VOLUME - III
ISSUE - XXVIII
JUL / AUG 2008

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

With a worldwide footprint, Rickettsiosis are diseases that are gaining increasing significance as important causes of morbidity and to an extent mortality too. Encompassed within these are two main groups, viz., Rickettsia spotted fever group and the Typhus group (they differ in their surface exposed protein and lipopolysaccharide antigens). A unique thing about these organisms is that, though they are gram-negative bacilli, they cannot be cultured in the traditional ways that we employ to culture regular bacteria. They need viable eukaryotic host cells and they require a vector too to complete their run up to the human host. Asia can boast of harbouring Epidemic typhus, Scrub typhus, Boutonneuse fever, North Asia Tick typhus, Oriental spotted fever and Q fever. The pathological feature in most of these fevers is involvement of the microvasculature (vasculitis/ perivasculitis at various locations). Most often, the clinical presentation initially is like Pyrexia of Unknown Origin. As they can't be cultured by the routine methods, the diagnostic approach left is serological assays. A simple to perform investigation is the Weil-Felix reaction that is based on the cross-reactive antigens of OX-19 and OX-2 strains of *Proteus vulgaris*. Diagnosed early, Rickettsiae can be effectively treated by the most basic antibiotics like tetracyclines/ doxycycline and chloramphenicol. Epidemiologically almost omnipresent, the DISEASE DIAGNOSIS segment of this issue comprehensively discusses Rickettsiae. Vector and reservoir control, however, is the best approach in any case.

There has been an array of Breast Tumour Markers that has littered the medical market. According to the latest International guidelines only a few of them are of actual clinical relevance. Only 9 of the available lot are said to be importance vis-à-vis prevention, screening, treatment and surveillance of breast cancers. INTERPRETATION section outlines the clinically important breast cancer markers.

MRSA will find its ending in the TROUBLESHOOTING section of this issue. The last of the preventive aspects are given for our day-to-day usage in the clinical settings.

Amongst all the serious talk, BOUQUET has not been forgotten, a few jokes, a few words of advise and 4 simple but tricky questions cap it all.

DISEASE DIAGNOSIS

RICKETTSIAE

INTRODUCTION

Rickettsiae are small, Gram-negative bacilli that have evolved in such close association with arthropod hosts that they are adapted to survive within the host cells. They represent a rather diverse collection of bacteria, and therefore listing characteristics that apply to the entire group is difficult. The common threads that hold the rickettsiae into a group are their epidemiology, their obligate intracellular lifestyle, and the laboratory technology required to work with them. In the laboratory, rickettsiae cannot be cultivated on agar plates or in broth, but only in viable eukaryotic host cells (e.g., in cell culture, embryonated eggs, or susceptible animals). The exception, which shows the artificial nature of using obligate intracellular parasitism as a defining phenotypic characteristic, is *Bartonella (Rochalimaea) quintana*, which is cultivable axenically, but was traditionally considered as a rickettsia. The diversity of rickettsiae is demonstrated in the variety of specific intracellular locations where they live and the remarkable differences in their major outer membrane proteins and genetic relatedness. An example of extreme adaptation is that the metabolic activity of *Coxiella burnetii* is greatly increased in the acidic environment of the phagolysosome, which is a harsh location for survival for most other organisms. Obligate intracellular parasitism among bacteria is not unique to rickettsiae. Chlamydiae also have evolved to occupy an intracellular niche, and numerous bacteria (e.g., *Mycobacteria*, *Legionella*, *Salmonella*, *Shigella*, *Francisella*, and *Brucella*) are facultative intracellular parasites. In contrast with chlamydiae, all rickettsiae can synthesize ATP. *Coxiella burnetii* is the only rickettsia that appears to have a developmental cycle.

Some organisms in the family *Rickettsiaceae* are closely related genetically (e.g., *Rickettsia rickettsii*, *R akari*, *R prowazekii*, and *R typhi*); others are related less closely to *Rickettsia* species (e.g., *Ehrlichia* and *Bartonella*); and others not related to *Rickettsia species* (e.g., *C burnetii*). The phenotypic traits of the medically important organism *Orientia* (*Rickettsia*) *tsutsugamushi* suggest that the species may be an example of convergent evolution in a similar ecologic niche. Rickettsioses are zoonoses that, except for Q fever, are usually transmitted to humans by arthropods (tick, mite, flea, louse, or chigger) . Therefore, their geographic distribution is determined by that of the infected arthropod, which for most rickettsial species is the reservoir host. Rickettsiae are important causes of human diseases in the United States (Rocky Mountain spotted fever, Q fever, murine typhus, sylvatic typhus, human monocytic ehrlichiosis, human granulocytic ehrlichiosis, and rickettsialpox) and around the world (Q fever, murine typhus, scrub typhus, epidemic typhus, boutonneuse fever, and other spotted fevers) (Table 1).

TABLE 1: Distinguishing Characteristics of Rickettsial Diseases

Disease	Organism	Geographic Distribution	Ecological Niche	Transmission to Human	Pathological Basis (Injury)	Rash	Eschar	Serological Diagnosis
Rickettsia spotted fever group								
Rocky mountain spotted fever	*R. ricktettsii*	North, Central and South America	Ticks	Tick bite	Microvascular	90%	Rare	IFA, LA, IHA, EIA, CF
Boutonneuse fever	*R.conorii*	Mediterenean Basin, Africa, Indian Subcontinent	Ticks	Tick bite	Microvascular	97%	50%	IFA, LA, CF
Rickettsial pox	*R.akari*	North America, Europe, Korea		Mite bite	Microvascular	100%	92%	IFA, CF
North-Asian tick typhus	*R.sibrica*	Russia China, Mongelia, Pakistan	Ticks	Tick bite	Microvascular	100%	77%	IFA, CF
Queensland tick typhus	*R. australis*	Australia	Ticks	Tick bite	Microvascular	92%	75%	CF
Oriental spotted fever	*R. japonica*	Japan	Unknown	Arthopod bite	Microvascular	100%	48%	IFA, CF
Typhus group								
Epidemic typhus	*R.prowazekii*	Africa, South America Mexico, Asia, Eastern United States	Humans, Flying Squirrels	Louse feces	Microvascular	100%	None	IFA, LA, IHA, EIA, CF
Murine typhus	*R.typhi*	Worldwide	Fleas, Rats	Flea feces	Microvascular	80%	None	IFA, LA, IHA, EIA, CF
Orientia Scrub typhus	*O.tsutsugamaushi*	Asia, South pacific, Australia	Chiggers	Chigger bite	Microvascular	50%	35%	IFA, EIA,
Sennetsu rickettissiosis	*E. sennetsu*	Japan	Unknown	Unknown	Lymphoid hyperplasia	Very Rare	None	IFA, CF
Human Monocytic ehrlichiosis	*E. chaffeenis*	North America, Europe, Africa	Deer	Tick bite	Granulomas	40%	None	IFA
Human granulocytic ehrlichiosis	*E.phagocytophila*	North America	Unknown	Tick bite	Unknown	Rare	None	IFA
Coxiella Q fever	*C.turnelli*	Worldwide	Ticks, Ungulates		Pneumonia, granulomas of liver and bone marrow, chronic endocarditis	Rare	None	IFA, EIA, CF
Bartonella Trench fever	*B.quintana*	North America, Europe, Africa	Humans	Louse bite or feces	Perivasculitis	Yes	None	IHA, EIA, CF
Cat scratch disease	*B.hensalae*	North America Worldwide	Cats	Cat scratch or bite	Granulomas, vascular proliferation	Rare	None	IFA, EIA
Oroyos fever	*B.bacilliformis*	South America	Humans	Sandfly bite	Acute hemolysis chronic vascular proliferation	Yes, Chronic phase	None	EIA

