriously, the most bizarre report of all, the curing of mental troubles by experimental infection (Blanc et al., 1948).

Rash

The highest proportion of patients reported to have a rash was four per cent. in a Northern Californian series (Clark et al., 1951b) although most series suggest figures between one per cent. and three per cent. (Gallacher, 1961). Sporadic rashes experienced by patients at various times of their illness have been reported as being red and punctuate, maculopapular, scarlatiniform, macular or just indefinite red spots (Derrick 1937; Beck et al., 1949; Clark et al., 1951b; LeLong et al., 1955; Report in Monthly Bull. Minist. Health, 1956; Gallacher, 1961; McKeil, 1964; Herbert et al., 1965).

Rabbit experiments demonstrated that the type of rash depended on the dose and number of inoculations. Numerous large doses produced nodules with necrotic centres, while smaller doses resulted in scarlatiniform exanthemata and fewer inoculations resulted in a localised maculopapular outbreak (Roger et al., 1956).
Other uncommon clinical features of acute infection:

Conjunctivitis and Uveitis

Derrick (1937) reported severe conjunctivitis lasting for 11 days in one patient and blood-shot eyes in another: they both complained of photophobia. A case of "Q" fever accompanied by uveitis has also been reported (Guyard et al., 1959).

Orchitis

Beck reported transient epididymitis and orchitis as a complication of one of the acute cases he described (Beck et al., 1949).

Thyroiditis

A woman with convalescent "Q" fever was found to have acute thyroiditis, associated with transient antithyroid antibodies (Somlo and Kovalik, 1966).

Placental infection

The isolation of *C. burnetii* from human placentae (Syrucek and Sobeslavsky, 1958; Wagstaff et al., 1965), at periods of up to three years after the initial infection, and Sidwell's demonstration of reactivation of latent "Q" fever by parturition (Sidwell and Gebhardt, 1966) suggest that intrauterine infection may occur. It is possible that this could be another cause of abortion in humans, similar to that reported due to infection with the ovine abortion
agent (Roberts et al., 1967), and Perez (1960) has reported a woman with *C. burnetii* infection who subsequently had recurrent abortions and neonatal deaths among her live born infants.

**Differential diagnosis of acute "Q" fever**

Because of its protean manifestations "Q" fever has been confused with many other clinical syndromes:

1) **Influenza:** The infrequency of coryza and sore throat differentiate "Q" fever from influenza, especially in sporadic cases (Derrick, 1937).

2) **Typhoid and Paratyphoid fevers:** Although the high fever and slow pulse are suggestive of enteric infections, the acute onset and negative Widal reaction make confusion with typhoid and paratyphoid fevers unlikely (Derrick, 1937).

3) **Typhus:** The acute onset and rapid fall of fever in most cases of "Q" fever, together with the febrile response induced in guinea-pigs, are very suggestive of typhus: however, the absence of rash on the patient or of scrotal reaction in guinea-pigs make the diagnosis unlikely, especially when convalescent serum from the patient or guinea-pig fails to agglutinate strains of *Proteus*. 
4) **Leptospirosis:** Leptospiral infection may be considered in the few cases of "Q" fever with severe conjunctivitis and jaundice. Diagnosis is only established by isolation of the causative organism or demonstration of specific antibodies (Derrick, 1937).

5) **Brucellosis:** Its association with animals and occasional recurrent fever make differentiation of "Q" fever from brucellosis very difficult without the use of specific diagnostic tests (Iarovoi et al., 1963). Occasionally there are antibodies to both organisms and, since cross reactions do not occur, double infections have been postulated (Renoux and Maurin, 1954).

6) **"Primary Atypical Pneumonia":** The pneumonitis caused by *C. burnetii* infection has often been confused with "atypical pneumonia" due to other agents and can only be differentiated by demonstration of specific antibodies in convalescent sera (Caughey and Dudgeon, 1947; Feinstein et al., 1946; Robbins et al., 1946a).

7) **Tuberculosis:** A case of "Q" fever was diagnosed retrospectively in an officer who had been invalided out of the navy as a case of pulmonary tuberculosis. He responded well to tetracycline therapy (Frazer et al., 1967).

8) **Meningitis:** The occurrence of vomiting in patients with severe headaches has often led to the misdiagnosis of meningitis, even in the absence of neck rigidity. Normal cerebrospinal fluid
helps to differentiate cases of "Q" fever (Stoker, 1954).

Mortality:

Deaths associated with "Q" fever have been reported in numerous outbreaks (Hornibrook and Nelson, 1940; Harman, 1949; Beck et al., 1949; Jadin and Giroud, 1950; Marmion et al., 1953; Stoker and Thompson, 1953; Nicolau, 1963). It is difficult to estimate the percentage mortality of acute "Q" fever as a clinical entity, because of the number of subclinical infections. Harman (1949) calculated the fatality rate in those with clinical infection to be about 0.2 per cent but Marmion observed that death occurred in 5.8 per cent of the 69 sporadic cases he studied (Marmion et al., 1953). By 1954 a combination of the sporadic and outbreak cases showed that the total number of British deaths due to diagnosed "Q" fever was four in 163 cases (Stoker, 1954). Although the first recorded death due to "Q" fever was on the 16th day of an uncomplicated pneumonitis (Lillie et al., 1941), very few of the patients reported to have died during acute "Q" fever infections were free from other debilitating conditions (Jadin and Giroud, 1950) such as bulbar palsy (Harman, 1949), carcinoma (Stoker and Thomson, 1953) Hodgkin's Disease and myocardial infarction (Perrin, 1949; Beck et al., 1949), so that the mortality rate will probably vary according
to the population at risk and be lowest in outbreaks amongst healthy young troops.

Pathological Features of Acute "Q" fever:

White Blood Count: In most cases of "Q" fever there is no alteration in the leucocyte count (Robbins and Ragan, 1946; Feinstein et al., 1946) but it has been noted that there is often a leucocytosis in protracted illness (Gallacher, 1961; Manderson, 1949) and in convalescence (Feinstein et al., 1946). Experimental evidence of an increase in D.N.A. synthesising mononuclear cells, shortly after the acute stages, suggested that these cells were required for secondary reparative processes (Gump and Fakety, 1967). Neutropenia was observed in the outbreak amongst art students (Harvey et al., 1951).

Other Laboratory Investigations: Erythrocyte Sedimentation Rate (E.S.R.), haemoglobin and cerebrospinal fluid are usually normal in acute "Q" fever, although the former has been reported to be slightly elevated in the early stages of severe illness (Feinstein et al., 1946). Liver function tests have been discussed.

Post Mortem Examination: Although death due to uncomplicated "Q" fever is uncommon, the post mortem findings have been documented.
(Lillie et al., 1941; Perrin, 1949). Macroscopically the lungs had patchy consolidated areas with alveolar exudates, which consisted microscopically of lymphocytes and mononuclear cells, and there was hyperplasia of the alveolar epithelium. The bronchial epithelium was noted to be desquamated and the interalveolar septa thickened.

Enlargement of the spleen and focal hypoplasia of the bone marrow have been the only other abnormal findings reported in uncomplicated deaths from pneumonitis; other lesions being related to underlying debilitating disease.

Microcolonies containing C. burnetii have been isolated from the blood vessel walls in patients dying of cardiovascular diseases but with unrecognised "Q" fever (Nicolau, 1963).

**Chronic "Q" Fever**

"Q" Fever Endocarditis: Although the occurrence of endocarditis in "Q" fever was first noted by Evans (Evans et al., 1959) it was first described as a clinical entity by Andrews and Marmion (1959). Their patient died after an illness which had started less than two years earlier, as an acute febrile illness in Panama and progressed, after a latent period, to be indistinguishable from subacute bacterial endocarditis.

All attempts to culture bacteria from the blood were negative.
negative and there were raised $\alpha_2$ and $\gamma$ globulins on electrophoresis. The patient's past history included a cardiac murmur but he could not recall having had rheumatic fever (Robson and Shimmin, 1959). During the illness $C.\ burnetii$ was isolated from his blood and he was found to have complement-fixing antibodies to both "phase I" and "phase 2" $C.\ burnetii$ antigens. At post mortem, death was found to be due to left ventricular failure following rupture of an aortic valve which had been weakened by friable vegetations from which large numbers of $C.\ burnetii$ were isolated. The organism was also isolated from the lungs and spleen.

The most significant finding in this case had been the first demonstration in man of "phase I" complement-fixing antibodies and these have subsequently been used as a diagnostic test of chronic "Q" fever infection.

A search for further cases of "Q" fever endocarditis amongst 56 patients with suspected subacute bacterial endocarditis, led to the diagnosis being made in another case who was found to have "phase I" complement-fixing antibodies: $C.\ burnetii$ were later isolated from his mitral valve at post mortem (Marmion et al., 1960). By 1963, eight cases of "Q" fever endocarditis had been reported and all but one had had "phase I" antibodies (Grist, 1963). In a later series of 16 cases, 15 had been known to have previous valvular disease. It was found that the aortic valve was involved in 81 per cent. of cases, while only 25 per cent. had mitral valve damage (Kristinnson and Bentall, 1967): These results contrasted with the
55 per cent, aortic and 86 per cent, mitral valve involvement in a series of cases with bacterial infective endocarditis (Lepeschkin, 1952). Grist (1963) noted the similarities to typhus endocarditis, including the grave prognosis.

A study of the incidence of "phase I" complement-fixing antibodies in 51 patients who had been ill two or more years before, suggested that the 15 patients with these antibodies had latent infection which could give later "chronic infection". The presence of these antibodies was unrelated to the duration of fever, height of E.S.R. or abnormal liver function tests, but was significantly related to the length of incapacity following the acute illness in patients over 30 years of age (Powell and Stallman, 1962). Marmion's observation, that organ involvement in "Q" fever was more likely to occur when there had been previous disease in that organ, would account for the fact that in patients with intact heart valves, latent infection with C. burnetii does not necessarily lead to endocarditis.

In addition to "phase I" complement-fixing antibodies, patients with "Q" fever endocarditis have been reported to have unusually high levels of the circulating immunoglobulin IgM, in their blood (Hobbs et al., 1967). Measurements of this immunoglobulin can be used to differentiate these patients from cases of "Q" fever without cardiac involvement and from cases of bacterial endocarditis, although they could still be confused with patients suffering from trypanosomiasis, bartonellosis and malaria where
viable organisms in the blood stream stimulate a similar immunological response (Hobbs et al., 1967).

Recent reports of combined medical and surgical treatment of chronic "Q" fever endocarditis appears to have altered the terrible prognosis for these cases: the number and follow-up period of the patients reported is still relatively small (Kristinnson and Bentall, 1967).

"Q" FEVER IN CHILDREN

One of the most significant features of many outbreaks of "Q" fever has been the total absence of the infection in children up to about the age of ten years. (Clark et al., 1951a and b; Marmion et al., 1953; Stoker and Thompson, 1953). Johnson (1966) reported cases in a few Australian children who lived on farms or were in direct contact with animals.

Obviously the number of children examined depends on the method of obtaining cases but Marmion considered that children were less at risk because occupational outbreaks were unlikely to involve them (Marmion et al., 1953). A farm outbreak has been reported where the only susceptible person who did not develop "Q" fever was a boy of five who was subsequently found to have antibodies, suggesting that he had had a subclinical infection (Stoker and Thompson, 1953). However, the lack of children with antibodies in many epidemiological surveys, even using the most sensitive diagnostic test, the radioiodine precipitation test (Luoto et al., 1965; Reed and Schnurrenburger
1966) suggested that in American children, infection is not occurring in subclinical or atypical forms which would be missed during outbreaks of classical "Q" fever. Dirk van Peenen and Reid's finding that children over the age of four years had approximately the same proportion with complement-fixing antibodies as the adult population suggested a different epidemiological pattern for "Q" fever in Egyptian Bedouin tribesmen (Dirk van Peenen and Reid, 1963), and another pattern of infection was shown by Pavilani's finding in Montreal of an unexpectedly high proportion of children aged one to four years with antibodies. This was accounted for by infection in milk, because in a subsequent survey there were no cases under one year of age, but the raw milk tested from five dairies was found not to contain C. burnetii (Pavilani et al., 1958).

Childhood cases of typical "Q" fever were reported in some surveys of infection (Beck et al., 1949; Evans, 1963; Johnson, 1966) and cases of childhood pneumonia due to C. burnetii have been reported from South Africa (Gear et al., 1950), Canada (McLean et al., 1960; Herbert et al., 1965), Scotland (Grist, 1966) and Russia (Kereev, 1959; Iarovoi et al., 1963). Brainerd (1955) noted that the course of the disease in children and young adults was much shorter, lasting only three to seven days, being confused with influenza and virus pneumonia rather than brucellosis or typhoid.

Encephalitis accompanying "Q" fever has been reported in a number of children (Forte and Capponi, 1954; LeLong et al., 1955; Gillet et al., 1963) including two cases in Belgium where "Q" fever is uncommon in all age groups. Most of these cases were accompanied
by other features of the illness such as pneumonia, rash and headaches but stupor and convulsions were the predominant signs.

No deaths due to "Q" fever have been reported in children.

Apart from the usual modes of infection by ingestion of contaminated milk and inhalation of infected dust, it has been postulated that transplacental infection may occur (Marmion, 1959). This was suggested because of the finding of large numbers of C. burnetii during early and late pregnancy in artificially infected pregnant guinea-pigs (Abinanti, 1957), and the isolation of organisms from human placentae at delivery (Syrueck and Sobeslavsky, 1959; Babudieri, 1953a).

THE EPIDEMIOLOGY OF HUMAN "Q" FEVER

AGE AND SEX DISTRIBUTION:

The clinical and epidemiological features of "Q" fever were carefully studied in Southern California and it was noted that there was a preponderance of male patients, aged between 20 and 59 years. Both age and sex variables were outwith the normal demographic pattern for the area and it was suggested that women and children may have had subclinical infections and therefore not be included in the survey (Beck et al., 1949).

Sporadic cases of "Q" fever have been observed to occur 12 times more commonly in men of working age than in any other group of humans (Marmion and Stoker, 1956) and this fact has always been
assumed to be due to occupational exposure. However, serological surveys of healthy blood donors (Harmon et al., 1956) indicate that this is not a true picture of *C. burnetii* infection, and the fact that in the outbreak amongst art students a smaller proportion of exposed females than exposed males developed clinical "Q" fever (Harvey et al., 1951) suggested that females may have a different constitutional reaction to the infection (Stoker, 1954).

**Epidemiological Features**

In studying the epidemiology of human "Q" fever, it is necessary to study the natural sources of *C. burnetii*, its immediate portals of entry to, and the intermediary steps it takes to the human host. (Derrick, 1953).

There is much evidence to show that direct or indirect contact with large domestic animals or their products is the chief natural source of human infection. (Derrick et al., 1942) and information that there was a positive association with cattle, pigeons, rats and dusty attics containing hay and straw, was an important finding related to the natural, as opposed to laboratory, infection of man. (Robbins et al., 1946).

**Natural Sources of C. burnetii**

**Cattle:** The observation that a majority of the original human cases of "Q" fever had occupational contact with cattle led Derrick to demonstrate that infection can be induced in calves and that a few
of the cattle in the Brisbane area had agglutinating antibodies to *C. burnetii* (Derrick et al., 1942). Epidemiological surveys in Southern California confirmed that there was occupational association with cattle in a large number of naturally acquired human infections (Beck et al., 1949; Bell et al., 1950). At that time there appeared to be no illness amongst cattle associated with *C. burnetii* infection, but Gallacher (1961) reports that Russian workers have since attributed anorexia, depression, decreased milk yield, rhinitis, conjunctivitis and abortion to the infection in cows. Jellison was able to demonstrate excretion of viable organisms from all four quarters of the udder of a naturally infected cow which was sacrificed when it was five months pregnant, but he failed to isolate *C. burnetii* from the amniotic fluid, amnion, blood or spleen of the same animal (Jellison et al., 1948b). The later demonstration of large numbers of *C. burnetii* in the placentae of seropositive cows at term (Luoto and Huebner, 1950) suggested that pregnant cattle may have the same relationship to *C. burnetii* as pregnant guinea-pigs, who were shown by Abinanti (1957) to excrete the organism in the products of conception only during late pregnancy.

