Animal Microbiology

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Volume 2: Rickettsias and Viruses

ANIMAL MICROBIOLOGY

VOLUME 2

Rickettsias and Viruses

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Preface

Our purpose in writing this book has been to collect together information on animal microbiology required both by undergraduates studying for their primary veterinary degrees and by veterinarians undertaking postgraduate courses. We also hope that the book will be of use to veterinary surgeons in different walks of professional life as well as being of interest to medical microbiologists and others requiring information on this subject. To cater for these various interests we have included discussions on the epidemiology, pathogenesis, clinical features, diagnosis, control, public health and other aspects of infectious diseases as well as on purely microbiological matters. We hope that the tables of contents at the beginning of each chapter, the textual headings and lists of further reading, together with the general index, will enable the reader to locate easily any required information.

In order to present the subject in a manner equally acceptable to all readers, we have supported the text with a selection of coloured photographs and drawings as well as monochromes illustrating the appearance of microbiology as seen in a diagnostic laboratory. For the benefit of postgraduate students, and particularly those in tropical and subtropical countries, we have tried to maintain a balance between our discussions on microbial diseases of animals occurring in warmer climates and those in more temperate zones. Consequent upon the recent expansion of fish farming throughout the world and as a result of the increasing importance of controlling and preventing diseases of fish, a chapter has been devoted to this specialist subject.

During the prolonged gestation period of this book we have become increasingly aware of the difficulties that can beset two authors when trying to ensure that a text covering such a wide canvas as animal microbiology is up-to-date in all its facets, while simultaneously avoiding undergraduate indigestion arising from the inclusion of too much detailed information in particular areas. It is inevitable in a first edition of a book of this size that some errors and omissions will have occurred and we hope that readers will call our attention to any shortcomings. While the preparation of the whole book has been the concern of both of us, the senior author has dealt in particular with the introductory chapters, bacteriology and the appendices and the junior author has been responsible for the sections on mycotic, rickettsial and viral infections.

During the writing and correction of the text we have received advice and encouragement from many people, and we should like to mention in particular Dr. G. H. K. Lawson for information on *Campylobacter*, Dr. W. J. Penhale on Immunology and Dr. J. E. Phillips on *Actinobacillus*. We are especially grateful to Dr. W. B. Martin for reading the whole of the manuscript on Virology and for making many valuable suggestions and corrections.

It is a special pleasure to acknowledge the apparently inexhaustible patience and skill of Mr. R. C. James, who was responsible for photographing most of the colour plates used in this book. We also hope that we have done justice to those who have contributed illustrative material and we accord our thanks to Mr. I. S. Beattie, Mr. K. W. Head and Mr. A. C. Rowland for allowing us to include some of their coloured transparencies of pathological lesions in the section on Bacteriology. Recognition of colleagues, research students and correspondents who have kindly provided us with many of the illustrations in the chapters

dealing with Mycology and Virology is recorded in the captions of the relevant plates. To all those and to others, too numerous to mention individually, who have helped in many ways, large and small, we offer our sincere thanks.

It is a pleasure to record the untiring assistance we have received from Miss M. Millar, who has not only typed the entire manuscript but has also helped in numerous other ways towards the completion of the final text.

Finally, our grateful thanks are due to our publisher, in particular Mr. Per Saugman, who invited us to write the book, and Mr. Nigel Palmer for his constant advice in matters relating to production.

A. Buxton
G. Fraser

Edinburgh, April 1977

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CHAPTER 36

The Rickettsias

The unicellular microorganisms can be arranged in order of decreasing size and complexity, viz: protozoa, yeasts and certain fungi, bacteria, mycoplasmas, rickettsiae and chlamydiae. Members belonging to these last two groups resemble bacteria in some ways and viruses in others, and are not immediately identifiable as either. They are generally smaller than bacteria but larger than viruses (300–500 nm), and most species are obligate intracellular parasites.

Most workers are now agreed that *Rickettsia* and *Chlamydia* (the psittacosis-lymphogranuloma venereum agents), have properties more closely related to bacteria than to viruses in that: (1) they contain both DNA and RNA, (2) multiplication is by binary fission or budding, and (3) particles possess bacterial cell wall constituents and certain growth enzyme systems which render them susceptible to some antibiotics. Thus, rickettsiae and chlamydiae are probably very small bacteria which have developed an ultra-parasitic mode of life.

The general properties of these organisms are compared with those of bacteria, mycoplasmas and viruses in Table 36.1.

THE RICKETTSIAE

Rickettsiae constitute a relatively small but important group of obligate, intracellular microorganisms that mostly occur naturally in the tissues of arthropods and are transmissible to man and animals in which they may cause disease. The name rickettsia was assigned to these organisms in memory of Dr H. T. Ricketts who died of typhus accidentally contracted while studying the disease.

Classification

Organisms of the order *Rickettsiales* may be classified into three families based on their morphology, serology, mode of transmission and whether they occur in the cytoplasm or nucleus of the cells they infect (Fig. 36.1). All are small, rod-shaped or coccoid and often pleomorphic microorganisms that are usually obligate intracellular parasites and grow only in living tissues. The one exception to this general rule is *R. quintana*, the causative agent of trench fever, which grows extracellularly in the louse

TABLE 36.1. Biological relationships of microorganisms.

