REVIEW

Neglected bacterial zoonoses

I. Chikeka and J. S. Dumler

Departments of Pathology and Microbiology & Immunology, University of Maryland School of Medicine, Baltimore, MD, USA

Abstract

Bacterial zoonoses comprise a group of diseases in humans or animals acquired by direct contact with or by oral consumption of contaminated animal materials, or via arthropod vectors. Among neglected infections, bacterial zoonoses are among the most neglected given emerging data on incidence and prevalence as causes of acute febrile illness, even in areas where recognized neglected tropical diseases occur frequently. Although many other bacterial infections could also be considered in this neglected category, five distinct infections stand out because they are globally distributed, are acute febrile diseases, have high rates of morbidity and case fatality, and are reported as commonly as malaria, typhoid or dengue virus infections in carefully designed studies in which broad-spectrum diagnoses are actively sought. This review will focus attention on leptospirosis, relapsing fever borreliosis and rickettsioses, including scrub typhus, murine typhus and spotted fever group rickettsiosis. Of greatest interest is the lack of distinguishing clinical features among these infections when in humans, which confounds diagnosis where laboratory confirmation is lacking, and in regions where clinical diagnosis is often attributed to one of several perceived more common threats. As diseases such as malaria come under improved control, the real impact of these common and under-recognized infections will become evident, as will the requirement for the strategies and allocation of resources for their control.

Keywords: Acute febrile illness, *Borrelia*, chiggers, fleas, *Leptospira*, *Orientia*, relapsing fever, *Rickettsia*, ticks, vector-borne bacteria

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Corresponding author: J.S. Dumler, Departments of Pathology and Microbiology & Immunology, University of Maryland School of Medicine, 685 W. Baltimore Street, HSFII 322D, Baltimore, MD 21218, USA

E-mail: sdumler@som.umaryland.edu

Introduction

Zoonoses comprise an array of infections, many known throughout man's history, and others that were only recently recognized to cause human or animal disease. The phrase 'neglected infectious disease' recently emerged based on the perception that neglected infections are largely tropical or affect large poverty-ridden populations, are chronic, and are perceived to take a steep toll on society and development [1]. However, the concept of neglected infections is neither new nor fixed, and depends on the interpretation context. Fig. 1 shows zoonoses in the USA as reported in *Morbidity Mortality Weekly Reports* spanning 1981 to 2012 [2] and lacks any priority neglected zoonosis as cited by the World Health Organization (WHO) or the U.S. CDC [3,4]. Depending on the source, bacterial infections defined as 'neglected' by the Bill & Melinda Gates Foundation include only trachoma and buruli ulcer [5], whereas WHO and CDC add yaws and leprosy, and the list expands to include infantile diarrhoea (enterotoxigenic *Escherichia coli*) in the European Union [3,4,6]. The Bill & Melinda Gates Foundation-sponsored Global Burden of Diseases, Injuries, and Risk Factors Study 2010 list of deaths from globally important infectious diseases includes only a small proportion that are zoonotic (Fig. 2) [7]. The biased data presentation occurs largely because neglected diseases cannot be compared if they are not accurately diagnosed and reported, or assessed based on disability-adjusted life-years if that factor is unable to be calculated given the absence of meaningful incidence or prevalence data. Rather the list reflects existing data shaped by infections for which diagnosis or reporting is easy, or based on

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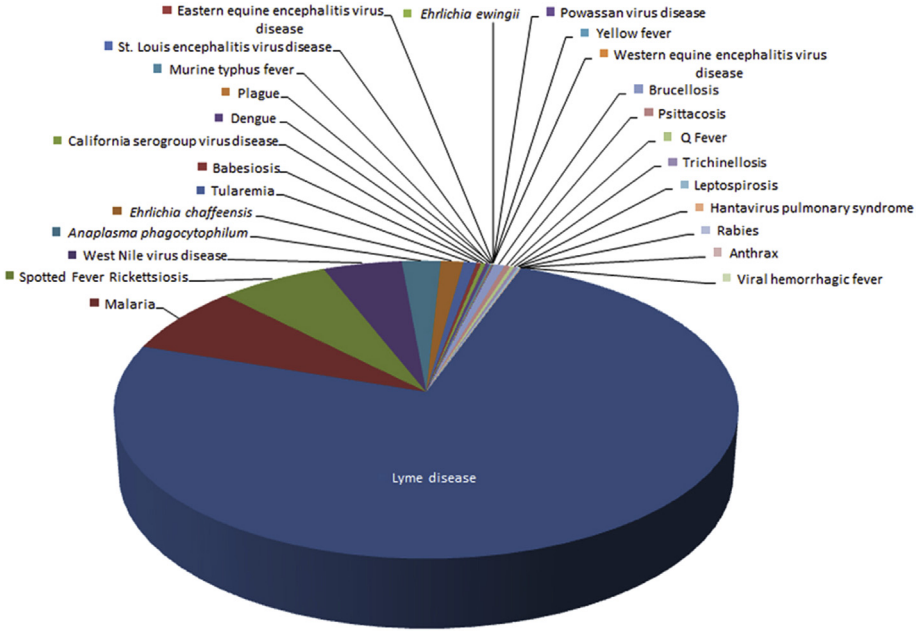

FIG. 1. Zoonoses reported to the CDC and published in *Morbidity Mortality Weekly Reports* between 1981 and 2012. Not all diseases were reportable over this interval, and some case definitions changed. No neglected infectious disease as cited by the CDC or WHO appears on this list. Aside from malaria, which is most often imported, the most commonly reported zoonoses in the USA over this interval were Lyme disease, spotted fever group rickettsioses, West Nile virus infection, *Anaplasma phagocytophilum* infection and *Ehrlichia chaffeensis* infection.

evidence estimates that could be biased given the lack of such data [8,9]. Much speculation has been made in recent years about priorities for allocation of resources as malaria comes under increasing control [10]. A growing number of investigations to discern the aetiology of acute febrile illness in developed and under-resourced regions illustrate major gaps in our knowledge, and these gaps identify a greater expanse of neglected infectious diseases, many of which are bacterial zoonoses [11–16]. Such investigations shed light on prevalence estimates and the likely high human toll of inattention to other infections, many bacterial, and the topic of this article.