IFA - Indirect Fluorescence Antibody Test, LA - Latex Assay, IHA - Immuno Heamagglutination Assay, EIA - Enzyme Immuno Assay, CF - Complement Fixation.

Rickettsiae of the Spotted Fever and Typhus Groups

The rickettsial diseases are arranged into several major categories (Table1), the first two of which are the spotted fever and typhus fever groups.

Clinical Manifestations

Rocky Mountain Spotted Fever: Rocky Mountain spotted fever is among the most severe of human infectious diseases, with a mortality of 20 to 25 percent unless treated with an appropriate antibiotic. The severity and mortality are greater for men, elderly persons, and black men with glucose-6-phosphate dehydrogenase deficiency. Although, in theory, the disease is always curable by early, appropriate treatment, the case fatality rate is still 4 percent. The incidence of disease parallels the geographic distribution of infected *Dermacentor variabilis* ticks in the eastern United States and *D andersoni* in the Rocky Mountain states, where the infection was first recognized. Rocky Mountain spotted fever was subsequently recognized in the eastern United States. The incidence has declined in the Rocky Mountain states and increased dramatically in the southeastern,

United States and Oklahoma. Currently most cases actually occur in the Atlantic states from Maryland to Georgia, as well as in Oklahoma, Missouri, Kansas, Ohio, Tennessee, Arkansas, and Texas, although cases are reported in nearly every state. In the southeastern states, the disease occurs during the seasonal activity of *D variabilis* ticks (April through September) and affects children more frequently than adults. Significant changes in incidence do occur. From a low of 199 cases reported in 1959, the annual number of cases rose steadily to a peak of 1,192 cases in 1981, with a subsequent decline and plateau of approximately 700 cases since 1985. The reasons for these fluctuations are unclear. The rickettsiae are maintained in nature principally by transovarial transmission from infected female ticks to infected ova that hatch into infected larval offspring (Fig.1). A low rate of acquisition of rickettsiae by uninfected ticks occurs when the ticks feed upon small mammals with enough rickettsiae in their blood to establish tick infection. This effect replenishes lines of infected ticks that are occasionally killed by massive rickettsial overgrowth. A recently observed factor of potential importance in this balance of nature is the interference phenomenon, by which infection of ticks with nonpathogenic spotted fever group rickettsiae prevents the establishment of infection by *R rickettsii*.

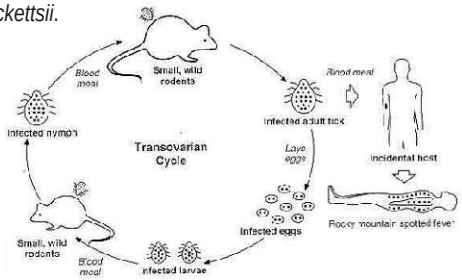

FIG. 1: Transovarian passage of *R rickettsii* in the tick vector is an important cycle in maintaining the infection in nature from one generation of tick to another.

Horizontal transmission (i.e., acquisition of the bacteria by uninfected ticks feeding on infected animals) occurs less often and is not shown. Humans become incidental hosts after being bitten by an infected adult tick.

The clinical gravity of Rocky Mountain spotted fever is due to severe damage to blood vessels by *R rickettsii*. This organism is unusual among rickettsiae in its ability to spread and invade vascular smooth muscle cells as well as endothelium. Damage to the blood vessels in the skin in locations of the rash leads to visible hemorrhages in one-half of all infected persons (Fig. 2). Attempted plugging of vascular wall destruction consumes platelets, with consequent thrombocytopenia also affecting approximately one-half of the patients.

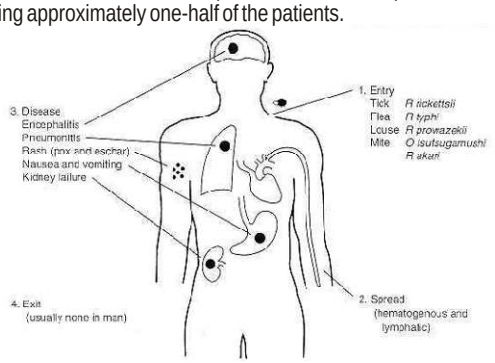

FIG. 2: Common clinical manifestations of the rickettsial diseases.

Rickettsialpox and Other Spotted Fevers

In the 1940s an epidemic of disease characterized by fever, rash, and cutaneous necrosis appeared in one area of New York City. The etiology was traced to *R akari* transmitted by the bite of mites (*Liponyssoides sanguineus*) that infested the numerous mice in an apartment house in this area. The disease was named rickettsialpox because many patients had blister-like rashes resembling those of chickenpox. Epidemics were diagnosed in other cities, and *R akari* has been isolated in other countries (e.g., the Ukraine). Perhaps because this nonfatal disease is seldom considered by physicians, or its incidence is truly low, the diagnosis is rarely made. Transovarial transmission in the mite and periodic documentation of cases assure us that the etiologic agent is still with us. Boutonneuse fever, so called because of the papular rash in some cases, has many synonyms, reflecting different geographic regions of occurrence (e.g., Mediterranean spotted fever, Kenya tick typhus, and South African tick bite fever). Cases are observed in the United States in travelers returning from endemic areas. The agent, *R conorii*, is closely related to *R. rickettsii*. Severe disease resembling Rocky Mountain spotted fever can cause death in high-risk groups (e.g., elderly, alcoholic, and glucose-6-phosphate dehydrogenase-deficient patients). Cutaneous necrosis caused by rickettsial vascular infection at the tick bite site of inoculation, known as an eschar or tache noire, is observed in only half the patients with boutonneuse fever. The curiously high prevalence of antibodies reactive with *R conorii* in healthy populations in endemic regions might be explained by missed diagnosis of prior illness, subclinical infection, infection with an antigenically related but less pathogenic rickettsia, or nonspecificity of the laboratory test. Other spotted fevers occur in geographic distributions of little concern to many physicians in the United States. North Asian tick typhus caused by *R sibirica*, Queensland tick typhus caused by *R australis*, and the recently discovered oriental spotted fever caused by *R japonica* demonstrate that spotted fever group rickettsiae occur worldwide.