	Bacteria	Mycoplasmas	Rickettsiae	Chlamydiae	Viruses
Visible by light microscopy	+	+	+	+	−†
Size (nm)	c. > 1000	Variable	c.300	c.300	< 300
Nucleic acids	DNA+RNA	DNA+RNA	DNA+RNA	DNA+RNA	Either DNA or RNA
Muramic acid	+	−	+	+	−
Ribosomes and other organelles	+	+	+	+	−
Growth in non-living media	+	+	−	−	−
Intracellular replication	−*	+	+	+	+
Mode of replication	Binary fission	Binary fission & budding	Binary fission	Binary fission & budding	Synthesis of viral NA and viral proteins
Eclipse phase	−	−	−	−(?)	+
Sensitivity to antibiotics	+	+	+	+	−
Sensitivity to interferon	−	−	−	+	+

* Some will replicate intracellularly, e.g. *Mycobacterium tuberculosis*

† Some are just visible, e.g. vaccinia.

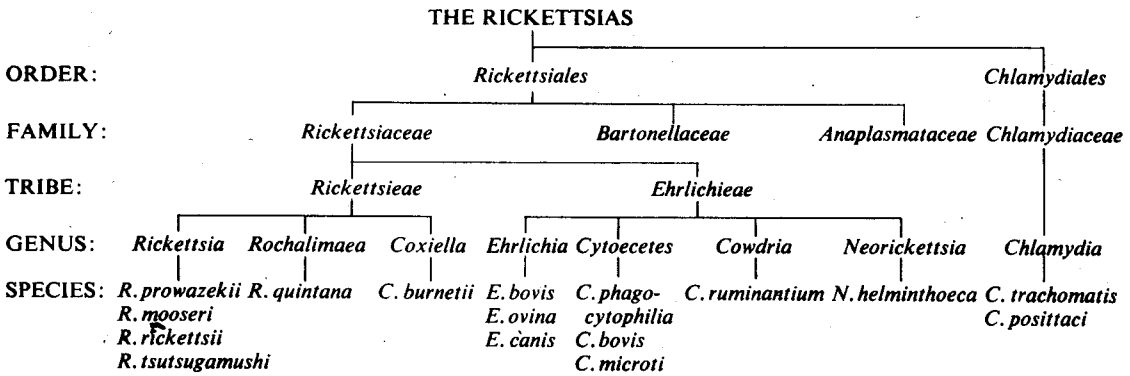

FIG. 36.1. A classification of the rickettsias.

gut and has been cultivated successfully on modified blood agar. Most but not all species of rickettsiae are transmitted by arthropod vectors and many cause diseases in man and animals.

Members of the family *Rickettsiaceae* usually inhabit the gut cells of the arthropods which transmit the microorganisms to other susceptible hosts, and those that affect mammals parasitize the cells of the vascular endothelium or other tissue cells. The properties of *Chlamydiaceae* are so similar to those of *Rickettsiaceae* that many authors prefer to classify them with rickettsiae even though they have no arthropod vectors. In contrast, members of the families *Bartonellaceae* and *Anaplasmataceae* primarily parasitize mammalian erythrocytes and may be transmitted by arthropod vectors. (Table 36.2).

The *Rickettsiaceae* may be divided into two tribes, the *Rickettsieae*, which are adapted to arthropods and cause disease in mammals, and the *Ehrlichieae*, some of which are adapted to arthropods and are pathogenic for mammals but not for man.

The *Rickettsieae* consist of three important genera, each of which contains pathogenic species that cause diseases of man and animals. Members of the genus *Rickettsia* are transmitted by lice, fleas, ticks or mites and include several important species, e.g. *R. prowazekii* of epidemic typhus, *R. mooseri* of endemic typhus or murine typhus, *R. tsutsugamushi* of scrub typhus, *R. conorii* of Mediterranean fever, and *R. rickettsii* of Rocky Mountain spotted fever. The genus *Coxiella* includes the important species *C. burnetii*, of Q fever, which is not dependent on arthropod transmission and does not produce a typhus-like rash on the skin of the patient. The third genus, *Rochalimaea*, includes *R. quintana* of trench fever.

The tribe *Ehrlichieae* includes four genera, viz. *Ehrlichia*, which are lymphocytic parasites associated with 'rickettsiosis' of cattle (*E. bovis*), sheep (*E. ovina*) and dogs (*E. canis*); *Cytoecetes*, which are present in the granular leucocytes and monocytes in cases of tick-borne fever in sheep (*C. phagocytophilia*) and cattle (*C. bovis*), and in a disease of voles (*C. microti*); *Cowdria*, which form characteristic clusters in the cytoplasm of vascular endothelial cells in heartwater fever of ruminants (*C. ruminantium*); and, *Neorickettsia*, occurring as short rods, crescents or rings in the cytoplasm of reticuloendothelial cells of mammals and in the tissues of fluke vectors of salmon disease of dogs (*N. helminthoeca*) (Table 36.3).

