Emerging Infections 3
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Chapter 12

The Emerging Role of Pathology in Infectious Diseases

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BACKGROUND AND OVERVIEW

In the United States, infectious diseases continue to produce extensive mortality and morbidity. On a global scale, infectious diseases are the leading cause of death (32, 80). During the past three decades, the world has witnessed the emergence of many new infectious diseases as well as the reemergence of previously known diseases. Notable recent infectious disease challenges faced by public health professionals include Legionnaires' disease, AIDS, cat scratch disease, *Helicobacter pylori*-associated disease, ehrlichiosis, hepatitis C, hantavirus pulmonary syndrome (HPS), Ebola virus hemorrhagic fever (EHF), leptospirosis, and new-variant Creutzfeldt-Jakob disease (9, 15, 31). Effective surveillance systems are essential for the timely recognition of emerging infections before they become major public health problems. These systems require a thorough understanding of the epidemiology of infectious diseases, good communication and collaboration among health care professionals and the public health community, and very importantly, effective diagnostic capabilities. Strengthening of the capacity of various health care professionals to diagnose infectious diseases is therefore one of the critical steps in an effective response to emerging microbial threats (9, 15).

Infectious disease pathologists have a long-standing history of contributing to the diagnosis and identification of infectious disease agents. Notable early contributions include landmark studies of *Rickettsia rickettsii* by the pathologists Howard Ricketts and S. Burt Wolbach (71). More recently, because of increased awareness of infectious diseases, there has been renewed interest and revitalization in infectious disease pathology (57, 59). Table 1 provides a sample of recently described diseases for which pathologists have had an important role in identifying the causative agent or describing the pathogenetic process.

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Table 1. Selected examples of emerging infectious diseases with significant contributions by pathology

Disease or syndrome	Agent(s)	Selected reference(s)
Cryptosporidiosis	*Cryptosporidium parvum*	29, 38
Legionellosis	*Legionella* spp.	79
Cat scratch disease	*Bartonella henselae*	1, 61, 75
Adult T-cell leukemia/ lymphoma	Human T-cell lymphotropic virus type 1	3
AIDS	Human immunodeficiency virus type 1	5, 19, 55
Gastroduodenal disease	*Helicobacter pylori*	47, 74, 81
Microsporidiosis	*Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*	7, 43, 58
Bacillary angiomatosis	*B. henselae*, *Bartonella quintana*	37, 50, 66
Human ehrlichioses	*Ehrlichia chaffeensis*, *Ehrlichia phagocytophila*	2, 23, 24, 72
Granulomatous amebic encephalitis	*Balamuthia mandrillaris*	70
Coccidian enteritis	*Cyclospora cayetanensis*	21, 67
Hantavirus pulmonary syndrome	SNV	28, 85, 86
Leptospirosis	*Leptospira* spp.	16, 69, 91
Kaposi's sarcoma	Human herpesvirus 8	4, 20
Ebola hemorrhagic fever	Ebola virus	27, 35, 39, 90
Viral encephalitides	Hendra virus, Nipah virus	17, 18, 44, 62, 73
	Cache Valley virus	63
	Enterovirus 71	13, 65
New-variant Creutzfeldt-Jakob disease	Prion	77

Pathologists are characteristically among the first health care workers involved in the recognition of infectious disease outbreaks and are in an excellent position to discover new pathogens and infectious disease syndromes. These discoveries have been accomplished by collaborative research with colleagues from other scientific disciplines, such as epidemiology, clinical care, veterinary medicine, and microbiology.

Traditionally, anatomic pathologists have relied on routine histopathology or special stains to arrive at a diagnosis. Some pathogens, such as certain species of fungi and bacteria, are morphologically distinct and can be readily identified by microscopy or with special stains. Additionally, viral pathogens, such as rabies virus, herpesviruses, parvovirus, and measles virus, produce characteristic intracellular inclusions which may support a specific diagnosis. Observations of patterns of tissue injury and host responses are also important because tissues often react to infections in specific and predictable ways, making it possible to suspect certain infections. Nonetheless, in many cases it is impossible to identify a specific infectious agent by morphologic observations alone. Recent experience at the Centers for Disease Control and Prevention (CDC) and other institutions has shown that use of a combination of traditional morphology with immunologic and molecular

pathology techniques is an extremely useful approach for the confirmation of diagnoses for patients with otherwise unexplained illnesses.