Epidemic Typhus and Brill-Zinsser Disease

Epidemics of louse-borne typhus fever have had important effects on the course of history; for example, typhus in one army but not in the opposing force has determined the outcome of wars. Populations have been decimated by epidemic typhus. During and immediately after World War I, 30 million cases occurred, with 3 million deaths. Unsanitary, crowded conditions in the wake of war, famine, flood, and other disasters and in poor countries today encourage human louse infestation and transmission of *R prowazekii*. Epidemics usually occur in cold months in poor highland areas, such as the Andes, Himalayas, Mexico, Central America, and Africa. Lice live in clothing, attach to the human host several times daily to take a blood meal, and become infected with *R prowazekii* if the host has rickettsiae circulating in the blood. If the infected louse infests another person, rickettsiae are deposited on the skin via the louse feces or in the crushed body of a louse. Scratching inoculates rickettsiae into the skin. Between epidemics *R prowazekii* persists as a latent human infection. Years later, when immunity is diminished, some persons suffer recrudescent typhus fever (Brill-Zinsser disease). These milder sporadic cases can ignite further epidemics in a susceptible louse-infested population. In the United States Brill-Zinsser disease is seen in immigrants who suffered typhus fever before entering the country. In the eastern United States, sporadic human cases of *R prowazekii* infection have been traced to a zoonotic cycle involving flying squirrels and their own species of lice and fleas.

Murine Typhus

Murine typhus is prevalent throughout the world, particularly in ports, countries with warm climates, and other locations where rat populations are high. *Rickettsia typhi* is associated with rats and fleas, particularly the oriental rat flea, although other ecologic cycles (e.g., opossums and cat fleas) have been implicated. Fleas are infected by transovarian transmission or by feeding on an animal with rickettsiae circulating in the blood. Rickettsiae are shed from fleas in the feces, from which humans acquire the infection through the skin, respiratory tract, or conjunctiva. During the 1940s more than 4,000 cases of murine typhus occurred annually in the United States. The incidence declined coincident with increased utilization of the insecticide DDT. Although the infection and clinical involvement affects the brain, lungs, and other visceral organs in addition to the skin, mortality in humans is less than 1 percent.

Structure, Classification, and Antigenic Types: *Rickettsia* species include two antigenically defined groups that are closely related genetically but differ in their surface-exposed protein and lipopolysaccharide antigens. These are the spotted fever and typhus groups. The organisms in these groups are smaller (0.3 μm by 1.0 μm) than most Gram-negative bacilli that live in the extracellular environment. They are surrounded by a poorly characterized structure that is observed as an electron-lucent zone by transmission electron microscopy and is considered to represent a polysaccharide-rich slime layer or capsule. The cell wall contains lipopolysaccharides, a major component that differs antigenically between the typhus group and the spotted fever group. These rickettsiae also contain major outer membrane proteins with both cross-reactive antigens and surface-exposed epitopes that are species specific and easily denatured by temperatures above 54°C. The major outer membrane protein of typhus group rickettsiae has an apparent molecular mass of 120,000 Da. Spotted fever group rickettsiae generally have a pair of analogous proteins with some diversity of their molecular masses. *Rickettsia prowazekii* has a transport mechanism that exchanges ATP for ADP in its intracellular environment, thus providing a means to usurp host cell energy sources under favorable circumstances. Rickettsiae also are able to synthesize ATP via metabolism of glutamate. Adaptation to the intracellular environment is further evidenced in a variety of transport mechanisms to obtain crucial substances such as particular amino acids from cytoplasmic pools in the host cell.

These adaptations and the presence of numerous independent metabolic activities demonstrate that rickettsiae are not degenerate forms of bacteria, but rather have evolved successfully for survival with an intracellular life-style.

Pathogenesis: Rickettsiae are transmitted to humans by the bite of infected ticks and mites and by the feces of infected lice and fleas. They enter via the skin and spread through the bloodstream to infect vascular endothelium in the skin, brain, lungs, heart, kidneys, liver, gastrointestinal tract, and other organs (Fig. 1). Rickettsial attachment to the endothelial cell membrane induces phagocytosis, soon followed by escape from the phagosome into the cytosol (Fig. 3). Rickettsiae divide inside the cell. *Rickettsia prowazekii* remains inside the apparently healthy host cell until massive quantities of intracellular rickettsiae accumulate and the host cell bursts, releasing the organisms. In contrast, *R rickettsii* leaves the host cell via long, thin cell projections (filopodia) after a few cycles of binary fission. Hence, relatively few *R rickettsii* organisms accumulate inside any particular cell, and rickettsial infection spreads rapidly to involve many other cells. Perhaps because of the numerous times the host cell membrane is traversed, there is an influx of water that is initially sequestered in cisternae of cytopathically dilated rough endoplasmic reticulum in the cells more heavily infected with *R rickettsii*.