General properties

Rickettsiae are mostly obligate intracellular parasites having properties that are intermediate between those of bacteria and viruses. They are usually smaller than bacteria and their size ranges from 0·3 to 2·0 μm in length by 0·3–0·5 μm in width. Most species are highly pleomorphic and their shape may vary from coccoid, ellipsoid or coccobacillary to rod-shaped and, occasionally, filamentous forms. They are Gram-negative and mostly stain poorly with watery solutions of aniline dyes. The most satisfactory methods for staining them are Giemsa, Leishman, Castaneda and Macchiavello; some, e.g. *R. prowazekii* and *C. burnetii* retain basic fuchsin but others, e.g. *C. ruminantium* and *C. phagocytophilia* do not. Electron micrographs show that the individual organism is surrounded by a plasma membrane and a rigid cell wall which is chemically and structurally very similar to that of *E. coli*. The cytoplasmic material is granular in appearance and contains irregular, filamentous bodies which are believed to constitute the nucleus.

Rickettsiae resemble bacteria in that they are composed of protein, neutral fat, phospholipids and carbohydrates and the cell wall contains amino-acids, polysaccharide and muramic acid. Unlike viruses which contain either DNA or RNA, but never both, the nucleic acid of rickettsiae consists of relatively constant amounts of DNA and variable quantities of RNA, which is usually about

TABLE 36.2. Some diseases associated with members of the families *Bartonellaceae* and *Anaplasmataceae*.

Organism	Host	Transmission	Natural disease	Activated by splenectomy	Remarks
BARTONELLACEAE (Four genera)					
Bartonella bacilliformis	Man	Sand fly (Phlebotomus)	(1) Oroya fever – anaemia (2) Verruga peruana – granulomatous skin disorder		Grows in non-living media
Haemobartonella muris	Rodents	Louse (*Polyplax spinulosum*)	Not primary pathogen	Yes	May grow slowly in non-living media
H. bovis	Cattle	?Insect vector	Not primary pathogen	Yes	
H. canis	Dogs	Not known	Severe anaemia, or none	Yes	
H. felis	Cats	?Insect vector	Fever and anaemia	?	
Grahamella spp.	Rodents	Not known	Not primary pathogen	?	
Eperythrozoa suis	Pigs	?Ectoparasites, e.g. lice	Icteroanaemia	?	not cultivated
E. parva	Pigs	?Ectoparasites	Not primary pathogen	Yes	
E. wenyoni	Cattle	?Ectoparasites	Not primary pathogen	Yes	
E. ovis	Sheep, goats	?Ectoparasites	Haemolytic anaemia	Yes	
E. felis	Cats	?	Anaemia or none	?	
E. coccoides	Laboratory mice	?	Not primary pathogen	Yes	
ANAPLASMATACEAE					
Anaplasma marginale	Cattle	Ticks (7 genera) (Biting flies – 19 species)	'Gall-sickness' – may be severe icteroanaemia		Widely distributed in the tropics and sub-tropics.
A. centrale	Cattle	Various arthropods	Mild anaemia		
A. ovis	Sheep, goats	Various arthropods	Mild anaemia		

TABLE 36.3. Some diseases caused by members of the family *Rickettsiaceae*.

Species	Disease	Geographical distribution	Host	Transmission	Straus Reaction	Weil-Felix Reaction
RICKETTSIA						
R. prowazekii	Epidemic typhus	Worldwide	Man	Body louse. Infected louse faeces into broken skin	—	OX-19
R. mooseri (*R. typhi*)	Endemic (murine) typhus	Worldwide	Rats, mice (man)	Flea. Infected flea faeces into broken skin	+	OX-19
R. tsutsugamushi	Scrub typhus	Asia, Far East.	Wild rodents (man)	Mite. Bite of trombiculid mite	—	OX-K
R. rickettsii	Rocky Mountain spotted fever	America	Wild rodents, dogs (man)	Tick. Bite of wood tick or dog tick (*Dermacentor* spp.)	+	OX-19, OX-2
COXIELLA						
C. burnetii	Q fever	Worldwide	Small animals, cattle, sheep, goats, man	Inhalation of dried infective material from placentas, discharges, etc. ?Ingestion, ?Tick bite or faeces	Rarely	—
EHRLICHIA						
E. canis	'Rickettsiosis' (dog typhus)	Africa, India.	Dogs	Tick. Bite of *Rhipicephalus sanguineus*	—	?OX-19, OX-K
CYTOECETES						
C. phagocytophilia *C. bovis*	Tick-borne fever	Europe.	Sheep, cattle	Tick. Bite of *Ixodes ricinus*	—	—
COWDRIA						
C. ruminantium	Heartwater	Africa, south of the Sahara.	Sheep, goats, cattle	Tick. Bite of *Amblyoma* spp.	—	—
NEORICKETTSIA						
N. helminthoeca	Salmon poisoning	N.W. Pacific seaboard	Dogs, foxes	Ingestion of raw fish containing fluke cercaria infected with neorickettsia. No arthropod transmission	—	—

three times that of DNA. Although rickettsiae possess their own enzyme systems which enable them to oxidise intermediate metabolites like pyruvate and succinate, they depend on living susceptible host cells for essential metabolic reactions and cannot, therefore, be cultivated in artificial media.