Natural infection amongst dairy cattle depends to a great extent on the movement of animals and the intensity of animal husbandry in any given area. Calves have been artificially infected by feeding them with grossly contaminated milk (Bell et al., 1949) but it is not yet known whether vertical infection occurs *in utero* from infected placentae and birth fluids (Stoker and Marmion, 1955b);
however, if recent suggestions are substantiated, that *C. burnetii* causes a significant number of abortions in cattle (Brotherston, 1967), there would be evidence of infection equivalent to the transovular infection of ticks. Experiments to infect heifers by the intranasal, intravenous, gastro-intestinal and vaginal routes all failed, although inoculation of large doses of *C. burnetii* to the lactiferous ducts of lactating cows produced mastitis and subsequent excretion of the organism in milk (Bell et al., 1949). Transmission of infection from cow to cow could not be shown to occur by means of mechanical milking machines (Stoenner and Lackman, 1952), and it has been assumed that cattle acquire their natural infection like sheep from the dust-laden air which has been contaminated by infected birth fluids and placentae (Delay et al., 1950; Luoto and Huebner, 1950; Derrick, 1953). Infection in British herds occurs at a much slower rate, than in California, probably because of the damp climate and subsequent reduction in infected dust (Slavin, 1954).

In the early 1960s infection amongst dairy cattle was shown to be spreading and increasing in many parts of North America (Luoto, 1960; Fish and Labzoffsky, 1960; Luoto and Pickens, 1961; McKel, 1964; Herbert et al., 1965) and appeared to be doing so in Scotland when the last cattle survey was performed in 1956 by Grist. Although not strictly comparable, he showed that 2.3 per cent. of cattle in South West Scotland were excreting infected milk compared with Slavin's (1952) finding of 0.8 per cent. in dairy herds from all
over Scotland. Slavin's findings amongst infected herds showed that about 10 per cent. of the cows were excreting viable organisms and that up to a quarter of the infected herds had complement-fixing antibodies to *C. burnetii*. Movement of infected animals and subsequent contamination of uninfected herds is bound to occur until all herds in any given country are affected or some method of controlling infection has been found. Treatment of infected dairy cattle with chlortetracycline was ineffective in eliminating *C. burnetii* from infected animals (Luoto et al., 1951) and, although vaccination was found to give a fivefold reduction in the rate of *C. burnetii* excretion amongst newly introduced seronegative heifers in infected herds (Luoto et al., 1952), this method has not been used to stop the spread of "Q" fever amongst the cattle population of the world.

Sheep and Goats: Caminopetros (1949) had been the first to incriminate goats in the epidemiology of "Q" fever, and in Northern California it was found that sheep and goats had the same relationship to human illness as cows had had in Australia and Southern California except that there was a seasonal preponderance of human infection in the late spring (Clark et al., 1951d); groups of animals which had been associated with human cases of "Q" fever were found to have a high percentage with complement-fixing antibodies to *C. burnetii* (Lennette et al., 1949). These findings were confirmed in Texas (Irons et al., 1949) and Idaho (Stoener et
al., 1959), and in outbreaks of illness amongst the crew of trans-Pacific cargo boats where an infection known as "goat-boat" fever was identified as "Q" fever. It was never established whether these infections were acquired by the unauthorised consumption of raw goat's milk or direct contact with infected material while playing with new born kids (Clark et al., 1951c).

The importance of the isolation of C. burnetii from the placentae (Welsh et al., 1951), birth fluids (Abinanti et al., 1953a), urine and faeces (Winn et al., 1953) of naturally infected sheep, and from the soil and surface water of their premises for periods of up to 150 days after contamination (Welsh et al., 1959b), could be seen when considering the development of infection in serologically negative animals by ingestion of infected material (Winn et al., 1961). Contamination of the premises by sheep and goats gave rise to the finding of viable organisms in naturally infected air-borne dust (Delay et al., 1950; Lennette and Welsh, 1951); and the demonstration of infection from contaminated air (Abinanti et al., 1953b) provided evidence of another route of natural infection within the flock although the infected animals did not develop pulmonary lesions and only excreted organisms when they became pregnant (Welsh et al., 1959).

The significant difference between the number of sheep per acre in Kent and East Anglia, and the British peaks of human infections during the spring and early summer, suggested that it was the activities involved in lambing and shearing which caused many cases
of human "Q" fever (Marmion et al., 1954; Marmion and Stoker, 1958).

Ovine infection could therefore be postulated in certain British outbreaks of human infection such as that amongst R.A.F. personnel (Holland et al., 1960) and in three Welsh villages (Report, Monthly Bull. Minist. Health, 1956) but the number of sheep with antibodies in South West Scotland - 0.7 per cent. in 1956 - did not suggest that they were an important factor in the aetiology of the disease in that area (Grist, 1956).

As with infected cattle, "Q" fever in sheep is probably a benign infection but abortion in infected sheep (Evenschick, 1963; Miller, 1967) and goats (Kilschperger and Wiesman, 1949) have both been reported and Caminopetrous induced pulmonary lesions and fever by experimental intranasal inoculation (The Commission on Acute Respiratory Diseases, 1946b).

Serological diagnosis of infection in sheep can be difficult because of anticomplementary activity. The microscopic slide agglutination test was shown in these animals to be more sensitive than other agglutination tests or the complement-fixation test (Welsh et al., 1956a); the latter test is also confused by the fact that sera from sheep living in some areas react better with the "Henzerling" strain of antigen, while sera from sheep in other locations give more sensitive results with "Nine Mile" strain (Stoker et al., 1955).

Other Domestic Mammals and Birds  Horses, donkeys and dogs have all been shown to have antibodies to *C. burnetii* (Jadin et al.,
They probably acquire infection by ingestion or inhalation of material contaminated by infected cattle, sheep and goats and have been noted to excrete viable organisms in their faeces. No clinical illness and no spread from these animals to man has been reported.

One outbreak of human disease was believed to be caused by the delivery of an infected sow, but no antibodies to *C. burnetii* could be demonstrated in the sow or her piglets and the organism was not isolated from her premises (Stoker and Thomson, 1953).

Amongst domesticated birds infection has been found to occur in the following – turkeys, geese, ducks and pigeons: and the organism has been isolated from hen stools between the seventh and 40th day after infection. (Syrucek and Raska, 1956; Zdrodovskij, 1964; Babudieri and Moscovici, 1952). No human illness has yet been directly linked with birds: an outbreak of "Q" fever in a combined meat and poultry abattoir had to be accounted for by the slaughter of pregnant cows since no evidence of *C. burnetii* infection could be obtained from the poultry breeders concerned or from chickens on their farms. (Patrick and Stallman, 1966).

Other Mammals and birds. *C. burnetii* or antibodies to the organism have been demonstrated in many wild animals and birds and their importance in maintaining natural infection varies in different parts of the world. There exists a large reservoir of
infection amongst the bandicoots in Australia (Freeman et al., 1940) and the merions and gerbils in Morocco (Blanc et al., 1946): the normal baboon (Kalter et al., 1964) and 19 different species reported from over 16,000 fauna tested in Western Utah (Vest et al., 1965) also showed evidence of infection. Infected wild birds have been found, especially in the neighbourhood of infected farms (Syrucek and Raska, 1956).

**Anthropods and Insects.** A large number of species of ticks have been reported to be infected, among them the Montana wood tick *Dermacentor andersoni* (Parker and Davis, 1938) and a British tick, *Haemaphysalis punctata* (Stoker and Marmion, 1955a). Infection of these arthropods survives from the larval to the nymphal and adult stages, where it is finally transmitted in the ova to succeeding generations (Parker and Davis, 1938). Tissues and faeces of all stages contain many millions of guinea-pig infective doses (Evans, 1963) and remain infective for a long time (Philip, 1948a). While ticks are able to transmit *C. burnetii* infection to their progeny, fleas only just survive and lice are killed by infection with such rickettsial-like organisms (Gelfand and Berney, 1953).

The experimental isolation of contaminating *C. burnetii* from houseflies (*Musca domestica*) and of ingested *C. burnetii* from *Aedes aegypti* were reported by Philip (1948a), and the suspected connection of mosquitoes with a Welsh outbreak of infection has already been mentioned (Evans and Baird, 1959). However, it seems
that even if the association had been proved the mosquito will remain a less satisfactory environment to the *C. burnetii* than the intestine of a tick, the reproductive tract of a cow or the respiratory tract of a man, which were all noted by Marmion (1953) to be equally good habitats for the organism of "Q" fever.

The immediate portals of entry to man.

Blanc compared the effects of experimental infection in man by three different routes and found that inhalation of *C. burnetii* was the most effective method of inducing clinical "Q" fever. Intramuscular and intradermal infections were effective in producing immunity but gave very little clinical illness (Blanc et al., 1948). Tigertt and Benenson confirmed the importance of inhalation as a mode of infection. They allowed young male volunteers to inhale varying doses of *C. burnetii* and observed that the incubation period was directly proportional to the dose given (Tigertt and Benenson, 1956). There is no evidence of per conjunctival infection in man but a case of percutaneous infection, from crushing body ticks, has been reported (Eklund et al., 1947) and the possibility that this may occur quite frequently during the handling of animal products has been considered (Derrick, 1953).

The ingestion of *C. burnetii* in infected milk must be accepted as a further route of entry into man. It explains only a minority of cases (Derrick, 1953) but was probably an important factor in the sporadic cases with no seasonal variation reported by Marmion and Stoker (1958).

Inhalation: The presence of infective *C. burnetii* in the air and
dust of animal premises has been discussed in relation to animal infection (Delay et al., 1950; Lennette and Welsh, 1951). The possibility of human infection from such infected air is obvious amongst patients who have direct contact with animals which contaminate the atmosphere, and occupational contact with cows was noted to be a significant feature in the original cases of human "Q" fever (Derrick et al., 1942). Since then the number of infections demonstrated amongst abattoir workers, especially in slaughter houses handling pregnant cows, has confirmed this finding (Irons et al., 1949; Strauss and Sulkin, 1949; Tonge and Kennedy, 1963; Patrick and Stallman, 1966; Schonell et al., 1966). Sheep shearers, shepherds, dairy workers and goat herders have all experienced similar infections, either as sporadic cases or in outbreaks (Beck et al., 1949; Forte and Capponi, 1954; Bell et al., 1950; Clark et al., 1951d; Johnson, 1966).

The ability of C. burnetii to withstand drying for long periods was shown by the finding that tick faeces remained infective for 586 days and dried guinea-pigs blood for 182 days at room temperature (Philip, 1948a; Parker et al., 1949). This property meant that indirect contact with animals could occur amongst many persons far removed from the original infected animal. Dried blood, used as a fertiliser (Evans, 1963), dusty packing straw (Wegman, 1948) and contaminated clothing (Oliphant et al., 1949; Beck et al., 1949) have been reported as intermediary steps to human infection where contact with the infected material was quite obvious.
Windborne infections have had rather less obvious links with the source of contamination, the classical example being Young's description of an outbreak of illness in Artesia, California, following a windstorm known as "Santa Ana" (Young, 1948). Windborne infections from infected stock are probably rather less common in Great Britain than in drier climates such as Italy and Los Angeles but even in this country residence in proximity to a glue and fertiliser factory has been reported to be of epidemiological significance (Stoker and Marmion, 1955; Marmion and Stoker, 1958).

Survival of *C. burnetii* for long periods of time also allows animal premises to remain contaminated when they are no longer being used for animals. Outbreaks of "Q" fever amongst troops billeted in animal quarters have been explained in this way (The Commission on Acute Respiratory Disease, 1946a; Caughey and Dudgeon, 1947; Robbins et al., 1946a; Holland et al., 1960; Frazer et al., 1960) and a case of "Q" fever in a European child in Africa was explained by temporary residence in a native hut near animals (Porte and Capponi, 1954).

Contamination of the laboratory air by *C. burnetii*, especially before its remarkable property of resistance to formalin had been described (Ransom and Huebner, 1951), has accounted for many laboratory cases of clinical "Q" fever (Burnet and Freeman, 1939; Smith et al., 1939; Dyer et al., 1940; Hornibrook and Nelson, 1940; Huebner, 1947; The Commission on Acute Respiratory Diseases, 1946d). Johnson and Kadull (1966) reviewed 50 of these
laboratory cases and noted that only 21 cases could be explained by
direct accidental contact with *C. burnetii*, especially during the
preparation of yolk-sac antigen. The other 29 cases occurred in
laboratory workers and clerks who worked in the same buildings.

Human contamination of the air is very rare but cases of
infection from this source has been reported and *C. burnetii* has
been isolated from urine (Derrick, 1937) sputum (Spicknall et al.,
1947) and garglings (Hengel et al., 1950). The source of an
outbreak at the Royal Cancer Hospital was a patient who died of
bronchopneumonia in association with bulbar palsy. Most of the
cases he infected acquired their infection at his post-mortem, but
one patient was a nurse who had had contact with him during life;
(Marmion and Stoker, 1950). Another report of infection at autopsy
resulted in 18 fresh cases (Babadieri, 1951), while a small outbreak
amongst R.A.F. personnel was attributed to a live patient who
infected four members of his sick bay staff (Holland et al., 1960).

**Ingestion:** Viable *C. burnetii* were recovered from raw milk in
the careful epidemiological surveys in Southern California, and
ingestion of infected raw milk from certain dairies was considered
to be a possible route of human infection (Huebner et al., 1948).
Further studies in Los Angeles showed that 10 per cent. of the dairy
cows were secreting viable organisms (Luoto et al., 1951), and other
workers have shown milk to be infected in such widely separated North
American states as Ontario, Maryland, Alberta and Ohio (Fish and
Demonstration of infection with *C. burnetii* following ingestion of the viable organism was first shown in calves and sheep (Bell et al., 1949; Winn et al., 1961). In 1963 the first human infection following ingestion of infected raw milk was demonstrated in a group of prisoners who developed complement-fixing antibodies to *C. burnetii* after drinking this milk, although they had no clinical evidence of infection and had had no contact with animals (Benson et al., 1963). This confirmed suspicions noted by Huebner and Marmion (Huebner et al., 1948; Marmion et al., 1953) but doubted by other workers. (Fonseca et al., 1949; Lackman et al., 1964); however, since none of the prisoners were ill, its significance as a common source of clinical illness remained in doubt. Because Huebner had shown that a significantly greater number of "Q" fever cases regularly drank raw milk, as compared with the rest of the population (Huebner et al., 1949), Marmion and Stoker investigated about 8,000 persons who drank infected raw milk. They found that 1,070 (12 per cent.) of these people had antibodies to *C. burnetii* although only 30 (0.4 per cent.) had had an illness retrospectively diagnosed as "Q" fever, giving a 1:35 ratio of overt to subclinical infections due to consumption of *C. burnetii* in their contaminated milk (Marmion and Stoker, 1958). To explain this high proportion of subclinical infections it has been suggested that whey antibodies to *C. burnetii* might inactivate the organism and give a combination
which was much less infectious to humans ingesting the organism (Abinanti and Marmion, 1957), or that the gastro-intestinal tract was less susceptible to infection than the lower respiratory tract (Marmion and Stoker, 1958). Studies showed that infection from raw milk accounted for cases of "Q" fever in urban areas and caused an equal male to female distribution with cases occurring at all seasons of the year (Stoenner et al., 1959; Marmion and Harvey, 1956; Marmion and Stoker, 1958); but in rural populations, where most of the raw milk is consumed, the relative importance of contact with animals and ingestion of raw milk is difficult to assess (Clark et al., 1951d).

The demonstration of viable \textit{C. burnetii} in milk which had been pasteurised meant that raw milk was not the only potential source of ingested organisms. It was found that 6.2 per cent. of milk samples pasteurised at 143°F (61.7°C) for 30 minutes, by the vat-holding method, stimulated antibody production in guinea-pigs but none of the samples pasteurised at 160.5°F (71.4°C) for 15 seconds, by the high temperature/short time method stimulated antibodies (Huebner et al., 1949). Marmion also demonstrated the inefficiency of pasteurisation in heavily contaminated milk. He infected milk artificially with doses which could be expected from a single cow, although this concentration would be unlikely from pooled herd milk unless all the cows were heavily infected. Unlike Huebner, he and other workers have found the high temperature/short time method, of 161°F (71.7°C) for 15 seconds which is the minimum British requirement, was less efficient at destroying \textit{C. burnetii} than the
vat-holding method: it is significant to note that in all specimens tested, the phosphatase test had been negative (Marmion et al., 1951; Lennette et al., 1952a).