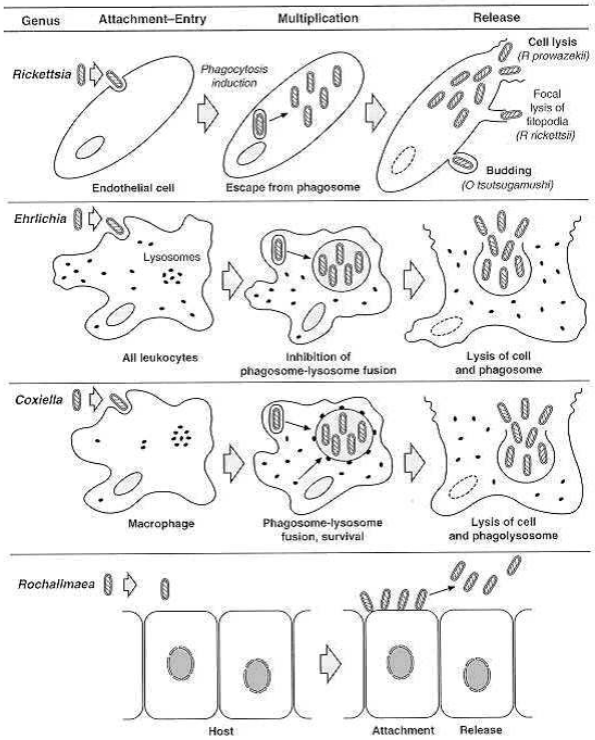

FIG. 3: Pathogenesis of the rickettsial agents illustrating unique aspects of their interactions with eukaryotic cells.

The bursting of endothelial cells infected with *R prowazekii* is a dramatic pathologic event. The mechanism is unclear, although phospholipase activity, possibly of rickettsial origin, has been suggested. Injury to endothelium and vascular smooth muscle cells infected by *R rickettsii* seems to be caused directly by the rickettsiae, possibly through the activity of a rickettsial phospholipase or rickettsial protease or through free-radical peroxidation of host cell membranes. Host immune, inflammatory, and coagulation systems are activated and appear to benefit the patient. Cytokines and inflammatory mediators account for an undefined part of the clinical signs. Rickettsial lipopolysaccharide is biologically relatively nontoxic and does not appear to cause the pathogenic effects of these rickettsial diseases. The pathologic effects of these rickettsial diseases originate from the multifocal areas of endothelial injury with loss of intravascular fluid into tissue spaces (edema), resultant low blood volume, reduced perfusion of the organs, and disordered function of the tissues with damaged blood vessels (e.g., encephalitis, pneumonitis, and hemorrhagic rash).

Diagnosis: Diagnosis of rickettsial infections is often difficult. The clinical signs and symptoms (e.g., fever, headache, nausea, vomiting, and muscle aches) resemble many other diseases during the early stages when antibiotic treatment is most effective. A history of exposure to the appropriate vector tick, louse, flea, or mite is helpful but cannot be relied upon. Observation of a rash, which usually appears on or after day 3 of illness, should suggest the possibility of a rickettsial infection but, of course, may occur in many other diseases also. Knowledge of the seasonal and geographic epidemiology of rickettsioses is useful, but is inconclusive for the individual patient. Except for epidemic louse-borne typhus, rickettsial diseases strike mostly as isolated single cases in any particular neighborhood. Therefore, clinico-epidemiologic diagnosis is ultimately a matter of suspicion, empirical treatment, and later laboratory confirmation of the specific diagnosis. Because rickettsiae are both fastidious and hazardous, few laboratories undertake their isolation and diagnostic identification (Fig. 4). Some laboratories are able to identify rickettsiae by immunohistology in skin biopsies as a timely, acute diagnostic procedure, but to establish the diagnosis physicians usually rely on serologic demonstration of the development of antibodies to rickettsial antigens in serum collected after the patient has recovered. Currently, assays that demonstrate antibodies to rickettsial antigens themselves i.e. Weil-Felix test that is based on the cross-reactive antigens of OX-19 and OX-2 strains of *Proteus vulgaris* is used.

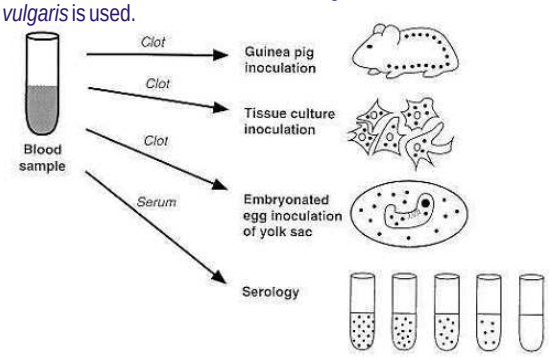

FIG. 4: Laboratory methods used in confirming a diagnosis of rickettsial infection. These bacteria can be cultivated as indicated, but use of serology is more common.

Control: Although early treatment with doxycycline, tetracycline, or chloramphenicol is effective in controlling the infection in the individual patient, this action has no effect on rickettsiae in their natural ecologic niches (e.g., ticks). Human infections are prevented by control of the vector and reservoir hosts. Massive delousing with insecticide can abort an epidemic of typhus fever. Prevention of attachment of ticks and their removal before they have injected rickettsiae into the skin reduces the likelihood of a tick-borne spotted fever. Control of rodent populations and of the access of rats and mice to homes and other buildings may reduce human exposure to *R typhi* and *R akari*. Vaccines against spotted fever and typhus group rickettsiae have been developed empirically by propagation of rickettsiae in ticks, lice, embryonated hen eggs, and cell culture. Vaccines containing killed organisms have provided incomplete protection. A live attenuated vaccine against epidemic typhus has proved successful, but is accompanied by a substantial incidence of side effects, including a mild form of typhus fever in some persons. The presence of strong immunity in convalescent subjects indicates that vaccine development is feasible, but it requires further study of rickettsial antigens and the effective anti-rickettsial immune response. T-lymphocyte-mediated immune mechanisms, including effects of the lymphokines, gamma interferon tumor necrosis factor, and interleukin-1, seem most important.

Orientia (Rickettsia) tsutsugmushi and Scrub Typhus: Although the agents of scrub typhus bear a single taxonomic name, *Orientia* (*Rickettsia*) *tsutsugamushi*, these interrelated organisms are somewhat heterogeneous and differ strikingly from *Rickettsia* species of the spotted fever and typhus groups.

Clinical Manifestations: Patients with scrub typhus often have only fever, headache, and swollen lymph nodes and in some cases myalgia, gastrointestinal complains, or cough beginning 6 to 21 days following exposure to the vector. Fewer than half of the patients have an eschar at the site where the larval mite fed and the classic rash. The mortality varies but averages 7 percent without anti-rickettsial treatment.

Structure, Classification, and Antigenic Types: *Orientia* (*Rickettsia*) *tsutsugamushi* is a very labile rickettsia that is particularly difficult to propagate and separate from the host cells in which it grows. In contrast with spotted fever group and typhus group rickettsiae, *O tsutsugamushi* does not seem to possess lipopolysaccharides, peptidoglycan, a slime layer, or other T-independent antigens. The rickettsial cell wall consists of proteins linked by disulfide bonds. Antigenically distinguishable strains represent only part of what seems to be a great antigenic mosaic. Immunity to infection with the homologous strain wanes within a few years; cross-protective immunity to heterologous strains disappears within a few months. The reasons for this lack of long-term immunity are unclear.