Most species of rickettsiae can survive only briefly outside the living cell and are quickly inactivated by chemical disinfection, drying, heat and other adverse environmental conditions, and their growth is inhibited by broad-spectrum antibiotics, e.g. tetracyclines. Recent isolates of rickettsiae are inactivated within a few hours at room temperature but may be preserved in 50 per cent glycerol-saline at 4°C or by freeze-drying emulsions of infected tissues suspended in sterile skimmed milk, serum albumen or other suitable stabilizer.

Pathogenicity

Most species of rickettsiae have a predilection for vascular endothelium and stimulate the cells lining the small capillaries to swell and divide. Hyperplasia of the endothelial cells, localised thrombus formation and the release of erythrocytes into the surrounding tissues give rise to some of the more prominent clinical manifestations of rickettsial infections such as petechial rash and stupor. Other rickettsia-like organisms, e.g. *Ehrlichia* spp. and *Cytoecetes* spp. mostly parasitize granular leucocytes and monocytes.

Cultivation

Most rickettsiae can be propagated in a wide range of laboratory animals, in the yolk sacs of embryonated hens' eggs and in various cell culture systems. Growth may occur in different parts of the host cell; members of the typhus group usually parasitize the cytoplasm while those of the spotted fever group are mostly to be found in the nucleus. So far, only one member of the *Rickettsiaceae*, *R. quintana*, which causes trench fever, has been shown to grow on cell-free media; all other species require living susceptible cells and grow best when the cell metabolism is depressed. Thus, their rate of multiplication in chicken embryos is enhanced when the temperature of incubation is reduced to 32°C. In many cases, enhanced growth is also obtained in the presence of sulphonamides.

A few species of rickettsiae are capable of producing lethal toxins. For example, if large numbers of *R. mooseri* or *R. prowazekii* are inoculated intravenously or intraperitoneally into mice, the animals usually die within 2–8 hours due to the direct effects of the toxins. It is significant that these toxins are not neutralized by antisera prepared against the rickettsial cell wall and that they cannot be separated from the intact living organism. Further evidence suggests that they are heat labile and are detoxified by formaldehyde. It is also interesting to note that yolk sac suspensions of *R. mooseri* and *R. prowazekii* can haemolyse sheep and rabbit red blood cells, *in vitro*, but have no effect on those of human origin.

Ecology

Rickettsial infections vary enormously in severity, from symptomless, mild and self-limiting illnesses to some of the most fulminating diseases known; and most are transmitted through the bite of infected lice, fleas, mites or ticks or by their infected faeces through broken skin.

The role of arthropods in the transmission of rickettsial infections is well illustrated in the case of epidemic typhus of man. The causative agent, *R. prowazekii*, is spread from man-to-man by the bite of the human body louse (*Pediculus corporis*) which acquires the infection when it bites either a patient suffering from typhus or a latent carrier in whom the organism has persisted for many months or years. When rickettsiae in the blood-meal reach the insect's gut they invade the epithelial cells and multiply therein. In due course the cells swell and rupture, releasing enormous quantities of organisms into the intestinal contents. The louse contaminates the skin with its infected faeces and the organisms are probably introduced into the human host through abrasions caused by scratching rather than by actual lousebites.

In contrast to epidemic typhus, the spotted fever group of diseases represent accidental infections in man following the bite of infected ticks. Several species of tick, e.g. wood ticks and dog ticks are the principal vectors. Some may carry the organism in all stages of their development but it is the adult tick that is mainly responsible for transmitting the infection to humans. In many cases the rickettsiae are maintained in various mammalian reservoir hosts.

A number of important rickettsial infections of domesticated animals are also transmitted by tick bite, e.g. *C. ruminantium* of heartwater by the bont tick, *Amblyoma hebraeum*, and *C. phagocytophilia* of tick-borne fever by *Ixodes ricinus*. On the other hand, although *C. burnetii* of Q fever is often conveyed from animal to animal by tick-bite, man usually acquires the infection by inhaling infected dust from straw and bedding soiled by animals or from fomites, placentae and uterine discharges and, perhaps, by drinking infected milk.

Diagnosis

The isolation of a known strain of rickettsia from a typically affected individual probably constitutes the best evidence that the organism is the cause of the disease but technical difficulties limit the usefulness

of the method, especially in human medicine. In many rickettsial infections of domesticated animals a tentative diagnosis can usually be made by demonstrating the rickettsial bodies in stained blood or tissue smears, e.g. within the granular leucocytes in tick-borne fever (Plate 36.1b), the endothelial cells of the capillaries in heartwater (Plate 36.1c) and in the uterine discharges and placentae of cattle and sheep infected with Q fever (Plate 36.1a, facing p. 388).

For confirmatory diagnosis a number of serological tests are available. These include the Weil-Felix agglutination reaction with Proteus OX antigens for certain human infections, specific agglutination tests, complement-fixation tests which can detect antibodies to both group and type specific antigens, neutralization tests performed in guinea-pigs or yolk sacs of developing chicken embryos, and fluorescent antibody staining.

Biological methods for the isolation and identification of rickettsiae are also available but, as the identification procedures are not always easy and many are extremely hazardous to perform, these should never be attempted except by highly trained personnel in suitably equipped laboratories.