Immunologic and molecular methods, such as immunohistochemistry (IHC), in situ hybridization (ISH), and PCR, have revolutionized the ability of pathologists to diagnose and study infectious diseases (88). These techniques allow detection of microbial antigen or nucleic acid sequences in formalin-fixed, paraffin-embedded tissues. In this context, unexplained diseases may be investigated prospectively, as well as retrospectively, with archival tissue samples. Traditionally, microbial identification has relied primarily on serologic assays and culture techniques. Serologic assays characteristically require the collection of paired serum samples to demonstrate rising titers of antibody against a specific pathogen. Certain circumstances, including immunocompromised states, early therapy, or rapid death, may preclude the development of diagnostic antibody responses. Finally, serologic tests may occasionally give nonspecific results, especially if only a single sample is available for evaluation. Visualization of microbial antigens or nucleic acids in the context of pathology allows the pathologist to assess the clinical significance of serologic test results or microbial isolation.

Culture of fastidious pathogens and obligate intracellular viruses and bacteria is often labor-intensive, may require biosafety facilities not readily available, and may take weeks or months to yield results. By comparison, the use of molecular pathology techniques offers several distinct advantages over traditional microbiologic methods, including speed, sensitivity, reduced risk of exposure of laboratory personnel to the agent, and tissue localization of pathogens. The unique role of molecular pathology in the confirmation of infection in seronegative patients and the detection of fastidious or nonculturable organisms cannot be overemphasized. A prompt, specific diagnosis can prevent the use of unnecessary therapeutic regimens, reduce the need for invasive diagnostic procedures, and alert the medical community to institute appropriate therapeutic and precautionary measures that can control or halt the spread of the disease.

The use of molecular techniques in infectious disease pathology has proven to be a powerful tool in the diagnosis of previously unexplained syndromes. By using a syndrome-based approach, the pathologist can narrow the diagnostic possibilities and perform targeted molecular testing for a limited number of organisms. A syndrome-based approach to diagnosis is currently used for CDC surveillance efforts for Unexplained Deaths due to Possibly Infectious Causes. This program represents the first attempt to conduct early detection of new infectious diseases in large segments of the U.S. population (49).

Table 2 lists the IHC and ISH tests available in laboratories of the Infectious Disease Pathology Activity (IDPA), which is CDC's organizational unit that serves as a scientific and technical resource for studies of the pathology and pathogenesis of infectious diseases. Epidemiologic, clinical, and histopathologic findings enable CDC pathologists to use the most appropriate initial immunologic and molecular pathology tests. This chapter highlights some of the contributions of CDC pathologists in addressing some of the challenges posed by new, emerging, and reemerging infectious diseases.

Table 2. IHC and ISH tests used by IDPA, CDC, for diagnosis of infectious disease agents

Pathogen[a]	Test availability	
	IHC	ISH
Viruses		
Adenovirus	+	+
Crimean-Congo hemorrhagic fever virus	+	+
Dengue virus	+	
Ebola virus	+	+
Eastern equine encephalitis virus	+	
Enterovirus 71	+	+
Guanarito virus	+	
Hantaviruses (cross-reactive and serotype-specific assays)	+	+
Hendra virus	+	+
Herpesviruses		
HHV-1, HHV-2 (HSV-1, HSV-2)		+
HHV-3 (VZV)		+
HHV-4 (EBV)		+
HHV-5 (CMV)	+	+
HHV-6		+
HHV-7		+
HIV-1	+	+
HTLV-1		+
Human papillomavirus		+
Human parvovirus	+	+
Influenza and parainfluenza viruses	+	
Japanese encephalitis virus	+	
JC virus		+
Junin virus	+	
La Crosse encephalitis virus	+	
Lymphocytic choriomeningitis virus		+
Machupo virus	+	
Marburg virus	+	
Measles virus	+	+
Nipah virus	+	+
Rabies virus	+	
Respiratory syncytial virus	+	
Rift Valley fever virus	+	
SIV		+
St. Louis encephalitis virus	+	
Venezuelan equine encephalitis virus	+	
Western equine encephalitis virus	+	
Yellow fever virus	+	
Rickettsiae and ehrlichiae		
Ehrlichia chaffeensis	+	+
Ehrlichia equi	+	+
Coxiella burnetii	+	
Spotted fever group rickettsiae	+	
Typhus group rickettsiae	+	

Table 2. *Continued*

Pathogen[a]	Test availability	
	IHC	ISH
Bacteria		
Bartonella henselae	+	
Bartonella quintana	+	
Brucella spp.	+	
Chlamydia spp.	+	+
Francisella tularensis	+	
Helicobacter pylori	+	
Legionella pneumophila (serogroups 1, 5, and 6)	+	
Leptospira spp.	+	
Listeria monocytogenes	+	
Mycoplasma pneumoniae	+	
Neisseria meningitidis (serogroup C)	+	
Streptococcus pneumoniae	+	
Treponema pallidum	+	
Yersinia pestis	+	
Parasites		
Acanthamoeba culbertsoni	+	
Balamuthia mandrillaris	+	
Naegleria fowleri	+	
Toxoplasma gondii	+	
Trypanosoma cruzi	+	
Fungi (*Aspergillus* spp.)	+	

[a]Abbreviations: HHV-1, human herpesvirus 1; HSV-1, herpes simplex virus type 1; VZV, varicella-zoster virus; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HIV-1, human immunodeficiency virus type 1; HTLV-1, human T-cell lymphotropic virus type 1; SIV, simian immunodeficiency virus.