Bacterial zoonoses occur with transmission via one of several mechanisms: 1) direct contact with animals or infected materials; 2) animal bites and scratches; 3) bites or mechanical transmission by arthropod vectors; and 4) consumption of contaminated foods (Table 1). The bacteria that cause the infections can sometimes be acquired by more than one transmission mechanism, complicating control measures. Most do not appear on lists of neglected infections, in part because of serious problems with definitive aetiological diagnosis and reporting: most acute febrile illnesses in Sub-Saharan Africa are diagnosed and reported on clinical grounds as malaria, and in South East Asia, as typhoid fever or dengue virus infection. When investigated objectively using pathogen-specific diagnostics, infections such as leptospirosis, rickettsioses and melioidosis are diagnosed as frequently as dengue, typhoid or malaria [11–13,16,17]. Owing to the biases toward the 'neglected diseases' and the paucity of objective data accumulated by major public health agencies, this article will focus on only selected bacterial zoonoses for which their recognition as important or relevant is not generally acknowledged, and for which diagnosis, treatment and research priorities are minimal globally. Despite the exclusion of certain bacterial zoonoses, the approach is intended to highlight what is known and not known as examples of why improvements in the study of these diseases are needed.

Leptospirosis

Leptospirosis is a zoonosis caused by pathogenic spirochaetes of the genus *Leptospira* [18–20]. Leptospires are spiral shaped, motile aerobic spirochaetes distinguished morphologically from other spirochaetes by characteristic hooked ends [19]. Members of the *Leptospira* genus were previously grouped according to antigenic determinants, with pathogenic leptospires belonging to the species *Leptospira interrogans* and non-pathogenic leptospires grouped under the species *Leptospira biflexa*. A new classification system proposes grouping of leptospires by DNA relatedness into 20 species: nine pathogenic, five of intermediate or unclear pathogenicity, and six non-pathogenic saprophytes [21,22].