Whole blood, emulsified blood clot or tissue suspensions are inoculated intraperitoneally into male guinea-pigs. Rectal temperatures are taken twice daily and, if the animals fail to show signs of clinical illness such as fever, scrotal swelling (Straus reaction), emaciation or death, serum samples should be obtained by cardiac puncture and examined for the presence of specific antibodies. At autopsy, carcases should be examined for macroscopic lesions such as swellings or other changes in the spleen, liver and other tissues. Impression smears of the viscera are stained and examined for the presence of rickettsiae and tissues may be passaged to other male guinea-pigs.

It is emphasised that the Straus reaction is not produced by all species of rickettsiae. Characteristic scrotal lesions, due to inflammatory changes in the *tunica vaginalis*, are generally found in guinea-pigs infected with Rocky Mountain spotted fever (*R. rickettsii*), Fièvre boutonneuse (*R. conorii*), South African tick-bite fever (*R. rickettsii var. pijperi*), Rickettsial pox (*R. akari*) and endemic typhus (*R. mooseri* or *R. typhi*) but not by epidemic typhus (*R. prowazekii*), scrub typhus (*R. tsutsugamushi*) and only very occasionally by Q fever (*C. burnetii*).

Antigenicity

Each species of rickettsia possesses its own antigens which stimulate the production of homologous antibodies. The presence of these antigens can be demonstrated by various serological tests including agglutination, serum neutralization, complement-fixation and immunofluorescence. All species except *C. burnetii* also possess a soluble group antigen which is probably derived from the mucoid envelope of the organism and is released when shaken with ether.

Antibodies formed in the course of typhus fever and certain other rickettsial infections have been found to react, fortuitously, with the polysaccharide somatic antigens of certain non-motile O variants of *Proteus*, the so-called OX strains. This is because these rickettsiae contain an alkali-stable carbohydrate hapten, identical to a somatic constituent of these particular proteus bacilli which are readily agglutinated by sera from convalescent cases of typhus. This agglutination test is usually referred to as the Weil-Felix reaction which forms the basis of a simple and safe diagnostic procedure since it does not require the use of dangerous rickettsial antigens. Three strains are used: *Proteus* OX-2, OX-19 and OX-K, and the agglutination reactions to all three help to differentiate the important human rickettsial diseases. The agglutinins tend to disappear a few months after recovery and thus a positive Weil-Felix reaction is a useful indication of a recent infection. On the other hand, complement-fixing antibodies persist for much longer periods and provide evidence of past infection.

Treatment

Chloramphenicol, erythromycin and especially tetracyclines are effective in treating many rickettsial diseases. Unfortunately, however, they are rickettsistatic rather than rickettsicidal and do not always cure the body of organisms but rather suppress their growth. Since recovery largely depends on immunity, the course of treatment should continue for several days after the temperature has returned to normal. It should also be noted that if antibiotics are given early in the disease before immunity has developed, relapses may occur unless a second course of treatment is given about a week after the original treatment was stopped. Sulphonamides enhance the growth of many strains of rickettsiae and are generally contra-indicated.

It is of interest that rickettsiae can survive in recovered patients for many months or years and in Brill's disease, which is a recrudescence of an old epidemic typhus infection due to *R. prowazekii*, the organisms can persist for as long as 20 years in the tissues without the patient showing symptoms of the illness.

Control

In rickettsial diseases, control is often best achieved by breaking the infection chain, by treatment with antibiotics and by immunization if suitable vaccines are available.

Q fever

Synonyms Abattoir fever, Query fever, Burnet's rickettsiosis.

Definition

An infectious but usually symptomless condition of cattle, sheep, goats and other animals that is transmissible to humans in whom it causes an acute, and sometimes serious illness characterised by sudden onset, severe headache, high fever and, frequently, an interstitial 'atypical' pneumonia.

History

Q fever was first recognised as a 'local' disease among slaughterhouse workers in Brisbane, Australia, in 1933. The original name, 'abattoir fever' was changed to 'query fever' until 1937 when the causative agent was isolated and identified as belonging to the genus *Rickettsia*. In 1939 the name of *Rickettsia burnetii* was proposed for this organism but this has now been changed to *Coxiella burnetii*. In 1940 it was shown that several species of wild-living animals carried *Rickettsia* and probably formed the natural reservoir of infection in Australia. In the same year it was found that ticks belonging to the genera *Ixodes, Ornithodorus* and *Rhipicephalus* could become infected from carrier animals and shed the agent in their faeces or transmit it by tick-bite. In America during the late 1930s, it was shown that an agent isolated in Montana from the tick *Dermacentor andersoni* was identical with *C. burnetii* and that an earlier isolation made in 1926 was probably of the same organism. Although a serious epidemic of Q fever occurred among laboratory workers at Bethesda, U.S.A., in 1940, it was only during the later years of the second world war, when numerous outbreaks of a febrile pulmonary disease occurred in German and Allied troops stationed in Italy and the Balkan countries, that Q fever was recognised as a serious health problem in human and veterinary medicine.

The disease was first reported in the U.K. at the end of 1945 and a serological survey carried out in 1956 showed that about 3 per cent of healthy blood donors gave positive reactions.