Evans found that 38 per cent of bulked raw and 5.5 per cent of bulked pasteurised milk contained viable _C. burnetii_, and that although 4.1 per cent of normal human serum in the area contained complement-fixing antibodies, he calculated that only 0.6 per cent of cases of "atypical pneumonia" could be explained by the ingestion of either form of contaminated milk (Evans, 1963). A suggestion which is unlikely is that antibodies are formed to ingested _C. burnetii_ which has been inactivated by efficient pasteurisation because no such antigenic stimulus occurs when heat-inactivated organisms are inoculated into guinea-pigs (Huebner et al., 1949).

Butter, a milk product, which was shown to maintain its infectivity even after 41 days refrigeration (Jellison et al., 1948) has not been shown to be of epidemiological significance in "Q" fever.

**Inoculation.** A Spanish lady (de Prada, 1950) an American man (McCurl and Williams, 1948) and Texan troops (Anigstein and Bader, 1943) have all been reported to have acquired their "Q" fever from tick bites: but although the number of species of ticks reported as having natural _C. burnetii_ infection is numerous, there are very few cases of tick bites preceding human illness (Derrick, 1953; Babudieri, 1951). This is possibly because the ticks that have been found to be infected are not normally human parasites (MacCallum
et al., 1949).

Mosquitoes have been infected by feeding on infected guineapigs (Philip, 1948a) but they have never been shown to transmit C. burnetii. The increased incidence of the British tick Ixodes ricinus during the spring and summer peaks of infection have also been considered as coincidental findings (Marmion, 1959).

Since 1 per cent. phenol has been shown to be relatively ineffective at destroying C. burnetii, it was suggested that the organism could survive as a contaminant in glycerol/phenol treated calf lymph for smallpox vaccination (Malloch and Stoker, 1952). Stoker (1953a) recommended that these vaccines should be screened for infectivity by inoculation into sheep.

**Intermediary Steps to the human host.**

**Epidemiological investigations.** While inhalation probably accounts for between 90 and 95 per cent. of human "Q" fever infections (Derrick, 1953), and ingestion or inoculation for the rest, there are many cases which cannot obviously be explained by any of these means. The aetiology of the infection in the case of the student wood cutter (Hesdorffer and Duffalo, 1941); the people of Cardiff, thought to have been infected by mosquitoes (Evans and Baird, 1959) and the art school students (Harvey et al., 1951) has never been satisfactorily explained despite extensive epidemiological investigations. The investigations used vary with every outbreak, but always involve close questioning of patients to determine any possible source.
of direct or indirect contact with infected animals.

Epidemiological surveys of the immunity status of specific population groups have been performed in various ways. Serological agglutination tests were originally used because they remained positive for several months after infection (Freeman et al., 1940) but in 1952 Lennette demonstrated that the complement-fixing antibodies could be detected even later (Lennette et al., 1952b; Babudieri 1953a). Surveys in most areas begin by assessing the presence of antibodies in the workers at abattoirs and fat rendering plants and later in the general population and animals (Kaplan and Hulse, 1953). The intradermal skin test has also been widely used in surveys (Giroud et al., 1951; Giroud et al., 1952), but none of the above methods can be assumed to give the total picture of past experience with the organism after the demonstration by Luoto of the marked superiority of the radio-iodine precipitation test.

Because of its ability to withstand adverse conditions, C. burnetii continues to have what Marmion (1953) described as a most remarkable success story, and its diverse and devious routes from animals to man will continue to baffle epidemiologists as they have done in the past (Derrick, 1953; Marmion, 1959).

CONTROL OF "Q" FEVER INFECTION

TREATMENT OF "Q" FEVER

Antibiotics and the standard fever regime of bed rest,
aspirin, adequate protein, calorie and vitamin intake and optional
cough sedative were recommended by Brainerd (1955) for the treatment
of "Q" fever, at a symposium of unusual infections in childhood.
The antibiotics that he suggested—chlortetracycline, oxytetracy-
cline, tetracycline and chloramphenicol—have been carefully studied
in "Q" fever infections. Wong and Cox (1948) first showed chlortet-
tracycline to be effective against *C. burnetii* in animals and this was
later confirmed in man. Clark compared the actions of chlortetra-
cycline and penicillin and found that the former produced a signifi-
cant reduction in the duration of fever. He compared different
doses and routes of administration of chlortetracycline and found
that all were equally effective in the majority of patients, although
a few patients failed to respond to any regime in the usual five
days (Clark et al., 1951a).

Ormsbee compared the effects of newer antibiotics on
experimental infections in chick embryos. He found that erythromy-
cin and chloramphenicol had only a slight effect on *C. burnetii*
although they were active against other rickettsiae. Oxytetracycline
and chlortetracycline had the highest and most consistent death-
delaying activity in chick embryos infected with *C. burnetii* or the
rickettsiae. (Ormsbee et al., 1955). None of these antibiotics
are coxiellicidal.

Tigertt and Benenson (1956) noted that treatment with the
tetracyclines was most effective early in the disease. They reported
that clinical disease could be prevented if these antibiotics were
administered late in the incubation period, but that earlier administration only resulted in delaying the onset of the disease. The failure of antibiotics to cure chronic infection was observed by Ferguson, whose patient died of "Q" fever endocarditis despite massive doses of tetracycline (Ferguson et al., 1962). Nicolau (1963) suggested that chronic "Q" fever infections should be treated with intermittent courses of tetracycline, rather than continuous therapy, but even this has proved disappointing where vegetations have become established on infected heart valves.

The report of a combined medical and surgical management for "Q" fever endocarditis by Kristinsson and Bentall (1967) gives us hope for those cases with a previously poor prognosis. Medical control was attempted by giving two grams of tetracycline daily for a month, followed by long term administration of one gram daily for six months or a year. If the disease was not controlled by this regime, surgical replacement of the infected valve - in their series, all aortic valves - by a prosthesis, was performed. Of their six patients, four required surgery and all but one were alive at periods of up to two years following operation. The patient who died had an occlusive coronary episode six months after his operation, and the other three continue to take prophylactic doses of tetracycline.

Despite these encouraging results of surgical management, latent and chronic "Q" fever infections are likely to remain a problem. Studies in naturally infected dairy cattle indicated that, despite its efficacy in treating early clinical "Q" fever, chlortetracycline,
given both intravenously and into the mammary ducts, did not eliminate C. burnetii from the infected animals. (Luoto et al., 1951).

Sidwell has shown in animals the danger of indiscriminate therapy with corticosteroid drugs and whole body irradiation in the absence of coxiellicidal chemotherapeutic agents. He found that a dose of whole body irradiation gave reactivation of latent "Q" fever infection in guinea-pigs. (Sidwell et al., 1964a). He also showed that a weeks course of cortisone injections reactivated latent infection in guinea-pigs and white mice so that they resumed excretion of C. burnetii in their urine and faeces. (Sidwell et al., 1964b)

Whether this alteration in host resistance is confined to small mammals or not, it is important to remember that drugs such as corticosteroids should be avoided, if possible, in patients who have had previous experience with C. burnetii.

PREVENTION OF "Q" FEVER.

1. Protection from infected animals.

In 1953 Derrick stated that, as with brucellosis, prevention of human "Q" fever will essentially depend on the control of the disease in animals. (Derrick, 1953). Luoto's failure to eliminate the causative organism from infected animals by antibiotic therapy led to attempts to prevent infection in antibody negative cows introduced to infected herds. Killed vaccine was given subcutaneously and the vaccinated animals were isolated from the herd until they had
calved. A significant decrease in animals producing complement-fixing antibodies, and an even greater reduction in the number excreting C. burnetii was achieved, but the results did not show 100 per cent. protection and vaccination of animals at risk to infection has not been widely used (Luoto et al., 1952).

Babudieri (1953a) advocated notification of proven animal cases and very elaborate improvements in animal husbandry, from the delousing of animals to the prohibition of animal movement through towns. Many of his suggestions, such as destruction of placentae and removal of large animals from human habitations, are common practice in some countries of the world, but would require revolutionary measures to enforce in many of the countries where "Q" fever is endemic.

Control of milk supplies is considered by some to be relatively easy by using efficient pasteurisation (Lennette et al., 1952a). Since it is believed that very little clinical "Q" fever has been acquired from infected milk, the compulsory testing of animals used for raw milk production, proposed by Babudieri (1953a) and practised in Montana (Lackman et al., 1964) may be superfluous.

2. Vaccination of Humans.

Since prevention of infection in animals and subsequent reduction in the source of infection to man does not yet seem feasible, it will be necessary to rely on vaccination of persons having
occupational contact with *C. burnetii*, either in the laboratory or from parturient animals. The first suggestion that vaccination might be possible was Blanc's finding that subjects who had been infected by the intradermal route acquired immunity although they gave no evidence of infection. (Blanc et al., 1948). Vaccination was considered necessary because of the appearance of "Q" fever as a disease of importance in Military medicine as well as among laboratory employees and a formalinised yolk-sac vaccine was prepared and tested in guinea-pigs who developed antibodies to homologous and heterologous strains of *C. burnetii*. The experiment was repeated in 28 human subjects and all but two developed antibodies while only one had a systemic reaction. (Smadel et al., 1948).

Preparation of *C. burnetii* vaccine will be described in the preparation of antigen for the intradermal sensitivity test (Lackman et al., 1962). Safety tests in embryonated eggs, using at least three passages, have been found to give the most sensitive results for the detection of small numbers of viable *C. burnetii* in the vaccine. In guinea-pigs inactivated organisms in the vaccine continue to act as an antigenic stimulus. (Berman et al., 1960).

The vaccine is administered subcutaneously or intracutaneously to skin test negative subjects, in doses ranging from one to 10 complement-fixing units, and is repeated after two weeks. If a follow-up skin test nine months after vaccination remains negative, a third dose of vaccine is given. Before the routine use of
pre-vaccination skin tests, local reactions to vaccine doses in previously sensitised persons produced severe local reactions, with sterile abscesses whose complement-fixing titres were up to 1:16,384 (Lackman et al., 1962). Because the intradermal sensitivity test is not as sensitive a measure of past "Q" fever infection as the radio-iodine precipitation test (Luoto et al., 1965), some previously sensitised persons have negative skin test reactions. Vaccination of these persons, and some with minimal skin test induration, has not resulted in serious local lesions because these people appear to possess a different antibody response and to be non-reactive to local antigen. (Bell et al., 1964; Philip, 1967; Lackman et al., 1967).

The development of antibodies following vaccination is very slow, due to the minimal sensitising doses used. The first antibodies to appear at about four to six weeks are those demonstrated by the capillary agglutination test (Luoto et al., 1963). The radio-iodine precipitation test is positive in 100 per cent of vaccinees by 60 days after immunisation (Tabert and Lackman, 1965), and about 80 per cent have positive skin tests after 40 weeks (Bell et al., 1964); complement-fixing antibodies do not usually appear in response to this small antigenic stimulus (Luoto et al., 1963).

Vaccination of laboratory workers, together with stricter laboratory precautions, has led to a marked reduction in the number of reported laboratory outbreaks. The value of vaccination as a prophylactic measure in the community will depend on the size of the infected animal reservoir and the number and severity of human...
infections but vaccination has been claimed as a successful prophylactic procedure. (Lackman et al., 1967).

**TESTS AVAILABLE FOR DIAGNOSIS OF INFECTION BY C. BURNETII**

In all microbial infections, essential evidence of infection with a specific agent must be provided by isolation and identification of the organism concerned from cases of clinical infection. Isolation of *C. burnetii* requires laboratory animals and although relatively straightforward, is hazardous, as indicated by the large number of laboratory outbreaks of "G" fever. (Burnet and Freeman, 1939a; Smith et al., 1939; Dyer et al., 1940; Hornibrook and Nelson, 1940; Huebner, 1947; Johnson and Kadull, 1966). However, it is not feasible to isolate organisms from patients who have been ill for some time or who have already recovered from a suggestive illness of unknown aetiology. It is for this reason that a number of serological tests – of varying sensitivity – have been developed, to identify specific antibodies to *C. burnetii* in man and animals after natural infection or vaccination. Some of these serological tests have also been applied to the demonstration of *C. burnetii* antibodies in milk, and an additional method of retrospective diagnosis of specific antibodies has been provided by an intradermal sensitivity test.
1. ISOLATION TESTS:

The effects of artificial *C. burnetii* infection in experimental animals has already been described.

2. SEROLOGICAL TESTS:

**Direct Agglutination Test.**

Burnet and Freeman (1937) used an emulsion of mouse spleen to demonstrate agglutination by homologous convalescent serum and by serum obtained from immune monkeys and guinea-pigs. Sera from uninfected guinea-pigs and humans and a case of typhus all failed to cause agglutination, so the first specific serological test for "Q" fever had been found. Its use as a means of diagnosing convalescent infection in guinea-pigs made unnecessary the sacrifice and challenge of infected animals. In these animals, agglutinating antibodies usually appeared 10 to 30 days after infection, depending on the number of organisms in the inoculum.

Subsequent agglutination tests have used an antigen prepared from yolk-sac material, which had been purified by ether extractions and centrifugation to prevent spontaneous clumping of organisms. (Babudieri, 1953b). The final washed suspension was inactivated with 0.25 per cent. formal saline at pH 7.0, and used as a 1:5 dilution for the test. (Wolfe and Kornefeld, 1949).

This test has been widely used for diagnosis of clinical
cases and for epidemiological surveys, but is extravagant with antigen.

**Microscopic Agglutination Test.**

Although it has not been widely used, an agglutination test on special slides has been described which uses only 0.02 ml. of antigen and equal amounts of each serum dilution in a physiological solution with 0.01 per cent. merthiolate. The test is left overnight in a damp chamber, dried out at 37°C, and the microscopic agglutination that occurs is demonstrated by Geimsa stain (Babudieri, 1953b). On comparative testing, this method was found superior to other serological tests for demonstrating antibodies to *C. burnetii* in sheep sera (Welsh et al., 1959).

**Capillary Agglutination Test.**

Because of the numerous reagents required for the complement-fixation test and the expense of the direct agglutination test, Luoto (1953) developed a capillary agglutination test using partially purified yolk-sac antigen stained with a Harris stain containing extra haematoxylin. He and Mason used the test to demonstrate antibodies in bovine sera, and in milk (Luoto and Mason, 1955) and found a high degree of reproducability and sensitivity with both substances. Milk samples can be tested whole, skimmed, as whey, diluted or frozen and thawed; the positive pattern - a blue black cream "ring" or scattered black agglomerates is dependent on the fat content of the sample under test. This test has been used for determining the
amount of infection in bulked raw milks from dairy herds, (Fish et al., 1960).

**Complement-Fixation Test.**

In 1941 Bengtson (1941b) first developed a complement-fixing antigen from infected embryonated yolk-sacs. She found that its strength was directly proportional to the number of organisms demonstrated in a smear and used harvests from the fifth and sixth passages. She used an Australian strain of *C. burnetii* and inoculated it into the yolk-sac of six to eight day fertile eggs. The infected yolk-sacs were harvested about three days after inoculation, macerated and centrifuged to remove coarse particles. Further high speed centrifugation, resuspension and sedimentation of remaining tissue produced an antigen for testing with known positive human and guinea-pig sera.

Sera, inactivated at 56°C for 30 minutes, were diluted in normal saline and 0.2ml. amounts were mixed with equal volumes of guinea-pig complement containing two units in 0.2ml.; 0.2ml. of antigen, diluted to two complement-fixing units according to a chequer-board test, was added and the tubes were incubated in a water-bath at 37°C, for an hour. 0.4ml. of 5 per cent. sensitised sheep erythrocytes were then added before a further hour of incubation in the 37°C water-bath. The highest dilution of serum giving fixation of complement was observed after overnight refrigeration. 50 per cent.
fixation of complement at dilutions greater than 1:8 were considered positive and controls of antigen, sera, complement and haemolytic system were all included in the test.

Since Bengtson's original work with the Australian strain, numerous other strains have been used for the production of antigen. Of these the Italian "Henzerling" and the Montana "Nine Mile" strains have been shown to give the best reactions with both homologous and heterologous antisera from most species. In Britain the "Nine Mile" strain has been found to react better with human sera although in some geographical areas, sheep sera react better with the "Henzerling" strain. (Stoker et al., 1955). Antigens are used in "phase 2" for routine testing, and "phase 1" is only used to diagnose cases of chronic "Q" fever.