HANTAVIRUS PULMONARY SYNDROME

In May 1993, the deaths of several previously healthy individuals baffled health care workers in the southwestern United States and at CDC. These patients developed an influenza-like illness, followed by rapidly progressive pulmonary edema, respiratory insufficiency, and shock (8, 34, 40). Initial laboratory testing of patient specimens for bacterial, viral, and toxic causes failed to identify an etiologic agent. Patient specimens were subsequently forwarded to CDC for further studies.

CDC quickly performed batteries of immunoassays for numerous bacterial and viral pathogens in addition to toxicologic testing. The first clue came when antibodies reactive with hantaviruses were detected in the serum of the patients. However, the suggestion that a hantavirus was the etiologic agent of this outbreak was met with skepticism for several reasons. First, the pattern of serologic reactivity was atypical for known hantaviruses, and patients resided in a geographic area where hantavirus disease had not previously been documented. Second, all patients with hantavirus-associated illnesses known prior to this outbreak shared various degrees of fever and renal involvement, with or without hemorrhagic manifestations. Finally, and most importantly, the pronounced pulmonary involvement in

patients involved in this outbreak had not been a prominent clinical feature for other patients with recognized hantavirus-associated disease.

An initial examination of coded specimens allowed correlation between the histopathologic features of patients with fatal disease and those patients with serologic evidence of hantavirus infection. Although this correlation led to increased confidence in the diagnosis during the initial days of the laboratory investigation, additional confirmation was needed to identify the nature of the hantavirus and to establish an etiologic relationship. Within days, demonstration of viral antigens in tissues from patients with HPS and amplification of genetic sequences from autopsy material established a previously unrecognized hantavirus as the causative agent (Color Plate 1A) (85). The newly identified agent, Sin Nombre virus (SNV), was subsequently isolated at CDC from a deer mouse, *Peromyscus maniculatus*, the natural reservoir and vector of this zoonotic virus (25). Although most cases of HPS in the United States are caused by SNV, other SNV-like hantaviruses have been implicated, including Bayou and Black Creek Canal viruses. Reports of HPS outside North America are increasing, primarily in South America (10, 68, 76, 78).

Correlation of clinicopathologic findings with the cellular tropism of SNV and its distribution within human tissues provided important insights into the pathogenesis of HPS (26, 28, 42). IHC analysis showed the widespread presence of hantaviral antigens in endothelial cells of the microvasculature, particularly in the lung. Hantaviral antigens were also observed within follicular dendritic cells, macrophages, and lymphocytes. The magnitude and extent of pulmonary microvascular involvement in HPS indicated that functional derangement of endothelial cells is central to the pathogenesis of the hemoconcentration, pulmonary edema, and shock seen in patients with HPS (82, 85).

Pathology was instrumental in answering questions early in the investigation related to the novelty of this infection. IHC analysis was used to identify retrospective cases in the United States that had occurred as early as 1978, predating the outbreak in the southwestern United States by 15 years (64, 83, 86). The occurrence of these earlier fatal cases of HPS caused by a virus or viruses antigenically related to SNV suggests that environmental and ecologic factors rather than genetic reassortment were responsible for the 1993 outbreak. These observations are in agreement with phylogenetic studies of the SNV genome.

LEPTOSPIROSIS

During the fall of 1995, more than 2,000 residents of northern Nicaragua contracted an acute febrile syndrome (16, 69, 91). Patients presented at local health centers with fevers, chills, headaches, and musculoskeletal pain. Among the most severely ill patients, additional clinical manifestations included severe abdominal pain, hypotension, respiratory insufficiency, and hemoptysis. At least 40 patients died with acute pulmonary hemorrhage and respiratory insufficiency. Because of hemorrhagic manifestations, the initial clinical speculation centered particularly on dengue hemorrhagic fever and other arthropod- and rodent-borne viral diseases. However, these conditions were excluded by using serologic, PCR, and IHC assays of serum and tissue specimens (91).