Distribution

At first, Q fever was thought to be confined to Australia but the results of both cultural and serological surveys have shown that infection occurs extensively in many countries and is probably worldwide in its distribution.

Hosts affected

C. burnetii is widely distributed in nature and has been isolated from a variety of ticks and from many animal species, both wild and domesticated, including cattle, sheep, goats, horses, camels, pigs, dogs, bandicoots and birds. Although the organism rarely produces clinical illness in animals several species, including domesticated cattle and sheep, are important reservoirs of infection for man.

Aetiology

C. burnetii occurs within the cytoplasm of infected cells as large masses (20–30μm in diameter) of tightly packed organisms. Each organism occurs as a very short rod or as a tiny paired coccus with a bipolar appearance: some of the particles may be as large as medium-sized bacteria, while others are so small (0·3 × 0·15 μm) that they will pass membrane filters with an average pore diameter of 0·4 μm. They are weakly Gram-negative but stain Gram-positive when alcoholic iodine is used as a mordant. They stain readily with Romanowsky stains, appearing reddish-blue or purple with Giemsa and bright red with Macchiavello or the modified Ziehl-Neelsen (Brucella differential) method. In this respect they closely resemble both *Brucella abortus* and the agent of enzootic abortion of ewes, and their presence in ruptured cells in stained smears of uterine discharges or fetal membranes may cause confusion in diagnosis. Best results are probably obtained by staining *Coxiella* with auramine and examining the smear under the fluorescence microscope.

Although *C. burnetii* possesses many of the morphological and cultural characters of rickettsiae and does not grow in non-living media, it is unusually stable outside the host cell. It resists dessication or putrefaction and remains viable for prolonged periods at room temperature in excreta, secretions, water and milk, and in tissues suspended in 50 per cent glycerol–saline. It is relatively resistant to many physical and chemical bactericidal agents and to heat, and is capable of withstanding a temperature of 70°C for several minutes. Heating of infected raw milk at 143°F for 30 minutes is not sufficient to destroy all viable organisms but the 'flash' method of pasteurization, at 162°F for 15 seconds, is highly efficient. It is emphasised, that *C. burnetii* may remain virulent for days or weeks in milk, cream, butter and cheese. Tick faeces have been shown to retain infectivity for up to 586 days and infected dried blood for 186 days. *C. burnetii* resists merthiolate at 1 :1000, hypochlorite solutions containing 100 mg/litre of active chlorine and phenol at 1 per cent. for 24 hours, but is inactivated by 2 per cent formalin, 1 per cent lysol, 5 per cent H_2O_2 and ethyl ether. Lyophilised yolk sacs and tissue suspensions retain their infectivity for years.

Cultivation

C. burnetii is an obligate intracellular parasite that cannot be grown in non-living media. In the labora

tory it is readily propagated by the intraperitoneal inoculation of guinea-pigs, rabbits, hamsters and mice, and less easily in the yolk-sacs of 6–8-day old embryonated hens' eggs incubated at 35°C. Experimentally infected animals usually develop a fever after 5–28 days but male animals rarely show a typical Straus reaction (orchitis). In chick embryos, the infection is confined to the yolk-sac and does not spread to other tissues. Infected yolk sacs are commonly used for the preparation of antigens for complement-fixation and agglutination tests but they are dangerous to handle and frequently cause infection of laboratory workers by inhalation. The agent can also be grown in cell cultures, producing colonies of organisms in the cytoplasm of the infected cell, but often without destructive cytopathic changes of the monolayer. Growth is improved after 14 days of incubation when the metabolic level of the host cell system is reduced.

Pathogenicity

Apart from an occasional report that *C. burnetii* has been found in association with bronchopneumonia in sheep and goats, and with abortions in sheep, goats and cattle, there is little evidence that it is responsible for any specific disease in domesticated animals. Indeed, most workers are of the opinion that Q fever infections of animals produce an astonishing lack of clinical signs. In naturally and artificially acquired infections of sheep and cattle there is a marked predilection for the mammae and placenta and, as a result, heavily infected uterine discharges and fetal membranes are probably the main sources of infection for other animals and for veterinarians, abattoir workers, farmers and others engaged in agriculture. Infection in chronically affected cows and goats resides in the udder and the agent may persist in the tissues for many months or years or be excreted in the milk over very long periods.

In man, on the other hand, Q fever causes a sudden onset of illness following an incubation period of between 14–28 days. The clinical picture is similar to that of influenza and is characterised by a sharp rise in temperature, a severe headache and photophobia, accompanied by symptoms of fever and chills; but there is no rash nor local lesion of the skin as in other rickettsial infections. Primary 'atypical' pneumonia is not uncommon and there may be some involvement of the central nervous system. During the febrile stage of the illness *C. burnetii* is present in the blood stream and may be excreted in the urine and sputum, and even in the milk, but inter-human spread is rare. The course of the disease is usually short but it may vary from a few days to several months. Although the mortality rate is low, recovered patients may require a prolonged period of convalescence, but subsequent immunity is fairly solid. Convalescence from Q fever is usually uneventful but chronic infections may occur and cases have been described at autopsy in which there is endocarditis, with vegetations on the heart valves containing rickettsiae. In man, and especially in children, the disease can also run a symptomless course which is only detectable by serological examinations.