Various modifications of the test have been advocated. In The World Health Organisation survey reported by Kaplan and Hulse (1953) sera were inactivated at 60°C for 30 minutes and a little magnesium sulphate was added to the diluent to sharpen reactivity. Stoker also inactivated his sera at 60°C, but used unbuffered saline and overnight fixation at 4°C before addition of the haemolytic system. Positive tests were those showing 75 per cent. fixation of complement at dilutions over 1:10. (Stoker et al., 1955). These workers also included a control "typhus vaccine", referred to by Marmion, to differentiate true positives from sera with non-specific reactions to a combination of yolk-sac, rickettsial suspension and
serum factors (Marmion et al., 1951). Babudieri (1953b) completed the preparation of his antigen by inactivation of the C. burnetii with formol and ultra-violet irradiation.

In her initial tests, Bengtson had reported that guinea-pig complement-fixing antibodies appeared nine days after infection and human ones after 13 days. Babudieri (1953a) found that nine per cent. of humans had developed complement-fixing antibodies, at dilutions of 1:8 or more, within the first week, 65 per cent. in the second and 100 per cent. by the third week after clinical "Q" fever. The maximum level was maintained for one to two months and then fell slowly over months or years. Lennette found that 97 per cent. of patients had complement-fixing antibodies by the third week after infection and that most patients still had antibodies after two years (Lennette et al., 1952b) while Tonge has reported positive results from 29 per cent. of patients 11 to 15 years after known clinical infection (Marmion et al., 1956).

Although the complement-fixation test has been shown to be a reliable diagnostic test in clinical "Q" fever, Luoto found that complement-fixing antibodies rarely developed after vaccination with small doses of C. burnetii (Luoto et al., 1963). Occasionally, non-specific reactions to "phase 2" C. burnetii antigen have been found to occur in patients with positive serological tests for syphilis (Robbins et al., 1946a; Strauss and Sulkin, 1949) and adenovirus pneumonia (Van der Veen and Heyen, 1966), so that its value as a test
of subclinical infections and in epidemiological surveys is therefore in doubt.

Comparison of the complement-fixation test and agglutination tests.

Opinions differ as to the relative merits of the direct agglutination and the complement-fixation tests. The former uses more antigen and is therefore expensive but it can be used with undiluted sera and there are no non-specific reactions. (Kaplan and Bertagna, 1955). Agglutinating antibodies appear sooner but also fall away faster, making the agglutination test less useful for retrospective epidemiological surveys. (Babudieri, 1953a; Lennette et al., 1952b). With sheep sera the agglutination test was found to give more sensitive results (Welsh et al., 1959), possibly because complement-fixing antibodies are relatively transient in these animals. (Babudieri, 1953a).

Agglutination tests with milk samples (Luoto and Mason, 1955) and cow's whey (Stoker and Marmion, 1952) proved very accurate, easy to read and more sensitive than complement-fixation tests.

Luoto (1956) also found the capillary agglutination test to be more sensitive than the complement-fixation test because the sera and milk did not need to be diluted. Later he confirmed its greater sensitivity by finding capillary agglutinating antibodies in most "Q" fever vaccinees four to nine weeks after inoculation.
(Luoto et al., 1963). Stallman (1965), however, found that although 74 per cent. of positive human sera he tested gave comparable results with both the capillary agglutination and complement-fixation tests, 22 per cent. were found to have only complement-fixing antibodies compared with four per cent. positive in the capillary agglutination test alone.

**Mouse Neutralisation Test.**

Derrick and the earliest workers with *C. burnetii* made use of "protective" sera in convalescent animals, but the development of the agglutination and complement-fixation tests made this animal challenge test redundant. Abinanti and Marmion (1957) revived it and developed a mouse neutralisation test where the amount of remaining live antigen, after overnight incubation at 4°C, with the serum under test, was proportional to the number of organisms obtained from the smears of mouse spleens from animals inoculated with the mixture. They found that some sera were positive although they had been negative in the capillary agglutination and complement-fixation tests and he suggested that neutralising antibodies must persist for longer than the others.

**Opsonin Test.**

The perfection of a quantitative method for measuring opsonin production in brucella infections was followed by application of the same principles to measurement of opsonins for the rickettsiae
and *C. burnetii*. The method involves phagocytosis of *C. burnetii* by polymorphonuclear leucocytes, in the presence of opsonins in the sera under test (Victori, 1952). Despite its relative ease, convenience and cheapness it has not been widely used as a diagnostic test.

**Antiglobulin Sensitisation Test.**

The measurement of antibodies to "Q" fever by the agglutination of a carefully washed antigen/antibody complex, incubated for at least two hours at 37°C, with rabbit anti-human globulin, provided a specific and sensitive test for measuring antibodies to *C. burnetii* (Coombs and Stoker, 1951). This test was particularly suitable for small particle antigens such as the rickettsiae and *C. burnetii* but it was extravagant with antigen and more time consuming than the complement-fixation test. It has not been widely used despite its greater sensitivity over other tests.

**Radio-Iodine Precipitation Test.**

A development of the antiglobulin test was made possible after Gerloff had demonstrated a method of measuring poliovirus antibodies by a radioactive precipitation test (Gerloff et al., 1961) and Grmsbee (1962) had described a method of preparing purified *C. burnetii* antigen, free of contaminating protein.

The purified antigen was coupled with radioactive-iodine and added to serial dilutions of the sera under test. The antigen/antibody complex so formed was incubated and then agglutinated by
rabbit antiglobulin. Centrifugation removed both the complex antiglobulin and other globulin / antiglobulin agglutinates and the test was considered positive if more than 50 per cent. of the radioactivity in the remaining supernatant had been removed. (Hoyer et al., 1964). It is important that sufficient antiglobulin is added to agglutinate the total complex formed, but only very small amounts of antigen are required. It is a highly specific test and has been found to detect low levels of antibody, even in deteriorating sera but unfortunately most laboratories do not have the specialised equipment required for the mechanical reading of radioactivity (Lackman et al., 1964).

The specificity of the test was confirmed by Tabert and Lackman (1965), who tested 176 natives of Alaska and found that only one had a positive reaction; this compared with a group of people who had been vaccinated with Escherichia coli and who were all positive. They also tested various groups of people who had occupational contact with animals in various parts of the United States and found that more had positive radio-iodine precipitation tests than had positive complement-fixing and capillary agglutination tests. By doing animal experiments, they determined that the radio-iodine precipitation test measured 7S antibodies and was therefore equal in specificity to the mouse neutralisation test. This has been confirmed in man by the resistance of these antibodies to treatment with 2-mercaptoethanol although the complement-fixing antibodies (19S) were inactivated (Lackman et al., 1967).

3. INTRADERMAL SENSITIVITY TEST.

While the range of reliable serological tests available for
diagnosis of present or past infection with *C. burnetii* is now very comprehensive, it is useful to have a non-serological method available for epidemiological investigations. A specially prepared chick embryo antigen was used by Giroud as an intradermal allergic test for *C. burnetii* in personnel associated with the meat industry in Urban-Ghari. (Giroud et al., 1951). This work was extended as an epidemiological survey with four of the rickettsial diseases in West Africa where a wide variation in the positive percentage rate to *C. burnetii* was found. 70 per cent. of the butchers had positive skin tests while people who had no contact with animals were all negative.

When comparing the allergic reaction to *C. burnetii* with those to *R. prowazekii, R. mooseri, R. rickettsii* and *R. tsutsugamushi* it was found that the maximum reaction to *C. burnetii* occurred later because it has no external membrane to give a soluble antigen. The absence of an external membrane component in the antigen provided complete specificity for this test of *C. burnetii* antibodies; for accurate results it was important that the patients were well nourished and without gross cutaneous lesions (Giroud et al., 1952).

Babudieri (1953b) used an allergic test in epidemiological studies of animals. He inoculated cows and sheep subcutaneously in the lower eyelid and observed swelling after three or four days in positive animals. He also noted that the allergic reaction remained positive after the complement-fixing antibodies had become immeasurable
but he could not determine how long it took for his allergic test to become positive after infection. (Baludieri, 1953a). Omul determined that in man a positive skin test developed within three to eight days of clinical infection and persisted for at least four or five years, giving a reliable method for retrospective epidemiological studies. (Ouml and Uysalefe, 1954).

In 1962 Lackman described the production of a purified vaccine in six day old embryonated eggs. 0.02 complement-fixing units were used as an intradermal antigen and the test was read after 48 hours; induration of more than four mm. was considered to be positive because of a high correlation with positive radio-iodine precipitin tests. (Lackman et al., 1967). There were very few local reactions to the dose used and the induration was absorbed gradually over the following weeks. (Lackman et al., 1962). This antigen has been used as a test of sensitivity to C. burnetii in persons due to be vaccinated against "Q" fever and has reduced the incidence of local reactions to vaccine doses of the same antigen from 45 per cent, in a series of 94 persons to zero in a further 250 vaccinees. (Lackman et al., 1967).

Luoto vaccinated skin test negative volunteers and considered the development of a positive skin test was a reproducible, accurate and sensitive criterion of exposure. Initially he considered any induration to be a positive result and graded them according to the amount of induration from Grade One (1 X 2mm.) to Grade Four (greater than 25mm.) (Luoto et al., 1963). Bell found that a very
small number of people who were known to have been vaccinated had negative skin tests after 48 hours, but developed measurable induration after seven days. He, therefore, reads the tests twice. (Bell et al., 1964).

Gelpi (1966) has used the same antigen, giving 0.01 complement-fixing units intradermally in an epidemiological survey in Saudi Arabia. He used it on Americans resident in Arabia as well as on the local Saudi population and found the latter to have a higher incidence of positive reactions than the long term American "oil" area residents.

The main advantages in using an intradermal allergic test in epidemiological surveys has been its specificity and known persistence after the disappearance of complement-fixing antibodies. It is, however, an expensive antigen, is difficult to read without experience and requires a second visit at 48 hours. (Kaplan and Bertagna, 1955). Maurin (1953) noted that in skin test negative persons complement-fixing antibodies have occasionally appeared following the test and Onul and Usalefe (1954) reported occasional mild systemic reactions.

Luoto compared the results of six diagnostic tests in an epidemiological survey of people living and working in infected Montana dairy premises. None had had clinical "Q" fever but 72.5 per cent. had a positive radio-iodine precipitation test to C. burnetii compared with only 3.5 per cent. with complement-fixing antibodies. The capillary agglutination test (14.7 per cent.), skin test (35 per cent.), direct agglutination test (52 per cent.) and mouse neutralisation test
(54.5 per cent.) all fell between these two extremes. This survey demonstrated the relative value of many of the preceding tests in epidemiological work, where minimal sensitising doses may have occurred. (Luoto et al., 1965). However, despite its relative insensitivity in the above survey, the complement-fixation test will continue to be universally available and of value in the diagnosis of clinical "Q" fever infections.
AIM OF THE PRESENT INVESTIGATION.
During a survey of the aetiology of respiratory diseases in children throughout the winter of 1965 and 1966, it was found that 15 per cent. of 208 young children in the Edinburgh area had complement-fixing antibodies to *C. burnetii*.

The purpose of the following study was to confirm this finding over a longer period of time, during which a total of 433 children were studied, and to establish that the complement-fixing antibodies were specific. The study also compares the incidence in children at the time of the study with adults at the same time and children in previous surveys in the same area and investigates possible sources of the infecting *C. burnetii*. 
MATERIALS AND METHODS.
Patients, families and animals.

The children in the study were all in-patients in a general medical ward at the Royal Hospital for Sick Children, Edinburgh, between 1st November 1965 and 31st March 1967. Their ages ranged from three days to 13 years but most were under five years of age.

Adult patients were in-patients in the Respiratory and Infectious Diseases Units of the City Hospital, Edinburgh.

A group of 25 children in a residential nursery was also examined.

The families studied were those of children found to have complement-fixing antibodies to C. burnetii.

The cows used in the epidemiological studies were all dry dairy cows being slaughtered at the Edinburgh abattoir. The sheep were also being slaughtered at the Edinburgh abattoir. They and the cows had come from all parts of the Borders and Lothians of Scotland.

Specimens.

Specimens of venous blood were taken from all the patients, the cows and the sheep. Where possible, paired samples were taken from the patients but only a single specimen was available from many of the children. Human sera were inactivated at 56°C for 30 minutes before being stored at -20°C. Inactivation at 60°C for 30 minutes was used for the animal sera. Complement-fixation tests
were done on paired sera or on a single specimen if the second specimen had not been available.

**Complement-fixation Test**

Complement-fixation tests on all the sera were based on the method described by Bradstreet and Taylor (1962).

**Antigen.** *C. burnetii* and adenovirus antigens used in all the tests were kindly supplied by Dr. C.M. Patricia Bradstreet of the Standards Laboratory, Colindale. "Phase 2* *C. burnetii* antigen was prepared in embryonated yolk-sacs from the "Nine Mile" strain. Antigen from the "Henzerling" strain was used in parallel with the "Nine-Mile" strain for testing the cow's and sheep's sera. Uninoculated yolk-sac antigen and "phase 1* *C. burnetii* antigen were included as controls in all the tests. Adenovirus antigen was prepared in HeLa cells. All the antigens were used at the concentration recommended by the Standards Laboratory.

**Sera.** The stored sera were diluted to 1:16 before being reinactivated at 56°C for a further 30 minutes. Positive and negative control sera were included in the tests.

**Complement.** Preserved guinea-pig complement, purchased from Burroughs-Wellcome and Co., was used for the tests.

**Haemolytic System.** A four per cent suspension of sheep erythrocytes was sensitised by the addition of an equal volume of horse haemolytic antiserum, and incubated at 37°C for 10 minutes
Procedure of the test. Overnight fixation of complement at 4°C. was used throughout the tests. Serum, antigen and cell controls were included and dilutions of sera giving 50 per cent. fixation of complement were considered positive.

Modifications to the test. A dilution of complement giving 2½ M.H.D. 50 was used throughout the tests. Positive children's sera were also tested after inactivation of a 1:16 dilution at 60°C. for 30 minutes. Cow and sheep sera were always inactivated at 60°C.

The radio-iodine precipitation test.

Dr. Leo Thomas of the Rocky Mountain Laboratory, Montana, U.S.A., kindly agreed to test 44 of the children's sera for antibodies to C. burnetii by the radio-iodine precipitation test.

The intradermal sensitivity test.

Patients and their families. This test was done on all the children with complement-fixing antibodies to C. burnetii who could be followed-up at home, and on as many of the other members of their families as was feasible. It was also used, in parallel with the complement-fixation test, on a group of 58 child and adult patients in hospital.

Antigen. The antigen was kindly supplied by Dr. Robert Philip at the Rocky Mountain Laboratory, Montana. Its preparation
has been described as a means of preparing "Q" fever vaccine by Lackman, Bell, Bell and Pickens in 1962. Yolk-sacs of six day old embryonated eggs were inoculated with a dose of C. burnetii which produced death of 50 per cent. of the embryos within seven to nine days. The remaining yolk-sacs were harvested, emulsified in N/1 sodium chloride and inactivated with 0.3 per cent. formalin and 0.75 per cent. phenol. After filtration and centrifugation, an antigen suspension in 0.1 per cent. formalin was obtained and the volume adjusted so that 20 grams of yolk-sac material were contained in 100 ml. Concentrated vaccine was finally obtained after overnight extraction with diethyl ether at 6°C. and used as a complement-fixing antigen with guinea-pig serum containing "phase 1" antibodies. On the basis of several of these tests the vaccine was diluted to contain ten complement-fixing units per ml. Before use, it was checked for total nitrogen and free formaldehyde content and for sterility and safety. For use as an intradermal antigen it was further diluted to contain 0.01 complement-fixing units in 0.1 ml.

Procedure for skin testing. After obtaining permission from the appropriate general practitioner by means of a letter (forms 1 and 2) and sending a warning letter (form 3) to the mother about the visit, the children with C. burnetii antibodies were visited at their homes. The skin test was carried out on all members of the family who were present.

The skin was cleansed and 0.1 ml. of the antigen inoculated intradermally on the volar surface of the left forearm. The tests
were read after 48 hours. The maximum diameter of the induration was measured and those over four millimetres were considered positive. Erythema alone was not considered positive.