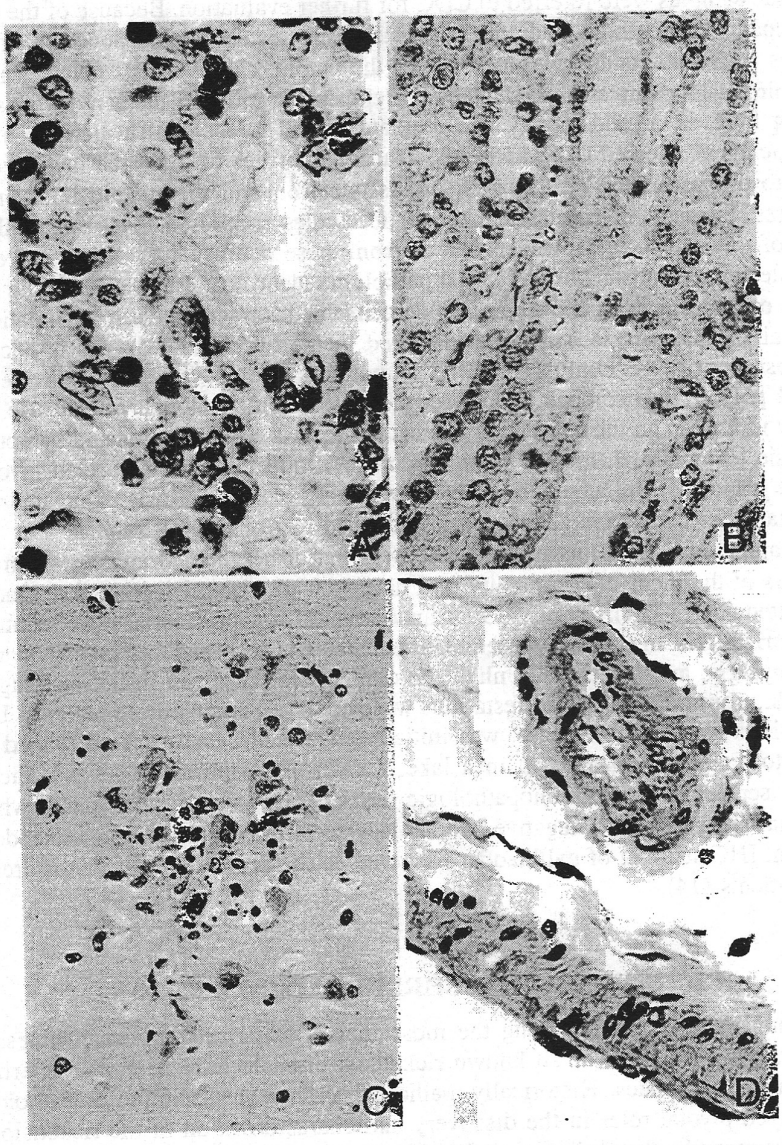
Tissue samples were referred to CDC for further evaluation. Because of the renal and hepatic histopathologic findings, tissues were tested for the presence of leptospires with IHC stains developed during the outbreak. These tests revealed intact leptospires and granular and filamentous leptospiral antigens in many organs, including lung, liver, and kidney (Color Plate 1B). The rapid identification of the etiologic agent allowed CDC and local health authorities to focus the investigation on leptospirosis-related risk factors, disease control, and therapeutic intervention measures. Subsequent culture and serologic studies supported the leptospiral etiology of this outbreak. However, results from these confirmatory assays were not available for several weeks after initial pathologic identification because of the slow growth of leptospires in culture and the time needed for seroconversion in patients.

Initially, leptospirosis was not considered in the differential diagnosis because the most impressive clinical findings in patients with severe disease were the marked pulmonary hemorrhage and respiratory difficulty, along with diffuse pulmonary infiltrates on the chest X rays. Furthermore, unlike the signs and symptoms of classic leptospirosis, there was no significant clinical evidence of renal involvement or jaundice. Similar reports of large outbreaks of leptospirosis associated with pulmonary hemorrhage have also been described in Korea and China.