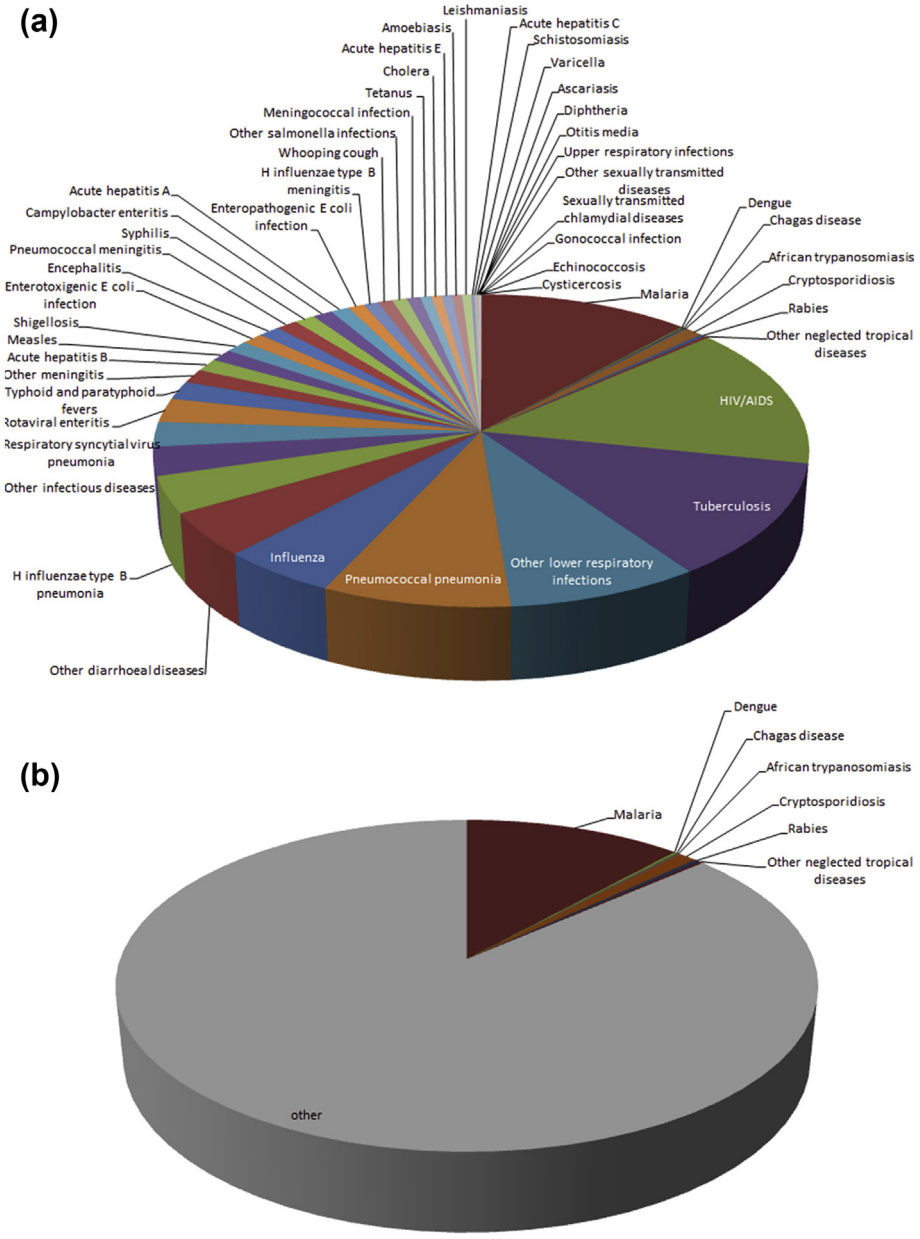

FIG. 2. The proportional contribution of various infectious disease fatalities as reported in the Global Burden of Diseases, Injuries and Risk Factors Study 2010 [7]. (a) Pie chart demonstrating all infectious diseases examined by the study. (b) Zoonoses occupy a minority of the reported deaths and disease burden, and include no bacterial zoonoses.

Animals serve as natural hosts and can be asymptomatic [19]. Leptospires are excreted in their urine, contaminating water and soil where they can remain viable for days to months. Rodents are the most significant reservoirs for transmission and when infected can shed the leptospires throughout their lifetime. Humans are accidental hosts, acquiring infection after exposure of mucous membranes and abraded skin to animal urine, contaminated water or soil, or infected animal tissue. After entering the bloodstream, the spirochaetes multiply in organs, most commonly the central nervous system (CNS), kidneys and liver. They are cleared from the blood and most tissues by the immune response but can persist and multiply in the tubules of the kidneys.

The distribution of leptospirosis is worldwide but it occurs with the greatest frequency in tropical and subtropical environments [18,20]. It is estimated that more than 10 million cases occur each year around the world and it is a significant cause of morbidity and mortality [21,23]. Leptospirosis is most

TABLE 1. Bacterial zoonoses by transmission mechanism and causative agent(s)

Bacterial zoonoses transmitted by direct contact with animals or infected animal materials	Causative agent(s)
Anthrax	*Bacillus anthracis*
Brucellosis	*Brucella* spp.
Cat scratch disease	*Bartonella* spp.
Erysipelothrix infections	*Erysipelothrix rhusiopathiae*
Glanders and melioidosis	*Burkholderia mallei* and *Burkholderia pseudomallei*
Leptospirosis	*Leptospira interrogans* spp.
Mycobacterioses	*Mycobacteria* spp.
Q fever	*Coxiella burnetii*
Bacterial zoonoses transmitted principally by animal bites or scratches	
Pasteurellosis	*Pasteurella multocida* and other spp.
Capnocytophaga infections	*Capnocytophaga canimorsus*
Cat scratch disease	*Bartonella henselae*
Rat bite fever	*Spirillum minus* and *Streptobacillus moniliformis*
Vector-borne bacterial zoonoses	
Lyme borreliosis	*Borrelia burgdorferi* sensu lato (incl. *Borrelia garinii*, *Borrelia afzelii*)
Tick- and louse-borne relapsing fever borreliosis	*Borrelia recurrentis*, *Borrelia turicatae*, *Borrelia hermsii*, others
Plague	*Yersinia pestis*
Tularaemia	*Francisella tularensis*
Rickettsioses	Spotted fever and typhus group *Rickettsia* species
Ehrlichiosis and Anaplasmosis	*Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*
Scrub typhus	*Orientia tsutsugamushi*
Foodborne bacterial zoonoses and intoxications	
Salmonellosis	*Salmonella enteritidis*
Campylobacteriosis	*Campylobacter* spp.
Listeriosis	*Listeria monocytogenes*
Escherichia coli O157:H7 infections	*Escherichia coli* STEC
Yersinia enterocolitica infections	*Yersinia enterocolitica*
Clostridium perfringens gastroenteritis	*Clostridium perfringens*
Botulism	*Clostridium botulinum*
Staphylococcal food poisoning	*Staphylococcus aureus*

common in developing urban and rural areas with inadequate sewage disposal and water treatment, yet outbreaks in temperate regions, including the USA and Europe are well documented [21,24]. The burden of disease is highest in the Caribbean, Central and South America, South East Asia, Oceania, India and South Asia, and Eastern Europe. The incidence in the Seychelles in the Indian Ocean is as high as 432 per 1 000 000 population and globalization continues to occur through international travel to these and other similar areas where leptospirosis is endemic [25]. Although well-validated global data on leptospirosis are lacking, the estimated global annual incidence of leptospirosis in temperate regions is 0.1–1 cases per 100 000 population while the incidence in tropical climates is > 10 cases per 100 000 population [26]. These are likely underestimates because of misdiagnosis and under-reporting, particularly in regions where other diseases with similar non-specific presentations such as dengue and malaria are prevalent. For example, a cross-sectional study among hospitalized febrile patients in northeastern Malaysia between August 2010 and February 2011 found an 8.4% seroprevalence; but among these, only 31% were correctly diagnosed using clinical criteria—the remainder were misdiagnosed usually as dengue/dengue haemorrhagic fever (38%), pneumonia (14%), or typhoid fever (7%) [27]. Among 3165 sera from acutely febrile patients with suspected dengue in Jamaica over 2007–2008, only 38.4% were confirmed to have dengue antibodies, whereas 6% were misdiagnosed and had leptospirosis instead, and 1.6% had serological responses consistent with both infections [28]. Perhaps more directly, Reller *et al.* showed separately in Sri Lanka and Nicaragua where leptospirosis is often suspected, that clinical diagnosis alone is poorly sensitive, 23% and 11%, respectively [14,29]. Likewise, in Sri Lanka, clinical diagnosis of other acute febrile illnesses is poor, 14% for dengue virus infection, 3% for rickettsial infections and 0% for Chikungunya virus infection [13,30,31].