**Epidemiological Tests.**

"Matched controls". In order to investigate the environments of the children with complement-fixing antibodies to *C. burnetii*, each child with antibodies was "matched" with another child in the survey who did not have these antibodies. The following criteria were used for the selection of controls in the order in which they are given:— a) age; b) month of admission to hospital; c) diagnosis.

**Environmental History.** During the visit when the skin test was done of the patients with antibodies, the environmental history was obtained from the child's mother. The information was used to complete form 4 and covered five main topics:— a) medical history; b) milk consumption history; c) direct and indirect animal contact; d) residence in relationship to animals and e) immunisation history.

In order to complete form 4 for the "matched controls", a questionnaire was devised and sent to the mothers of these children (form 5 and 6).

**Isolation of *C. burnetii*.**

No attempt was made to isolate *C. burnetii* from patients, sheep, cows or milk samples because facilities were inadequate for safe work.
FORM 1

Letter sent to General Practitioners.

Wellcome Laboratory,
City Hospital,
Greenbank Drive,
EDINBURGH 10.

Over the last two years a number of patients in the Royal
Hospital for Sick Children have been found to have antibodies to Q
fever. I am anxious to relate the significance of these antibodies
to each child's illness, subsequent progress and environment.

I would be most grateful for permission to follow-up your
patient ........................................

If you are agreeable, I shall then contact the child's parents
to ask when it would be convenient for me to visit the home to:-

   a) ask a few questions - e.g. about milk supplies
   b) do an intradermal skin test on as many of the family as
      possible.

(This is much less frightening than obtaining further samples
of blood).

I am sorry to add to your mail bag in this way but would be
most grateful for your reply on the enclosed slip of paper.

Yours sincerely,

Dr. Helen Zealley.
FORM 2.

I give my permission for Dr. Helen Zealley
do not give to visit my patient at home.

Signed

FORM 3

THE LABORATORY
CITY HOSPITAL
GREENBANK DRIVE
EDINBURGH 10

Letter sent to the patient's mothers.

I am following the progress of a group of children, including

, who have been in the Royal Hospital for Sick Children with different illnesses.

It is very important that I meet as many of the family as possible, so please try and be at home on

This work is very important and I am grateful for your help.

Yours sincerely,

Dr. Helen Zealley.
# Environmental History Form

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<table>
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<tr>
<th>URTI</th>
<th>7 Dog</th>
<th>8 Chicken</th>
<th>F/U</th>
<th>8 Chicken</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>M/En</th>
<th>9 Budgie</th>
<th>10 Pidgeon</th>
<th>Pre H</th>
<th>10 Pidgeon</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Conv</th>
<th>11 Nice Rab.</th>
<th>12 Raw</th>
<th>Milk F/U</th>
<th>12 Raw</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Pug</th>
<th>Sup.</th>
<th>10 Pidgeon</th>
<th>Milk Past</th>
<th>10 Pidgeon</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Oth</th>
<th>10 Pidgeon</th>
<th>10 Pidgeon</th>
<th>Occr</th>
<th>10 Pidgeon</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>11 Nice Rab.</th>
<th>11 Nice Rab.</th>
<th>M</th>
<th>F</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Arm @ 2/d</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Occup</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>
You will find with this letter a very unusual set of questions. They have been sent to you as part of an important study of a group of children who have been in the Royal Hospital for Sick Children in the past two years.

The name of your child ___________________________ was "pulled out of a hat" so there is no need for you to worry.

I will be most grateful if you would answer them carefully and return them to me as soon as possible.

I am very grateful for your help and hope you enjoy giving the answers.

Yours sincerely,

Dr. Helen Zealley.
FORM 6.

Questionnaire to the mothers of "matched controls".

1.

1. Please write the name, age, occupation and relationship to ............... of everyone who lives in your house.

* For the rest of the answers each person can be referred to by the number beside their name.

<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>Occupation</th>
<th>Relationship to ..........</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Have you lived anywhere else in the past 2 years? Yes/No.

(Do not include holidays). If Yes please state where and when.

........................................................................................................
........................................................................................................

3. Do you have any friends or relatives who are butchers. Yes/No.

If Yes please give details ...........................................................................
........................................................................................................

4. Where do you buy your family's milk? ...........................................
5. Is it pasteurised? Yes/No.

6. Please read this list of illnesses. Put beside each one the number (see question 1) of anybody who suffers or has suffered from them.

<table>
<thead>
<tr>
<th>Illness</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pneumonia</td>
<td></td>
</tr>
<tr>
<td>bronchitis</td>
<td></td>
</tr>
<tr>
<td>more &quot;colds&quot; than average</td>
<td></td>
</tr>
<tr>
<td>sore throats or ears</td>
<td></td>
</tr>
<tr>
<td>flu lasting more than 2 days</td>
<td></td>
</tr>
<tr>
<td>fever lasting more than 2 days</td>
<td></td>
</tr>
<tr>
<td>meningitis</td>
<td></td>
</tr>
<tr>
<td>inflammation of the brain</td>
<td></td>
</tr>
<tr>
<td>fits</td>
<td></td>
</tr>
<tr>
<td>jaundice</td>
<td></td>
</tr>
<tr>
<td>blood transfusion</td>
<td></td>
</tr>
<tr>
<td>other illness</td>
<td></td>
</tr>
</tbody>
</table>

7. Please read this list of animals, things, and places. Put beside each one the number (see question 1) of anybody who has handled, or visited them in the last 5 years.

<table>
<thead>
<tr>
<th>Animal/Place</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
</tr>
<tr>
<td>Chickens</td>
<td></td>
</tr>
<tr>
<td>Fidgeons</td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
</tr>
<tr>
<td>Budgies</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td></td>
</tr>
<tr>
<td>Rabbits</td>
<td></td>
</tr>
<tr>
<td>A farm</td>
<td></td>
</tr>
<tr>
<td>Slaughter house</td>
<td></td>
</tr>
<tr>
<td>Animal fertilisers e.g. bone meal, manure</td>
<td></td>
</tr>
</tbody>
</table>
8. Please tick the appropriate space to show what kind of milk has been drinking at different times in his / her life.

<table>
<thead>
<tr>
<th></th>
<th>As a baby</th>
<th>Before going to hospital in</th>
<th>Now</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostermilk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>National dried</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow &amp; Gate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows milk-boiled</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows milk-pasteurised</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows milk-raw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast milk</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. What age did he / she start drinking milk as it comes out of the bottle from the dairy? ........................

10. Is he / she a good, bad or average milk drinker? ...........

11. Please give the approximate age when was vaccinated against smallpox ........................

Thank you for your help.
RESULTS.
I. THE INCIDENCE OF COMPLEMENT-FIXING ANTIBODIES TO C. BURNETII IN EDINBURGH CHILDREN.

During the time of the study, from 1st November 1965 to 31st March 1967, blood specimens were obtained from 433 children aged between three days and 13 years in a general medical ward at the Royal Hospital for Sick Children, Edinburgh. Paired venous samples were taken, nine or more days apart, from 251 children and single specimens from 182 children who were discharged or died before a second specimen could be taken.

All the sera were tested for complement-fixing antibodies to "phase 2" C. burnetii antigen and Table 1 shows that 66 (15.3 per cent.) of the 433 children possessed these antibodies at titres of 1:16 or more after inactivation of their sera at 56°C for 30 minutes.

Table 1. The incidence of complement-fixing antibodies to C. burnetii at titres of 1:16 or more in Edinburgh children.

<table>
<thead>
<tr>
<th>Sera tested</th>
<th>Complement-fixing antibodies to C. burnetii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>251 paired</td>
<td>37</td>
</tr>
<tr>
<td>182 single</td>
<td>29</td>
</tr>
<tr>
<td>433 Total</td>
<td>66</td>
</tr>
</tbody>
</table>

Most of the children with antibodies are shown in Table 2 to have had low stable or single titres of 1:16 or 1:32 but there were nine who were found to have titres of 1:64 or more and two who
had a fourfold or greater rise in antibody titre. These last 11 children were all considered to have serological evidence of recent infection with \textit{C. burnetii}.

Table 2. Titres of complement-fixing antibodies to \textit{C. burnetii} found in 66 children.

<table>
<thead>
<tr>
<th>Reciprocal of antibody titre</th>
<th>X4 rise</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 32 64 128</td>
<td></td>
</tr>
<tr>
<td>stable titre</td>
<td>22 11 1 1</td>
</tr>
<tr>
<td>single titre</td>
<td>17 5 4 3</td>
</tr>
<tr>
<td>X4 rise</td>
<td>- - - 2</td>
</tr>
<tr>
<td>Total</td>
<td>39 16 5 4</td>
</tr>
</tbody>
</table>

The relationship of \textit{C. burnetii} antibodies to clinical diagnosis requiring hospital admission.

An attempt was made to relate the finding of \textit{C. burnetii} antibodies to the clinical diagnosis. Table 3 shows that there was no relationship to the following clinical groups:

1. Lower respiratory tract infections - pneumonia, bronchiolitis and bronchitis.
2. Upper respiratory tract infections - coryza, tonsillitis,
otitis media and conjunctivitis.
3. Encephalitis, aseptic meningitis or febrile convulsions.
4. Gastrointestinal disorders - including infections.
5. Bacterial infections - urinary infections, meningitis and septicaemia.
6. Pyrexia of unknown origin.
7. Cardiac disorders - including congenital heart disease.
8. Jaundice.
10. Other - poisoning, epilepsy, exanthemata, etc.

Table 3. The clinical diagnosis for admission to hospital in children with complement-fixing antibodies to C. burnetii.

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>Number tested</th>
<th>With C. burnetii antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>---------------</td>
<td>--------</td>
</tr>
<tr>
<td>1. L.R.T.I.</td>
<td>125</td>
<td>20</td>
</tr>
<tr>
<td>2. U.R.T.I.</td>
<td>63</td>
<td>9</td>
</tr>
<tr>
<td>3. Enceph./asep./men./feb./conv.</td>
<td>104</td>
<td>16</td>
</tr>
<tr>
<td>4. GI disorders</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>5. Bacterial infections</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>6. P.U.O.</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>7. Cardiac disorders</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>8. Jaundice</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>9. Purpura</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>10. Other</td>
<td>52</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>433</strong></td>
<td><strong>66</strong></td>
</tr>
</tbody>
</table>
Of the 11 children with rising or significantly high antibody titres suggesting recent infection with *C. burnetii*, five (45 per cent.) had been admitted to hospital because of encephalitis, aseptic meningitis or febrile convulsions. This seemed to be a greater proportion with central nervous system involvement than the 24 per cent. who came into this diagnostic category in the total group of 433 children although the numbers were too small for statistical analysis. Of these five children, two had rises in antibody titre: the first, whose antibody titre rose from less than 1:16 to 1:32, was a girl of 18 months with aseptic meningitis who recovered completely; but the other, a nine month old girl whose titre rose from less than 1:16 to 1:256, died after an encephalitis that lasted three months. In this second child the diagnosis was confused by the demonstration of a transient rise of complement-fixing antibodies to mumps V antigen and the isolation of an unidentified enterovirus from her stool. This girl's only full sibling had also died at the same age, following a similar encephalitic illness that was accompanied by rises of complement-fixing antibodies to the herpes simplex and mumps viruses; because four step-siblings remained well some genetic abnormality of response to infection was postulated. Unfortunately no post-mortem material was available for virological investigation but a pathology report confirmed the presence of encephalitis.

One of the other three children with central nervous involvement also had a maculo-papular rash and of the remaining six children with significant antibody levels three had pneumonia or bronchitis,
one had a persistent and unexplained pyrexia, one had myocarditis and one had an upper respiratory tract infection.

The relationship of *C. burnetii* antibodies to past clinical history.

Information relating to the past clinical history of the children with *C. burnetii* antibodies was compared with similar data from a group of selected "matched controls" that did not have these antibodies. The medical histories were examined under four categories which have all been reported as manifestations of clinical "Q" fever. Firstly, those who had had lower respiratory tract infections; then a group containing those who had had meningitis, encephalitis or any form of convulsions, followed by a group who had had jaundice and finally those who had had undiagnosed fevers or clinical "influenza" lasting two or more days.

Table 4. The relationship between the presence of complement-fixing antibodies to *C. burnetii* and past clinical history.

<table>
<thead>
<tr>
<th></th>
<th>With <em>C. burnetii</em> antibodies</th>
<th>&quot;matched controls&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>per cent.</td>
</tr>
<tr>
<td>Total number studied</td>
<td>53</td>
<td>100</td>
</tr>
<tr>
<td>L.R.T.I.</td>
<td>27</td>
<td>51</td>
</tr>
<tr>
<td>Men./Enceph./Convul.</td>
<td>18</td>
<td>34</td>
</tr>
<tr>
<td>Jaundice</td>
<td>7</td>
<td>13.2</td>
</tr>
<tr>
<td>P.U.O. or &quot;flu&quot;</td>
<td>6</td>
<td>11.3</td>
</tr>
</tbody>
</table>
Table 4 shows that no difference could be demonstrated between the children with complement-fixing antibodies to C. burnetii and their "matched controls" in the incidence of lower respiratory tract disease, central nervous system disorders or fevers and "influenza". Of the children with antibodies to C. burnetii seven had a history of jaundice but none of the "matched controls" did so; however, five of these seven had had a mild neonatal jaundice, leaving only two who had had a clinical illness accompanied by jaundice. These numbers were too small to be considered statistically.

The relationship of C. burnetii antibodies to age on admission to hospital.

Complement-fixing antibodies to C. burnetii were found in children of all ages but Table 5 shows that there were far fewer with antibodies in the group aged less than six months.

Table 5 and Figure 1 compare the incidence of C. burnetii antibodies to that of complement-fixing antibodies to the adenovirus group in the children under study. The proportion of children with adenovirus antibodies fell in the second three months of life, due perhaps to the loss of maternal antibodies and preceded a steady increase to the maximum proportion in children aged four years and over, as they acquired their own antibodies in response to natural infection.
Table 5. The percentage of children with complement-fixing antibodies to C. burnetii and the adenovirus group at different ages.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Total number tested</th>
<th>Complement-fixing antibodies to:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. burnetii</td>
<td>adenovirus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>number</td>
<td>per cent.</td>
<td>number</td>
</tr>
<tr>
<td>&lt; 3/12</td>
<td>49</td>
<td>2</td>
<td>4.1</td>
<td>27</td>
</tr>
<tr>
<td>3-5/12</td>
<td>49</td>
<td>3</td>
<td>5.1</td>
<td>9</td>
</tr>
<tr>
<td>6-11/12</td>
<td>59</td>
<td>9</td>
<td>15.3</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>75</td>
<td>15</td>
<td>20.0</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>13</td>
<td>19.7</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>8</td>
<td>19.5</td>
<td>29</td>
</tr>
<tr>
<td>4 and over</td>
<td>94</td>
<td>16</td>
<td>17.0</td>
<td>85</td>
</tr>
<tr>
<td>Total</td>
<td>433</td>
<td>66</td>
<td>15.3</td>
<td>257</td>
</tr>
</tbody>
</table>

Figure 1. Percentage of children in each age group with complement-fixing antibodies to C. burnetii and adenovirus.
This pattern contrasted markedly with that of *C. burnetii* antibodies. No fall occurred in the first few months due to the loss of maternal antibodies. A sudden increase in the proportion of children with these antibodies was found after the age of six months and reached a maximum level in those aged one year. This was followed by stable proportions until a slight falling off occurred in those aged four years and over.

The relationship of *C. burnetii* antibodies to the date of admission to hospital.

Specimens of blood were obtained during all the months of the survey. The numbers taken in each month are shown in Figure 2. Because of the small and fluctuating numbers in many of the months the number of children with antibodies to *C. burnetii* were analysed in six monthly groupings as shown in Table 6.

No difference in the proportion with antibodies could be related to the time of year in the children with low / stable titres to *C. burnetii*, but of those with rising or high titres a greater number were admitted to hospital during the winter months. The numbers were too small to be considered statistically.
Figure 2. The number of children's sera tested for *C. burnetii* antibodies during each month of the survey.

Table 6. Seasonal incidence of complement-fixing antibodies to *C. burnetii*.