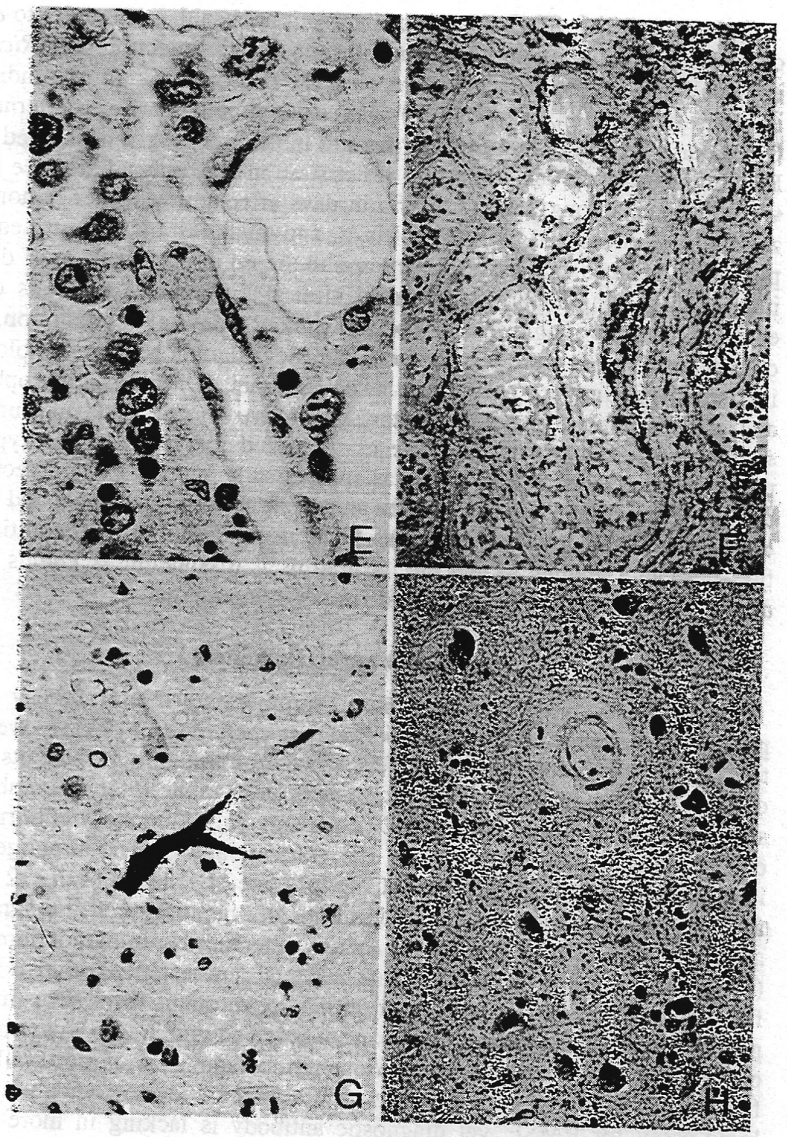
A major outcome of this investigation was that it heightened awareness among members of the medical community of a disease with global endemicity, including the United States (14). In July 1997, an outbreak of leptospirosis was identified among triathletes in the midwestern United States. These patients presented with fever, myalgia, and headache. Unlike the clinical features seen in the Nicaraguan outbreak, prominent renal manifestations were observed among these patients. Leptospirosis, a waterborne disease, was initially suspected because of prolonged exposure to water during a swim in a lake. Laboratory confirmation was achieved through serologic and immunopathologic assays. Two of the participants whose serum had initially tested negative had cholecystectomies because of acute abdominal pain. IHC staining of gallbladder tissue revealed the presence of leptospires in these patients (14).

RICKETTSIAL AND EHRLICHIAL INFECTIONS

Rickettsial diseases are among the most ancient of described human illnesses; however, more than half of all known rickettsial diseases have been characterized in the last two decades. Historically, pathologists and the discipline of pathology have played pivotal roles in the discovery and characterization of the rickettsioses and, more recently, the ehrlichioses (71, 72). Because rickettsiae and ehrlichiae can be isolated only in cell culture and because the diseases that they cause mimic a variety of other infectious processes, direct visualization of these small gram-negative bacteria in patient tissues and body fluids has been fundamental in the initial recognition of rickettsial and ehrlichial infections. Newly available techniques in pathology provide more sensitive and specific methods for diagnosis and for reassessment of the magnitude of morbidity and mortality attributable to these agents.



Color Plate 1. Emerging infectious diseases. Immunostaining of viral and rickettsial antigens in patient tissues by IHC is shown. (A) Hantavirus pulmonary syndrome, lung. Viral antigens are localized within the pulmonary microvasculature. (B) Leptospirosis, kidney. Intact and granular staining of leptospires within renal interstitium is shown. (C) Epidemic typhus, cerebral cortex. Staining of *Rickettsia prowazekii* within a characteristic glial nodule is shown. (D) Rocky Mountain spotted fever, leptomeninges. Spotted fever group rickettsiae are localized within the



endothelia of medium-sized vessels. (E) Ehrlichiosis, bone marrow. Morulae of *E. chaffeensis* are stained within mononuclear cells. (F) Ebola hemorrhagic fever, skin. Abundant viral antigens in connective tissue surrounding a sweat gland in dermis are shown. (G) Enterovirus 71 encephalitis, central nervous system. Immunostaining of viral antigens within a neuron is shown. (H) Nipah virus encephalitis, cerebral cortex. Viral antigens are present within neurons and glial cells. Note the abundant extracellular antigen.