Pathogenesis: *Orientia* (*Rickettsia*) *tsutsugamushi* is injected into the skin during feeding by a larval trombiculid mite (chigger). An eschar often forms at this location. Rickettsiae spread via the bloodstream and damage the microcirculation of the skin (rash), lungs (pneumonitis), brain (encephalitis), and other organs. The generalized enlargement of lymph nodes is unique among rickettsial diseases. *Orientia* (*Rickettsia*) *tsutsugamushi* is phagocytosed by the host cell, escapes from the phagosome into the cytosol, divides by binary fission, and is released from projections of the cell membrane. The pathogenic mechanism of *O tsutsugamushi* is not known.

Epidemiology: Scrub typhus occurs where chiggers infected with virulent rickettsial strains feed upon humans. *Leptotrombidium deliense* and other mites are found particularly in areas where regrowth of scrub vegetation harbors the *Rattus* species that are hosts for the mites. Some of these foci are quite small and have been referred to as mite islands. Because *O tsutsugamushi* is transmitted transovarially from one generation of mites to the next, these dangerous areas tend to persist for as long as the ecologic conditions, including scrub vegetation, persist. Truly one of the neglected diseases, scrub typhus occurs over a vast area, including Japan, China, the Philippines, New Guinea, Indonesia, other islands of the southwest Pacific Ocean, southeastern Asia, northern Australia, India, Sri Lanka, Pakistan, Russia, and Korea. Recognized in western countries mainly because of large numbers of infections of military personnel during World War II and the Vietnam War, scrub typhus perennially affects native populations. Reinfection and undiagnosed infections are highly prevalent. Mortality ranges from 0 to 35 percent and has not been correlated with any specific factor.

Diagnosis: Classic textbook cases with fever, headache, eschar, and rash are far outnumbered by cases that lack rash or eschar. Such cases are usually misdiagnosed. Laboratory diagnosis is unavailable in many areas where scrub typhus occurs. Isolation of rickettsiae requires inoculation of mice or cell culture. Serologic diagnosis is made by specific methods (indirect fluorescence antibody test or enzyme immunoassay) or by the older method of demonstrating cross-reactive antibodies that agglutinate the OXK strain of *P mirabilis*.

Control: Scrub typhus can be treated with doxycycline, tetracycline, or chloramphenicol. Chigger repellents may prevent exposure. Prophylaxis with weekly doses of doxycycline during and for 6 weeks after exposure protects against scrub typhus. Attempts to develop a safe, effective vaccine have failed.

Ehrlichia

According to the evolutionary scheme suggested by 16S rRNA sequence homology, ehrlichiae are genetically related to *Rickettsia* species. The genus *Ehrlichia* contains Gram-negative bacteria that reside in a cluster (morula) within membrane-bound cytoplasmic vacuoles of monocytes and macrophages, or polymorphonuclear leukocytes. Ehrlichiae have been implicated as the agents of diseases of horses (*E risticii* and *E equi*), dogs (*E canis*, *E ewingii* and *E platys*, a platelet pathogen), and other animals. *Ehrlichia sennetsu* causes a human disease in Japan resembling infectious mononucleosis. Ehrlichiae are unusual in their cell wall structure and they can establish persistent infections. In 1987 the first case of human ehrlichiosis was reported in the United States. A severely ill man with multiorgan system involvement had morula inclusions demonstrated in peripheral blood leukocytes. Subsequently, cases of human monocytic ehrlichiosis have been documented mainly in eastern and southern states between New Jersey and Texas. The infection has varied from severe and sometimes fatal, mimicking Rocky Mountain spotted fever, to oligosymptomatic and asymptomatic forms. A history of tick bite and the seasonal and geographic occurrence correlate with the predominant tick vector, *Amblyomma americanum*. Illness is often accompanied by leukopenia, thrombocytopenia, and damage to the liver. Lesions include perivasculitis in the central nervous system, kidney, heart, and lungs and granulomas in the bone marrow and liver. Clinical diagnosis is difficult. Laboratory diagnosis by indirect fluorescence antibody assay or polymerase chain reaction is not widely available. *Ehrlichia chaffeensis* morulae are difficult to detect in peripheral blood leukocytes. In 1994 another serious new infectious disease, human granulocytic ehrlichiosis, was reported. Ehrlichiae seen within morulae in neutrophils in smears of peripheral blood were identified as very closely related to *E phagocytophila* (a European tick-transmitted infection of sheep, cattle, goats, and deer) and *E equi*. The causative organism, like other granulocytic ehrlichiae, has never been cultivated. Human granulocytic ehrlichiosis has been associated with the deer tick, *Ixodes scapularis*, and thus is found as far north as Minnesota, Wisconsin, and New England. Laboratory diagnosis is practically achieved by visualizing morulae in neutrophils, as serology and polymerase chain reaction for the agent are presently research procedures. Sometimes fatal, human granulocytic ehrlichiosis, like *E chaffeensis* infection, can be treated effectively with doxycycline.

Coxiella burnetii and Q Fever

Coxiella burnetii is sufficiently different genetically from the other rickettsial agents that it is placed in a separate group. Unlike the other agents, it is very resistant to chemicals and dehydration. Additionally, its transmission to humans is by the aerosol route, although a tick vector is involved in spread of the bacteria among the reservoir animal hosts.

Clinical Manifestations: Q fever is a highly variable disease, ranging from asymptomatic infection to fatal chronic infective endocarditis. Some patients develop an acute febrile disease that is a nonspecific influenza-like illness or an atypical pneumonia. Other patients are diagnosed after identification of granulomas in their liver or bone marrow. The most serious clinical conditions are chronic *C burnetii* infections, which may involve cardiac valves, the central nervous system, and bone.

Clinical manifestations of Q fever.