Ecology

The most likely source of infection in the original cases of Q fever among abattoir workers in Queensland was an animal reservoir in bandicoots from which two species of tick were infected. It is believed that cattle acquired the infection from ticks and that the slaughtermen became infected by inhaling the organisms from contaminated hides. Despite the fact that many different varieties of ticks can carry the organism and spread it by contaminated faeces or tick-bites, it is now believed that they play only a minor role in the epidemiology of Q fever. There can be no doubt that *C. burnetii* is now firmly established among domestic animals and that animal-to-animal infection occurs readily by direct contact, without the necessity for intermediate vectors. In fact, the introduction of a single infected animal into a herd results in the rapid dissemination of infection among all the cattle; and several surveys have shown that in some areas as many as 50 per cent of cattle may become infected within a period of 6 months.

In man, infection by inhalation is undoubtedly the commonest route by which *C. burnetii* enters the body. In rural areas large numbers of organisms reach the external environment by means of infected uterine discharges, after-births, milk and other secretions and excretions. Much of this material becomes dried on exposure to air and sunlight and the tiny infectious particles, which are among the most resistant of the non-sporogenic organisms, become suspended in the air and may be transported for considerable distances before being inhaled by susceptible humans. Hay, straw, bedding and clothing contaminated by carrier sheep and cattle also remain infective for many weeks or months, and in Italy during the Second World War a number of severe outbreaks of pneumonia occurred among servicemen sleeping on straw palliases contaminated with *C. burnetii*.

It has also been suggested that dogs, especially sheepdogs, become infected by eating contaminated placentas and consequently spread the organisms over great distances via their infected faeces. Furthermore, in a recent epidemic in Germany it was found that dogs acquired infected ticks from fields grazed by cattle and subsequently carried the infection to a number of neighbouring farms.

Man may also become infected by the oral route and it has frequently been shown that milk samples from cows, sheep and goats contain rickettsiae and that the organisms can survive for considerable periods in cream, butter, cheese and other milk by-products. A survey conducted in the U.K. in 1952 showed that 7 per cent, 2 per cent and 1 per cent of the milk from farms in England, Wales and Scotland, respectively, were contaminated with *C. burnetii*. In an American survey carried out in an area where only 5 per cent of the community consumed raw milk, it was shown that 32 per cent of 300 people who had contracted Q fever were habitual drinkers of unprocessed milk. In an outbreak in England 41 per cent of cases were thought to have resulted from the patients drinking raw milk. While there is known to be a real risk of man contracting Q fever from animals, it is remarkable that the disease does not appear to occur in certain agricultural countries such as New Zealand and Scandinavia. Birds may also play a part in spreading *C. burnetii* and several cases of Q fever have been reported in human patients associated with pigeons.

Diagnosis

Laboratory diagnosis of Q fever includes the demonstration of the organisms in stained smears and the isolation and identification of *C. burnetii* by the inoculation of laboratory animals and chick embryos with blood and sputum from human sources, and with uterine discharges, placental material or other secretions and excretions from animal sources.

The direct microscopical examination of stained smears of pathological material from human patients has only limited application but the method is of value in veterinary medicine. The presence of large masses of red-coloured spherical or cocco-bacillary particles in preparations stained by Macchiavello or modified Ziehl-Neelsen methods is strong presumptive evidence of Q fever infection provided the organisms are not mistaken for *Brucella abortus* or psittacosis agents (Plate 36.1a, facing p. 388).

The isolation of *C. burnetii* by the intraperitoneal inoculation of guinea-pigs, rabbits, mice and hamsters or by the yolk sac route in embryonated hens' eggs has been described earlier (p. 366).

A confirmatory diagnosis may be obtained by serological methods including complement-fixation, agglutination and allergic tests.

Serology

Serological tests have confirmed the individuality of *C. burnetii* and there are no cross-reactions with other species of *Rickettsia* or with antigens of the PLGV group of organisms (*Chlamydia*).

Complement-fixation is the most widely used serological test for the diagnosis of Q fever in man and animals, and is generally carried out with antigens prepared from infected yolk sacs. In human patients, complement-fixing antibodies are present in from 60–100 per cent of cases after 2–3 weeks. The maximum reaction occurs after 1–2 months and then slowly declines, but sometimes high titres persist for many months. In cattle and sheep the complement-fixation test remains positive for shorter periods than in man. The test is also useful for detecting *C. burnetii* antibodies in the whey of infected milk.

Newly isolated strains of *C. burnetii* are characteristically in Phase I and react only with antibodies in late convalescent sera, but after repeated passages in eggs the organism converts to Phase II which reacts with antibodies in early convalescent-phase sera. This host-controlled variation resembles the S–R change in bacteria.

An agglutination test can also be used for the diagnosis of Q fever and was, in fact, the first serological test employed in the study of this disease. There are many techniques for performing this reaction but the same antigen that is used for the complement-fixation test may also be used for the agglutination test. For the microslide method a dilution ten times greater than that used for the macrotube agglutination method is desirable. The test is not so widely used as that of complement-fixation, largely on account of the difficulties of standardising the technique and because of the variable results that may arise. Specific agglutinins do not usually appear before the 10th day and up to a month may pass before all the animals react. An opsonic test may also be used, but the technique is complex and some workers are doubtful as to its specificity.