The clinical presentation of leptospirosis is highly variable and non-specific [21,23], explaining the poor predictive value of clinical assessment for establishing the diagnosis as a guide to appropriate therapy [14,29,32]. Severity ranges from subclinical to fatal. Clinical illness usually begins abruptly after an incubation period of 2–26 days. The less severe, anicteric form of leptospirosis resembles an influenza-like illness with fever, rigors, myalgias, headache, abdominal pain, non-productive cough and conjunctival suffusion, a distinguishing sign [20,21]. The severe icteric form, known as Weil's disease, occurs in a minority of patients and is associated with jaundice, hepatic dysfunction, myocarditis with arrhythmias, haemorrhage, uveitis and multi-organ failure. Both can occur in two phases: an acute septicaemic phase and an immune phase that can immediately follow. Routine laboratory tests are typically non-specific but can include leucocytosis with a left shift, increased erythrocyte sedimentation rate, mildly elevated transaminases, alkaline phosphatase and bilirubin, abnormal urinalysis and thrombocytopenia. Leptospirosis mimics many other tropical diseases and diagnosis requires a high degree of clinical suspicion [14,17,33]. The average annual hospitalization

rate for leptospirosis in the USA from 1998 to 2009 was 0.6/1 000 000 population and the average length of stay and hospital charges were higher in comparison to non-leptospirosis-associated hospitalizations [34]. In Brazil, untreated leptospirosis results in significant social costs in years of potential life lost and partial hospitalization costs when compared with early treatment and prevention [35]. Death occurs in 5–15% of infections if untreated, and the proportion increases with age.

Acute disease is the direct result of inflammatory responses to the presence of leptospires in tissues, including hepatitis and cholestasis, interstitial nephritis, and meningoencephalitis [21]. Pulmonary haemorrhage is increasingly observed, and the pathogenesis of this process is not defined, but relates to host immune response, the production of bacterial factors that lead to local coagulation abnormalities, or both [18,21]. Disease requires the ability of leptospires to enter organs and tissues and spread. *Leptospira* genome studies show that >1% of genes are dedicated to motility, and the presence of adhesins and invasins, such as the outer membrane protein Loa22 and the immunoglobulin-like LigA could contribute to this [21,36]. In addition, genes that could affect haemostasis and coagulation are present, including a platelet-activating factor acetylhydrolase-14 (LA2144, *paf*AH) and von Willebrand factor 15 type A domains (LB054 and LB055, *vwa*) [21,36]. The development of disseminated intravascular coagulation with multi-organ failure provides some evidence to support an immunological basis for severe leptospirosis including the pro-inflammatory response to bacterial lipopolysaccharide and lipoproteins that stimulate Toll-like receptor-2. Likewise, pulmonary haemorrhage is more frequent in persons with high antibody titres, and there is evidence that immunoglobulin and complement fixation on host cells are related to the expression of specific leptospiral haemostatic proteins [36]. The late occurrence of uveitis correlates with the 'immune' phase and with immunoglobulin and complement deposition [37].

Leptospirosis is diagnosed by serology, culture and molecular tests [18,19,38]. Culture is definitive but slow, lacks sensitivity, and requires specialized medium, and is therefore not very useful for diagnostic purposes. Immunohistochemistry has been used, but is relatively unavailable. Likewise, PCR is sensitive but requires broad-range primers that despite the high degree of genetic diversity, can amplify all variants. Serological tests are used most often, particularly the reference standard, the microscopic agglutination test. This is sensitive as early as 5–7 days after onset; a single high titre is acceptable, but seroconversion is preferred. The pitfall of microscopic agglutination test is the requirement for continued growth of multiple serovars, a daunting task for clinical laboratories, and the delay in detection because antibodies develop later in the course of illness.

A recent Cochrane review was unable to discern any significant advantages to the use of antibacterial treatments for active leptospirosis [39]. Yet many believe that antimicrobial therapy shortens illness duration and reduces shedding of leptospires in the urine [33]. Treatment options include oral doxycycline or azithromycin for mild disease and parenteral penicillin, doxycycline, or third-generation cephalosporin for severe disease. Supportive care may be required for Jarisch–Herxheimer reactions that result in a systemic inflammatory response after lysis of spirochaetes with treatment.

Prevention of leptospirosis focuses on public health efforts, chemoprophylaxis and vaccination. Public health interventions include identification and modification of risk factors through public education. Human and animal behavioural and environmental risk factors vary by region and include rodent infestations, particularly rats, exposure to contaminated surface water such as in rice paddies, and the presence of skin wounds. A recent study by Samarakoon and Gunawardena indicates that in endemic regions the knowledge of leptospirosis prevention is adequate but does not lead to implementation of personal protective measures [40]. Prophylaxis with doxycycline (200 mg once a week) reduces morbidity and mortality during outbreaks and other homeoprophylactic measures are reported to provide preventive benefit, but will require extensive evaluation [41,42]. There is no human vaccine available but vaccines do exist for animal reservoirs including dogs and cattle.