Structure,Classification, and Antigenic Type: *Coxiella burnetii* is an obligately intracellular bacterium with some peculiar characteristics. It is small, generally 0.25 μm by 0.5 to 1.25 μm. However, there is considerable ultrastructural pleomorphism, including small- and large-cell variants and possible endospore-like forms, suggesting a hypothetical developmental cycle. Among rickettsiae, *C burnetii* is the most resistant to environmental conditions, is the only species that resides in the phagolysosome, is activated metabolically by low pH, and has a plasmid. The extensive metabolic capacity of *C burnetii* suggests that its obligate intracellular parasitism is a highly evolved state rather than a degenerate condition. The cell wall is typical of Gram-negative bacteria and contains peptidoglycan, proteins, and lipopolysaccharide. When propagated under laboratory conditions in embryonated eggs or cell culture, *C burnetii* undergoes phase variation analogous to the smooth to rough lipopolysaccharide variation of members of the Enterobacteriaceae. Phase I is the form found in nature and in human infections. The phase II variant contains truncated lipopolysaccharide, is avirulent, and is a poor vaccine.

Pathogenesis: Human Q fever follows inhalation of aerosol particles derived from heavily infected placentas of sheep, goats, cattle, and other mammals. *Coxiella burnetii* proliferates in the lungs, causing atypical pneumonia in some patients. Hematogenous spread occurs, particularly to the liver, bone marrow, and spleen. The disease varies widely in severity, including asymptomatic, acute, subacute, or chronic febrile disease, granulomatous liver disease, and chronic infection of the heart valves. The target cells are macrophages in the lungs, liver, bone marrow, spleen, heart valves, and other organs. *Coxiella burnetii* is phagocytosed by Kupffer cells and other macrophages and divides by binary fission within phagolysosomes. Apparently it is minimally harmful to the infected macrophages. Different strains have genetic and phenotypic diversity. The lipopolysaccharides are relatively nonendotoxic. Host-mediated pathogenic mechanisms appear to be important, especially immune and inflammatory reactions, such as T-lymphocyte-mediated granuloma formation.

Epidemiology: *Coxiella burnetii* infects a wide variety of ticks, domestic livestock, and other wild and domestic mammals and birds throughout the world. Most human infections follow exposure to heavily infected birth products of sheep, goats, and cattle, as occurs on farms, in research laboratories, and in abattoirs. *Coxiella burnetii* is also shed in milk, urine, and feces of infected animals. Animals probably become infected by aerosol and by the bite of any of the 40 species of ticks that carry the organisms.

Diagnosis: Clinical diagnosis depends upon a high index of suspicion, careful evaluation of epidemiologic factors, and ultimately, confirmation by serologic testing. Although *C burnetii* can be isolated by inoculation of animals, embryonated hen eggs, and cell culture, very few laboratories undertake this biohazardous approach. Likewise, the diagnosis is seldom made by visualization of the organisms in infected tissues. Acute Q fever is diagnosed by demonstration of the development of antibodies to protein antigens of *C burnetii* phase II organisms. Chronic Q fever endocarditis is diagnosed by demonstration of a high titer of antibodies, particularly IgG and IgA, against the lipopolysaccharide antigens of *C burnetii* phase I organisms in patients with signs of endocarditis whose routine blood cultures contain no organisms.

Control: Antibiotic treatment is more successful in ameliorating acute, self-limited Q fever than in curing life-threatening chronic endocarditis. Reduction in exposure to these widespread organisms is difficult because some serologically screened animals that have no detectable antibodies to *C burnetii* still shed organisms at parturition. Persons with known occupational hazards (e.g., Australian abattoir workers) have benefitted from a vaccine composed of killed phase I organisms. This vaccine is not readily available, but offers promise for development of safe, effective immunization.

Bartonella

It has been recognized recently that organisms thought to be closely related to rickettsiae such as the louse-borne causative agent of trench fever, *Bartonella* (formerly *Rochalimaea*) *quintana*, in fact, belong in the genus *Bartonella*. These bacteria can be cultivated in cell-free medium and hence do not fit the criterion of definition of rickettsiae as obligately intracellular bacteria. *Bartonella quintana* infections were a serious medical problem during World War I. Soldiers in the trenches were infested with body lice that passed *B quintana* in their feces onto the skin. Individuals who have recovered from trench fever continue to have *R quintana* circulating in this stage of infection and may serve as sources of infection for lice, which can transmit the infection to others. In association with the AIDS epidemic, another species *B henselae* (in addition to *B quintana)* has been discovered to be the cause of opportunistic infections often masquerading as hemangioma-like lesions of skin and visceral organs, bacillary angiomatosis. *Bartonella henselae* was recognized subsequently to be the long sought after cause of cat scratch disease, which usually manifested as a self-limited enlargement and inflammation of lymph nodes of several months duration in the regional drainage of a cat scratch or bite. *Bartonella bacilliformis* transmitted by the sandfly in certain regions of Western South America invades human red blood cells, causing acute, often severe, hemolytic anemia. In chronic infections, there are skin lesions known as *verruga peruana* (Peruvian warts) that are similar to those of bacillary angiomatosis. A Peruvian medical student, Daniel Carrion, proved these lesions to be caused by an infectious agent in 1885 when he fatally inoculated himself with material from a *verruga peruana*. He died of the acute infectious hemolytic anemia known today as Oroya fever or, in his memory, Carrion's disease.

Microbiology

The *Bartonella* are small, Gram-negative aerobic bacilli that are difficult to grow in culture. They are found in many different animals but they cause no apparent disease in animals. Insects are thought to be vectors in human disease. Some species are able to infect erythrocytes while others simply attach to host cells. Table 2 summarizes the organisms and the diseases they cause.

Table 2

Organism	Disease
B. quintana (formerly *Rochalimaea quintana)*	Trench fever (shin-bone fever, 5 day fever), bacillary angiomatosis, bacillary peliosis endocarditis
B. henselae	Cat-scratch disease, bacillary angiomatosis, bacillary peliosis endocarditis
B. bacilliformis	Oroya fever (bartonellosis, Carrion's disease)
B. elizabethae	Endocarditis (rare)

B. quintana (Trench fever)

Epidemiology: Trench fever is a disease associated with war. The vector is the human body louse and there is no known reservoir except man. Transovarian transmission in the louse does not occur. The organism is found in the feces of the louse and is inoculated into humans by scratching. The cycle is human to louse to human.

Clinical syndromes: Infection with *B. quintana* can result in asymptomatic to severe debilitating illness. Symptoms include fever, chills, headache and severe pain in the tibia. A maculopapular rash may or may not appear on the trunk. The symptoms may reappear at five day intervals and thus the disease is also called five day fever. Mortality rates are very low.

Laboratory diagnosis: Serological tests are available but only in reference laboratories. PCR based tests have been developed.

Treatment, prevention and control: Various antibiotics have been used to treat trench fever. Measures to control the body louse are the best form of prevention.

B. henselae - (Cat-scratch disease)

Epidemiology: Cat-scratch disease is acquired after exposure to cats (scratches, bites, and possible cat fleas).