Epidemic Typhus

In January 1996, CDC alerted the international health community to an outbreak of epidemic typhus in Burundi by confirming a fatal infection with *Rickettsia prowazekii* in a European health care worker. The outbreak in Burundi ultimately involved over 43,000 persons (52). The sentinel patient was an International Red Cross nurse who provided care for inmates in the Ngozi Prison, situated in northern Burundi. During the last 2 months of her stay, an unexplained increase in mortality was observed among prisoners. Within days after returning to her home in Switzerland, she developed high fever, chills, and myalgias and was subsequently hospitalized. Because of her travel history and the rapid development of disseminated intravascular coagulopathy, the initial clinical differential diagnosis centered on exotic viral hemorrhagic fevers. The patient died 3 days after admission, and tissues obtained at autopsy were forwarded to CDC for evaluation. Histopathology revealed interstitial pneumonitis, myocarditis, portal triaditis, hepatic erythrophagocytosis, and glial nodules in the cerebral cortex, suggestive of a rickettsial infection. IHC staining for typhus group rickettsiae confirmed the diagnosis of typhus in this patient (Color Plate 1C), which was subsequently supported by detection of antibodies reactive with *R. prowazekii* in an acute-phase serum sample and by detection of *R. prowazekii* DNA in patient tissues by using PCR (92). This sentinel case and the subsequent epidemic in Burundi remind us that epidemic typhus remains endemic worldwide with the potential for periodic emergence.

Spotted Fever Rickettsioses

During 1996 and 1997, acute-phase serum samples and autopsy tissues from patients with suspected fatal Rocky Mountain spotted fever (RMSF) were submitted to CDC for confirmatory testing. RMSF was confirmed in 12 patients on the basis of IHC staining for spotted fever group rickettsiae alone or in combination with serologic testing and/or PCR. All 12 patients with laboratory-confirmed cases of disease had unequivocal evidence of rickettsiae and rickettsial antigens primarily in and around vascular endothelium (Color Plate 1D). Three of the 12 patients with fatal RMSF as confirmed by IHC additionally demonstrated a diagnostic titer of antibody reactive with *R. rickettsii* by using the indirect immunofluorescence assay (IFA). However, the remaining nine patients demonstrated positive IHC staining for rickettsiae in the absence of a confirmatory serologic result. This included seven patients from whom serum samples were obtained within 2 to 8 days after the onset of illness and two patients from whom serum samples were not available for confirmatory testing (45). IFA is the best recognized and most widely available serologic assay for RMSF, yet diagnostic antibody is lacking in more than 50% of patients in the first week of disease. Since at least half of all deaths as a result of RMSF occur in the first 8 days of the illness, serology alone will miss the majority of patients with fatal disease (45). In this context, immunopathology provides a powerful and much needed tool for better assessment of the magnitude of mortality attributable to *R. rickettsii*.

In recent years, the recognized emergence of several spotted fever group rickettsioses in new geographic areas has been facilitated by immunopathologic studies.

Between November 1993 and March 1994, a cluster of six pediatric patients with fever and rash was identified in Jujuy Province, Argentina. Although spotted fever had previously been documented in several countries in Central and South America, immunopathologic findings provided the first confirmatory evidence of the occurrence of spotted fever group rickettsial infections in Argentina (53).

The role of pathology in posttravel diagnosis of imported rickettsial disease is exemplified by the recognition of an emerging rickettsial infection that occurs in travelers from Africa. The disease, African tick bite fever, was described as a distinct entity in 1994 (33) and has recently been observed among U.S. travelers returning from southern Africa (12). In June 1997, a 66-year-old man returned to the United States after travel to Zimbabwe with fever, multiple skin lesions, and inguinal adenopathy. Skin biopsy specimens were forwarded to CDC for evaluation, by which IHC demonstrated abundant spotted fever group rickettsiae in areas of intense perivascular infiltrates and dermal necrosis. DNA of *Rickettsia africae*, the etiologic agent of African tick bite fever, was subsequently amplified from these tissues by PCR.

Infections Caused by *Ehrlichia* spp.

In June 1992, a 41-year-old human immunodeficiency virus-seropositive woman from Arkansas was hospitalized with an acute illness characterized by fever, diarrhea, and pancytopenia. Despite treatment with multiple broad-spectrum antibiotics, the patient died 2 weeks after the onset of her illness. Small, intracytoplasmic aggregates of bacteria were identified in the mononuclear cells in bone marrow, suggesting infection with an *Ehrlichia* sp. (46). IHC and PCR confirmed *Ehrlichia chaffeensis* as the etiologic agent (Color Plate 1E). Further confirmation of the etiologic agent was subsequently performed by using a novel ISH test for ehrlichiae (22). Ehrlichiae are small, obligately intracellular bacteria closely related to the rickettsia. Although well-recognized as pathogens of animals for over 60 years, these tick-borne agents have only recently been associated with disease in humans in the United States. These include ehrlichial infections caused by *E. chaffeensis* and the as-yet-unnamed agent of human granulocytic ehrlichiosis. As with the rickettsioses, these emerging infections can be difficult to diagnose by clinical criteria alone. Characteristically, the ehrlichioses require specialized serologic or molecular assays to confirm the disease. IHC and ISH have provided powerful adjuncts to the panel of confirmatory tests for ehrlichiae. In the past two decades, pathologists have played significant roles in the identification of these bacteria and in enhancing our knowledge of the expanding clinical spectrum of these diseases (72).