Relapsing fever borreliosis

Relapsing fever is an arthropod-borne disease caused by pathogenic spirochaetes of the genus *Borrelia* [43,44]. It occurs in two major forms: tick-borne relapsing fever (TBRF) and louse-borne relapsing fever (LBRF). LBRF is primarily seen in East and Central Africa and is a frequent cause of epidemics in areas of extreme poverty, war, natural disasters and overcrowding. It is widely perceived that there has been a decline in the incidence of LBRF secondary to improved standards of living and the introduction of the insecticide DDT. However, LBRF is still a major public health issue in East Africa, particularly in Ethiopia and in surrounding regions where louse infestation is commonplace. TBRF is found worldwide and is endemic in many countries including the Americas, Central Asia, the Mediterranean region and many parts of Africa. In most rural areas of Senegal, Mauritania and Mali, TBRF is a common cause of fever with incidence comparable to *Plasmodium falciparum* malaria and influenza [45]. The incidence of tick-borne relapsing fever peaks in summer but infection can occur year round, depending on local climate conditions.

Relapsing fever is caused by bacteria of the genus *Borrelia*. Borreliae are thin, helical, motile spirochaetes that are unique in their ability to change their outer membrane surface proteins by gene conversion to generate antigenic variation [43]. Each relapse is associated with the emergence of a unique antigenic bacterial clone that is subsequently controlled by the immune system, only to select for new antigenic variant clones and another relapse of fever and disease. In fact, some variant clones have a propensity for dissemination into tissues and organs such as the CNS.

Louse-borne relapsing fever is caused by *Borrelia recurrentis*, now believed to be a reduced-genome variant of the TBRF *Borrelia duttonii* [46]. The vector for LBRF is the human body louse (*Pediculus humanus*), so humans are the only known reservoir for this spirochaete [44]. TBRF is transmitted to humans through the bite of infected soft ticks of the genus *Ornithodoros*. There are many *Borrelia* species that cause TBRF, roughly divided into Old World and New World species, and they are associated with specific tick species. Unlike the human body louse that lives for several weeks, *Ornithodoros* ticks can live for many years between blood meals, harbour spirochaetes for prolonged periods, and can transmit the pathogen vertically to offspring [44].

Lice acquire the spirochaetes by feeding on infected humans whereas humans acquire LBRF by scratching infected haemolymph of a crushed louse into the skin [44,47]. Ticks become infected by feeding on infected wild rodents and then transmit the spirochaetes to humans through a tick bite. After entering the blood, the spirochaetes disseminate, seeding multiple organs including the CNS in the case of TBRF.

Relapsing fever is characterized by two or more episodes of high fever (usually >39°C), myalgias, arthralgias, nausea, vomiting, headache and non-productive cough [48,49]. Signs can include haemodynamic instability, rash, abdominal tenderness and bleeding. Laboratory studies can reveal mild elevations in bilirubin and aminotransferases, thrombocytopenia and anaemia. Later in the illness, jaundice, hepatosplenomegaly and myocarditis can occur [50]. The initial febrile episode typically lasts 3–6 days and is followed by an afebrile period of 4–14 days, after which fever and symptoms recur. Subsequent relapses are usually less severe and can follow at 1- to 2-week intervals.

Because the WHO advises a presumptive diagnosis of malaria in endemic regions, or collects 'verbal autopsy' reports for deaths, relapsing fever often goes undetected, particularly in parts of Africa where malaria is highly prevalent. A study by Nordstrand *et al.* in Togo, West Africa between 2002 and 2004 found that among febrile patients originally diagnosed and treated for malaria, the prevalence of TBRF was 8.8%, compared with 63.1% for malaria; 4.5% of patients had both malaria and TBRF [51]. Similarly, among a cohort of individuals participating in a study of mass treatment to eradicate trachoma in Tanzania, 17% of febrile episodes were caused by either *Plasmodium falciparum* or *Plasmodium vivax*, whereas 4% were attributed to relapsing fever [52], underscoring how uncorroborated clinical diagnosis can lead to erroneous reporting coupled with inflation of the incidence of reportable infections at the expense of other aetiologies.

Although the incidence of relapsing fever has declined in the developed world, it continues to be a formidable public health issue in certain regions. In Tanzania, for example, infection with TBRF is associated with significant morbidity and mortality, especially in women and children, where it results in high perinatal mortality (436/1000 births) and is frequently listed as one of the top ten causes of mortality in children under 5 years of age [53,54]. A retrospective study conducted between 2009 and 2012 of patients with LBRF-like symptoms who were admitted to a referral hospital in Ethiopia found the prevalence to be 4.9% and case fatality rates ranged from 2 to 6% [55]. Relapsing fever can account for up to 27% of hospitalizations in some regions [56]. There still exists the possibility of significant re-emergence, particularly as travel patterns continue to change and imported cases of relapsing fever are described. There is also evidence of a resurgence of louse infestation among certain groups. In Marseille, France, Brouqui *et al.* found a high prevalence of louse-borne infections in the homeless and a high level of exposure to tick-borne diseases [57].

Relapsing fever is diagnosed by visualization of spirochaetes in the blood using dark-field microscopy or Wright or Giemsa staining on thin and thick blood smears [49]. Serological tests were typically unreliable, but have improved with the development of assays based on detection of antibodies to relapsing fever spirochaete-specific GlpQ [58]. Culture requires techniques that are not available in most laboratories. Nucleic acid amplification tests are available in some public health facilities, but not generally accessible to primary healthcare workers, or accessible in under-resourced regions where relapsing fever is often present.