Allergic tests may be employed in animals for the diagnosis of Q fever. These include an intrapalpebral reaction which has proved useful in cattle, horses and sheep, and an intradermal test which has given good results in experimentally infected guinea-pigs. In the intrapalpebral tests the animal is inoculated with killed *Coxiella* antigen intracutaneously into the lower eyelid. In a positive reaction an intense swelling of the eyelid develops in 3–4 days and the animal becomes acutely febrile. Unfortunately, the allergic response develops later than either the complement-fixation or agglutination reaction and is not suitable, therefore, for early diagnosis of infection. Moreover, the allergic response may lead to the development of antibodies in the animals tested and these may subsequently interfere with agglutination tests carried out at a later date. Its use in uninfected animals does not lead to false results in later complement-fixation tests.

Other serological methods include neutralization, antiglobulin sensitization and conglutinating complement absorption tests, all of which are of value in

experimental studies but not for the diagnosis of disease under natural conditions.

Treatment
Antibiotic therapy is of very limited value for the treatment of animal infections, and attempts to eradicate *C. burnetii* from the milk of cows by intramammary and intravenous injections of tetracyclines have proved unsuccessful. It is of interest that cortisone administered to experimentally infected guinea-pigs is said to bring about a more rapid recovery.

In the medical field there is some evidence that chloramphenicol, tetracycline and lincomycin are active against *C. burnetii.* Unfortunately, this activity is bacteriostatic rather than bactericidal, and in several cases illness has recurred despite prolonged courses of treatment.

Control
Ether-extracted yolk sac vaccines and live attenuated vaccines have been used to protect laboratory workers, livestock attendants and abattoir workers against Q fever, but with varying degrees of success. Many of the preparations produce severe reactions including sterile abscess formation.

The use of inactivated yolk sac vaccines in cattle produces some immunity and may lead to a reduction in the incidence of infection in dairy herds, but unfortunately the method is not practicable on a large scale.

Raw milk is a likely source of infection and should be sterilized by the 'flash' method of pasteurization.

In infected herds every precaution should be taken to remove and destroy placentas, straw, bedding and other materials soiled by excretions and secretions. Contaminated utensils and vehicles used for animal transport should be thoroughly cleansed and disinfected.

THE EHRLICHIAE

Ehrlichieae, the second tribe of the family *Rickettsiaceae* consists of at least four genera, *Ehrlichia*, *Cytoecetes*, *Cowdria* and *Neorickettsia*. In contrast to rickettsiae, the ehrlichia group of organisms occur mostly in circulating monocytes and are pathogenic for animals but not for man. The genus *Ehrlichia* includes three species, *E. canis* which causes an important 'rickettsiosis' of dogs, and two other very similar species, *E. bovis* and *E. ovina*, which are associated with mild illnesses in ruminants.

About ten years after these three species were first described in 1935–37, organisms were observed in monocytes and occasionally lymphocytes of pigeons, and in monocytes and endothelial cells of pigs. It is possible that these latter two species will be included in the genus when more information becomes available.

Rickettsiosis of dogs

Synonyms
Canine rickettsiosis. Canine typhus. Nairobi bleeding disease.

Definition
An infectious, febrile, tick-borne disease of dogs. The severity of the clinical symptoms is greatly enhanced by concurrent infections with *Babesia*, *Leishmania* and *Bartonella*. Acute illness is characterised by hyperplasia of the lymph nodes, nasal and ocular discharges, emaciation and death.

History and Distribution
A virulent rickettsial infection of dogs was first described in Algeria in 1935 and in Kenya in 1937. The causative agent was identified in 1938 and the name *Rickettsia canis* was changed in 1945 to *Ehrlichia canis*. In subsequent years canine rickettsiosis has been reported from other parts of Africa including South Africa, Nigeria, Uganda, Zaire and the Sudan. It occurs along the Mediterranean littoral and may also be present in Iran, India, Sri Lanka and the U.S.A. Thus, canine rickettsiosis has a very wide distribution and it seems likely that the occurrence of the causative organism corresponds fairly closely with that of *E. bovis* and *E. ovina*.

Hosts affected
The natural disease affects both wild and domestic dogs, and symptomless infections can be produced in jackals and Macaca monkeys.

Aetiology
Ehrlichiae are present in the circulating blood of affected dogs and examination of stained blood films shows that they occur almost exclusively in the cytoplasm of the monocytes. There are a number of reports that they may occasionally parasitize neutrophils, histiocytes and endothelial cells of the meningeal vessels.

In most cases the organisms are found as clusters or colonies within the cytoplasm of infected cells. These colonies may be small and contain only a few deeply staining granules or they may be large, consisting of aggregates of amorphous particles which tend to fill the cytoplasm and indent the nucleus. The number of colonies in a cell is usually between one and 8 but may vary from one to as many as 20 or even 40. The individual particles are pleomorphic, spherical or cocco-bacillary bodies