EBOLA HEMORRHAGIC FEVER

In April and May 1995, health authorities in Zaire identified an outbreak of a rapidly progressive hemorrhagic fever-like illness associated with a high rate of mortality. At least two clusters of patients in two different hospitals in Kikwit, a large city located several hundred kilometers east of the capital Kinshasa, were identified (11, 56). Patients experienced fever, asthenia, abdominal pain, nausea,

vomiting, diarrhea, and headache (35). Initially, the disease was clinically diagnosed as epidemic dysentery; however, reports indicated that the disease had spread among health care workers involved in patient care and treatment, causing local medical providers to suspect a viral hemorrhagic fever (6, 35). Patient specimens were forwarded to CDC for laboratory testing. Immunoassay, PCR, and IHC testing identified Ebola virus as the etiologic agent of the outbreak (36, 87). The Kikwit epidemic of EHF was the first large outbreak of recognized filovirus disease among humans in nearly 20 years.

IHC and electron microscopic examination showed that endothelial cells, mononuclear phagocytes, and hepatocytes are the main targets for infection, and IHC showed an association of cellular damage with viral infection. However, while Ebola virus antigen can readily be detected by IHC in almost any part of the body, the amount and extent of immunostaining in the skin were remarkable in all patients with EHF examined (90) (Color Plate 1F). This finding provided an opportunity to develop and use an easy means of surveillance for EHF and, possibly, other hemorrhagic fevers.

Traditionally, laboratory diagnosis of EHF has been accomplished through virus isolation or serologic assays (36, 51, 84, 87, 89). Because of biosafety hazards associated with the handling and testing of Ebola virus, these assays can be performed only in a few specialized laboratories under biosafety level 4 containment conditions. Such testing requires transportation of potentially dangerous biological specimens to these laboratories from remote sites where the disease occurs by using a cold chain and rigorous international packaging and shipping procedures.

Small skin-snip biopsy specimens can be taken and placed in formalin, obviating the need for a cold chain and concerns about infectivity, and then moved to a central laboratory for IHC testing. In 1996, a surveillance program based on the test with skin-snip biopsy specimens was used to confirm an outbreak of EHF in Gabon and proved to be a more practical, cost-effective surveillance mechanism that could be managed easily in a large geographic area without on-site supervision and support (90).

Early detection of EHF is critical in stopping the spread of the disease. If EHF had been suspected or confirmed by the use of the test with skin-snip biopsy specimens for one of the patients with an early case of EHF in Kikwit, Zaire, from January to April 1995, the hospital might have taken steps earlier to improve infection control measures, such as the use of sterile needles, gloves, hand-washing, and isolation of suspected patients. These measures may not have prevented all cases of EHF transmission in the community, but they certainly may have helped protect the many health workers who contracted the disease (39).

ENTEROVIRUS 71 ENCEPHALOMYELITIS

CDC was involved in the investigation of two large outbreaks of hand, foot, and mouth disease in Malaysia and Taiwan in 1997 and 1998, respectively (13). Unique to these outbreaks was the rapid clinical deterioration and death (29 deaths in Malaysia, 55 deaths in Taiwan) among affected children. Initial clinical reports from Malaysia in 1997 suggested that myocarditis was the cause of death. However,

no histopathologic evidence of myocarditis was observed in tissues forwarded to CDC for evaluation. Furthermore, histopathologic examination of central nervous system tissue revealed a fulminant brain stem encephalomyelitis. These findings suggested neurogenic pulmonary edema rather than a primary cardiac process as the cause of death in these children. Neurons in areas of inflammation and tissue necrosis were positive for enterovirus 71 (EV71) by IHC testing (Color Plate 1G). Isolation of EV71 from several patients further supported the diagnosis. These findings were helpful in guiding the epidemiologic and laboratory investigations of the subsequent outbreak in Taiwan in 1998.

The etiologies of the deaths in Malaysia and Taiwan are still under investigation, and further studies are needed to determine whether EV71 infection alone was responsible for all deaths reported from these outbreaks. Case-control studies are under way in Taiwan to further assess the associations between EV71 infections and rapid death and to identify other potential factors or cofactors (e.g., toxins, medicines, or environmental exposures) that might have contributed to the disease process.