Clinical syndromes: The disease in usually benign, characterized by chronic regional lymphadenopathy.

Laboratory diagnosis: Serological tests are available.

Treatment: The disease does not appear to respond to antimicrobial therapy.

INTERPRETATION

BREAST TUMOUR MARKERS

The American Society of Clinical Oncology (ASCO) has updated its recommendations on November 13, 2007 for use of tumor markers in prevention, screening, treatment, and surveillance of breast cancer. Recommendations were based on significant health outcomes, namely overall survival, disease-free survival, quality of life, lesser toxicity, and cost-effectiveness. Of 13 categories of breast tumor markers considered, 6 were new to these guidelines. The categories recommended for use in practice, based on evidence of clinical use, were CA 15-3, CA 27.29, carcinoembryonic antigen (CEA), estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), urokinase plasminogen activator (uPA), plasminogen activator inhibitor 1 (PAI-1), and certain multiparameter assays for gene expression. However, not all applications for these markers were supported. Categories for which available evidence was insufficient to support routine use in clinical practice was DNA/ploidy by flow cytometry, p53, cathepsin D, cyclin E, proteomics, certain multiparameter assays, detection of bone marrow micrometastases, and circulating tumor cells (CTCs).

Specific changes from the 2000 guidelines are as follows:

ER and PgR should be measured on every primary invasive breast cancer and may be measured on metastatic lesions if the results would affect treatment planning. Steroid hormone receptor status should be used to identify patients most likely to benefit from endocrine therapies. In patients with ductal carcinoma in situ (DCIS) who are candidates for hormonal therapy, data are insufficient to recommend routinely measuring ER and PgR. Immunohistochemically based markers of proliferation are new to the guidelines. Present data are insufficient to recommend measuring markers of proliferation to assign patients to prognostic groups. These include Ki67, cyclin D, cyclin E, p27, p21, thymidine kinase, and topoisomerase II. To guide selection of trastuzumab in the adjuvant or metastatic setting, HER2 expression or amplification should be evaluated in every primary invasive breast cancer, either at the time of diagnosis or at the time of recurrence. HER2 may be useful to predict response to specific chemotherapeutic agents. Based on level II evidence, overexpression of HER2 (3+ by protein or > 2.0 fluorescent in situ hybridization [FISH] ratio by gene amplification) identifies patients who may benefit more from anthracycline-based adjuvant therapy. Assuming there are no contraindications to anthracycline therapy, and that chemotherapy is being considered for a patient with HER2-positive breast cancer, it is recommended that an anthracycline be strongly considered. In the context of trastuzumab therapy, there is level I evidence that a regimen without anthracycline may produce similar outcomes. The Update Committee does not currently recommend that HER2 be used to guide use of taxane chemotherapy in the adjuvant setting. uPA and PAI-1 as a marker for breast cancer is a new topic to the guidelines. In patients with newly diagnosed, node-negative breast cancer, uPA and PAI-1 measured by enzyme-linked immunosorbent assays (ELISAs) on 300 mg or more of fresh or frozen breast cancer tissue may be used to determine prognosis. Especially in hormone receptor positive women who will receive adjuvant endocrine therapy, low levels of both markers are associated with a sufficiently low risk for recurrence that chemotherapy will only confer minimal additional benefit. Compared with observation alone, cyclophosphamide, methotrexate, and 5-fluorouracil (CMF)-based adjuvant chemotherapy offers substantial benefit in patients with high risk for recurrence, based on high levels of uPA and PAI-1. Cyclin E fragments as markers for breast cancer is a new topic to the guidelines. Currently available data are insufficient to recommend use of whole-length or fragment measurements of cyclin E to manage patients with breast cancer. Proteomic analysis for breast cancer is new to the guidelines; present data are insufficient to support use of proteomic patterns to manage breast cancer. Multiparameter analysis of gene expression for breast cancer is new to the guidelines. The Onco*type* DX assay (Genomic Health Inc, Redwood City, California) can be used to predict the risk for recurrence in newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer who are treated with tamoxifen. Onco*type* DX may help identify patients who should most benefit from adjuvant tamoxifen and who may not require adjuvant chemotherapy. Patients with high recurrence scores seem to benefit relatively more from adjuvant chemotherapy with (C)MF than from tamoxifen. Bone marrow micrometastases as markers for breast cancer are a new topic to the guidelines. Currently available evidence is insufficient to recommend evaluation of bone marrow micrometastases for management of patients with breast cancer.

TROUBLESHOOTING

PREVENTION OF MRSA INFECTIONS

....contd.

FAQs For the Workplace

Contact Precautions

1) Patient placement

In Patient placement in hospitals and LTCFs, When single-patient rooms are available, assign priority for these rooms to patients with known or suspected MRSA colonization or infection. Give highest priority to those patients who have conditions that may facilitate transmission, e.g., uncontained secretions or excretions. When single-patient rooms are not available, cohort patients with the same MRSA in the same room or patient-care area. When cohorting patients with the same MRSA is not possible, place MRSA patients in rooms with patients who are at low risk for acquisition of MRSA and associated adverse outcomes from infection and are likely to have short lengths of stay. In general, in all types of healthcare facilities it is best to place patients requiring Contact Precautions in a single patient room.

2) Gloving

Wear gloves whenever touching the patient's intact skin or surfaces and articles in close proximity to the patient (e.g., medical equipment, bed rails). Don gloves upon entry into the room or cubicle.

3) Gowning

Don gown upon entry into the room or cubicle. Remove gown and observe hand hygiene before leaving the patient-care environment. After gown removal, ensure that clothing and skin do not contact potentially contaminated environmental surfaces that could result in possible transfer of microorganism to other patients or environmental surfaces.

4) Patient transport

In acute care hospitals and long-term care and other residential settings, limit transport and movement of patients outside of the room to medically-necessary purposes. When transport or movement in any healthcare setting is necessary, ensure that infected or colonized areas of the patient's body are contained and covered. Remove and dispose of contaminated PPE and perform hand hygiene prior to transporting patients on Contact Precautions. Don clean PPE to handle the patient at the transport destination.

5) Patient-care equipment and instuments/devices

In acute care hospitals and long-term care and other residential settings, use disposable noncritical patient-care equipment (e.g., blood pressure cuffs) or implement patient-dedicated use of such equipment. If common use of equipment for multiple patients is unavoidable, clean and disinfect such equipment before use on another patient. In home care settings limit the amount of non-disposable patient-care equipment brought into the home of patients on Contact Precautions. Whenever possible, leave patient-care equipment in the home until discharge from home care services. If noncritical patient-care equipment (e.g., stethoscope) cannot remain in the home, clean and disinfect items before taking them from the home using a low- to intermediate-level disinfectant. Alternatively, place contaminated reusable items in a plastic bag for transport.