<table>
<thead>
<tr>
<th>Time of year</th>
<th>Total number tested</th>
<th>Complement-fixing antibodies to <em>C. burnetii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>low / stable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>number</td>
</tr>
<tr>
<td>Nov 65-Mar 66</td>
<td>182</td>
<td>26</td>
</tr>
<tr>
<td>Ap 66-Sept 66</td>
<td>111</td>
<td>13</td>
</tr>
<tr>
<td>Oct 66-Mar 67</td>
<td>140</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>433</td>
<td>55</td>
</tr>
</tbody>
</table>
II. DETERMINATION OF THE SPECIFICITY OF COMPLEMENT-FIXING
ANTIBODIES TO C. BURNETII AT DILUTIONS OF 1:16 OR GREATER.

The sera containing complement-fixing antibodies to C. burnetii were subjected to further tests to determine the specificity of these antibodies.

Reaction of sera containing C. burnetii antibodies with "phase 1" C. burnetii antigen and with normal yolk-sac antigen.

The 66 sera containing complement-fixing antibodies to C. burnetii were all tested with an antigen prepared from uninfected yolk-sacs and with C. burnetii antigen in "phase 1". None of the sera fixed complement in the presence of these antigens.

Reaction of sera containing C. burnetii antibodies after the removal of heat labile factors.

Most of the sera containing complement-fixing antibodies to C. burnetii were checked after heat inactivation at 60°C, for 30 minutes. Sera from four children could not be tested because there was insufficient serum available and one because it had become anticomplementary on storage at -20°C.
Table 7. Complement-fixing antibody titres to *C. burnetii* after inactivation at 60°C, for 30 minutes in sera with antibody titres of 1:16 or more after inactivation at 56°C, for 30 minutes.

<table>
<thead>
<tr>
<th>Complement-fixing antibody titre after inactivation at 56°C.</th>
<th>Total number tested</th>
<th>Complement-fixing antibody titre to <em>C. burnetii</em> after inactivation at 60°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:16</td>
<td>34</td>
<td>&lt;1:8 1:8 1:16 1:32 1:64 1:128 1:256</td>
</tr>
<tr>
<td>1:32</td>
<td>17</td>
<td>- 1 4 12 - - -</td>
</tr>
<tr>
<td>1:64</td>
<td>5</td>
<td>- - 2 2 1 - -</td>
</tr>
<tr>
<td>1:128</td>
<td>4</td>
<td>- - - - - - 4 -</td>
</tr>
<tr>
<td>1:256</td>
<td>1</td>
<td>- - - - - - 1</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>6 8 27 14 1 4 1</td>
</tr>
</tbody>
</table>

Table 7 shows that 39 (65 per cent.) of the 61 sera tested after inactivation at 60°C, for 30 minutes maintained the same complement-fixing antibody titre to *C. burnetii* while 13 (21.3 per cent.) had a slight decrease (2-fold) in titre. Because heat inactivation is known to destroy a small proportion of the antibodies present, sera with complement-fixing antibodies to *C. burnetii* at a dilution of 1:8 were considered to be positive after inactivation at 60°C; antibodies at dilutions smaller than this could not be considered significant because of the presence of non-specific complement-fixing factors at these levels. Using titres of 1:8 or more as significant, it was found that only nine (14.8 per cent.) of the sera had complement-fixing antibodies to *C. burnetii* which could
be considered as heat labile because their concentration fell by fourfold or more after inactivation at 60°C for 30 minutes.

The relationship between the presence of complement-fixing antibodies to C. burnetii and antibodies to C. burnetii measured by the radio-iodine precipitation test.

The results of the radio-iodine precipitation test on 44 of the children's sera are not yet available.

The relationship of C. burnetii antibodies to the presence of adenovirus group complement-fixing antibodies.

All sera were tested for complement-fixing antibodies to the adenovirus group antigen. It was found that a significantly greater proportion of the children with antibodies to C. burnetii had complement-fixing antibodies to the adenovirus group when compared with those who did not have C. burnetii antibodies. Table 8 shows that this difference only occurred between the ages of three months and four years.

Table 8. The relationship between complement-fixing antibodies to C. burnetii and the adenovirus group at different ages.

<table>
<thead>
<tr>
<th>Age</th>
<th>With C. burnetii antibodies</th>
<th>Without C. burnetii antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total tested</td>
<td>With adenovirus antibodies</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>per cent.</td>
</tr>
<tr>
<td>&lt;3/12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3-5/12</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>6-11/12</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>4 and more</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>47</td>
</tr>
</tbody>
</table>

$\chi^2$ (1 degree of freedom) = 4.63; 0.05 > P > 0.01.
III. EXAMINATION OF THE CHILDREN WITH COMPLEMENT-FIXING ANTIBODIES TO C. BURNETII FOR OTHER ANTIBODIES TO THE SAME ORGANISM.

As a further means of confirming the specificity of the complement-fixing antibodies to C. burnetii another immunological test was carried out.

Relationship of complement-fixing antibodies to C. burnetii and antibodies measured by an intradermal sensitivity test.

a) In a group of hospital patients of all ages.

A comparison between complement-fixing antibodies to C. burnetii and positive intradermal sensitivity tests to C. burnetii antigen was made by performing these tests in parallel on a small group of hospital patients.

A group of 58 patients, with ages ranging from 1 year to 64 years, was investigated. Of these, 18 (31 per cent) were found to have induration at the inoculation site after 48 hours but only seven of these 18 (12.1 per cent.) had induration of more than four millimetres. Complement-fixing antibodies to C. burnetii at dilutions of 1:16 or more were also found in seven (12.1 per cent.) patients in the group although they did not all have positive skin tests. Because of the small numbers the children have not been considered as a special group.

No local or systemic reactions to the intradermal sensitivity tests were reported by these hospital patients.

b) In children known to have complement-fixing antibodies to C. burnetii.

The intradermal sensitivity test was used on 53 of the 66
children with complement-fixing antibodies to *C. burnetii*. The time interval between the initial complement-fixation test and the subsequent intradermal sensitivity test varied from seven to 23 months. Of the 13 children who were not tested, two had died, five had "moved house" with no notification of their new addresses and two had been adopted into new families.

Of the 53 children tested, 32 (60 per cent.) had induration after 48 hours of more than four millimetres at the antigen inoculation site and were therefore considered to have a positive intradermal sensitivity test for *C. burnetii*. This was a significantly higher proportion than had been found in the group of hospital patients.

\[ x^2_c (1 \text{ degree of freedom}) = 28.37; \, P < 0.001. \]

An attempt was made to relate the presence of a positive skin test to the following two factors:-- a) the heat stability of the complement-fixing antibodies to *C. burnetii* and b) the presence of complement-fixing antibodies to the adenovirus group.

Table 9 shows that the presence of a positive skin test for *C. burnetii* did not appear to be related to the heat stability of the complement-fixing antibodies although the numbers were too small for statistical analysis.

### Table 9. The relationship of positive skin tests in children with *C. burnetii* complement-fixing antibodies to the heat stability of their complement-fixing antibodies.

<table>
<thead>
<tr>
<th>Complement-fixing antibodies to <em>C. burnetii</em></th>
<th>total number tested</th>
<th><em>C. burnetii</em> skin test after 48 hours=5mm, or more</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>number</td>
</tr>
<tr>
<td>heat stable</td>
<td>43</td>
<td>34</td>
</tr>
<tr>
<td>heat labile</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>total</td>
<td>49</td>
<td>38</td>
</tr>
</tbody>
</table>
Statistical proof that the presence of a positive skin test for \textit{C. burnetii} was unrelated to the presence of complement-fixing adenovirus antibodies is shown in Table 10.

\textbf{Table 10.} The relationship of positive skin tests in children with complement-fixing antibodies to \textit{C. burnetii} to the presence of complement-fixing adenovirus antibodies.

<table>
<thead>
<tr>
<th>Complement-fixing antibodies to Adenovirus</th>
<th>Total number tested</th>
<th>\textit{C. burnetii} skin test after 48 hours=5mm, or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>present</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>absent</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>42</td>
</tr>
</tbody>
</table>

\(X^2\) (1 degree of freedom) = 1.25; \(P\) not significant at 5 per cent.

\textbf{IV. DETERMINATION OF THE SIGNIFICANCE IN CHILDREN OF COMPLEMENT-FIXING ANTIBODIES TO \textit{C. burnetii} AT THE TIME OF THE STUDY.}

A retrospective analysis of the laboratory records was used to demonstrate the number of patients in the years 1960 - 1967 with complement-fixing antibodies to \textit{C. burnetii} in the Respiratory and Infectious Diseases Units of the City Hospital, Edinburgh.


Sera had been routinely tested for complement-fixing antibodies to \textit{C. burnetii} since 1960. The number of patients aged 15 years and over whose sera were tested with this antigen had risen from 37 in 1960 to 551 in 1967 and the number of patients found to
have antibodies to *C. burnetii* had also risen from zero (0 per cent.) in 1960 to 33 (6 per cent.) in 1967.

Although the proportion of patients with these antibodies fluctuated from year to year, Table 11 shows that there has been a significant increase from 0.8 per cent. of 653 patients between 1960 and 1963 to 5.0 per cent. of 1,400 patients in the years 1964 to 1967.

\[ X^2 \text{ (1 degree of freedom)} = 22.77; \quad P < 0.001. \]

Table 11. The incidence of complement-fixing antibodies to *C. burnetii* in patients aged 15 and over in the years 1960-1967.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number tested</th>
<th>with complement-fixing <em>C. burnetii</em> antibodies</th>
<th>number</th>
<th>per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>37)</td>
<td></td>
<td>0)</td>
<td>0.0</td>
</tr>
<tr>
<td>1961</td>
<td>162) 653</td>
<td></td>
<td>2) 5</td>
<td>1.2 0.8</td>
</tr>
<tr>
<td>1962</td>
<td>253)</td>
<td></td>
<td>2) 1</td>
<td>0.8 0.5</td>
</tr>
<tr>
<td>1963</td>
<td>201)</td>
<td></td>
<td>12)</td>
<td>4.9 5.0</td>
</tr>
<tr>
<td>1964</td>
<td>244) 1400</td>
<td></td>
<td>7) 70</td>
<td>2.6 5.0</td>
</tr>
<tr>
<td>1965</td>
<td>269)</td>
<td></td>
<td>18)</td>
<td>5.4 6.0</td>
</tr>
<tr>
<td>1966</td>
<td>336)</td>
<td></td>
<td>33)</td>
<td>6.0</td>
</tr>
<tr>
<td>1967</td>
<td>351)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2053</td>
<td></td>
<td>75</td>
<td>3.7</td>
</tr>
</tbody>
</table>

During the three year period, 1965 - 1967, which covered the time when the children's sera were examined, 58 (4.8 per cent.) sera from 1158 adult patients were found to have *C. burnetii* antibodies. The overall incidence of 15.3 per cent. in the children under study showed a highly significant disproportion between the presence of
complement-fixing antibodies to C. burnetii in Edinburgh children during the time of the study when compared with adults at the same time and from the same area.

\[ X^2 (1 \text{ degree of freedom}) = 45.75; \quad P < 0.001. \]


The number of children's sera tested routinely in the laboratory before 1965 was small. However, two previous surveys of childhood respiratory infections in Edinburgh in 1961 and 1962 (Urquhart et al., 1965) allowed a comparison to be made between the incidence of complement-fixing antibodies to C. burnetii in children before and during the time of the present study.

Table 12 shows that only 1 (0.6 per cent.) of 161 children's sera tested between 1961 and 1964 contained these antibodies; a highly significant change had therefore occurred to account for 15.3 per cent. with antibodies in the sera from children during the present study.

\[ X^2 (1 \text{ degree of freedom}) = 25.6; \quad P < 0.001. \]

Table 12. The incidence of complement-fixing antibodies to C. burnetii in children before 1965.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number tested</th>
<th>With complement-fixing C. burnetii antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>number</td>
</tr>
<tr>
<td>1961</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>1962</td>
<td>97</td>
<td>1</td>
</tr>
<tr>
<td>1963</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>1964</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>161</td>
<td>1</td>
</tr>
</tbody>
</table>
V. STUDIES TO DETERMINE THE SOURCE OF INFECTION WITH C. BURNETII IN THE CHILDREN WITH COMPLEMENT-FIXING ANTIBODIES TO THIS ORGANISM

a) Family studies.

i) The incidence of positive intradermal sensitivity tests in the families of children with C. burnetii antibodies.

During the follow-up visit to perform the intradermal sensitivity test on 53 of the children with complement-fixing antibodies to C. burnetii, the opportunity was taken to use this test on as many other members of the family as possible, in order to assess their previous experience with C. burnetii. It was found that 62 (34 per cent.) of 183 other family members had positive skin tests of more than four millimetres induration after 48 hours. This was a significantly greater proportion than that found in the mixed hospital patients (12.1 per cent.), and suggested that these families had had greater previous experience with C. burnetii than the general population.

\[ X^2_c (1 \text{ degree of freedom}) = 10.52; \quad 0.005 > P > 0.001. \]

Mild complaints of sickness, fever or general malaise in their children were attributed to the intradermal skin test by four of the mothers; all but one of these complaints were in families where one or more members were mildly unwell when the test was performed. One mother, with a positive result, developed a purpuric rash (3 centimetres X 4 centimetres) the day after inoculation, about five centimetres proximal to the positive reaction. This rash faded
over the following two days as the skin test induration subsided and became less obvious.

Photograph to show purpuric reaction to intradermal test.

[Image of a skin test result showing purpuric reaction]

ii) The incidence of complement-fixing antibodies to *C. burnetii* in children living in a residential nursery that had been the "home" of a child found to have *C. burnetii* antibodies.

One child with complement-fixing antibodies to *C. burnetii* had been living in a children's residential nursery before her admission to hospital. She had subsequently been adopted and could not be skin tested because the family had moved to another part of the country. Sera were therefore collected from all 25 of the children remaining in the nursery and five (20 per cent.) of these were found to have complement-fixing antibodies to *C. burnetii* and,
if the ten aged less than six months were excluded, it was found that 33.3 per cent. of the others had these antibodies. Although the numbers were too small to be considered statistically it again suggested that this child's "family" had had more experience with the organism than would be expected in a general population of the same age.

b) Environmental Studies.

During the follow-up visits to skin test the families of 53 children with complement-fixing C. burnetii antibodies, information was obtained from the parents about the child and family's environment before hospitalisation of the index child. This was compared with the answers to similar questions received by post (on form 6) from the parents of 49 of the 66 "matched controls" who had been contacted. Of the "matched controls" whose parents did not reply, seven had "moved house" with no forwarding addresses and the other ten failed to return the questionnaire.

i) Direct contact with animals or their products.

From the results of the questions it was possible to assess how many of the children with C. burnetii antibodies and how many of their "matched controls" had had direct contact with animals or their products.
Table 13. The relationship between the presence of C. burnetii antibodies and direct or indirect animal contact.

<table>
<thead>
<tr>
<th>Total number studied</th>
<th>with C. burnetii antibodies</th>
<th>&quot;matched controls&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>index</td>
<td>family</td>
</tr>
<tr>
<td>abattoir</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>farm</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>butcher</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>fertiliser or manure</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>cows and/or sheep</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>dogs</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>chickens</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>budgerigars</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>pigeons</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>mice or abbits</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

There were 23 children with antibodies to C. burnetii who gave a history of direct contact with cows and/or sheep, because they had played in fields and pastures where these animals were grazing. This was significantly more than the 12 amongst the "matched controls" who had had such contact with these animals.  

\[ X^2_c (1 \text{ degree of freedom}) = 24.09; 0.005 > P > 0.001. \]

Table 13 shows that there was no appreciable difference between the two groups in the amount of contact with any of the following animals and birds: chickens, budgerigars, pigeons, mice and rabbits. Nor was there any difference in the numbers who had been at the abattoir, on farms or who had had contact with butchers or animal products used as fertilisers.
ii) Indirect contact with animals or their products.

Indirect animal contact was presumed to have occurred when another member of the same household as the children being studied had had direct contact with animals, animal products and butchers or who had been at a farm or the abattoir although the children themselves had not had this direct contact.

Table 13 shows that there was no significant difference in this indirect animal contact between the group of children with \textit{C. burnetii} antibodies and their "matched controls".

iii) Vaccination with calf lymph against smallpox.