Few careful studies of *in vitro* susceptibility for relapsing fever *Borrelia* species are published to guide treatment. LBRF was shown to be effectively treated with a single dose of oral tetracycline or doxycycline, or intramuscular penicillin G procaine [43,49], but over one-third of patients had recurrent fever, suggesting that a prolonged regimen is preferable. Similarly, the relapse rate of TBRF is at least 20% after single-dose therapy; hence, the treatment for TBRF is extended to 7–10 days. Relapses yield as many as 10^8 bacteria/mL of blood, so antibiotic therapy can trigger a Jarisch–Herxheimer reaction, particularly with LBRF, and its occurrence is associated with high mortality. Tetracyclines are highly effective at clearing

spirochaetes with no or few relapses, but their use is associated with more frequent and severe episodes of Jarisch–Herxheimer reactions [59,60]. Therefore, preferred treatment employs either single-dose doxycycline or tetracycline, or a single dose of procaine penicillin intramuscularly followed by 2 or more days of oral doxycycline or tetracycline. Prevention of infection requires good personal hygiene, delousing, laundering at high temperatures and application of insecticides to clothing and bedding to diminish louse infestations for LBRF. TBRF prevention includes exclusion of rodents that host the argasid tick vectors, avoiding habitats that harbour the ticks themselves, and the use of tick repellents.

Scrub typhus, murine typhus and spotted fever rickettsiosis

Bacteria in the genera *Orientia*, *Rickettsia*, *Ehrlichia* and *Anaplasma* are obligate intracellular α2 proteobacteria in the Order Rickettsiales and Families Rickettsiaceae and Anaplasmataceae [61]. Genome sequences illustrate that these agents adapted to an obligatory intracellular lifestyle through the loss of genes and pathways required for extracellular growth [62]. Regardless, each contains DNA, RNA, ribosomes, divides by binary fission and possesses a cell wall with ultrastructural characteristics of Gram-negative bacteria. Rickettsiaceae members (*Rickettsia* and *Orientia*) target endothelial cells in mammals and reside within the cytosol. In contrast, Anaplasmataceae family members usually reside in haematopoietic-derived cells, such as leucocytes in mammals where they propagate within pathogen-modified endosomes. All Rickettsiales have at least part of their life cycle in vectors, and for *Rickettsia*, *Orientia*, *Ehrlichia* and *Anaplasma* these are usually arthropods, often ticks, fleas, lice or mites.

The genus *Rickettsia* is divided into spotted fever (SFGR) and typhus groups. Genome studies illustrate diversity with SFGR, yet these cluster separately from typhus group rickettsiae [62]. Several species occupy intermediate positions sometimes called 'transitional' or 'ancestral'. There are over 25 recognized SFGR species and two typhus group species; most are human pathogens, yet at least several 'transitional' and 'ancestral' rickettsiae are questionably pathogenic or never associated with known human or animal disease. The genus *Orientia* has a similar degree of genetic diversity as for SFGR, yet has only two named species, *Orientia tsutsugamushi* and *Orientia chuto*, both human pathogens. Although many Anaplasmataceae are animal pathogens, only a limited range are human pathogens.

Among the rickettsial agents, the most neglected for which sufficient evidence exists that there are large global disease burdens include *O. tsutsugamushi*, the agent of scrub typhus [10,12,13,16], *Rickettsia typhi*, the aetiological agent of murine typhus [11–13], and various SFGR, especially *Rickettsia rickettsii* (Rocky Mountain spotted fever [RMSF]) [63,64], *Rickettsia conorii* (Mediterranean spotted fever), *Rickettsia africae* (African tick bite fever), and likely others in Asia and Australia (*Rickettsia sibirica*, *Rickettsia heilongjiangensis*, *Rickettsia japonica*, *Rickettsia honei* and *Rickettsia australis*) [65,66]. Although the emergence of ehrlichiosis and anaplasmosis argues for their importance as globally neglected infections [67], for simplicity, only scrub typhus, murine typhus and 'generic' SFGR will be considered here.

Scrub typhus

Orientia tsutsugamushi is transmitted by larval trombiculid mites (genus *Leptotrombidium*), which are distributed throughout Asia and parts of Australia. The range of scrub typhus extends from northeast Asia to Papua/New Guinea and Northern Australia in the southeast, the Maldives and Réunion Islands in the southwest and to Pakistan and Afghanistan in the northwest [68]. This distribution encompasses regions where over 2 billion people live. Several recent studies provide preliminary evidence that scrub typhus could be endemic outside the previously established ranges, and extend into Africa and as far as South America, although much more study is needed [10,69,70]. Based on the wide ecological distribution of the pathogen, the density of populations in these geographic regions and the high seroprevalence and incidence of infection, it is conservatively estimated that there are more than 1 million cases of scrub typhus yearly [10].

Scrub typhus usually presents as undifferentiated fever [68,71]. Patients also often demonstrate headache, myalgias, rash, among other manifestations, and an eschar at the site of the mite bite is a key sign (Table 2). Laboratory studies are usually not revealing, except for a normal leucocyte count and left shift with thrombocytopenia, and moderate increases in serum hepatic transaminase activities (Table 2), all common features of infections described here. The diagnosis of scrub typhus relies on clinical suspicion for early treatment [72]. Diagnostic approaches during acute disease include immunohistochemical demonstration of *O. tsutsugamushi* in eschar biopsies, and although much less sensitive, PCR amplification of *O. tsutsugamushi*-specific nucleic acids from blood [72,73]. Serology is most often employed and includes specific indirect immunofluorescence assays for *O. tsutsugamushi* IgG and IgM, although the latter is associated with false positive tests in the absence of a concurrent IgG seroconversion [60,74]. Point-of-care assays are not readily available or well validated. Patients with scrub typhus are best treated with doxycycline, and

TABLE 2. Clinical signs, symptoms and laboratory findings of neglected bacterial zoonoses compared with dengue virus infection (% of cases with finding)