NIPAH VIRUS ENCEPHALITIS

Between September 1998 and May 1999, over 250 residents of the states of Perak and Negri Sembilan in Malaysia contracted an acute febrile encephalitis. Over 100 deaths were associated with this outbreak (17, 18). A similar illness occurred among abattoir workers in Singapore during March 1999. In both Malaysia and Singapore, the disease occurred primarily among males who had been exposed to pigs. Concurrent with the human cases were illnesses and deaths among pigs from the same regions. Patients presented with 3 to 14 days of fever and headache, followed by drowsiness and disorientation. Patients with severe disease developed generalized seizures and coma within 24 to 48 h. In comparison, the disease in swine primarily involved the respiratory system, with manifestations including rapid and labored breathing and an explosive, nonproductive cough. Only a few pigs exhibited neurologic signs such as lethargy and aggressive behavior.

Initial clinical speculation focused on Japanese encephalitis (JE). However, several clinical and epidemiologic features of epidemic JE differed from the features of the outbreaks in Malaysia and Singapore, namely, (i) a predominance of cases in men, (ii) a strong association of disease with pig farming, (iii) the magnitude of disease in adult pigs, and (iv) a history of JE vaccination in many of the victims.

Virus isolates, serum samples, and autopsy tissues from several patients were forwarded to CDC for evaluation. Patient serum samples were negative for antibodies to JE virus. Electron microscopy studies of viral isolates revealed ultrastructural characteristics of a paramyxovirus. Immunofluorescent-antibody studies of infected cell cultures suggested infection with a Hendra virus ("equine morbillivirus") or a Hendra virus-like agent. Testing of patient serum samples by using a capture immunoglobulin M enzyme-linked immunosorbent assay demonstrated antibodies reactive with Hendra virus antigens. Histopathologic features of patients with fatal cases of disease, such as syncytial multinucleated endothelial cells and characteristic cytoplasmic viral inclusions, further supported the diagnosis of para-

myxovirus infection. IHC studies showed systemic distribution of viral antigens within the vascular endothelium, as well as within several cell types in the central nervous system, including neurons and glial cells (Color Plate 1H) (17, 18, 48).

Molecular evaluation of viral isolates and human tissue by PCR and nucleotide sequencing revealed a virus related to, but distinct from, the recently described Hendra virus (30, 41, 44, 54, 62, 73). This new virus was tentatively named Nipah virus after the region where the first cases of disease were observed. Testing of swine specimens by molecular, serologic, and immunohistologic tests also demonstrated infection with the same virus. The molecular and immunologic reagents developed after the 1994 discovery of Hendra virus were instrumental in elucidating the etiology of this disease and illustrate the need for preparedness in confronting emerging infections.

CONCLUSIONS

A fundamental principle in the concept of emerging infections is recognition of disease. As illustrated in this chapter, disease recognition by infectious disease pathologists can play a key role in the study of emerging infectious diseases. It must be emphasized that pathologists from other disciplines, including medical examiners and academic, community, and veterinary pathologists, have also made important contributions to these studies. Improved collaboration among pathologists will undoubtedly advance this field.

This review provides several examples of the frontline role of pathology in guiding the early phases of epidemiologic investigations of infectious diseases. The sciences of pathology and epidemiology are complementary disciplines. Each is largely based on the recognition of shared patterns, albeit one from a microscopic perspective and the other from a macroscopic approach. The increasing role of pathology as an active partner in surveillance activities (90) highlights the logical and synergistic combination of these two disciplines in the study of emerging and reemerging infections.

It is important to consider that in an era of increased awareness of potentially new and emerging infections, contemporary pathologic methods can be used effectively in the identification and diagnosis of new emerging infectious diseases (88). The use of newly available molecular and immunologic assays also allows new insights into the pathogenesis and epidemiology of historically familiar diseases. These may include recognition of atypical presentations, such as pulmonary insufficiency caused by hantaviruses or pulmonary hemorrhage caused by *Leptospira*, or better definition of morbidity and mortality attributable to a disease, e.g., RMSF (45, 85, 91). However, with the realization that these new molecular and immunologic methods facilitate investigations that were never before possible, it is imperative that a continued role for standard pathology methods be maintained: the use of autopsies for unexplained deaths, the use of routine histopathology to help focus and guide the subsequent application of specific specialized tests, and the need for systematic archiving of frozen and paraffin-embedded biopsy and autopsy specimens from patients with diseases of unknown etiology to facilitate the study of new clinical entities and their associated etiologic agents (60).

Further challenges are difficult to predict, but it is certain that additional infectious diseases remain to be discovered. As in the past, successful identification and characterization of these diseases will rely on a multidisciplinary approach. The abilities of pathologists to diagnose infectious diseases have been expanded remarkably in the past decade. Continued advancements in this discipline will contribute to the field of emerging infectious diseases.

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