Children in the Edinburgh area are usually vaccinated after the first 12 months of life. Because this was also the age at which the maximum proportion of children could be demonstrated to have complement-fixing antibodies to \textit{C. burnetii} and because it has been suggested that infected calf lymph could transmit \textit{C. burnetii}, a comparison was made of the vaccination status in the two groups of children.

Table 14 shows that there was no relationship between vaccination status and the presence of \textit{C. burnetii} antibodies.

Table 14. The relationship between the presence of \textit{C. burnetii} and previous vaccination against smallpox.

<table>
<thead>
<tr>
<th></th>
<th>Total number questioned</th>
<th>Vaccinated against smallpox number</th>
<th>per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>With \textit{C. burnetii} antibodies</td>
<td>53</td>
<td>26</td>
<td>49.0</td>
</tr>
<tr>
<td>&quot;matched controls&quot;</td>
<td>49</td>
<td>29</td>
<td>59.0</td>
</tr>
</tbody>
</table>
iv) Residential contact with cows and sheep.

Sketch maps (figures 3 and 4) of Edinburgh and its surrounding area were used to show that the presence of *C. burnetii* antibodies had no relationship to residential contact with cows and sheep. The maps show the situation within the city of a park where sheep are often grazed and the abattoir with its surrounding pastures.

Of the children with antibodies to *C. burnetii* 41 (62 per cent.) lived within the city boundary as did 35 (53 per cent.) of the "matched controls". The rest of the children in both groups lived in small towns, villages and farms in the rest of South East Scotland.
Figure 3. Sketch map to show the distribution of children with C. burnetii antibodies.

Figure 4. Sketch map to show the distribution of "matched controls"
v). Milk consumption by the children under study.

The milk drinking history of the children with \textit{C. burnetii} antibodies was compared with that of their "matched controls". Most of the children in the two groups were said to be good or average milk drinkers. Even those described as poor milk drinkers usually drank half a pint of milk every day.

In both groups of children about a quarter had initially been breast fed and one fifth drank or had drunk raw milk. Two thirds of both groups had had various powdered milks whilst four fifths were receiving pasteurised milk at the time of the study. Thus, as shown in Table 15, there was no significant difference between the numbers in each group who had drunk any special type or brand of milk.

Table 15. The relationship between the presence of \textit{C. burnetii} antibodies and the type of milk consumed.

<table>
<thead>
<tr>
<th>type of milk</th>
<th>with \textit{C. burnetii} antibodies</th>
<th>&quot;matched controls&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total number studied</td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td>53</td>
<td>49</td>
</tr>
<tr>
<td>evaporated</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>powdered total</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>national dried</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Ostermilk</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Cow &amp; Gate</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>S.M.A.</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>pasteurised</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>raw</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>number</th>
<th>per cent.</th>
<th>number</th>
<th>per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>breast</td>
<td>13</td>
<td>24.5</td>
<td>11</td>
<td>22.4</td>
</tr>
<tr>
<td>evaporated</td>
<td>6</td>
<td>11.3</td>
<td>7</td>
<td>14.3</td>
</tr>
<tr>
<td>powdered total</td>
<td>32</td>
<td>60.4</td>
<td>34</td>
<td>69.5</td>
</tr>
<tr>
<td>national dried</td>
<td>14</td>
<td>26.4</td>
<td>15</td>
<td>30.6</td>
</tr>
<tr>
<td>Ostermilk</td>
<td>15</td>
<td>28.4</td>
<td>16</td>
<td>32.7</td>
</tr>
<tr>
<td>Cow &amp; Gate</td>
<td>3</td>
<td>5.7</td>
<td>4</td>
<td>8.1</td>
</tr>
<tr>
<td>S.M.A.</td>
<td>2</td>
<td>3.8</td>
<td>1</td>
<td>2.2</td>
</tr>
<tr>
<td>pasteurised</td>
<td>40</td>
<td>74.5</td>
<td>38</td>
<td>76.6</td>
</tr>
<tr>
<td>raw</td>
<td>9</td>
<td>17.0</td>
<td>10</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Since so many of the children (76.4 per cent.) drank...
pasteurised milk, a comparison was made of the relationship between the presence of antibodies and the supply of pasteurised milk in Edinburgh and the surrounding district.

Table 16 shows that there was no marked difference in the distribution of pasteurised milk between the two groups of children.

**Table 16.** Source of pasteurised milk related to the presence of *C. burnetii* antibodies.

<table>
<thead>
<tr>
<th>Pasteurised milk supply:</th>
<th>Total drinking milk with <em>C. burnetii</em> antibodies</th>
<th>&quot;Matched controls&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Per cent.</td>
</tr>
<tr>
<td>dairy 1</td>
<td>10</td>
<td>25.0</td>
</tr>
<tr>
<td>dairy 2</td>
<td>8</td>
<td>20.0</td>
</tr>
<tr>
<td>dairy 3</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td>other</td>
<td>15</td>
<td>37.5</td>
</tr>
</tbody>
</table>

An informal visit was made to "dairy 2". Bulked raw milk is collected by them from farms in the Lothians, Borders and South West of Scotland. It is pasteurised by the high temperature-short time ("flash") method, using a temperature of 163.5°F. (73°C.) to ensure that the minimum British standard of 161.5°F. (71.9°C.) is always achieved. "Dairies 1 and 3" found it inconvenient to discuss their milk production at the time of the study.

**vi)** The presence of *C. burnetii* infection amongst dairy cows in the Edinburgh area.

Sera were collected from 180 dry dairy cows being
slaughtered at the Edinburgh abattoir between January and May 1967. All but four of the cows were from the areas supplying commercial milk to the city of Edinburgh and its surrounding neighbourhood. Of 178 sera tested, 26 (14.6 per cent.) were found after inactivation at 60°C to have complement-fixing antibodies to "phase 2" "Nine Mile" C. burnetii antigen at dilutions of 1:16 or more. None of these sera had antibodies to the "Henzerling" strain of antigen. Two of the sera could not be tested because one was grossly contaminated and the other was anticomplementary.

vii) The presence of C. burnetii infection amongst sheep in the Edinburgh area.

Sera were collected from 98 "lambs" being slaughtered at the Edinburgh abattoir in January 1968. All the animals were less than two years old and were from farms in the Lothians and Borders in South East Scotland. Of 97 sera tested, 16 (16.5 per cent.) were found after inactivation at 60°C to have complement-fixing antibodies to "phase 2" "Nine Mile" C. burnetii antigen at dilutions of 1:16 or more but only three (3.1 per cent.) had antibodies to the "Henzerling" strain.
DISCUSSION.
DISCUSSION

Before the incidence and aetiology of exposure to C. burnetii in children in the Edinburgh area can be discussed, it is necessary to consider the tests that have been used to measure this infection.

SPECIFICITY OF TESTS.

The specificity of diagnostic tests can be demonstrated in two ways: first, by showing that positive results occur more commonly in persons known to have had "Q" fever in the past than in the general population being studied; and secondly, by the exclusion of technical errors known to complicate the tests (Marmion et al., 1956).

In this study it was impossible to examine children known to have had "Q" fever in the past because only one childhood case has previously been reported in Scotland (Crist, 1956).

The specificity of each test and its possible technical errors will be considered separately.

The Complement-Fixation Test.

In her original complement-fixation test for C. burnetii, Bengtson (1941b) confirmed Dyer's (1938) finding that convalescent sera from guinea-pigs with typhus and Rocky Mountain spotted fever failed to react with C. burnetii antigens. The test was considered to be so specific for C. burnetii that in 1947 Huebner wrote - "no other agent produces antibodies to C. burnetii" and it is partly the failure of C. burnetii to stimulate agglutinins to Proteus organisms
(Burnet and Freeman, 1937) that differentiates it from the rickettsiae.

Cross reactions with members of the rickettsial group were not the only non-specific reactions to be examined. Renoux and Maurin (1954) have shown that where antibodies to both C. burnetii and brucella organisms occur in the same patient a double infection must have occurred.

However, non-specific complement-fixation reactions at low dilutions have been reported. Babudieri and Secchi (1952) observed that patients with pyogenic abscesses occasionally have antibodies to C. burnetii and other workers have noted a significant correlation between these antibodies and positive serological tests for syphilis (Robbins et al., 1946b; Strauss and Sulkin, 1949). Despite these findings, it is presumed that the relatively small number of patients with syphilis in the general population will not have accounted for many of the positive results for "Q" fever antibodies in serological studies. The converse finding was that of Clark, who found that a number of patients with clinical "Q" fever had transiently positive tests for syphilis during their acute illness (Clark et al., 1951b).

Stoker noted that, despite elaborate purification of the C. burnetii used as complement-fixing antigen, a small amount of egg material can remain and react non-specifically with some sera under test. Although these sera did not appear to be anticomplementary in control tests the summation of anticomplementary activity in both the antigen and serum produced non-specific reactions (Stoker et al., 1955).

In the present study all sera found to contain complemente-
fixing antibodies to C. burnetii were tested with normal yolk-sac antigen and with C. burnetii antigen in "phase 1" which contained approximately the same concentration of organisms as the "phase 2" antigen. None of the positive sera reacted with these antigens. Thus the presence of combined anticomplementary activity in the sera and antigen and non-specific reactions to remaining egg material could both be discounted in the sera under test.

Complement-fixing antibodies to C. burnetii have recently been reported in children with adenovirus pneumonia. Van der Veen and Heyen (1966) demonstrated rises in these antibodies in 18 of 67 children with adenovirus pneumonia but in only one of 61 children with parainfluenza and respiratory syncytial virus infections. Since "Q" fever is very uncommon in the Netherlands and because the sera also reacted with normal mouse lung antigen, normal yolk-sac and human lung antigens, the authors presumed that these must have been non-specific antibodies. They postulated that the adenovirus had caused abnormal destruction of cells rendering them antigenic to the patient and found that in 13 (72 per cent.) of the 18 cases studied, complement-fixing reactions to C. burnetii required high concentrations of antigen; they also noted that sera from three of these patients lost all reactivity to C. burnetii after heating at 60°C, for 30 minutes unlike sera from patients known to have had "Q" fever.

In the present study a significantly greater proportion of children with complement-fixing antibodies to C. burnetii were found to have complement-fixing antibodies to the adenovirus group but
none of the sera required high concentrations of \textit{C. burnetii} antigen to demonstrate the antibodies and only nine sera (14.8 per cent.) had a fourfold or greater fall in \textit{C. burnetii} titre after heat inactivation for 30 minutes at 60°C.

\textbf{The Intradermal Sensitivity Test.}

\textit{Lackman, in his early report of the use of a purified "phase 1" "Q" fever vaccine as the skin test antigen used in the present study, considered any induration 48 hours after inoculation as evidence of previous exposure to \textit{C. burnetii}. He divided positive reactors into four grades and noted that most of them had no history of "Q" fever infection. The skin test was thus a valuable screening test of people with \textit{C. burnetii} antibodies who should not be vaccinated because of the risk of local reaction to vaccine doses of the antigen in persons with immunity (Lackman et al., 1962). Using these criteria Luoto found that 35 per cent. of residents living in infected dairy premises had positive skin tests although only 3.5 per cent. had complement-fixing antibodies to \textit{C. burnetii} (Luoto et al., 1965). Recently however, skin tests have only been considered positive when, after 48 hours, there was more than four millimetres of induration at the inoculation site, because this gave a better correlation with positive radio-iodine precipitation tests (Lackman et al., 1967).}

The present study compared the results of skin test induration with the presence of complement-fixing antibodies to \textit{C. burnetii} in a group of hospital patients. Of the 58 tested 18
(31 per cent.) had induration at the inoculation site but only seven (12.1 per cent.) had induration greater than four millimetres. This gave a very good numerical correlation with positive complement-fixation tests in the same patients.

Follow-up skin tests on the children known to have complement-fixing antibodies to *C. burnetii* and their families were only considered positive if the induration measured five millimetres or more. Of the 53 children with complement-fixing antibody titres to *C. burnetii* of 1:16 or more who were later skin tested, 32 (60 per cent.) had induration of five millimetres or more. This was significantly more than the 12.1 per cent. of hospital patients found to have positive skin tests and was considered evidence that the presence of complement-fixing antibodies in these children gave specific evidence of past experience with *C. burnetii*. However, Lackman has shown that the skin test only has limited usefulness for general epidemiological studies because some people who are known to have had "Q" fever appear to be skin test non-reactors (Lackman et al., 1967; Philip 1967).

The Radio-Iodine Precipitation Test.

The remarkable specificity of the radio-iodine precipitation test was first noted by Luoto and his coworkers in Montana (1965). Dr. Leo Thomas has kindly agreed to submit 44 of the Edinburgh children's sera to this test. He is testing both sera with complement-fixing antibodies to *C. burnetii* at dilutions of 1:16 or more.
and sera without such antibodies as a further means of determining the specificity of the complement-fixation test used in this study.

The results of these tests are not yet available.

THE SIGNIFICANCE OF COMPLEMENT-FIXING ANTIBODIES TO C. BURNETII IN CHILDREN DURING THE TIME OF THE STUDY.

Having accepted that the complement-fixing antibodies at titres of 1:16 or greater obtained in the present study can be taken as evidence of past exposure to C. burnetii it is important to compare the incidence of these antibodies in 66 (15.3 per cent.) of the 433 children under study with other groups of patients.

In all series of clinical "Q" fever cases and most serological surveys for C. burnetii antibodies, (including two using the radio-iodine precipitation test,) there has been a remarkable absence of children (Clark et al., 1951a and b; Marmion et al., 1953; Stoker and Thomson, 1953; Luoto et al., 1965; Reed and Schmurrenberger, 1966). However, one epidemiological survey in Montreal showed an unexpectedly high proportion of children to have these antibodies (Pavilanis et al., 1958) and occasional childhood cases of infection - either clinical or subclinical - have been reported from elsewhere (Beck et al., 1949; Gear et al., 1950; Stoker and Thomson, 1953; Porte and Capponi, 1954; LeLong et al., 1955; Orist, 1956; Kereev, 1959; McLean et al., 1960; Herbert et al., 1965).

The results of the present study show that children between November 1965 and March 1967 aged less than 13 years in the Edinburgh area had a far greater risk of infection with C. burnetii than adults.
It would appear that this is a recent difference because only one (0.6 per cent.) of 161 children's sera tested in the same laboratory between 1960 and 1964 had complement-fixing antibodies at this level.

In contrast to the findings of Marmion and his colleagues (1956) investigating adult blood donors in South East England, no association could be found between the presence of complement-fixing antibodies in the children under study and a previous history of a clinical syndrome suggestive of "Q" fever, except that of jaundice. However, of the seven children with antibodies who were reported to have had jaundice, five had had mild neonatal jaundice leaving only two who had had a clinical icteric illness. This was not considered to be significantly important.

No correlation was found between the presence of low titre, stable complement-fixing antibodies to C. burnetii and the clinical diagnosis on admission to hospital. In the 11 children who were considered to have significant serological evidence of recent experience with C. burnetii only three (27 per cent.) had lower respiratory tract infections while five (45 per cent.) had illnesses with central nervous system involvement. Amongst the latter group of children, one also had an undiagnosed maculo-papular rash and the remaining three were respectively diagnosed as having myocarditis, upper respiratory tract infection and pyrexia of unknown origin.

The high proportion of children with central nervous system involvement was greater than the proportion of the total group of children with such a diagnosis. This finding is of interest because some of the few childhood cases of "Q" fever that have previously been
reported were notable for the predominance of central nervous system involvement (Forte and Capponi, 1954; LeLong et al., 1955; Gillet et al., 1963).

In the present study one of the 11 children with a significant antibody level died. She was a girl of nine months with encephalitis and a complement-fixing antibody rise to C. burnetii from less than 1:16 to 1:256. Her diagnosis was confused by numerous factors and because it was not possible to study post-mortem material, no proof that this was the first childhood death due to "Q" fever could be obtained.

THE EPIDEMIOLOGY OF COMPLEMENT-FIXING ANTIBODIES TO C. BURNETII IN THE CHILDREN UNDER TEST.

Age.

Children of all age groups were found to have antibodies but in those aged less than six months there was no significant difference in the proportion with antibodies when compared with hospital adults tested over the same period of time.