History, signs, or symptoms	Leptospirosis (*Leptospira interrogans*)	Scrub typhus (*O. tsutsugamushi*)	RMSF (*R. rickettsii*)	Murine typhus (*R. typhi*)	Dengue virus infection
Clinical findings					
Fever	100	100	100	99	100
Headache	85	100	91	59	78
Myalgia	77	32	72	46	77
Rash	5	49[a]	90	37	11-53
Rash on palms and soles	na	na[b]	82	na	na
Nausea or vomiting	45	28	60	40	53
Abdominal pain	33	na	43	17	na
Conjunctivitis	61	29	30	na	na
Pneumonitis	37	28	15	15	35
Any severe neurological complication	<25	10	26	6	1-6
Laboratory findings					
Leucocytosis (white blood cell count) > 10×10^9/L	39	34	28	22	6
Leucopenia (white blood cells) < 5×10^9/L	8	3	24	13	25
Platelet count <150×10^9/L	26	25	44	46	59
Elevated ALT or AST	78	70	50	57	64

[a]Includes eschars.
[b]na, data not available.

although questions about doxycycline resistance in some Thai strains are not resolved, azithromycin seems to be an effective alternative [75]. Severe outcomes include pneumonia, renal failure, meningoencephalitis, shock, gastrointestinal bleeding, myocarditis and death [76].

In some Asia-Pacific regions, scrub typhus can account for up to 23% of all febrile illness in hospitalized patients [77]. A study by Chanyasanha *et al*. of febrile patients in Thailand who presented to malaria clinics found the seroprevalence of scrub typhus to be close to 60% [78]. A meta-analysis using the search terms 'scrub typhus incidence' identified 132 publications that examined acutely febrile subjects between 2009 and 2015, for which 23 contained sufficient details of study design, patient enrolment, diagnostic approach, and individualized data to discern incidence and prevalence (see Supplementary material, Table S1). All studies were conducted in Asia (Lao PDR, Thailand, Vietnam, China, India, Sri Lanka, Indonesia, Bangladesh and Malaysia). This analysis accumulated data from 10 192 subjects with acute febrile illness, and identified 1881 incident scrub typhus infections, for a median of 14% (interquartile range 8–31%) infection rate. One recent study by Varghese *et al*. conducted between 2005 and 2010 on patients with scrub typhus in South India found the overall case fatality rate to be 9% with shock, renal failure and CNS involvement associated with a higher mortality [79]. The case fatality rate varies in contemporary studies from 0.5% to 24% [80] to historical highs between 30 and 50% [81], such that the mortality among 1 million infections in a single year is probably enormous.

Scrub typhus remains a serious public health issue. In regions such as India, Sri Lanka and China where it was thought to have declined, scrub typhus has experienced a resurgence, and it is increasingly identified as an emerging infection in new foci [10]. It is unclear whether this is the result of improved diagnostic methods and increased awareness, expansion of farmlands and population growth, or changes in travel and migration patterns. Misdiagnosis and under-diagnosis are also known to occur through the lack of availability of diagnostic tests and the non-specific nature of symptoms, especially when the characteristic eschar is not present. The economic impact of scrub typhus has only recently begun to be explored in regions of China where the disease has rapidly re-emerged in recent years [82].

Murine typhus and spotted fever rickettsiosis

Murine typhus occurs when the rat flea (*Xenopsylla cheopis*) or the cat flea (*Ctenocephalides felis*) transmits *R. typhi* via infected flea faeces into the flea bite wound [83]. The apparent obligatory relationship of rats or domestic pets that harbour fleas with human domiciles makes murine typhus a risk in any location with human activities, and especially in regions with warm weather and year-round flea activity. Murine typhus has been reported on every continent (except Antarctica), and is a particular risk in tropical regions of South East Asia, Africa, Central and South America, and even in southern parts of the USA and Europe. Well-established prevalence data are lacking in almost all regions owing to neglect of this disease, yet seroprevalence studies continue to demonstrate high rates in many locations and have implicated murine typhus as the causative agent in increasingly large proportions of patients with febrile illness. Hamaguchi *et al*. [97] studied acute undifferentiated fever in hospitalized patients in Northern Vietnam, where 33% were seropositive for *R. typhi*. Certain communities, notably, homeless populations, have higher seroprevalence rates, ranging from 9.6% in the USA to 22% in Marseille, France, and to 67% in Bangladesh [84] and 21% in Lao PDR [85]. A pilot

meta-analysis conducted using the search terms 'murine typhus + incidence', 'murine typhus + acute febrile illness', and 'murine typhus' retrieved 880 articles since 1934 and only the 325 articles dating to 2001 were reviewed for adequacy of details and methodology, including geographic location for inclusion in the study, and with sole focus on studies of incidence among prospective acute febrile disease studies (see Supplementary material, Table S2). Selected studies originated from every continent except Antarctica. Of the 325 articles reviewed, 26 were selected for inclusion, comprising a total of 8642 patients, among which 1486 (17.2%) were deemed to have murine typhus based on serology and PCR and culture. The median proportion of murine typhus cases among the studies was 7.9% (interquartile range 4.2–14.5%).

As with scrub typhus, the clinical presentation is most often undifferentiated fever, but lacking an eschar (Table 2) [86–88]. A rash can develop in half of infected patients. Complications are similar to those of other rickettsioses. The case fatality rate is variably between 0.5 and 4% in contemporary series and most infections have a benign clinical course; however, morbidity can be significant particularly in cases of delayed diagnosis and in the elderly who, even with proper treatment, suffer a greater number of complications.