6) Environmental measures

Ensure that rooms of patients on Contact Precautions are prioritized for frequent cleaning and disinfection (e.g., at least daily) with a focus on frequently-touched surfaces (e.g., bed rails, overbed table, bedside commode, lavatory surfaces in patient bathrooms, doorknobs) and equipment in the immediate vicinity of the patient.

7) Discontinuation of Contact Precautions

No recommendation can be made regarding when to discontinue Contact Precautions.

BOUQUET

In Lighter Vein

A guy walks into a bar, sits down, and asks, "Bartender, got any specials today?" Bartender answers, "Yes, as a matter of Fact we have a new drink, invented by A gynecologist patron of ours. It's a mix of Pabst Blue Ribbon Beer and Smirnoff Vodka."
The guy asks, "Good grief, what do you call that?"
The bartender replied, "It's a "Pabst Smir."

A guy is stranded on an island with only a Doberman and a pig for company. There's plenty of food and water, and the weather is beautiful, so he's doing alright - but after a few months he gets lonely...
The pig starts to look more and more attractive - soft, pink flesh and attractive figure. But every time this poor guy makes an advance towards the pig, the Doberman snarls at him and once almost bit his leg. Very frustrating.
One day the guy sees a speck on the horizon, so he swims out there and it turns out to be a dinghy, cast adrift, and in the bottom of the boat is a beautiful woman, unconscious. He drags her to shore and brings her into his hut and slowly nurses her back health. Finally she is well enough to walk and she says to him "Thank you, thank you for saving my life. I don't know how I can ever repay you. I'll do anything for you, anything, just name it."
The guy thinks for a minute and says, "Would you mind taking my dog for a walk?"

A man who had spent his whole life in the desert visited a friend. He'd never seen a train or the tracks they run on. While standing in the middle of the RR tracks, he heard a whistle, but didn't know what it was. Predictably, he's hit and is thrown, ass-over-tea-kettle, to the side of the tracks, with some minor internal injuries, a few broken bones, and some bruises.
After weeks in the hospital recovering, he's at his friend's house attending a party. While in the kitchen, he suddenly hears the teakettle whistling. He grabs a baseball bat from the nearby closet and proceeds to batter and bash the teakettle into an unrecognizable lump of metal. His friend, hearing the ruckus, rushes into the kitchen, sees what's happened and asks the desert man, "Why'd you ruin my good tea kettle?"
The desert man replies, "Man, you gotta kill these things when they're small."

Wisdom Whispers

- In the small matters trust the mind, in the large ones the heart.
- From error to error one discovers the entire truth.
- Being entirely honest with oneself is a good exercise.
- When inspiration does not come to me, I go halfway to meet it.
- Yesterday I was very irritable; you should have been here so as to wish you weren't.
- He did each single thing as if he did nothing else.
- Never close your lips to those to whom you have opened your heart.
- Regrets are the natural property of grey hairs.

Brain Teasers

1. Individuals with which blood group are at higher risk of getting carcinoma stomach?
   (a) A (b) B (c) O (d) AB
2. In a male gastric carcinoma metastases do not present as
   (a) Secondary carcinoma of bone (c) Krukengerg tumour
   (b) Secondary carcinoma in liver (d) Local nodal metastases
3. Sago spleen is described in
   (a) Hodgkin's lymphoma (c) Fibro-congestive splemegaly
   (b) Amyloidosis (d) Thalassemia
4. At autopsy heart should be opened under water to confirm
   (a) Fat embolism (b) Air embolism (c) MI (d) Pericarditis

Answers: 1. A, 2. B, 3. C, 4. B.

TULIP NEWS

Tulip group introduces a diagnostic test for screening of beta thalassemia

In the Disease Diagnosis section of the last issue of The Crux (Issue No 27, May-Jun 2008), we had dealt with Beta thalassemia, a major genetic disorder.

It is now evident that this disorder is spreading at an alarming rate due to

- Lack of awareness coupled with non existent premarital counselling.
- Non availability of a reliable testing system.

The implications of this disorder is severe and if not controlled may drain out blood bank supplies in the future.

BETA THALASSEMIA

Beta thalassemia in its severest form, thalassemia major is transfusion dependent. Many thalassemic major children are born every year and require regular transfusions to sustain life. Untreated thalassemia major eventually leads to death usually by heart failure, therefore birth screening is very important.

Though this disorder has emerged from the Mediterranean region and therefore the name 'thalasa' meaning sea, it is catching up in India and other Asian countries. In India the spread of this hereditary disorder is mainly due the practice of consanguineous marriages (marriages within families), a well- accepted social norm widely prevalent in India.

Almost 25 million people in India are carriers of the beta thalassemia gene and 6000 to 8000 children are born every year with thalassemia major. The only cure available today is the bone marrow transplantation, which is not affordable to almost all patients. The birth of a thalassemic child, thus places considerable physical and economic strain, not only on the affected child and its family, but also on the community and nation at large. With these limitations, emphasis must shift from treatment to prevention of such births in future. Prospective prevention, which includes population education, mass screening, genetic counseling, premarital testing and prenatal diagnosis is the only totally effective way to cope successfully with this disease.

After reviewing the current system of diagnosis of beta thalassemia

Our objective is

To provide a diagnostic method that overcomes the limitations of currently used diagnostic tests.
To provide a diagnostic kit of high sensitivity, which enables the detection of all people carrying the beta-thalassemia trait, without any false negative result.
To provide a diagnostic method which may be carried out in a short time for a number of people with great ease.
To provide an economical alternate method for prenatal and premarital testing of beta-thalassemia carriers
To provide a quantitative screening test which enables the results to be recorded and is not subjective like the osmotic fragility which is less precise and reliable.

TULIP GROUP PRESENTS

THALVUE

Turbidimetry test for screening of beta thalassemia

25 TESTS

Printed and published by D.G. Tripathi, Edited by Dr. R.J. Sood M.D. (path.) for and on behalf of Tulip Diagnostics Private Ltd, Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh Alto Santacruz, Bambolim Complex Post Office, Goa - 403202, INDIA.
Fax: (0832) 2458544, E-mail: sales@tulipgroup.com. Website: www.tulipgroup.com