Two explanations of this seem likely; first, that these are maternal antibodies persisting for approximately the first six months of life; and secondly, that these children had less exposure to the organism than older children. Because no details of maternal complement-fixing antibodies to C. burnetii have been recorded, the results in the present study were compared with the pattern of adenovirus group complement-fixing antibodies in the first six months of life. A steady loss of maternal antibodies to the adenovirus group was suggested by a fall from 55 per cent. with these antibodies in
children aged less than three months of life to 18.3 per cent. in those aged three to five months. This preceded a gradual increase in the proportion of children with adenovirus complement-fixing antibodies, stimulated by adenovirus infections, to the maximum figure of 87.6 per cent. in those aged four years and over. Such a pattern did not occur with complement-fixing antibodies to C. burnetii: no loss of antibodies was demonstrated in the early months of life and a sudden increase in the number of children with antibodies occurred from the age of six months. Because the highest proportion with antibodies was in those aged one year it suggested that children over the age of six months were particularly susceptible to infection with C. burnetii and that only a few children had had exposure to the organism before this age. These findings correspond with Pavilanis' survey where most of the children with complement-fixing antibodies to C. burnetii were aged between one and four years of age (Pavilanis et al., 1958).

Sex.

It was not considered relevant to relate the presence of complement-fixing antibodies to C. burnetii in British children to a difference in sex. In this country children of both sexes are reared in similar epidemiological surroundings.

Infection in the rest of the Family.

After assessing that the sensitivity of the intradermal sensitivity test for C. burnetii was the same as the complement-fixation test in a group of hospital patients, this test was used to study the families of 53 of the children who had been found to have
complement-fixing antibodies to *C. burnetii*. This test was chosen in preference to a serological test because, despite the necessity for a second visit, it was easier to do on relations of all ages during a domiciliary visit.

Induration of five millimetres or more at 48 hours was considered evidence of past infection with *C. burnetii*.

The contacts of one child who had lived in a children's home before admission to hospital had blood specimens taken, with the help of a nurse, and the complement-fixation test was used for these sera.

The results of both these surveys showed that a significantly higher proportion of other family members had had experience with *C. burnetii* than the general population, and suggested that the children with antibodies in the original study had been infected by *C. burnetii* in the family setting. Since so few cases of direct human to human infection have been reported (Marmion and Stoker, 1950; Babudieri, 1951; Holland et al., 1960), a common environmental source of infection was postulated.

**Occupation and contact with animals.**

Unlike studies on adults with antibodies to *C. burnetii* it was not possible to connect the presence of complement-fixing antibodies to *C. burnetii* in children with occupations involving contact with cattle and sheep or their products (Derrick et al., 1942; Beck et al., 1949; Strauss and Sulkin, 1949; Bell et al., 1950; Clark et al., 1951d; Marmion et al., 1956; Schonell et al., 1966).

Because Beck had reported a family infection from the contaminated clothes of the father who was a calf buyer (Beck et al.,
1949), a comparison was made in the present study between parental involvement - either occupational or leisure - with animals and their products in the group of children with antibodies and a group of children in the same study without complement-fixing antibodies who acted as "matched controls". No connection could be made between the presence of antibodies and parental involvement with animals and their products. Nor was there an association with residence on or visits to farms by the children or their families although Johnson (1966) reported that children who lived on Australian farms had a relatively high proportion of antibodies to \textit{C. burnetii} when compared with other children.

The results of comparative studies with the "matched controls" showed that a significantly greater proportion of the children with antibodies to \textit{C. burnetii} had had direct contact with cows and sheep by playing in fields or pastures where these animals were grazing. A serological study of cows and sheep being slaughtered at the Edinburgh abattoir showed that in South East Scotland these animals are infected with \textit{C. burnetii}. These results were considered to be very significant since so many series of patients with \textit{C. burnetii} antibodies, with and without clinical illness, have stressed the positive association with cattle and / or sheep (Derrick et al., 1942; Beck et al., 1949; Bell et al., 1950; Clark et al., 1951d; Stoker, 1953a; Marmion et al., 1954; Luoto et al., 1965; Schonell et al., 1966).

It is possible that the amount of sheep farming in this area (114 sheep per 100 acres) is an important factor because in the epidemiology of "C" fever in Britain a higher incidence of infection
among cattle and man has been related to intensive sheep farming, such as occurs in the Romney Marshes in Kent, than to farming where crops and cattle predominate (Marmion and Stoker, 1956).

The relative importance of contact with cattle and sheep has been differentiated by the seasonal incidence of acute "Q" fever infections. Infection from sheep occurs mainly in the spring and early summer months in association with lambing and shearing activities (Clark et al., 1951d; Marmion et al., 1953; Marmion and Stoker, 1956; Marmion and Stoker, 1958) while human patients infected by cattle, either directly or via infected milk, became ill at any season of the year (Beck et al., 1949; Marmion and Harvey, 1956; Marmion and Stoker 1958). In the present study, ten of the 11 children with serological evidence of recent infection with C. burnetii were admitted to hospital in the winter months but because the organism has been shown to survive for long periods of time in a dessicated form (Philips, 1948a; Welsh et al., 1959) this discrepancy was probably due to a different form of animal contact than usually occurs in adults.

A negative finding in the present study was that it was not possible to connect the presence of complement-fixing antibodies to C. burnetii to contact with any of the following animals and birds which have been shown to carry the organism; dogs (Jadin and Giroud; 1950; Zdrodovskij, 1964), mice and rabbits (Derrick, 1937; Burnet and Freeman, 1937), domestic chickens and pigeons (Syruci and Ruska, 1956; Babudieri and Moscovici, 1952; Marmion and Stoker, 1958).

Place of Residence.

In contrast to the findings of Californian workers, no correlation could be made in the children being studied between the
presence of \textit{C. burnetii} antibodies and residence near farms or other premises involving large animals and their products (Bell et al., 1950). Marmion has previously noted that conditions of animal husbandry and climate in Great Britain are so unlike the conditions prevailing in California that it is not surprising that the epidemiology of "Q" fever infections should be different (Marmion et al., 1956; Marmion and Stoker, 1959).

\textbf{Ingestion of Milk.}

Because the children in the present study showed a sudden increase in the proportion with \textit{C. burnetii} antibodies after the age of six months of age, a connection with weaning to unboiled dairy milk was postulated as a significant aetiological factor. However, unlike many surveys of "Q" fever infection in adults living in urban areas, where a significant number of the population with antibodies to \textit{C. burnetii} were regular users of raw milk (Beck et al., 1949; Bell et al., 1950; Marmion and Harvey, 1958; Stoenner et al., 1959) the present study did not show that the presence of antibodies in children in the Edinburgh area could be accounted for by ingestion of infected raw milk. This absence of association with raw milk consumption is possibly related to the fact that the amount of raw milk consumed in Great Britain is declining. Only 18 per cent. of the children in the present study either drank or had drunk raw milk regularly as compared with 70 per cent. of the group studied in a survey of "Q" fever infection in an urban area of Kent in 1956 (Marmion and Harvey, 1956).

Negative results were also obtained in relation to the consumption of breast milk, proprietary evaporated and powdered milks. Because \textit{C. burnetii} has been demonstrated to withstand
pasteurisation (Huebner et al., 1949; Marmion et al., 1951; Evans, 1963) an attempt was made to relate the presence of antibodies to the consumption of pasteurised milk. Pasteurised milk in the city of Edinburgh is supplied by three main dairies and in the surrounding area by a number of smaller dairies. There was no association between the presence of antibodies and the consumption of milk from any of these sources. Although no attempt was made to isolate the organism from raw or pasteurised milk it was concluded that ingestion of *C. burnetii* in milk was not an important aetiological factor in the stimulation of complement-fixing antibodies to that organism in the children under study.

A final negative finding related to Malloch and Stoker's (1952) suggestion that *C. burnetii* could be transmitted to children from infected calves by the glycerol/phenol treated calf lymph used for smallpox vaccination. Comparative investigations with the "matched controls" showed that this was not of epidemiological importance in the present study.

**CONCLUSIONS.**

As a result of laboratory and epidemiological investigations the presence of complement-fixing antibodies to *C. burnetii* in a group of children in the Edinburgh area appeared to be related to two factors: firstly, the presence of complement-fixing antibodies to the
adenovirus group and secondly to direct physical contact with sheep and cows, or their pastures.

On the first count, 85 per cent. of the complement-fixing antibodies to C. burnetii in the present study were heat stable and none required high concentrations of antigen to demonstrate their presence unlike those reported by Van der Veen and Heyen (1966). However, none of the Dutch cases were studied for more than 72 days following the initial adenovirus pneumonia and it is possible that non-specific complement-fixing titres to C. burnetii became more stable with the passage of time. If this hypothesis is correct it is possible that a few of the low titre complement-fixing antibody titres to C. burnetii in the children in the present study resulted from past adenovirus infection. However, because a significant number also had positive skin tests to "Q" fever antigen, and there was evidence of an increased experience with C. burnetii in the families of children with complement-fixing antibodies to C. burnetii, the presence of these antibodies was considered to indicate specific infection with the organism under discussion.

The second observation, that the presence of antibodies was significantly related to contact with cows and sheep suggested a source of infection for a proportion of the children. It is disappointing that no particular milk supply could be connected with the presence of antibodies in children who had had no contact with animals. This would also have explained the lack of correlation with a clinical illness suggestive of "Q" fever since it has been shown that ingested C. burnetii are less pathogenic to man than inhaled organisms (Marmion
et al., 1956; Benson et al., 1963; Evans, 1963). However, it is possible that, because of the presence of infection amongst dairy cattle in the areas supplying milk to the city of Edinburgh, all the major milk supplies are or have been infected from time to time. It is unfortunate that it was not possible to attempt isolation of the organism from these sources in an attempt to account for the presence of antibodies in children who did not have contact with cows and sheep. So because Derrick's (1953) advice on the subject of "Q" fever was that "one should be cautious in accepting conclusions ......... who can tell what surprises yet lie ahead" the final statement of this study makes no claim to have answered the query posed so long ago (Derrick, 1937). It is that specific complement-fixing antibodies to C. burnetii have recently been found in a greater proportion of small children in the Edinburgh area than in the same adult population and that while some cases could be explained by infection from cows and sheep the rest add yet another problem to the "unsolved problems" discussed by Marmion in 1959.
SUMMARY.
"Q" fever and its causative organism C. burnetii have been reviewed with reference to the epidemiology and clinical manifestations of infection in children.

The present study was planned:

a) to confirm the finding that 15 per cent. of young children in the Edinburgh area had complement-fixing antibodies to "phase 2" C. burnetii,

b) to assess the specificity of these antibodies, and

c) to study the epidemiological features of the infections.

a) Complement-fixing antibodies to C. burnetii in Edinburgh children.

The study showed that 66 (15.3 per cent.) of 433 hospitalised children had antibodies to C. burnetii. This was significantly more than adult hospital patients tested over the same period of time in the same area and appeared to be a new finding because previous childhood surveys in Edinburgh had not shown this difference.

Antibodies were present in children of all ages but particularly in those aged more than five months. No relationship to present or past illness could be demonstrated in those with low, stable or single titres; but of 11 children with titres suggestive of recent "Q" fever infection, five were noted to have central nervous system disease, including one girl who died.

b) The specificity of the complement-fixing antibodies to C. burnetii.

None of the sera containing C. burnetii antibodies fixed complement with normal yolk-sac antigen or C. burnetii antigen in
"phase 1", and 85.2 per cent. of 61 that were inactivated at 60°C for 30 minutes were found to have heat stable antibodies.

Further evidence that these antibodies were specific was obtained by finding that significantly more of the children with antibodies had positive intradermal sensitivity tests for C. burnetii when compared with another group of hospital patients of mixed ages.

However, a relationship was found between the presence of antibodies to C. burnetii and antibodies to the adenovirus group, suggesting that the C. burnetii antibodies in some of the children may have been a non-specific reaction to cell damage during adenovirus infection as demonstrated by Van der Veen and Heyen (1966).

_c) Epidemiological features._

A domestic source of infection with C. burnetii was postulated because a significant number of other family members were shown to have C. burnetii antibodies by the intradermal sensitivity test.

The activities and environments of 53 children with antibodies were compared with those of 49 "matched controls" who did not have C. burnetii antibodies.

Although occupational contact with animals could not be considered in this study, it was found that significantly more of the children with antibodies had had contact with cows and / or sheep by playing on pastures where these animals were grazing. Further studies showed that both cows and sheep in the Edinburgh area had complement-fixing antibodies to C. burnetii and increasing infection in these animals is presumed to account for the change in C. burnetii infection in Edinburgh children.

No relationship could be found between contact with other
animals and birds or with residence near large animals: nor could indirect contact with large animals through other members of the family be related to the presence of antibodies.

No association was found between the presence of *C. burnetii* antibodies and the ingestion of raw milk or any other particular milk supply although many of the children developed antibodies at an age when they were weaned on to unboiled cow's milk. Unfortunately no attempt could be made to isolate the organism from milk supplies and it is possible that all Edinburgh milk supplies—raw and pasteurised—contain viable *C. burnetii*. 
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REFERENCES.


Babudieri, B. (1953a) W.H.O. Monograph Series. 19: 157

Babudieri, B. (1953b) W.H.O. Monograph Series. 19: 193


Babudieri, B. and Secchi, P. (1952) Rend. ist super sanità 15: 78


Berge, T.C. and Lennette, E.H. (1953b) Amer. J. Hyg. 57: 144


Cheney, G. and Geib, W.A. (1946) Amer. J. Hyg. 44: 158


Clark, W.H., Lennette, E.H., Romer, M.S. (1951c) Amer. J. Hyg. 54: 35

Clark, W.H., Lennette, E.H., Romer, M.S. (1951d) Amer. J. Hyg. 54: 319
Evans, A.D. (1963) Practitioner 191: 605
Feinstein, M., Yesner, R., Marks, J.L. (1946) Amer. J. Hyg. 44: 72


Harvey, M.S., Forbes, G.B., Marmion, B.P. (1951) Lancet 2: 1152


Kilschperger, G. and Wiesman, E. (1949) Schweiz Arch. Tierheilk 91: 553


Lennette, E.H. and Welsh, H.H. (1951) Amer. J. Hyg. 54: 44
Marmion, B.P. and Harvey, M.S. (1956) J. Hyg., Camb. 54: 533


Marmion, B.P. and Stoker, M.G.P. (1956) J. Hyg., Camb. 54: 547


Miller, K. (1967) Personal communication.


Noguchi, H. (1926a) J. Exp. Med. 43: 515

Noguchi, H. (1926b) J. Exp. Med. 44: 1


Perrin, T.L. (1949) Arch. Path. 47: 361
Philip, C.B. (1948a) J. Parasitol. 34: 457
Philip, R.N. (1967) Personal communication
Ransom, S.L. and Huebner, R.J. (1951) Amer. J. Hyg. 53: 110
Reed, C.F. and Schnurrenberger, P.R. (1966) Amer. J. Epidem. 84: 234
Robbins, F.C., Rustigan, R., Snyder, M.J., Smadel, J.E. (1946b) Amer. J. Hyg. 44: 51


Sidwell, R.W., Thorpe, B.D., Gebhardt, L.P. (1964a) Amer. J. Hyg. 79: 113

Sidwell, R.W., Thorpe, B.D., Gebhardt, L.P. (1964b) Amer. J. Hyg. 79: 320

Slavin, G. (1952) Vet. Rec. 64: 743


Stoker, M.G.P. (1953b) J. Hyg., Camb. 51: 311


Stoker, M.G.P. and Marmion, B.P. (1952) J. Hyg., Camb. 50: 1

Stoker, M.G.P. and Marmion, B.P. (1955a) J. Hyg., Camb. 53: 322
The Commission on Acute Respiratory Diseases, Fort Bragg, North Carolina. (1946a) Amer.J.Hyg. 44: 88
The Commission on Acute Respiratory Diseases, Fort Bragg, North Carolina. (1946b) Amer.J.Hyg. 44: 103
The Commission on Acute Respiratory Diseases, Fort Bragg, North Carolina. (1946c) Amer.J.Hyg. 44: 110
The Commission on Acute Respiratory Diseases, Fort Bragg, North Carolina. (1946d) Amer.J.Hyg. 44: 123
Therapeutic Notes (1967) 41: 88


Welsh, H.H., Jensen, F.W., Lennette, E.H. (1959a) Amer. J. Hyg. 70: 1