Spotted fever group rickettsiosis is best known because of the severity of RMSF (*R. rickettsii* infection) [89–91]. Whereas RMSF occurs only in the western hemisphere, other SFGR occur globally. Individual species vary in pathogenicity, but the underlying pathology for SFGR is endothelial cell infection, vasculitis and increased systemic and pulmonary vascular permeability [92]. The majority of SFGR are transmitted by tick bites; for *R. rickettsii* this includes *Dermacentor variabilis*, *Dermacentor andersoni* and *Rhipicephalus sanguineus* in North America, and *Amblyomma* species in Central and South America. *Rickettsia conorii* is vectored by *Rhipicephalus sanguineus* in the Mediterranean region, whereas *R. africae* is transmitted via *Amblyomma* tick bites in Sub-Saharan Africa. A few SFGR are vectored by other arthropods (mites for rickettsial pox (*Rickettsia akari*); fleas for cat flea typhus (*Rickettsia felis*)).

Spotted fever group rickettsioses are among the most virulent of all human infections, especially RMSF for which historical case fatality rates of 25–80% are recorded [92]. Contemporary data describe case fatality rates between 0.5 and 8–12% in the USA and up to 35% in Brazil. In Beja, Portugal in 1997, the case fatality rate in hospitalized patients with Mediterranean spotted fever was 32.3% and in the USA, the American Indian population is especially affected by RMSF with both incidence and case fatality rates significantly higher than that of other racial groups; this incidence continues to increase at a disproportionate rate. Of particular interest is the high seroprevalence of SFGR in many regions around the world where human SFGR are not known to exist [91,93]. In southern Taiwan, a region where spotted fever is poorly characterized, an investigation of 413 febrile patients found that 49 (11.9%) were seropositive for spotted fever group rickettsiae. Spotted fever is also increasingly diagnosed in South Asia, including Sri Lanka where spotted fever rickettsiosis occurred in 10% of febrile patients, second in number only to leptospirosis [13], and the seroprevalence was 33% for any rickettsial infection. Despite inadequate data collection, reported cases of SFGR are at historical highs in the USA and globally [64,91]. The reasons for this are not clear, but could include greater clinical suspicion, case definition and diagnostic test changes, or real increases in disease incidence and prevalence. The latter is in part likely given the increasingly defined distribution of infected vectors [91].

Clinical manifestations of RMSF and other SFGR include fever, and variably headache, myalgias, rash, (macular, maculopapular or petechial), abdominal pain, nausea, vomiting, diarrhoea, and in severe cases, renal failure, non-cardiogenic pulmonary oedema, shock and multi-organ failure, and CNS involvement (meningoencephalitis, cerebral oedema, herniation) (Table 2). Most SFGR do not demonstrate this degree of severity, but it is possible in any single case. Many patients have eschars (except in RMSF), and some develop vesicular rashes. Major factors for ineffective diagnosis and delayed therapy include absence of a typical rash, presentation during non-peak tick activity season, and presentation during first 3 days of illness when a rash may not be present [94].

Murine typhus and SFGR diagnosis requires clinical suspicion because adequate diagnostic tests in the acute phase of disease are not available [95]. If rash is present, rickettsiae can be demonstrated by immunohistochemistry in rash lesion biopsies in SFGR and murine typhus. Although PCR sensitivity in blood is low (<25%), accumulating data suggest that skin biopsy PCR could be sensitive [91]. The most sensitive diagnostic test, immunofluorescence assay, is only confirmatory because it requires acute and convalescent serum antibody titres. However, in general, immunofluorescence assays cannot distinguish SFGR species or cross-reactions with typhus group rickettsiae. Cultivation is not timely and is generally considered dangerous and unacceptable for clinical laboratories. Perhaps most challenging of all is the fact that serodiagnosis only works well with paired sera separated by at least 14 days, precluding its use for diagnosis and management at the time of active infection. Given the marked but mostly unrecognized incidence of these infections worldwide, and their high case fatality rates, readily available accurate and sensitive point-of-care diagnostic devices are desperately needed.

Both SFGR and murine typhus are treated with doxycycline; however, in a study that examined treatment practices in the USA, fewer than 40% of healthcare providers correctly chose

doxycycline as the treatment of choice for RMSF for children <8 years of age, a potential cause of the increased case fatality rates in this age category [95]. Controlled clinical trials have not been conducted to adequately define efficacy among antibiotics except for *R. conorii* but some evidence exists to support the use of fluoroquinolones for murine typhus and forms of SFGR other than RMSF, though their use has become controversial [96]. Currently, no vaccines exist for spotted fever rickettsiosis, murine typhus or scrub typhus. Prevention requires avoidance of bites by ticks, fleas and chiggers by avoiding infested locations, by wearing clothing that potentially excludes their ability to access the skin, or by use of repellents. Prophylactic antibiotic treatment is not well assessed and currently cannot be advocated.

Conclusions

Other than leptospirosis, relapsing fever, scrub typhus and rickettsioses are not yet recognized as neglected diseases by the WHO, despite data that illustrate their high incidence and prevalence and the potentially dramatic morbidity and costs to human life. Future goals should include establishing their incidence and prevalence comparative to other infections that seemingly occur at similar rates. Development of easily deployed point-of-care diagnostics will be critical to assist in accurately collecting these data and in directing appropriate care and treatments. Careful clinical and epidemiological/ecological studies, including the roles of animal or vector reservoirs that increase risk will identify opportunities to prevent infection. Study of human infections could identify new locations where other preventative methods, including vaccination might be used. A basic analysis of disability-adjusted life-years will depend on careful collection of such data and could drive deployment of critically short resources in areas where these infections cause more harm than others commonly believed to have the greatest impact on humans and their environments.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.04.022.

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